Spatial distributions of phytoplankton in rock pools:

metacommunities to molecules

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Abstract

Ecological studies on how populations and communities assemble and function traditionally focussed on the effects of local environmental conditions and species interactions. Still, no species, the consequences of its actions or interactions, are confined to a single spatial scale. The importance of spatial processes, such as dispersal, immigration, emigration, habitat connectivity and availability, was eventually realised through metapopulation and -community ecology. Accordingly, species distributions are deciphered at large scales, whereas processes at small scales reveal the mechanisms behind the observed patterns.

Spatial distributions of microorganisms are generally considered to be wide, even global. This implies that fundamental patterns and processes, such as food web structures, primary production, or carbon cycling governed by these organisms are structured by local processes. Applying the metacommunity concept on microorganism assemblages has loosened the perception of them as closed systems strongly structured by local environmental conditions. However, uncertain species identities and dispersal modes impede the comprehension of their patterns and processes. Moreover, an anthropocentric scale is often applied on microorganisms. The scale of fundamental microorganism assemblage forming processes is generally difficult to perceive, leading to inappropriate extrapolations of small-scale patterns.

The papers summarised in this thesis assess spatial distributions of phytoplankton in rock pools. Rock pools form natural metacommunities. They have well-delineated boundaries, occur in high numbers within a limited area, and their species assemblages are connected by dispersal. The main aim of the thesis was to explore the mechanisms underlying observed spatial patterns. Descriptions of phytoplankton species, functional groups and traits were included. Variables structuring phytoplankton assemblages were studied at regional and local scales. Centimetre scale vertical distributions of phytoplankton were considered, which added a spatial dimension ecologically relevant for phytoplankton. Molecular properties of a species isolated from the rock pool habitat were characterised. Their potential ecological implications were considered, as molecular processes cannot be neglected in a mechanistic understanding of phytoplankton patterns. Finally, the importance of an ecologically meaningful sampled scale in phytoplankton studies was emphasised.

The phytoplankton assemblages in the studied rock pool metacommunity were characterised by acclimatising and colonising species adapted to a light abundant and well-mixed habitat. An overarching structuring effect of habitat stability on the phytoplankton assemblages was discovered. The effects of pool stability were not explained by mere local environmental conditions, as these were similar among the pools. Regionally, the observed distributional patterns suggested competition-colonising trade-offs. At this metacommunity scale, assemblages in stable pools were likely influenced by monopolising and priority effects, whereas effective outcompeting and colonising were important for assemblages among unstable pools.

Vertical distributions of phytoplankton implied a local structuring effect of turbulence, diverging between stable, deep and unstable, shallow pools. Despite well-mixed water columns, the vertical species-specific distributions were structured. Thus, by influencing fundamental community forming processes, small-scale turbulence non-randomly formed the local species assemblages, with implications at the metacommunity scale.

At the molecular level, the chemotypic characterisation of a rock pool species revealed production of bioactive compounds. The molecular effects, inhibition of enzymes present in both prokaryotes and eukaryotes, implied consequences beyond the individual scale. An ambiguous morphologic and genetic species identification exemplified the bottleneck in disentangling microorganism patterns. Furthermore, the unknown biological and ecological functions of the molecules demonstrated the difficulties in comprehension of their large-scale impacts.

Unexpectedly structured species-specific centimetre distributions in the well-mixed rock pools were discovered by precision sampling. Generalisations of such small-scale patterns with conventional phytoplankton sampling methods might lead to an underestimation of their effects at large scales.

Overall, the results in this thesis highlight the ineffectiveness of imposing a single spatial scale on microorganisms. A meaningful understanding of microorganism patterns and processes requires a widening of the traditional local scale, but also a comprehension that small-scale processes have importance for large-scale microorganism patterns. Throughout, the sampled scale should be non-arbitrarily considered. The findings in this thesis also exemplified the vulnerability of ambiently influenced rock pool ecosystems. The results can be considered in phytoplankton research and monitoring, as well as nature conservation and management.

Keywords

· Chemotype · Metacommunity · Phytoplankton ·
· Precision sampling · Rock pool · Spatial scale ·

Sammanfattning (Swedish abstract)

Ekologiska studier av arters och samhällens uppkomst och funktion har traditionellt fokuserat på effekter av lokala miljöförhållanden och artinteraktioner. Ändå är ingen art, följderna av dess handlingar eller interaktioner begränsade till en enda rumslig, spatial, skala. Betydelsen av spatiala processer, som exempelvis spridning, invandring, utvandring, habitatfragmentering och -tillgång, insågs i och med uppkomsten av metapopulations- och metasamhällsekologi. Enligt dessa utreds en arts utbredning på stora spatiala skalor, medan mekanismerna bakom den iakttagna utbredningen avslöjas genom studier på små spatiala skalor.

Generellt anses mikroorganismer ha en mycket bred, till och med global, utbredning. Det här antyder att grundläggande strukturer och processer som styrs av dessa organismer, exempelvis näringsvävar, primärproduktion, eller kolets kretslopp, övervägande påverkas av processer på en lokal skala. Ett metasamhällsekologiskt perspektiv på mikroorganismer har luckrat upp uppfattningen om deras samhällen som stängda system, starkt styrda av lokala förhållanden. Men förståelsen för de strukturer och processer som mikroorganismer upprätthåller försvåras av osäkerheter kring artidentifiering och spridningssätt. Dessutom tillämpas vanligen en antropocentrisk skala i studier av mikroorganismer. Gällande mikroorganismer är det i regel svårt att uppfatta skalan på vilken flera fundamentala samhällsformande processer sker, vilket kan leda till felaktiga tolkningar av småskaliga mönster.

I den här avhandlingen sammanfattas arbeten om den spatiala utbredningen av växtplankton i hällkar. Hällkar bildar naturliga metasamhällen. De är välavgränsade, förekommer ofta talrikt inom ett begränsat område och deras organismsamhällen sammankopplas av spridning. Det huvudsakliga målet med avhandlingen var att utforska mekanismer bakom iakttagna spatiala mönster. Beskrivningar av växtplanktonarter, funktionella grupper och artegenskaper inkluderades. Variabler som strukturerar växtplanktonsamhällen studerades på en regional och lokal skala. Växtplanktons vertikala fördelning undersöktes på en centimeterskala, därmed adderades ett för växtplankton ekologiskt relevant spatialt perspektiv. Molekylära egenskaper hos en art som isolerats från ett hällkar karakteriserades. Molekylernas potentiella ekologiska konsekvenser beaktades, eftersom processer på en molekylär skala har en central betydelse för växtplankton. Slutligen betonades betydelsen av en ekologiskt meningsfull provtagningsskala i studier av växtplankton.

Växtplanktonarterna i det studerade hällkarsmetasamhället karakteriserades av acklimatiserande och koloniserande arter, anpassade till ett ljusrikligt, välomblandat habitat. En övervägande effekt av hällkarens stabilitet på artstrukturen upptäcktes. Miljöförhållandena i hällkaren var likartade och effekterna av stabilitet kunde således inte förklaras enbart med lokala processer. På en regional skala kunde strukturen i arternas utbredning härledas till en kompromiss mellan effektivt konkurrerande och koloniserande arter. På den här metasamhällsskalan influerades växtplankton i stabila

hällkar troligen av monopoliserande effekter och grundareffekter (priority effects), medan effektiv konkurrens och kolonisering var centrala i ostabila hällkar.

Vertikala fördelningar av växtplankton antydde en lokal strukturerande effekt av turbulens, vilken skilde sig mellan stabila, djupa, och ostabila, grunda hällkar. Trots välomblandade förhållanden var den vertikala, artspecifika fördelningen av växtplankton klart strukturerad. Genom att icke-slumpmässigt påverka fundamentala samhällsformande processer kunde småskaliga fysikaliska vattenrörelser följaktligen forma de lokala samhällena, vilket även hade följder på en metasamhällskala.

På en molekylär nivå upptäcktes produktion av bioaktiva kemiska föreningar hos arten som isolerats från hällkarsmiljön. Molekylernas bioaktiva egenskaper, inhibition av enzymer som finns i både prokaryoter och eukaryoter, antydde effekter utöver den individuella skalan. En osäker morfologisk och genetisk identifiering av den isolerade arten exemplifierade flaskhalsar i utredningar av mikroorganismers spatiala förekomster. Molekylernas alltjämt okända biologiska och ekologiska funktioner demonstrerade dessutom svårigheter i att förstå deras storskaliga effekter.

Oväntat strukturerade artspecifika vertikala växtplanktonfördelningar på en centimeterskala i de välomblandade hällkaren upptäcktes med precisionsprovtagning. Generaliseringar av dylika småskaliga mönster med traditionella provtagningsmetoder kan leda att deras storskaliga betydelse underskattas.

En övergripande slutsats i den här avhandlingen är att det är ineffektivt att pålägga en enda spatial skala på mikroorganismer. En meningsfull förståelse för mikroorganismers strukturer och processer kräver en utvidgning från den traditionella lokala skalan, men även uppfattningen att småskaliga processer har central betydelse på en stor skala. Ett konsekvent beaktande av skalan som iakttas borde genomsyra studier av mikroorganismer. Med den här avhandlingen exemplifierades även sårbarheten hos hällkarsekosystem, som starkt påverkas av externa förhållanden. Resultaten kan beaktas i växtplanktonforskning och miljötillsyn, -skydd och -förvaltning.

Nyckelord

· Hällkar · Kemotyp · Metasamhälle ·
· Precisionsprovtagning · Växtplankton · Spatial skala ·

Contents

List of original papers

I **Häggqvist K.** & Lindholm T. (2015) Phytoplankton communities in rock pools on the Åland Islands, SW Finland – environmental variables, functional groups and strategies. *Biodiversity* 16:15–26.

II **Häggqvist K.** & Lindholm T. (2016) Phytoplankton, physical and chemical microscale variations in three brackish rock pools. *Phycological Research* 64:241–250.

III **Häggqvist K.**, Toruńska-Sitarz A., Błaszczyk A., Mazur-Marzec H. & Meriluoto J. (2016) Morphologic, phylogenetic and chemical characterization of a brackish colonial picocyanobacterium (Coelosphaeriaceae) with bioactive properties. *Toxins* 8:1–17.

IV **Häggqvist K.** & Lindholm T. Precision sampling increases the ecological relevance of phytoplankton studies. *Manuscript*.

Additional publications not included in the thesis

Häggqvist K. & Lindholm T. (2012) Phytoplankton dynamics in a shallow lake dominated by common water milfoil. *Inland Waters* 2:137–146.

Häggqvist K., Akçaalan R., Fastner J., Horecká M., Humbert J-F., Izydorczyk K., Jurczak T., Kokociński M., Lindholm T., Mankiewicz-Boczek J., Quesada A., Salmaso N., Quiblier C. & Echenique I. (2017) Case studies of environmental sampling, detection and monitoring of potentially toxic cyanobacteria. In: *Handbook of cyanobacterial monitoring and cyanotoxin analysis.* Meriluoto J., Spoof L. & Codd G.A. (Eds), John Wiley & Sons, United Kingdom, p. 70–83.

Salmaso N., Bernard C., Humbert J-F., Akçaalan R., Albay M., Ballot A., Catherine A., **Häggqvist K.**, Horecka M., Izydorczyk K., Köker L., Komárek J., Maloufi S., Mankiewicz-Boczek J., Metcalf J., Quesada A., Quiblier C. & Yéprémian C. (2017) Basic guide to detection and monitoring of potentially toxic cyanobacteria. In: *Handbook of cyanobacterial monitoring and cyanotoxin analysis.* Meriluoto J., Spoof L. & Codd G.A. (Eds), John Wiley & Sons, United Kingdom, p. 46–69.

Savela H., Spoof L., Perälä N., Preede M., Lamminmäki U., Nybom S., **Häggqvist K.**, Meriluoto J., Vehniäinen M. (2015) Detection of cyanobacterial *sxt* genes and paralytic shellfish toxins in freshwater lakes and brackish waters on Åland Islands, Finland. *Harmful Algae* 46:1–10.

1. Introduction

Distribution of species in nature and how their distributions are related to abiotic and biotic variables constitute fundamental community ecology. Intrinsic to this relation between a species and its habitat is that the species, its activities, or their consequences are not confined to a single spatial scale. Rather, the life of an organism involves processes at nested spatial scales, where its distributional patterns are resolved by studies at large scales and their underlying mechanisms appear by studies at small scales.

The importance of scale dependency in ecology was comparatively recently realised. Studies have focussed on patterns and processes at the local scale, especially concerning microorganisms. This restriction of spatial scale has been justified by the traditional perception that microorganisms are omnipresent, strongly structured by local environmental conditions. However, increasing evidence points to non-random distributions of microorganisms, at both large and small spatial scales. Therefore, in addition to local conditions, spatial processes, e.g. dispersal, habitat connectivity and availability, may impact microorganisms and their ecosystem functions. These ecosystem functions include crucial global processes related to primary production, food web structures, and carbon cycles, and also phenomena such as harmful algal blooms and species endemism.

This thesis summarises four papers on phytoplankton in rock pools and their distributions at regional and local scales. Rock pool ecosystems are well suited for exploring species distributions, as their inhabiting species in addition to environmental conditions are impacted by dispersal between the pools. Mechanisms behind observed distributional patterns were investigated at small spatial scales, necessitating consideration of molecular properties and adequate sampling techniques.

1. 1. Regional and local processes set the scene for metacommunities

Broadly, the presence or absence of a species in a habitat is driven by dispersal, environmental conditions and species interactions (Belyea & Lancaster 1999). Traditionally, studies in community ecology focussed on local processes and patterns (Ricklefs 1987) and the perception that communities are an outcome of interactions at different spatial scales is fairly recent (Ricklefs 1987, Wiens 1989, Levin 1992). A demand to reassess the traditional approach by realising that spatial scale matters in community ecology rose from the increasing concern of species extinction and habitat destruction, and the failure of established ecological theories to explain and predict the changes (Ricklefs 1987, Lawton 1999, Holyoak et al. 2005).

Spatial scale is integrated in community ecology through a hierarchical approach, where large-scale patterns and processes give the context for species distributions, whereas the underlying mechanisms are resolved by small-scale studies (Wiens 1989). For example, climatic conditions determine the context of species presence or absence, dispersal structures species occurrence, whereas habitat characteristics (abiotic) and species interactions

(biotic) constrain species abundances. Observed local species communities are those succeeding in passing through filters at each hierarchical level (Levin 1992). Thus, spatial variables, e.g. geographical areas or habitats, are comparable to environmental variables in structuring ecological communities (Ricklefs 1987).

Recognition of spatial scale as a fundamental part of ecological patterns and processes led to the development of metapopulation (Hanski & Gilpin 1991) and metacommunity ecology (Holyoak et al. 2005). A metacommunity is defined as local assemblages that share the same species pool through dispersal (Hanski & Gilpin 1991). Dynamics within and between the connected assemblages, as well as interactions of these local and regional dynamics, are central in structuring the metacommunity. Variability in local communities cannot be fully understood without recognising this scale dependency. Hence, species distributions, abundances and interactions are put in a spatial context (Holyoak et al. 2005).

By extending the spatial scale, metacommunity ecology spurred new ways of thinking about communities and how they are structured. Traditionally, four principal conceptual models are considered influencing metacommunities: neutral, species sorting, patch dynamics and mass effects (Leibold et al. 2004, Holyoak et al. 2005). Essentially, the main difference between these four models is the strength of regional (dispersal) versus local (abiotic and biotic) processes in structuring the metacommunity.

In the *neutral perspective*, local environmental conditions and species traits are equal. Local low rate dispersal equalises communities in close proximity and structures the metacommunity. The local and regional processes are random and consequently, there is no spatial dependence between communities and habitat characteristics.

In the *patch dynamics perspective*, local environmental conditions are considered identical, whereas species traits differ in a distinct way. At the regional scale, a trade-off between colonisation and competition structures the metacommunity. Colonisers will prevail in recently disturbed communities, whereas competitors will dominate in stable communities.

In the *species sorting perspective*, local abiotic and biotic conditions structure the metacommunity, as local environmental conditions diverge and species traits vary. Thereby, the species sorting perspective assumes a spatial dependence between community structure and habitat characteristics. Consequently, dispersal rates must be sufficient to allow species to spread to all potential habitats.

In the *mass effects perspective*, local environmental conditions as well as species traits differ and dispersal rates are high. High dispersal in combination with variations in species traits enables source-sink dynamics, where large masses of individuals disperse from source to sink communities. The spatial dependence between communities and habitat characteristics is not definite, as with high dispersal rates, species also spread to suboptimal habitats. This will eventually lead to homogenisation of the metacommunity.

Considering the varying strengths of regional and local processes, the four perspectives can be simplified by regarding metacommunities as neutral or structured by environmental conditions in

combination with dispersal (Winegardner et al. 2012). Dispersal can be limiting (as for certain species in patch dynamics), efficient (as for the majority of species in species sorting), or high (as for some species in mass effects). The structures of real metacommunities are determined by interactions between processes described in these four conceptual models (Leibold et al. 2004). Thus, the main objective is not assigning a conceptual model to a particular system, but rather to understand the mechanisms behind the dynamics structuring the ecological communities (Holyoak et al. 2005).

1. 2. Are metacommunity processes relevant for phytoplankton?

The traditional perception of microorganisms as widely, even globally distributed (Finlay & Fenchel 2004) has substantiated the attention on local patterns and processes in microorganism ecology. In fact, it is practically impossible to confirm the absence of a microorganism taxon, and most studies concerning their distribution focus on the relative abundance of each species (Martiny et al. 2006). Thus, following the well-known idea of Beijerinck and Baas Becking, it may be argued that microorganisms are omnipresent, however most occur at densities below our limit of detection. Accordingly, the environment selects which species are abundant, i.e. observed (Baas Becking & Canfield 2015). However, the mere difficulty in identifying microorganisms, to the degree of obscuring the species concept, and their largely unknown dispersal modes, contribute to a warranted uncertainty regarding the actual distribution of different species. Consequently, recent studies have questioned a global occurrence and random distributions of microorganisms (Hillebrand et al. 2001, Martiny et al. 2006, Padisák et al. 2016).

Phytoplankton are microorganisms at the base of aquatic food webs, which contribute to over half of the global net primary production (Field et al. 1998) and have an irreplaceable role in aquatic energy fluxes. In addition, their short generation times enable them to quickly respond to environmental change. Studies of patterns and processes shaping phytoplankton assemblages thus contribute to the understanding of aquatic ecosystems.

Through traditional community ecology, the recognition of top-down (grazing) and bottom-up (abiotic) processes in structuring phytoplankton assemblages is well established. The importance of species (biotic) interactions is less studied, but undoubtedly also a valid contributor (Griffiths et al. 2016). However, the singlehanded dominance of random local conditions in influencing phytoplankton assemblages has been challenged, opposing them as closed systems (Incagnone et al. 2015, Naselli-Flores & Padisák 2016). Indeed, there are indications of regional processes overriding local structural impacts on phytoplankton (Heino et al. 2010, Ptacnik et al. 2010, Meier et al. 2015). Still, extending the spatial scale at which phytoplankton assemblages are observed provide no clear-cut patterns and has also suggested a greater effect of the local environment over regional processes (De Bie et al. 2012, Gallego et al. 2014, Padial et al. 2014, Santos et al. 2016), or a structuring effect of the two in combination (Soininen et al. 2007, 2011). Some

studies support none of these and propose importance of unmeasured environmental variables, stochastic processes, or fine scale variations (Beisner et al. 2006, Nabout et al. 2009, Nogueira et al. 2010, Lopes et al. 2011). There are also studies supporting phytoplankton assemblages as closed systems, structured by environmental conditions alone (Vanormelingen et al. 2008, Mazaris et al. 2010).

Trying to resolve processes behind observed phytoplankton distributions is, as for other microorganisms, complicated. Undersampling of certain species and taxonomic ambiguities cause major challenges. Above all, dispersal, a fundamental part of the metacommunity concept, is largely unknown for phytoplankton (Padisák et al. 2016). Moreover, given their short regeneration times, phytoplankton metacommunity structures may be revealed by variability below the species level. For example, regional distributions among genotypes of the cyanobacterium *Nodularia* appeared unrelated to environmental conditions (Hayes & Barker 1997). Environmental conditions and regional dispersal barriers impacted the distributions of genetically differentiated populations of the diatom *Skeletonema marinoi* (Sjöqvist et al. 2015).

Studies considering phytoplankton assemblages as metacommunities have often focussed on the importance of local versus regional effects, whereas underlying mechanisms of the observed patterns have been overlooked. Possibly because a mechanistic approach in phytoplankton ecology requires a significant reduction of spatial scale, setting constraints on *in situ* sampling methods and moving from ecological to molecular processes. Still, processes at these small scales are ecologically relevant for phytoplankton and can also have impacts at the scale of the metacommunity. For example, distributions of prevalent cyanobacteria chemotypes among lakes were related to dispersal efficiency, not local environmental conditions (Rohrlack et al. 2009).

1. 3. Consideration of the sampled scale

There is no obvious, instinctive scale at which species or ecological phenomena should be studied (Levin 1992). The studied scale depends on the organism in focus, where the individual units of observation (grain) set the lower resolution and the spatial or temporal scale over which comparisons are made (extent) the upper resolution (Wiens 1989). As patterns and processes are scale dependent, their magnitude and direction may change if the unit of observation or scale is changed. Decreasing the unit of observation reveals variability that would be averaged out at larger units of observation (Levin 1992), whereas enlarging the studied scale might reveal distributional patterns (Wiens 1989, Levin 1992). For example, sampling at scales well above the individual units of observation average out the microhabitat heterogeneity contributing to phytoplankton species coexistence (Siegel 1998).

The observed unit, spatial and temporal scales are generally subjectively and anthropocentrically chosen (Wiens 1989). This is particularly evident in studies of microorganisms, which exist on a scale that is difficult to comprehend (Stocker 2015). Yet, central to explaining patterns and processes in ecological communities is that the response of the

organism, the observations and measurements overlap (Wiens 1989). Assuming that microorganisms are everywhere, strongly structured by local environmental conditions, implies that the ecological demands of a microorganism are to be identified to understand its distribution (Fontaneto & Brodie 2011). However, microorganisms are consistently characterised by bulk samplings well above the scale at which they experience environmental conditions. Consequently, there is a mismatch in the sampled scale and organism perceived scale, which might lead to incorrect conclusions when comparing patterns and processes across spatial scales.

1. 4. Rock pools form metacommunities in the terrestrial landscape

Small ponds and pools are ecologically and functionally different from lakes (Søndergaard et al. 2005) and marine systems. Due to their small volume in relation to surface area they are exposed to ambient variations. Hourly, daily, seasonally, vertical and horizontal variations are realistic in these habitats. Consequently, their flora and fauna generally have traits and strategies allowing them to endure, and even successfully exploit, the variability. Small ponds and pools form discrete, unique patches, which greatly increase landscape heterogeneity, as well as local and regional species diversity (Williams et al. 2004).

Through functioning as migration corridors or stepping stones, the impact of small aquatic systems at the large scale might be considerable (De Meester et al. 2005). Therefore, their conservation value may be surprisingly high, both due to their characteristic flora and fauna, as well as their function at the ecosystem level (De Meester et al. 2005). Even at global scales the importance of small aquatic habitats might be considerable (Downing 2010). Still, these habitats have generally been overlooked in research and nature conservation, their importance only recently getting acknowledged (Boix et al. 2012). For example, temporary ponds are recognised as a wetland type of international importance (Ramsar Convention 2002). In Finland, rock pools are included in the Finnish national environmental protection plan (Raunio et al. 2008). Including these small aquatic habitats in nature conservation is especially important considering their vulnerability to climate change.

Rock pools, "relatively small permanently, or temporarily water-filled depressions in the rock" (Hällfors 1984), form one distinct type of small aquatic systems, often present on, but not restricted to, seashores. Rock pools are very abundant on the over ten thousand islands in the Finnish archipelago. Here, the combination of bare bedrock, glacial landscape formations and postglacial land upheaval create a suitable setting for rock pool formation (Hällfors 1984). In fact, a large part of the rock pools in Europe occur in this area (Raunio et al. 2008). Perhaps as a result of their abundance in the archipelago landscape, they have intrigued researchers in this area for over 100 years (references in Hällfors 1984). Many of these rock pool studies concern microalgae and are predominantly descriptive, or e.g. relating microalgae distribution to pool location on the shore, or bottom material and chemical properties of the water. Few studies

concern rock pools on the Åland Islands (Cedercreutz 1934, Lindholm 1996).

Because of their special features, e.g. ephemerality, or simple food web structure, small ponds and pools are appealing study systems concerning habitat properties and species interactions (De Meester et al. 2005). Often many rock pools occur within a limited area, which facilitate sampling, experimental manipulation and replication (De Meester et al. 2005).

Small aquatic habitats form well-delineated islands in the terrestrial landscape, with clear boundaries for their local species assemblages (De Meester et al. 2005). Thus, in a spatial context, a set of individual pools constitutes a metacommunity. Indeed, studies in these systems have increased the understanding of metacommunity processes, but often related to their faunal components (Jocque et al. 2010). On the contrary, studies of patterns and structuring processes related to rock pool phytoplankton are scarce. Knowledge of these patterns can be utilised in conservation of small aquatic habitats *per se*, but also gives insights into how phytoplankton assemble and function and their importance for ecosystem processes.

2. Aims of the thesis

The overall aim of this thesis was to explore spatial patterns of phytoplankton assemblages in rock pools and identify mechanisms behind observed patterns (Fig. 1). Additionally, methodological aspects in phytoplankton ecology were emphasised. The studies were grounded in metacommunity ecology. However, instead of determining the importance of regional versus local processes, which are likely to diverge based on the studied scale, mechanisms underlying the observed spatial patterns were in focus.

The aim of papers I–II was to identify variables, and through them mechanisms, structuring the observed spatial distributions of rock pool phytoplankton. Relationships between measured physical and chemical variables and the observed spatial phytoplankton distributions were assessed, principally assuming structuring effects on the phytoplankton assemblages.

In paper I, a regional scale was considered by studying 34 rock pools covering an area 12 km in length and 30 km in width (Fig. 1).

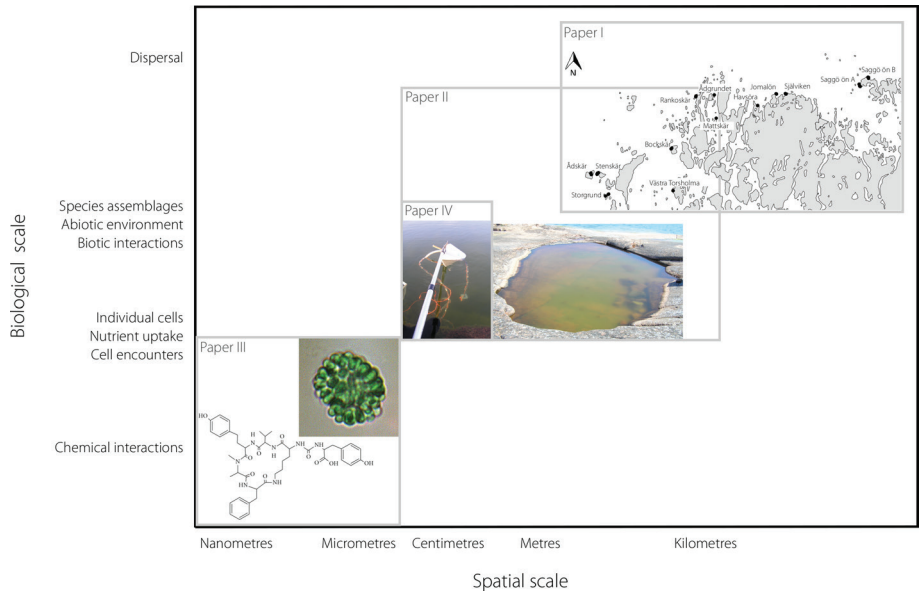

Figure 1. The spatial scales of the papers summarised in this thesis and corresponding relevant biological processes. Based on an idea by Prairie et al. (2012).

The purpose of paper I was partly also descriptive, aiming at defining characteristic species, functional groups and traits among rock pool phytoplankton. In paper II, the local vertical fine scale conditions in individual rock pools were considered. By this the objective was to focus on a local spatial scale relevant to phytoplankton.

The chosen mechanistic approach required species-specific (paper II) and molecular studies (paper III), but also methodological considerations (paper IV). In paper III, the aim was to identify a species from the rock pool habitat and characterise its chemotypic properties with reference to their ecological implications.

By presenting modifications of a precision sampling method, the aim of paper IV was to stress the importance of fine scale sampling for a realistic understanding of mechanisms behind observed phytoplankton patterns and processes. The leading questions in each study are summarised in Fig. 2.

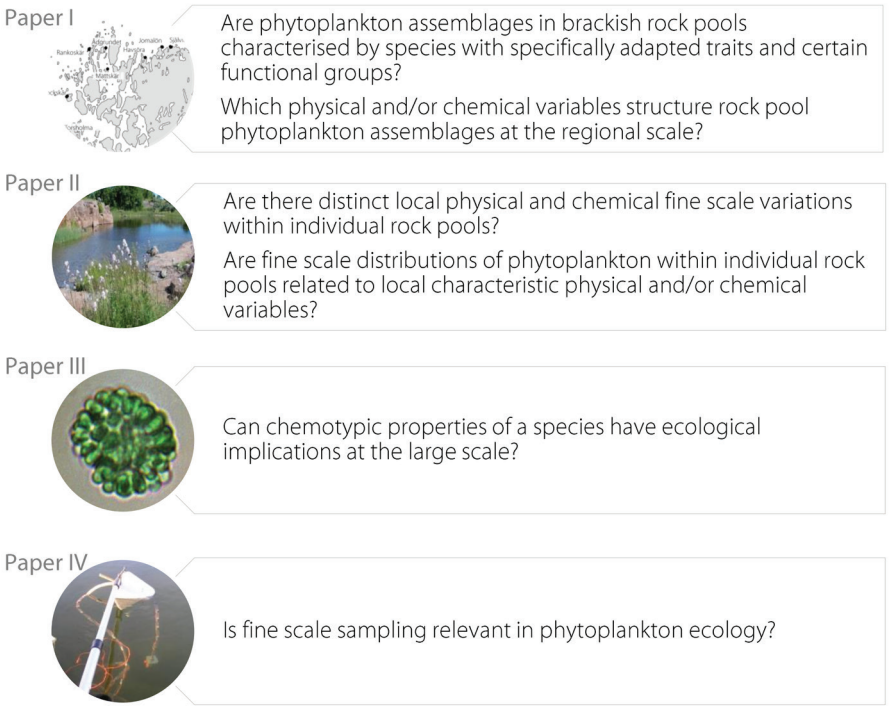

Figure 2. The leading questions of the four papers summarised in this thesis.

3. Material and methods

The studies summarised in this thesis included field samplings, revealing patterns kilometres and centimetres apart, characterisations of phytoplankton assemblages, monocultures, and individual cells, isolations of single cells, as well as extractions of DNA and chemical compounds. Applied methods mirrored the focus on spatial patterns and the objective to include regional, local, species, and molecular levels. Detailed small-scale studies represented the intention to understand mechanisms behind observed patterns.

Patterns at regional and local scales were studied by different sampling designs (papers I–II). An available precision sampling method was modified to explore fine scale phytoplankton structures in the shallow rock pools. This fine scale sampling revealed intricate patterns in the pools (paper II). Thus, to emphasise the importance of precision sampling in phytoplankton studies, the applied method was further described in in paper IV. To present modifications of the precision sampler and its functionality in contrasting aquatic habitats, results from studies done in a shallow, densely vegetated lake (Häggqvist 2007, Häggqvist & Lindholm 2012) and among *Phragmites australis* (unpublished data) were included in addition to rock pool data.

Basic analytical methods, concerning both abiotic and biotic variables, were similar in papers I–II. The analytical methods used in paper III, in which molecular structures were studied, highly diverged from those used in papers I–II, and included both genetic and biochemical analyses. Detailed descriptions of materials and methods are included in the individual papers (papers I–IV). Next, the studied area, sampling, analytical and statistical methods are described.

3. 1. Study area

The studied rock pools are located in the north-western outer archipelago of Åland Islands (Fig. 1). Of the >10 000 islands forming the south-western Finnish archipelago, about 6700 belong to the Åland Islands. Here, rock pools are a characteristic feature in the landscape; in Finland, 89% of rock pools ≤1 ha can be found in the south-western area (Raunio et al. 2008). Brackish pools (salinity ≥0.5‰) located on bare rock, which on the Åland Islands mainly is rapakivi granite, were considered. Large fluctuations in sea water level due to tidal movements do not occur in the Baltic Sea. Thus, the influence of the nearby sea in rock pools may be sporadic and indirect, e.g. through sea splash during strong winds, or regular and direct when pools are connected to the sea. Both types were included in this study. The diameter of the studied pools was 0.5–30 m and they were 0.2–2.5 m deep. Complete desiccation of the smallest pools was possible, however during this study the pools were permanent during the summer months.

The 34 rock pools included in paper I were randomly chosen from twelve islands, seven were on small peninsulas connected to the main island of Åland Islands (the pools on Havsöra, Jomalön and Själviken). The islands were chosen

based on their accessibility and existing data (Lindholm 1996). In addition to collection of material for paper I, samples from the 34 rock pools were also collected for establishment of phytoplankton cultures for the work in paper III. The three rock pools studied in paper II were chosen based on paper I, intentionally emphasising differences in area and depth. These three pools were located on islands (Storgrund, Ådgrundet and Ådskär) in the middle and western part of the study area, covering a 15 km distance. The species in focus in paper III was isolated from a rock pool on Storgrund, which was characterised in paper I, II and IV.

3. 2. Sampling rock pools at regional and local scales

The sampling designs of papers I–II represented the strong spatial focus of the studies. In paper I, each of the 34 rock pools were sampled once during the growth season. Five subsamples (1 L) were taken at points evenly distributed along the sides of the pools and combined. From this integrated sample the representative sample was taken. An integrated sample was sufficient for identifying main phytoplankton taxa in the rock pools, but this spatial resolution was too coarse for discovering local fine scale structures aimed at in paper II. Therefore, an altered version of a precision sampling method (based on Lindholm 1979) with a centimetre resolution was used.

The precision sampler was modified to retrieve vertical samples in the shallow rock pool habitat. A plastic hose (ø 1 cm), float and hose clamp were the main parts of the sampler. Its essential functional principle was based on pressure difference between atmosphere and water in combination with placement of the sampling outlet below the water surface. By carefully regulating the depth, samples were retrieved at intervals of centimetres. Thus, instead of averaging across a vertical scale of 30 cm (common length of conventional water samplers), the precision sampler averaged across a vertical scale of about 1 cm. More detailed description of the precision sampler and its modifications can be found in paper IV.

Precision sampling at a scale ecologically relevant to phytoplankton enabled exploration of potential mechanisms behind observed spatial patterns. By comparing among pool differences, the regional scale was also briefly considered in paper II. Spatial patterns were the main focus throughout this work, however the sampling for paper II was repeated throughout the growth season, which indirectly allowed brief consideration of temporal patterns.

3. 3. Analyses of abiotic variables

Measured abiotic variables (papers I–II) were considered to influence the rock pool phytoplankton assemblages. In paper I, depth, distance from the sea, height above sea level and area were included as physical variables. Macrophytes were considered as present (>50% cover) or absent (<<50% cover). Similarly to phytoplankton, macrophytes are primary producers and thus potential competitors for available resources. However, macrophytes also modify the habitat physically and chemically.

In papers I–II, the environmental conditions in the rock pools were characterised by dissolved oxygen

concentration, nutrients (total nitrogen, total phosphorus), pH and salinity. Dissolved oxygen was measured according to the Winkler method (paper I) or directly in the field with an YSI probe (paper II). Temperature was measured directly, salinity and pH in the laboratory. Nutrient concentrations were analysed according to Koroleff (1983a, b) (papers I–II) and Finnish standards (SFS 1998) (paper II). In paper II, the colour of the rock pool water was included as a potential influencing variable on the fine scale distributions of phytoplankton and was measured spectrophotometrically. The degree of mixing in the three pools in paper II was characterised by relative water column stability (RWCS) according to Padisák et al. (2003).

3. 4. Analyses of biotic variables

Identifications of phytoplankton in papers I–II were based on morphological features. Available literature was used for the identifications (mainly Tikkanen 1986, John et al. 2002, Pliński & Komárek 2007, Hoppenrath et al. 2009). Taxonomy and scientific names were verified with databases (Guiry & Guiry 2016, WoRMS 2016). In addition, species were categorised into functional groups (*sensu* Reynolds et al. 2002, Padisák et al. 2009).

Compared to species, polyphyletic assemblages representing different functional groups give a more telling picture of habitat conditions and might provide indications of underlying mechanisms, e.g. why some species are more successful than others (Belyea & Lancaster 1999, Reynolds et al. 2002). The phytoplankton functional group concept contains many functional assemblages (Reynolds et al. 2002, Padisák et al. 2009), which complicates the detection of main dividing characters among the groups. Therefore species were also categorised according to their life strategies (*sensu* Reynolds 2006), with the intention to focus on a few clearly distinct traits among the strategies. These characterisations were part of paper I.

Phytoplankton abundances and biomasses were used for discovering patterns at regional and local scales. Abundances were determined according to the Utermöhl method using an inverted light microscope. Chlorophyll *a* was used as a proxy for phytoplankton biomass. Species-specific biovolumes were additionally used as detailed alternatives to phytoplankton biomass. Merely numerical characterisations of phytoplankton assemblages will emphasise small species, which often occur in large numbers, and underestimate the contribution of large species. The work in paper I was based on 197 taxa in eight phyla and the work in paper II on 147 taxa in eight phyla. In all, 243 taxa in eight phyla were identified in this work.

3. 5. Species-specific and molecular analyses

In paper III, species-specific and molecular properties of a species isolated from the rock pool habitat were studied. The objective of the study was two-parted 1) characterise chemotypic properties, i.e. identify metabolites, and 2) confirm morphological identification by phylogenetic methods. Ultimately, the intention was to explore the importance of small-scale structures for patterns at large scales.

Establishment of monospecies cultures and production of biomass were central parts of this work. Species were isolated, cultures established, biomass produced and harvested. In subsequent analyses the cultured species were narrowed down to one candidate. First, biomass from the cultures was extracted to obtain initial concentrated samples containing compounds produced by the species. These crude extracts were screened to find certain metabolites. Crude extracts, in which presence of such metabolites was indicated, were fractionated, i.e. concentrated.

In order to confirm the presence of the suggested metabolites, the fractions were tested in bioactivity assays, after which the alternatives could be narrowed down to one strong candidate. In addition to confirming the presence of desired metabolites, analyses of bioactivity also enabled a consideration of biological and ecological functions of the compounds. The metabolites in the fractions of the final candidate were characterised and its phylogenetic position determined.

Next, the methods used in these steps are briefly described. Detailed descriptions of the crude extraction, fractionation, bioactivity assays, characterisation of metabolites, phylogenetic analyses (including DNA-extraction and amplification of genetic material) can be found in paper III.

Establishment of cultures, production of biomass, harvest

Throughout the field sampling for material to paper I, samples were collected for isolation of phytoplankton in order to establish monospecies cultures. Using a sterile micropipette, single cells (colonies/filaments) were isolated from net samples (mesh size 25 µm) taken in the rock pools. The cells were washed in three drops of filtered, sterile seawater before they were transferred to diluted growth media in 24 cell-well plates. Species were selected for isolation according to their prevalence in the samples; abundant species were chosen, focussing on cyanobacteria and dinoflagellates.

In total 750 single cells (colonies/filaments) were isolated from the rock pool samples, 45% of these were dinoflagellates, 39% cyanobacteria, 13% chlorophytes, 3% euglenophytes and 0.3% cryptophytes. Viable cells were regularly transferred to higher volumes of decreasingly diluted fresh growth media. The isolations resulted in 39 cultures of ten species, five cyanobacteria and five chlorophytes. The cyanobacteria were cultured in Z8 medium with nitrogen and the chlorophytes in f/2 medium without silica, both media were based on artificial seawater (6‰).

Growth conditions for the cultures varied: temperature was 21–23°C and light 35–50 $\mu Em^2 s^{-1}$, the light:dark cycle was always 16:8 h. The cultures were maintained in 30 mL or 100 mL volumes. To acquire enough biomass for the biochemical analyses, the culture volumes were increased, up to 5 L in polyethylene plastic bags, or up to 25 L in BIOSTAT CultiBag RM bioreactors (Fig. 3). Culture volumes of all five cyanobacteria species and three chlorophyte species were increased. Biomass was harvested by filtration (Whatman GF/A), freeze-dried directly on the filter and stored at -18°C.

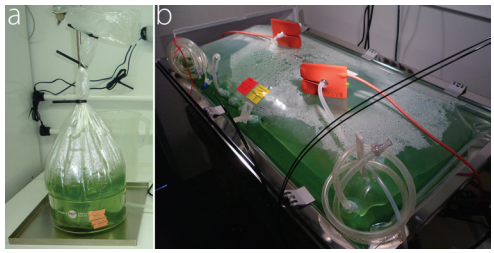

Figure 3. Large volume (a) 5 L, (b) 25 L cultures prepared for the work in paper III. Photo (b) by Jaana Rikkinen (2012).

Analyses of metabolites and bioactivity assays

The extraction and detection methods used in this part of the work aimed at discovering metabolites with low molecular weight. Biomass harvested from the large volume cultures was extracted in 80% ethanol, which is a solvent of medium polarity, resulting in extracts containing various compounds. The crude extracts were dried and redissolved in 30% methanol.

Presence of polar, organic molecules in the methanolic samples were screened with Agilent 1200 (Agilent Technologies) liquid chromatography tandem mass spectrometry (LC-MS/MS) system coupled online to a hybrid triple quadrupole/linear ion trap mass spectrometer (QTRAP5500, Applied Biosystems, Sciex). In LC-MS/MS, compounds are first separated according to their physicochemical properties (usually polarity) by high-performance liquid chromatography. The separated compounds, eluting at varying times, are ionised. The ions are optionally fragmented in the QTRAP and detected, in which the detection provides information on the mass-to-charge (*m/z*) ratio of the ions.

The LC-MS/MS screening of crude extracts indicated presence of organic, polar compounds in two of the eight analysed cultures, both cyanobacteria. The crude extracts of these two cyanobacteria, morphologically identified as *Cylindrospermum* sp. and *Woronichinia* sp., were dissolved in water and 0.5% dimethyl sulfoxide (enhancing the solubility), and further fractionated by solid-phase extraction with C18 SPE cartridges (1 g C18(EC) 3 mL, ISOLUTE). In this extraction, the desired small molecules were adsorbed on the nonpolar cartridges. To release the desired molecules from the cartridges, a solvent with decreasing polarity (aqueous methanol of increasing concentration) was used for elution of fractions in which the desired small molecules were concentrated. The obtained fractions were divided and used in bioactivity assays and characterisation of metabolites in the final candidate.

Some of the organic, polar compounds present in the crude extracts of the two cyanobacteria were identified as nonribosomal oligopeptides. These compounds are known inhibitors of various enzymes (Agha & Quesada 2014). Therefore, in order to confirm their production by the two cyanobacteria species, i.e. their presence in the fractions, their activity against enzymes were tested in inhibition assays. The inhibition of the fractions against six enzymes, carboxypeptidase A, chymotrypsin, elastase, protein phosphatase I, protein phosphatase 2A, thrombin, and trypsin were tested. All tests were done as colorimetric assays, in which enzymes, substrates and reaction buffers were combined with dilutions of the fractions, standard inhibitors (positive controls) or no inhibitors (zero controls).

In colorimetric assays, the enzymatic reaction and release of product results

in a visible colour. Presence of an inhibitory compound in the sample prevents the enzyme from binding to its substrate and, consequently, no colour is developed. Inhibitory activity in the fractions was measured as a decrease in absorbance (Opsys MR microplate reader, Dynex Technologies, or Varioskan Flash, Thermo Scientific). The half maximum inhibitory concentration (IC_{50}) of fractions with significant inhibitory activity was calculated and used as an indication, as well as an intensity proxy, of inhibitory compounds in the fractions. Detailed descriptions of each assay can be found in paper III.

Based on the inhibition assays, significant activity against enzymes was found in fractions of the species morphologically identified as *Woronichinia* sp., which was chosen as the final candidate for metabolite characterisation. Structures of the metabolites were tentatively characterised with the described LC-MS/MS system. An information dependent acquisition method (IDA) was used in these analyses. In the IDA method the data from the MS is analysed as it is being acquired and the next MS scan is dependent on the results of the previous scan. Criteria can be set as to when dependent scans should be acquired. Here, the IDA was run in positive ionisation mode and the retrieved enhanced product ion spectra were limited to the *m/z* 50–1000 range, which sufficiently detects polar, organic molecules. The fragmentation patterns were analysed in complementary software (Analyst QS 1.5.1, Applied Biosystems, and PeakView 2.1 and 2.2, AB Sciex). To identify and characterise compound structures, their *m/z* and fragmentation patterns were compared with those corresponding to known compounds.

Phylogenetic analyses

The final candidate species after metabolite screening and bioactivity assays was a colony forming cyanobacterium, which based on morphological characters was identified as *Woronichinia* sp. (strain 06S067). After one year in culture, this species no longer formed colonies, which prevented a reliable identification based on morphology. Metabolite profiles, chemotypes, are species-specific and may therefore be used for identification (Fastner et al. 2001, Rohrlack et al. 2008). However, chemotypic characterisations of *Woronichinia* species, or the family Coelosphaeriaceae to which this genus belongs, are too sparse for useful comparisons. Moreover, a polyphasic approach, taking into account ecology, morphology, molecular profile and genetic features, is highly desired in phytoplankton identifications (Komárek 2006). Consequently, the phylogeny of the isolated species was determined.

A kit (FastDNA SPIN kit for soil, MP Biomedicals) was used for the extraction of DNA from a frozen (-80°C) sample of the *Woronichinia* sp. culture and the presence of DNA was confirmed by gel electrophoresis. The phylogenetic identification was based on part of the 16S rRNA gene. This gene codes for subunits in prokaryotic ribosomal RNA and is frequently used in phylogenetic studies of prokaryotic organisms as it contains both highly stable and variable regions. Here, the 16S rRNA, part of the 23S rRNA gene, and spaces between the genes were amplified by polymerase chain reaction (PCR) using primers targeting stable

end regions of the sequence. Part of the variable region between the primer sites was used for phylogenetic comparisons.

In the sequencing (performed by Genomed, Warszawa) the 16S rRNA gene in the amplified gene sequence was targeted. The sequenced part (895 bp) was deposited in the publicly available database GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and can be found by the accession number KU533863. The obtained sequence was compared to other 16S rRNA sequences using the basic local alignment search tool (BLAST) and those showing high similarity (>93%) were chosen for further comparisons.

Base pairs in the sequences were aligned by pairwise and multiple comparisons in Clustal X (version 2.1, Conway Institute). Truncated sequences (substantially shorter) and nonhomologous regions (substantially longer) were manually removed using BioEdit (version 7.2.5, Ibis Biosciences). Phylogenetic trees were constructed based on neighbour-joining, maximum parsimony and maximum likelihood analyses in PHYLIP (version 3.695, University of Washington). Detailed descriptions of the phylogenetic analyses can be found in paper III.

3. 6. Statistical analyses

In paper I, relationships between abiotic variables and phytoplankton (abundance and biomass) were analysed with nonparametric Spearman rank correlations. One correlation matrix was acquired to analyse relationships in the complete data set. False discovery rate (FDR) of the *p*-values was corrected. The relationship between macrophyte presence-absence and phytoplankton (abundance and biomass) was analysed using logistic regression. In paper II, nonparametric Spearman rank correlations between abiotic variables and phytoplankton (abundance, biomass and dominating species) were separately studied for each sampling date in each rock pool. The *p*-values were FDR-corrected for multiple tests across all the correlations in each rock pool. Differences in abiotic and phytoplankton (abundance and biomass) fine scale patterns between the rock pools were analysed in Friedman tests and Mann-Whitney post hocs (paper II). Correlation, regression and difference analyses were done using SPSS 21 (IBM).

In paper I, the influence of selected abiotic variables on the regional distribution of phytoplankton was analysed with constrained canonical correspondence analysis (CCA) using the package vegan (Oksanen et al. 2013) in R (version 3.0.2, R Core Team 2013). Only species with ≥100 counted individuals were included, therefore the phytoplankton abundances were not transformed to equalise differences between rare and abundant species.

In paper IV, causal impacts of abiotic variables on rock pool phytoplankton based on precision (centimetre) sampling were compared with sampling corresponding to a metre scale in piecewise structural equation models (pSEMs). Data acquired for the work in paper II was used in the analyses. Phytoplankton were divided in groups or phyla and their abundances were square root transformed. Groups/phyla occurring only one sample date were omitted from the analyses. The models were fit according to generalised linear mixed models with penalised likelihoods, Gaussian distributions and link identity function. To account for non-independent samples, depth was set as a

random factor.

The models were constructed applying the package piecewiseSEM (Lefcheck 2016) in R (version 3.2.3). Spearman rank correlations among the variables were analysed in SPSS 23 (IBM) and strong, significant correlations were omitted from the models. Conceptual models were constructed assuming an effect of every abiotic variable on every phytoplankton group/phyla. Additionally, all significant missing paths following Shipley's test of directed separation were included in the conceptual models. These were reduced based on Akaike corrected information criterion (AICc) by stepwise elimination of non-significant paths.

To summarise results from papers I–II, differences among rock pools based on Bray-Curtis dissimilarity in phytoplankton abundances using non-metric multidimensional scaling (NDMS) is visualised here. The NDMS was done applying the vegan package (Oksanen et al. 2013) in R (version 3.2.3), in which phytoplankton abundances were transformed by square root and Wisconsin double standardisation.

The complexity of small-scale patterns, focussing on causal effects of abiotic and biotic variables on *Woronichinia* sp. is also presented here by applying pSEMs according to the procedure described in paper IV. For these analyses data acquired in the rock pool on Storgrund, from where *Woronichinia* sp. was isolated, were used. In these analyses species occurring ≥3 sampling occasions were included and their abundances were square root transformed. The associated conceptual and final pSEMs, β-coefficients, as well as individual model fits for the final model are included in the supplement.

4. Results and discussion

In this thesis, rock pool phytoplankton were studied at different spatial scales, with the aim to understand possible mechanisms underlying the patterns. Additionally, rock pool conditions and phytoplankton species, functional groups and traits were described. The covered scales were regional (kilometres), local and species-specific (centimetres and molecular level).

At the regional and local scales influences and relationships between conditions in the rock pools and the phytoplankton assemblages were assessed (papers I–II). At the molecular level, chemotypic properties of one species isolated from the rock pool habitat were explored for identification purposes and to emphasise their ecological connection (paper III). Spatial scale was also considered in a methodological perspective by describing precision sampling and its relevance in phytoplankton ecology (paper IV).

In the studied rock pools, the characteristic phytoplankton flora represented species adapted to a variable habitat. Variations in pool morphology influenced pool stability, with structural impacts on the regional distribution of species (paper I). Observed regional patterns indicated influences of monopolising and priority effects in stable pools, as well as patch dynamics in unstable pools. At the local scale, the effect of pool morphology on pool stability resulted in varying small-scale physical patterns, with structural impacts on the fine scale phytoplankton distributions, ultimately impacting local species assemblages in individual pools (paper II). Observed patterns were possible during the prevailing weather conditions, which weakened strong environmental filtering at both regional and local scales (papers I–II).

Structured fine scale phytoplankton distributions in these shallow well-mixed systems, and their consequences on both local and regional scales, highlighted the importance of species-specific studies (paper III) and appropriate sampling methods (paper IV) in phytoplankton ecology. Complexities related to species-specific studies and chemical ecology of phytoplankton was revealed in the phylogenetic and chemotypic characterisation of the isolated species (paper III). Still, the bioactive properties of the species indicated effects beyond the individual scale and the ecological relevance of considering these patterns.

Precision sampling in various aquatic habitats revealed phytoplankton patterns typically not discovered with conventional sampling and supported the perception that spatial distributions of phytoplankton are structured, not random, at small scales, even in well-mixed systems (paper IV). Thus, conventional phytoplankton sampling methods might not correctly reflect the mechanisms behind observed patterns and therefore inaccurately predict phytoplankton patterns at large scales.

4. 1. Ambiently influenced systems with an adapted phytoplankton flora

Weather clearly influenced environmental conditions in the rock pools (papers I–II). Comparatively moderate summer temperatures and high precipitation, or frequent

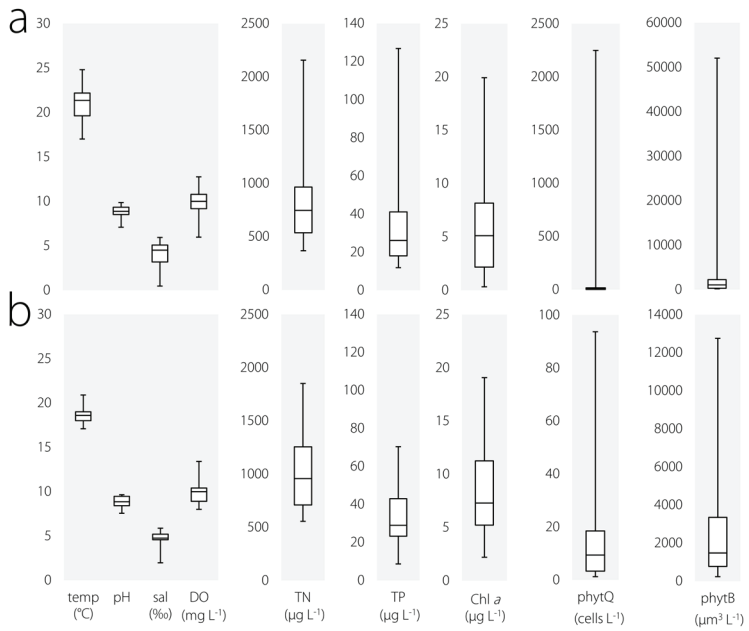

Figure 4. Regional variations in abiotic and biotic variables among (a) 34 rock pools sampled once (paper I) and (b) three rock pools sampled repeatedly during the growth season (paper II). Median values (line in boxes), first and third quartiles (top and bottom of boxes), minimum and maximum values (error bars) are shown. Temperature: temp, salinity: sal, dissolved oxygen: DO, total nitrogen: TN, total phosphorus: TP, Chlorophyll *a*: Chl *a*, phytoplankton abundance: phytQ, and phytoplankton biovolume: phytB.

downpours, levelled out large regional differences in environmental conditions (i.e. oxygen concentration, pH, salinity, temperature) among the pools (Fig. 4). Exceptionally high temperatures or salinities, possible in shallow pools during calm and warm weather (Ganning 1971), were not measured. Dissolved oxygen concentration and pH, both connected to primary production, were generally high. The pools were often oxygen saturated.

In comparison, the nutrient concentrations (total nitrogen, total phosphorus) varied between pools and were relatively high (Fig. 4, papers I–II). Thus, by flushing nutrients from surrounding rocks, precipitation probably contributed to nutrient variability among the pools. The rock pools were well mixed (paper II). That is, in addition to levelling out differences in environmental conditions between pools, prevailing weather prevented large vertical differences within pools, which during calm and warm weather may show substantial vertical stratification (Ganning 1971). During the growth season, the environmental conditions varied only moderately (paper II).

The phytoplankton biomass (Fig. 4) was within the range expected for very shallow systems (Phillips et al. 2008). Charophytes (order Zygnematales), small

Table 1. Infrequently dominating (dominating in 1–2 pools), common (present in ≥50% of the pools) and prevalent (present in ≥50% of the pools and occasionally dominating) species (paper I), as well as their classification into functional groups (FG, *sensu* Reynolds et al. 2002, Padisák et al. 2009) and life strategies (LS, according to the range of msv^{-1}; ratio of surface area × maximum linear dimension to biovolume, in Reynolds 2006).

Infrequently dominating			Common			Prevalent		
Species	FG	LS	Species	FG	LS	Species	FG	LS
Aphanizomenon flosaquae	H1	CR	*Chroococcus* spp.	L_O	C	*Anabaena* spp.	H1	CR
Chaetoceros tenuissimus		C	*Cosmarium* spp.	N		*Monoraphidium minutum*	X1	CR
Chlamydomonas sp.	X2	C	*Cryptomonas* spp.	Y	C	*Oocystis borgei*	F	C
Chlorococcaleans		C	*Merismopedia* spp.	L_O	CR	*Peridiniopsis polonicum*	L_O	CR
Chroomonas coerulea	X2	C	*Monoraphidium contortum*	X1	CR	*Pseudopedinella elastica*	X2	C
Flagellates		C	*Peridiniopsis balticum*	L_O	CR			
Gymnodinium simplex	Y	C	*Plagioselmis/Rhodomonas* sp.	X2	C			
Haematococcus pluvialis		C	*Pyramimonas* sp.	X2	C			
Halamphora coffeaeformis		C	*Scenedesmus ecornis*	J	C			
Heterocapsa triquetra		C	*Snowella lacustris*	L_O	CR			
Kryptoperidinium foliaceum		C	*Tetraedron minimum*	J				
Levanderina fissa		S						
Nodularia spumigena		CR						
Oxyrrhis marina		C						
Pandorina morum	G	CR						
Peridiniopsis borgei	L_O	S						
Picoplankton								
Spirogyra sp.	R							
Spondylosium planum	N	C						

flagellates, cyanobacteria and chlorophytes were abundant, whereas majority of the biomass consisted of charophytes, small flagellates and dinoflagellates. A detailed species list is included in paper I.

In most rock pools one or two species contributed to more than half of the total phytoplankton abundance and/or biomass (Table 1). This corresponds with expected species compositions in variable habitats, where dominance of a few tolerant species tend to cause uneven species abundance distributions (Passy 2016). However, at the regional scale the occurrence of these dominating species was infrequent. On the contrary, species that were noted in most of the pools seldom dominated, with a few exceptions (Table 1).

The majority of the phytoplankton flora was represented by 16 functional groups, of which the most common were F, L_O, and X2, assemblages typical of well-mixed, shallow, meso-eu-hypertrophic habitats with good light conditions (paper I). Characteristic traits among the species were adaptability, competitiveness, disturbance tolerance, invasiveness, efficient nutrient uptake in high nutrient concentrations, and effective dispersal (C-, R- and CR-strategists *sensu* Reynolds 2006). In comparison, acquisitive species (S-strategists *sensu* Reynolds 2006) with slow growth and efficient nutrient uptake in low nutrient concentrations, or alternative resource acquisition (e.g. mixotrophy) were rare, and in the pools represented by dinoflagellates.

Accordingly, the characteristic rock pool phytoplankton flora consisted of species adopting traits useful in a variable, unstable habitat. These traits were well represented among infrequently dominating, common and prevalent species, i.e. there were no apparent functional differences among these species. Small aquatic habitats are

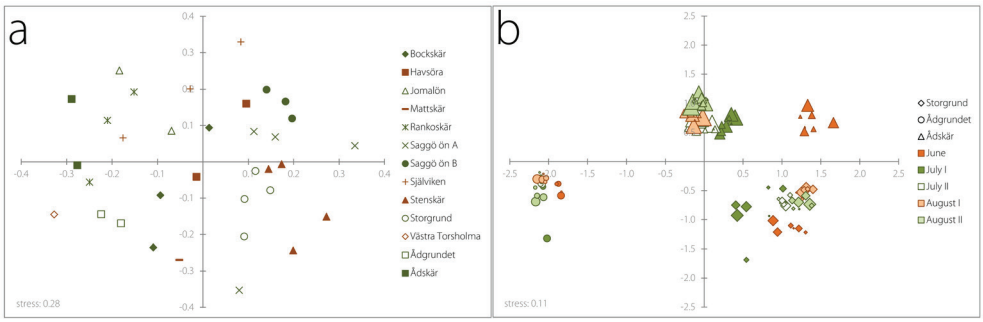

Figure 5. NDMS-ordinations based on species abundances. (a) Among the 34 rock pools in paper I. (b) In the three rock pools in paper II, depth increases with decreasing marker size.

exposed to ambient variations and the observed moderate regional and local spatiotemporal variations did not exclude significant variations at other scales. For example, daily vertical variations in brackish rock pools can be considerable (Ganning 1971).

Phytoplankton abundance and biomass varied largely among the rock pools (Fig. 4, papers I–II). Spatially, species assemblages among the pools varied substantially, even in pools on the same island (Fig. 5a). Temporally, the species assemblages early in the study period differed from those appearing later. Moreover, in each pool, the assemblages became more similar at the end of the season (Fig. 5b). Contrary to what would be expected in well-mixed conditions, the species-specific vertical fine scale distributions of phytoplankton were regularly clearly structured (paper II).

4. 2. Regional patterns of patch dynamics and priority effects

Regionally, phytoplankton biomass in the rock pools was related to nutrient concentrations (see Fig. 3 in paper I). However, the species assemblages were influenced by depth and macrophytes (Fig. 6). Depth and macrophytes are related to habitat morphology and influence pool stability. Generally, the likelihood of disturbance increases with decreasing system size (Borics et al. 2016). Specifically, conditions in deep rock pools are less variable compared to those in shallow pools (Ganning 1971). In the studied rock pools, macrophytes were mainly associated with shallow pools (Fig. 6), where their presence likely reflected more stable conditions. The structuring effect of vegetation in shallow habitats may be strong, e.g. emergent vegetation decrease pool permanency through increased evaporation (Altermatt et al. 2009). In absence of fish, macrophytes in shallow aquatic habitats influence phytoplankton assemblages through both abiotic and biotic mechanisms (Declerck et al. 2007).

Aquatic vegetation physically modifies habitats by adding structural complexity, which increases microhabitat heterogeneity and provides more colonisable area. Additionally, their shading may benefit phytoplankton in shallow, light abundant habitats. Consequently, a positive impact of macrophytes on phytoplankton diversity and biomass was observed in

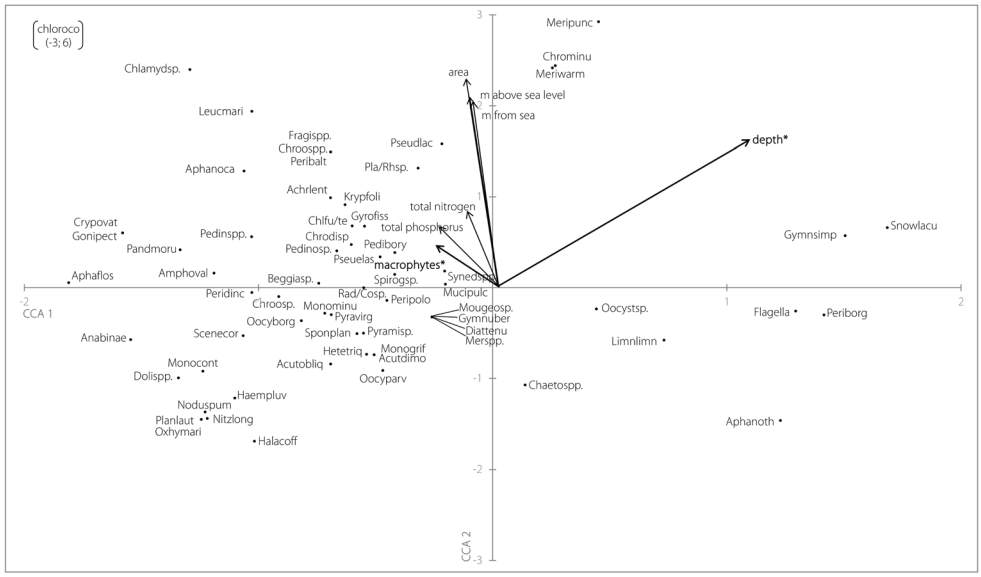

Figure 6. CCA-biplot based on species abundances and variables influencing the distribution of species among the 34 pools in paper I (*p<0.05). Species abbreviations as in paper I. Reprinted with permission from Taylor & Francis Group.

shallow habitats (Declerck et al. 2007, Naselli-Flores et al. 2016).

With increasing pool permanency, i.e. stability, the importance of biotic interactions in structuring species assemblages will increase, whereas environmental influence increases with decreasing pool permanency (Schneider & Frost 1996). The slow growth and acquisitive traits of S-strategists make them superior competitors in stable conditions, whereas the adaptability, disturbance tolerance, and invasiveness of C-, R-, and CR-strategists make them better competitors in unstable environments. For example, Gallego et al. (2014) found more S-strategists among phytoplankton in natural ponds compared to ponds with frequent management disturbances, in which colonisers were prevalent.

In stable systems, strong, structured biotic interactions have time to develop, leading to priority and monopolising effects by the established species against colonisers (De Meester et al. 2002). The sediments in deep rock pools are more stable compared to those in shallow pools (Ganning 1971), supporting seed banks providing resilience to new colonisers and a buffer against decreases in population size, enabling adaptation to the local habitat. For example, invertebrate assemblages in stable rock pools were more persistent compared to unstable pools (Therriault & Kolasa 2001). Such monopolisation effects are thought to be common among microorganisms due to their fast reproduction rates and may lead to differences in species assemblages already at small spatial scales (De Meester 2011). Thus, in addition to their regional influence (through dispersal), resting stages may have strong local influence.

In the studied rock pools, local versus regional effects of resting stages was concretely exemplified by the distribution of two cyst forming species with contrasting life strategies. The regional distribution of the dinoflagellate *Peridiniopsis borgei* (S-strategist) was limited, however it was conspicuous in one of the deepest pools (Ådskär, paper II). Here, the stability of the pool sediments probably ensured its continuing existence. In fact, *P. borgei* was noted in this pool also in 1995 (Lindholm 1996). On the contrary, the chlorophyte *Oocystis borgei* (C-strategist) was regionally prevalent (Table 1). Potentially, unstable sediments of shallow pools ensured a wide distribution of its cysts.

S-strategists were regionally limitedly distributed, indicating that they occupied patches in an incompatible environment of unstable pools. This supported an increased isolation and possibly augmented dispersal limitation (De Meester 2011). The C-, R-, and CR-strategists were regionally widely distributed. Compared to deep pools, passive dispersal by winds or birds (Incagnone et al. 2015, Padisák et al. 2016) may be more efficient in shallow, unstable pools, which are inclined to dry out (Altermatt et al. 2009). Species assemblages in unstable pools therefore tend to be strongly impacted by recolonisations (Therriault & Kolasa 2001, Vanschoenwinkel et al. 2013).

Presumably, the distribution of efficiently dispersing species is more influenced by local processes compared to species with limited dispersal (De Meester et al. 2005). For example, compared to rare species, stronger environmental filtering was observed for widely distributed, both common and abundant, bacteria in rock pools (Székely & Langenheder 2014). However, among the studied pools the measured environmental conditions were comparable (paper I), likely contributing to a moderate effect of environmental filtering at the regional scale. A strong environmental influence on the phytoplankton would, under the observed conditions, have resulted in similar assemblages, especially among the widely distributed colonisers. Thus, the regional distribution differences among infrequently dominating, common and prevalent C-, R-, and CR-strategists (Table 1) indicated variations in colonisation success and dispersal efficiency. Following Dethier (1984), stochastic disturbances potentially prevented competitive exclusion by the prevalent species (i.e. competitive dominants) and dominance in all pools they were able to colonise, supporting occasional dominance by inferior competitors (infrequently dominating species). More variable conditions, e.g. those caused by periodic warm and dry weather, would undoubtedly increase the effect of environmental filtering among the pools, also influencing distributional patterns at the regional scale (Vanormelingen et al. 2008, Östman et al. 2010).

The significant structuring effects of depth and macrophytes at the regional level contradicted a strong effect of random processes in forming the phytoplankton metacommunity, challenging the neutral perspective. Comparable environmental conditions among the rock pools suggested moderate regional influences of species sorting and mass effects. Rather, the observed regional patterns resembled patch dynamics (*sensu* Winegardner et al. 2012). Consideration

of species traits in combination with the effect of pool stability provided insight into processes underlying the observed regional patterns. Possible explanations for the observed limited regional distribution of acquiring S-strategists compared to invasive and acclimating C-, R-, and CR-strategists were monopolising and priority effects. This would further suggest that S-strategists were better competitors in stable pools (which were few in the studied rock pool metacommunity). The regional distribution of C-, R-, and CR-strategists appeared to be related to effective colonising and outcompeting activities in unstable pools, potentially driven by stochastic disturbances. Thus, among pool dynamics seemed especially important for phytoplankton in unstable pools.

In rock pools with distinctly differentiated environmental conditions, the patch dynamic perspective was not considered a realistic metacommunity forming process (Kolasa & Romanuk 2005). Therefore, prevailing weather conditions during these studies, leading to comparable conditions among pools, possibly enabled metacommunity processes resembling patch dynamics. In the studied rock pools, stochastic disturbances might have accounted for the occurrence of the infrequently dominating species. The limitation of snapshot sampling in these systems is a high risk of not including variables potentially structuring the species assemblages (Nabout et al. 2009). For example, zooplankton, which are more persistent in deep pools compared to shallow ones (Pajunen & Pajunen 2007), were omitted from the studies.

Recognition of species traits was emphasised in several recent metacommunity studies (De Bie et al. 2012, Algarte et al. 2014, Padial et al. 2014, Santos et al. 2016). For example, Santos et al. (2016) found strong environmental filtering of phytoplankton assemblages at large spatial scales (1500 km), but when accounting for species traits, some species were more strongly influenced by spatial processes. Consideration of the varying regional distributions of C-, R-, CR-, and S-strategists provided insight into metacommunity dynamics in the studied rock pools, and supported a trait-based approach.

4. 3. Locally small-scale turbulence modifies species assemblages

In paper II, local patterns in three rock pools with varying depth, exposure and area were explored by the fine scale vertical distributions of their phytoplankton assemblages. Similarly to results in paper I, the environmental conditions among the pools did not considerably differ (Fig. 4b). Despite the comparable environmental conditions, the phytoplankton abundance, biomass (Fig. 4b) and assemblages (Fig. 5b) diverged between the pools. Moreover, their vertical fine scale phytoplankton distributions differed by varying less in the deep pool compared to the shallow ones. These results were in accordance with those in paper I, where variables related to pool stability (depth, macrophyte presence-absence) influenced the regional phytoplankton distribution. They additionally supported the perception that deep pools were more stable compared to shallow pools.

Contrary to what would be expected in well-mixed conditions, the species-specific fine scale distributions of phytoplankton

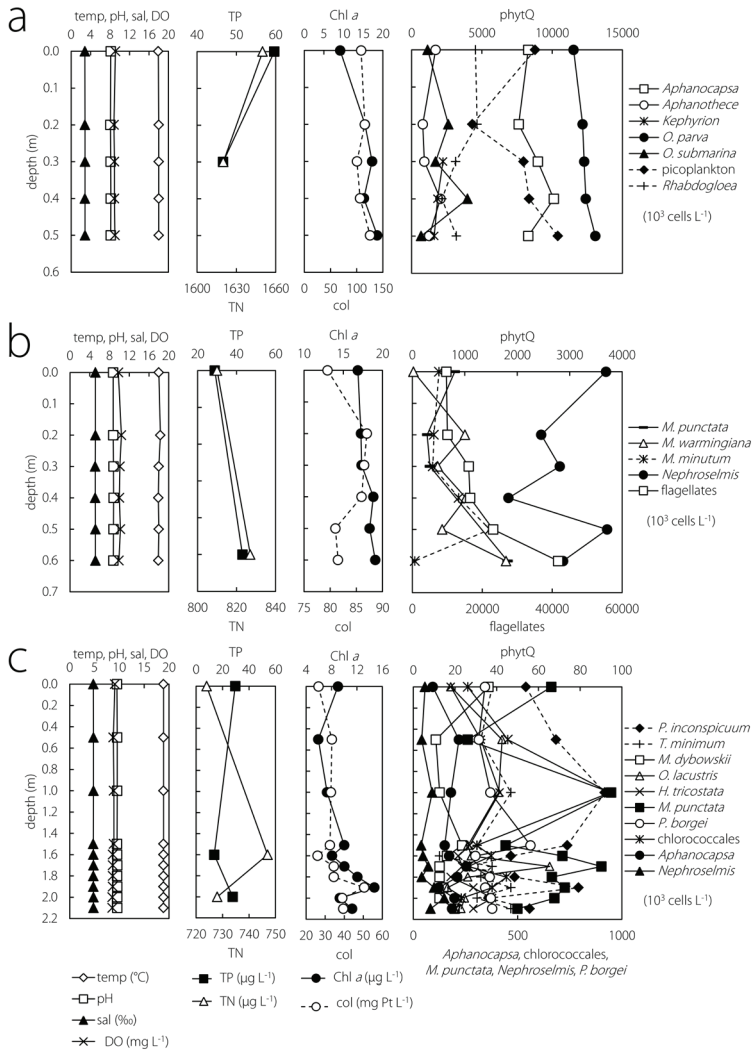

Figure 7. Local vertical fine scale profiles in three well-mixed rock pools (during lowest measured RWCS) (paper II). Abiotic and biotic variables in a (a) shallow and small (RWCS 1), (b) shallow and large (RWCS 3), and (c) deep and large rock pool (RWCS 3). Abbreviations as in Fig. 4, colour: col, *Helicopedinella tricostata*: *H. tricostata*, *Merismopedia punctata*: *M. punctata*, *Merismopedia warmingiana*: *M. warmingiana*, *Monoraphidium dybowskii*: *M. dybowskii*, *Monoraphidium minutum*: *M. minutum*, *Oocystis lacustris*: *O. lacustris*, *Oocystis parva*: *O. parva*, *Oocystis submarina*: *O. submarina*, *Parvodinium inconspicuum*: *P. inconspicuum*, *Peridiniopsis borgei*: *P. borgei*, *Tetraedron minimum*: *T. minimum*. The species are those contributing to ≥90% of total phytQ.

were structured in all three pools (Fig. 7). Phytoplankton abundance and biomass, or individual dominating species, were only occasionally related to local environmental conditions (dissolved oxygen, nutrients, pH, salinity). Several of these occasional relationships were discovered during seasonal minima or maxima in the measured variables. Consequently, the influence of these local environmental conditions on phytoplankton was only marginal and mostly due to stochastic disturbances. Non-random small-scale spatial distributions of phytoplankton, not related to environmental conditions, are well known in marine habitats (Durham & Stocker 2012), recognised in freshwater systems (Caron et al. 2008) and have also been noted in well-mixed upper ocean layers (Mitchell et al. 2008, Doubell et al. 2014). As indicated by the findings in paper II, they were also present in shallow well-mixed habitats.

Jointly, the marginal effect of environmental conditions, the structured fine scale species distributions despite well-mixed water columns, and their higher variability the shallow pools, strongly indicated an effect of turbulence on the observed local phytoplankton patterns. Small-scale physical processes associated with turbulence substantially impact phytoplankton (Doubell et al. 2014). Gravitational and rotational forces, caused by wind and vertical movements, may cause thin layers of intense shear, keeping phytoplankton cells trapped (Durham et al. 2009). Aggregations of phytoplankton may form between parcels of high turbulence (Mitchell et al. 2008). Considering that small-scale phytoplankton aggregations are formed at ecologically relevant time scales, enough for e.g. nutrient acquisition, competition, grazing, or infection (Mitchell et al. 2008), their impact is highly relevant. Consequently, turbulence modifies and affects key ecological processes (Prairie et al. 2012).

At small scales, turbulence decreases the boundary layer surrounding individual cells, making it possible for the cell to increase its nutrient uptake and growth (Prairie et al. 2012). This is highly dependent on cell size, shape, buoyancy, abundance, and swimming speed, which are species-specific characteristics that depend on the physiological condition of the cells (Waters & Mitchell 2002). Small-scale processes related to turbulence are therefore highly taxa specific, influencing the success of individual species. Thus, turbulence has a selective influence on phytoplankton assemblages. Consequently, the effects of small-scale turbulence on microorganisms are non-random with deterministic consequences for their species assemblages (Niederdorfer et al. 2016).

In the studied rock pools, the fine scale vertical phytoplankton distributions varied more in the shallow pools compared to the deep one (paper II), indicating a stronger effect of turbulence. Wind causes turbulence at the water surface, whereas friction causes turbulence near the bottom. In shallow systems these two turbulence layers may coalesce (Jumars et al. 2009) leading to an unstable water column. In the two shallow pools the dominating taxa, contributing strongly to the observed fine scale vertical phytoplankton distributions, were *Oocystis* spp. and small flagellates (both C-strategists). In the deep pool *Peridiniopsis borgei* (S-strategist) was not abundant, but

dominated the phytoplankton biomass and the observed phytoplankton fine scale vertical distribution strongly reflected the *P. borgei* distribution. Possibly, high turbulence in the shallow pools caused unstable conditions supporting C-, R- and CR-strategists, which contributed to the proposed effective colonising and outcompeting regional processes among unstable pools. Weak turbulence in the deep pools potentially led to stable conditions upholding S-strategists and promoting biotic interactions, leading to the proposed regional priority effects of S-strategists versus C-, R-, and CR-strategists.

The distribution of phytoplankton in the water column adds another dimension to their spatial patterns and processes, which especially requires consideration of physical features in the water column (Naselli-Flores & Padisák 2016). The findings in paper II indicated that spatial distributions within habitats were crucial for understanding both regional and local patterns of phytoplankton in shallow systems. Small-scale patterns require consideration of species-specific characters and demand an appropriate sampling technique, which are considered in the following two sections.

4. 4. The complexity of small-scale processes underlying observed patterns

Physical processes overarch processes and patterns at all spatial scales and naturally, ecological interactions do not violate physical laws, but these are not sufficient to explain observed variability in ecological communities (Ricklefs 1987). Similarly to bacteria (Azam & Malfatti 2007), patterns and processes related to phytoplankton cannot only be understood by studying them at large scales. Microorganisms influence their environments through molecular interactions and insights into processes at a molecular level provide fundamental biochemical models for the interactions. Consequently, the spatial distributions of microorganism assemblages are to some extent self-organising because of the metabolic interactions (Hellweger et al. 2016). Thus, in paper III, molecular properties of a species isolated from the rock pool habitat were characterised.

The species in focus in paper III, morphologically identified as *Woronichinia* sp., was a consistent part of the phytoplankton assemblage in a rock pool at Storgrund (noted during a field sampling in 2010, present in 2011 and throughout the season in 2012), although usually contributing to <0.2% of total phytoplankton abundance and biomass.

Cyanobacteria in the family Coelosphaeriaceae are a regular part of phytoplankton assemblages in many aquatic environments. However, phylogenetic and chemical characterisations of cyanobacteria in this family are scarce and have often been based on individuals in natural, uncultured, samples. Seven of the 48 species included in this family have been genetically sequenced and, excluding paper III, one study of the phylogenetic relationships between species in the family has been published (Rajaniemi-Wacklin et al. 2006). The results presented in paper III include the first chemotypic characterisation of a cultured species in the family Coelosphaeriaceae isolated from a brackish habitat.

The limited existence of comparable genetic and chemotypic information

Table 2. Chemotypic properties of *Woronichinia* sp. (strain 06S067) isolated from a rock pool (paper III). Identified anabaenopeptins (AP) in methanol (MeOH) fractions of the species. The fraction with the most intense ion signal of corresponding AP in bold. Oscillamide: OSC, molecular mass is included as suffix of suggested new analogs. Schematic structures of the amino acid residue sequences follow Welker & von Döhren (2006), amino acids are given in common three letter codes, [] denotes a ring.

Fraction (% MeOH)	*m/z*	Proposed anabaenopeptin	Amino acid residue sequence
40, 50, **60**	803	AP 802	Arg+CO+[Lys+Val+Htyr+*N*-MeAla+Ile/Leu]
30, 40	810	AP 809	Tyr+CO+[Lys+Val+Htyr+*N*-MeAla+Ile/Leu]
40	828	AP 827	Tyr+CO+[Lys+Val+Hph+*N*-MeAla+Phe]
20, **30**, 40, 50, 60	844	AP A	Tyr+CO+[Lys+Val+Htyr+*N*-MeAla+Phe]
40, **50**, 60	837	AP B	Arg+CO+[Lys+Val+Htyr+*N*-MeAla+Phe]
60	851	AP F	Arg+CO+[Lys+Ile+Htyr+*N*-MeAla+Phe]
30, **40**, 50	858	OSC Y	Tyr+CO+[Lys+Ile+Htyr+*N*-MeAla+Phe]

complicated the identification of the isolated *Woronichinia* sp., which in the phylogenetic analyses aligned with the closely related *Snowella* genus (see Fig. 3 paper III). Although 16S rRNA is a commonly used genetic marker for cyanobacteria (Moreira et al. 2013), its taxonomic resolution at the species level is low, which may cause ambiguities for closely related species (Fox et al. 1992). Inclusion of several genetic markers increases the reliability of phylogenetic characterisations, but also the cost and work (Moreira et al. 2013). Moreover, the attained phylogenetic tree can vary based on the used genetic marker (Moreira et al. 2013). The results stressed one major difficulty in deciphering microorganism patterns and processes – that of species identification. Still, the polyphasic identification approach increased ways in which the results may be utilised for future elucidations of species in the family.

Table 3. Enzyme inhibition of methanol (MeOH) fractions from *Woronichinia* sp. (strain 06S067). Modified from Table 2 in paper III.

Enzyme	Fraction (% MeOH)	Inhibition (%)
Carboxypeptidase A	30	13
	40	8
	50	1
Chymotrypsin	50	5
	60	14†
Elastase	90	15†
	100	–‡
Protein phosphatase 1	40	<1
	50	<1
Protein phosphatase 2A	30	<1
	40	<1
	50	<1
Thrombin	60	49†
	70	44
Trypsin	60	94
	70	26†

†>50% mean relative error of applied regression.
‡Negative dose-response curve.

The metabolite characterisation of *Woronichinia* sp. indicated a rich profile of small, cyclic nonribosomal oligopeptides that are produced by numerous cyanobacteria (Agha & Quesada 2014). The oligopeptides were mainly identified as anabaenopeptins, differing only in one amino acid residue (Table 2). Many cyanobacterial nonribosomal oligopeptides, also those produced by *Woronichinia* sp. (Table 3), show activity against proteolytic enzymes. However, despite the seemingly ubiquitous presence of these metabolites

in cyanobacteria, their biological and ecological functions are yet to be discovered.

Considering microorganisms, the impact of ecological interactions, such as competition, infection or grazing, increases at small scales, where these processes actually take place. The importance of ecological interactions at small scales is also reflected in proposed suggestions for functions of cyanobacteria nonribosomal oligopeptides. Given their activity against proteolytic enzymes, found in both prokaryotes and eukaryotes, their production has been connected to protection against chytrids (Rohrlack et al. 2013) and grazers (Czarnecki et al. 2006), as well as allelopathy (Engström-Öst et al. 2011). There is also evidence of their potential roles as info-chemicals (Briand et al. 2016), and in bloom termination (Sedmak et al. 2008).

The roles of bioactive nonribosomal oligopeptides in ecosystem dynamics are not easily disentangled; although their biological purpose may be defence against chytrids, they may also cause side effects in grazers by hampering their digestive activity (Czarnecki et al. 2006) or molting (Rohrlack et al. 2004). Moreover, even though the nonribosomal oligopeptides characterised in *Woronichinia* sp. are regarded as non-toxic, they have been shown to accumulate in mussels and crustaceans (Mazur-Marzec et al. 2015). This bioaccumulation adds to the complexity of their ecological consequences, especially considering the presence of *Woronichinia* spp. in various aquatic habitats, or the overall global increase in cyanobacteria (Paerl & Paul 2012).

Although comparatively small organic molecules, the production of nonribosomal oligopeptides requires big enzyme complexes, which are encoded by large gene clusters, thus their production is costly (Rohrlack et al. 2009). Different chemotypes may show varying responses to environmental conditions and chemotype profiles can be considered as species traits (Fastner et al. 2001, Rohrlack et al. 2008), which influence the distribution of species (Rohrlack et al. 2009, Kyle et al. 2015). For example, there are indications of distinct cyanobacteria chemotypes in deep and shallow lakes (Kurmayer et al. 2015). In combination with studies describing cyanobacteria chemotypes in many geographic areas and various aquatic habitats, this offers an intriguing aspect in relating chemotype distributions to metapopulation, or -community processes, and even biogeographical patterns (Padisák et al. 2016).

The degree of species interactions profoundly impact metacommunity dynamics (Holyoak et al. 2005) and molecular properties may strongly impact the way in which species, especially microorganisms, interact. Thus, specific structures at the molecular scale presented in paper III underline that phytoplankton assemblages consist of individual species, which in addition to being structured by processes at regional and local scales, respond to these processes also at a molecular level. Reciprocally, consequences of molecular properties are transferred to large scales. Effects of toxic compounds are perhaps the most conspicuous considering cyanobacteria, but processes related to phytoplankton molecular properties are complex, e.g. involving behavioural responses of grazers

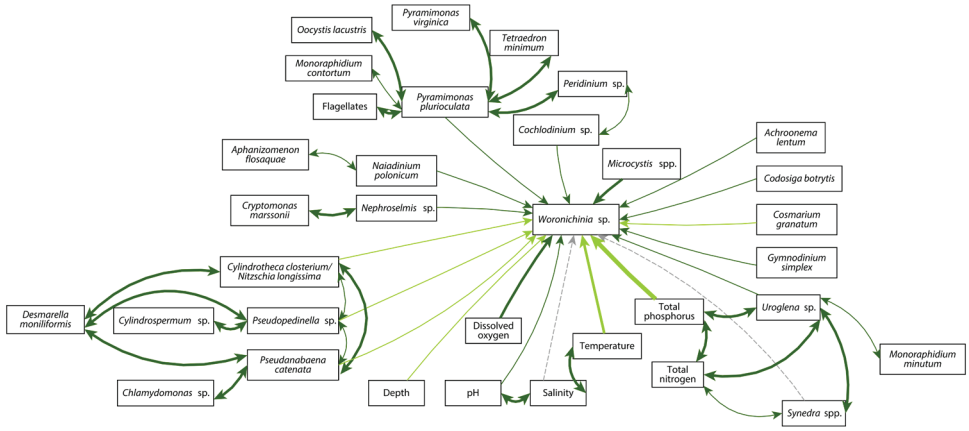

Figure 8. Piecewise structural equation model of positive and negative (light green arrows) abiotic and biotic interactions influencing *Woronichinia* sp. in a rock pool. The thickness of the causal paths was scaled according to the strength of their standardised regression coefficients (Supplement). Two-headed arrows indicate correlations. Non-significant dashed paths in grey. Fischer C_{33} = 19.9, *p*-value = 0.07, AICc = -281.

(Wolfe 2000). Complexity increases with decreasing scale (Fig. 8), but as exemplified by the findings in paper II, studies of small-scale processes improve the understanding of mechanisms behind observed patterns. Still, the greatest risk of a reductionist approach in ecological studies might not be associated with an excess of data, but rather with the difficulties in relating small-scale patterns and processes to those at large scales (Wiens 1989).

4. 5. Precision sampling considers scales relevant to phytoplankton

In paper II, a centimetre characterisation of phytoplankton distributions in rock pools facilitated the understanding of observed local and regional patterns. Coarse sampling, e.g. integrated sampling as in paper I, do not reveal fine scale patterns, but is often used in studies of small aquatic systems (De Meester et al. 2005). Moreover, phytoplankton in well-mixed, turbulent environments have generally been considered homogeneously and randomly dispersed (Doubell et al. 2014). Accordingly, fine scale spatial distributions of phytoplankton have not been studied in well-mixed, shallow habitats. Still, spatial structures formed by phytoplankton are anticipated to spread across smaller scales in well-mixed environments compared to those in stable conditions (Waters & Mitchell 2002), indicating that precision sampling is needed to fully understand phytoplankton ecology in well-mixed systems.

Concerning microorganisms, the sampled scale is generally anthropocentric, ignoring the scale at which several community forming processes occur. For example, conventional samplers, which are regularly used for phytoplankton characterisations, are generally >2 L in volume and >30 cm long, consequently combining species and conditions that are spatially separated *in situ* (Donaghay et al.

1992). The inconsistency between sampled scale and organism perceived scale inhibit extrapolation of mechanistic processes at small scales to large-scale patterns. However, these cross-scale comparisons are ecologically highly relevant as they increase the predictability and comparability across ecosystems (Levin 1992), but only knowledge of correct mechanisms lead to correct predictions (Hellweger et al. 2016).

Technical developments enable studies of microorganisms at very high resolutions, e.g. processes between a cell and its immediate surroundings (Kreft et al. 2013), and the use of individual based models is an interesting and promising approach in extrapolating from micro- to macroscales (Hellweger et al. 2016). Additionally, exact, minute physical and chemical variations can be measured with sensors. These advancements are highly useful in understanding mechanisms behind small-scale structures, but they are often expensive and may not be applicable in all types of habitats. Moreover, sample withdrawal, usually not possible with sensors, is needed in species quantification. Thus, these applications are of limited use in phytoplankton field studies, where inclusion of precision samplings, even a few, may increase the accuracy of causal conclusions (Dann et al. 2016, paper IV).

The importance of precision sampling in phytoplankton ecology, especially in stratified environments, was early recognised. Developed precision samplers have generally been complicated, consisting of many parts, with considerable limits in sample withdrawal volume, possibly leading to their limited reported use in field studies. In comparison, the precision sampler applied by Lindholm (1979) and presented in paper IV, has several advantages, which makes it a realistic alternative in various phytoplankton field studies. These advantages relate to its simple construction of off-the-shelf, low-cost parts; sampling at centimetre scales; sample withdrawal at litre volumes; functionality in sub-zero air temperatures; potential for near bottom phytoplankton characterisations; applicability in dense subsurface vegetation and shallow habitats. By applying this method, species-specific phytoplankton patterns at centimetre scales were acquired below lake ice, among *Phragmites australis* and dense submerged *Myriophyllum sibiricum*, as well as in shallow rock pools (paper IV). These are all habitats where conventional sampling is difficult.

Rather than indicating correlative relationships, precision sampling increased the number of causal relationships (see Fig. 6 in paper IV), emphasising its potential for a mechanistic understanding of observed phytoplankton patterns. Consistent with the findings in paper II, structured fine scale phytoplankton distributions were discovered in the various aquatic habitats (see Fig. 2–5 in paper IV). Moreover, the precision sampling revealed small-scale spatial patterns that contradicted light or nutrient limited phytoplankton assemblages among dense aquatic vegetation. Thus, conventional sampling might underestimate phytoplankton abundance, biomass and primary production in these habitats.

Fine scale structured spatial distributions of phytoplankton, hot spots of biological activity, challenge the perception of primary production taking

place in an environment where nutrients are homogeneously distributed (Azam & Malfatti 2007). Likewise, in well-mixed environments turbulence is generally considered to homogenise phytoplankton distributions, likely a result of applied coarse sampling methods (Fig. 9). However, the fine scale spatially structured distributions of phytoplankton in mixed water columns (Mitchell et al. 2008, paper II), and consequences of interactions in these hot spots, are underestimated in models assuming random distributions of phytoplankton (Doubell et al. 2014).

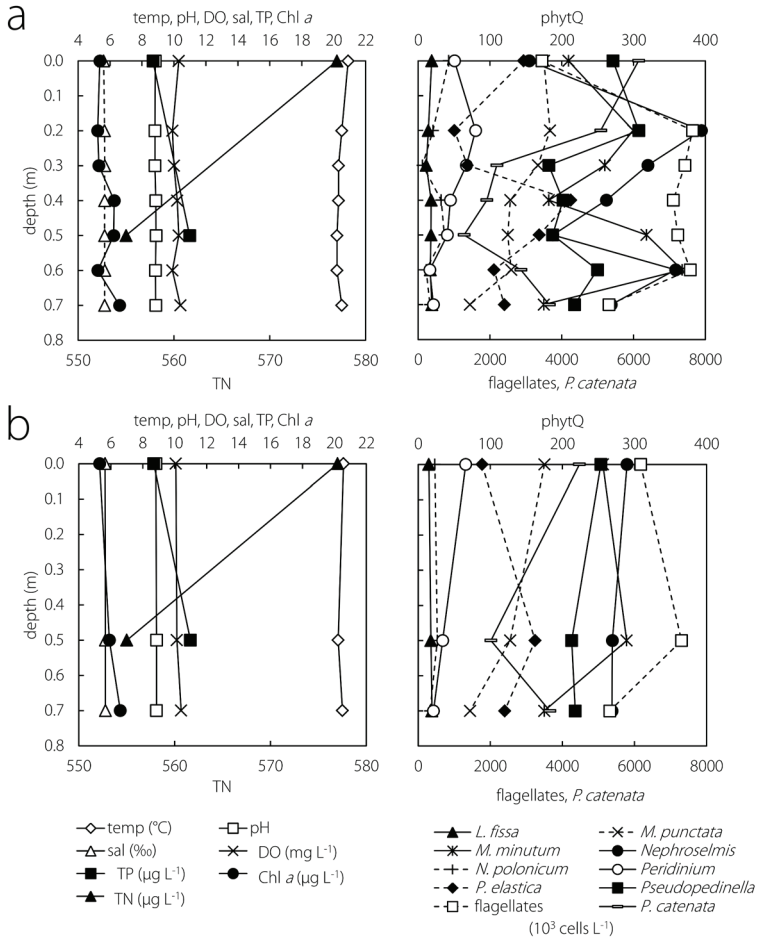

Figure 9. The influence of sampling method on observed phytoplankton patterns in a well-mixed rock pool. (a) Vertical profiles attained with precision sampling, modified from Fig. 5 in paper IV. (b) Corresponding vertical profiles with values averaged as in paper IV (with the exception of TN and TP), comparable to conventional sampling (sampler 30 cm in height). Abbreviations as in Fig. 4. *Levanderina fissa*: *L. fissa*, *Merismopedia puncata*: *M. punctata*, *Monoraphidium minutum*: *M. minutum*, *Naiadinium polonicum*: *N. polonicum*, *Pseudopedinella elastica*: *P. elastica*, *Pseudanabena catenata*: *P. catenata*.

Species-specific profiles attained with the precision sampler showed the substantial variations behind single chlorophyll profiles (Fig. 9a), which often are used for characterisation of phytoplankton. Detailed species profiles may provide insights into functional traits such as sinking rates, as well as inter- and intraspecific relationships (Fig. 8). Rather than studying such biotic relationships at scales of metres, they should be explored at their scale of occurrence, thereby increasing the reliability of causal and associative relationships. For example, patterns and processes related to global carbon cycles, species diversity, and spatial distribution have been explained by studying bacteria at their scale of existence (Azam & Malfatti 2007). In combination with characterisations of structures at the molecular scale, as for example in paper III, such species-specific patterns may be highly informative in species autecology.

5. Main conclusions

The phytoplankton assemblages in the studied rock pools were characterised by C-, R- and CR-strategists, which are species with acclimatising and colonising traits, adapted to a variable and unstable habitat. The most conspicuous functional groups (F, L_O, X2) were assemblages typical of light abundant and well-mixed habitats. The regional distribution of the conspicuous species was wide, but their occurrence was divided into infrequently dominating, common and prevailing species. On the contrary, the regional distribution of acquiring species (S-strategists) was limited.

Depth and macrophytes, both connected to pool stability, influenced the regional distribution of species. Stochastic disturbances likely enabled effective outcompeting and colonising activities among acclimatisers and colonisers in shallow, unstable pools. Moreover, macrophytes were primarily associated with shallow pools. The regional distribution of acquiring species seemed to be related to their monopolising and priority effects in stable, deep pools. Observed regional patterns in the studied rock pool metacommunity resembled patch dynamic processes. Thus, trade-offs among colonisers and competitors between stable and unstable pools, as well as in unstable pools, likely influenced the regional species distribution.

Locally, measured abiotic fine scale vertical variations were not distinct among rock pools with diverging morphology. Vertical fine scale distributions of phytoplankton varied less in the deep pool compared to shallow pools, but were predominantly not related to measured abiotic variables. Ultimately, differences in species assemblages among deep and shallow (stable and unstable) pools appeared to be related to variations in turbulence patterns. Structured fine scale spatial distributions of phytoplankton within individual pools, despite well-mixed conditions, indicated that these physical processes shaped the species assemblages non-randomly. Thus, by influencing fundamental community forming processes as nutrient uptake and cell encounter rates, small-scale turbulence impacted the metacommunity from local scale up. A moderate structuring effect of variables typically influencing phytoplankton, e.g. nutrients, oxygen concentration, pH, was possible during the prevailing weather conditions.

At the scale of assemblage influencing small-scale physical processes, species interactions, i.e. for phytoplankton molecular processes, come into play. However, chemical ecology, and even species identification, of microorganisms is complex, as exemplified by the inconclusive phylogenetic and chemotypic characterisation of a cyanobacterial species isolated from the rock pool habitat. Nonetheless, these molecular based processes are undoubtedly contributing to observed patterns. Compounds with potent activity against certain proteolytic enzymes were discovered in the isolated cyanobacterium. These bioactive properties indicated ecological implications beyond the individual scale.

Unanticipatedly structured phytoplankton fine scale distributions within

the well-mixed rock pools were discovered by precision sampling. Detection of these small-scale patterns emphasised the importance of matching measurements with organism responses to correctly decipher mechanisms underlying observed patterns. Thus, by sampling at a scale relevant to phytoplankton, precision sampling increased the predictive value of the study.

To summarise, the findings in papers I–IV suggested that traditional metre interval characterisations of phytoplankton assemblages limited to a local scale might not adequately explain observed patterns. Merely local conditions could not satisfactorily explain the observed phytoplankton spatial patterns in the studied rock pools. Species patterns revealed in papers I–II illustrated a need to enlarge the extent, i.e. spatial scale, at which phytoplankton assemblages were studied. On the contrary, an understanding of mechanisms behind observed patterns required a decrease of the grain (papers II–III), i.e. unit of observation, which necessitated sampling at appropriate scales (papers II and IV).

6. Significance of results and outlook

The findings in this thesis emphasised the need for a non-arbitrary consideration of spatial scale in phytoplankton ecology. Abandoning the traditional view of phytoplankton assemblages as randomly structured closed systems will likely be advantageous in future studies. Focussing on species traits and underlying mechanisms improved the understanding of observed patterns, but identification ambiguities and complex molecular processes challenged a mechanistic approach. The vulnerability of small aquatic systems, which provide unique ecosystem services, was additionally highlighted in this thesis.

6. 1. The implications of studies in small aquatic habitats for nature conservation

Small aquatic habitats constituting metacommunities are biodiversity refuges, contributing to regional biodiversity (Williams et al. 2004) and are important to consider in nature conservation (Naselli-Flores et al. 2016). Conditions in these weather-influenced systems are distinctly different from those in lakes and oceans, implying the existence of unique species assemblages, concerning geno-, pheno- and chemotypes. In agricultural areas small ponds contribute to increased crop yields, fish production, groundwater recharges, reduction in flooding, supplies of domestic water and are highly important where water is scarce (Mushtaq et al. 2007). Thus, the value of small ponds and pools in nature conservation is increasingly recognised (Raunio et al. 2008, Downing 2010, Boix et al. 2012). Traditionally, studies of waterbodies in the terrestrial landscape have been type specific with little comparisons among systems and concentrated on streams, rivers and lakes. However, consideration of different aquatic systems is needed in order to attain a successful catchment management. In a landscape context, the knowledge of patterns and processes in small aquatic systems, e.g. pools, ponds, ditches, springs, is usually very limited. Nevertheless, at the landscape catchment level small aquatic habitats may be especially important in maintaining species diversity (Williams et al. 2004). This also indicates that species diversity can be enhanced relatively easily by increasing the number of small aquatic habitats in an area (Williams et al. 2004), which also is cost-beneficial (Mushtaq et al. 2007). Additionally, the studies summarised in this thesis exemplify how small and shallow aquatic systems can be used for exploration of ecological theories (papers I–II) and method development (paper IV).

Spatial scales used in policy-making and ecological studies often diverge (Keskitalo et al. 2016). Generally, results from ecological studies are extrapolated to meet the demands of large-scale generalisations in policy-making. Incorporating processes that structures species assemblages at different scales in monitoring and assessment programmes is important. There is a limited gain in imposing a single scale on all organisms in a community (Wiens 1989). Exemplified by the local and regional influences on phytoplankton distributions in studies summarised in

this thesis, similar environmental conditions or close geographical proximity of habitats do not satisfactorily predict their species assemblages. For example, phytoplankton may be strongly influenced by environmental processes at habitat boundaries (Meier et al. 2015), which should have implications in monitoring programmes.

The impacts of climate change and habitat destruction are alarming issues in shallow aquatic systems. Due to the overarching effect of weather, climate change profoundly impacts these shallow systems. At small scales, the dynamic response to disturbances is fast. Thus, the consequences of disturbance might be considerable in small systems compared to large ones (Wu & Loucks 1995), which in small systems ultimately leads to disappearance. Their exposure to ambient conditions makes shallow aquatic habitats suitable study systems of effects of climate change. For example, by increasing storm frequencies, climate change has high local impact on water turbulence (Jumars et al. 2009). Considering the impact of small-scale physical processes on phytoplankton assemblages in the studied rock pools (paper II), climate change undoubtedly modifies local phytoplankton assemblages as well as regional distribution patterns.

6. 2. To comprehend microorganisms and their spatial scale

Complexities involved in microorganism identification impair an understanding of their spatial distributions at various scales (Fontaneto & Brodie 2011), as exemplified by the ambiguous results in paper III. The conception that microorganisms are ubiquitous has been based on morphological features (Finlay & Fenchel 2004). Therefore, the use of genetic and chemotypic characteristics provide an interesting aspect in microorganism metacommunity ecology and biogeography (Fontaneto & Brodie 2011). For example, based on phylogeography, the cyanobacterium *Microcystis aeruginosa* is considered to be cosmopolitan (Van Gremberghe et al. 2011). On the contrary, both regional (Hayes & Barker 1997) and global (Bolch et al. 1999) distribution of *Nodularia* cyanobacteria differed among genotypes. Diverging distributional patterns of different species demonstrate the peril of considering microorganisms as one coherent group in trying to resolve their spatial patterns. Findings in this thesis demonstrated that the spatial distribution of phytoplankton with distinct traits, but also among species with similar traits, differed (paper I). Hence, already recognition of species traits might provide a better insight into the spatial distributions of microorganisms (De Bie et al. 2012, Algarte et al. 2014, Padial et al. 2014, Santos et al. 2016).

Methods such as remote sensing and geographical information systems offer possibilities in describing large-scale patterns and processes, required in nature conservation. However, the significance and consequences of large-scale patterns cannot be fully understood without consideration of patterns and processes at scales relevant to the species (Belyea & Lancaster 1999, Miller et al. 2004). After all, observed species patterns are ultimately a result of individual responses to the environment (Levin 1992). This thesis showed the significance of large-scale patterns in exploring how species assemble (paper I) and how small-scale

patterns increased the understanding of underlying mechanisms (paper II). Concerning microorganisms, a bottleneck in a mechanistic approach is the difficulties to comprehend the relevant scale of these processes.

A mismatch between sampled scale and the scale of microorganism existence has likely contributed to the general perception that microorganisms are randomly distributed at small scales. In accordance with Azam & Malfatti (2007), Doubell et al. (2014) and Dann et al. (2016), precision sampling studies in this thesis (papers II, IV) supported the perception that microorganisms are not randomly distributed at small spatial scales (micro- to centimetres). Conventional phytoplankton sampling methods (e.g. volumes of 2 L at 0.5 m depth intervals) therefore likely underestimate the consequences of small-scale phytoplankton structures (Doubell et al. 2014). Consequently, it is highly likely that consideration of fine scale patterns, especially those related to physical processes, increase the understanding of large-scale microorganism patterns in aquatic systems. Concurrenlty, a consideration of bioactive molecular properties, with potential for ecological implications, will likely increase the understanding of fine scale patterns.

The combination of cell specific studies (e.g. Kreft et al. 2013) with *in situ* methods, such as the described precision sampler (paper IV), and individual based models (Hellweger et al. 2016) might appear effective in relating small-scale mechanisms to large-scale microorganism distributional patterns. Instead of merely quantifying a change, these methods permit a comprehension of how the change is taking place. Revealing such mechanisms can increase the predictability of phytoplankton processes, e.g. global carbon cycles, or harmful algal blooms, and their implications.

6. 3. The relevance of studying phytoplankton distributions

In contrast to traditional phytoplankton studies, phytoplankton patterns at multiple spatial scales were explored in this study. The results showed that cross-scale studies increase the understanding of ecological patterns, as no organism is isolated to a particular scale. Thus, in accordance with Lawton (1999), valuable ecological generalisations did not appear by concentrating on one spatial scale.

The structure of the studied rock pool phytoplankton metacommunity was related to habitat stability at both regional and local scales during moderate environmental filtering enabled by the prevailing weather. Differences in regional distribution of species with distinct traits suggested impacts of metacommunity processes, as they could not be explained by measured environmental conditions. The results thus challenged an environmentally strongly structured omnipresence of microorganisms.

The ultimate goal in studying species patterns and processes is to understand their relation to ecosystem functioning (Holyoak et al. 2005). Through primary production, carbon dioxide respiration and decomposition, phytoplankton have important roles in ecosystem functioning. These fundamental processes are dependent on the structure of the phytoplankton assemblages (Naeem & Li 1997). An understanding of what impacts these

assemblages and the mechanisms behind is essential to predict consequences of changes in ecological systems. Recognising that both local and regional processes structure phytoplankton assemblages, leads to the conclusion that these central ecosystem processes are not only upheld by the local assemblages, but also influenced by regional processes (Ptacnik et al. 2010). Spatially structured distributions of microorganisms also indicate that microorganism assemblages in different geographical areas may perform differently under similar environmental conditions (Martiny et al. 2006). Consequently, regional processes might need to be considered in e.g. phytoplankton responses to environmental change or frequency of harmful algal blooms (Ptacnik et al. 2010). In this thesis, absence of extreme environmental summer conditions seemed to increase among patch dynamics (paper I), whereas environmental filtering would likely be more noticeable during more variable and extreme climate.

It appears undeniable that phytoplankton assemblages are influenced by both environmental and spatial variables. The strength of their influence is dependent on environmental conditions, system and scale, both temporal and spatial (Heino et al. 2010, Östman et al. 2010, Soininen et al. 2011). Consequently, conclusions in studies aiming at discerning if spatial or environmental processes control the distribution of phytoplankton differ based on studied environmental conditions, systems and scales (Beisner et al. 2006, Soininen et al. 2007, 2011, Vanormelingen et al. 2008, Nabout et al. 2009, Heino et al. 2010, Mazaris et al. 2010, Nogueira et al. 2010, Ptacnik et al. 2010, Lopes et al. 2011, De Bie et al. 2012, Gallego et al. 2014, Padial et al. 2014, Meier et al. 2015, Santos et al. 2016). Moreover, considering phytoplankton as one coherent group may be detrimental. Instead of focussing on which of these processes predominate, underlying mechanisms of the observed patterns should be studied. For phytoplankton, a correct understanding of these mechanisms requires a conception of small scales, but also the comprehension that the consequences of these mechanisms are not confined to small scales.

The failure of ecological theories based solely on local community ecology in explaining and predicting species extinctions and habitat destruction led to apprehension of scale and development of metapopulation and -community ecology. Similarly, the inability or inaccuracy in ecological models predicting microorganism patterns and processes may need to consider the scales relevant for these organisms. Thus, in line with studies of macroorganisms, it is essential to resolve "who does what, when, where and next to whom" (Hellweger et al. 2016) also for microorganisms. Such individual based studies may even provide insight into microorganism biogeographical patterns (Hellweger et al. 2016).

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Supplement

Conceptual model

Significant Spearman rank correlations ($R_s \geq 0.700$, p*=0.000)*

"temp~~sal","pH~~sal","sal~~Aphanot","TP~~TN","TP~~Monmin","TP~~Urogle","TN~~Monmin","TN~~Synedr","TN~~Urogle","Aphani~~Dolflo","Aphani~~Naiadi","Chaneo~~Tetrae","Chaten~~Pseela","Chlamy~~Psecat","Cochlo~~Peridi","Crymar~~Nephro","Crymar~~Plagio","Crypha~~Perbal","Cylspe~~Desmar","Cylspe~~Dolflo","Cylspe~~Flagel","Cylspe~~Peridi","Cylspe~~Pseudo","Cy_Ni~~Desmar","Cy_Ni~~Psecat","Cy_Ni~~Pseudo","Desmar~~Peridi","Desmar~~Psecat","Desmar~~Pseudo","Dolflo~~Flagel","Dolflo~~Parumb","Dolflo~~Peridi","Flagel~~Moncon","Flagel~~Ooclac","Flagel~~Peridi","Flagel~~Pyrplu","Flagel~~Pyrvir","Flagel~~Tetrae","Gymube~~Monmin","Moncon~~Peridi","Moncon~~Pyrplu","Moncon~~Tetrae","Monmin~~Urogle","Ooclac~~Pyrplu","Ooclac~~Pyrvir","Peridi~~Pyrplu","Peridi~~Pyrvir","Psecat~~Pseudo","Pyrplu~~Pyrvir","Pyrplu~~Tetrae","Synedr~~Urogle"

Component models

```
Woronichinia_2012=list(
wor_1<-glmmPQL(Woroni~depth+temp+sal+pH+DO+TN+TP+Urogle+Microc+Cod
osi+Aphanot+Synedr+Sceeco+Cosgra+Scecom+Cochlo+Zygnem+Perbal+Neph
ro+Achlen,random=~1|spatial,family="gaussian",data=woronichinia),
wor_2<-glmmPQL(Woroni~Anabae,*      wor_27<-glmmPQL(Woroni~Levand,*
wor_3<-glmmPQL(Woroni~Aphani,*      wor_28<-glmmPQL(Woroni~Lyngby,*
wor_4<-glmmPQL(Woroni~Aphanoc,*     wor_29<-glmmPQL(Woroni~Merpun,*
wor_5<-glmmPQL(Woroni~Botryo,*      wor_30<-glmmPQL(Woroni~Merism,*
wor_6<-glmmPQL(Woroni~Carter,*      wor_31<-glmmPQL(Woroni~Merwar,*
wor_7<-glmmPQL(Woroni~Chaneo,*      wor_32<-glmmPQL(Woroni~Moncon,*
wor_8<-glmmPQL(Woroni~Chaten,*      wor_33<-glmmPQL(Woroni~Monmin,*
wor_9<-glmmPQL(Woroni~Chlamy,*      wor_34<-glmmPQL(Woroni~Naiadi,*
wor_10<-glmmPQL(Woroni~Chrmin,*     wor_35<-glmmPQL(Woroni~Nitzsc,*
wor_11<-glmmPQL(Woroni~Chrtur,*     wor_36<-glmmPQL(Woroni~Nodula,*
wor_12<-glmmPQL(Woroni~Crycur,*     wor_37<-glmmPQL(Woroni~Oocbor,*
wor_13<-glmmPQL(Woroni~Crymar,*     wor_38<-glmmPQL(Woroni~Ooclac,*
wor_14<-glmmPQL(Woroni~Crypha,*     wor_39<-glmmPQL(Woroni~Oscill,*
wor_15<-glmmPQL(Woroni~Cyanod,*     wor_40<-glmmPQL(Woroni~Parumb,*
wor_16<-glmmPQL(Woroni~Cylspe,*     wor_41<-glmmPQL(Woroni~Peridi,*
wor_17<-glmmPQL(Woroni~Cy_Ni,*      wor_42<-glmmPQL(Woroni~Plagio,*
wor_18<-glmmPQL(Woroni~Desmar,*     wor_43<-glmmPQL(Woroni~Pleuro,*
wor_19<-glmmPQL(Woroni~Diatom,*     wor_44<-glmmPQL(Woroni~Proroc,*
wor_20<-glmmPQL(Woroni~Dolflo,*     wor_45<-glmmPQL(Woroni~Psecat,*
wor_21<-glmmPQL(Woroni~Dolich,*     wor_46<-glmmPQL(Woroni~Pseela,*
wor_22<-glmmPQL(Woroni~Entomo,*     wor_47<-glmmPQL(Woroni~Pseudo,*
wor_23<-glmmPQL(Woroni~Flagel,*     wor_48<-glmmPQL(Woroni~Pyrplu,*
wor_24<-glmmPQL(Woroni~Gymsim,*     wor_49<-glmmPQL(Woroni~Pyrvir,*
wor_25<-glmmPQL(Woroni~Gymube,*     wor_50<-glmmPQL(Woroni~Rholen,*
wor_26<-glmmPQL(Woroni~Krypto,*
wor_51<-glmmPQL(Woroni~Tetrae,random=~1|spatial,family="gaussian",
data=woronichinia))
```

*random=~1|spatial,family="gaussian",data= woronichinia)

Final model

Significant Spearman rank correlations ($R_s \geq 0.700$, p=0.000)

```
"temp~~sal","pH~~sal","TP~~TN","TP~~Urogle","TN~~Synedr","TN~~Urogle","
Aphani~~Naiadi","Chlamy~~Psecat","Cochlo~~Peridi","Crymar~~Nephro","Cyl
spe~~Pseudo","Cy_Ni~~Desmar","Cy_Ni~~Psecat","Cy_Ni~~Pseudo","Desmar~~P
secat","Desmar~~Pseudo","Flagel~~Pyrplu","Moncon~~Pyrplu","Monmin~~Urog
le","Ooclac~~Pyrplu","Peridi~~Pyrplu","Psecat~~Pseudo","Pyrplu~~Pyrvir"
,"Pyrplu~~Tetrae","Synedr~~Urogle"
```

Component models

```
Woronichinia_final=list(
wor_1f<-glmmPQL(Woroni~depth+temp+sal+pH+DO+TP+Urogle+Microc+Codosi+
Synedr+Cosgra+Cochlo+Nephro+Achlen,random=~1|spatial,family=
"gaussian",data=woronichinia)
wor_17f<-glmmPQL(Woroni~Cy_Ni,*   | wor_45f<-glmmPQL(Woroni~Psecat,*
wor_24f<-glmmPQL(Woroni~Gymsim,*  | wor_47f<-glmmPQL(Woroni~Pseudo,*
wor_34f<-glmmPQL(Woroni~Naiadi,*  |
wor_48f<-glmmPQL(Woroni~Pyrplu,random=~1|spatial,family="gaussian",
data=woronichinia))
*random=~1|spatial,family="gaussian",data=woronichinia)
```

Regression coefficients for individual predictors on the response variable *Woronichinia* sp. in the final model

Unstandardised, standardised β-coefficients and p*-values of regressions*

Predictor	Unstandardised β	Standardised β	*p*-value
Achroonema lentum	0.185	0.239	0.016
Cochlodinium sp.	2.674	0.442	0.000
Codosiga botrytis	0.848	0.431	0.011
Cosmarium granatum	-0.935	-0.244	0.040
Cylindrotheca closterium/ Nitzschia longissima	-0.365	-0.352	0.045
Depth	-117.9	-0.314	0.001
Dissolved oxygen	110.7	1.103	0.000
Gymnodinium simplex	0.441	0.372	0.033
Microcystis spp.	0.834	0.790	0.000
Naiadinium polonicum	0.420	0.538	0.001
Nephroselmis sp.	0.088	0.653	0.000
pH	173.7	0.377	0.033
Pseudanabaena catenata	-0.026	-0.383	0.028
Pseudopedinella sp.	-0.147	-0.346	0.049
Pyramimonas plurioculata	0.237	0.401	0.021
Salinity	-74.87	-0.332	0.210
Synedra spp.	0.471	0.215	0.197
Temperature	-74.78	-0.904	0.001
Total phosphorus	-8.400	-1.600	0.000
Uroglena sp.	0.054	0.577	0.009

Correlation coefficients for significant correlations in the final model

Standardised β-coefficients and p*-values of correlations*

Correlated errors	Standardised β	*p*-value
Aphanizomenon flosaquae~~*Naiadinium polonicum*	0.677	0.000
Chlamydomonas sp.~~*Pseudanabaena catenata*	0.779	0.000
Cochlodinium sp.~~*Peridinium* sp.	0.607	0.000
Cryptomonas marssonii~~*Nephroselmis* sp.	0.797	0.000
Cylindrospermum sp.~~*Pseudopedinella* sp.	0.735	0.000
Cylindrotheca closterium/Nitzschia longissima~~ *Desmarella moniliformis*	0.801	0.000
Cylindrotheca closterium/Nitzschia longissima~~ *Pseudanabaena catenata*	0.918	0.000
Cylindrotheca closterium/Nitzschia longissima~~ *Pseudopedinella* sp.	0.665	0.000
Desmarella moniliformis~~*Pseudanabaena catenata*	0.714	0.000
Desmarella moniliformis~~*Pseudopedinella* sp.	0.686	0.000
Flagellates~~*Pyramimonas plurioculata*	0.820	0.000
Monoraphidium contortum~~ *Pyramimonas plurioculata*	0.669	0.000
Monoraphidium minutum~~*Uroglena* sp.	0.549	0.001
Oocystis lacustris~~*Pyramimonas plurioculata*	0.681	0.000
Peridinium sp.~~*Pyramimonas plurioculata*	0.740	0.000
pH~~Salinity	0.856	0.000
Pseudanabaena catenata~~*Pseudopedinella* sp.	0.669	0.000
Pyramimonas plurioculata~~*Pyramimonas virginica*	0.742	0.000
Pyramimonas plurioculata~~*Tetraedron minimum*	0.681	0.000
Salinity~~Temperature	0.817	0.000
Synedra spp.~~*Uroglena* sp.	0.857	0.000
Total nitrogen~~*Synedra* spp.	0.649	0.000
Total nitrogen~~Total phosphorus	0.911	0.000
Total nitrogen~~*Uroglena* sp.	0.751	0.000
Total phosphorus~~*Uroglena* sp.	0.692	0.000

Component model fits for the final model

Conditional (R^2_c) and marginal (R^2_m) R^2-values

Component model	Predictor	R^2_c	R^2_m
	Depth		
	Achroonema lentum		
	Cochlodinium sp.		
	Codosiga botrytis		
	Cosmarium granatum		
	Dissolved oxygen		
wor_1f	***Microcystis*** spp.	0.996	0.956
	Nephroselmis sp.		
	pH		
	Salinity		
	Synedra spp.		
	Total phosphorus		
	Uroglena sp.		
wor_17f	***Cylindrotheca closterium/Nitzschia longissima***	0.892	0.127
wor_24f	***Gymnodinium simplex***	0.894	0.142
wor_34f	***Naiadinium polonicum***	0.913	0.296
wor_45f	***Pseudanabaena catenata***	0.895	0.151
wor_47f	***Pseudopedinella*** sp.	0.892	0.123
wor_48f	***Pyramimonas plurioculata***	0.897	0.165

Spatial distributions of phytoplankton in rock pools:

metacommunities to molecules

This thesis describes phytoplankton structures in rock pools at regional, local and molecular scales. Additionally, an ecologically relevant phytoplankton sampling technique is evaluated. The results suggest that processes at both regional and local scales influence phytoplankton assemblages in rock pool metacommunities. Moreover, they imply that molecular processes may have consequences beyond the individual scale. The thesis highlight that a meaningful understanding of microorganism patterns and processes requires a widening from the local scale, but also a comprehension that small-scale processes have importance for large-scale microorganism patterns. Throughout, the sampled scale should be non-arbitrarily considered.

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