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Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF LESC EXPLORATORY WORKSHOP

1st European Workshop on Aquatic Phage Ecology (EWAPE-1)

Scientific Report



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EWAPE-1
Thonon, France

EWAPE-1 scientific report

ESF Exploratory Workshops
EW04-065 (LESC)

Executive summary

Viruses infecting bacteria and/or cyanobacteria are an essential biological compartment in aquatic microbial food webs. They are important controlling agents for planktonic communities, playing a key role in cell mortality, nutrient cycles, microbial diversification and diversity. However, still little is known on how this viral activity is linked to diversity and ecosystem functioning. **This first workshop in Europe** has provided the opportunity for promoting this field of research through discussion and presentation of our past and most recent results, of the various approaches and methods we use and the problems we face, for reinforcing or developing collaboration, and *in fine* for developing strategies for future research at national and European scales.

Viruses are the most abundant and likely the most diverse biological entities in aquatic ecosystems. They can exceed abundances of 10 million per millilitre. In aquatic systems, the majority of viruses are mainly thought to be bacteriophages and cyanophages (i.e. viruses infecting bacteria and cyanobacteria later referred to as phages). To place this into context, in the world's oceans, there is an estimated 27-270 and 2,740-13,700 megatons of viral and bacterial carbon, respectively! The role of phages in aquatic environments has been the subject of increased investigation during the last 15 years. The development of techniques to study viral populations and communities has also progressed tremendously. This has been due to the recognition of phages as a key biological component of aquatic ecosystems. From the first studies (1988-1992), viruses were shown to be very abundant. More recent studies have demonstrated that they play crucial roles in bacterial and/or cyanobacterial mortality and water column trophodynamics. There is increasing evidence that phages exert significant pressure on bacterial and cyanobacterial community structure and diversity. However, research related to this pressure, and research to address general aspects of viral diversity is still in its infancy. One of the problems with current research approaches is that scientists working in individual and independent laboratories can only make limited advances in the field. One of the propositions with this workshop was to talk about the necessity of a much more collaborative approach.

The aims of this **ESF Exploratory Workshop** were to target and explore this challenging scientific topic, which would greatly benefit from a collaborative European approach to foster trans-national and interdisciplinary links as well as to develop future collaborative research projects and programmes. This workshop has focused on virus-

bacterial and virus-cyanobacterial interactions in aquatic systems, and how these interactions affect the structure and functioning of microbial food webs and their participation in important biogeochemical processes such as the carbon cycle, as well as viral diversity. I proposed this workshop to be the first of future ones, and so referred to it as the **1st European Workshop on Aquatic Phage Ecology (EWAPE-1)**.

This workshop has been very useful to the whole European community because it highlighted what has been currently done in Europe and what we should do in the future. Discussions dealt with:

- The impact of viral lysis on the structure of host communities and the responses of these hosts;
- The impact of viral lysis and its relationship to protozoan grazing;
- The mechanism of host defence against infection (resistance acquisition) as well as the survival and dissemination strategies (lysogeny, pseudolysogeny);
- The influence of changing environmental factors (UV for example) on the following parameters: abundance, integrity, infectivity, production;
- The role of viruses in gene transfer, among microbial populations, and also the role of bacteriophage in evolution and diversity of bacterial communities;
- The roles of cyanophages vs. bacteriophages in the structuring of microbial communities, including the formation and termination of cyanobacterial blooms;
- Making real attempts at the study of viral diversity in freshwater, estuarine and marine environments, with transnational database development;
- Non-traditional ways to pursue isolation of viruses specific to *Archaea*, for which no known virus-host systems have been developed;
- Understanding viral genomics, including conserved viral nucleic acid sequences, and their importance to viral replication, infection and production;
- Understanding the impact of suspended particles on virus-related processes, a field largely ignored in virus ecology;
- Understanding the role between water column bacteriophage and sediment based bacteriophage. The dynamics of sediment-water column microbial interactions are not well known, and there have been several controversial studies on distribution and abundances of viruses in sediments, especially due to associated methodological difficulties.

The objective of the workshop on behalf of ESF was to focus on virus-bacteria and virus-cyanobacteria interactions, focusing on dynamics, diversity, gene transfer, nutrient and organic matter recycling, and ecosystem function. I made the attempt to invite all members of the European scientific community that are currently working in this field of research for freshwater and marine ecosystems. We are still convinced that a collaborative approach is necessary for studying viral impacts on aquatic systems. With a collaborative effort, phage diversity can be more clearly understood and placed into the context of evolutionary processes. It is apparent that a well-organized collaborative effort will be necessary to move forward. The scale of research contributions required to provide an adequate foundation for understanding phage diversity is clearly beyond that of a single research group, or single national effort. Simultaneously to our studies of phage diversity, information will be generated to improve our understanding of microbial diversity and ecosystem functioning in aquatic environments.

Scientific content of the event

During 3 days, participants could hear about 25 talks and see 18 posters. Plenty of time was available for discussion and propositions. See the details below.

Assessment of the results, contribution to the future direction of the field, outcome

In this paragraph, I will talk about:

- The French virus group (A)
- The European virus network (B)
- The next meeting (C)
- The special issue of *Aquatic Microbial Ecology* (D)
- The EWAPE-1 website (E)
- The opinions sent to me about and after the workshop (F)

A – The French virus group

This workshop gave the opportunity to the entire French community working on the ecology of aquatic viruses to meet for the very first time. This community is a very young one with very dynamic scientists below 45 years old. We have decided to create a French network and a first meeting will be held in May in Montpellier. The French teams and responsible are:

- Dr Stéphan JACQUET (INRA, Thonon)
- Dr Markus WEINBAUER (CNRS, Villefranche-sur-mer)
- Dr Thierry BOUVIER (CNRS, Montpellier)
- Dr Téléphore SIME-NGANDO (CNRS, Clermont-Ferrand)
- Dr Philippe LEBARON (CNRS, Banyuls-sur-mer)
- Dr Yvan BETTAREL (IRD, Sénégal)

B – The European virus network

When I concluded this meeting, I proposed to the participants we could try to create a European scientific network dealing with aquatic viral ecology (dynamics, diversity and role). As it has been outlined above, the idea will be to reinforce or develop collaboration for developing strategies for future research at both national and European scales (Exchange of expertise, equipment, student, Development of common projects, Field workshops, Meetings). As a SCOR group on marine viruses already exists, it has been proposed to enlarge the community with the 'freshwaters'. The proposition to link our group to the one working more specifically on algal viruses will also be discussed in April in Amsterdam during the 4th Algal Virus Workshop.

In the frame work of this European scientific network, I also proposed it could be named as follows: EUROPHAGE for EUROpean network on aquatic PHage Genomics and Ecology. I will make in a few weeks a proposition to use one of the ESF possibilities such as the *Scientific Programs*, i.e. medium- to long-term activities focused on specific themes with the idea to bring together substantive research projects carried out by multinational teams of researchers.

Last but not least, one of the main point in this network will be the formation of a European data basis on viruses (methodologies, abundances, burst-size, FIC, FVIC, VICM, sequences).

C – The next meeting

Will there be a EWAPE-2? It seems that everybody agrees with this idea (see 'Opinions' below). However, EWAPE-2 is likely to be included in a larger European workshop or meeting following what has been mentioned above.

D – The special issue of *Aquatic Microbial Ecology*

A few weeks before the workshop, Dr Mark GESSNER, one of the participants and chief editor of special issues of the revue *Freshwater Biology*, proposed to me that a special issue could be realized following the workshop. This news was posted on the EWAPE-1 website and has been discussed during the conclusion I gave to the meeting. Some people were interested but a large majority was not. The main reason why was that this journal is not of real interest for the marine

community. Recently, I had another proposition by *Aquatic Microbial Ecology*. Many participants said they were more interested and this is probably what we will do in forthcoming months.

E – The EWAPE-1 website

At the beginning of this adventure, I made a website for this workshop. It is still alive and some information is regularly posted for our community. All the presentations are online but their access is only restricted to participants. A gallery of pictures has also been created. I invite you to go and visit: <http://www.thonon.inra.fr/EWAPE1/index.htm>

F - Opinions

At the end of the workshop, I asked participants to send me back their feeling about this meeting and I simply reproduced below what they sent me:

From Markus WEINBAUER

I would like to thank YOU for that (i.e. excellent atmosphere during the 3 days) !!

It was an excellent experience. All experts together in one spot for a couple of days!! I (and lots of others) prefer this to the mega-meetings.

The content was well balanced. I just realized during EWAPE how much ahead cyanophage ecologists are in some aspects. You happy guys have a good sequence data base and cyanophages are relatively easy to isolate. (meta)genomics was less well represented, although the UK people are using this in a very clever and innovative way.

No complaints at all about the organization

The menu was fantastic. The only thing missing was an additional hour of digestion!

Coming to Thonon is not very easy, but also not very complicated. The castle is certainly a very stimulating environment.

The perspectives proposed: four topics: continuation of EWAPE (or a similar attempt) in relation to continuing a conference, EWAPE as an integration of phage ecologists in Europe, EUROPHAGE and publication.

1. I think we should try to continue with EWAPE as a regular (and small!!) conference. If the algal virus people join, it would be perfect!! If they do not want, I suggest we try to continue with what you have initiated. Virus sessions are covered in other meetings (as Jed and Gunnar have stressed). This is important to remain connected to microbial ecology and biogeochemistry. But it is also vital for our field to have our own little familiar meetings. I also believe that ESF will not finance continuous workshops in our field for a long period. Therefore, my suggestions would be to make an open EWAPE workshop (maybe some invited speakers but most have to pay for their own). If we call it EWAPE we can get money from European Organizations (ESF, EU, FEMS....) but it could still be possible to allow for international contributions.

2. We should try a lot of networking here. For example the connection to MARBEF. Exchange of students, methods workshops, etc. Whenever ESF has a possibility to give as money, let's tap it. Please send your list of possibilities you presented, so that I can make suggestions.

3. Difficult. Maybe we should try point 2 first and try to find some lobbying for a EUROPHAGE project (sexy idea though). What would be the community added value? I think we have to find a good answer for this question before we start trying that. I'll ask Fereidoun and John about AME but I am bit skeptical about that. The option of Freshwater Biology doesn't seem to be very attractive for the marine people. We would have to find a way to assure that this volume is made available to them like sending out pdfs (are they for free?). Also, we would have to apply a rigorous review process (and promise that!!!!) to make sure it is not just a help for buddies to get crappy data published). I like the proposition of Mark but it will need a strong effort to convince the marine guys.

From Branko Velimirov

I think it was a pleasure for all of us to be there, certainly for me. I only felt sorry to have to leave a few hours earlier. I have to thank you for the excellent atmosphere prevailing during the workshop.

- the meeting WAS absolutely SUPER
- the content WAS certainly up to get close to the actual state of art
- the organization Was perfect
- the menu was pure luxury
- the place: It was a very special place for a very special meeting - great class!

From Guenter Jost

As far as I remember you wanted to have some kind of resume about the conference. I think that such small meetings concentrating on a certain topic are in most cases more interesting than the large conferences. I guess we got a good state of the art in aquatic phage ecology not only from Europe. I was surprised that the guys working with cyanophages seems to have more phage host systems than we working with heterotrophic bacteria and their phages.

From the new techniques the PFGE seems to become something like the DGGE for phages. But I am a little bit sceptics about it. I guess that using FCM becomes more and more convenient even for phages. But having more of this kind of counting will not improve our understanding. In some cases we will be in the typical range of the bacteria to phages ratio and in some cases not. Surprisingly, the same range is found not only nearly in all pelagic environments but also in sediment samples from different environments. So, what may behind this ratio?

It is hard to find arguments against such a hypothesis like "killing the winner". That makes it even more surprising that Jed had to state we have nearly as much studies supporting it as studies disproving it. And this describes a little bit where we might stay at the moment - we know a little bit but we are in front of a fog. Maybe we have some visions what is behind but nobody really knows.

Therefore, a continuation would be great. Concerning further founding by ESF I would like to have a modification. Since they normally found it for about 5 years I would like to have the same amount of founding for ten years and instead of every year a meeting a meeting every second year would be fine. In between there should be a possibility to exchange students between different labs.

And summing all up in a sentence - you did a great job!

From Lillian CARLSSON

Being a master student it was my first meeting, so I don't have anything to compare with, but my overall impression was really good. As you said there was a really nice atmosphere, it was much more relaxed than I had expected it to be. It was relaxed but at the same time, very professionally arranged, and it seemed to me like everything was as it should be. Of course we all will remember this meeting for it's excellent food - I must say, who ever said that French are awesome at cooking were so right. It was so delicious, but maybe a little too much with four dishes you were so exhausted after dinner that you just felt like sleeping :-). Besides that I also enjoyed the poster session, which for me was very useful, as I'm defending my thesis in a month. I got more response on my poster than expected - I guess that is because it was such a small conference! I think it was a really good programme, with some really great presentations!

For the future, I think it would be nice to have a meeting like this again; however I got the impression that it would be too much with another meeting, as there are already so many meetings. So as you discussed it would probably be better to join with the SCOR or algal virus meetings.

Finally I would like to thank you very much for making such a great meeting - it was a really nice break away from my thesis, in the perfect settings with the most excellent food!

From Ludwig Jardillier

Merci pour ton invitation à ce workshop qui m'a non seulement permis de prendre des contacts, mais aussi de me donner envie de faire si possible un post-doc sur les virus, mais également de me remotiver. Thank's a lot!

From Thierry Bouvier

Encore bravo et merci pour ce workshop.

From Téléphore SIME-NGANDO

Encore merci pour la sublime réunion de la semaine passée, très bien joué, très bien organisé, chapeau.

From Johanna Laybourn-Parry

Once again thank you for all your hard work in organising the meeting. Having done that sort of thing myself I know how big a job it is.

- the meeting - excellent organisation, and a great opportunity for people to meet and discuss viruses. As I have only worked on viruses for a short time and have only published two papers on viruses I found it very useful to talk to others who are more established in the field.
- the content - well balanced range of presentations and an opportunity for research students to present as well as more established scientists. So the training aspect was met as giving papers are conferences it an important part of postgraduate training.
- the organisation - superb!
- the menu ! - also superb. Good food certainly encourages people to relax and talk about science.
- the place - lovely venue which encouraged networking.
- the perspectives proposed - I think that further meetings would be a good idea. Having formed a network I think it would be a pity not to exploit it for EC funding opportunities through the Marie Curie programmes or through the other grant initiatives.

From Dolors Vaqué

I enjoyed to see all of you and I learned a lot from the meeting.

The meeting was good, with a lot of discussion and sharing opinions among the participants I Have to think a bit more in the future of EWAPE, but for sure we need an EWAPE-2, perhaps as you say in 2008

From Marjolijn Tijdens

Thanks again for organizing this great meeting. Here comes a short impression of the meeting, I hope you can use it. The meeting, in its size and setup, gave me an excellent opportunity to meet and discuss with other people working with viral ecology in aquatic environments. In the three days of the meeting I have obtained a good overview of the progress that is being made in this field and the questions that will be important in the coming years. The organization arranged the accommodation, location and transport very well and especially the lunches during the workshop were amazing. I am very enthusiastic about the plans to set up an European aquatic viral ecology network, including a website. I think that such a network would greatly enhance and encourage international cooperation.

So I am very positive about this meeting and I am looking forward to the next one,

From Martin AGIS

First of all, i really enjoyed the meeting and I really think you did a great job! It was good to see the faces of the people of whom I ususally only know the publications. The organisation was excellent from the beginning until the end (including your efforts concerning transport from the hotel to the chateau and to the airport, bus station etc.). All the people are working in the same field and it was a good opportunity to learn from their experiences using the same or different methods and to hear about their findings in different systems. Phage ecology is an important topic and it was a good idea to bring them all together. The atmosphere was very 'agreeable' because of the way you organised the meeting, because of the people that participated and also because of the location you have chosen. About the menu is nothing more to say than 'excellent'. To join together again in the future (maybe even with the algal virus people) would be a good idea.

From Olivier Zemb

I have found the meeting very interesting and the atmosphere was really excellent. The relatively small number of participant makes it easier to talk with everybody. This was a really nice way to know the current works about viruses in aquatic systems.

The content was very well adapted to my needs and I've learned a lot !

I thank you once more for this no-mistake organisation.

Yes! French food has a reputation, and I think this meeting confirm it. So good food ! I will make a diet for two weeks.

The place was so nice. The French castle also have a reputation, and once more, it's confirmed with this workshop. I didn't found the room cold or anything. Oh ! but the hotel rooms were very hot (and quite a bit comfortable !)

From Jed Fuhrman

I had a very enjoyable meeting, and thank you very much for all your work and effort. My responses are below:

- the meeting

Excellent group of colleagues from broad swath of the field. Size was suitable for good interactions throughout.

- the content

Very good mix of freshwater, marine, estuary, and bacterial, cyanobacterial, and even archaeal. Also good mix of different perspectives from young to very established (=old) investigators. I learned something from every presentation - that's unusual.

- the organisation

Very well organized, from meeting schedule, housing, meals, breaks, small tour, stc.

- the menu !

Fantastic. I gained 1 kilo in one week!

- the place

Beautiful surroundings, room suitable for that group, poster session small but OK. Small issues: It would have been a little nicer to have warm water in the cold bathroom, and I would have benefited from a high speed internet connection (to avoid having so many emails accumulate while I was away - but maybe I should have invested in a PDA cell phone).

- the perspectives proposed

Very useful broad perspectives.

From Mathias Middelboe

Thanks again for at great meeting. I really enjoyed it. I think the whole concept was great and very nice and constructive to have a meeting with such a small group of people. The discussions are relaxed and because we were all of us together throughout the meeting, there was time to follow up on discussions. It is also a nice forum for students to get introduced to such meetings and present their work in. I know my students were very happy about it.

Also, all my compliments to your organization, the place and the food. I really think this was all excellent.

Even though it is a large variety of topics that are investigated in the different labs and which were presented at the meeting, I found it interesting to get an overview of peoples work and I think this might help us to increase the interactions between the European virus-groups.

I realize that there is a problem regarding the overlap in people and topics between this meeting and the Algal virus workshop, and this has to be solved somehow. It might be a good idea to combine the meetings, although the idea about having a European meeting then falls apart. I really think that there would be a point in establishing a European network as a follow-up on this, and if we decide to try and do that, perhaps through some EU funding, I will be willing to be involved with that.

Having thought more about it, I don't think that a special issue in Freshwater Biology based on the presentations at the meeting is such a good idea. At least I would prefer to publish my work elsewhere.

From Caroline Jenkins

I very much enjoyed the workshop, and I believe it was the most useful and productive meeting I have attended so far (so well done!).

I found the small group size allowed me to talk to each of the people I needed to, which is in direct comparison with the last meeting I attended where it was impossible to locate the relevant people. I also appreciated the atmosphere of co-operation and interaction that was generated.

I'm not sure that it is such a good idea to merge the European phage group with the international eukaryotic algal virus group, as it would double the numbers, shift the focus of the meetings, and perhaps increase travel costs. Although people pointed out that there are several virus meetings in the year, the emphasis of each one ranges from veterinary/medical pathogens and antimicrobials to structural/functional genetics/evolution and proteomics which are useful but the methodology is not directly relevant to ecologists. For example I will attend this year the EWAPE1, VEG (uk), and possibly the Evergreen International Phage Biology Meeting (USA).

The organisation was very good, and the pace of the meeting was appropriate. Although other meetings I have attended have had evening sessions, I found that dining together in smaller groups at each hotel allowed topics to be persuaded further.

The menu was excellent and I very much enjoyed telling Paul all about what he missed when I got back! However I did find it very hard not to fall asleep during the afternoon session following such a huge lunch.

The castle was an appropriate venue, although travel to and from Thonon was quite complicated.

I look forward to seeing what you do with the additions to the website.

I don't feel that publishing my work in the freshwater biology journal mentioned at the end of the meeting would be appropriate, as I don't read this journal.

From Karel Simek

The meeting was very nice, everything on a schedule with enough time to discuss many details with my colleagues. Overall, I was very happy with all the points you raised. As for perspectives - I guess it rely more on "core" and "big people" in viruses job. I am much interested in this topic, but more from the side of interactions with other microbes in plankton.

From Ludwig Jardillier

The First European Workshop on Aquatic Phage Ecology, organised by Stéphan Jacquet at Thonon-les-bains, was, from my point of view as young scientist, a real success. Indeed, everything was well organised. It allow to many European researcher of the same research area to meet together. The level of all communications was high, with the major scientists on this topic. Thus, for young researcher like me, it was a great opportunity to discuss about our studies and results, but also to keep contact for future collaborations like post-doctorat positions. Generally, I enjoy to have taking part to this great workshop and thank's organisers and partners.

From Martin Muhling

I had looking back on last week's meeting in Thonon. I have to say that I have only good memories. Thanks for your hard work; the meeting was really fantastic in every sense.

The atmosphere throughout the workshop was very good and extremely friendly. The location in the French Alps with the conference being held in the beautiful setting of Chateau Ripaille certainly contributed to the overall ambience.

The content of both oral and poster presentations were of outstanding quality and stimulated intense discussions.

The mix of participants was well balanced. Beside the pioneers of the field (Prof. Gunnar Bratbak, Prof. Jed Fuhrman) and other, well established colleagues there were also young scientists, post-doctoral and post-graduate. And due to the good atmosphere everyone felt confident to get involved in the scientific discussions, if not directly after the presentations then over coffee, lunch and dinner. I also met several colleagues which I only knew from their publications or not at all, and had interesting exchanges of research ideas and solutions to scientific problems.

The organisation of the whole event was flawless thanks to the hard work and enthusiasm of Stephan Jacquet: the hotels were adequate, transport from the hotels to the conference venue and back, as well as the lunches and dinners were perfectly organised and always on time. And the quality of the food just confirmed that the French Cuisine is worth its reputation.

In conclusion, this meeting was a solid "three-day brain-storming" in a fantastic scenery and atmosphere, from which I took good ideas for further work as well as new contacts to European colleagues in the field. And I hope that this workshop was only the first in a soon established series of European Workshops on Aquatic Phage Ecology.

From Ruth-Anne Sandaa

I found the meeting scientifically very interesting. A lot of new and exciting results were presented. The size of the group of researchers was optimal as everybody got the opportunity to talk with everybody. In this way knowledge was shared between scientists from different fields of study and localities. However, it might be possible to enlarge the group to approx 100 people without destroying this atmosphere. The locality of the meeting was also perfect for creating a good atmosphere. The combination of extremely good food, wine, and nice surroundings really loosens up people's mind making it easy to start talking and thereby creating contact that might be very important in a scientific view. A good workshop should not only allow presentations of good talks, but also give the opportunity for a lot of good discussions also outside the conference room.

My suggestion is that we should try to continue organising this kind of workshop, but we might try to enlarge the group to approx 100 people. The frame of the meeting should be the same focusing on viral ecology in water systems; however we might include people working with algae viruses as we share common interests. I do not think we should include virologists, as I am afraid that that will create a too large group of people. However, what I suggest is that we might enlarge the scientific focus by inviting key-speakers that are specialised in some fields that are of interest for the group, e.g. virologists etc.

From Dolores Vaqué

My feelings of the excellent meeting

- the meeting was excellent. From the point of view of science it was the right size to discuss and learn a lot from the different types of research on virus. From the point of view of the participants there was a very good atmosphere among all of us
- the content. It was quite balanced between molecular biology (diversity) and microbial ecology, freshwater and marine systems. I enjoyed and I learned a lot from my colleagues and friends
- the organisation. It was excellent. Congratulations to Stephan Jacquet and collaborators that participated in the organisation of the meeting.
- the menu: it was very accurate and well prepared, as in the castle as well as in the hotel (Arc en Ciel)
- the place: Fantastic, gorges, beautiful!!!!
- the perspectives proposed: I would like to have, and participate actively in the future EWAPE meetings. As you said probably it would be nice to have a periodicity between two and three years. Seems fine to me to celebrate next in 2008 (time flies!!!!).

From Mark Gessner

Thanks for your excellent organization of the meeting. I'm hoping it will spark further activities in the field.

the meeting: excellent overall

- - the content: good balance; as an ecologist, I appreciated the prevailing ecological perspectives throughout, as opposed to focussing purely on technical issues
- - the organisation: first class; allocating a bit more official time for posters might have been good but since the programme wasn't very tight (an good idea in my opinion), this turned out to be no problem
- - the menu ! just lousy
- - the place: phantastic
- - the perspectives proposed:
 - 1) Evidently, getting an ESF programme established would be an excellent opportunity for developing aquatic phage ecology in Europe and to foster scientific collaboration
 - 2) I would still be happy to work on a special issue for Freshwater Biology, although I do see Jed Fuhrmann's point. It will be interesting to hear what everybody else thinks.
 - 3) As I said at the meeting, writing a separate short synthesis/perspectives paper for Trends in Ecology & Evolution or Trends in Microbiology may be worthwhile. So maybe you want to explore that option, too, and perhaps get some of the established people in aquatic virus ecology on board (e.g. Markus Weinbauer, Jed, etc.). I'd be happy to get involved if that seems useful.
 - 4) I forgot to mention the idea to organize a postgraduate training course. This may be an item to include in an ESF programme or be organized independently.
 - 5) I am curious to see how things evolve.

From Christian Winter

- the meeting

I think that this meeting was a good place to meet people working in our field. Since there were about 40 participants it was possible to talk to nearly everyone during the meeting and finally fill in the last, as of then unknown, faces to the known names. It sort of felt being home while actually being far away from home because everybody is working on the same subject, thus, complicated introductions are not necessary and it is possible to 'shoot' away right from the start of conversations.

- the content

Since this was a workshop it is perfectly ok even desirable to present 'unfinished business' or even contradictory stories that would spark some exchange of ideas and discussions. Many participants took advantage of that, thus, one could get an idea of what's going on in european phage ecology from the front row.

- the organisation

No flaw found, very well done!!

- the menu !

I think that I never have been on a meeting before that actually advertised the menu to be served on the corresponding web-page. But then again, this was happening in France...:). The menu was very good and tasty (I still wonder about some of the ingredients since I never tasted them before).

- the place

Although it was a bit complicated to reach Thonon, it was well worth it.

- the perspectives proposed

I really enjoyed the cosy, almost familiar atmosphere created by the place in combination with the relatively small number of participants. However, I do see why Gunnar Bratbak and Markus argued for combining it with the algal virus workshop. Meaning, I definitely would like to see EWAPE2 or alternatively a combined workshop for both algal and prokaryotic viruses.

As for a special issue about EWAPE: I am probably the wrong person to ask since I cannot really contribute to such an issue.

From Gunnar Bratbak

Here are a few words about the meeting as you requested

I really enjoyed it, thank you for organizing it - excellent job!!!

- the meeting

The meeting was a success in that it assembled most of the European scientists that are active in this field and also a number of younger scientists. The number of participants was suitable for a focused meeting like this.

- the content

The meeting focused on virus and mainly bacteriophage ecology. It is fruitful to keep a tight focus when a new field is established and expanding, but over time it may be fruitful to consider a broader view and include virus of other aquatic micro organisms.

- the organisation

Excellent - no problems.

- the menu !

Excellent.

- the place

Very nice. It may be an idea for future meeting to have all participants in one hotel to facilitate social interactions.

- the perspectives proposed

Great. This kind of meeting as informal forum is very important for developing a field of research. It is an idea to merge EWAPE with the Algal virus workshop but this involves many non-Europeans and it also broadens the perspective of the group.

I am uncertain about publishing the presentations in a special issue of a journal as what is publishable will be published anyway in relevant journals addressing the adequate audience.

Web-publishing: I would need to edit my presentation as it contains personal communications and unpublished material that I can use in the talk but not necessarily put out on a (restricted) webpage.

From Lasse Riemann

Thank you for a great time in Thonon. I think the meeting was a great success. 3 days was perfect, the organization and the number of people was perfect. I found the meeting much more giving than larger conferences. It was a privilege to be among all of these bright people.

Concerning food, place etc. everything was perfect.

Scientifically, I find that hearing talks on so many aspects of viral ecology has been very giving - something I can use in my research and future project planning.

I would definitely support a second EWAPE in the future.

Thank you for your tremendous effort!!!!

From Nick MANN

I would like to compliment you on the excellent organization of the recent EWAPE-1 meeting. I thought that it succeeded at every level. Most importantly the scientific content was extremely high and this merely serves to emphasize the increase in importance that attaches to phage ecology these days. The meeting also served the very useful function of bringing together the European community of scientists working in this area and I hope very much that these meetings continue in order to maintain and develop this community. The venue was very well chosen and I would particularly like to comment on the superb lunches.

I feel that the continuation of these meetings is scientifically very important and would like to offer you any help or support that I can provide.

From Peter PEDUZZI

Regarding the questions: my answer is to give an A+ to all of the respective points (with the exception that I've caught a really bad flu and am still trying to recover). One minor suggestion for a next meeting would be to include well organized round-table discussions which should make sense for a workshop.

From Ulrike FISCHER

I want to thank you for inviting me to your workshop; it was really a fantastic idea to organize such an event!!!!!! It was interesting to see what's going on in "phage science" in Europe and to have the opportunity to exchange experiences, opinions..... I really enjoyed it very much!!!!!!

about the place: you couldn't have found a better one!!!!!!

the menu: phenomenal!!!!

the organisation: PERFECT!!!!!! CONGRATULATIONS!!!!!!

special thanks for organising the "taxi" for Branko and me to the airport, this was really a great help! Please apologise that we had to leave before the end of the meeting and thanks for sending the pdf about the conclusions. I think online presentations, a special issue of freshwater biology, "EUROPHAGE" (especially the creation of a European data basis on viruses!!) etc. are very good ideas and would be helpful/useful to all of us.

From Eleanor HARRISON

As it was my first conference I found it a very beneficial experience. It was a particularly good place to meet and converse with people in my field other than my supervisors. It gave me a different perspective on the subject. It was a relaxed and friendly environment that made it easy to meet people and exchange ideas. I found the content of the meeting was focused and concise, as it was all within the field of aquatic phage ecology. There was a good proportion of work presented on Cyanophage, my own area of study.

The conference organisation was well thought through, the website and instructions being particularly useful in getting us there! Everything ran to schedule and the transport to and from the hotels was fast and efficient. Even the menu was excellent, there was almost too much food to eat, and fortunately I did not mind that.

The setting and venue were exceptionally nice, if a little distracting. The one negative point about the venue was that it was a little tucked away and getting between Geneva airport and Thonon-le-Bain took a little time as we had to take a train and a coach and then navigate to the hotel.

The continuation and development of the website will be a great resource as it will be good to maintain the exchange of ideas.

Thank you for organising such a great event

For Corina BRUSSARRD**Responsible for the Algal Virus Workshop**

Hi Stephan,

I am just back from my cruise and checking emails and noticed also your emails. I see from internet that EWAPE was a success - congratulations. Personally, I would like to see a continuation of EWAPE but then with the focus on Europe and including all aquatic viruses. Combining with AVW will make this a non-European meeting. I would feel it would be best to have a European forum that meets regularly - every 3 years for example.

Final programme (oral communications)**2 February, Morning session part 1****Virus-phytoplankton interactions**

Chaired by Ruth-Anne SANDAA + Markus WEINBAUER

9h00 - 9h30

BRATBAK Gunnar

An overview of algal viruses

9h30 - 10h00

ZINGONE Adriana

Do viruses control phytoplankton blooms? The case of *Micromonas pusilla*

10h00 - 10h30

MANN Nicolas

Impact of phage infection on photosynthesis in the marine picoplankton

Coffee break (10h30-11h00)

2 February, Morning session part 2

Virus-phytoplankton interactions

Chaired by Ruth-Anne SANDAA + Markus WEINBAUER

11h00 - 11h30

MILLARD Andrew

An investigation into cyanophages in the Red Sea

11h30 - 12h00

MUHLING Martin

Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton

12h00 - 12h30

PARRY Jackie

Picocyanobacteria – the sweetcorn of the microbial world?

Lunch in the castle (12h30-14h00)

2 February, Afternoon session part 1

Virus genomics and diversity

Chaired by Ulrike FISHER + Nicolas MANN

14h00 - 14h30

CLOKIE Martha

Photosynthesis genes in marine cyanophages: acquisition and physiological significance

15h00 - 15h30

DORIGO Urusla

Cyanophage isolation and diversity of the largest natural French lake

15h30 - 16h00

JACQUET Stéphan

A new virus infecting freshwater PE-rich picocyanobacteria of Lakes Annecy and Bourget (France)

Coffee break (15h30-16h00)

2 February, Afternoon session part 2

16h00 - 17h30

Free discussion & Poster session

3 February, Morning session part 1

Role of viruses for bacterial diversity and population dynamics

Chaired by Isabelle DOMAIZON + Peter PEDUZZI

9h00 - 9h30

LAYBOURN-PARRY Johanna

Annual viral dynamics in contrasting Antarctic lakes

9h30 - 10h00

RIEMANN Lasse

Prevalence of plasmids and lysogenic viruses in Baltic Sea bacterioplankton

10h00 - 10h30

SANDAA Ruth-Anne

Viral diversity in the marine environment. Effects on the species richness and activity of planktonic organisms

Coffee break (10h30-11h00)

3 February, Morning session part 2

Visit of the castle and wine tasting (11h00-12h30)

Lunch in the castle (12h30-14h00)

3 February, Afternoon session part 1

Role of viruses for bacterial diversity and population dynamics

Chaired by Adriana ZINGONE + Jed FUHRMAN

14h00 - 14h30

WEINBAUER Markus

Viruses as driving forces of microbial diversity and ecosystem functioning

14h30 - 15h00

WINTER Christian

Influence of planktonic bacteriophages on prokaryotic community composition

15h00 - 15h30

VELIMIROV Branko

New transducing particle types or are we still missing the essential?

Coffee break (15h30-16h00)

3 February, Afternoon session part 2

Role of viruses for bacterial diversity and population dynamics

Chaired by Adriana ZINGONE + Jed FUHRMAN

16h00 - 16h30

BOUVIER Thierry

Absence of virus releases dominance of rare marine prokaryotic groups

16h30 - 17h00

JOST Gunter

Autochthonous phage-host systems – do we need them?

17h00 - 17h30

SIME-NGANDO Télesphore

Seasonal phage bacteriolysis equals protistan bacterivory in a freshwater reservoir: coupling with the physical environment

4 February, Morning session part 1

Viral dynamics. Role of viruses in ecosystem function. Viral vs. protist control of microbial communities

Chaired by JACKIE PARRY + Gunnar BRATBAK

9h00 - 9h30

MIDDELBOE Mathias

Virus dynamics in marine sediments: implications for benthic carbon cycling.

9h30-10h00

GESSNER Mark

High abundance but low impact of viruses on benthic bacteria

10h00 - 10h30

PEDUZZI Peter

Ecology of viruses in river systems

Coffee break (10h30-11h00)

4 February, Morning session part 2

Viral dynamics. Role of viruses in ecosystem function. Viral vs. protist control of microbial communities

Chaired by JACKIE PARRY + Gunnar BRATBAK

11h00 - 11h30

FUHRMAN Jed

Interactions between marine bacterial and viral community composition

11h30 - 12h00

SIMEK Karel

Comparing the effects of resource enrichment and grazing on a bacterial community and viral dynamics in a freshwater reservoir

12h00 - 12h30

BETTAREL Yvan

Nanoflagellate 'virivory' in two lakes of the French Massif Central

12h30 - 13h00

LYMER David

The importance of viral induced bacterial mortality compared to bacterial grazing in lakes of different trophic status

Lunch in the castle (13h00-14h30)

4 February, Afternoon session = conclusions

14h30 - 16h00

Conclusions by Jed FUHRMAN & Stéphan JACQUET

Propositions for the future by Stéphan JACQUET

5 February, morning

Visit of the *INRA Hydrobiological Station of Thonon*

Final programme (Posters)

AGIS Martin

Variability of virioplankton diversity in a river floodplain system

BETTAREL Yvan

Isolation and characterisation of a small nuclear inclusion virus infecting the diatom *Chaetoceros* c.f. *gracilis*

BOUVIER Thierry

Response of viral communities to rain and river waters in coastal mesocosms

CARLSSON Lillian

Spatial variations in viral production rates and microbial activity in surface sediments along a depth transect in the Øresund, Denmark

FILIPPINI Manuela

Viral diversity in different benthic ecosystems

FISHER Ulrike

Impact of abiotic factors on virus adsorption to particles in aquatic sediments

HARRISON Eleanor

Molecular studies on the diversity of marine *Prochlorococcus* strains and their co-occurring phages

JARDILLIER Ludwig

Effects of viruses and predators on prokaryotic community composition

JENKINS Caroline

Characterization of cyanophages associated with the filamentous cyanobacterium *Nodularia*

KERNEGGER Lisa

Interaction between bacteria, viruses and suspended particles: an experimental approach

LUEF Birgit

Imaging aquatic viruses on suspended particles: a non-solvable problem?

MAGAGNINI Mirko

Viral production and decay in aquatic sediments

MOESENEDER Markus

Virioplankton in a river-floodplain system: layout of a FWF-research project

PERSONNIC Sébastien

Dynamics and interactions of microbial communities in Lake Geneva with an emphasis on bacteriophages

SIEM-JORGENSEN Maria

The seasonal dynamic of benthic viruses and bacteria

TIJDENS Marjolijn

Viral ecology of a shallow, eutrophic lake

VAQUE Dolors

Prokaryotic mortality by protists and viruses: A case study in Antarctic waters

ZEMB Oliver

A protocol to isolate the virus of a specific strain

Abstracts

Bettarel Y, Chen F, Wommack E, Coats W

Nanoflagellate 'virivory' in two lakes of the French Massif Central

Seasonal and depth-related variability in the grazing activity of heterotrophic nanoflagellates (HNF) on viruses was examined in the oligo-mesotrophic Lake Pavin and in the eutrophic Lake Aydat, between May and November 2000. Ingestion rates (IR) were determined using 50 nm diameter fluorescent microspheres, as virus analogues. In both lakes, highest grazing activities on virus-size particles were recorded in the metalimnion, at the beginning and the end of the thermal stratification period. Estimated IRs in Lake Pavin (mean = 0.4 viruses HNF⁻¹ h⁻¹, CV = 38.0%) and in Lake Aydat (mean = 0.3 viruses HNF⁻¹ h⁻¹, CV = 35.6%) were not significantly different, in contrast to clearance rates (CR) that were significantly higher in the oligomesotrophic (2.3×10^{-2} nL HNF⁻¹h⁻¹) than in the eutrophic lake (0.7×10^{-2} nL HNF⁻¹h⁻¹). CRs for viruses were correlated with CRs for bacteria in Lake Aydat but not in Lake Pavin, suggesting a greater occurrence of virus-feeder specialists within the HNF assemblages in rather oligotrophic conditions. We estimated that 4.1 and 0.8% of viral production were grazed by HNF in Lake Pavin and Lake Aydat, respectively. Finally,

although viruses seem to represent a minor food source for HNF (i.e. compared to bacteria), they still may not be inconsequential for HNF diet, especially in low productivity ecosystems.

Bouvier T, del Giorgio PA

Absence of virus releases dominance of rare marine prokaryotic groups

Aquatic bacterial communities are composed of phylogenetic groups whose relative importance often changes dramatically among ecosystems and along environmental gradients. Although we can describe these changes in bacterioplankton composition with increasing detail using nucleic-acid based techniques, our understanding of the mechanisms underlying these fundamental shifts in community structure is still very limited. Viral infection is thought to play an important role in determining community composition, although the strength of this effect and the mechanisms underlying this regulation are still unclear. Here we report that incubations of marine bacterioplankton in the absence of viruses produced an unexpected and dramatic increase in the relative abundance of phylogenetic groups that are often undetectable in the ambient assemblages, such as Beta-proteobacteria and the Actinobacteria. Our results suggest that susceptibility rather than numerical dominance, may determine the impact of viruses on marine bacterioplankton composition, and that the “phage kills winner” hypothesis may not always be the dominant form of viral/bacterial interaction in marine microbial communities.

Bratbak G

An overview of algal viruses

A number of studies indicate that both lytic and latent viruses are important in the life cycles of many, if not all, algae. The lines of evidence include a large number of occasional observations, electron microscopy, molecular and environmental studies, and deliberate search for and isolation of new viruses. Despite the accumulating evidence of the ecological importance of algal viruses, only about 15 viruses that infect microalgae have been cultured and even fewer, subjected to minimal characterization. Most of these viruses are large polyhedral (120 - 200 nm in diameter) particles containing huge dsDNA genomes, up to ~560 kb. Recently, however, several viruses have been described that do not fit this general description, e.g. small (30-80nm in diameter) dsRNA, ssRNA and ssDNA viruses. The ecological significance of algal viruses with respect to termination of phytoplankton blooms, population dynamics, diversity and nutrient flow has been investigated in field and experimental studies or inferred from their general properties. The abundance of some bloom forming species, e.g. *Emiliana huxleyi* and *Heterosigma akashiwo*, seem to be controlled by viral infections as termination of blooms frequently are observed to be associated with massive virus production. Viruses may also prevent some species from blooming altogether and it is tempting to suggest that the difference between blooming and non-blooming species, at least in some cases, may be explained by the virulence of their respective viruses. Some viruses are recognized to have a significant impact on population dynamics, nutrient flow and food web structure, but we have a long way to go before we are able to make a general assessment of the ecological significance of algal viruses as such. The short term ecological effects of virus may be important enough, but their ability to transfer genetic information between host-cells may, at least in an evolutionary perspective, be far more important.

Clokier MRJ, Mann N

Photosynthesis genes in marine cyanophages: acquisition and physiological significance

Cyanobacteria from the genus *Synechococcus* make a significant contribution to the primary productivity of the oceans. We recently sequenced the genome of the phage S-PM2 which infects a number of different strains of marine *Synechococcus*. A genomic analysis showed the presence of photosynthesis genes in the genome *psbA* and *psbB* which code for the proteins D1 and D2. These genes appear to have been acquired horizontally from their cyanobacterial hosts. Under normal growth conditions D1 is one of the most rapidly turned over proteins in *Synechococcus* and this turnover is essential for photosynthetic activity. In other phage-host interactions host genes are switched off during phage infection. Our hypothesis is that S-PM2 encodes an alternative transcript of *psbA* which maintains photosynthesis during infection. We tested this hypothesis by using real-time PCR experiments that measure the specific expression of host and phage *psbA* throughout a phage infection cycle. This is coupled with an analysis of the photosynthetic capacity (relative photoinhibition) of infected *Synechococcus* at different time points during infection by PAM fluorimetry. We show that photosynthesis is indeed maintained during much of the infection cycle, and that at later time points, phage encoded *psbA* genes are highly expressed whereas host expression is low.

Dorigo U, Jacquet S, Humbert JF

Cyanophage isolation and diversity of the largest natural French lake

The genetic diversity of the natural freshwater community of cyanophages and its variations over time have been investigated for the first time in the surface waters of the largest natural French lake. Water was collected at the reference station of the lake located in the middle and deepest part of the lake, at a depth of approximately 5 m, once a month from September 2002 to January 2003. 20-L of GF/F prefiltered lake water was then concentrated at least 100 fold by tangential flow filtration using a mini-ultrasette with a 100 kDa cut-off membrane. The diversity of the cyanophage community was assessed by random screening of clone libraries established by using primers targeting the g20 gene and by denaturing gradient gel electrophoresis (DGGE). Nucleotide sequence analysis revealed 35 distinct cyanomyovirus g20 genotypes among the 47 sequences analyzed. Phylogenetic analyses showed that these sequences fell into 7 genetically distinct Operational Taxonomic Units (OTUs). The distances between these OTUs were comparable to those reported between marine clusters. Moreover, some of these freshwater cyanophage sequences were genetically more closely related to marine cyanophage sequences than to other freshwater sequences. Both approaches on the g20 gene (sequencing and DGGE analysis) showed that there was a clear seasonal pattern of variation in the composition of the cyanophage community that could reflect changes in its biological, chemical and/or physical environment.

Filippini M, Buesing N, Bettarel Y, Sime-Ngando T, Gessner MO

High abundance but low impact of viruses on benthic bacteria

The discovery of a large and diverse viral community in lakes and oceans has profoundly reshaped ideas about aquatic carbon and nutrient fluxes, food-web dynamics and maintenance of biodiversity. To explore whether these ideas are also applicable to benthic environments, we used epifluorescence microscopy to examine the abundance of free viruses in four microhabitats of a littoral freshwater marsh over an annual cycle. In addition, we used transmission electron microscopy and data on bacterial production to estimate viral infection rates (FVIC, FIC), virus-induced mortality (VIM), burst size and viral production at four occasions corresponding to the

meteorological middle of winter, spring, summer and autumn. The abundance of bacteria was high in all habitats, with virus-to-bacteria ratios (VBR) invariably >1 except in biofilms on plant surfaces. Seasonal patterns in viral abundance and VBR differed among habitats. In the marsh water column, infection rates (FVIC, FIC) and VIM were well in the range of values known from pelagic environments. Values varied among seasons, VIM from as much as 66% in the autumn to a minimum of 10% in winter. Infection of bacteria in benthic habitats, in contrast, was nearly undetectable. Only 4 out of a total of 14,384 bacterial cells inspected contained mature phages. VIM and viral production were thus negligible. This low incidence of benthic virus-bacteria interactions would suggest that the role of viruses in controlling microbial diversity, food-web dynamics and biogeochemical cycles in benthic environments is significantly smaller than would be predicted based on data from the pelagic zones of lake and oceans and from the abundances of free benthic viruses.

Fuhrman J, Hewson I, Schwalbach M

Interactions between marine bacterial and viral community composition

For several years, microbial ecologists have considered how viruses may affect microbial community composition, and the dominant idea has been the “kill the winner” hypothesis, whereby viruses are thought to help promote diversity by tending to infect and kill the most common organisms (winners of competition for resources). We have addressed this question by manipulation of field samples of plankton and sediment. Samples have come from the open sea as well as coastal waters. Experiments have included those where viruses are (1) enhanced by ultrafiltration and then added to the sample, or (2) reduced by filtration and dilution. We use sensitive molecular biological assays, usually ARISA community fingerprints, to observe community composition, and have also made measurements of specific rare groups, such as nitrogen fixers. One difficulty in such experiments is that they take so much time to observe expected effects (a few generations, typically several days) that there are often substantial community shifts from containment (“bottle”) effects in the controls, and this may mask many virus effects. In practice, we have usually found some virus effects that are consistently different from control treatments, but they are often subtle. In retrospect, it is not so surprising to see a lack of dramatic effects in experiments where we typically just make incremental changes to the virus community, and have only a few days to observe the effects. Meta-analysis of several such experiments confirms that, as expected from the “kill the winner” hypothesis, viruses have a tendency to reduce the abundance of the most common taxa and increase the relative proportions of minor taxa. But in a given single experiment, the results are usually not so clear cut. Looking at the other direction of interactions, it is reasonable to ask what controls the community composition of the viruses, and is there feedback to the bacteria again? Of course we expect viruses to in some way follow their hosts, and it may be reasonable to expect some sort of predator-prey-like cycles of shifting community composition. We have looked for such cycles on scales of days and months in a marine plankton time series study, but so far there is no strong evidence of them.

Jacquet S, Dorigo U, Delmas B, Six D, Leberre B

A new virus infecting freshwater PE-rich picocyanobacteria of Lakes Annecy and Bourget (France)

After isolation and characterization of two phycoerythrin-rich species of *Synechococcus* from Lake Annecy and Bourget (France) using flow cytometry, HPLC pigment analysis and fluorescence properties, an attempt was made to isolate one or several viruses infecting these strains. A virus was

isolated and this talk will deal with its characterization (using Flow cytometry, transmission electron microscopy, protein sequence analysis) and some basic properties (burst size, host range, etc).

Jost G, Witzel, K-P

Autochthonous phage-host systems – do we need them?

Usually, there are about 10^6 bacteria per mL and in many cases a tenfold concentration of phages in aquatic ecosystems. Compared to this there are only a few reports about direct phage detection using autochthonous bacterial strains. Moreover, mostly the specific phage concentrations ranged from 1 to 10 plaque-forming units per mL. A main limitation for phage-host systems is probably the availability of host strains, recognizing the low efficiency of culturing autochthonous bacteria. Consequently, this leads to the question if the currently accessible bacterial isolates may be a sufficient basis?

Different culture-independent techniques and experimental approaches are now available to answer questions at the community level. For example, the mean burst size under different nutrient conditions could be estimated by counting VLP in individual bacterial cells using transmission electron microscopy. Or the existence of mean threshold concentrations of phages to infect their host cells could be detected by dilution or phage enrichment experiments. Previously, such questions were typically studied by using phage-host systems.

But how can we observe a specific phage-host interaction under environmental conditions? How many different phages are able to infect a certain host bacteria? What about the evolution of different phage-host systems under natural conditions?

A short review about the published studies using autochthonous phage-host systems is presented and some examples may illustrate the potential and limitations of this approach.

And what about combining specific phage-host systems with more general community approaches in the future?

Laybourn-Parry J, Madan N, Marshall C

Annual viral dynamics in contrasting Antarctic lakes

Viruses are a common feature of the microbial plankton of Antarctic lakes, which are systems dominated by microbial plankton with few or no zooplankton. The annual patterns of viral abundance, levels of lysogeny and production were investigated in three marine derived lakes in the Vestfold Hills (68°S). These were meromictic Ace Lake (18-20‰), Pendant Lake 18-19 ‰ and Highway Lake (4-5 ‰). Virus like particles (VLP) showed no clear seasonal pattern, with high concentrations occurring in both winter and summer, though numbers were consistently high over the winter. Pendant Lake supported the highest bacterial biomass resulting in higher bacteria: virus ratios in Ace Lake (18.6 – 126.7) and Highway Lake (30.6 – 80.0). These values are at the upper end of the spectrum of VBRs reported for marine and freshwater systems. Lysogenic bacteriophage showed a seasonal pattern with highest levels in winter (maximum 72% in Ace Lake and 32% in Pendant Lake), falling to non-detectable in summer. Corresponding VLP concentrations in the lakes suggest that the lytic cycle was occurring in winter, but viral decay rates were probably low in cold, dark waters. We found a strong negative correlation between VLP and photosynthetically active radiation (PAR) and temperature, suggesting that as PAR and temperature increased, the ice-covers thinned and disappeared causing an increase in viral decay. Viral production during mid summer ranged between 0.202 to $0.823 \times 10^6 \text{ ml}^{-1} \text{ h}^{-1}$ in Pendant Lake and $0.176 - 0.424 \times 10^6 \text{ mL}^{-1} \text{ h}^{-1}$ in Ace Lake. Bacterial concentrations and production showed only limited correlation with

VLP concentrations. Overall VLP dynamics in saline Antarctic lakes appear to differ from other lakes and marine systems in a number of ways: in having high VLP numbers in winter and high VBR.

Leitet C, Riemann L, Hagström Å

Prevalence of plasmids and lysogenic viruses in Baltic Sea bacterioplankton

The pelagic ocean is an ever-changing environment in which the ability to adapt is a prerequisite for survival. Acquisition of new phenotypic traits may be one means of adaptation. Ecological and whole-genome studies suggest that plasmids and other extrachromosomal elements such as viruses are important for the movement of genes, and thereby traits, between bacterial populations. Current knowledge on the prevalence and diversity of plasmids and lysogenic viruses in marine bacterioplankton is, however, limited. Using the Kieser method and Mitomycin C induction, 84 bacterial strains isolated from the central Baltic proper in spring, summer and autumn were screened for the presence of plasmids and lysogenic viruses, respectively. We found that 14% of the isolates contained plasmids and 34% contained lysogenic viruses. No significant seasonal variation was seen, although fewer isolates tended to contain plasmids/viruses during summer. Plasmids and viruses were found with genome sizes in the range of 1.8 – 6.5 kb and 9 – 87 kb, respectively, and seemed to be randomly distributed among the bacterial taxa examined. Viruses with identical genome sizes were found among most bacterial taxa examined and 2 - 3 different viruses were commonly observed within one host. The widespread distribution of viruses and plasmids among Baltic isolates emphasize the potential importance of these extrachromosomal elements for bacterial adaptation. Future work will include characterizations of the isolated plasmids/viruses and comparisons to host phylogeny.

Mann N

Impact of phage infection on photosynthesis in the marine picoplankton

The prokaryotic component of the marine picoplankton is dominated by unicellular cyanobacteria belonging to the genera *Synechococcus* and *Prochlorococcus* and makes a significant contribution to primary production, particularly in the oligotrophic regions of the world's oceans. Phages infecting these organisms were first characterized in 1993 and are now thought to affect the genetic diversity and abundance of their hosts and to drive a significant fraction of fixed carbon into the microbial loop. Two of the key factors determining the nature of phage host interactions are host population density and host quality and a theoretical consideration of these factors can yield interesting predictions regarding the pressures that have shaped the evolutionary trajectory of these phages and consequently determine the current nature of their interactions with hosts. Evidence from a very different approach, genomics, also provides novel insights into phage-host interactions and extends current ideas and models based solely on the study of phages infecting heterotrophic bacteria. In particular, phages infecting *Synechococcus* and *Prochlorococcus* appear to be capable of subverting and modifying the host's photosynthetic physiology in a variety of ways.

Middelboe M, Glud RN

Virus dynamics in marine sediments: implications for benthic carbon cycling

The recognition of viral influence on pelagic processes has in the past few years led to an increased focus on the role of viruses in benthic environments. Such studies have verified that viruses are abundant, diverse and dynamic players also in benthic communities. There are 10^7 - 10^9 viruses cm^{-3} surface sediment, which is 10-100 times higher than densities usually found in the overlying water column of the same area, and viruses therefore potentially influence bacterial mortality and biogeochemical processes in aquatic sediments. The presentation will include data on benthic viral production and distribution and discuss the impact of viruses on bacterial mortality and benthic carbon cycling in coastal and deep sea environments. Virus net production ranged from 2 to 7×10^6 VLP $\text{cm}^{-3} \text{h}^{-1}$ in anaerobic sediment incubations (> 2 cm below sediment surface) and was closely coupled with bacterial respiration. At a coastal site, dynamic viral communities were detected even below 150 cm depth in the sediment, in association with the sulfate-methane transition zone with high rates of bacterial sulfate reduction. Doubling times of benthic viral communities of 0.5 to 6 days, suggested that viral lysis constitute an important loss factor for benthic bacteria (up to 40 % of bacterial production). However, estimated inputs of viral lysates could in general only explained <10 % of total bacterial metabolism in anaerobic incubations at various sites, thus suggesting that viral activity had only minor impact on carbon cycling in marine benthos.

Millard A, Mann NH

An investigation into cyanophages in the Red Sea

Cyanophages infecting marine *Synechococcus* have been studied for just over a decade. They are known to be abundant throughout the world's oceans. Numerous studies have examined their diversity using cyanophage-specific PCR probes. This study aimed to further investigate the abundance of cyanophages over an annual cycle on a temporal and spatial scale in the Gulf of Aqaba in the Red Sea. Cyanophages were enumerated from surface waters down to a depth of 150 m. The results showed that cyanophage abundance changes both temporally and spatially throughout the annual cycle. With a maximum in cyanophage numbers in the months of July, August and September at a depth of 30m. As well as cyanophage enumeration a number of cyanophages were isolated for further characterisation. A total of 87 cyanophages were isolated and purified. These cyanophages were further characterised in terms of genome size, morphology, host range and the presence of the photosynthetic gene *psbA*. This was achieved by the use of techniques including PFGE, TEM and PCR. These cyanophages have genomes in the range of 150-210 kb. The majority of cyanophages were capable of infecting multiple strains of *Synechococcus* while others could only infect a single strain. It was also found that the photosynthetic gene *psbA* was widespread in the cyanophages isolated.

Mühling M, Fuller NJ, Millard A, Somerfield PJ, Marie D, Wilson WH, Scanlan DJ, Post AF, Joint AF, Mann NH

Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton

Unicellular cyanobacteria of the genus *Synechococcus* are a major component of the picophytoplankton and make a substantial contribution to primary productivity in the oceans. This presentation provides evidence that supports the hypothesis that virus infection can play an important role in determining the success of different *Synechococcus* genotypes and hence of seasonal succession. In a study of the oligotrophic Gulf of Aqaba, Red Sea, we showed a succession of *Synechococcus* genotypes over an annual cycle. There were large changes in the genetic diversity of *Synechococcus*, as determined by RFLP analysis of a 403 bp *rpoC1* gene fragment, which was

reduced to one dominant genotype in July. The abundance of co-occurring cyanophage capable of infecting marine *Synechococcus* was determined by plaque assays and their genetic diversity was determined by DGGE analysis of a 118 bp *g20* gene fragment. The results indicate that both abundance and genetic diversity of cyanophage co-varied with that of *Synechococcus*. Multivariate statistical analyses show a significant relationship between cyanophage assemblage structure and that of *Synechococcus*. These observations are consistent with cyanophage infection being a major controlling factor in picophytoplankton succession.

Parry J, Thurman J, Pickup Z, Dillon A, Drinkall J

Picocyanobacteria – the sweetcorn of the microbial world?

Protozoa have long been considered the major predators of bacteria, playing an important role in the transfer of bacterial carbon along the aquatic foodweb. There is no doubt that many bacterial strains are adequately digested by these predators, however, it has often been assumed that the level of prey ingestion is proportional to the level of prey digestion. Our work has shown this not to be true, particularly for the picocyanobacteria. Although many protozoa can ingest vast numbers of these bacterial cells many are unable to digest them and the picocyanobacterial cells are excreted in a viable form. Thus, many protozoa do not appear to effectively transfer picocyanobacterial carbon along the food-web, they just re-package it. Attention has thus turned to the potential role of viruses in controlling the mortality of this bacterial group and freshwater cyanophage are currently being isolated and characterised.

Peduzzi P

Ecology of viruses in river systems

Lotic systems are well known as critical links in global organic matter cycles, and in particular rivers and their riparian zones are hot spots of both carbon cycling and biodiversity. Natural floodplains are known to increase the spatial and temporal heterogeneity of a river system, can serve as sinks, sources or transformers of organic material, inorganic nutrients and biota, thus providing highest species richness and habitat diversity. However, in many populated regions these environments are, due to river regulation, among the world's most degraded ecosystems. Although viruses are well recognized for playing a significant role in marine and lake waters, it is a surprising fact that most aspects of viral ecology are largely ignored in research on the functioning of river systems.

As an example, studies of a lotic-lentic inland water network in tropical Sri Lanka revealed that most virus parameters, such as abundance or frequency of visibly infected cells, were positively linked to prokaryotic abundance and production but also to organic nitrogen or some phosphorus species. We calculated that between 13–46% of the prokaryotic standing stock was subjected to virus-mediated mortality. As another example, ongoing studies in the river Danube attempt to understand the relevance of hydrological connectivity within a river-floodplain system. There are indications that the abundance of viruses is highest under hydrological isolated conditions, whereas lowest abundance can be detected in the main channel. Furthermore, we have found that a very variable but occasionally significant proportion of the virus population (between 0.2–44%) is associated with seston material, and the quality of suspended particles seems to be of relevance. A first screening of the virus community structure in the Danube river-floodplain system, using pulsed field gel electrophoresis (PFGE), revealed that various habitats (main channel, isolated pools, dynamically connected sections) are apparently inhabited by distinct viral communities showing characteristic dynamics of viral diversity. In a recently launched program we aim to further

understand the impact of hydrology on viral diversity and investigate various effects of viral lysis (“viral shunt”) on the carbon cycling in this human-impacted river-floodplain system.

Sandaa, R-A, Larsen A, Thingstad F

Viral diversity in the marine environment. Effects on the species richness and activity of planktonic organisms

Virioplankton are the most commonly found biological agents in the marine environment. The diversity and function of these plankton organisms in the aquatic food web remains a relatively unexplored field of study. In this talk we will focus on the ecological importance of viruses in the marine ecosystem demonstrated by different studies carried out on seasonal samples from coastal water and samples from different mesocosms. In these studies we employed flow cytometry (FCM) to monitor the succession and numbers of dominating algal, bacterial and viral populations, while the relationship and changes in the bacterial and viral community structure were investigated by denaturant gradient gel electrophoresis (DGGE) and pulse field gel electrophoresis (PFGE). Some dominating PFGE and DGGE bands were further characterised by PCR and sequencing. Our study demonstrates how changes in the viral community correlates with changes in the bacterial and phytoplankton community structure, and the experimental results will be discussed in light of theories for biodiversity control.

Šimek K, Hornák K, Jezbera J, Nedoma J, Weinbauer M, Dolan JR, Gasol JP

Comparing the effects of resource enrichment and grazing on a bacterial community and viral dynamics in a freshwater reservoir

We studied the effects of different top-down factors (bacterial consumers and viruses) and nutrient availability on bacterial dynamics and bacterial community composition (BCC) in a series of experiments conducted in a freshwater reservoir. We investigated the effects of protistan grazing and viral lysis on BCC using a size-fractionation approach. Dialysis bags containing water with only bacteria and viruses (<0.8 μm), or bacteria, viruses and heterotrophic nanoflagellates (HNF, <5 μm), or whole water, were incubated in situ. Top-down manipulations (size-fractionation) were also combined with bottom-up manipulations, i.e., transplantation of samples to the reservoir inflow area, which markedly differed in phosphorus (P) availability. Significant genotypical and morphological shifts in BCC occurred with high rates of protistan bacterivory as well as with the sample transplantation into P-richer areas of the reservoir as indicated by means of FISH and DGGE. Overall, our data from the manipulation experiments indicate that viral abundance and infection rates increased in parallel with HNF bacterivory and P-availability. Besides using different FISH probes, we found that 10-50% of total bacteria were members of a phylogenetically small group of the *Rhodospirillum rubrum* sp. BAL47 cluster (β -proteobacteria, targeted with the probe R-BT065). These rod-shaped cells of very uniform size and morphology were vulnerable to predation, showed the highest growth rates of all studied bacterial groups, and responded markedly to the different experimental manipulations. Moreover, the relative abundance of the members of the R-BT065 cluster was tightly correlated with bacterial bulk parameters and proportions of high nucleic acid content bacteria, thus indicating the principal role of this group in the overall bacterioplankton dynamics.

Sime-Ngando T, Pradeep Ram AS, Boucher D, Debroas D, Romagoux JC

Seasonal phage bacteriolysis equals protistan bacterivory in a freshwater reservoir: coupling with the physical environment

Phage abundance and infection of bacterioplankton were studied from March to November 2003 in the Sep Reservoir (Massif Central, France), together with temperature, chlorophyll, bacteria (abundance and production) and heterotrophic nanoflagellates (abundance and potential bacterivory). Virus abundance (VA) ranged from 0.6 to 13×10^{10} viruses l^{-1} , exceeding bacterial abundance (BA) by about six fold on average. In terms of carbon, viruses corresponded to up to 25% of bacterial biomass. A multiple regression model indicated that BA was the best predictor for VA ($R^2 = 0.75$). The frequency of infected bacteria (estimated from the percentage of visibly infected cells) varied from 1 to 32% and was best explained by a combination of temperature ($R^2 = 0.20$) and bacterial production ($R^2 = 0.25$). Viruses and flagellates contributed about equally to bacterial mortality. Both factors destroyed 55% of bacterial production, with a shift from phage bacteriolysis in early spring to protistan bacterivory in late summer. The vertical differences in most of the biological variables were not significant, contrasting with the seasonal differences (i.e. spring vs summer-autumn). All biological variables under study were indeed significantly coupled to temperature. We regarded this to be the consequence of the enhanced discharge of the reservoir in 2003 (compared to previous years). This substantially weakened the stability and the thermal inertia of the water column, thereby establishing temperature as a stronger forcing factor in setting the conditions for optimal metabolic activity of microbial communities.

Velimirov B, Hiroshi CX, Kazuhiro K, Fischer UR

New transducing particle types or are we still missing the essential?

Virus mediated transfer of genetic elements among bacteria in nature is a major research topic in viral ecology. Along with conjugation and transformation, transduction is a well-known mechanism resulting in horizontal gene transfer in procaryotic organisms. Both virulent and temperate phages have the capability to induce general transduction.

In a number of pilot studies we have reported the presence of virus-like-particles (VLPs) that are released spontaneously from bacterial cells of strains from *Agrobacterium*, *Flavobacterium* sp 16-04, and *Aquificales* and which displayed the following features. All observed particles could transfer genes to the recipient *E. coli* AB 1157, they remained infectious after UV treatment and produced only low lethality among the recipient cells. Also, a first analysis revealed that nucleic acid species encapsulated in such particles were DNA.

Within the frame of a study on intergeneric phage-mediated gene transfer between marine bacteria and enteric bacteria, and using the auxotrophic mutant of *Escherichia coli* (AB1157 : F; thr-1leuB6thi-1lacY1falK2ara-14xyl-5mtl-1proA2his-4argE3rpsL31tsx-33supE44) we used virus like particles (VLPs) from an oligotrophic marine environment (Mediterranean Sea, West coast of Corsica), obtained high gene transfer frequencies (10^{-2} to 10^{-6} per viral particle). It was assumed that some of the VLPs obtained via ultrafiltration from surface seawater have the capability to induce general transduction. Obtained transductants could produce new VLPs which were again able to induce transduction. This observation and a number of characteristics concerning the obtained particles were not compatible with the classical concept of general transduction. According to these findings we have to assume the existence of transducing particles which are not related to the viral concept.

Weinbauer M, Rassoulzadegan F

Viruses as driving forces of microbial diversity and ecosystem functioning

Viruses are ca. 10-100 times more abundant in aquatic systems than their most significant host, the prokaryotes, and they cause prokaryotic mortality that is on average as significant that due to grazing. A comparatively large data set has accumulated over the last 15 years on viral control of bacteria. Typically, viruses cause an increase in bacterial respiration and growth efficiency, whereas the effects on bacterial biomass and production are variable. A longer retention time of nutrients in the euphotic zone should be the consequence of this viral activity. There is also indication that the growth of cyanobacteria is dependant on virus-mediated recycling of heterotrophic prokaryotic production. Although there are numerous mechanisms, how phage infection can influence prokaryotic diversity, only few hard data are available. Overall, in situ and experimental data modestly support the 'killing the winner' hypothesis, i. e. a reciprocal mechanisms, in which viruses control the most dominant phylotypes and viral production is controlled by the activity of dominant types. There are almost no studies on the effect of viruses on the link between prokaryotic diversity and ecosystem functioning. We provide evidence for a complex interplay between bottom-up (nutrient availability) and top-down factors (viral lysis and disturbance by turbulence) on prokaryotic biomass, production and richness.

Winter C, Smit A, Herndl GJ, Weinbauer MG

Influence of planktonic bacteriophages on prokaryotic community composition

We determined the influence of viroplankton on prokaryotic communities in experiments and the co-variation of prokaryotic community richness with prokaryotic production and viral abundance *in situ*. Batch culture experiments were performed during cruises in the tropical Atlantic Ocean and the North Sea. Prokaryotic cells equivalent to 10-100% of the *in situ* abundance were inoculated into virus-free seawater and viruses equivalent to 35-360% of the *in situ* abundance were added. Batch cultures with microwave-inactivated viruses and without viruses served as controls. Archaeal and bacterial richness was determined as the number of peaks detected by terminal restriction fragment length polymorphism (T-RFLP) analysis of PCR amplified 16S rRNA gene fragments. The effects of virus amendment were detected at the level of individual operational taxonomic units (OTUs) of both groups, *Archaea* and *Bacteria*. One group of OTUs was detected in the control treatments but was absent in the virus treatments. This negative response of OTUs to virus amendment is probably caused by viral lysis. Additionally, we found OTUs not responding to the amendments and several OTUs exhibited a variable response to the addition of inactive or active viruses. *In situ* studies were conducted during 6 cruises in the North Sea. Bacterial richness decreased with viral abundance and total and cell-specific prokaryotic production in the free-living and the total community. Stepwise multiple regression analysis revealed that temperature also had an influence on bacterial richness. Archaeal richness was not related to any other parameter. The data suggest that high prokaryotic productivity was sustained by a relatively small number of highly active bacterial populations that also maintained high viral abundance.

Zingone A, Biffali E, Borra M, Forlani G, Natale F, Sarno D

Do viruses control phytoplankton blooms? The case of *Micromonas pusilla*

Over the past decades an increasing number of eukaryotic microalgae have been shown to be attacked by viruses. In some cases, a high number of viruses and infected host cells have been detected during the final phases of blooms, which has suggested that viruses may be responsible for the decline of microalgal populations at sea. In the case of *Micromonas pusilla*, viruses may have

different morphological, molecular and infectivity features, whereas host populations show different susceptibility to infections by the same viral strains. The presence of resistant host strains, either natural or by infections, indicate that viruses are unlikely to be responsible for bloom termination. However, they can significantly contribute to the genetic make-up of natural host populations by abating selected strains. A challenge for future research is the assessment of the cryptic diversity of viruses and hosts which underlies their complex relationships. In addition, a methodology is needed to estimate the actual rate of algal deaths due to viral infection in the natural environment.

Agis M, Luef B, Peduzzi P

Variability of viroplankton diversity in a river floodplain system

Floodplains are extremely diverse ecosystems with habitats, which vary strongly with respect to the influence of the river such as caused by flooding events and water exchange (connectivity). No studies have been performed on the diversity of viruses (mainly infecting bacteria) in rivers or floodplains. Thus, we investigated the diversity of viroplankton in following three habitats: The river Danube close to Vienna (Austria), a floodplain pool characterized by high connectivity (HC) with the river and a low connectivity (LC) habitat subject to river water only during flooding events. Samples were collected biweekly from the mid of March to begin of August 2003 and viral diversity was assessed by pulsed field gel electrophoresis (PFGE) by quantifying the banding pattern and band intensity of viral genome size groups. Viral diversity in the river usually varied moderately between consecutive sampling times and the number of bands was rather low (up to 10). In the LC habitat, band numbers were higher compared to the river and different size classes were observed. Moreover, banding patterns remained relatively stable over 1-2 months and then changed rapidly to enter another phase of stability. These changes seem to be related to changing ecological conditions during spring. In the HC environment, the number of bands was highest (up to 17 bands) as can be expected from the frequent mixing of the indigenous viroplankton community with the river community in the beginning of the sampling period. Variability of diversity was even higher than in LC pool. Overall, the various habitats were inhabited by specific viral communities and showed a characteristic dynamic of viral diversity. These data will be compared with parameters measured simultaneously such as temperature, nutrient load, bacterial production and diversity. The study confirms that PFGE is a suitable method for assessing viral diversity and studying its dynamics in floodplain habitats.

Bettarel Y, Chen F, Wommack E, Coats W

Isolation and characterisation of a small nuclear inclusion virus infecting the diatom *Chaetoceros* c.f. *gracilis*

A novel virus (*Chaetoceros* nuclear inclusion virus: ChaNIV) causing lysis of a culture of the diatom *Chaetoceros* c.f. *gracilis* was isolated from the Chesapeake Bay, USA, in April 2003. Transmission electron microscopy of ultrathin sections of infected *Chaetoceros* revealed that ChaNIV proliferates within the nucleus and forms paracrystalline arrays corresponding to the alignment of icosahedral viral particles of about 25 nm diameter. ChaNIV shows some strong similarities with *Heterosigma akashiwo* nuclear inclusion virus (HaNIV) (cf. Lawrence *et al.* 2001). The latent period of ChaNIV is less than 12 hours, and we estimated that approximately 10^4 viral particles are released during host cell lysis. The most widespread occurrence of *Chaetoceros* viruses in Chesapeake Bay was recorded in April 2003, roughly one month after the winter-spring *Chaetoceros* bloom. However, results indicate that ChaNIV remain infectious no longer than a

month after the disappearance of their host. Thus, our results reinforce the idea that viruses other than those belonging to the family of Phycodnaviridae are also significant in virus-mediated mortality of phytoplankton. Overall, the present article provides additional information on algal viral ecology by showing direct evidences of the vulnerability of diatoms to viral attack.

Carlsson LM, Glud RN & Middelboe M

Spatial variations in viral production rates and microbial activity in surface sediments along a depth transect in the Øresund, Denmark

Production rates of viruses and bacteria were measured in relation to aerobic microbial activity in homogenized and slurried oxic surface sediment from Øresund, Denmark (56°05'N, 12°30'W). To elucidate spatial and temporal variations in viral and bacterial activity, the rates were measured at four sites covering a range in water depth from 0.5 to 28 m, in March, April and August 2004. Production rates ranged from undetectable to 2.3×10^7 viruses $\text{cm}^{-3} \text{h}^{-1}$ and from 1.6×10^5 to 4.2×10^6 bacteria $\text{cm}^{-3} \text{h}^{-1}$, which was significantly lower than most previous measurements. Viral and bacterial abundances and production rates exhibited significant spatial variation, and were generally highest at the most shallow eutrophic coastal site (station A), and lowest in the sandy sediment (station B). Viral production rates were coupled to microbial activity, indicating that benthic viruses are dynamic components of the benthic microbial community. Simultaneous measurements of bacterial respiration rates in the homogenized sediment, during incubation and in parallel intact sediment cores at *in situ* conditions indicated, that bacterial activity was not stimulated by the manipulations in the production experiments. We suggest, therefore, that the measured viral production rates are representative for *in situ* viral activity in the oxic surface sediment at the investigated sites. Turn over times of the viral community (13-23 h) were relatively low compared to typical values for viroplankton (~24-48 h). Virus induced mortality rates corresponded to 3 – 68 % of total bacterial production. However, the input of viral lysates only corresponded to 1-14 % of the total dissolved inorganic carbon produced, suggesting that benthic viruses in contrast to pelagic viruses play a minor role in benthic carbon cycling.

Filippini M, Middelboe M & Gessner M

Viral diversity in different benthic ecosystems

The viral community diversity in water and sediment samples of freshwater, brackish and marine ecosystems was analysed by the Pulsed Field Gel Electrophoresis (PFGE) method. Viruses were extracted and concentrated first by ultracentrifugation through a glycerol gradient and then, prior running the gel, through a cesium chloride (CsCl) gradient. This last step allowed better band resolution and eliminated some of the smear effect on the agarose gel. Three genome size classes dominated the viral community in the analysed ecosystems: (i) size class around 190 kb, (ii) size class between 50 and 60 kb and (iii) size class between 29 and 38 kb. These three genome size classes were present in all the analysed ecosystems. In addition to these, the freshwater ecosystem contained a fourth size class with smaller genome size (12-19 kb). In general, viral diversity was higher in the water samples than in the sediment and no changes in viral community with sediment depth were observed. The present study shows that the PFGE method can be applied to assess viral community diversity also in limnetic and benthic ecosystems.

Fischer UR, Velimirov B

Impact of abiotic factors on virus adsorption to particles in aquatic sediments

Viral degradation plays an essential role in the balance of the microbial food webs and in the flow of genetic information within the prokaryote compartment in aquatic systems, because it influences e.g. the viral-mediated mortality of bacterio-, phyto- and zooplankton, the free DNA / RNA pool, the concentration of peptides and free and combined amino acids. Knowing the environmental factors that cause the destruction of viral particles and regulate virus concentrations in the water column and sediments is therefore critical to elucidating the role(s) of viruses in aquatic systems. Attachment of viruses to particles is stated to have a major impact on their persistence, leading to stabilization of viral structure by electrostatic forces, and / or trapping of the virus in a surface opening, thus being less vulnerable to antagonistic substances such as extracellular enzymes. The aim of the present investigations was to determine the role of abiotic factors (temperature and pH) on the adsorption of benthic viral particles. Sediment samples were incubated at different temperatures (5 - 25°C) and different pH-values (5 - 9) for 1 hr. The fraction of benthic viruses adsorbed to particles was compared to that of free viruses in the pore water. Our experiments revealed that the adsorption of viruses significantly depended on the pH-value of the sediment, whereas temperature had no obvious impact. The fraction of viruses attached to particles ranged from 36% to 71%, with the following correlation: the higher the pH-value of the sediment, the lower was the fraction of viruses attached to particles. Since micro-environmental conditions at the sites of enzyme activities, (particularly at living cell surfaces) can be totally different from the bulk water, the pH of the cell surface of an algae or bacterium is expected to vary by 2 to 3 units within hours under normal diel cycles. These changes in pH may influence the adsorption of viral particles in the close vicinity of these cells. This, in turn, will have a direct effect on the enzyme-induced degradation of the protein capsid of non-enveloped viruses.

Harrison E

Molecular studies on the diversity of marine *Prochlorococcus* strains and their co-occurring phages

Prochlorococcus contributes significantly to global primary production. It has the ability to survive at irradiance levels spanning 4 orders of magnitude, due to the presence of multiple genetically different populations. Cyanophages have been isolated that infect *Prochlorococcus*. Viral infection of bacteria, conversely, increases overall bacterial diversity. Due to the fast generation times of bacteria, recombination through processes such as virus facilitated transduction, can quickly alter the diversity of genotypes. This may account for the presence of multiple distinct *Prochlorococcus* phenotypes. Little is known about *Prochlorococcus* phages, due to their recent isolation. This project will provide a valuable insight into *Prochlorococcus* phage diversity. The diversity of *Prochlorococcus* communities and co-occurring cyanophages will be studied through amplification of marker genes using PCR combined with high throughput fingerprinting techniques. Abundance of *Prochlorococcus* infecting phage will be estimated through plaque assay.

Jenkins C, Hayes P

Characterization of cyanophages associated with the filamentous cyanobacterium *Nodularia*.

The Baltic Sea supports a large population of buoyant toxic *Nodularia* (cyanobacteria) filaments, which form harmful surface blooms during calm summer months. Recent studies have shown that the horizontal transfer of genetic information within the Baltic Sea population of the cyanobacterium *Nodularia spumigena* has generated a large pool of genotypes (Barker *et al*, 2000). Here, we report on our investigations to isolate and characterise cyanophages from the Baltic Sea,

with a view to exploring the role of cyanophage-host interactions in the transfer of genetic information within the host community, via transduction. This is the first report of cyanophages able to infect *Nodularia*; we have isolated a collection of 16 lytic cyanophages from the brackish Baltic Sea and 6 putatively temperate cyanophages from spontaneous auto-plaquing that has been observed for about 50 % of our clonal *N. spumigena* isolates. Cyanophages examined in the TEM have diverse morphology, and particle sizes range up to three times that of previously characterised cyanophages. Investigations into the cyanophage host range have indicated that the phenomenon of superinfection immunity may be protecting certain cyanobacterial strains from lysis. Though collected during summer months, a high proportion of the *Nodularia* strains appear to be lysogenised, and carry prophages. The genetic diversity of these cyanophages was investigated through the amplification and partial sequencing of a T4 *g20* homologue (encodes the portal vertex protein) and T4 *g23* homologue (encodes the major capsid protein). Phylogenetic analyses of the two structural genes has shown that the Baltic cyanophages are more closely related to each other than to cyanophages isolated from other marine environments, and that isolates that infect phylogenetically related hosts are also closely related. The observation that temperate and lytic *Nodularia*-specific cyanophage are present in the Baltic Sea leads us to the obvious conclusion that both specific and generalised transduction may be important in the movement of genes within the *Nodularia* population. The unique marine cyanophage-host systems presented here provide a model system to study the role of cyanophages in cyanobacterial bloom communities.

Kernegger L, Peduzzi P

Interaction between bacteria, viruses and suspended particles: an experimental approach

The River Danube downstream of Vienna offers one of the last remnants of European river floodplain systems exhibits. High concentrations of suspended inorganic and organic particles are a characteristic feature of many large rivers.

The present study is an experimental approach to highlight how particles different of different quality, that are abundant in the study area, influence bacteria and viruses in the water as well as on particles. Another major goal of this study was to investigate how variable phage abundance influences the BSP (bacterial secondary production) of free-living and particle-associated heterotrophic bacteria.

Laboratory culture experiments with manipulated virus abundances and different particle qualities were conducted. Different parameters like BSP, specific BSP, bacterial and viral abundance in the ambient water and on particles were measured over a time period of 60 hours.

We demonstrate that different “particle qualities” influenced microbial abundance and their activity on particles and in the ambient water. Furthermore, different virus abundances in the ambient water influenced the BSP and specific BSP in the ambient water but had no influence on the BSP and the specific BSP on particles.

Luef B, Neu T, Peduzzi P

Imaging aquatic viruses on suspended particles: a non-solvable problem?

The development of accurate methods for detection and enumeration of viruses is an important issue in aquatic microbial ecology. In particular “viruses attached to aquatic particles” is until now a largely ignored field. The occurrence of phages in sediments and on particles is already documented. However, data on total abundance and the colonization of aggregates by viruses are rare because of methodological difficulties. The goal of our work is to improve methods for precise quantification of viruses associated with aquatic aggregates. We present preliminary results and

discuss problems when using different methods such as sonication, direct counting of viruses on particles by epifluorescence microscopy, transmission electron microscopy and confocal laser scanning microscopy to obtain information on the abundance and spatial distribution of viruses on suspended particles and aggregates.

Magagnini M., Bongiorni R, Armeni M, Noble R, Danovaro R

Viral production and decay along a trophic gradient on North Adriatic Sea

Although the relationships between trophic conditions and viral dynamics have been largely explored in different pelagic environments, there are no attempts of independent estimates of viral production and decay in order to evaluate factors controlling their net balance. In this study, viral production and decay, together with the analysis of life strategies (inducible lysogenic infections) were investigated along a trophic gradient of the North Adriatic basin. The relative importance of nanoflagellate grazing and viral infection on prokaryote mortality was also investigated. Our results indicate that viral production exceeds considerably viral decay, determining a strong increase of the number of viral particles. Such effect increased along with the increasing trophic conditions, reaching higher values in eutrophic waters. Therefore, the assumption of steady state in terms of viral abundance could be wrong if other controlling factors are lacking. Our study experiments also indicated that nanoflagellates exerted a significant control on prokaryote and viral abundance, but only in eutrophic environments. Other factors can, therefore, be involved in the removal of viral particles from the water column in steady-state conditions.

Moeseneder M, Peduzzi P

Virioplankton in a river-floodplain system: layout of a FWF-research project

Work on marine systems indicates that viral lysis can profoundly affect the carbon cycle by converting cells into dissolved organic matter. Such "rapidly cycling carbon" could be of particular relevance in river systems with a usually high proportion of aged and recalcitrant carbon. Surprisingly, the role of biotic interactions between viral and bacterial potamoplankton is poorly studied, and almost nothing is known about the effects of viral lysis on the carbon cycle in riverine systems. Natural floodplains are known to increase the heterogeneity of a river system. Here, the driving force for lateral exchange processes is hydrological connectivity, which profoundly influences biological processes within the system. Almost all large rivers in Europe are greatly affected by pollution, alterations in the catchment, damming and regulation, some large rivers even being among the world's most degraded ecosystems.

The proposed study aims to contribute to our understanding of the structure and function of riverine microbial communities and of carbon cycling in human-impacted river-floodplains by tackling the following issues:

- impact of variable hydrology (e.g. flooding) on virally-induced mortality of prokaryotes
- impact of variable hydrology (e.g. flooding) on the percentage of lysogenized prokaryotes
- consequences of viral lysis of prokaryotes on the microbial carbon cycle in different floodplain subsystems under variable hydrological situations
- influence of varying hydrology on the bacterial and viral community structures.

The planned project attempts to combine important issues of microbial and river ecology and is therefore thought to contribute significantly to the field of aquatic ecology.

Personnic S, Domaizon I, Jacquet S

Dynamics and interactions of microbial communities in Lake Geneva with an emphasis on bacteriophages

It is now well established that viruses play a crucial role in the regulation of aquatic microbial populations. Viruses are the most abundant particles in both marine and freshwater environments (with typically 10^7 - 10^9 part.ml⁻¹), and may exert an important control on microbial population dynamics and diversity. There is a general agreement in saying that viral studies are fundamental to a better comprehension of the microbial food web. Studies taking into account the whole microbial community are required to understand the dynamics and the role of viral populations at the basis of microbial food webs. In this study we investigated the distribution of virioplankton, bacterioplankton, protozoan (ciliates and flagellates) and small phytoplankton (cynobacteria and small eukaryotes) over two years (2003-2004) along the water column in the largest occidental European lake (Lake Geneva) using flow cytometry and epifluorescence microscopy. *In situ* experiments were performed in order to assess flagellate protozoan and virus-induced mortality of heterotrophic bacteria. Our results reveal that flagellates and viruses could be responsible for between 31 and 42% of the total daily mortality of heterotrophic bacteria. In May 2004, viruses could explain up to 10% of the mortality of the bacteria whereas flagellates were responsible for 32% of bacterioplankton removal.

Siem-Jørgensen M, Middelboe M, Glud RN

The seasonal dynamic of benthic viruses and bacteria

Seasonal variations in benthic viral and bacterial abundance were investigated in the oxic surface sediment and in the deeper anaerobic zone (6-10 cm) at a location in Øresund, Denmark (55°58'N, 12°42'W). Viral and bacterial abundance showed a strong seasonal pattern in both oxic and anaerobic sediment, which was closely coupled with the sedimentation and subsequent benthic turnover of the phytoplankton spring bloom. Viral abundance in the oxic and anaerobic zone increased from 1.53 and 1.35 x 10⁸ VLP cm⁻³, respectively in January to a maximum of 6.45 and 4.5 x 10⁸ VLP cm⁻³, respectively in May. During the same period, the bacterial abundance increased by a factor ~2.1. Sedimentation of phytoplankton in spring and autumn was reflected in increased benthic bacterial activity, followed by an increase in benthic viral abundance. This increased microbial activity also resulted in a doubling in the virus:bacteria ratio (VBR) in the surface sediment from 4.1 to 9.8. A similar, but temporal delayed respond in VBR was observed in the anaerobic zone (6-10 cm). The net increase in viral and bacterial abundance in response to the sedimentation event was significantly higher in the oxic than in the anaerobic zone, with accumulation rates of 7.0 x 10⁶ VLP cm⁻³ d⁻¹ and 10.4 x 10⁵ bacteria cm⁻³ d⁻¹ and 3.9 x 10⁶ VLP cm⁻³ d⁻¹ and 5.1 x 10⁵ bacteria cm⁻³ d⁻¹, respectively. The present work is the first to determine *in situ* dynamic of benthic viruses and bacteria and its coupling to microbial activity on a seasonal scale.

Tijdens M, Gons H

Viral ecology of a shallow, eutrophic lake

We describe the seasonal dynamics of virioplankton in a filamentous-cyanobacteria-dominated lake. The virioplankton community in this shallow, eutrophic lake (Lake Loosdrecht, The Netherlands) is especially interesting since earlier research repeatedly showed a dramatic collapse of the cyanobacterial community associated with viral activity during lake water enclosure experiments. Furthermore the presence of virally infected cyanobacteria in Lake Loosdrecht has been observed and it is known that grazing only accounts for part of the

cyanobacterial mortality. These observations suggest that the viral community could play a significant role in shallow, eutrophic lakes. During March till November 2003, samples were taken every two weeks to describe the virioplankton community, its possible hosts and physical parameters. We describe the viral community dynamics in relation to the bacterial, cyanobacterial, algal and flagellate communities and to physical parameters. We discuss the environmental variables controlling viral abundance and the importance of the virioplankton community in Lake Loosdrecht. We also discuss the possible role of flagellates in the decay of the viral community in this lake.

Vaqué D, Guixa-Boixereu N

Prokaryotic mortality by protists and viruses: A case study in Antarctic waters

The distribution abundance of viruses, prokaryotes and protists, and virus decay rates together with prokaryotic heterotrophic production and mortality due to viral infection and protists bacterivory, were determined in Antarctic waters over three months cruise, on Board of the R/V BIO-Hespérides, during the Austral spring - summer (1995-1996). The study area was characterised by strong production gradients, between highly productive zones (parts of the Bransfield and Gerlache Straits, the southern boundary of the Antarctic circumpolar current in the Bellingshausen Sea), and oligotrophic zones (eastern part of the Bellingshausen Sea and parts of the Western Bransfield Basin). We investigated the impact of prokaryotic mortality due to protist bacterivory compared to viral infection in these zones with different productivity. Virus, prokaryotes and heterotrophic nanoflagellate abundance (HNF) were determined by epifluorescence microscopy, previously stained with YO-PRO and DAPI, respectively. Prokaryotic heterotrophic production (PHP) was estimated using the ^3H -leucine incorporation. Viral decay rates were obtained using KCN (potassium cyanamid). Viral mortality of prokaryotes was calculated throughout viral decay rates. Finally, protists prokaryotic predation was determined by the disappearance of fluorescent minicells over 48h incubations. Viruses were abundant in all sampled areas ($1.0 \times 10^9 \text{ l}^{-1}$ - $8.0 \times 10^{10} \text{ l}^{-1}$) and exceeded the prokaryotes ($1.3 \times 10^8 \text{ l}^{-1}$ - $1.3 \times 10^9 \text{ l}^{-1}$) by one order of magnitude on average. Abundance of HNF varied between $0.06 \times 10^6 \text{ l}^{-1}$ and $5.01 \times 10^6 \text{ l}^{-1}$. Prokaryotes exceeded the HNF by three orders of magnitude on average. Abundance of all these variables decreased with depth. Concentration of prokaryotes was correlated with virus ($r = 0.30$, $n = 155$, $p < 0.001$) and HNF ($r = 0.30$, $n = 252$, $p < 0.0001$). PHP and virus decay rates ranged between 0.029 and $4.4 \mu\text{g C l}^{-1} \text{ d}^{-1}$, and 0.006 and 0.260 h^{-1} respectively. When comparing viral lysis and protist bacterivory at the stations where both processes were measured, viruses always accounted for a percentage of the PHP and abundance larger than bacterivory. Thus, viral infection accounted for the total PHP in the two stations located in Bellingshausen Sea and Gerlache Strait (eutrophic) and for half of the PHP in Bransfield Strait (oligotrophic). Protist bacterivory accounted for 38%, 40% and 1% of PHP (d^{-1}), respectively. Despite the uncertainties of our estimation of prokaryotic mortality due to viruses, our study indicate, that in Antarctic waters, viral lysis presented a higher impact on prokaryotes than protists bacterivory, and this was more important in eutrophic areas than in oligotrophic ones.

Zemb O, Lebaron P

A protocol to isolate the virus of a specific strain

We used tangential ultrafiltration and we have done an optimisation from the classical Moebus method based on lysis plaga. We are coupling this classical method with a PEG8000 purification. We have isolated some host-bacterial systems with this method.

Final list of participants

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Statistical information on participants (Age bracket, countries of origin, discipline represented)

Age bracket

20-30	30-40	40-50	50-60
16	12	7	8

Countries of origin

14 countries were represented including US with the invited expert and on colleague from Africa

The 12 European countries were:

- France (10)
- UK (10)
- Austria (6)
- Denmark (4)
- Italy (2)
- Norway (2)
- Sweden (2)
- Czech republic (1)
- The Netherlands (1)
- Germany (1)
- Switzerland (1)
- Spain (1)

Disciplines represented

Biology of organisms

Biology of populations

Biology of communities

Molecular Biology and Ecology

Experimental ecology

Microbial diversity and genomics