Viral Loop Dynamics in Temperate and Polar Freshwaters

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Abstract

All cellular organisms in aquatic environments are susceptible to virus attack. Viruses are the smallest but most abundant biological entities in freshwaters. This thesis describes interactions between viruses and bacteria in temperate and polar freshwaters with particular emphasis on Arctic and Antarctic aquatic systems. Free virus-like particles in freshwaters are vulnerable and exposed and as parasites their survival is dependent on the existence of a suitable host. I found that high concentrations of humic substances in lakes had a negative impact on viral abundance as viruses may be adsorbed to humic matter and replication or viral infectivity may be lost. This indicates that virus-bacteria interactions in humic lakes may differ from those in clear water lakes. Furthermore, there was a high incidence of lysogenic bacteria in humic lakes when compared to other temperate freshwaters which could indicate that lysogeny may act as a viral survival strategy in environments where high rates of destruction and inactivation may occur. On the contrary, I found that lysogenic bacteria were not common in the Arctic and Antarctic freshwaters and during a seasonal study conducted on two Antarctic ultra-oligotrophic lakes lysogenic bacteria were only detected on 3 out of 10 sampling occasions.

As viruses are parasites the metabolic state of their host will largely determine how successful they are in replicating. I found that bacterial growth in the Arctic and Antarctic freshwaters was primarily limited by phosphorus however even though an increase in phosphorus had a positive effect on bacteria this was not reflected in the virus abundance. Using electron microscopy I established that a high percentage of the Arctic and Antarctic bacteria population was visibly infected and that the average number of virus-like particles per cell was low relative to previous reported data. This indicates that virus-bacteria interactions in polar freshwaters differ significantly from those in temperate freshwaters.

In microbially dominated Antarctic freshwater lakes the major pathway of energy and carbon flow is through the microbial loop. Viral lysis disrupts this flow and shunts organic carbon back into the dissolved organic carbon (DOC) pool making it available for the uninfected bacterial community. I found that viral lysis of bacterial cells could contribute significantly to the DOC pool in Antarctic freshwater lakes. I estimated that over 60% of the carbon supplied to the DOC pool originated from viral lysis during the winter months while during the summer period less than 20% originated from viral lysis. This implies that viral processes in extreme Antarctic lakes may be of quantitative significance with respect to carbon flow.

Key words
Virus, bacteria, Antarctic, Arctic, humic, dissolved organic carbon, lysogeny, bacterial mortality, nutrient limitation, freshwaters, polar, temperate

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Viral loop dynamics in temperate and polar freshwaters

Introduction

Viruses can be found wherever life is present and are the most abundant biological entities on our planet. Viruses have caused major devastation in human societies and continue to do so as new viral diseases are frequently discovered, like HIV, SARS and avian flu. This has led to an in depth knowledge of the general properties and biology of viruses which has in turn greatly contributed to the discipline of molecular biology where viruses are used as tools for the microbial geneticist.

Virology is today a well-studied field. On the other hand viral ecology is still a largely unknown field, where the natural occurrence and activities of viruses in ecosystems are just beginning to be unravelled. The presence of viruses in aquatic ecosystems was first discovered in the 1950's [40] and high estimates of virus abundance were later reported in marine systems by Torella and Morita in 1979 [45]. Viruses role in the aquatic food web and their ecological significance was not questioned until 10 years later when Bergh *et al.* [2] published a letter in Nature showing high natural abundance of viruses, the majority classified as bacteriophages (bacterial viruses), in unpolluted waters. This implied that viruses could be a significant mortality factor in the microbial plankton world that had so far been overlooked. Seventeen years later and it is now clear that viruses are a crucial component of the aquatic food web capable of infecting two large important groups of the microbial plankton; phytoplankton and bacterioplankton. Bacterioplankton recycle dissolved organic matter (DOM) in the aquatic food web through the microbial loop [1] (Fig. 1). In the microbial loop, bacteria consume dissolved organic material that cannot directly be ingested by larger organisms. The bacteria are in turn grazed upon by protozoa, which are then ingested by zooplankton and then ultimately recycled back to higher trophic levels. Viral lysis of bacterioplankton disrupts this flow of energy and organic matter by forming a "viral loop" among bacteria, viruses and the DOM pool (Fig. 1). It has been suggested that as much as 30% of bacterial production can be channelled through the viral loop in aquatic systems [7]. The net effect of this is an increase in respiration and recycling in the lower parts of the food web and a less efficient organic matter transfer to higher trophic levels [41]. There is also evidence that viruses can influence bacterial community and genetic diversity structure by killing of the most dominant species and allowing for co-existence of less competitive species, and through viral-mediated transfer of genes between species [10, 7, 48, 54]. Most of the studies on aquatic viruses have been conducted in marine environments, whereas studies in freshwaters are still lagging behind. This thesis looks at viruses in freshwater environments from both temperate and polar regions, with the largest emphasis being on viruses in Antarctic and Arctic freshwa-

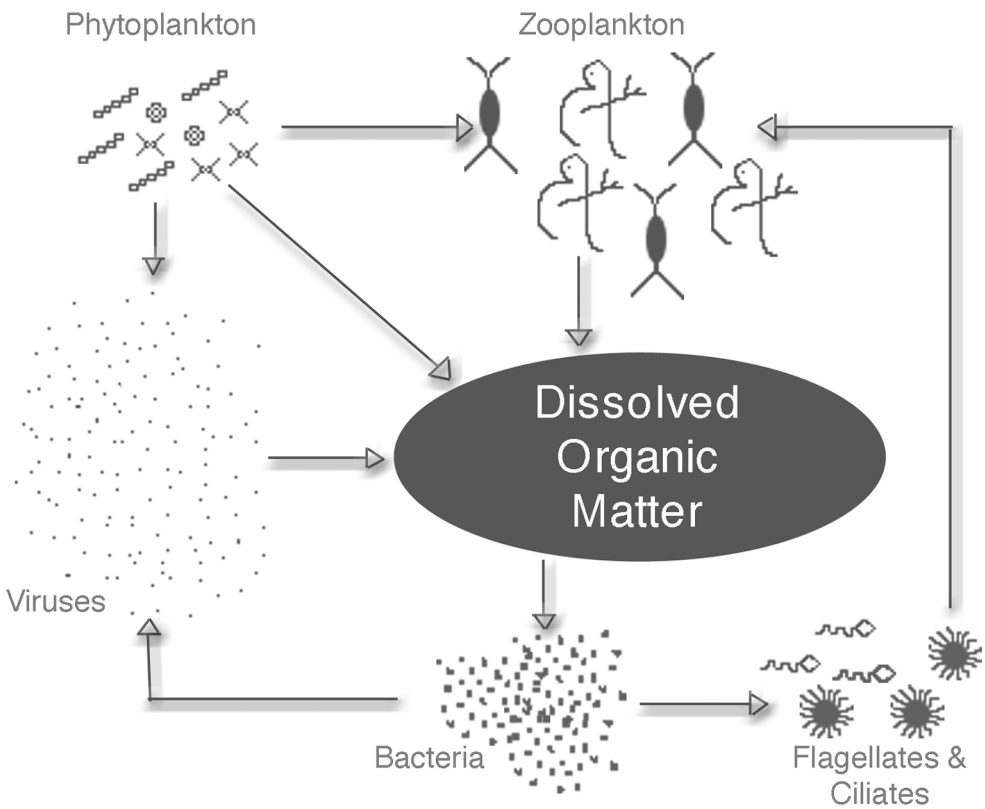

Fig. 1. The microbial loop including the viral loop which is formed between bacteria, viruses and dissolved organic matter.

ters. The thesis describes interactions between viruses and bacteria and the factors that may influence these interactions in freshwaters. In addition, it examines what proportion of the bacterioplankton in Arctic and Antarctic freshwaters is infected with viruses and the seasonal relevance of the viral loop in ultra-oligotrophic Antarctic lakes.

Viral regulation and replication in freshwaters

The regulation of viruses in aquatic systems is dependent on the release of new viral particles from the host and the survival of free viral particles in the water. The estimation of the abundance of free viral particles in natural waters rely on direct counts of virus-like particles (VLP), using either transmission or epifluorescence microscopy. Direct counting of viruses shows that the highest abundance of viruses is found in freshwater rather than marine systems and viral abundance typically exceeds bacterial abundance in both systems [25, 61].

Free viral particles are vulnerable and exposed in the water media and their survival is dependent on encountering a suitable host. Information on the removal and inactivation processes of viruses in natural waters is limited. However, studies have shown that solar radiation can have a significant negative impact on the number of infective viral particles in waters [56, 62]. On the other hand, grazing on viral particles appears to be of minor importance in viral removal [16, 42]. Decay processes of free viral particles in natural waters can occur via adsorption to large particles and high molecular weight dissolved organic matter (HMW

DOM) [32, 35]. Inorganic and organic particles present in the freshwater medium can have a large impact on viral processes [32]. For instance, brown-water lakes or humic lakes contain high concentrations of humic substances which are complex mixtures containing aliphatic and aromatic carboxyl and hydroxyl functional groups that have important binding properties which may inactivate viruses in freshwaters [34]. Humic-like substances have been found to have a negative effect on viral infectivity and replication in both the human immunodeficiency virus and the influenza virus [24, 39, 49]. However, humic substances have a net positive effect on bacterial growth and several studies [46, 47] have shown that bacteria can utilise humic substances as an additional/alternative energy source. Thus, one would predict that the positive effects of humic substances on bacteria could reflect positively on their parasite; viruses. A field and laboratory study was conducted to determine the effects of humic substances on the interactions between viruses and bacteria in freshwaters (**Paper I**). The results from **Paper I** showed that humic substances had a positive effect on bacterial growth but a negative impact on the abundance of viruses. This suggests that infection and/or replication rates of viruses in freshwaters with high concentrations of humic substances may be significantly different relative to clear water lakes.

There are two principal mechanisms of virus replication; lytic and lysogenic. In the lytic cycle, the virus infects its host and takes over the host nucleic acid and protein synthetic machinery and turns the host cell into an efficient factory for the production of new viral particles. After a latent period during which the new viral particles are formed and assembled, the host cell is destroyed (lysis) resulting in the release of the viral particles into the environment [5, 60]. In the lysogenic cycle the viral nucleic acid is incorporated into the host genome (lysogens) but remains inactive until some external factor induces the lytic cycle. The factors that induces the lytic cycle are largely unknown in aquatic systems but studies have shown that environmental factors such as UV irradiation, chemicals and nutrients – in particular phosphate – may trigger the lytic cycle [60]. Viruses that are able to use the lysogenic cycle are called temperate and over 90% of known bacteriophages are temperate [14]. Temperate phages have been found in both marine and freshwater environments [44, 55, 58] and it has been hypothesised that temperate phages may have an advantage over virulent phages (viruses only using the lytic cycle) in aquatic ecosystems. A virulent phage in an oligotrophic environment with a slow-growing host present in small numbers could run the chance of being eliminated as it would literally run out of host cells to infect. On the contrary, a temperate phage could "survive" harsh periods of low host abundance and bide its time until better conditions prevail. The prediction would then be that temperate phages should predominate in oligotrophic aquatic systems [60].

Paper I, II, IV used a direct approach to estimate the occurrence of lysogens in the natural bacterioplankton community by monitoring the viral abundance in water samples treated with a potent inducing agent (the antibiotic Mitomycin C) and comparing it with untreated control samples [33]. The susceptibility of natural bacterioplankton communities to Mitomycin C may vary between environments and bacterial species. Thus there is the possibility that

Table 1. Percentage of lysogens in the total bacterial population in temperate and polar freshwater environments.

Environment	Lysogeny (%)
Lake Skärlen	ND
Lake Feresjön	31.8
Lake Homehultsjön	13.5
Lake Stråken	23.1
Lake Skärshultsjön	18.1
Arctic Lake 1	ND
Arctic Lake 2	ND
Cryoconite hole	ND
Lake Druzhby (17/12/03)	22.3
Crooked Lake (11/08/04)	73.0

In Crooked Lake and Lake Druzhby lysogens were detected on 3 out of 10 sampling occasions and the value presented in the table is the highest percentage of lysogens detected during season 2003/2004. ND = Not detected.

only a part of the lysogenic bacterial population is affected, thereby resulting in an underestimation of the occurrence of lysogens. Four of five lakes in Småland, Sweden (**Paper I**) showed presence of lysogens, indicating that lysogeny might be an important ecological strategy in humic lakes (Table 1). One may speculate that lysogeny is a strategy for viruses to survive in humic lakes, where high rates of destruction and inactivation of viruses is likely to take place due to the presence of HMW DOM. On the contrary, findings from the Arctic and the Antarctic freshwater environment (**Paper II, IV**) showed that lysogens were uncommon in polar freshwaters and that lysogenic viral production was of minor importance (Table 1).

Virus and bacteria interactions in freshwaters

Viruses are parasites and their survival is dependent on the existence of a suitable host. Host encounter occurs via passive diffusion (Brownian motion) and hence, at low host density the opportunity for a virus to make contact with a bacterial cell is reduced. In most marine and freshwater environments the microbial plankton, in this case referring to the bacterioplankton, are living under less than optimal conditions, where low nutrient levels and temperatures restricts their growth, often resulting in a slow-growing population at low density. In the extremes of the Arctic and Antarctic freshwaters microbial life is pushed to its limits in regards to temperatures, light- and nutrient levels [23]. These freshwater ecosystems have low trophic complexity and are mainly made up of the microbial loop, where the bacterioplankton play a crucial role as recyclers of the available nutrients [22] (Fig. 1). The bacteria in polar systems most likely live in a state of starvation with a large proportion of the bacteria being inactive or/and dormant, which will ultimately have a negative effect on the production of viruses. Studies have shown that the metabolic state of the host cell and its surrounding environment can affect the number of virus particles released upon lysis (burst size) and the length of the latent period [18, 27, 36]. Often there is a positive correlation between bacterial and viral abundance in natural aquatic systems (**Paper II**), indicating that these two components are closely linked and that parameters which control bacterial production may also influence viral processes [61]. Several studies have shown that freshwater bacterioplankton growth is primarily limited by phosphorus [8, 9, 11, 12, 30, 37, 51] and that carbon may on occasion act as a secondary limiting nutrient [17]. **Paper II** investigated how nutrient addition and temperature influenced virus-bacteria interactions in the Arctic and Antarctic freshwater environment. The results of **Paper II** adds to a growing set of evidence that freshwater bacterioplankton growth is primarily limited by phosphorus, in both tropical, temperate and polar environments. Furthermore, Arctic bacteria seemed to have a temperature-substrate dependent growth with quicker response to nutrient amendments at high temperatures relative to low temperatures. Whereas, Antarctic bacteria were well adapted to low temperatures. Even though an increase in phosphorus concentration had a positive effect on bacteria this was not reflected in the virus abundance, which stayed fairly stable throughout the study. Results from both **Paper I** and **II** indicate that viral abundances are not necessarily affected by the same parameters that control bacterial production in freshwaters.

Results from **Paper II** showed that the bacterioplankton growth was severely restricted by the *in situ* environment in polar aquatic ecosystems. With a host living under low nutrient and temperature constraints the question arises as to how an active virus population can be maintained. To answer this question a bi-polar investigation was conducted (**Paper III**) in the Antarctic and Arctic freshwaters. Transmission electron microscopy (TEM) was used to visualise virus-infected bacterioplankton in water samples taken from two ultra-oligotrophic Antarctic freshwater lakes (Crooked Lake and Lake Druzhby Fig. 2A, B) and from cryoconite holes (small, water-filled melt holes that form on the surface of the glacier) on the Midre Løvenbreen glacier in the high Arctic (Fig. 2C). TEM has been used extensively to study the morphological diversity of aquatic viruses [20, 52] as well as

Fig. 2. Photographs of the two study lakes in the Antarctic and of cryoconite holes in the Arctic. **A:** Crooked Lake. **B:** Lake Druzhby **C:** Cryoconite holes on Midre Løvenbreen glacier in the Arctic.

the frequency of visibly infected bacterial cells (FVIB) and burst sizes [3, 13, 19, 20, 21, 26, 50, 52, 59]. In temperate freshwaters FVIB ranges between 0.6 to 5% with an average of 26 phages per cell [3, 13, 19, 20, 21, 26, 50, 52, 59]. Virus particles within the host cell become visible with TEM only towards the end of the latent period, thus the total fraction of infected bacterial cells (FIB) will be much greater than the number of visibly infected bacterial cells viewed under the microscope. Correction factors to convert FVIB to FIB vary and require estimates of what portion of the latent period cells are visibly infected [4, 57]. As mentioned earlier, the length of the latent period can vary but is often assumed to be equal to the bacterial generation time [36]. Results from **Paper III** indicate that virus-bacteria interactions in polar regions differ significantly from those in temperate regions, as the study reveals the highest FVIB (range 8.1% to 66.7%) and the lowest burst sizes (average 4) ever reported in the literature. Long generation times in the polar bacterioplankton, in particularly in the Antarctic ultra-oligotrophic lakes where the generation time can be as high as 2.5 days (**Paper III**), may result in low burst sizes and long latent periods. For instance, results from studies of a marine virus-host system showed that an increase in the bacterial growth rate (i.e. shorter generation times) resulted in a higher burst size and a shorter latent period [27]. **Paper III**, shows that even in ultra-oligotrophic environments, where host density is low and often slow growing with extremely long generation times, it is possible to find an active viral community. Furthermore, high bacterial infection rates in the Arctic and Antarctic indicate that the lytic cycle may prevail in polar freshwater environments. This statement is further strengthened by the fact that lysogens were infrequently detected in polar freshwaters (**Paper II, IV**). One may further speculate that a high frequency of visibly infected cells may offer a novel way for the viral population to survive when there are only a few viruses released per infected cells. Moreover, high infection rates means that there is frequent virus-bacteria encounter and for this to occur in

an environment with low bacterial density the virus must be either capable of infecting multiple bacterial species or bacterial diversity is low with only a few virus-host systems.

Impact of viruses on the microbial loop

Viruses are contributors to the element cycling within the microbial loop as they act as catalysts that accelerate the transformation of particulate organic matter into dissolved organic matter [41]. Both experimental and theoretical studies [6, 15, 29, 31] have shown that viruses may have a significant impact on the nutrient and carbon cycling in microbial communities. When bacteria or phytoplankton are destroyed by viruses new viral particles are released along with dissolved and colloidal organic matter, referred to as lysate products [29]. These lysate products contain both labile and refractory products which can potentially be used by the uninfected microbial community. Fischer and Velimirov [13] estimated that viral lysis of bacterial cells in an eutrophic lake could potentially release 15.2 $\mu g\ C\ l^{-1}\ d^{-1}$ which corresponded to 46% of the bacterial production in the water column. Other studies conducted by Middelboe *et al.* [27, 28] indicated that lysate products could be used by the uninfected bacterial community but required more energy for assimilation into bacterial biomass, which was indicated by the decrease in bacterial growth efficiency (the ratio of biomass produced to substrate utilised).

In microbially dominated Antarctic freshwater lakes the major pathway of energy and carbon flow is through the microbial loop. Viral lysis of bacteria can disrupt this flow and shunt organic carbon back into the dissolved organic carbon (DOC) pool (Fig. 1), having the net effect of increasing the DOC pool and making it available for incorporation into new bacterial biomass. The two study lakes, Lake Druzhby and Crooked Lake (**Paper IV**) are ultra-oligotrophic with a small DOC pool where virtually all the carbon is said to originate from authochtonous sources, such as primary production [22]. Efficient recycling of the available DOC within the lakes would thus be crucial for sustaining an active bacterial population throughout the season. The results from **Paper IV** showed that viral activity contributed significantly to the recycling of DOC in the study lakes with over 60% of the estimated DOC pool being derived from viral activity in the dark winter months. The findings presented in both **Paper III** and **Paper IV** add to a growing set of evidence that the greatest viral impact occurs in low productivity lake ecosystems, ranging from temperate to polar environments [3, 31].

Summary of papers I–IV

Objectives

The overall objective of this thesis was to examine virus-bacteria interactions in temperate and polar freshwaters and what factors may influence the temporal and seasonal dynamics of viruses and their bacterial host in freshwaters. Specifically the thesis addresses the following issues:

- The influence of inorganic and organic nutrients on virus-bacteria interactions in temperate and oligotrophic polar freshwaters (**Paper I, II**).
- The role of lysogeny in temperate and oligotrophic polar freshwaters (**Paper I, II, IV**).
- The frequency of visibly infected bacterial cells and the average number of virus-like particles per cell in the Arctic and Antarctic freshwaters (**Paper III**).
- The role of the viral loop in microbially dominated Antarctic freshwaters and the relevance of viral-induced carbon transfer in Antarctic ultra-oligotrophic lakes (**Paper IV**).

Study sites and methods

Fieldwork was carried out in temperate and polar freshwater environments. In **Paper I**, a lake survey of 24 lakes in southern Sweden

(57°10′N) was conducted to identify any relationships between viruses, bacteria and DOC concentrations. The lakes were chosen to represent a DOC gradient from clear water to humic water and the DOC concentrations ranged from 3 to 19 mg of C liter^{-1}. Water samples were analysed for viral and bacterial abundance, DOC concentrations, Chl *a* levels and for total nitrogen and phosphorus. The occurrence of lysogeny was determined in samples from five lakes using Mitomycin C as an inducing agent of cells containing prophages. In addition, grazer-free water from Lake Skärlen (with relative low DOC concentration of 3.3 mg of C liter^{-1}) was used in a short-term (15 days) laboratory experiment to look at the effects of different sources of carbon (i.e. glucose and fulvic acids) and nutrients on virus-bacteria interactions. Microbial parameters, including viral and bacterial abundance and bacterial production, were monitored throughout the experiment whereas lysogeny was only analysed on a subset of samples.

In **Paper II**, fieldwork was carried out near Ny-Ålesund, northwest Svalbard (78°56′N, 11°56′E) and the Antarctic (Vestfold Hills, Eastern Antarctica (68°35′S, 78°20′E). Water was collected from a variety of freshwater environments, including crycoconite holes, glacier runoffs, melt pools and freshwater lakes. I conducted a field survey of several Arctic freshwater environments to examine the virus-bacteria interactions in these systems. Water samples were analysed for bacterial and viral abundance, bacterial production, DOC and dissolved nitrogen concentrations, chl *a* levels and occurrence of lysogeny. In addition, I set up several nutrient enrichment experiments with water from both the Arctic and the Antarctic to elucidate what factors (i.e., nutrients and temperature) limits bacterial growth and how these factors influence virus-bacteria interactions in polar freshwaters. The changes in bacterial and viral abundance and bacterial production were monitored throughout the experiments.

In **Paper III**, I used electron microscopy to estimate what percentage of the Arctic and Antarctic freshwater bacteria population was visibly infected by viruses and the average number of virus-like particles within each bacterial cell. Virus-infected bacterial cells were investigated with a slightly modified method relative to the one previously described by Suttle in 1993 [42]. In brief, water samples were centrifuged onto electron microscope grids at a relative low centrifugation speed (9000 × g) which only allowed for bacterial cells to be harvested, hence improving the estimation of infected bacterial cells as all viruses found were within the bacterial cells.

To investigate the importance of viruses and their role in carbon cycling in polar freshwaters, I conducted a seasonal study in the Antarctic on two ultra-oligotrophic lakes (Fig. 2A, B). The two lakes were sampled fortnightly and water samples were analysed for DOC, inorganic nutrients, chl *a*, bacterial and viral abundance and bacterial production whereas occurrence of lysogeny in each lake was analysed on a monthly basis. Electron microscopy data from **Paper III** was used in combination with the seasonal data set from **Paper IV** to work out the fraction of bacterial mortality caused by viral lysis and the contribution of viral-induced DOC release in Lake Druzhby and Crooked Lake.

Results and conclusions

- Parameters that had a positive net effect on bacterial growth and abundance, such as phosphorus, humic substances and temperature, did not cause a positive response in viral abundance. This shows that viral and bacterial abundances are not always positively correlated and that the presence of a healthy and high density host cannot be used as a sole indicator of viral success in freshwaters.

- Humic substances influenced viral abundance and virus to bacteria ratio negatively. This demonstrates that virus-bacteria interactions in humic lakes may be significant different relative to clear water lakes. Factors such as binding to HMW substances, destruction, lower infectivity or lower replication rates are likely scenarios taking place in humic lakes.

- The incidence of lysogeny was high in the humic lakes of Småland, which shows that lysogeny might be an important mechanism in these aquatic systems. On the contrary, lysogeny was uncommon in polar freshwaters where the lytic cycle seemed to prevail.

- The frequency of visibly infected bacterial cells was exceptionally high in both the Arctic and Antarctic freshwater environment and the average number of virus-like particles per cell was very low relative to previous reported data from temperate freshwaters. It is evident that virus-bacteria interactions in microbially dominated polar freshwaters differ significantly to temperate freshwaters.

- Viruses were important players in the Antarctic ultra-oligotrophic lakes. A large proportion of bacterial mortality was due to viral lysis and viral induced carbon transfer seemed to be of quantitative significance in both Lake Druzhby and Crooked Lake, particularly during the winter months.

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Virusinfekterade vatten

Populärvetenskaplig sammanfattning av avhandlingen

Alla organismer i våra sjöar är mottagliga för virusattacker. Virus är de minsta men vanligaste biologiska enheterna i sjöar. En klunk sjövatten innehåller till exempel miljardtals virus! De är väldigt små och för att begripa hur små de är kan man föreställa sig ett knappnålshuvud där över 20 miljarder virus kan få plats.

Då de är så små behöver man speciell utrustning för att kunna se dem och det var inte förrän i mitten av 50-talet som man fick in den första rapporten om bakteriofager (virus som attackerar bakterier) i havet. Den ekologiska betydelsen av virus började inte diskuteras förrän i slutet av 80-talet, då en norsk forskargrupp använde sig av elektronmikroskopi och hittade över 10 miljoner virus per ml i havsvatten. De självklara frågor som dök upp var: ”varför finns de så många virus i våra hav och vad gör de egentligen?” Detta sporrade forskningen inom akvatisk virusekologi och nu 17 år senare vet vi ganska mycket mer, men långt ifrån tillräckligt, om virusens betydelse i sjöar och hav. Virus kan till exempel infektera två mycket viktiga organismgrupper i sjöar och hav, växtplankton och bakterier. Bakterier är efter virus de biologiska enheter som förekommer i högst antal och de är viktiga i sjöar och hav då de omvandlar löst organiskt kol till partikulärt som sedan blir tillgängligt till organismer högre upp i födokedjan.

Virus är parasiter som är totalt beroende av sin värd för sin överlevnad och då bakterier finns i så stort antal är de ett bra byte för virus. Virus reproducerar sig genom att infektera sin värdcell och sedan tvingar de värdcellen att producera nya viruspartiklar vilka släpps ut från cellen när den exploderar (lytisk virusinfektion). Men virus kan även använda sig av en annan typ av reproduktion som innebär att värdcellen hålls vid liv och att virusarvsmassan kopieras tillsammans med bakteriearvsmassan när nya bakterier tillverkas (icke-lytisk virusinfektion). Den icke-lytiska virusinfektionen används oftast när värdcellen inte lever under optimala förhållanden.

Syftet med avhandling har varit att undersöka interaktioner mellan virus och bakterier i olika typer av näringsfattiga sötvatten. Jag har undersökt bruna skogssjöar i Småland, glaciärer och vattendrag i Arktis och extremt näringsfattiga sjöar i Antarktis.

Bakterier i bruna sjöar kan använda sig av det organiska materialet, som finns i höga koncentrationer. Då bakterier trivs i bruna sjöar med mycket organiskt material (humöst material) skulle man kunna tänka sig att virusen också skulle gilla att leva där, eftersom det då finns gott om värdbakterier. Till min förvåning visade sig detta vara osant och höga koncentrationer av organiskt material påverkade viruspartiklarna negativt då de stora komplexa molekylerna verkade binda eller inaktivera virusen. Detta visar att fria viruspartiklar i vatten är ganska utsatta och sårbara och deras överlevnadstid i sjöar påverkas av hur snabbt de hittar en bra värd men också av miljön i sjön. Jag konstaterade även att i bruna skogssjöar var den icke-lytiska virusinfektionen vanlig vilket kan vara ett sätt för viru-

sen att kompensera för de negativa effekterna av att leva i en miljö med hög virusförstörelse.

Sjöbakterier lever oftast under väldigt svåra förhållanden och får nöja sig med lite näring och oftast låga temperaturer. I extrema akvatiska miljöer som i Arktis och Antarktis lever mikroorganismer på gränsen till vad som är möjligt med konstant låga temperaturer, ljus- och näringshalter. Jag undersökte vad det var som begränsade tillväxten av bakterier i olika sötvatten i både Arktis och Antarktis. Det visade sig att bakterierna främst var begränsade av tillgången på fosfor medan temperaturen hade en mindre betydelse för tillväxten. Jag ställde mig alltså frågan hur en aktiv viruspopulation kan överleva då deras värd lever under sådana svåra förhållanden. Det visade sig att andelen virusinfekterade bakterier i Antarktis och Arktis sötvatten var mycket hög jämfört med vanliga sjöar, men å andra sidan innehöll varje cell bara ett fåtal viruspartiklar. Jag konstaterade att interaktioner mellan virus och bakterier i sötvatten från polarområden skiljer sig signifikant från sötvatten i t ex sjöar i södra Sverige. Det är möjligt att polarvirus har utvecklat unika strategier för att överleva i en extrem miljö. Då bara ett fåtal viruspartiklar släpps ut per cell måste tex virusen infektera flera bakterier för att upprätthålla en konstant viruspopulation. Dessutom betyder en hög infektionsfrekvens att fria viruspartiklar måste stöta ihop ofta med bakterier, vilket resulterar i infektion. Men i en miljö med få bakterier tyder det på att virusen antingen kan infektera mer än en bakterieart eller att det bara finns ett fåtal bakteriearter. Jag hade förväntat mig att icke-lytiska virusinfektioner skulle vara vanliga i både Arktis och Antarktis då bakterier lever under extrema förhållanden men detta antagande visade sig vara fel då lytiska virusinfektioner verkade dominera i både Arktis och Antarktis.

Ett annat syfte med min avhandling har varit att undersöka hur viktiga virus är i Antarktiska sjöar där födokjedjan är kort och domineras av mikroorganismer. En studie jag gjorde i två sjöar under ett år i Antarktis visade att virus var väsentliga för näringsåtervinning. Virusaktiviteten bidrog med mycket näring till de icke infekterade bakterierna, speciellt under de kalla och mörka vintermånaderna då det inte sker någon fotosyntes p g a totalt mörker.

Sammanfattningsvis kunde jag konstatera att interaktioner mellan virus och bakterier skiljer sig mellan olika typer av sötvatten vilket berodde både på värdcellens tillstånd och på kvalitén på vattnet. I vatten med korta födokedjor, dvs i Antarktis och Arktis, var virus extremt viktiga då deras aktivitet kunde hjälpa till med återvinning av näringsämnen, som finns i extremt låga koncentrationer i Arktis och Antarktis.

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