



LUND UNIVERSITY

Viral Loop Dynamics in Temperate and Polar Freshwaters

Sävström, Christin

2006

[Link to publication](#)

Citation for published version (APA):

Sävström, C. (2006). *Viral Loop Dynamics in Temperate and Polar Freshwaters*. [Doctoral Thesis (compilation), Department of Biology]. Department of Ecology, Lund University.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Viral loop dynamics in temperate and polar freshwaters

Viral loop dynamics in temperate and polar freshwaters

Christin Sävström

Akademisk avhandling, som för avläggande av filosofie doktorsexamen vid Naturvetenskapliga fakulteten, Lunds Universitet, kommer att offentligens försvaras vid Ekologiska institutionen, Avdelningen för limnologi, fredagen den 28 april 2006, kl 10.00.

Plats: Blå hallen, Ekologihuset, Sölvegatan 37, Lund

Fakultetsopponent: Associate Professor Mathias Middelboe, Marine Biological Laboratory, University of Copenhagen, Denmark.

Avhandlingen kommer att försvaras på engelska.

A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (in press, submitted or in ms).

Front cover and chapter separator photos by Christin Sävström

Layout: Gunilla Andersson/ZooBoTech & GrafikGruppen
Proofreading: Christin Sävström
Printed by Xanto Grafiska AB, S Sandby

ISBN 91-7105-235-6
SE-LUNBDS/NBLI-06/1060+76 pp

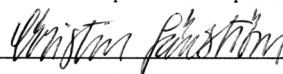
Contents	Page
Viral loop dynamics in temperate and polar freshwaters	7
Virusinfekterade vatten – Populärvetenskaplig sammanfattning av avhandlingen	17
Acknowledgments	19
I Anesio, A. M., Hollas, C., Granéli, W. and Laybourn-Parry, J. 2004. Influence of humic substances on bacterial and viral dynamics in freshwaters. – <i>Appl. Environ. Microbiol.</i> 70: 4848–4854.	23
II Sävström, C., Laybourn-Parry, J., Granéli, W. and Anesio, A. M. 2006. Nutrient and temperature control of bacterioplankton and virioplankton in the Arctic and Antarctic freshwater environments. – Submitted.	33
III Sävström, C., Granéli, W., Laybourn-Parry, J. and Anesio, A. 2006. High viral infection rates in Antarctic and Arctic bacterioplankton. – Submitted.	49
IV Sävström, C., Anesio, A. M., Granéli, W. and Laybourn-Parry, J. 2006. Seasonal viral loop dynamics in two large ultra-oligotrophic Antarctic freshwater lakes. – Submitted.	59

Paper I is reprinted with permission from the publisher.

Organization LUND UNIVERSITY Department of Ecology, Limnology Ecology Building SE-223 62 Lund Sweden	Document name DOCTORAL DISSERTATION
Author(s) Christin Säwström	Date of issue 28 April 2006
Sponsoring organization	
Title and subtitle Viral loop dynamics in temperate and polar freshwaters	
<p>Abstract</p> <p>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.</p> <p>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.</p> <p>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.</p>	
Key words Virus, bacteria, Antarctic, Arctic, humic, dissolved organic carbon, lysogeny, bacterial mortality, nutrient limitation, freshwaters, polar, temperate	
Classification system and/or index terms (if any)	
Supplementary bibliographical information	Language English
ISSN and key title	ISBN 91-7105-235-6
Recipient's notes	Number of pages 76
	Price
	Security classification

Distribution by (name and address)

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature 

Date March 6, 2006

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-

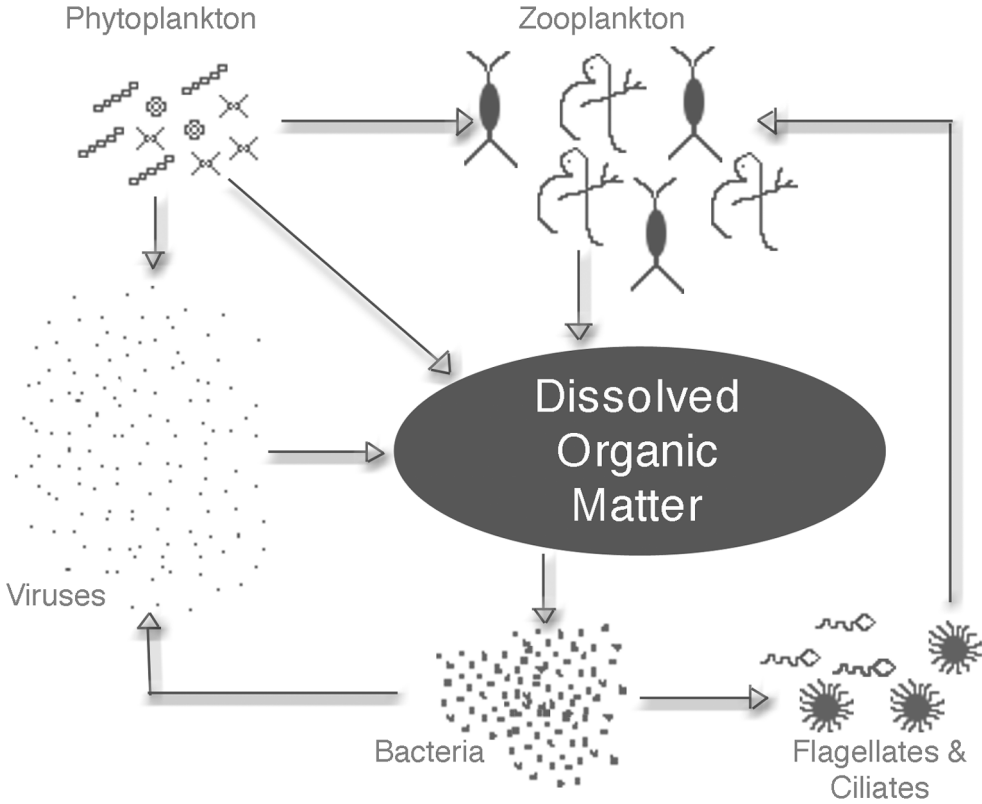


Fig. 1. The microbial loop including the viral loop which is formed between bacteria, viruses and dissolved organic matter.

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 la-

tent 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 ul-

tra-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 $15.2 \mu\text{g C l}^{-1} \text{d}^{-1}$ 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 autochthonous 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 Skärilen (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 cryoconite 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. Vi-

rus-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.

References

1. Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad TF (1983) The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser* 10:257–263
2. Bergh Ø, Børsheim KY, Bratbak G, Haldal M (1989) High abundance of viruses found in aquatic environments. *Nature* 340:467–468
3. Bettarel Y, Sime-Ngando T, Amblard C, Dolan J (2004) Viral activity in two contrasting lake ecosystems. *Appl Environ Microbiol* 70:2941–2951
4. Binder B (1999) Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells. *Aquat Microb Ecol* 18:207–215
5. Bohannon BJM, Lenski RE (2000) Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecology Letters* 3:362–377
6. Bratbak G, Haldal M, Thingstad TF, Riemann B, Haslund OH (1992) Incorporation of viruses into the budget of microbial C-transfer. A first approach. *Mar Ecol Prog Ser* 83:273–280
7. Bratbak G, Haldal M (2000) Viruses rule the waves – the smallest and most abundant members of marine ecosystems. *Microbiol Today* 27:171–173
8. Brett MT, Lubnow FS, Villar-Argaiz M, Müller-Solger A, Goldman CR (1999) Nutrient control of bacterioplankton and phytoplankton dynamics. *Aquat Ecol* 33:135–145
9. Carlsson P, Caron DA (2001) Seasonal variation of phosphorous limitation of bacterial growth in a small lake. *Limnol Oceanogr* 48:108–120
10. Chiura HX (1997) Generalized gene transfer by virus-like particles from marine bacteria. *Aquat Microb Ecol* 13:75–83
11. Elser JJ, Stabler LB, Hasset RP (1995) Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: a comparative study. *Aquat Microb Ecol* 9:105–110
12. Farjalla VF, Esteves FA, Bozelli RL, Roland F (2002) Nutrient limitation of bacterial production in clear water Amazonian ecosystems. *Hydrobiol* 489:197–205
13. Fischer UR, Velimirov B (2002) High control of bacterial production by viruses in a eutrophic oxbow lake. *Aquat Microb Ecol* 27:1–12
14. Freifelder D (1987) *Molecular biology: a comprehensive introduction to prokaryotes and eukaryotes*. Jones and Bartlett Publishers Inc, Boston pp 665–700
15. Gobler CJ, Hutchins DA, Fisher NS, Cosper EM, Sañudo-Wilhelmy SA (1997) Release and bioavailability of C, N, P, Se and Fe following viral lysis of a marine chrysophyte. *Limnol Oceanogr* 42:1492–1504
16. González JM, Suttle CA (1993) Grazing by marine nanoflagellates on viruses and virus-sized particles: ingestion and digestion. *Mar Ecol Prog Ser* 94:1–10
17. Granéli W, Bertilsson S, Philibert A (2004) Phosphorous limitation of bacterial growth in high Arctic lakes and ponds. *Aquatic Sciences*, 66:430–439
18. Hadas H, Einav M, Fishov I, Zaritsky A (1997) Bacteriophage T4 development depends on the physiology of its host *Escherichia coli*. *Microbiol* 143:179–185
19. Hennes KP, Simon M (1995) Significance of bacteriophages for controlling bacterioplankton growth in a mesotrophic lake. *Appl Environ Microbiol* 61:333–340
20. Hofer JS, Sommaruga R (2001) Seasonal dynamics of viruses in an alpine lake: importance of filamentous forms. *Aquat Microb Ecol* 26:1–11

21. Jaquet S, Domaizon I, Personnic S, Ram ASP, Heldal M, Duhamel S, Sime-Ngando T. (2005) Estimates of protozoan- and viral-mediated mortality of bacterioplankton in Lake Bourget (France). *Freshwater Biol* 50:627–645
22. Laybourn-Parry J (1997) The microbial loop in the Antarctic lakes. In: Lyons WB, Howard-Williams C, Hawes I (Eds.) *Ecosystem Processes in Antarctic Ice-free landscapes*, pp 231–240
23. Laybourn-Parry J (2002) Survival mechanisms in Antarctic lakes. *Phil Trans R Soc Lond B* 357:863–869
24. Lu FJ, Tseng SN, Li ML, Shih SR (2002) In vitro anti-influenza virus activity of synthetic humate analogues derived from protocatechuic acid. *Arch Virol* 147:273–284
25. Maranger R, Bird DF (1995) Viral abundance in aquatic systems: a comparison between marine and fresh waters. *Mar Ecol Prog Ser* 121: 217–226
26. Mathias CB, Kirschner AKT, Velimirov B (1995) Seasonal variations of virus abundance and viral control of the bacterial production in a backwater system of the Danube river. *Appl Environ Microbiol* 61:3734–3740
27. Middelboe M (2000) Bacterial growth rate and marine virus-host dynamics. *Microb Ecol* 40: 114–124
28. Middelboe M, Jørgensen NOG, Kroer N (1996) Effects of Viruses on Nutrient Turnover and Growth Efficiency of Noninfected Marine Bacterioplankton. *Appl Environ Microbiol* 62:1991–1997
29. Middelboe M, Lyck PG (2002) Regeneration of dissolved organic matter by viral lysis in marine microbial communities. *Aquat Microb Ecol* 27:187–194
30. Morris DP, Lewis WM (1992) Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. *Limnol Oceanogr* 37:1179–1192
31. Murray AG, Eldridge PM (1994) Marine viral ecology: incorporation of bacteriophage into the microbial planktonic food web paradigm. *J Plankton Res* 16:627–641
32. Noble RT, Fuhrman JA (1997) Viral decay and its causes in coastal waters. *Appl Environ Microbiol* 63:77–83
33. Paul JH, Jiang SC (2001) Lysogeny and transduction. In: Paul JH (Ed.) *Marine microbiology – methods in microbiology*. Academic Press, London, pp 105–125
34. Perdue EM (1998) Chemical composition structure and metal binding properties. In: Hessen DO, Tranvik L (Eds.) *Aquatic humic substances: ecology and biogeochemistry*. Ecological studies, vol. 133. Springer-Verlag, Berlin, Germany, pp 41–61
35. Proctor LM, Fuhrman JA (1991) Roles of viral infection in organic particle flux. *Mar Ecol Prog Ser* 69:133–142
36. Proctor LM, Okubo A, Fuhrman JA (1993) Calibrating estimates of phage-induced mortality in marine bacteria: ultrastructural studies of marine bacteriophage development from one-step growth experiments. *Microb Ecol* 25:161–182
37. Rivkin RB, Anderson MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. *Limnol Oceanogr* 42:730–740
38. Sala MM, Peters F, Gasol JM, Pedrós-Alió C, Marrasé C, Vaqué D (2002) Seasonal and spatial variation in the nutrient limitation of bacterioplankton growth in the Northwestern Mediterranean. *Aquat Microb Ecol* 27:47–56
39. Schneider J, Weis R, Männer C, Kary B, Werner A, Seubert BJ, Riede UN (1996) Inhibition of HIV-1 in cell culture by synthetic humate analogues derived from hydroquinone: mechanism of inhibition. *Virology* 218:389–395
40. Spencer R (1955) A marine bacteriophage. *Nature* 175:690–691
41. Suttle CA (2005) Viruses in the sea. *Nature* 437:356–361
42. Suttle CA (1993) Enumeration and isolation of viruses. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (Eds.) *Handbook of methods in aquatic microbial ecology*. CRC Press, Boca Raton, FL, USA pp 127–129
43. Suttle CA, Chen F (1992) Mechanisms and rates of decay of marine viruses in seawater. *Appl Environ Microbiol* 58:3721–3729
44. Tapper MA, Randall EH (1998) Temperate viruses and lysogeny in Lake Superior bacterioplankton. *Limnol Oceanogr* 43:95–103
45. Torrella F, Morita RY (1979) Evidence by electron micrographs for a high incidence of bacteriophage particles in the waters of Yaquina Bay, Oregon: ecological and taxonomical implications. *Appl Environ Microbiol* 37:774–778
46. Tranvik L (1989) Bacterioplankton growth, grazing mortality and quantitative relationship to primary production in a humic and a Clearwater lake. *J Plankton Res* 11:985–1000
47. Tranvik L (1998) Degradation of dissolved organic matter in humic waters by bacteria. In: Hessen DO, Tranvik L (Eds.) *Aquatic humic substances: ecology and biogeochemistry*. Ecological studies, vol. 133. Springer-Verlag, Heidelberg, Germany pp 259–284
48. Van Hannen EJ, Zwart G, Van Agterveld MP, Gons HJ, Ebert J, Laanbroek HJ (1999) Chang-

- es in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associate with viruses. *Appl Environ Microbiol* 65:795–801
49. Van Rensburg CEJ, Dekker J, Weis R, Smith TL, Van Rensburg EJ, Schneider J (2002) Investigation of the anti-HIV properties of oxihumate. *Chemotherapy* 48:138–143
 50. Vrede K, Stensdotter U, Lindström ES (2003) Viral and bacterioplankton dynamics in two lakes with different humic contents. *Microb Ecol* 46:406–415
 51. Ward BB, Granger J, Maldonado MT, Wells ML (2003) What limits bacterial production in the suboxic region of permanently ice-covered Lake Bonney, Antarctica? *Aquat Microb Ecol* 31:33–47
 52. Weinbauer MG, Höfle MG (1998) Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Appl Environ Microbiol* 64:431–438
 53. Weinbauer MG, Peduzzi P (1994) Frequency, size and distribution of bacteriophages in different marine bacterial morphotypes. *Mar Ecol Prog Ser* 108:11–20
 54. Weinbauer MG, Rassoulzadegan F (2004) Are viruses driving microbial diversification and diversity? *Environ Microbiol* 6:1–11
 55. Weinbauer MG, Suttle CA (1999) Lysogeny and prophage induction in coastal and offshore bacterial communities. *Aquat Microb Ecol* 18:217–225
 56. Weinbauer MG, Wilhelm SW, Suttle CA, Garza DR (1997) Photoreactivation compensates for UV damage and restores infectivity to natural marine virus communities. *Appl Environ Microbiol* 63:2200–2205
 57. Weinbauer MG, Winter C, Höfle MG (2002) Reconsidering transmission electron microscopy based estimates of viral infection of bacterioplankton using conversion factors derived from natural communities. *Aquat Microb Ecol* 27:103–110
 58. Williamson SJ, Houchin LA, McDaniel L, Paul JH (2002) Seasonal variation in lysogeny as depicted by prophage induction in Tampa Bay, Florida. *Appl Environ Microbiol* 68:4307–4314
 59. Wilhelm SW, Smith REH (2000) Bacterial carbon production in Lake Erie is influenced by viruses and solar radiation. *Can J Fish Aquat Sci* 57:317–326
 60. Wilson WH, Mann NH (1997) Lysogenic and lytic viral production in marine microbial communities. *Aquat Microb Ecol* 13:95–100
 61. Wommack KE, Colwell RR (2000) Virioplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev* 64:69–114
 62. Wommack KE, Hill RT, Muller TA, Colwell RR (1996) Effects of sunlight on bacteriophage viability and structure. *Appl Environ Microbiol* 62:1336–1341

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 sjövattnet 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 värdceller. 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.



Acknowledgments

Four years have passed by quickly and I have more or less managed to travel around the world and back. During my PhD I have realised that with some help from other people anything is possible and as long as you show determination and stubbornness you can achieve amazing things. I have been extremely lucky in being able to visit two of the most fascinating places in the world; the Arctic and the Antarctic. This could never have been achieved without the support from my two main supervisors Wilhelm Granéli and Johanna Laybourn-Parry. I am grateful to both of you for all your help with manuscript preparations, financial and scientific support and most of all for believing in me and encouraging me to become an independent scientist. I learnt incredibly much during my year in the Antarctic, both good and bad things about science and also life. I met a remarkable bunch of people in the Antarctic you were my family for a year and all helped me through highs and lows and most importantly made it possible for me to sample my two lakes consistently throughout the year. I'd like to mention a few that were my closest friends; Imojen – for being a brilliant scientist and friend whom without I would have gone insane in the isolated lab, Tb – for excellent support in the field, delivering thai curry to the doorstep and Friday g + t's, what more can you ask for?, Benny – for excellent support in the field and for a great friendship, coffee making in the met building and endless of hours talking rubbish on bundy, Winkle – for constantly being happy and always having new plans for exiting things to do on an isolated station, Macca – for entertainment on the harmonica and for feeding us all winter, Watto – for playing “waiting for an an-

gel” with Ben Harper on request, Eddie – for being understanding of Friday duff duff from your neighbour. In addition I also like to thank Bob, our trusty station leader in Antarctic 2004 for logistical support and encouragement. In our Antarctic yearbook of 2004 Bob put in a quote by Len Sales that was published in the New Zealand Antarctic Society Bulletin in 1964 which I think summarise very nicely the impact of Antarctic on a person. I quote, “Those who have spent an Antarctic night bear an indelible mark on their innermost self, a feeling which a person finds hard to define and explain to another”.

The Limnology department in Lund is an amazing scientific place and to be part of this scientific community has been an honour and I thank you all for making me feel welcomed. Carina and Emma thank you for letting me ask stupid questions and for giving me good scientific advice. Kelly and Cesar, thank you both for being great friends and listeners. Johanna thank you for your help on anything to do with maths. Anders N thanks for help with statistics. My special thanks to Alexandre for your constructive reviews of manuscripts and scientific discussions, you have been a great inspiration to me and your constant positive attitude and scientific knowledge has helped me throughout my entire PhD. Ramiro, Alice and Astrid, thanks for being good friends and always being up for going out in Lund.

Thanks are also due to Gerry Nash at the Australian Antarctic Division and Rita Wallen at the Zoology department at Lund University for help with transmission electron microscopy.

Thanks to all my friends that kept me in touch with the real world and allowed me to forget about viruses from time to time. Ben King for being a great friend and support throughout my whole PhD, I will always remember your fascination for the Antarctic and I hope you will get to go there eventually. Vicky for being an awesome friend and for all our girlie talks. To all my party friends out there, without you there would have been no party and no party makes Christin sad so thanks for keeping me happy! A big shout goes out to my old party crew in Nottingham (Dom, Monica, Peter, Toby, Wendi &

Adam) remember the good old days at the Atomic Jam and the Garvey, priceless memories.

To my mother, you have been a constant support in my life and without you I would never have become the person I am today. You are the best!

If I have forgot to acknowledge someone it was not my intention to and to be on the safe side I wish to express thanks to all the people that I have ever met in my entire life as you have all influenced me in one way or another.