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## Linking changes in microbial communities to trophic variations in a tropical mangrove

Agnes Bouchez, Noemie Pascault, Cécile Chardon, Marc Bouvy, Philippe Cecchi, Mélanie Herteman, Luc Lambs, Christophe Leboulanger

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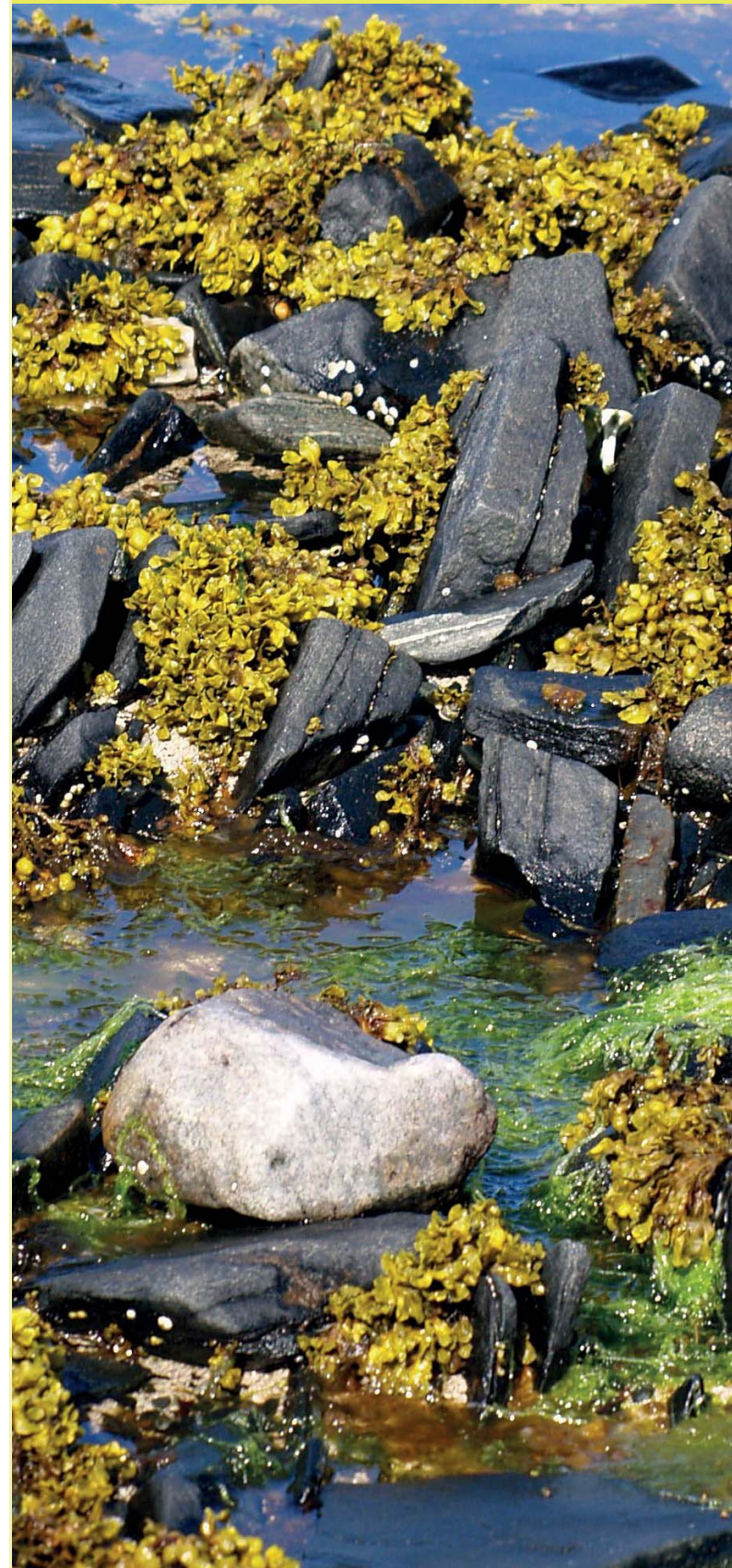
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The 12<sup>th</sup> Symposium on Aquatic Microbial Ecology



# SAME12 2011

from August 28<sup>th</sup>  
to September 2<sup>nd</sup>

12<sup>th</sup> Symposium  
on Aquatic Microbial Ecology

Germany  
Rostock–Warnemünde

12<sup>th</sup> Symposium on Aquatic Microbial Ecology  
August 28 – September 02, 2011



# Abstract Book

Editors: Klaus Jürgens and Matthias Labrenz



[www.io-warnemuende.de/same12](http://www.io-warnemuende.de/same12)

**The 12<sup>th</sup> Symposium on Aquatic Microbial Ecology**

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Symposium on Aquatic Microbial Ecology

Abstract book / SAME12 – The 12<sup>th</sup> Symposium on Aquatic Microbial Ecology,

August 28 – September 02, 2011, Rostock-Warnemünde, Germany

SAME secretariat IOW - Leibniz Institute for Baltic Sea Research Warnemünde (IOW)

Seestraße 15, 18119 Rostock, Germany

e-mail address: same12@io-warnemuende.de

On behalf of the Leibniz Institute for Baltic Sea Research Warnemünde (IOW), the University of Rostock and the Local Organizing Committee I am pleased to welcome you to the 12<sup>th</sup> Symposium on Aquatic Microbial Ecology (SAME12) in Rostock, from August 28<sup>th</sup> to September 2<sup>nd</sup>, 2011!

The SAME conference series derived from the merging of the “International workshop on the measurement of microbial activities in the carbon cycle in aquatic environments” and the “European Marine Microbiology Symposium (EMMS)” after the 7th EMMS meeting in Noordwijkerhout (NL) in 2000. Since then SAME symposia, numbered 8-11 following the EMMS numbering, have been held in Taormina (Italy), Helsinki (Finland), Faro (Portugal) and the last one, SAME11, in Piran (Slovenia).

The SAME conferences are organized in a rather informal way by the scientific community of aquatic microbiologist, without the background of an established scientific organisation. Despite the fact that some of the scientific themes encompassed are also present in other large international conferences, the frame of SAME meetings is still unique as it focuses entirely on Aquatic Microbial Ecology. Scientists dealing with diverse aspects of microorganisms in all kinds of aquatic environments have the opportunity to share ideas and newest results in the frame of a conference which has the right size to promote many personal interactions and to allow attendance to all presentations. This year, we are happy to welcome over 200 participants from 25 countries, with a good mixture of students and senior scientists, who will come together to present their newest results and ideas in this field.

Although we have tried to cover most recent developments in aquatic microbiology with the invitation of our key note speakers, the final thematic sessions are wholly the result of the contributions all of you have submitted. They therefore represent a major part of the diversity of topics within the active aquatic microbiology community today. The SAME12 conference will probably also be, for many of you, the first visit to this part of North-Eastern Germany, on the Baltic Sea coast. During the conference you will commute between the Hanseatic city and the University of Rostock and, for some social events, Warnemünde, the sea resort of Rostock. Here you will find beautiful beaches and an enchanting harbor, nice cafes and bars, and the IOW. Rostock itself is the major city in one of the most beautiful tourist areas in Germany, and during our excursions you will have the chance to discover some of the surroundings.

The IOW was the main marine institute of the former GDR and was newly founded after the German reunion, becoming a modern, interdisciplinary marine research institute, studying marginal and coastal seas, with a focus on the ecosystem of the Baltic Sea. The University of Rostock, which has a special research focus on maritime systems, has a long and important standing in the Baltic Sea area, being founded in 1419, 73 years before Columbus discovered America.

We hope you will enjoy the SAME12 conference, the presentations, discussions and personal contacts with many colleagues from around the globe, and that you will leave with a great memory of Rostock and Warnemünde!

Warnemünde, August 2011, Klaus Jürgens

## General Information

### SYMPOSIUM VENUE

The conference will take place in the **Audimax** lecture hall on the Campus of the University of Rostock (Ulmenstraße 69) in Rostock (in the map *city centre of Rostock* included in your SAME bag).

The Audimax is in short walking distance to the next urban railway station (S-Bahn station Parkstraße) connecting Warnemünde and Rostock.

### LANGUAGE

The official language of the symposium is English.

### SYMPOSIUM OFFICE

The symposium office will be situated at the entrance to the Audimax. The office will be open daily from 8:00 to 18:00. All participants will receive a name badge upon registration. Participants are kindly requested to wear the name badge when attending the symposium or social gatherings.

### WEBSITE

[www.io-warnemuende.de/same12](http://www.io-warnemuende.de/same12)

### CONTACT INFORMATION

IOW - Leibniz Institute for Baltic Sea Research Warnemünde  
Seestrasse 15  
18119 Rostock, Germany

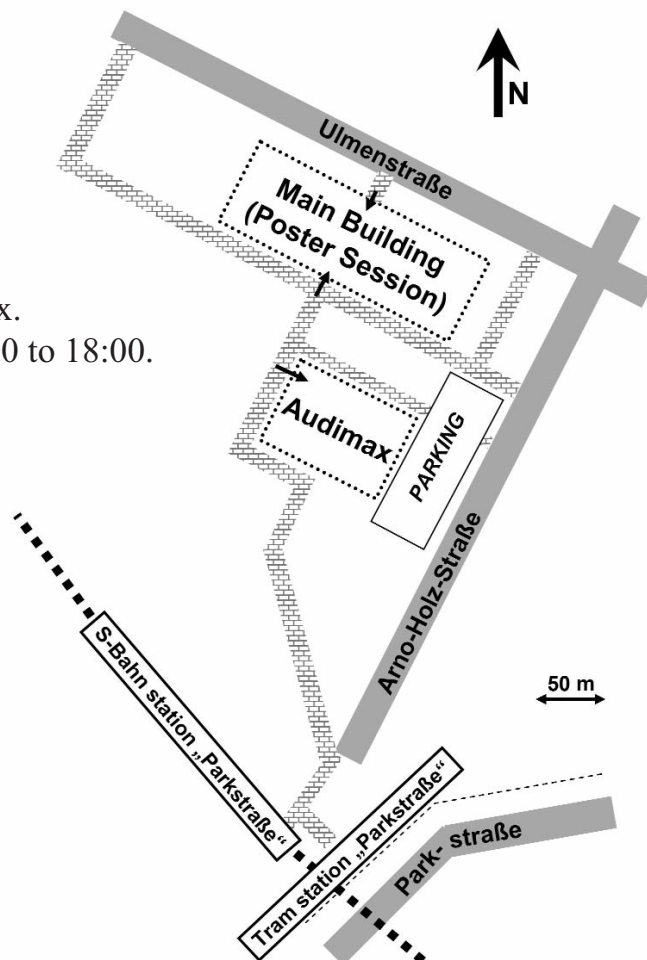
If you have any questions concerning SAME12, please contact us using the following e-mail address: [same12@io-warnemuende.de](mailto:same12@io-warnemuende.de)

### COMPUTERS AND INTERNET ACCESS

At the Audimax Rostock (main venue) and at the Leibniz Institute for Baltic Sea Research Warnemünde (Icebreaker and Opening Ceremony & Lecture) computers with access to the internet will be provided. In addition, a password for WiFi will be provided at the symposium office.

### INSTRUCTIONS FOR ORAL PRESENTATIONS

Talks are scheduled as 15 minutes time slots. Please prepare your talk to fit, including approximately 5 minutes for discussion. The chairs of each session will strictly follow the time target.



Laptops and projectors as well as assistance for technical support are available in the lecture hall. We recommend all speakers to check their presentation in advance. Please submit your presentation in the break before your session at latest. All presentations will be loaded onto our computers and deleted after the talks.

### INSTRUCTIONS FOR POSTER PRESENTATIONS

The poster session takes place at the main building and posters will be grouped by sessions. Poster numbers are provided in the abstract book and the respective numbers will be placed on each board, designating your specific location. Fastenings are provided at the site.

The poster session schedule is distributed to all participants during conference check-in. The poster session (including wine and beer) will be on Tuesday; however, posters are exhibited throughout the entire conference. The presenting author should be present at the poster during the Poster Session from 16:30 to 18:15 (PS I) or from 18:15 to 20:00 (PS II) depending on poster number.

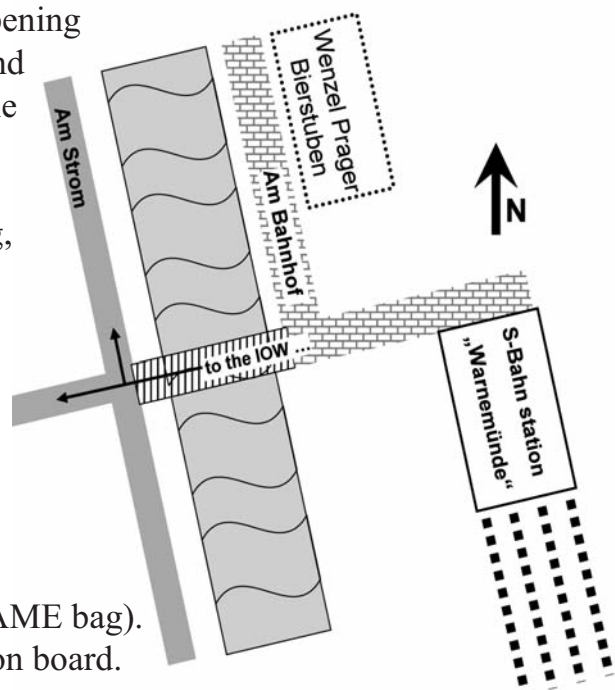
### SOCIAL EVENTS

#### Welcome Reception

On Sunday, August 28<sup>th</sup> 2011, after the opening lecture, all participants are invited to attend the Welcome Reception (Icebreaker) in the IOW located vis-à-vis the Baltic Sea in **Warnemünde** (see the map *sea resort Warnemünde* included in your SAME bag, Seestraße near “Kurhaus”).

#### Cruise on the Warnow River

On Monday, August 29<sup>th</sup> 2011, all participants are invited to attend the cruise on the Warnow River at 19:00 at the harbour of **Rostock** (Stadthafen, Schnickmannstraße, point (7) in the map *city centre of Rostock* included in your SAME bag). Drinks and sandwiches will be provided on board.



#### Conference Dinner

The Conference Dinner starts at 18:30 with a champagne reception in the Wenzel Prager Bierstuben (**Warnemünde**) on Thursday, September 1<sup>st</sup> 2011. The Prager Bierstuben is well known for its Czech food and beer specialities. There will be plenty of music to enjoy. Attendance is free for all SAME participants.

## General Information

### EXCURSIONS

There are four different excursions arranged for you on Wednesday, August 31<sup>st</sup> 2011, starting at 14:00.

You can book the tours at the registration desk until 18:00 on Monday 29<sup>th</sup> of August. The fees will be charged at booking in cash (Euro).

#### *Visiting the Oceanographic Museum (Ozeaneum) in Stralsund*

This recently built oceanographic museum, with its impressive aquariums, lets you experience up close the marine life of the colder regions on earth, ranging from the Baltic to the North Polar Sea. The largest aquarium, containing 2.6 million litres of water, is a real highlight, harbouring inhabitants of the open Atlantic Ocean. In addition, you can visit the museum's various exhibits on the ecosystem ocean.

To arrive, we will take a one-hour train ride to the historic hanseatic city of Stralsund. The museum is located within walking distance (20 min.) from the station. A guided tour of approximately one hour at the museum is included. Afterwards, there will be plenty of time to explore the museum on your own, as well as the beautiful old town of Stralsund. The train back to Rostock leaves hourly, giving you the opportunity to further explore the historic centre of Stralsund which is a UNESCO world heritage site and leave whenever you like.

*Price:* 25 Euro or 19 Euro (student) (including train rides, museum entrance and guided tour)

#### *Guided tour through Rostock downtown*

Take the guided tour (approximately 1.5 hour) through the picturesque old town of Rostock, a hanseatic city with a rich history dating back to the 11<sup>th</sup> century, and learn more about its interesting past. We will visit the tower of the Petri church (built in 1252) from where you can have a wonderful view above the roofs of Rostock – if weather permits you may even see the Baltic Sea and Warnemünde.

*Price:* 7 Euro

#### *Canoeing on the Warnow River*

Rostock is located near the Warnow River which we can explore by canoe (approximately 2-3 hours). We will bring you out to the nearby town of Pölchow and then make our way downstream towards Rostock. Don't worry – you don't need any experience to paddle this calm and broad stream. When we arrive in Rostock we will round off the trip with genuine German Bratwurst and beer.

*Price:* 25 Euro (including canoe trip, transportation and "Bratwurst")

#### *Guided tour through IOW and Günter Grass exhibition*

Take a look at the Baltic Sea from an unusual perspective by visiting the Günter Grass exhibition in our institute. The exhibition combines an artist's and a scientist's perspectives on the Baltic Sea, including etchings and sculptures of the famous



German author Günter Grass, who won the Nobel Prize for literature in 1999. You can combine this with visiting to the beautiful beach of Warnemünde or its old-town; both within a short walking distance (5min).

*Price: free*

### LOCAL TRANSPORT

Local transport to the Audimax: Take either the local train (S-Bahn) to the station **Parkstraße** or the tram No. 6 to the stop **Parkstraße**. A map for local transportation (trains, trams and buses map for Rostock) is included in your SAME bag.

Local transport to Warnemünde (Icebreaker, Conference Dinner): In Rostock City you can enter the local train (S-Bahn) at the stations **Hauptbahnhof, Parkstraße** or **Holbeinplatz**. Leave the train at the final station **Warnemünde** (take care not to mistake this stop with the previous one, called Warnemünde Werft). Please take a look at the map to get the directions to the IOW (Icebreaker) and Wenzel Prager Bierstuben (Conference Dinner).

### TICKET INFORMATION

You can either buy single tickets (1.70 €) or a weekly pass (17 €) for public transport. Single tickets have to be validated before you enter the train (orange boxes) for local trains (S-Bahn), and once inside for the trams. You can travel around Rostock City and to Warnemünde with these tickets.

Tickets can be bought at ticket machines of the Deutsche Bahn, at the tram stations at ticket machines (RSAG ticket machines) or inside the trams (single tickets only).

*How to buy a ticket:*

*Deutsche Bahn ticket machines:*

*Choose Transport association Verkehrsverbund Warnow → Rostock city entire HRO network → Weekly passes or Single journey*

*RSAG ticket machines:*

*Choose either Single ticket or Season tickets Rostock → 1 week Travel Card*

### TAXI

+49 (0) 3 81 68 58 58

4 96 89 66

6 66 13 58

## Committees

### **LOCAL ORGANIZING COMMITTEE**

Klaus Jürgens (Leibniz Institute for Baltic Sea Research Warnemünde, Germany)  
Carlo Berg (Leibniz Institute for Baltic Sea Research Warnemünde, Germany)  
Christian Bruckner (Leibniz Institute for Baltic Sea Research Warnemünde, Germany)  
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Claudia Wylezich (Leibniz Institute for Baltic Sea Research Warnemünde, Germany)

IOW webmaster: Steffen Bock

### **INTERNATIONAL SCIENTIFIC COMMITTEE**

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Karel Šimek (AS CR České Budějovice, Czech Republic)  
Meinhard Simon (ICBM Oldenburg, Germany)  
Lars Tranvik (University Uppsala, Sweden)

## **Sunday, August 28, 2011**

Conference Registration  
Opening Ceremony  
Opening Lecture  
Welcome Reception (Icebreaker)

## **Monday, August 29, 2011**

NITROGEN CYCLING IN AQUATIC ENVIRONMENTS  
NOVEL TECHNOLOGIES AND METHODS  
Cruise on the Warnow River

## **Tuesday, August 30, 2011**

MICROBIAL COMMUNITY STRUCTURE AND FUNCTION  
MICROBIAL BIOGEOCHEMISTRY  
Poster Session

## **Wednesday, August 31, 2011**

MICROBIAL INTERACTIONS, SYMBIOSIS AND COMMUNICATION  
Excursions

## **Thursday, September 1, 2011**

ECOLOGICAL PRINCIPLES  
ORGANIC MATTER TRANSFORMATIONS  
Conference Dinner

## **Friday, September 2, 2011**

MICROBIAL EUKARYOTES  
MICROBIAL PHOTOHETEROTROPHY  
Closing Lecture  
Farewell

## Plenary speakers

### **Sunday, August 28 - Opening Lecture**

THE RECENT EVOLUTION OF AQUATIC MICROBIAL ECOLOGY

*Jed Fuhrman* (University of Southern California, USA)

### **Monday, August 29**

OCEANOGRAPHIC DYNAMICS ASSOCIATED WITH NITROGEN CYCLING

MICROORGANISMS

*Matthew Church* (University of Hawai`i at Manoa, USA)

NITROGEN CYCLING IN OXYGEN DEFICIENT WATERS

*Marcel Kuypers* (Max Planck Institute for Marine Microbiology, Bremen, Germany)

HIGH THROUGHPUT SEQUENCING IN AQUATIC MICROBIAL ECOLOGY

*Anders Andersson* (KTH Royal Institute of Technology, Sweden)

### **Tuesday, August 30**

SINGLE CELL GENOMICS: DECODING THE MICROBIAL "DARK MATTER"

*Ramunas Stepanauskas* (Bigelow Single Cell Genomics Center, USA)

FROM FJORDS TO OPEN SEAS: ECOLOGICAL GENOMICS

OF EXPANDING OXYGEN MINIMUM ZONES

*Steven Hallam* (University of British Columbia, Canada)

### **Wednesday, August 31**

THE 'OMICS' OF SYMBIOTIC ASSOCIATIONS BETWEEN MARINE INVERTEBRATES  
AND CHEMOSYNTHETIC BACTERIA

*Nicole Dubilier* (Max Planck Institute for Marine Microbiology, Bremen, Germany)

### **Thursday, September 1**

HOW DO BACTERIAL COMMUNITIES ASSEMBLE AND DOES IT MATTER FOR THEIR  
FUNCTION IN ECOSYSTEMS?

*Eva Lindström* (Uppsala University, Sweden)

LINKING DISSOLVED ORGANIC MATTER AND MICROORGANISMS: TOWARDS A  
HOLISTIC SYSTEMS BIOLOGY APPROACH IN BIOGEOSCIENCE

***Thorsten Dittmar*** (Max Planck Institute for Marine Microbiology, Bremen,  
Germany; Institute for Chemistry and Biology of the Marine Environment,  
Oldenburg, Germany)

### **Friday, September 2**

TIME FOR PROTISTOMICS

***Colomban de Vargas*** (Station Biologique Roscoff, France)

PHOTOHETEROTROPHY AND THE PROCESSING OF DISSOLVED ORGANIC MATERIAL IN  
COASTAL OCEANS

***David Kirchman*** (University of Delaware, USA)

# Symposium Programme

Time	Sunday (28.08.2011)	Monday (29.08.2011)	Tuesday (30.08.2011)
8:00 - 8:30		Registration at Audimax (University of Rostock)	
8:30 - 8:45			<b>Structure &amp; Function</b>
8:45 - 9:00		<b>Welcome</b>	K-4 Keynote lecture: <b>Stepanauskas, R.</b>
9:00 - 9:15		<b>Nitrogen Cycle</b>	OS-1 Takasu, H.
9:15 - 9:30		K-1 Keynote lecture: <b>Church, M.J.</b>	OS-2 Hugoni, M.
9:30 - 9:45		ON-1 Sintes, E.	OS-3 Galand, P.E.
9:45 - 10:00		ON-2 Rissanen, A.J.	OS-4 Baltar, F.
10:00 - 10:15		ON-3 Auguet, J.C.	OS-5 Borrego,
10:15 - 10:30		ON-4 Llirós, M.	OS-6 Pop Ristova, P.
10:30 - 10:45		<b>Coffee break</b>	<b>Coffee break</b>
10:45 - 11:00			
11:00 - 11:15		<b>Nitrogen Cycle</b>	OS-7 Sebastian, M.
11:15 - 11:30		K-2 Keynote lecture: <b>Kuypers, M.M.M.</b>	OS-8 Moraes, P.C.
11:30 - 11:45		ON-5 Nunoura, T.	OS-9 Lindh, M.V.
11:45 - 12:00		ON-6 Jäntti, H.	OS-10 Kamjunke, N.
12:00 - 12:15		ON-7 Farnelid, H.	OS-11 Salcher, M.M.
12:15 - 12:30		ON-8 Großkopf, T.	OS-12 Glaeser, J.
12:30 - 12:45		<b>Lunch break</b>	<b>Lunch break</b>
12:45 - 13:00			
13:00 - 13:15			
13:15 - 13:30		IS-1 Lunch seminar: Nyakatura, G. (LGC Genomics)	IS-2 Lunch seminar: Speck, U. (BD Biosciences)
13:30 - 13:45			
13:45 - 14:00			
14:00 - 14:15		<b>Novel Technologies</b>	<b>Biogeochemistry</b>
14:15 - 14:30		K-3 Keynote lecture: <b>Andersson, A.</b>	K-5 Keynote lecture: <b>Hallam, S.</b>
14:30 - 14:45		OT-1 Severin, I.	OB-1 Reinthaler, T.
14:45 - 15:00		OT-2 Bienhold, C.	OB-2 Vadstein, O.
15:00 - 15:15		OT-3 Fagervold, S.K.	OB-3 Mau, S.
15:15 - 15:30		OT-4 Logares, R.	OB-4 Frindte, K.
15:30 - 15:45		OT-5 Bochdanský, A.B.	OB-5 Grünke, S.
15:45 - 16:00		<b>Coffee break</b>	OB-6 Zippel, B.
16:00 - 16:15			<b>Coffee break</b>
16:15 - 16:30	Registration at IOW (Warnemünde)	OT-6 Vila-Costa, M.	
16:30 - 16:45		OT-7 Schunck, H.	<b>Poster session I</b>
16:45 - 17:00		OT-8 Lundin, D.	
17:00 - 17:15		OT-9 Takaki, Y.	Beer and wine will be served
17:15 - 17:30			
17:30 - 17:45			
17:45 - 18:00			
18:00 - 18:15	<b>Opening Ceremony</b>		
18:15 - 18:30			<b>Poster session II</b>
18:30 - 18:45	<b>Opening Lecture</b>		
18:45 - 19:00	OL <b>Fuhrman, J.</b>		Beer and wine will be served
19:00 - 19:15		<b>Cruise on the Warnow River</b>	
19:15 - 19:30			
19:30 - 19:45	<b>Ice breaker</b>		
19:45 - 20:00	Open end	End: 21:00	End: 20:00

# Symposium Programme

Wednesday (31.08.2011)	Thursday (01.09.2011)	Friday (02.09.2011)	Time
<b>Microbial Interactions</b>	<b>Ecological Principles</b>		8:00 - 8:30
K-6 Keynote lecture: <b>Dubilier, N.</b>	K-7 Keynote lecture: <b>Lindström, E.S.</b>		8:30 - 8:45
OM-1 Freese, H.M.	OE-1 Langenheder, S.	<b>Microbial Eukaryotes</b>	8:45 - 9:00
OM-2 Wegner, K.M.	OE-2 Comte, J.	K-9 Keynote lecture: <b>De Vargas, C.</b>	9:00 - 9:15
OM-3 Grossart, H.-P.	OE-3 Fahlgren, C.	OME-1 Bachy, C.	9:15 - 9:30
OM-4 Šimek, K.	OE-4 Peter, H.	OME-2 Massana, R.	9:30 - 9:45
OM-5 Ullrich, M.	OE-5 Berga, M.	OME-3 Clivot, H.	9:45 - 10:00
OM-6 Seebah, S.	OE-6 Brek-Laitinen, G.	OME-4 Straškrábová, V.	10:00 - 10:15
<b>Coffee break</b>	<b>Coffee break</b>	OME-5 Macek, M.	10:15 - 10:30
OM-7 Simon, H.	OE-7 Pernthaler, J.	<b>Coffee break</b>	10:30 - 10:45
OM-8 Corno, G.	OE-8 Eiler, A.		10:45 - 11:00
OM-9 Jezbera, J.	OE-9 Winter, C.	<b>Photoheterotrophy</b>	11:00 - 11:15
OM-10 Hohmann, T.	OE-10 Romani, A.M.	K-10 Keynote lecture: <b>Kirchman, D.L.</b>	11:15 - 11:30
OM-11 Diaz Villanueva, V.	OE-11 Galvão, H.M.	OP-1 Koblížek, M.	11:30 - 11:45
OM-12 Van den Wyngaert, S.		OP-2 Medová, H.	11:45 - 12:00
<b>Lunch break</b>	<b>Lunch break</b>	OP-3 Boeuf, D.	12:00 - 12:15
		OP-4 Simon, M.	12:15 - 12:30
		OP-5 Akram, N.	12:30 - 12:45
		<b>Farewell Lecture</b>	12:45 - 13:00
		<b>Herndl, G.J.</b>	13:00 - 13:15
			13:15 - 13:30
			13:30 - 13:45
			13:45 - 14:00
<b>Excursions</b>	<b>Organic Matter Transformations</b>		14:00 - 14:15
	K-8 Keynote lecture: <b>Dittmar, T.</b>		14:15 - 14:30
	OO-1 Ruiz-González, C.		14:30 - 14:45
	OO-2 Dinasquet, J.		14:45 - 15:00
	OO-3 Endres, S.		15:00 - 15:15
	OO-4 Ziervogel, K.		15:15 - 15:30
	OO-5 Sieczko, A.		15:30 - 15:45
	OO-6 del Giorgio, P.A.		15:45 - 16:00
	<b>Coffee break</b>		16:00 - 16:15
			16:15 - 16:30
			16:30 - 16:45
			16:45 - 17:00
			17:00 - 17:15
			17:15 - 17:30
			17:30 - 17:45
			17:45 - 18:00
			18:00 - 18:15
			18:15 - 18:30
	<b>Conference dinner</b>		18:30 - 18:45
			18:45 - 19:00
	Location: Prager Bierstuben, Warnemünde		19:00 - 19:15
			19:15 - 19:30
			19:30 - 19:45
			19:45 - 20:00

## Symposium Programme: Sunday/Monday

### Sunday, August 28, 2011

16.00 – 18.00 Registration at IOW (Warnemünde)

18.00 – 19.30 **Opening Ceremony – Opening Lecture**  
OL Fuhrman, J.

19.30 – Icebreaker  
Open end

### Monday, August 29, 2011

08.00 – 08.45 Registration at Audimax (University of Rostock)

08.45 – 09.00 Welcome

#### **Nitrogen Cycling in Aquatic Environments I (chair: Herndl, G. J.)**

09.00 – 09.30 K-1 Keynote lecture: Church, M.J.

09.30 – 09.45 ON-1 Sintes, E.

09.45 – 10.00 ON-2 Rissanen, A.J.

10.00 – 10.15 ON-3 Auguet, J.C.

10.15 – 10.30 ON-4 Llíros, M

10.30 – 11.00 *Coffee break*

#### **Nitrogen Cycling in Aquatic Environments II (chair: Riemann, L.)**

11.00 – 11.30 K-2 Keynote lecture: Kuypers, M.M.M.

11.30 – 11.45 ON-5 Nunoura, T.

11.45 – 12.00 ON-6 Jäntti, H.

12.00 – 12.15 ON-7 Farnelid, H.

12.15 – 12.30 ON-8 Großkopf, T.

12.30 – 14.00 *Lunch break*

13.15 – 13.45 IS-1 Lunch Seminar: Nyakatura, G. (LGC Genomics)

#### **Novel Technologies and Methods I (chair: Simon, M.)**

14.00 – 14.30 K-3 Keynote lecture: Andersson, A.

14.30 – 14.45 OT-1 Severin, I.

14.45 – 15.00 OT-2 Bienhold, C.

15.00 – 15.15 OT-3 Fagervold, S.K.

15.15 – 15.30 OT-4 Logares, R.

15.30 – 15.45 OT-5 Bochdansky, A.B.

15.45 – 16.15 *Coffee break*

#### **Novel Technologies and Methods II (chair: Grossart, H.-P.)**

16.15 – 16.30 OT-6 Vila-Costa, M.

16.30 – 16.45 OT-7 Schunck, H.

16.45 – 17.00 OT-8 Lundin, D.

17.00 – 17.15 OT-9 Takaki, Y.

19.00 *Cruise on the Warnow River (Rostock - Warnemünde - Rostock)*  
*End: 21:00*



# Symposium Programme: Tuesday

**Tuesday, August 30, 2011**

## **Structure & Function I (chair: Gasol, J.M.)**

- 08.30 – 09.00 K-4 Keynote lecture: Stepanauskas, R.  
09.00 – 09.15 OS-1 Takasu, H.  
09.15 – 09.30 OS-2 Hugoni, M.  
09.30 – 09.45 OS-3 Galand, P.E.  
09.45 – 10.00 OS-4 Baltar, F.  
10.00 – 10.15 OS-5 Borrego, C.M.  
10.15 – 10.30 OS-6 Pop Ristova, P.

10.30 – 11.00 *Coffee break*

## **Structure & Function II (chair: del Giorgio, P. A.)**

- 11.00 – 11.15 OS-7 Sebastian, M.  
11.15 – 11.30 OS-8 Moraes, P.C.  
11.30 – 11.45 OS-9 Lindh, M.V.  
11.45 – 12.00 OS-10 Kamjunke, N.  
12.00 – 12.15 OS-11 Salcher, M.M.  
12.15 – 12.30 OS-12 Glaeser, J.

12.30 – 14.00 *Lunch break*

- 13.15 – 13.45 IS-2 Lunch Seminar: Speck, U. (BD Biosciences)

## **Biogeochemistry (chair: Schulz-Vogt, H.N.)**

- 14.00 – 14.30 K-5 Keynote lecture: Hallam, S.  
14.30 – 14.45 OB-1 Reinthaler, T.  
14.45 – 15.00 OB-2 Vadstein, O.  
15.00 – 15.15 OB-3 Mau, S.  
15.15 – 15.30 OB-4 Frindte, K.  
15.30 – 15.45 OB-5 Grünke, S.  
15.45 – 16.00 OB-6 Zippel, B.

16.00 – 16.30 *Coffee break*

16.30 – 18.15 **Poster Session - I**

Beer and wine will be served during both Poster Sessions

18.15 – 20.00 **Poster Session - II**

# Symposium Programme: Wednesday

**Wednesday, August 31, 2011**

## **Microbial Interactions I (chair: Rassoulzadegan, F.)**

- 08.30 – 09.00 K-6 Keynote lecture: Dubilier, N.  
09.00 – 09.15 OM-1 Freese, H.M.  
09.15 – 09.30 OM-2 Wegner, K.M.  
09.30 – 09.45 OM-3 Grossart, H.-P.  
09.45 – 10.00 OM-4 Šimek, K.  
10.00 – 10.15 OM-5 Ullrich, M.  
10.15 – 10.30 OM-6 Seebah, S.  
10.30 – 11.00 *Coffee break*

## **Microbial Interactions II (chair: Posch, T.)**

- 11.00 – 11.15 OM-7 Simon, H.  
11.15 – 11.30 OM-8 Corno, G.  
11.30 – 11.45 OM-9 Jezbera, J.  
11.45 – 12.00 OM-10 Hohmann, T.  
12.00 – 12.15 OM-11 Diaz Villanueva, V.  
12.15 – 12.30 OM-12 Van den Wyngaert, S.  
12.30 – 14.00 *Lunch break*

- 14.00 Excursions

# Symposium Programme: Thursday

**Thursday, September 01, 2011**

## **Ecological Principles I (chair: Iriberry, J.)**

- 08.30 – 09.00 K-7 Keynote lecture: Lindström, E.S.  
09.00 – 09.15 OE-1 Langenheder, S.  
09.15 – 09.30 OE-2 Comte, J.  
09.30 – 09.45 OE-3 Fahlgren, C.  
09.45 – 10.00 OE-4 Peter, H.  
10.00 – 10.15 OE-5 Berga, M.  
10.15 – 10.30 OE-6 Brek-Laitinen, G.

10.30 – 11.00 *Coffee break*

## **Ecological Principles II (chair: Langenheder, S.)**

- 11.00 – 11.30 OE-7 Pernthaler, J.  
  
11.30 – 11.45 OE-8 Eiler, A.  
11.45 – 12.00 OE-9 Winter, C.  
12.00 – 12.15 OE-10 Romaní, A.M.  
12.15 – 12.30 OE-11 Galvão, H.M.

12.30 – 14.00 *Lunch break*

## **Organic Matter Transformations (chair: Reinthaler, T.)**

- 14.00 – 14.30 K-8 Keynote lecture: Dittmar, T.  
14.30 – 14.45 OO-1 Ruiz-González, C.  
14.45 – 15.00 OO-2 Dinasquet, J.  
15.00 – 15.15 OO-3 Endres, S.  
15.15 – 15.30 OO-4 Ziervogel, K.  
15.30 – 15.45 OO-5 Sieczko, A.  
15.45 – 16.00 OO-6 del Giorgio, P.A.

16.00 – 16.30 *Coffee break*

18.30 *Conference Dinner*  
Location: Wenzel Prager Bierstuben, Warnemünde

*End: 24:00*

## Symposium Programme: Friday

**Friday, September 02, 2011**

### **Microbial Eukaryotes (chair: Šimek, K.)**

- 09.00 – 09.30 K-9 Keynote lecture: De Vargas, C.  
09.30 – 09.45 OME-1 Bachy, C.  
09.45 – 10.00 OME-2 Massana, R.  
10.00 – 10.15 OME-3 Clivot, H.  
10.15 – 10.30 OME-4 Straškrábová, V.  
10.30 – 10.45 OME-5 Macek, M.  
10.45 – 11.15 *Coffee break*

### **Microbial Photoheterotrophy (chair: Pinhassi, J.)**

- 11.15 – 11.45 K-10 Keynote lecture: Kirchman, D.L.  
11.45 – 12.00 OP-1 Koblížek, M.  
12.00 – 12.15 OP-2 Medová, H.  
12.15 – 12.30 OP-3 Boeuf, D.  
12.30 – 12.45 OP-4 Simon, M.  
12.45 – 13.00 OP-5 Akram, N.  
13.00 – 13.30 **Farewell Lecture**  
Herndl, G.J.

## Ecological Principles

- Size-dependent responses of phytoplankton to thermal stratification and mixing in two boreal lakes of contrasting humic matter content** P-1  
Peltomaa, E., Brek-Laitinen, G., and Ojala, A.
- Benthic microbial community responses to pesticides in lake littoral zones** P-2  
Larras, F., Bouchez, A., Montuelle, B.
- Growth efficiency evidences bacterial community adaptation to the ecosystem** P-3  
Baña, Z., Uranga, A., Abad, N., Artolozaga, I, Ayo, B., and Iriberry, J.
- Changes in Bacterioplankton Community Composition and Functionality in Response to Increasing Selection Pressure** P-4  
Sjöstedt, J., Pontarp, M., Tinta, T., Alfredsson, H., Canbäck, B., Tunlid, A., Lundberg, P., Hagström, Å., Turk, V., and Riemann, L.
- Bacterioplankton patterns in shallow lakes from the Pampa Plain (Argentina) with contrasting alternative steady states** P-5  
Llames, M.E., del Giorgio, P., Izaguirre, I., Ferraro, M., and Zagarese, H.E.
- Assessment of the impact of tebuconazol (fungicide) on lake and stream bacterial communities using a 16S rRNA pyrosequencing method** P-6  
Pascault, N., Artigas, J. , Roux, S., Tadonleke, R., Ter Halle, A., Mailhot, G., Pesce, S., Humbert, J.F., Debroas, D., and Bouchez, A.
- Linking changes in microbial communities to trophic variations in a tropical mangrove** P-7  
Bouchez, A., Pascault, N., Chardon, C., Bouvy, M., Cecchi, P., Herteman, M., Lambs, L., Fromard, F., and Leboulanger, C.
- Microbial activities, dynamics and diversity in a changing Arctic Ocean (Fram Strait)** P-8  
Wurst, M., E. Killias, H.-A. Giebel, E.-M. Nöthig, K. Metfies, and A. Engel
- Does ocean dumping affect marine microbial communities? Investigations at a dumping site in the german bight** P-9  
Störmer, R., Wichels, A., and Gerdts, G.
- Linking of OTC-resistance with ampicillin-resistance in bacteria from coastal seawater of Korea and Japan** P-10  
Soo-jin Kim, Farzana Ashrafi Neela, Satoru Suzuki and Myung-Joo Oh
- Salinity as a major factor shaping bacterial phylogenetic composition in the Baltic Sea** P-11  
Herlemann, D.P.R., Labrenz, M., Andersson, A.F., and Jürgens, K.
- Phytoplankton diversity and nutrients in mesocosm** P-12  
Paul, C., Schumann, R. and Feike, M.

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- P-13 Bacterial abundances and pigments on sediments from Southwest Atlantic**  
Cabral, A.S., Pinto, F.N., Curbelo, M.P., Lavrado, H.P., Falcão, A.P., Paranhos, R.
- P-14 Does terrestrial particulate organic matter affect benthic microbial metabolism and carbon flow?**  
Attermeyer, K., Grossart, H.-P., Premke, K.
- P-15 Determining bacterial carbon contribution to sediment organic matter in the Seto Inland Sea of Japan**  
Kunihiro, T., Shibata, J., Hamaoka, H., Sogabe, A., Moriya, K., Kuwae, M., Ito, K., Tsutsumi, H., Omori, K.
- P-16 Activity of methane oxidizing bacteria in different depths of water column along the River Elbe downstream to its estuary**  
Matousu, A., Bussmann, I., Simek, K.
- P-17 Polyphosphate storage in *Beggiatoa alba***  
Havemeyer, S., Schulz-Vogt, H. N.
- P-18 Spatial and Temporal variation of methane production and emission from a tropical lake (Pantanal, Brazil)**  
Barreto, D.P., Conrad, R., Batsviken, D., Peixoto, R., Cristina-Silva, E., Claus, P., Klose, M., Enrich-Prast, A.
- P-19 Linking biogeochemical zonation in hydrocarbon contaminated aquifers to microbial activity**  
Richert, I.; Wendeberg A.
- P-20 Identification of acetate incorporating *Arcobacter* spp. as potential manganese reducers in pelagic redoxclines of the central Baltic Sea via 16S rRNA based <sup>13</sup>C stable isotope probing**  
Berg, C., Labrenz, M., Beckmann, S., Jost, G., Jürgens, K.

### Microbial Community Structure and Function

- P-21 Distribution of *Synechococcus* and *Prochlorococcus* in the central and southern Adriatic Sea**  
Šantić, D., Krstulović, N., Šolić, M., and Grozdan Kušpilić
- P-22 Macrophyte drive the variation of bacterioplankton community composition in shallow lakes**  
Wu, Q.L., Zeng, J., Bian, Y., Xing, P.
- P-23 Occurrence of *Roseobacter* subclusters in the German Bight of the North Sea**  
Billerbeck, S., Giebel, H.A., Simon, M.
- P-24 Contribution of major prokaryotic groups in the Romanche Fracture Zone of the tropical Atlantic**  
Lekunberri, I., Sintes, E., Yokokawa, T., Herndl, G.J.

- Heterotrophic activity of Archaea and Bacteria throughout water column of the eastern Atlantic** P-25  
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- Changes in bacterial diversity during incubations of surface and mesopelagic Atlantic waters** P-29  
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- Microdiversity and habitat preferences of freshwater bacteria of the genus Limnohabitans (Betaproteobacteria)** P-30  
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- ROS formation by photochemical reactions affect BCC in a humic lake and induce adaptive responses in abundant bacteria** P-31  
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- Bacterial community structure from the sediments of Jiulong River Estuary in China** P-32  
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- Seasonal changes in the growth potential of bacterial populations in Lake Zurich** P-34  
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- Peculiar space-time dynamics of prokaryotic Picoplankton in a volcanic tropical lake** P-35  
Hernández-Avilés, J.S, Arellano, P. J. A., González, F. E., Macek, M.
- Space-time distribution of the bacterial community in the brackish meromictic Lake Faro (Messina, Italy)** P-36  
Raffa, C., Interdonato, F., Lo Giudice, A., De domenico, E., De Domenico, M., Strous, M., Michaud, L.

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Porporato E.M.D., Michaud L., Spanò N., De Domenico E., De Domenico M., De Domenico F., Mangano M.C., Raffa C., Lo Giudice A.

**P-38 Diel and tidal influence on planktonic microbial communities in a coastal brackish lake (Lake Ganzirri, Italy)**

Michaud, L., Pulicanò, G., Lo Giudice, A., Raffa, C., Guerrini, F., Pistocchi R., Vanucci, S.

### Microbial Eukaryotes

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Shi, X. L., Lepère, C., Scanlan D. J., and Vaulot, D.

**P-40 Mesoscale distribution of nanoflagellates in the first-year sea-ice of the eastern Canadian Arctic Archipelago, in spring**

Piwosz, K., Wiktor, J., Michel, C., Niemi, A., and Tatarek, A.

**P-41 Distribution patterns of key components of microbial food web in meromictic saline lake**

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**P-42 Allelopathy and Mixotrophy in *Prymnesium parvum* do not like it salty**

Weissbach, A., Gast, O., Legrand, C.

**P-43 Protist diversity in suboxic and sulfidic waters of the Black Sea**

Wylezich, C. and Jürgens, K.

**P-44 Community structure of autotrophic flagellates in waters of the west Spitsbergen (Svalbard, Norway)**

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**P-45 Testate amoeba community structure and distribution in lake sediments**

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**P-46 Life in a biofilm: unraveling the eukaryotic biodiversity at S. Domingos AMD in the south of Portugal**

Valente, T., Esteves, F., Reis, M.P., and Fonseca, F.

**P-47 Taxonomic novelty of heterotrophic protists revealed by unamended brackish water incubations from the Baltic Sea**

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- Grazing on the cyanobacterium *Microcystis aeruginosa* by the heterotrophic flagellate *Collodictyon triciliatum* in an experimental pond** P-50  
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- Succession of N-acetyl-glucosamine consuming bacterioplankton taxa with distinct life strategies in a freshwater lake** P-53  
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- Growth and release of extracellular organic compounds by benthic diatoms depend on interactions with bacteria** P-56  
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- Feeding of ciliates on toxic filamentous cyanobacteria in Lake Zurich (Switzerland)** P-57  
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- Trophic coupling between heterotrophic flagellates and bacteria in hypertrophic shallow lakes** P-58  
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- P-61 Links between viral and prokaryotic abundance and production throughout the water column along a latitudinal transect in the North Atlantic**  
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- P-62 Importance of protist grazing on a chemolithoautotrophic key player ('*Sulfurimonas*' GD17) of Baltic Sea redoxclines**  
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- P-63 The impact of jellyfish carcasses on the bacterial community**  
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- P-65 Isotopic composition of nitrate in the redoxcline of the Baltic Sea**  
Frey, C., Voss, M.
- P-66 Nitrate turnover during spring outflow from the nitrate-rich Curonian and Szczecin lagoon**  
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- P-67 Microbial composition of various components of closed aquaculture systems: analysis of biofilters and indigenous microbiota of fish intestines.**  
van Kessel, M.A.H.J., Flik, G., Jetten, M.S.M., Klaren, P.H.M., and Op den Camp, H.J.M.
- P-68 Anaerobic ammonium oxidation in the ocean's oxygen minimum zones is dominated by a single genus - '*Candidatus Scalindua* sp.'**  
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- P-69 Molecular characterization of nitrogen-fixing bacteria and their colonization pattern in mangrove roots**  
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- Ammonia-Oxidising Bacteria and Archaea in deep water of an oligotrophic subalpine lake** P-70  
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- Community composition and abundance of aerobic and anaerobic ammonia oxidizers in an agriculturally impacted sandy aquifer** P-71  
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- Can we trust on PCR-dependent microbial community analyses?** P-74  
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- Proteomics of ‘Gramella forsetii’ show its versatility in degradation of algae-derived biopolymers** P-75  
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- Diversity patterns of cultured marine bacteria using different organic matter sources as compared to Single Amplified Genomes** P-79  
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- P-82 Proteome based comparative analysis of exponentially growing and stationary phase marine bacteria**  
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- P-83 Identification of active bacterial assemblages by a new PCR independent 16S rRNA single strand conformation polymorphism (SSCP) approach**  
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### Organic Matter Transformations

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- P-85 Novel Clostridium populations involved in the anaerobic degradation of Microcystis blooms**  
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- P-87 Oxygen demand in surficial sediments during resuspension events**  
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- P-88 Aluminium, pH, or phosphorus: what really limits microbial leaf litter decomposition in acidified headwater streams ?**  
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- P-89 Ecological significance of assimilable organic carbon measurements in alpine karst aquifers**  
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- P-91 Linking phytoplankton and bacterial diversity through dissolved organic carbon: insights from continuous culture experiments**  
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- Ocean acidification and its impact on growth and activity of heterotrophic bacteria - first results from a coupled phytoplankton growth and bacterial degradation experiment.** P-94  
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- Temperature-mediated virus-induced bacterial mortality** P-95  
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# Oral Presentations

## THE RECENT EVOLUTION OF AQUATIC MICROBIAL ECOLOGY

Fuhrman, J.A.<sup>1</sup>

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Aquatic Microbial Ecology has evolved considerably over recent decades, from the exciting initial recognition of the Microbial Loop, through the discoveries of the importance of cyanobacteria, viruses and archaea, to the latest discoveries in "omics." A major driving force has been the development and application of new technologies like epifluorescence microscopy, flow cytometry, remote sensing, different generations of sequencing techniques, genomics, and bioinformatics. I will discuss these developments and how "pushing the envelope" keeps leading to new exciting discoveries. However, as the field matures, we need to be cognizant that many of the older approaches and fundamental questions are still very relevant today, but sometimes seem to be pushed aside by the latest "fads." The current generation of aquatic microbial ecology students are in the challenging position of not only needing to learn the latest dizzying molecular biology, microbiology, and bioinformatics, but also should be familiar with several decades worth of prior work in biological (and to some extent, chemical and physical) aquatic sciences, as well as classical ecology theory and practice. Our tools have been developed to a point where we can often study microbes in the same ways that animals and plants have been studied for so long, and can benefit from what such studies have learned.



## **OCEANOGRAPHIC DYNAMICS ASSOCIATED WITH NITROGEN CYCLING MICROORGANISMS**

Church, M.J.<sup>1</sup>

*<sup>1</sup>University of Hawaii, Department of Oceanography*

The ocean nitrogen cycle is controlled by the metabolic activities of diverse microorganisms. In the aerobic waters of the open sea, nitrogen (N<sub>2</sub>) fixation and nitrification are two major processes governing the transformation of nitrogen-containing compounds. N<sub>2</sub> fixing microorganisms (termed diazotrophs) introduce bioavailable sources of nitrogen to plankton food webs, while nitrifying microorganisms oxidize ammonia to nitrite, and ultimately nitrate. Our understanding how these microorganisms influence ocean biogeochemistry has improved dramatically over the past several decades; however, to date, we have only limited information on the ecology and population dynamics of the microorganisms driving these processes. By merging measurements on rates of nitrogen transformation with molecular-characterizations of the diversity and abundances of nitrogen cycling microorganisms, our understanding of the processes regulating the microbial ecology underlying the ocean nitrogen cycle continues to improve. This presentation will highlight temporal dynamics in the population structure of diazotrophs and nitrifying microorganisms based on work conducted at Station ALOHA, the field site for the Hawaii Ocean Time-series (HOT) program. Results from these time-series studies suggest physical dynamics, including episodic forcing by mesoscale eddies and seasonally-driven convective mixing, play an important role in controlling the physiology and population structure of nitrogen cycling microbes in this ecosystem.

### **ARCHAEAL amoA GENE DIVERSITY INDICATES NICHE SEPARATION OF AMMONIA-OXIDIZING CRENARCHAEOTA IN THE OCEAN**

Sintes, E.<sup>1</sup>, Bergauer, K.<sup>1</sup>, De Corte, D.<sup>2</sup>, and Herndl, G.J.<sup>1</sup>

<sup>1</sup>*University of Vienna, Austria,*

<sup>2</sup>*University of Groningen, The Netherlands*

Mesophilic ammonia oxidizing Archaea (AOA) are abundant in a diverse range of marine environments, including the deep ocean, as revealed by the quantification of the archaeal amoA gene encoding the alpha-subunit of the ammonia monooxygenase. Using two different amoA primer sets in combination with q-PCR, evidence was found for the occurrence of two distinct ecotypes of marine archaeal ammonia oxidizers (AOA) in the tropical Atlantic and the coastal Arctic. One ecotype dominated the coastal Arctic and the top 300 m layer of the open equatorial Atlantic where ammonia concentrations are variable but measurable with conventional spectrophotometric methods, while the other ecotype dominated the lower meso- and bathypelagic waters of the tropical Atlantic where ammonia concentrations are well below the detection limit of conventional methods. A latitudinal transect in the North Atlantic, from 70°N to 0°N confirmed the distinct biogeography of the two ecotypes throughout the Atlantic related to ammonia availability. Cluster analysis of the sequences from the clone libraries obtained by the two amoA primer sets revealed two phylogenetically distinct clusters. Moreover, the translated amino acid sequences of the two AOA ecotypes suggest the presence of two types of ammonia monooxygenase corresponding to the medium and low ammonia concentration in the shallow and the deep waters, respectively. Hence, we conclude that there is an apparent niche separation in MCGI, with distinct ammonia monooxygenase ecotypes dominating at different ammonia concentration environments.

**DENITRIFICATION AND ANAMMOX PROCESSES IN NORTHERN LAKES**Rissanen, A.J.<sup>1</sup>, Tiirola, M.<sup>1</sup>, Hietanen, S.<sup>2</sup>, Tulonen, T.<sup>2</sup>, and Ojala, A.<sup>2</sup><sup>1</sup>*University of Jyväskylä, Finland,*<sup>2</sup>*University of Helsinki, Finland*

The economic feasibility of actions against nitrogen discharges from wastewaters and agricultural drainage waters is dependent on the capabilities of water systems to remove nitrogen via natural removal processes, i.e. denitrification and anaerobic ammonium oxidation (anammox). These processes have not received much attention in studies of lakes of the northern Europe, e.g. of Finland, where, however, the fate of anthropogenic nitrogen emissions is currently a hot topic. The purpose of this study was to assess the seasonal and spatial variations in nitrogen removal of Finnish lakes and to identify physico-chemical and biological factors affecting this variation. The studies were conducted using sediment samples from three eutrophic and one oligo-mesotrophic lake and using water samples from nine oxygen-stratified humic-acid rich lakes. Sediment cores were incubated in laboratory for assessment of denitrification and/or anammox rates using isotope pairing techniques and oxygen-stratified lakes were studied for their vertical N<sub>2</sub> gas profiles ( $\delta^{15}\text{N}$  and concentration of N<sub>2</sub>). Physico-chemical properties of sediment and water overlying the sediment and the structure of nitrite-reducing bacterial community (nirK-gene-PCR-DGGE) and anammox community (cloning/sequencing of the 16S rRNA genes) in sediments were studied concurrently. Sediment denitrification rates varied between 50 to 600  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ , showed marked seasonal and spatial variations and were positively dependent on nitrate concentration of the water. Variations in the nirK-carrying bacterial community of sediments did not affect denitrification rates. Anammox was not active in the sediments of studied lakes, although bacteria related to known anammox species were present in two of the lakes. Denitrification was indicated by the oversaturation and slightly (1‰) depleted  $\delta^{15}\text{N}$  values of the dissolved N<sub>2</sub> gas in the suboxic zones of some of the oxygen-stratified lakes.

### SEASONAL CHANGES OF FRESHWATER AMMONIA-OXIDIZING ARCHAEAL ASSEMBLAGES IN OLIGOTROPHIC ALPINE LAKES

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The annual changes in the composition and abundance of ammonia-oxidizing archaea (AOA) were analyzed monthly in the water column of four high mountain lakes within the Limnological Observatory of the Pyrenees (LOOP, NE Spain) using both 16S rRNA and functional (ammonia monooxygenase gene, *amoA*) genes sequencing, and quantitative PCR amplification, respectively. The set of biological data was related to changes in nitrogen species and to other relevant environmental variables. The whole archaeal assemblage was dominated by phylotypes closely related to the crenarchaeal 1.1a group ( $58 \pm 18$  % of total 16S rRNA gene sequences), and consistent structural changes were detected along the study. Water temperature was the environmental variable that better explained spring, summer, and winter (ice-covered lakes) archaeal assemblage structure. The *amoA* gene was detected all around the year and seasonal changes in *amoA* gene composition were well correlated with changes in the archaeal 16S rRNA gene pool. In addition, copy numbers of both the specific 1.1a group 16 rRNA and archaeal *amoA* genes were well correlated, suggesting that most freshwater 1.1a Crenarchaeota had the potential to carry out ammonia oxidation. Seasonal changes in the diversity and abundance of AOA (i.e. *amoA* gene) were better explained by temporal changes in ammonium, the substrate for nitrification, and mostly nitrite, the product of ammonia oxidation. Lacustrine *amoA* gene sequences grouped in coherent freshwater phylogenetic clusters suggesting that freshwater habitats harbor typical *amoA*-containing ecotypes different from soils and seas. We observed within the freshwater *amoA* gene sequences pool a high genetic divergence (translated to up to 32 % amino acid divergence) between the spring and the remaining AOA assemblages. This suggests that different AOA ecotypes would be adapted to different temporal ecological niches in these lakes.

**MICROBIAL DIVERSITY AND PROCESSES IN LAKE KIVU (EAST AFRICA)**

Llirós, M.<sup>1</sup>, Darchambeau, F.<sup>2</sup>, Garcia-Armisen, T.<sup>3</sup>, Morana, C.<sup>4</sup>, Plasencia, A.<sup>5</sup>, Gich, F.<sup>5</sup>, Leporcq, B.<sup>1</sup>, Libert, X.<sup>1</sup>, Schmid, M.<sup>6</sup>, Servais, P.<sup>3</sup>, Borges, A. V.<sup>2</sup>, Bouillon, S.<sup>4</sup>, Borrego, C.M.<sup>5</sup>, and Descy, J. P.<sup>1</sup>

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Lake Kivu is a deep meromictic and oligotrophic tropical African lake with a permanent thermal- and haline stratification with huge accumulations of dissolved CO<sub>2</sub> and CH<sub>4</sub> (ca. 300 km<sup>3</sup> and 60 km<sup>3</sup>, respectively) in the deep anoxic monimolimnion (from 60 to 480 m depth). Although there is a wealth of information on the ecology of small eukaryotes and their trophic role on Kivu, available information on prokaryotic planktonic assemblages is scarce. Molecular analysis of archaeal and bacterial communities showed a vertical segregation imposed by the permanent redoxcline. In relation to Bacteria, Actinobacteria, Betaproteobacteria, Green Sulfur Bacteria and Bacteroidetes were the most commonly retrieved groups. For Archaea, a marked dominance of Thaumarchaeota and Crenarchaeota (75% of all archaeal OTUs) over Euryarchaeota was observed. In the anoxic hypolimnion, Euryarchaeota (Methanosarcinales and Methanocellales) lineages together with Miscellaneous Crenarchaeotic Group phylotypes were mainly recovered. In turn, Thaumarchaeota phylotypes were recovered in oxic and suboxic waters. CARD-FISH analyses over the first 100 m revealed the dominance of Bacteria (51.4% – 95.7% of DAPI-stained cells), especially Actinobacteria (epilimnion), Betaproteobacteria (oxic-anoxic interface) and Bacteroidetes (upper hypolimnion), over Archaea (1.0% – 4.5%; maximum abundances at the oxic-anoxic interface). In turn, flow cytometry evidenced the dominance of HNA cells in the euphotic layer, whereas the proportion of LNA cells increased with depth. HNA and LNA populations were still observed in the anoxic hypolimnion suggesting facultative or strict anaerobic metabolisms. The detection of distinct depth maxima of nitrate, nitrite, archaeal *amoA* and Marine Thaumarchaeota 16S gene copy numbers together with regularly detection of deep maxima of 3H-Thymidine uptake, and the presence of low-light adapted GSB species point towards a strong link of N, C, and S cycles in the redoxcline of Lake Kivu.

## K-2 Nitrogen Cycling in Aquatic Environments

### NITROGEN CYCLING IN OXYGEN DEFICIENT WATERS

Kuypers, M.M.M.<sup>1</sup>

<sup>1</sup>*Max Planck Institute for Marine Microbiology*

Globally, 30–50% of oceanic N-loss occurs in oxygen deficient waters known as oxygen minimum zones (OMZs). For decades, oceanic N-loss has been attributed entirely to heterotrophic denitrification. However, recent findings from incubation experiments with <sup>15</sup>N-labeled compounds indicated substantial N-loss due to anammox in the OMZs off Peru, Chile and Namibia, while denitrification was generally below detection. The occurrence and regulation of anammox, as well as its interactions with other N-cycling processes, were investigated in various OMZs as well as in the laboratory. We qualitatively and quantitatively compared the genetic potentials and active expressions of functional genes of various N transformations. The identified active processes were further verified by <sup>15</sup>N incubation experiments. Using this multidisciplinary approach, we found that in OMZ waters, the majority of nitrite for anammox came from nitrate reduction, whereas up to one-third came from aerobic ammonia oxidation. Anammox bacteria acquired ammonium from the degradation of organic matter associated with nitrate reduction, but also from dissimilatory nitrate reduction to ammonium.

**UNIQUE NITROGEN CYCLE IN THE HADOPELAGIC SEDIMENTS AT THE BOTTOM OF THE OGASAWARA TRENCH**

Nunoura, T.<sup>1</sup>, Nishizawa, M.<sup>1</sup>, Kikuchi, T.<sup>2</sup>, Tsubouchi, T.<sup>1</sup>, Hirai, M.<sup>1</sup>, Koide, O.<sup>1</sup>, Miyazaki, J.<sup>1</sup>, and Takai, K.<sup>1</sup>

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Nitrogen cycle in deep-sea sediments is still uncertain while that in the aphotic oceanic water has intensively been investigated. A hadopelagic sediment core (1.1m in length) was taken by the *ROV ABISMO* from the Ogasawara Trench at a depth of 9760m. Interstitial water chemistry indicates that nitrate reduction occurred in the upper parts of the sediment core, but significant sulfate reduction did not even at the bottom of the core column. Comprehensive molecular analyses including clone analyses and quantitative PCR for SSU rRNA genes and functional genes (*amoA*, *nirK*, *hao/hzo*) present unique distribution patterns of nitrifiers, denitrifiers and anammox in the nitrate reduction zone. The maximum abundance of both aerobic nitrifiers (archaeal and proteobacterial ammonia oxidizers, and nitrite oxidizing bacteria) and proteobacterial denitrifiers occurred in a same horizon, and stable isotopic analyses for nitrate also suggest the occurrence of nitrification in the nitrate reduction zone. On the other hand, anammox population decreased with increasing depth. These results suggest that the abundance of aerobic nitrifiers is regulated by both oxygen and ammonium concentrations, and the anammox population is suppressed as a result of competition for nitrite with nitrite oxidizers and denitrifiers except for surface layer in the hadopelagic sediment.

### NITROGEN CYCLING AND THE AMMONIA OXIDIZING COMMUNITY IN THE BALTIC SEA WATER COLUMN

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The Baltic Sea is one of the largest brackish water bodies in the world. It has large anoxic basins and the redoxclines above them are optimal sites for intense nitrogen cycling, because they allow coupling of the nitrogen redox processes. The chain of processes, where ammonium is first oxidized to nitrite or nitrate in the nitrification process and then reduced to dinitrogen gas by denitrification or anammox is referred to as the natural nitrogen removal system. There is a negative correlation between the dissolved inorganic nitrogen (DIN) pool and the volume of hypoxic water ( $O_2$  2 ml l<sup>-1</sup>) in the Baltic Sea. Because the sediment nitrogen removal rates decrease in hypoxia, nitrogen removal must intensify in the water column when hypoxia expands, in order for the negative relationship between DIN pool and volume of hypoxic water to occur. However, the nitrogen cycling rates in the Baltic Sea redoxcline are largely unknown. Our results from the Baltic Sea redoxcline show that the oxic-anoxic interface in the water column is highly dynamic and that the conditions fluctuate even within hours. Nitrification was active in a layer where oxygen was available in low concentrations. Peculiarly, nitrification was found to be active even when hydrogen sulfide (H<sub>2</sub>S) was present, suggesting that the ammonia oxidizers are archaea. This hypothesis will be tested by comparing ammonia oxidizer assemblages across the redoxcline with the microarray analyses. High denitrification potential was found 10–25 m below the active nitrification layer suggesting that intensive nitrogen removal can occur if nitrate becomes available in the deep, anoxic water layers. However, the frequency of this is still unknown. Including the nitrogen cycling in the water column in the calculation of the nitrogen budget will help to fill the gaps of understanding the nitrogen cycling of the entire Baltic Sea.



**NITROGENASE GENE AMPLICONS FROM GLOBAL MARINE SURFACE WATERS ARE DOMINATED BY GENES OF NON-CYANOBACTERIA**

Farnelid, H.<sup>1</sup>, Andersson, A. F.<sup>2</sup>, Bertilsson, S.<sup>3</sup>, Abu Al-Soud, W.<sup>4</sup>, Hansen, L. H.<sup>4</sup>, Sørensen, S.<sup>4</sup>, Steward, G. F.<sup>5</sup>, and Riemann, L.<sup>6</sup>

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Cyanobacteria are thought to be the main N<sub>2</sub>-fixing organisms (diazotrophs) in marine pelagic waters, but recent molecular analyses indicate that non-cyanobacterial diazotrophs are also present and active. Existing data are, however, restricted geographically and by limited sequencing depths. Our analysis of 79 090 nitrogenase (*nifH*) PCR amplicons encoding 7 468 unique proteins from surface samples (ten DNA samples and two RNA samples) collected at ten marine locations world-wide provides the first in-depth survey of a functional bacterial gene and yield insights into the composition and diversity of the *nifH* gene pool in marine waters. Great divergence in *nifH* composition was observed between sites. Cyanobacteria-like genes were most frequent among amplicons from the warmest waters, but overall the data set was dominated by *nifH* sequences most closely related to non-cyanobacteria. Clusters related to Alpha-, Beta-, Gamma-, and Delta-Proteobacteria were most common and showed distinct geographic distributions. Sequences related to anaerobic bacteria (*nifH* Cluster III) were generally rare, but preponderant in cold waters, especially in the Arctic. Although the two transcript samples were dominated by unicellular cyanobacteria, 42% of the identified non-cyanobacterial *nifH* clusters from the corresponding DNA samples were also detected in cDNA. The study indicates that non-cyanobacteria account for a substantial part of the *nifH* gene pool in marine surface waters and that these genes are at least occasionally expressed. The contribution of non-cyanobacterial diazotrophs to the global N<sub>2</sub> fixation budget cannot be inferred from sequence data alone, but the prevalence of non-cyanobacterial *nifH* genes and transcripts suggest that these bacteria are ecologically significant.

### THE “DARK MATTER” OF NITROGEN FIXATION

Großkopf, T.<sup>1</sup>, Mohr, W.<sup>1</sup>, Baustian, T.<sup>1</sup>, Schunck, H.<sup>1</sup>, Gill, D.<sup>1</sup>, Kuypers, M.M.M.<sup>2</sup>, Lavik, G.<sup>2</sup>, Schmitz, R.A.<sup>3</sup>, Wallace, D.W.R.<sup>1</sup>, and LaRoche, J.<sup>1</sup>

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Biological nitrogen fixation is the largest input of fixed nitrogen into the oceans and thus a key parameter in controlling primary productivity. Despite the importance of nitrogen fixation there is major controversy about its magnitude on a global scale, due to a gap in the marine nitrogen cycle on the input side. While this gap suggests that the nitrogen cycle is currently not in balance and the oceans are losing more nitrogen than they gain, stable isotope measurements from sediment cores suggest that the nitrogen cycle has been in balance over the last 3000 years. To resolve this paradox it has been suggested that marine nitrogen fixation is currently underestimated.

We used a revised method to measure nitrogen fixation and compared it with the prior, widely applied method. Our study reveals that over the whole Atlantic Ocean the prior method underestimated nitrogen fixation rates. In certain areas the mean fixation rate increased over six fold when measured with the revised protocol. The magnitude of the difference is not stable but rather highly variable on a coarse geographic scale. We suspected that species composition has a great influence on the magnitude of underestimation of nitrogen fixation rates by the prior method, a theory we could confirm with a laboratory experiment.

Taken together, our results imply that there is an urgent need to agree on a common protocol for nitrogen fixation rate measurements to assess the true potential of this nitrogen input process and be able to model the future development, given man-made climate changes.

**NEXT GENERATION SEQUENCING – NEW POSSIBILITIES IN HIGH THROUGHPUT SEQUENCING**

Nyakatura, G.<sup>1</sup>

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We shall briefly introduce the comprehensive LGC Genomics' sequencing portfolio, give a short overview of next generation sequencing technologies but will devote most of the talk to an in depth overview of LGC Genomics' capabilities and services in next generation sequencing especially using the Roche/454 GS FLX and the illumina HiSeq2000 platforms.

A proprietary novel approach to whole transcriptome sequencing will also be introduced.

Although "next generation sequencing" has radically reduced the cost of DNA sequencing primarily by dramatically increasing the output of sequencing runs, the cost of sequencing whole genomes is still well above the financial means of most research groups. We shall describe here a cost effective method to analyze the total gene content of most organisms.

This novel approach involves the generation of a full length optimized normalized cDNA library. This is followed by directed tandem ligation of the individual transcripts into high molecular weight concatamers. The concatamers are then sheared randomly and are subsequently shotgun sequenced (pyrosequencing) using the Roche/454 Genome Sequencer FLX with the Titanium chemistry. The individual reads are then assembled to complete transcripts with either the Roche/454 Newbler software or with other suitable software e.g. Staden Package to yield the total gene content of the organism of interest.

### HIGH THROUGHPUT SEQUENCING IN AQUATIC MICROBIAL ECOLOGY

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The emergence of high throughput sequencing technologies has boosted the field of microbial ecology by enabling deep sequencing of taxonomic marker genes from a large number of samples in parallel, which allows researchers to address fundamental questions about how microbial communities are structured in time and space on an unprecedented scale. A multitude of high throughput sequencing technologies are now available, each with different limitations and strengths. In this talk I will review recent advances in sequencing technology and bioinformatics with applications in aquatic microbial ecology. Finally, I will give examples on how we are employing high throughput sequencing for exploring the Baltic Sea microbiome.

**THE AMOUNT OF SEQUENCES NEEDED TO DESCRIBE BETA-DIVERSITY –  
A MULTI LAKE-STUDY**

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Microbial biogeography, the study of the distribution of microbial diversity in space, requires the identification of the members of the microbial community as well as the comparison of differences in diversity between sites (beta-diversity). Technical advances have enabled the identification of an increasing number of individuals by sequencing. Still, budget constraints impose a trade-off between the number of sequences obtained per sample and the number of samples (sites) to be included in a study. Ideally the number of sites should be maximized but the enormous diversity and the large number of individuals in microbial communities also calls for an identification of as many individuals as possible per site. Nonetheless, uncertainties remain concerning the extent of sequencing needed to sufficiently describe beta-diversity of communities in different samples. The necessary sequencing effort can vary, depending on the diversity and the evenness of the microbial community, which in turn may depend on a variety of factors, e.g., nutrient availability.

In this study we analyzed 454-sequencing data of the 16S rRNA gene with regard to the amount of sequences needed to accurately describe beta-diversity of bacteria in streams, lakes and lake sediments with different physico-chemical properties. Statistical evaluation revealed a difference between the amount of sequences needed for an accurate beta-diversity description in the stream-and lake-systems as well as for the lake sediments. Underlying factors for this difference were identified.

This study provides a rationale for the sequencing effort needed for different freshwater ecosystems with regard to describing beta-diversity.

**GLOBAL DISTRIBUTION PATTERNS OF SEAFLOOR BACTERIA IN THE DEEP SEA**Bienhold, C.<sup>1</sup>, Zinger, L.<sup>1</sup>, Boetius, A.<sup>2</sup>, and Ramette, A.<sup>1</sup><sup>1</sup>*Max Planck Institute for Marine Microbiology,*<sup>2</sup>*Alfred Wegener Institute for Polar and Marine Research*

Increasing evidence suggests that bacterial communities, similar to macro-organisms, exhibit large-scale, biogeographic patterns, which have been linked to ecosystem properties as well as historical events. The few studies that have investigated marine bacterial distribution patterns at the global scale have so far only focused on the euphotic zone of the ocean (e.g. Pommier et al. 2007, *Mol. Ecol.* 16:867-880; Fuhrman et al. 2008, *Proc. Natl. Acad. Sci. USA* 105:7774-7778). A global comparison between marine pelagic and benthic bacterial communities (Zinger et al., submitted) has evidenced higher provincialism for benthic than for pelagic communities which may be attributed to lower physical mixing, resulting in spatial isolation. Here, we analysed the biogeographic patterns of bacterial communities in deep-sea surface sediments covering nine oceanic regions across the globe, using 454 massively parallel tag sequencing. Our goal was to provide a first snapshot of typical deep seafloor bacterial communities and to further explore their degree of endemism as a possible consequence of dispersal limitation. Sequences of typical representatives of marine benthic communities, e.g. Gammaproteobacteria, Actinobacteria, were abundant in all samples and globally dispersed. Nevertheless, we observed clear differences in bacterial communities between oceanic regions, with the number of shared OTUs (operational taxonomic units defined at 97% sequence identity) decreasing with distance, both on small and large scales. In contrast to broad taxonomic levels (e.g. phylum, class), OTUs at fine taxonomic resolution levels displayed high degree of endemism, suggesting dispersal limitation and that bacterial communities in the deep sea may be actively shaped by ecological and evolutionary processes. In this context, we will discuss the role of environmental factors and other biogeographic barriers on the distribution of bacterial populations in the deep sea.

**DIVERSE BACTERIAL COMMUNITIES ASSOCIATED TO SUNKEN WOODS IN A MEDITERRANEAN DEEP SEA CANYON**

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Submarine canyons can trap and concentrate organic falls like terrestrial debris exported to the deep sea especially during storm events. In a changing climate these events are thought to increase, and understanding the faith of terrestrial organic matter transferred to the deep is more important than ever. Sunken woods are important organic substrates on the deep sea floor creating unique chemosynthetic ecosystems due to the sustained decay of the wood matrix, eventually creating anaerobic conditions. However, the microbial communities responsible for degrading the wood, thus representing the starting point of a community succession, remain poorly studied. Our aim was to examine the microbial community involved in wood degradation by comparing pine and oak wood samples experimentally submerged in the Blanes Canyon (NW Mediterranean). We analyzed the communities by pyrosequencing the V1 and V2 region of bacterial 16S rRNA genes from wood recovered after 9 and 12 months of deployment. We compared woods from different depths (1200, 1500 m) in the canyon. We also analyzed the microbial communities found in the "fecal matter" produced by wood-boring bivalves. The microbial diversity was relatively high in all samples and the number of shared OTUs between samples was small. Further analysis showed that "fecal matter," oak and pine formed separate clusters, suggesting different microbial communities. All samples were dominated by Proteobacteria, but Planctomycetes and Actinobacteria sequences were more present in the wood matrix, while bivalve borrows contained considerably more Bacteroidetes. Finally most samples contained a large amount of "unclassified" sequences, suggesting an unexplored microbial diversity and possible existence of undescribed microbial metabolic functions in sunken wood.

### COMBINING TRADITIONAL AND NEXT GENERATION SEQUENCING TO INVESTIGATE MARINE PROTIST DIVERSITY WITH EMPHASIS IN STRAMENOPILES

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Among microbes, the diversity of protists (microeukaryotes) is probably the less studied. Here, we have combined traditional (Sanger) with Next Generation (454-Titanium) sequence data (18S rRNA) to investigate the diversity patterns within a major protist lineage, the stramenopiles. The 454 sequences were generated through a large-scale sequencing initiative (BioMarKs) carried out in European coastal waters. Five geographic locations were sampled extensively at different times, taking into account different size fractions, DNA/cDNA and water-column/sediments. About 9 million 454 protist sequences (V4 region, ca. 400 bp) were produced and stramenopiles were extracted. Specifically, we focused in the less known but ecologically very important free-living heterotrophic groups known as MAST (MARine STRamenopiles). Our main aims were twofold, to a) characterize the phylogenetic lineages belonging to these groups by using trees based on thousands of longer Sanger sequences and b) to populate those Sanger-based core trees with shorter 454 sequences. As 454 sequencing can recover a large fraction of diversity, we aimed at determining whether or not new major lineages were missing. Furthermore, we analyzed the relationships between phylogeny and sample metadata. So far, no major lineages were found, but important associations between phylogeny and environment/activity were evidenced.



**APPLICATION OF DIGITAL INLINE HOLOGRAPHY FOR THE STUDY OF PARTICLES AND MICROPLANKTON IN THE OCEAN**Bochdansky, A. B.<sup>1</sup> and Herndl, G.J.<sup>2</sup>*<sup>1</sup>Old Dominion University, Norfolk, Virginia, USA, <sup>2</sup>University of Vienna, Austria*

In situ microscopy in aquatic systems is typically limited by the depth of field and consequently by a low image volume. This makes traditional microscopy unsuitable for concentrations at which most aquatic microorganisms occur. Digital Inline Holographic Microscopy (DIHM) overcomes this problem by utilizing large conical fields of view containing up to several milliliters of water in one image. Resolution (limited by the wavelength of the laser, the size of the point source and the resolution of the chip of the lensless camera) can reach into the micrometer range under ideal conditions. Here we present for the first time the application of DIHM on a basin-scale oceanographic expedition to the tropical and subtropical Atlantic Ocean, from the surface to a maximum depth of 6000 m. The DIHM was mounted on the frame of a CTD rosette, and thus became part of routine casts without the requirement of separate deployments. Even at speeds of 1 m per sec through the water, our instrument yielded clearly identifiable particles such as *Trichodesmium* colonies, radiolarians and tintinnids. Approximately 7 images with sizes of 2048x2048 pixels each were taken for every meter, each image representing a volume of 1.3 ml. An additional important advantage of holographic images is that they allow for the precise determination of sizes and the locations of particles in three-dimensional space (i.e., X, Y, Z coordinates). In this presentation we will show the principles of DIHM, our adaptations for oceanographic environments, and show in live demonstrations how images of microplankton and amorphous particles are reconstructed from the original holograms.

**DIEL GENE EXPRESSION PROFILES OF A PHOSPHORUS LIMITED HIGH-ALTITUDE LAKE THROUGH METATRANSCRIPTOMICS**

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Contrastingly to the increasing advances in marine ecosystems, bacterial functionality of freshwater systems remains largely unexplored despite their relevance in the biogeochemical cycles. In this work, we used metatranscriptomic sequencing to analyze day and night gene expression profiles of the bacterial assemblages from the oligotrophic high altitude Lake Llebreta (1620 m above sea level) in the Limnological Observatory of the Pyrenees (LOOP, Central Pyrenees, 42° 33'3"N, 0° 53'25"E). The goal of the study was to obtain clues about the main biogeochemical processes carried out by bacteria in a high altitude lake and explore unique biogeochemical features of this freshwater system. An average of 37871 unique reads were obtained per treatment after using 454 pyrosequencing technology to sequence the extracted and amplified messenger RNA (mRNA). Bacteroidetes and betaproteobacteria were the most actively transcribing phyla of the community and they showed different strategies to process limiting nutrients like phosphorus (P). Thus, whereas bacteroidetes mostly obtained P by hydrolyzation of stored polyphosphates and pyrophosphates generally at night and used proteorhodopsins as extra source of energy during the day, betaproteobacteria accounted for most of the phosphate membrane uptake systems and generated additional ATP molecules after CO oxidation during the day. Compared to marine diel studies, lake Llebreta showed an overabundance of transcripts related to lipid metabolism and specifically, an overrepresentation of transcripts related to degradation of polycyclic aromatic hydrocarbons (PAHs), which was overrepresented at night. This study represents the first attempt to characterize the bacterial functionality of a freshwater system.

**METAGENOMIC AND METATRANSCRIPTOMIC ANALYSIS OF SULFIDIC WATERS  
WITHIN THE OXYGEN MINIMUM ZONE OFF PERU**

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Oxygen minimum zones are hotspots of biogeochemical turnover processes. Nitrate and nitrite are usually thought to be the most favourable terminal electron acceptors in the absence of oxygen. But also the cycling of sulfur species can contribute largely to the energy flux in oxygen depleted environments. So far, only few studies have investigated the microbial community in sulfidic waters. Hence, the knowledge of many bacterial and archaeal taxa and their contribution to biogeochemical cycles is still very limited.

Here we present sequencing data from a sulfidic water column on the continental shelf off Peru within the oxygen minimum zone in the Eastern Tropical South Pacific. Approximately 3.5 million DNA and cDNA sequences were obtained from six depths using the Roche GS-FLX sequencing technology.

The sequence data shows a diverse microbial community with (in some depths) almost 40% of the sequences belonging to organisms which actively contribute to the sulfur cycle. Although the organisms able to oxidize sulfur were dominating, a considerable number of sulfate reducers were identified in the water column as well. The most abundant microorganisms in the sulfidic waters could be identified as gammaproteobacteria affiliated with uncultured chemoautotrophic gill symbionts of deep-sea clams, in some depths accounting for up to 30% of the whole microbial community. Recent studies have postulated that these organisms can couple sulfide oxidation with nitrate reduction and thus potentially contribute largely to the loss of fixed nitrogen.

The comparison of our data to those of non-sulfidic oxygen minimum zone waters will deepen the understanding of the metabolic activity of microorganisms and their contribution to biogeochemical transformations within the oxygen deficient waters.

### **MICROBIAL DIVERSITY ALONG AN OXYGEN GRADIENT – COMMUNITY GENOMES FROM LANDSORT DEEP IN THE BALTIC SEA**

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The Landsort Deep in the Baltic Sea south east of Stockholm, has a steep oxygen gradient from fully oxic surface waters, via an hypoxic zone at the halocline between 60 and 80m, down to anoxic conditions further down. We have characterised the microbial communities at three depths along the water column (10m, 75m and 400m) plus the sediment, using community genome shotgun 454 sequencing, yielding a total of 350Mb DNA. Bacteria are dominating all samples except for high frequencies of potentially ammonium oxidising Thaumarchaea in the 75m and 400m samples. Along the depth profile not only salinity and oxygen content varies, but also concentrations of e.g. phosphate, nitrate and sulfide. This is reflected in the taxonomical profile of our samples, with e.g. high frequencies of sulfate reducers in the anaerobic 400m sample. Furthermore, the degree of similarity, both in terms of taxonomical composition and functional coding capacity, between the 10m and 75m samples compared with the anaerobic samples, suggest that the ranges of many bacterial populations stretch down to the halocline. We also find a striking similarity between Landsort Deep 400m and sediment samples and other marine sediment and terrestrial soil community genomes.

**ISOLATION, CHARACTERIZATION AND GENOMIC FEATURES OF SULFUR-OXIDIZING GAMMAPROTEOBACTERIA, A CLOSE RELATIVE STRAIN OF CHEMOAUTOTROPHIC ENDOSYMBIONTS**

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Shimamura, S.<sup>1</sup>, and Takai, K.<sup>1</sup>

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A novel sulfur-oxidizing bacterium was isolated from a deep-sea hydrothermal field in the Middle Okinawa Trough. The strain grows chemolithoautotrophically using elemental sulfur or thiosulfate as an electron donor and oxygen or nitrate as an electron acceptor. Mixotrophic growth with proteincious compound or organic acid as a sole carbon source was also observed while final cell concentrations were less than 20 % of those under chemolithoautotrophic growth conditions. The strain formed biofilm on elemental sulfur and grew under the film. The 16S rRNA gene sequence indicates that the strain belongs to the previously uncultivated lineage of *Gammaproteobacteria* consisting of chemoautotrophic endosymbionts in gastropods, bivalves and tubeworms, and is especially closely related to gastropod endosymbionts (94–97 % homology). The genome sequence of this strain was obtained by the 454 FLX sequencer, and gap-regions were analyzed by the Sanger sequencing. The strain has one chromosome (3.13 Mb) and two plasmids (11 and 13 kb). The genomic information suggests the occurrence of the Calvin Benson cycle for the carbon assimilation, and complete Dsr and incomplete Sox pathways for sulfur oxidation in this strain. The strain also harbors diverse chemotaxis and two component regulatory systems. Unique physiology and genomic traits of this strain closely related with endosymbionts will provide novel insight into the host-symbiont relationship in deep-sea chemosynthetic ecosystem.

### **SINGLE CELL GENOMICS: DECODING THE MICROBIAL “DARK MATTER”**

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The vast majority of microbial taxa remains uncultured and is therefore inaccessible to classical microbiology methods. Cultivation-independent sequencing of microbial community DNA, such as metagenomics, enables gene discovery, but has limited capacity to reconstruct discrete genomes or metabolic pathways. In contrast, single cell genomics allows, for the first time, the study of multiple genes or entire genomes of uncultured microorganisms, independent of the complexity of their communities.

Single cell genomics relies on the physical separation of individual cells, followed by their lysis, whole genome amplification, and subsequent DNA sequencing. Bigelow Laboratory for Ocean Sciences pioneered the development of single cell genomics technology and established the first shared-user facility, based on an integration of fluorescence-activated cell sorting and high-throughput molecular biology tools. During its first year in operation, the Bigelow Laboratory Single Cell Genomics Center (SCGC) contributed to cutting-edge research at over 20 organizations around the globe. Over 150,000 individual cells have been analyzed by SCGC so far, providing unique access to genomic DNA, without cultivation biases, from microorganisms representing over 60 phyla of bacteria, archaea and protists. Projects performed at SCGC span genomic studies of the uncultured prokaryotes, protists and viruses from marine, freshwater, subsurface, and other environments. Research result highlights include identification of C-fixing microorganisms in the ocean twilight zone (mesopelagic), identification of predominant photoheterotrophs in freshwater lakes and individual-based protist prey and viral infection identification. Thus, single cell genomics is emerging as a transformative research approach in diverse areas of biological research.

**RELATIVE BIOMASS OF MAJOR PHYLOGENETIC BACTERIAL GROUPS AND BIOLOGICAL VARIABLES IN MESOTROPHIC LAKE BIWA, JAPAN**Takasu, H.<sup>1</sup>, Kunihiro, T.<sup>2</sup>, and Nakano, S.<sup>1</sup><sup>1</sup>Center for Ecological Research (CER), Kyoto University, Japan,<sup>2</sup>Center for Marine Environmental Studies (CMES), Ehime University, Japan

Abundance of bacterial phylogenetic groups in aquatic environments has been determined using some molecular biological techniques. Those techniques provide us numerical information about each group. However, our knowledge of the role of each bacterial phylogenetic group in carbon cycling of microbial food webs is still limited. To include each bacterial phylogenetic group in the carbon cycling, they must be determined as the carbon biomass. In the present study, we determined biomass of bacterial phylogenetic groups using quinone profiling at epilimnion (5 m) and hypolimnion (70 m) in the pelagic area of Lake Biwa during thermal stratification (from June to December 2010) and mixing period (from January to March 2011). Amount and composition of respiratory quinones (ubiquinone, UQ; menaquinone, MK) were used to estimate carbon biomass of major phylogenetic bacterial groups. *Betaproteobacteria* (UQ-8,  $38.4 \pm 9.8$  %) predominated, and relative biomass of *Actinobacteria* (MK-9~11 and partially hydrogenated MKs), *Cytophaga-Flavobacterium-Bacteroides* (CFB) (MK-6~8) and *Alphaproteobacteria* (UQ-10) was  $28.9 \pm 3.7\%$ ,  $17.9 \pm 6.6\%$  and  $10.0 \pm 5.1\%$ , respectively. *Betaproteobacteria* and *Actinobacteria* were the two important bacterial groups in terms of biomass in both the epilimnion and the hypolimnion. It is interesting to note that positive correlations were observed between alphaproteobacterial biomass and dissolved organic carbon concentration, and between CFB biomass and chlorophyll *a* concentration. Biomass of *Alphaproteobacteria* and CFB were also positively correlated with cell density of heterotrophic nanoflagellates. Thus, *Alphaproteobacteria* and CFB might also play important roles in the carbon cycling through utilization of organic matter and its transfer to higher trophic level organisms, though their biomass remained low during the study period.

### DIVERSITY AND SPATIO-TEMPORAL DYNAMICS OF ARCHAEA AND AMMONIA OXIDIZING COMMUNITIES IN THE PHOTIC ZONE OF TEMPERATE LAKES

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Crenarchaeota have recently been recognized as main drivers of the oxidation of ammonia to nitrite in many marine and terrestrial ecosystems. However, the study of Ammonia Oxidizing Archaea (AOA) still suffers from incomplete assessment of their diversity and roles in lacustrine ecosystems. In this study, four lakes from ultraoligotrophic to eutrophic trophic status (Godivelle, Sep, Bourget and Aydat) were sampled to investigate the diversity and distribution of planktonic Archaea and their potential role in nitrogen cycle. Analyses of archaeal 16S rDNA and ammonia monooxygenase  $\alpha$ -subunit (*amoA*) gene clone libraries from water samples of the different lakes revealed the presence of both euryarchaeal and crenarchaeal OTUs that were equally distributed among the lakes. The majority of 16S rDNA crenarchaeal sequences were affiliated to clade I.1a and *amoA* phylogeny highlighted the presence of a “Freshwater lake and hot spring A3” enriched clade in addition to a well-established “Freshwater and low salinity lake A1.1” clade. The seasonal change in the abundance of archaeal and bacterial *amoA* genes was estimated using quantitative PCR in three lakes, revealing different community patterns according to the ecosystem studied. In the eutrophic lake (Aydat), only Ammonia oxidizing Bacteria (AOB) were detected by this method while the mesotrophic lake (Bourget) was characterized by a shift in ammonia-oxidizers communities over the season, with AOA predominant during homothermy. Finally, in the Sep reservoir, only a few AOA were detected, whereas bacterial *amoA* genes predominated the ecosystem all year round. This study provides therefore the first temporal changes of ammonia-oxidizing communities among lakes.



**PHYLOGENETIC AND FUNCTIONAL DIVERSITY OF BACTERIA AND ARCHAEA IN THE STRATIFIED LAGOON OF THE CLIPPERTON ATOLL (N PACIFIC)**

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The Clipperton lagoon in the North Pacific Ocean has been isolated from the surrounding sea for ca. 160 years. It has a stratified water column that comprises an oxic and brackish upper water layer (mixolimnion) and a deep sulfuric anoxic saline layer (monimolimnion), separated by a steep pycnocline. Here we test if the Clipperton lagoon with its distinctive physico-chemical features, geographic isolation, recent water column stratification and large nutrient input, harbors original microbial communities. The combination of capillary electrophoresis single-strand polymorphism (CE-SSCP) fingerprinting and cloning sequencing of bacterial and archaeal 16S rRNA genes, and functional genes for methanogenesis (*mcrA*), methanotrophy (*pmoA*) and sulfate reduction (*dsrAB*), revealed that microbial communities and pathways were highly stratified down the water column. The mixolimnion contained ubiquitous freshwater clades of Alpha- and Betaproteobacteria while the pycnocline contained mostly green sulfur bacteria (phylum Chlorobi). Upper layers sequences were closely related to sequences found in other aquatic ecosystems, suggesting that they have a strong potential for dispersal and colonization. In contrast, the monimolimnion contained new deeply branching bacterial divisions within the OP11 cluster and Bacteroidetes, and was the most diverse of the layers. The unique environmental conditions characterizing the deep layers of the lagoon may explain the novelty of the microbial communities found at the Clipperton atoll.

### BACTERIAL VERSUS ARCHAEAL ORIGIN OF EXTRACELLULAR ENZYMATIC ACTIVITY IN THE NORTHEAST ATLANTIC DEEP WATERS

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Total and dissolved extracellular enzymatic activity (EEA) were determined in the epi-, meso- and bathypelagic waters of the subtropical Northeast Atlantic. Additionally, we determined two glycolytic enzymes ( $\alpha$ -glucosidase and  $\beta$ -glucosidase [AGase and BGase]), one proteolytic enzyme (leucine aminopeptidase [LAPase]) and alkaline phosphatase [APase] activities. In addition, EEA was determined in treatments in which bacterial EEA was inhibited by erythromycin (which inhibits specifically Bacteria but leaves Archaea unaffected) and EEA decay experiments were performed with surface and deep waters to determine EEA life-times in both water masses. The proportion of dissolved to total EEA was generally higher than the cell-associated (i.e. particulate) EEA (66-89%, 44-88%, 86-100%, 57-82% for AGase, BGase, LAPase and APase, respectively). The percentage of dissolved to total EEA was inversely proportional to the percentage of erythromycin-inhibited to total EEA, and since essentially all the total EEA was recovered in our measurements (i.e., erythromycin-inhibited plus dissolved EEA) this indicates that EEA in the open oceanic water column is almost exclusively of bacterial origin. The decay constants of dissolved EEA were in the range of 0.002–0.047 h<sup>-1</sup> depending on the type of extracellular enzyme, and temperature and depth in the water column. Our results indicate that the bacterially produced dissolved EEA fraction dominates total EEA in the open subtropical Northeast Atlantic down to bathypelagic layers and that mesophilic Archaea contribute only marginally to the EEA in the ocean.

**ANOXIA SELECTS FOR THE ARCHAEOAL COMMUNITY COMPOSITION IN SULFIDIC WATERS OF STRATIFIED KARSTIC LAKES**Borrego, C.<sup>1</sup>, Fillol, M.<sup>1</sup>, Plasencia, A.<sup>1</sup>, and Auguet, O.<sup>1</sup><sup>1</sup>*Group of Molecular Microbial Ecology, Institute of Aquatic Ecology, University of Girona, Spain*

Karstic lakes are characterized by an active sulfur cycle that affects the type and composition of their microbial communities. In these environments, stratification favours the generation of anoxia and accumulation of sulfide and ammonia as by-products of microbial metabolisms. Albeit the diversity and dynamics of bacterial communities inhabiting these lakes are known, less information is available on their archaeal counterparts, especially in the deep, anoxic, sulfide-rich waters. To fill this gap, we have studied the archaeal assemblages in several stratified lakes of the Banyoles Karstic System. Archaeal 16S rDNA clone libraries from oxic/anoxic interfaces, the anoxic hypolimnia and sediments were compared to investigate habitat segregation patterns according to environmental variables. Most of the recovered clones (75%) affiliated to *Crenarchaeota* (mainly to *Miscellaneous Crenarchaeotic Group*, MCG) and *Thaumarchaeota*. The remaining clones affiliated to several lineages of uncultured *Euryarchaeota*, such as the *Marine Benthic Group D*, the *Miscellaneous Euryarchaeotic Group* (MEG) and the *Deep Sea Euryarchaeotic Group* (DSEG). Remarkably, half of the clones from anoxic, sulfidic waters affiliated to MCG, a lineage composed of putative heterotrophic anaerobes recovered from very diverse environments. In clear contrast, 95% of the clones from the oligotrophic basin C-II (Lake Banyoles) were assigned to a single OTU affiliated to *Marine Group I.1A*. Identification of archaeal *amoA* genes provided further support to the presence of archaeal nitrifiers in this basin. Euryarchaeotal clones were only dominant in basin IV, where the accumulation of reduced iron and sulfur compounds draws some similarities with deep sea hydrothermal vents. The identification of prevalent archaeal lineages in these anoxic, dark and cold water masses contributes to the understanding of their ecology and may be of interest when studying other aquatic systems with similar environmental conditions.

### SPATIAL VARIATIONS OF BACTERIAL COMMUNITIES AND RELATED BIOGEOCHEMICAL ACTIVITY OF COLD SEEP SITES IN THE EASTERN MEDITERRANEAN DEEP SEA

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Cold seeps are characterized by emissions of the potential greenhouse gas methane from the seafloor to the hydrosphere, and represent biogeochemical hot spot ecosystems of the deep sea. They host highly dynamic habitats that are spatially fragmented and temporarily variable. Cold seep habitats harbour high microbial biomass, comprising primary producers of carbon and energy via mediation of core processes such as anaerobic oxidation of methane and sulphide oxidation. Despite their crucial role, little is known about the spatial scales on which the alpha and beta diversity of microbial communities vary at cold seeps, and how the geological, geochemical and biogenic habitat heterogeneity influences seep biodiversity.

The deep Eastern Mediterranean sea, encompassing numerous geologically different cold seep sites, offers a unique opportunity for the study of spatial scaling of microbial biodiversity patterns and interconnectivity of microbial communities. In this study we present data on the comparison of bacterial community structures on large (100 km) and small (0.01–100 m) spatial scales, i.e. between and within different cold seep ecosystems, such as the Amon mud volcano, the Amsterdam mud volcano and the Central Pockmark area. Furthermore, we specifically compare the bacterial community structures, as derived from ARISA and 454 pyrosequencing, and investigate how they relate to *in situ* biogeochemical fluxes.

**INORGANIC AND ORGANIC PHOSPHORUS USE BY DIFFERENT BACTERIAL PHYLOGENETIC GROUPS IN THE EASTERN MEDITERRANEAN SEA**

Sebastian, M.<sup>1</sup>, Pitta, P.<sup>2</sup>, Thingstad, T.F.<sup>3</sup>, and Gasol, J.M.<sup>1</sup>

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The Eastern Mediterranean is considered one of the most oligotrophic and P-starved marine systems on earth, where P limitation of bacterioplankton has been shown to be a generalized phenomenon. This system was the site for CYCLOPS, the only large-scale P-addition experiment conducted to date, in which the phytoplankton community was found to be N- and P-co-limited, and the heterotrophic community was P-limited. A mesocosm experiment with Eastern Mediterranean water was carried out to study the effect of Pi addition on the microbial community in this P-starved system. The use of Pi and DOP (ATP) among different bacterial phylogenetic groups was studied using microautoradiography combined with catalyzed reporter deposition fluorescence in situ hybridization (MARFISH). The low Pi turnover times in the control mesocosms and the increase in bacterial abundance and activity upon P addition, confirmed that the bacterial community was originally P-stressed. All the bacterial groups analyzed were considerably active in the uptake of Pi and ATP in the control mesocosms, whereas Bacteroidetes and Synechococcus were relatively inactive in the use of leucine. After 1 day of P addition Gammaproteobacteria, Roseobacter and Bacteroidetes still showed a high number of cells active in the uptake of <sup>33</sup>P-Pi, suggesting that these groups have higher requirements than SAR11 and Synechococcus and need more time to replenish their P quotas. In contrast, the number of cells active in the uptake of ATP was significantly reduced. Gammaproteobacterial cells seem to be the major contributor to Pi and ATP uptake, whereas SAR11 were the major contributor to the use of leucine.

### EFFECT OF PLANKTON-DERIVED PARTICULATE ORGANIC MATTER ON THE MICROBIAL COMMUNITY OF SEDIMENT

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An experimental study was carried out in Nov/Dec 2010 to observe the microbial response to two different POM inputs in a coastal sedimentary community of Ubatuba, São Paulo, Brazil. The study area is subjected to two distinct seasonal patterns: (1) an increase of primary production and diatom blooms owing to the advection of the deep, nutrient-rich South Atlantic Central Water (SACW) into the euphotic zone; and (2) re-suspension of the sediment due to cold fronts and predominance of nanoflagelates. The organic enrichment experiment was conducted in order to test experimentally the stimulus of the sediment prokaryotic community after the input of labile material. To test this hypothesis planktonic algae was added to the sediment surface to simulate an algal bloom reaching the sea floor. A total of 57 corers (two treatments: the diatom *Phaeodactylum tricornutum* and the nanoflagellate *Tetrasselmis* sp. and a control) were maintained for a total of 30 days in constant temperature (19°C), circulation and oxygenation. After the algae addition the corers were sampled six times for the microbial and biogeochemistry analysis. The microbial community was counted by the Live/Dead backlight viability kit and DGGE analyses. A genomic library was done with one sample in each treatment. After the addition of algae it was observed an increase in oxygen consumption and CO<sub>2</sub> production, accompanied by an increase of prokaryotic density, showing an immediate response from the community to the input of labile material in the sediment. The DGGE and cluster analysis of the bacterial community performed with the treatments and control showed that, in general, time was more important for the change of bacterial community than the treatments. Treatment with *Tetrasselmis* appears to cause more differences in the community than the treatment with *Phaeodactylum*, especially in the first two days after the addition of algae.

**CONSEQUENCES OF INCREASED TEMPERATURE AND ACIDIFICATION  
ON BACTERIOPLANKTON COMMUNITY COMPOSITION DURING A MESOCOSM  
SPRING PHYTOPLANKTON BLOOM IN THE BALTIC SEA**

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Climate change in the sense of increased seawater temperature and acidification is projected to substantially impact the marine ecosystem. Despite the paramount importance of bacteria in determining global carbon cycling and other biogeochemical cycles, little is known about the potential effects of climate change on these key organisms. The consequences of the projected climate change on bacterioplankton community dynamics were investigated in a Baltic Sea spring phytoplankton bloom mesocosm experiment. Climate change was simulated by increasing temperature with 3°C and decreasing pH by 0.4 units via CO<sub>2</sub> addition. Analysis of microbial DNA by denaturing gradient gel electrophoresis (DGGE) of amplified 16S rRNA gene fragments revealed substantial compositional changes during the experiment. Temperature was the major driver of community shifts e.g. several bacterial phylotypes belonging to Comamonadaceae were predominant at ambient temperature and were replaced by members of Cryomorphaceae at high temperature. There was a limited impact of acidification on phylogenetic composition, but when combined with increased temperature an effect on specific phylotypes occurred e.g. a member of Oxalobacteraceae appeared in the high temperature/low pH mesocosm. Our results suggest that although temperature is an important driver in structuring bacterioplankton composition, evaluation of the combined effects of temperature and acidification is necessary to fully appreciate future consequences of climate change on marine bacteria and their ecosystem function.

**SIMILAR BACTERIAL COMMUNITY COMPOSITION BUT DIFFERENT  
BACTERIAL PRODUCTION IN ACIDIC MINING LAKES WITH DIFFERENT pH  
AND LAKE CHEMISTRY**

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As extreme environmental conditions strongly affect bacterial community composition (BCC) we examined whether differences in pH -even at low pH- as well as in iron and sulfate concentrations lead to changes in BCC of acidic mining lakes. Thereby we tested the following hypotheses: (1) diversity of the bacterial community in acidic lakes decreases with reducing pH, (2) BCC differs between epilimnion and hypolimnion, (3) BCC in extremely acidic environments does not vary much over time, and (4) heterotrophic bacterial production (BP) decreases with declining pH. Therefore, we investigated the BCC of three acidic lakes with different pH and iron concentration (mean pH 2.3-3.2, 23-500 mg Fe L<sup>-1</sup>) by denaturing gradient gel electrophoresis (DGGE) and subsequent sequencing of DGGE bands as well as catalyzed reporter deposition-FISH (CARD-FISH). BCC did not significantly vary among the studied lakes nor differ much between water layers. In contrast, BCC significantly changed over time, which is contradictory to our hypotheses. Bacterial communities were dominated by Alpha-, Beta-, and Gammaproteobacteria, whereas Actino- and Acidobacteria rarely occurred. Cell numbers of both free and attached bacteria were positively related to DOC concentration. Bacterial biomass and BP increased with decreasing pH. Light stimulated the formation of ferrous iron, changed the DOC composition, and increased the BP in laboratory experiments suggesting that iron photoreduction caused DOC degradation. This may explain why we found the highest BP in the most acidic and most iron-rich lake. Overall, low pH and extreme chemical conditions of the studied lakes led to similar assemblages of bacteria with pronounced temporal differences. This notion indicates that temporal changes in environmental conditions including food web structure also affect unique communities of bacteria thriving at low pH.



**INSIGHT IN THE IN-SITU SUBSTRATE PREFERENCES OF IMPORTANT FRESHWATER BACTERIOPLANKTON POPULATIONS**Salcher, M.M.<sup>1</sup>, Posch, T.<sup>1</sup>, and Pernthaler, J.<sup>1</sup><sup>1</sup>*Limnological Station, University of Zurich, Switzerland*

Physiological traits of typical freshwater bacterioplankton populations of Lake Zurich, Switzerland, were studied via microautoradiography coupled with fluorescence *in situ* hybridization (MAR-FISH). Fourteen different radiolabelled tracers were used to determine microbial low-molecular-weight (LMW) substance acquisition and substrate specialization. The most abundant microbes, small *Actinobacteria* of the ac1-lineage, were highly involved in amino acid (especially leucine) but also thymidine and glucose assimilation, whereas *Cytophaga-Flavobacteria* of the *Bacteroidetes* did not show a significant incorporation of any of the offered substrates. Microbes of the LD12 lineage (the freshwater sister clade of SAR11, *Alphaproteobacteria*) had a distinct preference for glutamine and glutamate, but only low acquisition of the widely used tracers leucine and thymidine. *Betaproteobacteria* in general represented a highly active fraction of the bacterioplankton. More than 92% of *Betaproteobacteria* could be assigned to 8 species- to genus-like populations with differing substrate specialization. *Limnohabitans* sp. (R-BT) were the most abundant and active *Betaproteobacteria* with high incorporation of almost all tracers, while three other populations differed substantially in their substrate acquisition patterns only sharing high acetate and thymidine uptake. *Polynucleobacter acidiphobus* (PnecB) were also highly specialized for acetate and thymidine, but also for various amino acids. Uncultured LD28 bacteria (beta IV) did not show any incorporation at all, pointing to a methylotrophic lifestyle. Our data shows, that contrary to theoretical considerations about bacteria in oligotrophic habitats, different microbial taxa seem to be highly specialized in the assimilation of LMW compounds. Glutamate and glutamine were utilized by almost all bacterial populations and, therefore, seem to be more suitable for measurements of microbial bulk activity than leucine or thymidine.

### INHIBITION OF HETEROTROPHIC BACTERIA IN SURFACE WATERS OF A HUMIC LAKE IS PARALLELED BY THE FORMATION OF REACTIVE OXYGEN SPECIES

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Photochemical reactions caused by colored dissolved organic matter (cDOM) leads to the production of low molecular weight (LMW) substrates that stimulate bacterial activity and the formation of inhibitory reactive oxygen species (ROS). In order to investigate the impact of ROS generation on bacterial activity we monitored diurnal cycles of ROS formation and bacterial activity in the humic south-west basin of Lake Grosse Fuchskuhle. High solar radiation caused strong inhibition of bacterial <sup>14</sup>C-leucine and <sup>14</sup>C-acetate uptake in surface waters and increased the fraction of membrane-damaged cells assessed by life/dead staining. The inhibition was paralleled by the formation of ROS, which very likely are the agents causing bacterial inhibition. In order to verify our data, cultures representing predominant bacterial phylotypes of the SW basin were incubated in the surface water layer by using dialysis bags. Acetate and leucine uptake and the fraction of membrane-damaged cells were monitored in those cultures. *Novosphingobium acidiphilum* (Alphaproteobacteria) represents a persistent species of the SW basin and was not hampered in activity by solar radiation. In contrast, the activity of *Polynucleobacter necessarius* a predominant Beta-proteobacteria representative was strongly inhibited by high solar radiation as indicated by a low uptake of acetate and leucine compared to early morning samples. Cultures of both strains showed a very high fraction of life cells that did not decrease during daytime hours. Hence, we conclude that *N. acidiphilum* and *P. necessarius* have efficient mechanisms to cope with inhibitory products of photochemical reactions with respect to maintenance of cell integrity. Interestingly, solar radiation mediated formation of inhibitory substances leads to very low activity of *P. necessarius*, but not of *N. acidiphilum*. Hence, photochemical reactions that generate inhibitory ROS affect predominant bacteria of a humic lake in a species-specific manner.

**FLOW CYTOMETRY MEETS MARINE BIOLOGY**Speck, U.<sup>1</sup> and Engel, P.<sup>1</sup><sup>1</sup>*BD Biosciences, Tullastraße 8–12, 69126 Heidelberg, Germany*

Flow Cytometry is used since more than 20 years in aquatic sciences. Since the first detection of *Prochlorococcus marinus* (Chisholm et al, 1988 ) with a FACSCalibur, flow cytometry and sorting has experienced a broad acceptance in aquatic sciences and became an important tool for analyzing single-celled marine and freshwater organisms (bacteria and algae) and even very small marine viruses. Furthermore, several of the well-known fluorochromes in immunology are pigments from marine algae, e.g. PE Phycoerythrin from *Synechococcus*, APC Allophycocyanin (Cyanobacteria) and PerCP Peridinchlorophyll-Protein (Dinoflagellates). Therefore “normal equipped” flow cytometers like the BD FACSCalibur™, BD FACSCanto™, BD LSR II, BD LSRFortessa™ and cell sorters, like BD FACS Aria™ and BD Influx™ are optimal equipped for aquatic sciences in their standard configuration. Many efforts were made in scientific projects to adapt flow cytometry to the special requirements of aquatic sciences. Here the flexibility in the choice of lasers and filter configurations is given with many BD instruments. The BD Influx™ provides several additional upgrades to meet the needs in marine biology: The Small Particle FSC Detector with a high numerical aperture objective lens and a pinhole for discrimination of small particles down to 100 µm. The Polarization Module can measure the depolarization of the exciting laser light by coccolithophores (e.g. *Emiliana huxleyi*) through 2 different orientated FSC-PMTs under the so-called Brewster Angle. Polarization can therefore be used as an additional parameter to discriminate different populations. An especially designed Spectrafluorometer (Monochromator) is able to measure the spectra of photosynthetic pigments of certain populations. Flow Cytometry and Sorting is therefore an appropriate tool to study either single cells and their food uptake (algae, protists, bacteria etc.), its diversity and interaction (ecology) in symbioses and food webs. Sorting of aquatic organisms furthermore enables the possibility to sort unknown populations from field sample, put them either into cultivation or do direct analysis for further studies.

### FROM FJORDS TO OPEN SEAS: ECOLOGICAL GENOMICS OF EXPANDING OXYGEN MINIMUM ZONES

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Regions of low dissolved oxygen known as oxygen minimum zones (OMZs) are widespread oceanographic features currently expanding due to global warming. Although inhospitable to metazoan life, OMZs support a thriving but cryptic microbiota whose combined metabolic activity is intimately connected to nutrient and trace gas cycling within the global ocean. Therefore, OMZ expansion and intensification represents an emerging ecological phenomenon with potentially harmful effects on ocean health and climate balance. In order to understand, respond to, or mitigate these transitions, studies monitoring and modeling dynamics and systems metabolism of OMZ microbiota in relation to physical and chemical oceanographic parameters are imperative. To this end we are using environmental genomic approaches to chart microbial community responses to changing levels of water column oxygen-deficiency in the northeastern subarctic Pacific Ocean (NESAP). The NESAP is one of the world's most extensive OMZs and provides an exceptional model system for long-term observation and process-oriented studies of OMZ phenotypes.

**LINKING PROKARYOTIC ACTIVITY AND BIOGEOCHEMISTRY IN THE NORTH ATLANTIC DEEP WATER USING OPTIMUM MULTIPARAMETER ANALYSIS**

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During three cruises conducted in the eastern North Atlantic, we followed the North Atlantic Deep Water (NADW) from 65°N to 5°S and examined the development of nutrient concentrations and prokaryotic activity in the NADW. The NADW is a mixture of the Iceland Scotland Overflow (ISOW), Labrador Sea (LSW), Lower Deep (LDW) and Mediterranean Overflow (MOW) water types. Using an optimum multiparameter model (OMP) including salinity, temperature and silicate we partitioned the mixture into the proportions of the source water types along the transect. The relationship between geochemical (dissolved oxygen, organic and inorganic nutrients) and microbial (prokaryotic biomass and prokaryotic production) variables in the salinity maximum of the North Atlantic was examined with regression models corrected for the effect of mixing. Hence the comparison of uncorrected measurements and the modelled data yields the variability in the NADW that is not due to the influence of mixing from the different water masses. Uncorrected prokaryotic biomass and prokaryotic heterotrophic production decreased exponentially in the NADW from the North towards the equator, reflecting the generally higher productivity in the subpolar Atlantic. A significant fraction of the variability (30-50%) in the corrected biological parameters was not explained by the mixing model. However, N:P ratios of remineralized organic matter were similar to Redfield suggesting that particulate organic matter is the major substrate for the prokaryotic community in the NADW. Overall the variability in the prokaryotic turnover time was related to the variability of high DNA (HDNA) containing cells indicating that the fraction of HDNA cells dominate the local pattern of prokaryotic activity found in the NADW.

**CARBON, NITROGEN AND PHOSPHORUS RESOURCE SUPPLY AND UTILIZATION  
FOR COASTAL PLANKTONIC HETEROTROPHIC BACTERIA IN A GRADIENT OF  
NUTRIENT ENRICHMENT: RESULTS FROM INVERSE MODELLING OF  
A MESOCOSM EXPERIMENT**

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DOM in the sea is a large C reservoir affecting the global C cycle. Progress in our understanding of the DOM dynamics is hampered by lack of methods. We present data from seven mesocosms representing a gradient in daily nutrient loading where we have used inverse modelling to estimate all C, N and P flows in an idealized food web with seven functional biotic groups. The gradient in nutrient addition created an autotrophic production of 7 to 314  $\mu\text{g C L}^{-1} \text{ day}^{-1}$  (mean for 20 days). The production of DOC decreased from 240 to 40% of the autotrophic production, and was mainly by heterotrophs (80–58%). Half of it was produced by heterotrophic bacteria (BAC; 48±8%), either directly (excretion) or indirectly (viral lysis). The consumption of DOC by BAC decreased from 133 to 86% of the production within the gradient, and DOC accumulated at nutrient loading from natural to high. Despite the large consumption of DOC by BAC, the microbial loop supplied only 5.5 to 0.2% of the C consumed by copepods (average 2.3%) and  $\frac{3}{4}$  came the long way via ciliates. The production of dissolved N and P was mainly by heterotrophs (92±2% of DN, 92±4% of DP), even though autotrophs produced a significant part of organic N and P (26±7% of DON, 21±8% of DOP). The relative significance of the functional groups to DON and DOP production was similar to the DOC production. For the production of inorganic N and P BAC predators produced 50% of DIN and 70% of DIP at low and moderate loadings, with averages of 37±16% and 66±14%, respectively. The contribution by BAC was low for DIN (12±6%) and zero for DIP, and BAC competed for DIP. For the remineralisation of N and P the significance of BAC is as N and especially P rich food particles. DOP accumulated to a higher degree than DOC, despite high demand for P. Turnover time of DOM decreased from around 80 to 12 days with increasing loading. These data provide new insight into the role of the various functional groups of organisms for the cycling of DOM.

**UNDERSTANDING MICROPHYTOBENTHIC INDUCED CALCIFICATION –  
A MODELING APPROACH**

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Benthic (de)calcification is a microbially driven process, which was thought to be affected by changes in the overlying water pH caused by ocean acidification. However, recent studies have revealed that benthic microorganisms in sediment and mats are rather resilient to pH changes, as they generate large local pH shifts due to photosynthesis and respiration. Microsensor studies in diatom-dominated carbonate sediment from Bait Reef and Heron Island, Australia, as well as cyanobacterial biofilms from karst water creeks, show pH variations between 7.5 and 9.5 due to microbial processes. The shift of pH from night to day seems to be consistent, although the settings differ. Moreover, pH-changes and calcification appear to be not necessarily tightly coupled to the day-night cycle of photosynthesis and respiration. In some systems pH remains elevated and calcification continues although oxygen profiles indicate the cessation of photosynthesis. In order to understand these observations, we have constructed a reaction-transport model including photosynthesis, aerobic respiration, sulfate reduction, sulfide re-oxidation, calcification and dissolution of calcite as reactions and diffusion as transport term. Acid-base reactions also included in the model allow calculating of pH-variations. So far, our dynamic model indicates that carbon dioxide limitation of photosynthesis and accompanied buffer reactions cause the pH to rise to approximately 9.5, but commonly not higher during the day. Further work will focus on the decoupling of calcification and photosynthesis. The model, once completed and tested, will provide a tool to understand benthic (de)calcification and its relation to environmental changes such as ocean acidification.

### INFLUENCE OF PERIODIC REDOX CHANGES ON CHEMISTRY AND MICROBIAL ACTIVITY AT THE SEDIMENT-WATER-INTERFACE (SWI)

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Short-term redox changes typically occur in sublittoral sediments exposed to internal wave forcing. While physical processes related to seiches have been well studied, little is known about biogeochemical consequences to frequently altering redox intensities. In response to this quest, we performed mesocosm studies with intact sediment cores from oligotrophic Lake Stechlin that were incubated under controlled redox conditions. While one core each was kept permanently oxic (A) and anoxic (B), the SWI of the third (C) core was exposed to several daily shifts between oxic and sulfidic overlying water under continuous monitoring of oxygen, redox potential, pH, sulfide and ammonium. To study changes in activity of the microbial community, we took sediment subsamples for molecular analyses. After ten days, cores A, B and C were dissected for the analysis of oxic vs. anoxic sedimentary bacterial protein production (BPP) at the SWI. Additionally, porewater profiles of dissolved organic carbon, methane, phosphate and ammonium concentrations were measured. Our preliminary results indicate that periodic alteration of redox conditions results in significant changes of methane and phosphate depth profiles. Oxic BPP was highest at the SWI of core A compared to the anoxic BBP of core B. In contrast, in core C bacterial production was always high either under oxic or anoxic incubation conditions. Our first results show that periodic changes in redox conditions lead to an overall increased bacterial activity in freshwater sediments. However, more work is needed for quantifying these effects in situ and for a better understanding of their ecological consequences for lake carbon and energy cycling.



**ECOLOGICAL NICHES OF MAT-FORMING SULFIDE-OXIDIZING BACTERIA AT DEEP-SEA COLD SEEPS**

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In marine chemosynthetic ecosystems, mat-forming sulfide-oxidizing bacteria mostly thrive at chemoclines where energy-rich, reduced substances (H<sub>2</sub>S) rising from the seafloor get into contact with electron acceptors (O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>) contained in the bottom water. There, these bacteria can significantly contribute to chemosynthetic primary production and may represent efficient benthic filters against the toxic gas hydrogen sulfide when occurring in high biomasses. Here, we investigated dense assemblages of four prominent members of mat-forming sulfide oxidizers associated with cold seeps on the Nile Deep Sea Fan (Eastern Mediterranean): (I) giant *Beggiatoa* filaments (20–135 µm diameter) co-occurring with (II) vacuolate-attached filaments (VAF; 40–60 µm diameter), (III) giant spherical *Thiomargarita* cells (24–65 µm diameter) and (IV) small *Arcobacter* spp. (2 µm). The mats were found in different habitats of the Amon mud volcano (*Beggiatoa* and VAF, *Thiomargarita*) and in a pockmark-dominated area (*Arcobacter*). In situ microsensor measurements of sulfide and oxygen gradients revealed that each of the dominating bacteria occupied a different ecological niche. Filamentous sulfide oxidizers were associated with steep gradients of oxygen and sulfide in the sediment. *Thiomargarita* outcompeted other sulfide oxidizers when oxygen and sulfide concentrations varied temporarily, the fluctuations most likely caused by periodic overflows of sulfidic brine. *Arcobacter* dominated when oxygen and sulfide overlapped above the seafloor, but were also found in lower numbers beneath the filamentous and *Thiomargarita* mats. Concluding, this study significantly enhances our understanding of the ecological potential and genetic diversity of mat-forming sulfide-oxidizing bacteria in the deep sea.

**CHARACTERIZATION OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS)  
ASSOCIATED WITH CYANOBACTERIA IN LACUSTRINE, HYPERSALINE MICROBIAL  
MATS (KIRITIMATI ATOLL, CENTRAL PACIFIC)**

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In microbial mats, oxygenic phototrophs are the primary producers, coupling light energy to CO<sub>2</sub> fixation, and sometimes fix N<sub>2</sub>. Through EPS production, they have an important role in precipitation processes of calcium carbonate. Microbial mats of hypersaline lakes on the atoll of Kiritimati were investigated in order to analyze the structural composition of EPS glycoconjugates in different zones. Steep gradients of oxygen, pH, and calcium as well as very strong light attenuation were measured *in situ* within thick (up to 15 cm) microbial mats of Lake 21 in March 2011. The mats display a clear colour-zonation, and microbialites with a unique reticulate fabric were located below the microbial mats. The photosynthetic layer was dominated by cyanobacteria (genus *Cyanothece*). Analysis of EPS glycoconjugates in the photosynthetic, green layer was done by using fluorescent lectin-binding analysis (FLBA) in combination with laser scanning microscopy (LSM). All commercially available lectins were applied to unfixed, cryo-sectioned samples. An unexpected high number of lectins (60 out of 73) bind to EPS glycoconjugates in the photosynthetic layer. Cell associated glycoconjugates were differentiated in intracellular, envelopes, and coverage structures. In case of microcolonies, some lectins specific for mannose, glucose, and amino sugars bind preferentially to granules located in the intercellular space. Other lectins bind to glycoconjugates which occupied the whole intercellular space of the microcolonies. Apart from cyanobacteria-associated glycoconjugates, filamentous and rosette-like glycoconjugate structures were detected within the organic matrix of this layer. These results show that EPS in the photosynthetic layer of the microbial mats may have different functions, (i) protection against physicochemical fluctuations, (ii) substrate for metabolism of other microorganisms, and (iii) inhibition of calcium carbonate precipitation by binding of calcium ions.

**THE 'OMICS' OF SYMBIOTIC ASSOCIATIONS BETWEEN MARINE INVERTEBRATES AND CHEMOSYNTHETIC BACTERIA**Dubilier, N.<sup>1</sup><sup>1</sup>*Max Planck Institute for Marine Microbiology*

There is an emerging recognition that symbiotic associations with bacteria play a fundamental role in the nutrition, development, ecology and evolution of marine invertebrates. Metagenomic analyses have provided numerous insights into the metabolic interactions that are possible between animals and their microbial consortia, but metaproteomic and metabolomic analyses can go a step further by providing detailed information on the actual metabolic and physiological processes that occur in symbiotic associations. Combining metaproteomic and metabolomic analyses is a particularly powerful approach to the functional analysis of microbial communities but has so far only very rarely been applied.

The symbiotic association between the gutless marine worm *Olavius algarvensis* and its five bacterial symbionts is an ideal model system for the study of microbial consortia because of its specificity, temporal stability, and relatively low diversity. Metagenomic analyses of the bacterial symbionts of this worm revealed a number of hypotheses on how the symbionts have enabled their hosts to completely reduce their digestive and excretory systems (Woyke et al. Nature 2006). However, many questions were left unanswered, in particular how the symbiosis can successfully thrive in the extremely energy and nutrient poor Mediterranean sediments inhabited by these worms. In my talk, I will describe how a combined metaproteomic and metabolomic approach has enabled us to gain novel insights into the metabolic processes and interactions that drive the symbiotic association between *O. algarvensis* and its microbial consortium. I will focus on our discovery that the symbionts use energy sources not previously known to play a role in marine invertebrate symbioses, namely hydrogen and carbon monoxide, and describe how our in situ analyses of the *O. algarvensis* habitat have revealed the sources of these unusual energy sources.

## THE MICROBIAL COMMUNITY IN THE DIGESTIVE TRACT OF THE SMALL FRESHWATER CRUSTACEAN *DAPHNIA MAGNA*

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In aquatic systems, bacteria play a key role in the carbon cycle but their importance in zooplankton guts remains mostly unknown, although their presence was repeatedly documented. Recently, denitrification by ingested bacteria in anoxic guts of benthic aquatic invertebrates was demonstrated indicating their possible symbiotic participation in digestion. However, the guts of most important zooplankton organisms, e.g. *Daphnia* spp. which are a significant trophic link in freshwater systems, are much smaller and thus probably only partly anoxic if at all. In addition, *Daphnia* constantly take up bacteria from the surrounding environment. This leads to the question how the microorganisms interact with their host, i.e., whether they symbiotically participate in digestion, whether they prevent success of pathogens or whether they compete for food. The aim of this study was to characterise the intestinal microbial community of *Daphnia* and to estimate if it is considerably influenced by the surrounding environment. Therefore, the intestinal microbial community of *D. magna* clones was analysed via 16S rDNA clone libraries. To investigate the variability of their microbiota, *Daphnia* were incubated under different conditions (food sources, exposure to bacteria and environmental water) while changes in the intestinal community composition were followed by T-RFLP. The *D. magna* microbiota was dominated by clones affiliated to the  $\beta$ -proteobacterium *Limnohabitans* sp., common planktonic freshwater bacteria, which were described to respond rapidly to environmental changes. Overall, the intestinal microbial community did not contain known fermentative or obligately anaerobic gut bacteria. *Limnohabitans* spp. were also always prominent in the T-RFLP profiles despite changing food sources, thus indicating that they are specialised stable community members. Other intestinal microorganisms were stimulated by varying food sources but never dominated the community.

**WHEN SYMBIOSIS TURNS BAD – OPPORTUNISTIC BACTERIAL PATHOGENS IN COASTAL ECOSYSTEMS**

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One of the projected consequences of global warming is that an increased magnitude of thermal stress will increase the frequency and intensity of disease. Bacteria are often involved in such diseases, but many are opportunistic pathogens and express their virulence only under certain environmental conditions that facilitate themselves and/or weaken the host's defenses. One example is "summer disease syndrome" of Pacific Oyster populations caused by an interaction of viral and bacterial agents at high temperatures and that has been shown to affect invasive oyster populations in the European Wadden Sea. We identified environment sensitive parts of the microbial community associated with oyster tissue from several oyster populations using next generation sequencing and then focused on the genus *Vibrio* sp. as a temperature sensitive pathogenic candidate. Using bacterial isolates obtained from hemolymph we conducted several laboratory infection experiments determining the environmental conditions under which candidate pathogens express their virulence in relation to host stress status. We complemented this data by following the microbial community change during an acute infection with *V. tubiashii* to clarify the role of the resident hemolymph microbial community for determining the outcome of an infection.

### INTERACTIONS BETWEEN THE CILIATE *STENTOR AMETHYSTINUS*, GREEN ALGAE AND PROKARYOTES IN LAKE STECHLIN

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*Stentor amethystinus* usually lives in the benthos of freshwater ecosystems. In Lake Stechlin it also occurs in the pelagic zone and seasonally can substantially contribute to pelagic primary production (up to 60%). *S. amethystinus* cells are 250-500 microns long and have a spherical macronucleus (20-30 microns long) with many (20) micronuclei. Ciliates (e.g. *Paramecium aurelia*) can harbour endosymbiotic bacteria in their nucleus (Müller 1856), which is also the case for *S. amethystinus*. While being heterotrophic, *S. amethystinus* often associates itself with green algae to form a symbiosis with algae. Interestingly, *S. amethystinus* is associated with *Chlorella* in North America, but the Lake Stechlin population contains *Micractenium* species. The alga-ciliate system can be also inhabited by cyanobacteria and heterotrophic bacteria, thus forming a complex symbiotic community. Since we are able to grow the cyanobacteria endosymbiont as well as the major bacterial endosymbiont in pure or enrichment cultures we assume that these endosymbioses are still *in statu nascendi*. In this presentation, we will highlight specific interactions between the host and its symbionts and point to their functional consequences.

**STRAIN-SPECIFIC DIFFERENCES IN FOOD VALUE OF CLOSELY RELATED BACTERIA AFFILIATED WITH THE GENUS LIMNOHABITANS FOR NATURAL FLAGELLATE ASSEMBLAGES**

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Different bacterial lineages can have different value as food of heterotrophic flagellates (HNF), thus differently modulating HNF growth and community composition. Four strains of the genus *Limnohabitans* (the R-BT065 subcluster of Betaproteobacteria) of different cell size and shape, one *Polynucleobacter necessarius* strain (PnecC, Betaproteobacteria) and one strain affiliated with the Luna 2 cluster of Actinobacteria were fed to (i) a natural HNF community originating from a freshwater reservoir and pregrown in 5 µm filtrate for two days, and (ii) to an axenic culture of a mixotroph, *Poterioochromonas* sp. The amount of the respective prey bacteria added into the HNF community represented approximately 30-fold the background bacterial biomass (mostly grazing-resistant flocs and filaments) present in the HNF treatments, but were the only particulate prey food for *Poterioochromonas* sp. All the *Limnohabitans* strains as well as the *Polynucleobacter* strain yielded significant positive HNF population growth while the actinobacterial strain did not. In contrast, *Poterioochromonas* sp. showed marked positive growth only when fed by the *Limnohabitans* strains. Notably, there were significant prey-related differences in HNF and *Poterioochromonas* sp. growth parameters (growth rate, lag phase), which cannot be simply related only to size or biovolume of the bacterial prey. Overall, this study showed high food values of all the *Limnohabitans* strains, generally omnipresent in circum-neutral freshwater ecosystems, as flagellate food. Thus, the genus *Limnohabitans* obviously play a crucial role in channeling a significant part of bacterial carbon to higher trophic levels. However, we also documented significant bacterial prey-specific growth responses of flagellates reflecting also a possible shift in their community composition.

**MARINOBACTER ADHAERENS HP15 IS REQUIRED FOR AGGREGATION OF THE DIATOM, THALASSIOSIRA WEISSFLOGII**

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Aggregation of diatoms is an important process in marine ecosystems leading to the settling of particulate organic carbon predominantly in the form of marine snow. Exudation products of phytoplankton form transparent exopolymer particles (TEP), which act as adhesives for particle aggregation. Heterotrophic bacteria interacting with phytoplankton may influence TEP formation and phytoplankton aggregation. This bacterial impact has not been explored in detail. We hypothesized that bacteria attaching to *Thalassiosira weissflogii* might interact in a yet-to-be determined manner, which could impact TEP formation and aggregate abundance. The role of individual *T. weissflogii*-attaching and free-living new bacterial isolates for TEP production and diatom aggregation was investigated *in vitro*. *T. weissflogii* did not aggregate in axenic culture, and striking differences in aggregation dynamics and TEP abundance were observed when diatom cultures were inoculated with either diatom-attaching, i.e. *Marinobacter adhaerens* HP15, or free-living bacteria. The data indicated that free-living bacteria may not influence aggregation whereas bacteria such as *M. adhaerens* HP15 may increase aggregate formation. Interestingly, photosynthetically inactivated *T. weissflogii* cells did not aggregate regardless of the presence of bacteria. Comparison of aggregate formation, TEP production, aggregate sinking velocity, and solid hydrated density revealed remarkable differences. Both, photosynthetically active *T. weissflogii* and specific diatom-attaching bacteria were required for aggregation. It was concluded that interactions between heterotrophic bacteria and diatoms increased aggregate formation and particle sinking and thus may enhance the efficiency of the biological pump. *M. adhaerens* HP15 has become a genetically accessible model organism, which allows the molecular dissection of the diatom-bacteria interaction.



**ROLE OF MARINOBACTER ADHAERENS HP15 FLAGELLUM DURING ITS INTERACTION WITH THALASSIOSIRA WEISSFLOGII**

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The vertical sinking of marine aggregates and their subsequent sequestration to the sea floor majorly contribute to the oceanic carbon sink. Understanding the processes that influence the dynamics of marine aggregate formation therefore, is of major relevance to further our understanding of the functioning of the oceanic carbon cycle. Recent studies have shown a direct correlation between aggregated volume and the concentration of transparent exopolymeric particles (TEP). This further strengthens other studies on TEP where these particles have been described to impact the dynamics of marine aggregate formation.

Phytoplankton and bacteria are known to produce TEP precursors and TEP. The interactions between *Thalassiosira weissflogii* and *Marinobacter adhaerens* HP15 have been shown to be instrumental to enhance TEP production and marine aggregate formation. Our current research aims at understanding micro-scale phytoplankton-bacteria interactions in marine aggregate formation. It is hypothesised that bacterial motility – mediated by flagella and Type IV pili – might enable the interaction and ultimately impact TEP production and aggregate formation. In this study, we work with the established bilateral model system between *M. adhaerens* HP15 and *T. weissflogii*. By creating *M. adhaerens* HP15 flagellum-defective ( $\Delta$ fliC) and flagellum-deficient ( $\Delta$ fliG) mutants, we examine their behavior in terms of attachment to the diatom and with respect to TEP production and marine aggregate formation.

### AGGREGATION OF DIATOMS – EFFECTS OF BACTERIA AND TEMPERATURE

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Aggregation of diatoms is one of the most important processes for the export of particulate organic material (POM) from the euphotic zone in marine pelagic ecosystems. Heterotrophic bacteria play an active role in the aggregation of diatoms and transformation of aggregated POM. As heterotrophic microbial processes are favoured more by increasing temperature than autotrophic processes, we hypothesize that aggregation enhances with increasing temperature.

To test this hypothesis, we analyzed the aggregation and bacterial colonization of a mesocosm-generated spring diatom bloom at Helgoland during the exponential and stationary phase at the ambient temperature (6°C) and at 11°C in rolling tank experiments. Further, we examined the aggregation potential of *Thalassiosira rotula* as a function of temperature between 6 and 16°C and the natural bacterial community.

In the exponential growth phase of the bloom and at both temperatures aggregates were small and rather compact whereas later aggregates became larger and more fluffy. The addition of cells of *T. rotula* enhanced aggregation at the higher temperature and in particular towards the end of the bloom. Sphingobacteria and Flavobacteria always dominated on the aggregates and irrespective of temperature but in the stationary phase Gammaproteobacteria constituted significantly higher proportions than in the exponential phase. These results indicate that increasing temperature enhances aggregation of the diatom-dominated spring phytoplankton, especially in *T. rotula*-dominated blooms.

In more detailed experiments with *T. rotula* aggregation was positively correlated to temperatures only under xenic conditions and greatly enhanced in the presence of bacteria relative to axenic algal cells. Hence, the associated bacteria appear to greatly affect the aggregation of diatoms and that temperature has only little direct effect on aggregation.

**BACTERIAL COAGGREGATION IN WATERS: WHEN COOPERATION  
SUPPORT GROWTH**

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Aggregative behavior is a well known anti-predator strategy of many animals (primates, herbivores, birds, fish, zooplankton) in air, water and soil. The advantages against predation given by grouping are various, and in several cases compensate or exceed the disadvantages given by living in a spatially limited and overcrowded environment (e.g., intraspecific competition, increased costs of motility, higher transmission of diseases and parasites, reduced oxygen availability). Aggregation is also a successful anti-predation strategy for free-living aquatic bacteria to escape from grazing by heterotrophic nanoflagellates. Coaggregation of different species has been detected between organisms from a variety of taxa (algae, bacteria, protozoa); it may influence a number of ecological variables (from diversity to the control of C fluxes in oceans). We demonstrate that a bacterium with limited aggregation potential (*Brevundimonas* sp.) could form large aggregates only with the participation of a second strain (*Arthrobacter agilis*). The coaggregation of the two strains provided *Brevundimonas* with a means to better avoid predation by a flagellate, whereas *Arthrobacter* seemed to profit from reduced competition. Moreover, the coaggregates apparently provided a surface for the attachment of the predator, potentially allowing for a more efficient foraging on planktonic cells, as reflected in substantially higher flagellate densities. Altogether, the cooperative 3 species experimental system supported the formation of significantly more total biomass than every single two species competitive or predator-prey system.

**MAJOR FRESHWATER BACTERIOPLANKTON GROUPS: TRENDS IN DISTRIBUTION OF LIMNOHABITANS AND POLYNUCLEOBACTER ALONG A pH GRADIENT OF 72 LAKES AND PONDS**

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The major goals of the presented study were (i) to reveal the distribution of the key subgroups of *Betaproteobacteria*, namely of *Polynucleobacter*, *Limnohabitans* and *Methylophilus* genus, by employing following fluorescence in situ hybridization (FISH) probes: BET42a, PnecABCD-445, PnecC-16S-445, PnecB-23S-166, R-BT065 cluster (part of *Limnohabitans* genus) and Met1217 on a large set of different habitats located in Central Europe (ii) to identify environmental parameters that determine the distribution and abundance of the groups in their natural habitats (iii) to identify conditions under which the two major genera, *Polynucleobacter* and *Limnohabitans*, co-occur and if they are competing for certain resources (iv) to reveal why these groups are able to co-exist, what is the factor allowing them to co-exist, and last but not least (v) what proportion of the total *Betaproteobacteria* they constitute together.

*Betaproteobacteria* formed on average almost one third of all the heterotrophic bacteria along the pH gradient of the 72 sampled habitats. *Polynucleobacter* bacteria of the C subcluster (PnecC) displayed a clear preference to low pH habitats, where its abundance ranged between 5 to 60% of all detected bacteria, although the PnecC was also detectable (in much lower percentages) in the alkaline habitats. *Limnohabitans* genus, approximated by the R-BT065 oligonucleotide probe (on average less than 9% of all bacteria), showed a clear trend of increasing abundance with the incrementing pH, however it was present in lower quantities also in the habitats of very low pH (close to 3.8). Beta IV clade (*Methylophilus* probe MET1217) was never abundant, reaching on average 0.9% of all bacteria, displaying no clear trend across the investigated habitats. By using the general probes for *Polynucleobacter*, *Limnohabitans* and *Methylophilus*, we were able to cover almost three quarters (72.3%) of all *Betaproteobacteria* averaged across the wide range of sampled habitats.

**COORDINATED MOVEMENT OF BEGGIATOA FILAMENTS IN OXYGEN/SULFIDE GRADIENTS AND THE EFFECT OF BLUE/GREEN LIGHT**

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The filamentous sulfide oxidizing bacteria of the genus *Beggiatoa* are gradient organisms. When grown autotrophically in opposing gradients of oxygen and sulfide, the filaments establish a thin and well defined mat in the overlapping zone of oxygen and sulfide. We found that several factors modify or disturb this typical behavior. First, when inoculated into fresh medium, *Beggiatoa* filaments exhibit movement in a coordinated fashion and form one mat even if they are offered two overlapping zones of oxygen and sulfide. This mat formation is disturbed when cyclic adenosine monophosphate (cAMP) or N-acyl-homoserine lactone (AHL) is added to the culture. Second, in older cultures, when grown under steep sulfide gradients, a part of the population (ca. 10–30% of all filaments) move out of the oxic/anoxic interface into the anoxic zone below the mat. We could show that these filaments use internal sulfur as electron acceptor for the oxidation of internally stored PHA. Possibly, the sulfur is respired in the anoxic zone to avoid cell disruption due to excessive amounts of sulfur accumulated in the oxic zone by sulfide oxidation. Third, this downward movement of filaments can be induced by light in the blue to green region. Video recordings of moving filaments show that passing filaments can “drag” other filaments along, leading to a swarm-like movement of filaments. Freshly inoculated cultures, which are exposed to blue light, are not able to form a mat and die. Our results indicate possible communication amongst *Beggiatoa* filaments, which is influenced by filament density, cAMP, AHL and blue/green light.

**BIOFILM INTERACTIONS: DOES TEMPERATURE MATTERS AT THE BEGINNING OF A RELATIONSHIP?**

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Organisms in biofilms compete for space and nutrients, bacteria make use of algal exudates, ciliates feed on bacteria, and they communicate. Communication among bacteria through the production of signal molecules (quorum sensing) is used to monitor density. We investigated if quorum sensing is affected by autotrophic organisms in biofilm at two temperatures. We also tested temperature effects on the algal-bacteria interaction and functional diversity. A laboratory experiment was designed to analyze the effect of temperature on the first steps of colonization (2-35 days) under dark and light conditions, considering: low temperature (11-14°C) and high temperature (14-17°C). Quorum sensing was determined using two strains as exogenous signal inducers. Functional diversity was studied by Biolog Ecoplates. The effect of temperature on bacterial colonization depended on the light condition. On day 35, bacteria were more abundant at low temperature treatments and algal growth was higher at high temperature, when also photosynthetic efficiency was higher. Ciliates were negatively affected by temperature while rotifers were positively affected. Quorum sensing was detected on day 2, only in high temperature-light treatments. The heterotrophic use of polysaccharides ( $\beta$ -glucosidase) and organic phosphorus (phosphatase) were not affected by temperature but they were higher at light conditions. Peptidase activity was enhanced by temperature at light. Functional diversity was affected by temperature and light and communities were more different on day 28 than on day 7. The results suggest that increases in river water temperature up to 3°C might determine changes in river biofilm dynamics; and interactions among the different communities, as some effects on bacteria depended on the presence of light. Temperature changes in the activity of microbial communities and the diversity of heterotrophic capabilities might have immediate or lasting effects on ecosystem functioning.

**INTERACTIONS UNDER ENVIRONMENTAL STRESS**

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Although still largely understudied, it is known that many phytoplankton species have obligate host-specific parasites that may influence plankton dynamics. In addition, phytoplankton are non-target organisms for a multitude of pesticides, especially for herbicides, that enter aquatic ecosystems via run-off from the land. Conventional wisdom holds that organisms exposed to chemical stressors lack energy for mounting up defenses against parasite attack and therefore become more susceptible to infection. However, ecosystem stressors may reduce host density and/or host quality and therefore have negative effects on parasite fitness. Furthermore, hosts may harbor genetic variation for traits involved in infection and pollution resistance. We investigated these complex interplays experimentally using the microbial model system *Asterionella formosa* (freshwater diatom) and *Zygorhizidium planktonicum* (chytrid fungus).

A cross-infection experiment was conducted with 5 host genotypes, 1 parasite genotype and a sublethal concentration of the herbicide diuron. We found no evidence for genetic variation for resistance traits. Instead, there was a clear effect of host phenotype on stress response. Although the outcome of the experiment resulted in a higher parasite specific growth rate and higher prevalence of infection in the presence of diuron, the infection dynamic differed between treatments. We investigated this further with experiments where we disentangled the effects of environmental variation on single parasite fitness traits such as transmission efficiency, development time and zoospore production. It is discussed how temporal variation of the host environment can interact with environmental stressors to modify parasite life history traits and how this may have implications for disease dynamics.

### HOW DO BACTERIAL COMMUNITIES ASSEMBLE AND DOES IT MATTER FOR THEIR FUNCTION IN ECOSYSTEMS?

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The classic view of microbial communities is that “Everything is everywhere but the environment selects”, i.e. representatives of microbial taxa are readily dispersed among sites but the contemporary local conditions determine community composition. This view has, in recent years, been challenged and it has been proposed that community composition can be influenced also by the limitation of dispersal or massive dispersal of microbial cells between sites. I will in this talk summarise evidence from different studies of mainly inland water bacterial communities regarding the importance of different mechanisms for community assembly. Further, I will present how these results relate to statistical as well as theoretical models, for instance, within the metacommunity framework. One interesting issue of microbial diversity is its potential importance for the functioning of bacteria in ecosystems. However, disparate results have been obtained regarding the strength of the coupling between community diversity and functioning. Recent research has shown that metacommunity processes may be of importance not only for diversity but also for the functioning of bacterial communities. Therefore, I will also discuss how dispersal of bacteria among communities can affect ecosystem functioning and the coupling between community diversity and functioning.



**TEMPORAL VARIATION OF ASSEMBLY MECHANISMS  
IN A BACTERIAL METACOMMUNITY**

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The compositional variability of communities across space, i.e.,  $\beta$  diversity, is influenced by different assembly mechanisms, which place varying weight on local within-habitat factors, such as environmental conditions or species interactions, and regional factors that are related to dispersal from a regional species pool to the local community. Recently it has become clear that several assembly mechanisms act simultaneously, however, little is known about how they change over time. Here we implemented a field survey where we sampled a bacterial metacommunity consisting of 17 rock pools located at the Swedish Baltic Sea coast at 11 occasions during one year and determined to which extent communities were structured by local species sorting processes (selection by local habitat conditions) and neutral processes due to stochastic migration of taxa from within and outside the metacommunity. We found that neutral processes were relatively more important during times when environmental conditions were homogeneous and dispersal estimated to be high, resulting in low  $\beta$  diversity. On the contrary,  $\beta$  diversity was highest at times of high overall productivity and environmental heterogeneity in the metacommunity, possibly due to stronger species sorting as well as founder effects. In summary, our study clearly suggests that there are seasonal differences in the relative importance of different assembly mechanisms, which further results in temporal changes in  $\beta$  diversity in the metacommunity.

### RECRUITMENT SOURCES OF FRESHWATER BACTERIOPLANKTON TAXA ALONG A MARINE GRADIENT

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It is now well described that aquatic bacterial communities are extremely diverse. Currently there is an intense debate whether bacterial biogeography is determined exclusively by local contemporary environmental conditions, or whether dispersal hinders exists. For instance, it is clear that freshwater and marine communities differ with regards to the dominant phylotypes, but less is known about the rare taxa. Here, we explore the possibility of the existence of seed banks of marine bacteria among the rare bacteria in freshwater environments by exploring whether marine bacterial taxa can be retrieved from lake communities after exposure to marine conditions. We focus on two potential dispersal origins of marine bacteria: sediments resuspension and air deposition. To do this, we constructed batch cultures where lake, air and sediment bacterial communities were regrown for 3 weeks in their respective lake water set at different salinities (100, 75, 50, 25 and 0% marine water; vol/vol). We sampled three lakes of comparable trophic status (meso-oligotrophic) and longitude but differing in their coastal proximity: Lake Jonsvatn (Norway) is close to the Norwegian sea (35 ppt) coast, Lake Åresjön is located in the inland (Jämtland, Sweden) and Lake Hassellasjön (Medelpad, Sweden) is close to the Baltic sea (4 ppt) coast. Bacterial community composition was determined using 454 pyrosequencing at the beginning (3 lakes, Norwegian and Baltic sea) and the end of the experiment, i.e. after the marine perturbation. We hypothesize that we should retrieve marine taxa in lake water if these are present or stored as a seed bank in the lake water or sediments, or rapidly dispersed via atmospheric deposition. With regard to the latter, the different lakes may present different patterns in their response to marine perturbation due to differences in air deposition from the sea, so that marine taxa might be retrieved to a greater extent in lakes closer to the sea coast.

**AEROSOLIZATION OF MARINE BACTERIA: A MESOCOSM STUDY.**Fahlgren, C.<sup>1</sup><sup>1</sup>*Linnaeus University Kalmar*

Sea spray from Surface Ocean is one of the key physical processes contributing with aerosols to the atmosphere. The potential of different bacterial species to be ejected and transported to the atmosphere is poorly understood. We analyzed the bacterial diversity in seawater and experimentally generated aerosols derived from three locations in Kongsfjorden, Svalbard. Construction of 16S rRNA gene clone libraries from paired seawater and aerosol samples resulted in a total of 1311 sequences that clustered in 149 bacterial OTUs and 36 phytoplankton OTUs. The fjord water displayed a community composition that varied substantially. Dominant members of both seawater and aerosols were Bacteroidetes, Alphaproteobacteria (notably SAR11 clade bacteria) and Gammaproteobacteria along with Betaproteobacteria, Verucomicrobia and Actinobacteria. Comparisons of the entire diversity data set from four experiments revealed that the aerosol samples differed greatly from their paired seawater sample in three cases, while in one case the paired samples were similar. Further, occurrence of particular OTUs revealed that, although in a majority of cases OTUs present in the seawater were also found in the aerosols, at any particular time many OTUs were either selectively enriched or not at all aerosolized. Curiously, analysis of cultured bacteria revealed a strikingly high proportion of pigmented bacteria in the aerosols compared to the source seawater. Thus, selection during aerosol formation could provide an explanation to the frequently observed high proportions of pigmented bacteria in the natural atmospheric environment. In conclusion, a wide diversity of bacteria and phytoplankton can be emitted from seawater to the atmosphere, and that the transfer efficiency for specific OTUs can vary over time and space. Our findings suggest that determining the identity and diversity of microorganisms in bioaerosols is critical for deducing their role in atmospheric processes.

### FRAGILITY OF AQUATIC MICROBIAL COMMUNITIES TOWARDS A PULSE PERTURBATION

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The longstanding debate around the relationship of diversity and stability of communities and ecosystems has resulted in numerous measures and definitions of stability. Stability is often found to increase with diversity, but the mechanisms behind this correlation are poorly understood. To address the fragility towards breakdown of ecosystem functioning in a bacterial community, we manipulated its diversity using a dilution to extinction approach. Communities along a diversity gradient were grown under constant environmental conditions in chemostats. After two weeks of continuous cultivation, a pulse perturbation was applied by the addition of NaCl. We measured community composition by molecular fingerprinting of the 16S rRNA gene and community abundance using flow cytometry during the course of the experiment. Local Similarity Analysis was applied to extract the network of association patterns of operational taxonomic units in the communities before and after the perturbation. We found that the pulse perturbation leads to a fragmentation of the association network and to a shift in community abundance. Our results suggest that species interactions play an important role in the relationship of diversity and stability in single-trophic communities.

**BACTERIAL COMMUNITY COMPOSITION AND FUNCTION DEPEND ON  
DISTURBANCE INTENSITY AND FREQUENCY**Berga, M.<sup>1</sup>, Székely, A.J.<sup>1</sup> and Langenheder, S.<sup>1</sup><sup>1</sup>*Department of Ecology and Genetics, Limnology. Uppsala University. Sweden*

Little is known about how bacterial communities respond, functionally and compositionally, to perturbations. Here we implemented two transplant experiments with dialysis bags to separately investigate the effect of the intensity and frequency of a short-term pulse disturbance. Bacterial abundance, activity, community substrate utilisation profiles and bacterial community composition were determined at three time points during each experiment: before, directly after the disturbance, and at the end of the experiment. In the first experiment, bacterial communities were exposed to six different levels of salinity, changing from 0 to 3, 5, 10, 15 or 20 psu, respectively. Dialysis bags were incubated for 36 hours in the different disturbance treatments and afterwards moved back to the 0 psu tank and incubated for another 36 hours. The most pronounced functional changes occurred at the highest salinities (10-20 psu) directly after the disturbance and a tendency to recover to pre-disturbance levels was observed at the end of the experiment. However, changes in bacterial community composition were only observed at the latest time point. In the second experiment, bacterial communities were exposed to different disturbance frequencies by swapping dialysis bags between the 0 psu and the 20 psu treatment, 1, 2, 3 or 4 times over a 64 h period. Functional changes were detectable in the treatment with the highest frequency already directly after the disturbance, whereas changes in the other treatments were not discernable before the end of the experiment. Interestingly, disturbance frequency induced changes in community composition already directly after the disturbance. Our results show that studies investigating disturbance effects on bacterial communities need to consider the intensity and frequency of a perturbation. The results also suggest that both community function and composition are affected by disturbances, however, at different speed and magnitude.

**IMPACTS OF FISH MANIPULATION ON PLANKTONIC FOOD WEB IN HUMIC AND CLEARWATER BOREAL LAKE**

Brek-Laitinen, G.<sup>1</sup>, Linnaluoma, J.<sup>1</sup>, Huotari, J.<sup>1</sup>, Peltomaa, E.<sup>1</sup>, and Ojala, A.<sup>1</sup>

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A whole-lake food web manipulation suggested that planktivorous fish play an important role in regulating the pelagic food web structure of mesotrophic lakes. In this study, we compared the effects of perch, *Perca fluviatilis* L., on planktonic food web in a deep humic lake and in a shallow clearwater lake. We specifically asked whether predictions, derived from food chain theory of linear responses are valid only for total biomasses, or if these responses can be tracked also at finer taxonomic levels. We hypothesised that fish manipulation in clearwater lake will effect biomass, whereas in humic lake, due to higher levels of omnivory, changes will be mainly in the community composition. Responses of zooplankton, phytoplankton and microbial community (ciliates, nanoflagellates, bacteria and autrophic picoplankton) were tested in summers of two consecutive years in in situ enclosures with and without fish. Although fish addition resulted in similar loading of planktivore biomass in both lakes, the response of zooplankton was contradictory, i.e., fish addition increased total zooplankton in humic lake and decreased it in clearwater lake. Observed changes in zooplankton were mainly due to the protozoans and rotifers, not the crustaceans. Regardless of fish treatment, phytoplankton diversity decreased and, in both lakes, chrysophytes dominated the community. Fish also enriched enclosures with nutrients. In response, in humic lake, both auto- and heterotrophic productivity was enhanced and herbivorous ciliates developed. In clearwater lake, fish-mediated fertilization neither supported higher phytoplankton biomass and productivity nor influenced the activity of microbial community. Instead, the enclosure effect itself prevailed. In both lakes, fish influenced significantly the diversity of individual trophic levels, and, surprisingly, the primary path of response was rather through the bottom-up than the top-down control.

**SMALL AND LARGE POTS MAKE DIFFERENT SOUPS: WHY ARE THERE SO FEW MARINE BACTERIAL TAXA IN FRESHWATER HABITATS?**Pernthaler, J.<sup>1</sup><sup>1</sup>*University of Zurich, Switzerland*

In many aspects, heterotrophic prokaryotes in the pelagic zone of freshwaters seem to live in a comparable environment and to perform similar ecosystem functions as their marine counterparts. Surprisingly, there is almost no phylogenetic overlap between the microbial assemblages in the two biomes, and transitions seem to be rare: So far, there is only one known genus of numerically important freshwater bacteria with an equally common, closely related marine sister group. At a closer look, one can, however, readily identify specific properties of lakes and rivers (besides salinity and productivity) that together set a fundamentally different stage for the success of heterotrophic microbes. For one, freshwater habitats are more strongly embedded in catchment-related processes, as reflected in the access of microbes to organic matter of terrestrial origin and its seasonal variation. Consequently, there are co-existing groups of freshwater bacteria responsible for specific ecosystem processes such as the turnover of organic substances from auto- or allochthonous sources. Moreover, microbes in many lacustrine habitats are exposed to high within-system variability, e.g. due to mixing processes that break up horizontal and vertical gradients. This is believed to create numerous temporal and spatial niches for bacteria with an ‘opportunistic’ growth strategy. Finally, freshwater bacterioplankton taxa exist as discontinuous metapopulations across a physicochemically variable landscape, i.e., there are ‘biogeographic’ distribution patterns of specialist and generalist microbial populations across freshwater bodies with particular combinations of limnological properties. In my presentation, I shall try to interpret our current knowledge about freshwater microbial communities in the context of the above described perspective.

### COMPLEXITY AND DYNAMIC NATURE OF INTERACTIONS AMONG FRESHWATERS BACTERIOPLANKTON TAXA

Eiler, A.<sup>1</sup>, Bertilsson, S.<sup>1</sup>, Heinrich, F.<sup>1</sup>, Jones, R. I.<sup>2</sup>, Peura, S.<sup>2</sup>, and Tirola, M.<sup>2</sup>

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Bacteria play important roles in freshwater food webs and in cycling of elements in these ecosystems. Yet specific ecological features of individual phylogenetic groups and interactions among these are largely unknown. To explore ecological interactions within freshwater bacterioplankton communities we used temporally and spatially resolved 454 pyrosequencing data of 16S rRNA genes from a humic and eutrophic lake. After clustering denoised sequence data into operational taxonomic units (OTUs), interdependencies among freshwater taxa were resolved by time-dependent correlations. The complex relationships within the bacterioplankton communities were visualized in association networks allowing to infer the unique ecological features for each OTU and to reveal the natural history of abundant cultured and uncultured freshwater bacteria. Numerous taxa with a high degree of association were detected which could represent “keystones” as predicted from network theory.

Besides that these networks possess small world properties as indicated by higher clustering compared to random networks and short path lengths, they are dynamic over space and time. The evolution of networks such as changes in the composition of nodes and links was followed during a whole lake glucose enrichment five years of data. This facilitated the formulation of concepts and hypotheses about freshwater microbial ecology such as the propagation of species loss through complex bacterioplankton communities and community adaptations in response to environmental change. Furthermore, if generalizations about diversity and stability are supported by network analysis are discussed.



**BACTERIA, ARCHAEA, AND VIRUSES RESPOND INDEPENDENTLY TO THE SPATIAL STRUCTURE IN MARINE ENVIRONMENTS**

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A cruise from Halifax to Kugluktuk (Canada) covering 13 sampling stations and a depth range of 5–1000 m provided an opportunity to investigate the influence of local environmental parameters and dispersal on bacterial, archaeal, and viral community composition in the Labrador Sea, Baffin Bay, and the Arctic Archipelago. Prokaryotic and viral abundance varied between  $1.0\text{--}26.7 \times 10^5 \text{ ml}^{-1}$  and  $1.1\text{--}12.3 \times 10^6 \text{ ml}^{-1}$ , respectively. The relative abundance of Bacteria varied between 17–84% of total prokaryotic abundance. Prokaryotic and viral abundance was positively correlated in the Labrador Sea and Baffin Bay but not in the Arctic Archipelago. Temperature, salinity, the relative abundance of Bacteria determined by CARD-FISH and of flow cytometrically-distinct populations of prokaryotes and viruses, and the number of viral bands distinguished by RAPD-PCR differed significantly between the three sampling regions. The data indicate that the influence of local environmental conditions and dispersal on community composition changed with the organismal group and sampling region. Overall, the composition of prokaryotic and viral communities was largely determined by local environmental conditions in the Labrador Sea, whereas, dispersal constitutes a major factor in the Arctic Archipelago. Widespread dispersal of prokaryotes and viruses, as indicated by our data, represents an alternative solution to the problem that prokaryotic resistance to viral infection develops fast in experimental studies with isolates, yet the ocean is full of viruses. In other words, depending on the strength of dispersal, the development of resistance towards viral infection may be counteracted by an influx of new prokaryotic and virus types into local communities.

**POSITIVE AUTOTROPHIC-HETEROTROPHIC ECOLOGICAL INTERACTIONS  
MAY BE GOOD REASONS TO LIVE IN A BIOFILM**

Romaní, A.M.<sup>1</sup>, Díaz Villanueva, V.<sup>2</sup>, Font, J.<sup>1</sup>, and Ylla, I.<sup>1</sup>  
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Biofilms develop on any solid wet surface. The biofilm community under light conditions is mainly composed by microbial autotrophs and heterotrophs which make use of their respective capabilities. The biofilm gives physical refuge and available organic molecules from within the polymeric matrix. At the same time, biofilm microorganisms may compete for the resources from the flowing water and/or the main biofilm. Although it is not always clear when microorganisms became benthic, results suggest that when possible they prefer to be attached in biofilms.

The autotrophic-heterotrophic interactions in biofilms is analysed based on empirical results and under the view of the known ecological interactions usually described for higher organisms (facilitation, mutualism, competition). Biofilm autotrophic-heterotrophic interactions have been mainly defined in one direction, describing the positive effect of algae on bacteria by providing algal exudates. However, algae can also benefit from bacteria. Algae require special vitamins for their growth which are basically synthesized by heterotrophic bacteria. Bacteria may also release extracellular enzymes that degrade complex organic molecules not transportable across cell membranes, making organic and inorganic compounds available. This algal-bacterial relationship has been generally defined as mutualism or co-operation within the biofilm. However, at the initial biofilm formation phase, the great difficulties observed for lonely algae (with no bacteria) to form a biofilm suggest that a facilitation interaction occurs at this first step. Bacterial colonization may facilitate algal colonization providing a sticky active surface similarly than the facilitation mechanism described for higher plants where the first colonizers provide shadow. During biofilm formation autotrophic-heterotrophic ecological interactions may change from facilitation to mutualism, while competition may also occur specially in mature biofilms.

**ECOLOGICAL TOOLS FOR THE MANAGEMENT OF CYANOBACTERIA BLOOMS IN THE GUADIANA RIVER WATERSHED, SOUTHWEST IBERIA**

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The Guadiana River, running along the Southern border between Portugal and Spain has the fourth largest drainage basin of Iberian rivers (67840 km<sup>2</sup>), but a series of dams, have severely restricted its freshwater flow (ca. 75 %), and the recent construction of the large Alqueva dam increased flow regulation up to 81% of the total catchment area (55000 km<sup>2</sup>) starting in 2003.

Cyanobacteria blooms have been reported in the Guadiana River in association with seasons and/or years of low freshwater flow. Microbial ecology studies carried out from 1996 to 1998 showed a well defined chlorophyll maximum in the upper estuary. Changes in freshwater flow lead to alterations in water quality and hydrography, thus affecting phytoplankton composition and succession. River flow after completion of the Alqueva dam was severely restricted even during winter months with high rainfall. During the period of dam construction (1999-2000), the sediment load transported downstream increased dramatically causing severe photolimitation for the phytoplankton which resulted in the absence of blooms and very low chlorophyll values throughout spring and summer. Afterwards, during dam filling (2002–2003), nutrient concentrations increased, as well as cyanobacteria abundance, while diatom abundance remained. After this period, total phytoplankton abundance and succession followed the typical trend observed before dam filling. However, specific diversity and chlorophyll concentration tended to decrease after 2002.

The main goal of this work is to evaluate recent water management strategies adopted for the Guadiana watershed, comparing different criteria for ecological status classification, based on long-term ecological data series for Guadiana Estuary (1997–2010)

### LINKING DISSOLVED ORGANIC MATTER AND MICROORGANISMS: TOWARDS A HOLISTIC SYSTEMS BIOLOGY APPROACH IN BIOGEOSCIENCE

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Dissolved organic matter (DOM) in the ocean contains as much carbon as Earth's atmosphere. This huge pool of energy- and nutrient-rich compounds provides an important base for microbial life in the water column. The microbial community shapes DOM composition (and vice versa), thereby determining the dynamics of individual molecules and the entity of DOM. We propose "geo-metabolomics" as the principle that a most comprehensive characterization of molecular DOM composition and its biotic and abiotic sources and sinks reveals a correlation of properties and behavior, ultimately allowing for the prediction of function and reactivity. Geo-metabolomics considers the entity of DOM as a population of compounds, each characterized by a specific function and reactivity in the cycling of energy and elements.

We present results from a long-term study of the DOM geo-metabolome in the open North Sea off Helgoland Island. The geo-metabolome consisted of several thousand molecular formulae identified in DOM by ultrahigh resolution mass spectrometry (FT-ICR-MS, Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry). The DOM pool in the North Sea was highly dynamic and influenced by a complex interplay of processes that produced, transformed and degraded dissolved molecules. The temporal variability of individual molecular formulae in the geo-metabolome provided novel information on their function and reactivity. The dynamics of individual molecules was linked to abiotic environmental factors and the abundance and composition of phytoplankton and microbial communities. Phytoplankton blooms significantly changed the molecular composition of DOM, strongly increasing the inherent molecular diversity. This study, together with ongoing identifications of molecular structures, is a first step towards a mechanistic understanding of DOM dynamics and interactions.

**SUNLIGHT AND HETEROTROPHIC BACTERIAL PRODUCTION: COMPLEX INTERACTIONS IN VARIOUS ENVIRONMENTS**

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Sunlight quality and quantity may affect the functioning of aquatic ecosystems through differential effects on the components of natural bacterial communities. The involved mechanisms, however, are complex, and may interact in multiple directions: light might stimulate some bacterial groups and inhibit some others, might affect predators and viruses, and also affect the chemical nature of dissolved organic matter, photoautotrophs might act as heterotrophs under some circumstances, and photoorganoheterotrophs be active only under some ecological scenarios. These responses are potentially modulated by the environment type, the radiation dose received, the previous light-exposure history of the community, and the community composition. We used several strategies to try to disentangle these various effects in waters of the NW Mediterranean and the Arctic and Antarctica. Together with bulk measurements, we used single-cell (or group-specific) measurements, such as cell sorting or MAR-CARDFISH. We also tried to measure bacterial heterotrophic production in conditions as similar as possible to the in situ environment perceived by the bacteria, e.g., in UV-transparent vials and with the added radioactive tracer. We also followed several diel cycles of bacterial activity, and established experiments where light was manipulated. We will present experimental evidence that sunlight modulates bacterial use of DOM at both the daily and seasonal scales i) through coupling to circadian rhythms of other organisms, ii) as a function of the dose received, iii) depending on the previous light exposure history, and iv) changing depending on the composition of the community. We will also show experimental evidence of light modulation of the competition for some organic substrates between heterotrophic bacteria and cyanobacteria, and that this effect varies in different ecosystems, probably because different bacterial groups display different responses to light, yet variable both seasonally and spatially.

**COMMUNITY SUCCESSION IN DOC UTILIZATION EXPERIMENT REFLECTS FUNCTIONAL SPECIALIZATION BY BACTERIAL PHYLOTYPES**

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Dissolved Organic Carbon (DOC) in marine waters is one of the largest pools of carbon on Earth and is primarily processed by bacterioplankton. Studies have indicated that bacterial species utilize different fractions of the complex DOC pool, but evidence is rather circumstantial. If true, then a gradient in DOC bioavailability would elicit a bacterial community succession reflecting functional adaptations of species to more or less accessible fractions of the DOC. To examine this, a DOC utilization assay was set up, where we incubated four 1-L water samples obtained at four time points from a Baltic Sea grazer-manipulated mesocosm experiment. DOC utilization, O<sub>2</sub> consumption, and bacterial abundance, activity and community composition were followed over time (10 days). As bacterial abundance increased in the incubators, cell-specific enzyme activities decreased and a relative change from protein degradation to carbohydrate utilization was seen. 454 pyrosequencing of 16S rRNA genes showed the same general community succession in all incubators from a diverse composition dominated by Actinobacteria, Bacteroidetes and Alphaproteobacteria to a less diverse community dominated by Gammaproteobacteria. Different gamma-proteobacterial groups appeared in each incubator. Towards the end, the proportion of Beta- and Alpha-proteobacteria increased. The time-course changes in ectoenzymatic activities, the community successions, and the differences in community composition between the 4 incubators suggest that changes in DOC composition are imprinted in bacterial activity and community composition. Consequently, that bacterial subgroups show different adaptations to the utilization of DOC. Our further analyses of the dataset will focus on the identification of phylogenetic groups of bacteria specialized to grow on easy-degradable or semi-labile fractions of the DOC pool, respectively.

**MICROBIAL DEGRADATION OF ORGANIC MATTER PRODUCED BY *T. WEISSFLOGII*  
UNDER DIFFERENT  $p\text{CO}_2$  LEVELS**

Endres, S.<sup>1</sup>, Unger, J.<sup>2</sup>, Wannicke, N.<sup>2</sup>, Nausch, M.<sup>2</sup>, Voss, M.<sup>2</sup>,  
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Ocean acidification is expected to affect enzymatic hydrolysis, resulting in changes in microbial exopolymer decomposition. The effects of increasing  $\text{CO}_2$  concentrations on bacterial degradation of organic matter was studied during a combined chemostat and batch experiment in the frame work of the BIOACID project (Biological Impact of Ocean Acidification). Here, we report on the effect of  $p\text{CO}_2$  on the activity rates of extracellular enzymes. These process organic matter degradation as well as nutrient regeneration and hence play an important role in the turnover of dissolved organic matter (DOM).

The diatom *Thalassiosira weissflogii* was pre-adapted in chemostat chambers under nitrogen limitation and different  $\text{CO}_2$  partial pressures (180, 380 and 780 ppm representing past, present-day, and future atmospheric  $p\text{CO}_2$ , respectively). After that exudation was enhanced by growing the algae in  $p\text{CO}_2$  controlled batch cultures for three days. A natural bacterial community then degraded the produced exudates during a four-day incubation in the dark. Enzyme activities as well as concentration and composition of organic material were determined.

In accordance with nutrient availability in the medium, we measured high aminopeptidase activity in all treatment but no phosphatase activity. Beta-glucosidase and aminopeptidase activity increased with  $p\text{CO}_2$  during the batch phase and at the beginning of the degradation phase. We discuss whether this effect derives from a change in biomass production in the high  $p\text{CO}_2$  treatments or whether it was a direct pH effect on enzyme activities. After 48h of degradation, no enzyme activities were detectable anymore and bacterial cell counts decreased significantly. These findings suggest that the active bacterial community degraded all labile organic matter within 48h and perished subsequently.

**MICROBIAL ACTIVITIES AND DISSOLVED ORGANIC MATTER DYNAMICS IN OIL-CONTAMINATED SEAWATER FROM THE DEEPWATER HORIZON OIL SPILL SITE**

Ziervogel, K.<sup>1</sup>, McKay, L.<sup>1</sup>, Rodes, B.<sup>1</sup>, Osburn, C.L.<sup>2</sup>, Dickson-Brown, J.<sup>2</sup>, Arnosti, C.<sup>1</sup>, and Teske, A.<sup>1</sup>

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The Deepwater Horizon oil spill triggered a complex cascade of microbial responses that reshaped the dynamics of heterotrophic carbon degradation and the turnover of dissolved organic carbon (DOC), as well as the transport behavior of crude oil in the water column. A 21-day laboratory incubation of oil-contaminated water in rotating glass bottles demonstrated that oil-degrading bacteria associated with an oil slick from the spill site rapidly catalyzed the formation of macroscopic aggregates with incorporated oil droplets (oil aggregates). Oil aggregates were densely colonized by heterotrophic bacteria that showed elevated rates of enzymatic activity (lipase hydrolysis) indicative for oil degradation. We also found in bottle waters enhanced microbial growth and activities ( $\beta$ -glucosidase, leucine aminopeptidase, and two of the six polysaccharide hydrolase activities monitored here, i.e. xylanase and laminarinase) not directly associated with primary oil-degradation, as well as a twofold increase in DOC. Concurrent changes in fluorescence properties of colored dissolved organic matter (CDOM) indicated an increase in oil-derived, alkylated polycyclic aromatic hydrocarbons (PAHs) in the DOC pool. Our data show that microbial activities, together with physical mixing, enhance the formation of oil aggregates in oil-contaminated surface seawater. These aggregates likely mediate, by two distinct mechanisms, the transfer of hydrocarbons to the deep sea: a microbially-derived flux of PAHs from sinking oil aggregates into the ambient water column, and rapid sedimentation of the oil aggregates themselves, leading to massive accumulation of oily particulate matter, as observed on the seafloor around the spill site several months post-oil spill.



**ORIGIN AND FATE OF FLOOD-INTRODUCED DISSOLVED ORGANIC MATTER IN A RIVER-FLOODPLAIN SYSTEM**Sieczko A.<sup>1</sup> and Peduzzi, P.<sup>1</sup><sup>1</sup>*Department of Limnology, University of Vienna*

River-floodplain systems play a significant role in organic matter cycling and carbon (C) mineralization. However, their role in C flux remains understudied, particularly the linkage between the hydrological and C cycle in floodplain lakes has been hardly addressed so far.

We applied spectroscopic techniques and extracellular enzyme activity measurements together with assessment of primary and secondary production in order to elucidate flood-pulse-linked differences in C sources and related microbial processes. The sampling was conducted in a floodplain near Vienna (Austria), both when backwaters were disconnected and connected to the main stem, with the focus on flood event. The importance and bioavailability of allochthonous DOM was emphasized.

The flood introduced significantly large amounts of DOC that contained a great share of allochthonous, aromatic, higher molecular weight compounds. Bacterial enzymatic response, as a proxy to track changes in the DOM pool, indicated elevated utilization of imported allochthonous material. Autochthonous material supported bacterial secondary production (BSP) significantly only during the connected phase. During connection glucosidase and protease expression were dominant in bacterial metabolism while during disconnected periods a switch to lignin degradation (phenol oxidase) was observed. No significant differences in BSP ( $p=0.09$ ) between two phases were detected, indicating that heterogeneous sources of C could sufficiently support BSP.

Prolonged surface connectivity had minor influence on the assimilation efficiency of hydrolyzed products thus implying very adaptive metabolic capacity of the system. However, our results suggest that with the onset of the flood a greater share of the DOM load was respired rather than assimilated. Our study demonstrates that floods are important for delivering DOM which, in spite of allochthonous origin, is reactive and can be effectively utilized by aquatic bacteria in such a river-floodplain system.

**A LARGE-SCALE COMPARATIVE STUDY OF BACTERIOPLANKTON RESPIRATORY QUOTIENTS IN BOREAL LAKES**

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In most studies of bacterioplankton carbon metabolism, the need to assume a respiratory quotient (RQ; mole of CO<sub>2</sub> produced per mole of O<sub>2</sub> consumed) introduces a fundamental source of uncertainty. Many studies have assumed a fixed value, often close to 1, but this assumption has little empirical support, and ignores potential variability in RQ caused by physiological mechanisms and by varying oxygen contents of respired substrates. Here we present over 50 direct measurements of bacterioplankton RQ that we have carried out in epilimnetic samples of lakes distributed across the temperate and boreal regions of Québec, using O<sub>2</sub> and CO<sub>2</sub> sensitive mini sensors attached to the inside of closed incubation flasks. RQ was mostly in the range of 0.5–2, with the lowest values in net autotrophic and the highest in net heterotrophic systems. A plausible explanation is that bacteria were mainly utilizing oxygen-poor phytoplankton exudates in the net autotrophic lakes and oxygen-rich allochthonous organic acids, e.g., formed by photo-chemical processes, in the net heterotrophic lakes. The results suggest that respiration measured with the O<sub>2</sub> consumption method assuming RQ=1 may underestimate the role of bacterial respiration as cause of lake net heterotrophy.

**TIME FOR PROTISTOMICS**de Vargas, C.<sup>1</sup><sup>1</sup>*Station Biologique Roscoff*

Modern unicellular eukaryotes (or protists) are divided into at least 8 mega-divisions whose radiation appears to have occurred not more than 1 billion years ago. If the large majority of redox reactions that constantly recycle the bioessential atoms (CHNOPS) across the biosphere essentially belongs to prokaryotes and their phages, protists have fundamentally impacted biogeochemical cycles through their neomuran revolution. The invention of active membranes enclosing DNA and allowing phagocytosis has dramatically and irreversibly enhanced, through continual symbioses, the complexity of genomes, ultrastructures, and behaviors in living biota. I will review several steps in protistan symbiogenesis and irremediable complexification which may have significantly impacted the evolution of the Earth system. However, the recognition of the biogeochemical significance of protists has just started and is blocked by our still very limited knowledge of protistan diversity and ecosystem functions. The current revolution in high-throughput sequencing and imaging technologies allow us to start recognizing the frontiers of protistan total genomic and organismic biodiversity. Although certainly much bigger than previously recognized, total protistan biodiversity appears to be a finite compartment whose mode and rate of evolution could be incorporate into global ecological models.

**MORPHOLOGICAL- VERSUS MOLECULAR-BASED DIVERSITY SURVEYS OF PLANKTONIC MICROBIAL EUKARYOTES: THE CASE OF TINTINNID CILIATES**Bachy, C.<sup>1</sup>, Lopez-Garcia, P.<sup>1</sup>, Dolan, J.<sup>2</sup>, and Moreira, D.<sup>1</sup><sup>1</sup>*Unité d'Ecologie, Systématique et Evolution, Université Paris-Sud, France ,*<sup>2</sup>*Microbial Ecology, Laboratoire d'Océanographie de Villefranche-sur-Mer, Station Zoologique, France*

During the last decade, the use of culture-independent molecular approaches to describe the communities of microbial eukaryotes present in natural environments has led to the discovery of a huge diversity of these organisms. However, these analyses have rarely taken into account the temporal pattern of variation of diversity and most often lacked of a comparison of morphological- and molecular-based estimates. In this context, we have studied the temporal pattern of genetic diversity of tintinnids (Ciliophora) over a two-year survey in a Mediterranean Sea location (Villefranche-sur-Mer, France). The species-rich order Tintinnida contains freshwater and marine ciliates easily distinguishable based on morphological characters, in particular their conspicuous organic or inorganic tests. This allowed us to couple morphological observations with a double molecular approach (using *single cells and environmental DNA*) to analyse the *SSU-rRNA* and the *ITS coding regions*. Using a fingerprinting technique (DGGE), we detected a strong relationship between the structure of the tintinnid communities and the sampling depth. Despite an extensive work of single-cell isolation, identification, and subsequent *SSU-rRNA* and *ITS* sequencing, the analysis of tintinnid communities by direct PCR amplification and sequencing of rRNA genes from plankton samples revealed a number of phylotypes without any closely related known species. Conversely, several sequences from single-cell analyses were never found in the environmental sequence libraries. Using this well-characterized protist group, we discuss the limitations of morphological- and molecular-based studies to assess the diversity and temporal dynamics of microbial eukaryotic communities.

**MAST-4: A MODEL MARINE HETEROTROPHIC FLAGELLATE**Massana, R.<sup>1</sup><sup>1</sup>*Institut de Ciències del Mar, CSIC, Catalonia, Spain*

Heterotrophic flagellates are central in marine food webs, as was already recognized in the seminal papers conceptualizing the microbial loop. Indeed, if planktonic bacteria were abundant, growing actively and with balanced abundances, an equally significant bacterial loss mechanism was required. We know now that grazing by small heterotrophic (and mixotrophic) flagellates and viral lysis are the main mortality factors for marine bacteria. Despite this recognized ecological role, and contrasting with the significant advances achieved with marine bacteria, little is known on the diversity of marine heterotrophic flagellates. This heterogeneous group of cells is poorly captured by microscopic and culturing approaches, and has been recently targeted by environmental molecular surveys. These have illuminated an uncultured clade, MAST-4 (Marine Stramenopiles clade 4), formed by tiny cells (2-3  $\mu\text{m}$ ) that are widely distributed in the photic skin of the oceans. MAST-4 actively graze on bacteria and other picosized cells and on average account for ~10% of marine heterotrophic flagellates. MAST-4 displays low genetic variability, being formed by only five relatively homogeneous clades, indicating this widely successful group has suffered low evolutionary diversification. Future research based on novel isolation attempts, single cells analyses and in situ experiments combined with FISH are promising to reveal the true extent of their ecological and evolutionary significance.

**DIVERSITY AND ACTIVITIES OF FUNGAL COMMUNITIES ASSOCIATED WITH DECAYING LEAVES IN ACIDIFIED HEADWATER STREAMS.**

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Anthropogenic acidification has impaired numerous freshwater ecosystems throughout the Northern hemisphere, notably forested low-order streams that are particularly sensitive. Deleterious effects of low pH and elevated aluminium concentrations have been reported on the diversity of aquatic hyphomycetes and on litter breakdown in which these fungi are involved. Such impairments in turn can affect higher trophic levels.

Two litter-bag experiments have been carried out in the Vosges Mountains (North-eastern France) in order to better understand the effects of acidification on fungal diversity and activities during leaf processing.

The aim of the first study was to assess the impact of acidification on fungal diversity in both benthic and hyporheic zones of streams. During the initial stages of leaf decomposition, fungal richness was measured by both traditional (conidial identification) and molecular methods in five streams along an acidity gradient. The species richness of sporulating aquatic hyphomycetes decreased in both zones along the acidity gradient, whereas the ribotype richness investigated by PCR-DGGE was comparatively higher and was unaffected by the acidity. These results show that traditional methods could underestimate fungal diversity and/or suggest that some aquatic hyphomycete species might not be in good conditions for reproductive activity under acidic conditions.

The aim of the second study was to highlight possible shifts in microbial activities that could explain reduced leaf litter breakdown in acidified streams. Potential extracellular enzymatic activities and nutrient uptake on decaying leaves were compared between six sites during a 10-week period. While the microbial processing of leaf litter was depressed by high Al concentrations, enzyme production and N uptake appeared unaltered. By contrast, P uptake was significantly and negatively correlated with Al concentrations, suggesting that leaf decomposition could be constrained by P limitation.

**GROWTH RATES OF PELAGIC BACTERIAL COMMUNITY MEMBERS AND THEIR FLAGELLATE GRAZERS IN DIVERSE AQUATIC ECOSYSTEMS**Straškrábová, V.<sup>1</sup>, Šimek, K.<sup>1</sup>, and Kasalický, V.<sup>1</sup><sup>1</sup>*Biology Centre, Academy of Sci of the Czech Rep., Institute of Hydrobiology*

Using FISH-probes in microcosms with and without protozoan grazers, we studied abundances of alpha-, beta-, gamma-Proteobacteria (ALF, BET, GAM), Actinobacteria (ACT), Archaea (ARC), Bacteroidetes (CF), and of the Polynucleobacter cluster (Pnec – combined ABCD) and the R-BT lineage within the Limnohabitans genus. Samples from ecosystems with different phosphorus (P) concentrations, conductivity and organic matter contents were processed from shallow and riverine type reservoirs in the upper Volga River catchment (Russia) – 14 sites, and also from wetland pools in Hortobágyi puszta (Hungary) – 2 sites. We compared our results with published data from the canyon-shaped reservoir Římov in the Czech Republic (Šimek et al. 2006). Heterotrophic nanoflagellates (HNF, in 5- $\mu\text{m}$  filtered treatment) usually reached high growth rates of 0.56 – 2.84  $\text{d}^{-1}$ , especially in samples, where they were scarce initially, being in situ controlled by filtering zooplankton. Among samples from the Volga River system, however, there were several cases with a high initial abundance of HNF from sites poor in zooplankton. In such cases, they did not grow fast during the incubation, though their grazing activity was high. The bacterial highest growth rates (per day) found in the Volga system and in the wetlands, respectively, were as follows: ALF 0.57 and 0.93, BET 0.64 and 1.10, GAM 1.04 and 1.05, CF 0.82 and 0.78, ACT 0.14 and 0.24, R-BT 0.47 and 1.43, Pnec (not determined) and 0.95, ARC 0.59 and 0.95. In the Římov Reservoir, the proportions of BET, GAM, CF, ACT and R-BT were examined in series of incubations at concentrations of soluble reactive phosphorus from 1.9 to 96  $\mu\text{g L}^{-1}$ . Growth rates detected correlated with P concentrations and their maxima were in the range 0.8 to 1.2  $\text{d}^{-1}$  for all the groups including ACT. Compared to the Římov, in the Volga River system there was rather high percentage of ACT in total bacterial abundance and rather low R-BT.

### CILIATE MIXOTROPHY OR PICO- AND NANO-PLANKTON PREDATION IN SUBOXIC/ANOXIC LAYERS OF STRATIFIED LAKES?

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Fine-scale stratification (0.25 m) of ciliate assemblages (DAPI and quantitative protargol staining) and their autotrophic prey (picocyanobacteria, PCY; nanoflagellates, ANF – via autofluorescence) were studied in suboxic/anoxic layers of two meromictic lakes, Waldsee, WS (Lusatia, Germany) and Laguna de la Cruz, LC (Cuenca, Spain), and in a warm-monomictic lake, Alchichica, AL (Puebla, Mexico). An estimation of grazing rates upon PCY using Fluorescently Labelled Bacteria (FLB) method was performed in LC and AL.

Despite of very big differences in lake limnology (alkaline, deep and warm-monomictic AL vs. karstic meromictic LC and shallow, iron meromictic WS), the anoxic boundary showed very similar pattern in microorganism distribution; APP were found in elevated concentrations (between  $10^5$  to  $10^7$  cells ml<sup>-1</sup>). Among apparent mixotrophs, *Pelagothrix* and/or *Holophrya* spp. were identified in all lakes (WS-6; LC- 50; AL- 5 cells ml<sup>-1</sup>), *Euplotes* spp. in WS and AL (5 and 30 cells ml<sup>-1</sup>) and *Coleps* sp. in WS and LC (37 and 18 cells ml<sup>-1</sup>). Ingested PCY were observed in peritrichs (LC, AL) and in microaerophilic scuticociliates *Cyclidium* sp. (AL), *Ctedoctema* sp. (LC) but not in *Dexiotricha* sp. (WS). *Euplotes* and scuticociliates were feeding upon FLB in all assays but *Spirostomum teres*, biomass-dominating anoxic boundary (LC-17; AL-0.5 cells ml<sup>-1</sup>) only in anaerostat assays (He atmosphere). Anaerobic ciliates such as odontostomatids did not ingest PCY.

Generally, both mixotrophic and PCY-feeding ciliates were concentrated at an suboxic / anoxic boundary at about 2% oxygen saturation and upon irradiation of 0.2 to 0.1% PAR. Mixotrophic *Coleps* sp. and *Pelagothrix* sp. were observed also upon higher PAR levels but APP feeding mixotrophic *Euplotes* sp. and *Spirostomum teres* only below 0.2% PAR. It is hypothesized that optimum use of mixotrophy – combined use of PCY-photosynthesis products and digestion – in the suboxic conditions occurs in an optimum PAR below 0.2%.



## PHOTOHETEROTROPHY AND THE PROCESSING OF DISSOLVED ORGANIC MATERIAL IN COASTAL OCEANS

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Photoheterotrophic bacteria are capable of using dissolved organic material (DOM) as well as having light-harvesting mechanisms for potentially gaining another source of energy from sunlight. Two types of photoheterotrophic bacteria, aerobic anoxygenic phototrophic bacteria and proteorhodopsin-bearing bacteria, are abundant in the oceans and are diverse, with representatives in many of the major bacterial taxa found in oceanic communities. Although the diversity of these bacteria has been extensively explored, their contribution to DOM uptake and to biogeochemical processes in general is still unclear. This presentation will review recent studies of the function of photoheterotrophic bacteria in DOM uptake and in the marine carbon cycle.

### PHOTOHETEROTROPHIC METABOLISM AND CARBON UTILIZATION IN AEROBIC ANOXYGENIC PHOTOTROPHS

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Aerobic anoxygenic phototrophs are only recently discovered group of marine bacterioplankton. They contain bacteriochlorophyll-containing reaction centers and perform photoheterotrophic metabolism. We investigated efficiency of their carbon metabolism in carbon-limited chemostat cultures. When grown in light-dark cycle these bacteria converted 30–55% on of the supplied carbon (depending on the substrate) into the biomass. In contrast, when grown in the dark the efficiency dropped to 15–40%, which equal to yields usually reported for heterotrophic bacteria. The interplay of respiration and photosynthesis was further investigated by oxygen consumption measurements and infra-red kinetic fluorometry. The results showed that exposure to light strongly inhibited the respiration as it was replaced by light-derived energy. The enhanced efficiency of aerobic anoxygenic phototrophs in carbon utilization might be an important factor in tropical regions of the world ocean, which are generally considered as carbon sinks.

**REMARKABLE THERMAL STABILITY OF PHOTOSYNTHETIC REACTION CENTRES  
IN PHOTOTROPHIC BACTERIA**

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Photosynthetic bacteria are one of the oldest life forms on Earth. The physiological optimum of most of these organisms is at the temperature between 15 – 30 °C. We used kinetic fluorometry and molecular dynamics simulations to investigate the stability of photosynthetic reaction centres at the thermal range of 0 – 90 °C. Fourteen bacterial strains (eleven mesophilic, two thermophilic, one psychrophilic) belonging to aerobic anoxygenic phototrophic bacteria, purple nonsulphur, purple sulphur, and green nonsulphur bacteria were chosen for our study. The bacterial reaction centers of all tested strains were photosynthetically active up to the temperature of 65 °C, five of them up to 80 °C. The electron transport was functional up to 50 °C. The molecular dynamics simulations of interaction between L and H protein subunits showed that the helix-helix contact area and the probability of intrahelical hydrogen bond formation were larger in aerobic phototrophic bacteria than in purple and green nonsulphur bacteria. This indicates that the intrahelical H-bonds rather than interhelical H-bonds contribute to the thermal stability in aerobic phototrophic bacteria. The unexpected thermal stability of anoxygenic reaction centers raises the question about their evolutionary origin. It might reflect the fact that the anoxygenic phototrophs evolved in the Archean ocean under much warmer temperatures than they experience under today's conditions.

### ECOLOGICAL SIGNIFICANCE OF AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA IN THE ARCTIC OCEAN

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Photoheterotrophy, the ability to utilize organic substrates and harvest light energy, occurs in a broad range of microbes. Aerobic anoxygenic phototrophs (AAP) are photoheterotrophs that require oxygen for their growth and for bacteriochlorophyll *a* (Bchl *a*) synthesis. In the Arctic Ocean, we actually observe an increase in solar radiation and in export of organic carbon to the ocean due to permafrost thawing and increased river-runoff. The hybrid metabolism of photoheterotrophs (respiration and phototrophy) might probably make them key players in this changing environment.

Using infrared microscopy, cultural and molecular (*pufM* DNA libraries) approaches, we analyzed the AAP abundance and diversity in the MacKenzie river/Beaufort Sea system in samples collected during the MALINA cruise (July–August 2009). Very low relative abundances were recorded from the North Pacific to the Beaufort Sea (1% of the total prokaryotic community). However, high contributions were obtained in the MacKenzie plume (6-14% of the total prokaryotes).

A low cultural AAP diversity exclusively composed of Alphaproteobacteria of genera *Sulfitobacter* and *Loktanella* was identified in a collection of 145 isolates. Molecular analyses of thirteen *pufM* libraries (365 sequences) revealed the dominance of Alphaproteobacteria and Betaproteobacteria. Alphaproteobacterial *pufM* sequences were mostly recovered from oceanic oligotrophic samples whereas samples influenced by the MacKenzie plume were dominated by betaproteobacterial sequences.

Since BChl *a* synthesis is inhibited by light in AAPs, we hypothesized that the infrared numeration method used previously could have underestimated the actual numbers of these microorganisms at the time of sampling (long periods of sunlight in summer at high latitudes). Quantitative PCR experiments targeting the most abundant AAP clade confirmed the significance of betaproteobacteria in the MacKenzie plume and the low abundance of marine AAPs in the Beaufort Sea.

**PHYSIOLOGY AND INTERACTIONS OF PHYTOPLANKTON-ASSOCIATED ROSEOBACTERS**

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The *Roseobacter* clade has been shown to be a prominent component of the bacterioplankton in marine surface waters, predominantly in temperate to polar regions and in particular in the course of phytoplankton blooms. Several distinct clusters with typical pelagic representatives have been identified in which phylotypes and a few isolated strains occur. Available metagenomic, meta-transcriptomic and genomic data provide most valuable insights into the metabolic potential and gene expression patterns of these roseobacters. However, surprisingly little work has been done so far on the physiology of representative model organisms to better understand growth and interactions with other bacterioplankton components and with phytoplankton and thus specific biogeochemical roles of these organisms. We obtained several isolates of *Roseobacter* clusters typical for the water column from the North Sea and examined growth and various physiological properties, including substrate spectra and interactions with phytoplankton algae. Isolates belong to the RCA cluster, the SH6-1 cluster which is most closely related to the NAC11-6 cluster, and others are affiliated closely to the genera *Sulfitobacter*, *Loktanella* and *Oceanibulbus*. The results show that the isolates have rather distinct preferences for selected amino acids, monosaccharides and oligosaccharides, and that they can respond very differently to substrates released from individual algal species. Distinct algal species exhibit pronounced differences in the release of dissolved carbohydrates and amino acids explaining to a certain extent the interactions of the *Roseobacter* strains with the algae. Some of these observations are in line with the seasonal occurrence of these strains in the North Sea in the course of phytoplankton blooms and may explain some aspects of their growth control. The isolates provide a valuable basis for detailed analyses of specific processes in the organic matter cycling in pelagic systems and complement metagenomic and metatranscriptomic analyses. They may further be used for systems biology approaches in microbial oceanography.

### **PROTEORHODOPSIN GENE EXPRESSION IN THE MARINE BACTERIUM *VIBRIO* SP. AND4 IS DIFFERENTIALLY REGULATED BY NUTRIENT AVAILABILITY.**

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Proteorhodopsin (PR) is an abundant and widely distributed bacterial protein that is highly expressed in marine environments. Working as a light driven proton pump, this photoprotein is predicted to have an important role in the ecology of the oceans by supplying energy for microbial metabolism. Curiously, there appears to be differences in the ecological role of PR phototrophy, coinciding with unexplained variability in PR gene expression, among phylogenetically distinct marine bacteria under different growth conditions. The study reported here is an attempt to identify factors that regulate PR gene expression, in order to provide insights into the underlying causes of such variability. In earlier work, we showed that PR phototrophy promotes survival in the bacterium *Vibrio* sp. AND4, but knowledge on the regulation of this survival response was lacking. Our current results demonstrate that the PR induced survival response occurs only with cells from stationary phase and not with exponentially growing cells. Using real time quantitative PCR to determine the relative expression of the PR gene along the growth curve, we found that PR gene expression was differentially regulated through different phases of growth. PR gene expression values were very low in exponential phase, showed a pronounced peak at the intersection between exponential and stationary phase and declined in stationary phase. Further experiments revealed that nutrient limitation, not light exposure, regulated this differential PR gene expression in AND4. Thus, PR gene expression at the entry into stationary phase preceded, and therefore largely explained, the stationary phase light-induced survival response in this *Vibrio* species. These findings suggest that analyses of PR gene expression under different environmental conditions could provide crucial insights into when and where the potential for PR phototrophy is realized and how this phototrophy affects the ecology of different marine bacteria.

# Poster Presentations

### SIZE-DEPENDENT RESPONSES OF PHYTOPLANKTON TO THERMAL STRATIFICATION AND MIXING IN TWO BOREAL LAKES OF CONTRASTING HUMIC MATTER CONTENT

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We studied the size-specific phytoplankton production and chlorophyll-based biomass under stratified and mixing conditions in humic Lake Valkea-Kotinen and clearwater Lake Vesijärvi in southern Finland. We hypothesized that summer stratification resulting in oligotrophication of epilimnion favours small phytoplankton species, while autumn mixing replenishes nutrients and supports growth of large species. We sampled both lakes in 2005 and, in addition to size-fractionated production and biomass, we also identified the species and calculated the biovolume-based biomass estimates. In contrast to our hypothesis, during stratification, in both lakes most of the production was composed of a few large cell size taxa, i.e., flagellated *Gonyostomum semen* dominated in humic lake and colonial diatom *Tabellaria flocculosa* prevailed in clearwater lake. The large microplankton was the dominant primary producers contributing 44% and 42% of the total primary production in humic and clearwater lake, respectively. Large fraction was also superior in terms of chlorophyll-specific photosynthetic rate, whereas picophytoplankton was less efficient and thus less successful. With autumn mixing, community shifted towards *Peridinium* spp. dominance in humic lake and cyanobacterium *Woronichinia naegeliana* became most frequent phytoplankton in clearwater lake. The share of smaller fraction increased in production in autumn as well: in the humic lake up to 64% and in the clear water lake 22% of production was originated from picophytoplankton. Although the community composition differed between lakes, the large-sized phytoplankton was in general dominant and could largely overcome nutrient limitation. Therefore, light and temperature were presumably the main drivers for the production of phytoplankton.



**BENTHIC MICROBIAL COMMUNITY RESPONSES TO PESTICIDES IN LAKE LITTORAL ZONES**Larras, F.<sup>1</sup>, Bouchez, A.<sup>1</sup>, Montuelle, B.<sup>1</sup>*<sup>1</sup>Institut National de la Recherche Agronomique, CARRTEL, 74200 Thonon-Les-Bains, France*

Herbicides contamination of lake ecosystems is characterized by mixture of several molecules at low concentration. Their effect on microorganisms, especially benthic communities, is not well known. With the view of assessing the ecological risk of herbicides for aquatic microorganisms, some models (Species sensitivity distribution-SSD, Independent Action, Concentration Addition) have been developed on phytoplanktonic species of the pelagic zone. They predict protective concentrations for a species or a community exposed to a substance or a mixture. Lacustrine littoral zones are often defined by higher concentrations and diversity of substances. Benthic microalgae (biofilm) are, in these areas, an essential element of food web (biomass, function). The specific biofilm structure may modify their exposition and their sensibility. Among biofilm communities, diatoms are mainly used as standardized bioindicators for trophic pressure (Biological Diatoms Index) and this could be used for prediction of ecotoxicological effect. To assess if SSD models remain valid for benthic diatoms, the first step is to build a data set of benchmarks (as NOEC: No Observed Effect Concentration). Few data are available in the literature for microphytobenthos and often for the same species. So, we have tested, in monospecific bioassays, a dozen of lacustrine diatoms and eight herbicides with different way of action. The adaptation of monospecific tests (normalized and firstly used on planktonic algae) to benthic algae provides a panel of benchmarks depending on the species sensibility. Dose-response bioassays were carried out, using specific growth rate as endpoint. Data such EC<sub>50</sub>, NOEC or LOEC allow to assess the ecotoxicological response of each tested species and to elaborate adapted SSD models. Finally, these models should help to assess the phyto-benthic diversity changes and to put forward reliable benchmarks for herbicide risk assessment on the littoral zone.

### GROWTH EFFICIENCY EVIDENCES BACTERIAL COMMUNITY ADAPTATION TO THE ECOSYSTEM

Baña, Z.<sup>1</sup>, Uranga, A.<sup>1</sup>, Abad, N.<sup>1</sup>, Artolozaga, I.<sup>1</sup>, Ayo, B.<sup>1</sup>, and Iriberry, J.<sup>1</sup>  
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The aim of this study was to explore the factors regulating bacterial growth efficiency (BGE) in a marine coastal ecosystem. Our working strategy based on a seasonal study in order to obtain BGE data from different bacterial communities that occur over time under different environmental and biological conditions.

We distinguished two contrasting situations (periods of high and low production, HP and LP, respectively) in the ecosystem that alternated in time and were linked through very brief transitional periods. The carbon flow in periods of HP was significantly higher than in periods of LP, due mainly to the level of resources and biological abundance and activity that characterized each period. Also two major groups, Bacteroidetes and Alpha-Proteobacteria, followed trends that matched with the two situations, although alternate and opposite each other. Despite these compositional and physiological differences between the bacterial communities in the two situations, the BGE remained stable and relatively high throughout the year. Noticeably, BGE only fell to very low levels during the brief transitional periods. Our results suggest that the BGE does not depend on a single physical, chemical or biological factor, but indicates the degree of global adaptation of the bacterial community to the ecosystem. Thus, very different communities can grow with similar and high efficiencies provided they are adapted to the resources and biological components of their environment. However, when conditions change, the community must re-adapt to the new situation and during this transition period the BGE dramatically decreases. This would explain partially the contradictions found by other authors in other studies.

**CHANGES IN BACTERIOPLANKTON COMMUNITY COMPOSITION AND FUNCTIONALITY IN RESPONSE TO INCREASING SELECTION PRESSURE**

Sjöstedt, J.<sup>1</sup>, Pontarp, M.<sup>2</sup>, Tinta, T.<sup>3</sup>, Alfredsson, H.<sup>1</sup>, Canbäck, B.<sup>4</sup>, Tunlid, A.<sup>4</sup>,  
Lundberg, P.<sup>2</sup>, Hagström, Å.<sup>1</sup>, Turk, V.<sup>3</sup>, and Riemann, L.<sup>5</sup>

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Organic matter processing by bacterioplankton is assumed to be linked to bacterial community composition. This notion is conceivably based on information gained from work on cultivated strains combined with observations of extensive community dynamics elicited by changes in environmental drivers. However, to date it is not known to what extent bacterioplankton species are functionally redundant; i.e. whether different bacterial communities can carry out a similar ecosystem function. To address this we investigated how bacterial community composition, diversity and functionality were affected by increasing selection pressure. Natural bacterial communities from the Gulf of Trieste, Northern Adriatic Sea, were exposed to an increasing number of selection factors in a continuous culture setup. In the first treatment, the original community was exposed to decreased temperature (8°C below in situ). In the second treatment, in addition to decreased temperature the bacterial community was exposed to C- or N-limitation by adding phosphate to the seawater. Finally, in the third treatment, in addition to temperature and C- or N- limitation, the culture was bubbled with nitrogen gas to lower the oxygen level. Bacterial community composition was analysed using DGGE and 454-sequencing. The functionality of the resulting bacterial assemblages was arbitrarily defined by the ability to utilize natural dissolved organic carbon (DOC). Bacterial and viral abundances were highest in the two treatments with added phosphate indicating that phosphorous was a limiting factor in the natural environment. Despite that bacterial community compositions at the end of the experiment differed between treatments, which indicate ecological response to the selection pressure, DOC was utilized to the same extent in all treatments except for the culture incubated at lower temperature and with addition of phosphate. This bacterial community showed a more efficient utilization of DOC (p 0.05).

**BACTERIOPLANKTON PATTERNS IN SHALLOW LAKES FROM THE PAMPA PLAIN (ARGENTINA) WITH CONTRASTING ALTERNATIVE STEADY STATES**

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The main objectives of the present work were to evaluate the influence of spatial and environmental factors in shaping bacterial community structure in a set of shallow lakes characterized by contrasting alternative states (*sensu* Scheffer et al.1993) and to assess the impact of different taxonomic resolution on the biogeographical pattern observed.

Six temperate shallow lakes from the Pampa Plain (Argentina) were sampled every two months during a whole year. Within the framework of the Metacommunity concept we compared two molecular analyses with different genetic resolution: a 16S rDNA analysis (lower genetic resolution) was performed by means of DGGE profiles and an ITS analysis (higher genetic resolution) was conducted by means of ARISA profiles.

The results revealed marked differences in the community structure among clear-vegetated, inorganic-turbid and phytoplankton-turbid shallow lakes. Moreover, the alternative equilibrium state that characterized the different shallow lakes played a major role in structuring the community while seasonality resulted of a lesser importance. Interestingly, the higher the genetic resolution of the analysis, the tighter the relationship found between community composition and alternative steady state. On the other hand, the spatial effects evidenced by means of DGGE reflected the non-random distribution of the systems and were lost when the analysis was performed at a higher genetic resolution level. Altogether, these results indicated that the Species Sorting perspective best explained the regional pattern observed.

**ASSESSMENT OF THE IMPACT OF TEBUCONAZOL (FUNGICIDE) ON LAKE AND STREAM BACTERIAL COMMUNITIES USING A 16S rRNA PYROSEQUENCING METHOD.**

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The pollution of lakes and rivers by the agricultural use of pesticides is a growing problem worldwide. As these substances have complex effects on ecosystems, we need to improve our understanding of their fate and their impact on biological communities. With this in mind, we carried out experimental study of the responses of planktonic bacterial communities from lakes and benthic bacterial communities from streams to the fungicide tebuconazol, and of its degradation by these different communities. For each type of ecosystem, communities were collected from pristine environments (Lake Aiguebelette and the upstream segment of the river Morcille, France) and pre-exposed environments (Lake Léman and the downstream segment of the river Morcille, France in which tebuconazol was found). The experiments were carried out in 20L microcosms in which communities from each of the four environments were either not exposed (control), or exposed to a low (2µg/L) or high (20µg/L) concentration of the fungicide, in order to simulate the contamination levels encountered in aquatic ecosystems. Results obtained after three weeks of incubation showed that 60 to 70% of the initial concentration of tebuconazol had been degraded in the stream microcosms, whereas only minor degradation had occurred in the lake microcosms (15-20%). The fungicide had no significant impact on benthic bacterial abundance, although the proportion of dead bacteria increased. The changes observed in planktonic bacterial abundance and dead bacteria were transient and depended on previous exposure. To complete these findings, the bacterial diversity is characterizing by pyrosequencing of the gene coding for 16S rRNA. This high-throughput sequencing technology provides a deep inventory of the diversity in microbial communities. All the resulting findings will be discussed with regard to the fungicide history of the different communities studied, and to the rate at which these communities degrade the fungicide.

### LINKING CHANGES IN MICROBIAL COMMUNITIES TO TROPHIC VARIATIONS IN A TROPICAL MANGROVE

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Many coastal lagoons in the tropics harbor mangrove formations settled in intertidal zones, which exhibit high diversity and complexity. However, these ecosystems are under severe threat due to increasing anthropogenic pressures. Microorganisms are among the most efficient organisms involved in mangrove ecosystem processes. On solid substrates, they are organized into biofilms consisting mainly of heterotrophic bacteria and autotrophic eukaryotes. Changes in trophic conditions are known to modify the functioning and diversity of these communities. The present study investigated the relationship between changes in nutrient concentrations and changes in microbial communities in a tropical mangrove located in Chirongui Bay (Mayotte Island, France). One site, which was exposed to pre-treated wastewater and considered as impacted, was compared to a pristine control site. Microbial communities sampled from sediments, water and biofilms (colonized on roots or on artificial substrates) were subjected to functional, structural, and diversity measurements. At the impacted site, cell densities (flow cytometry) were significantly higher in the bacterial community, whereas neither the biomass values, nor the photosynthetic performance of the phototrophic community (PhytoPAM) revealed any difference between the 2 sites. The diversity of the phototrophic community (PhytoPAM) showed that the contribution of green algae and diatoms was higher at the impacted site. Molecular fingerprinting (DGGE) of eukaryotic and prokaryotic communities highlighted the presence of contrasting diversity patterns, depending on whether they originated from an impacted site or not. Pre-treated effluents impacted both the structure and the diversity of the microbial communities. Insight into functional diversity of these communities is required in order to improve our understanding of their ability to tolerate effluent discharges, and to assess their biological capacity to remove anthropogenic nutrients.

**MICROBIAL ACTIVITIES, DYNAMICS AND DIVERSITY IN A CHANGING ARCTIC OCEAN (FRAM STRAIT)**

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As climate change is expected to be extremely intense in the Arctic Ocean there is an utmost need to study food-web interactions to contribute to a better understanding of the direction and strength of biogeochemical and microbiological feedback processes. Climate change induced alterations will directly affect food-web structures and ecosystem functioning. Recent studies indicate that environmental changes like increasing temperatures as well as freshening of surface waters promote a shift in the phytoplankton community towards a dominance of smaller cells, especially of eukaryotic picoplankton. The response of oceanic ecosystems and marine carbon cycling to these changes is particularly determined by microbial loop activity. Heterotrophic bacteria, as part of the microbial loop and a crucial component of marine food webs, have a key role in controlling carbon fluxes in the oceans. Microbial activities, dynamics and diversity were studied in the area of the deep-sea long-term observatory HAUSGARTEN of the Alfred-Wegener-Institute (Fram Strait) in July 2009. The investigation area is located within a transition zone between the northern North Atlantic and the central Arctic Ocean, which separates the warm and cold water masses originating from the West Spitzbergen and the East Greenland currents. While bacterial abundance and chlorophyll a were tightly coupled, differences of the planktonic and bacterial community structures are most likely due to the heterogeneous hydrography. Warmer water masses comprise a higher genetic diversity of picoplankton, as it is also expected for bacteria. A shift towards a dominance of smaller plankton species can potentially affect the quality of organic matter and subsequently microbial cycling. Here we present data on bacterial abundance, biomass and protein production, hydrolytical enzyme activities and community structure within different size classes with respect to changing biotic and abiotic conditions in the Fram Strait.

**DOES OCEAN DUMPING AFFECT MARINE MICROBIAL COMMUNITIES?  
INVESTIGATIONS AT A DUMPING SITE IN THE GERMAN BIGHT**

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Bacteria of marine sediments play a crucial role in carbon and nutrient cycles. Surprisingly little is known about the natural succession and the response to environmental changes of benthic bacterial communities in the German Bight. As being very eclectic, the bacterial genome enables its carrier to adapt rapidly to these changes. Thus investigating the bacterial community allows following environmental changes, often caused by anthropogenic activities, very early. Waterways represent important connections in transport systems. Due to tidal pumping effects, sediment often accumulates in coastal rivers, inhibiting them. Dumping of these accumulations into the sea is a common procedure. But the impact of these potentially polluted sediments on bacterial communities of pristine environments is barely investigated. In this study the dumping site “Tonne E3” in the German Bight (North Sea, Germany) was examined. From 2005 to 2010 5 Mio m<sup>3</sup> of excavated material from the Hamburg port area have been dumped into the North Sea. In 2009 and 2010 sediment samples were taken at “Tonne E3” to analyze the bacterial community via automated ribosomal intergenic spacer analysis (ARISA). The dumping area and a reference region were investigated. ARISA focuses on the highly conservative ITS region in the bacterial genome possessing a species specific length polymorphism. ARISA profiles of samples at the dumping area in 2009 and 2010 revealed different bacterial communities changing over time. The analyses of community data in conjunction with environmental data indicate sedimentological factors but also contaminants affecting the communities significantly. In 2009 highly heterogenic community patterns in the vicinity of the dumping area were observed. This interesting fact will be further investigated by performing 454 sequencing of the highly conserved 16S region for representative samples.



**LINKING OF OTC-RESISTANCE WITH AMPICILLIN-RESISTANCE IN BACTERIA FROM COASTAL SEAWATER OF KOREA AND JAPAN**

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Antimicrobial agents have been extensively applied in aquaculture to prevent and control bacterial diseases. Oxytetracycline (OTC) is one of the most popular antibiotics that is used in aquaculture systems because it is effective over a broad spectrum of pathogens and has a low cost. Antibiotic resistance arises by the acquisition of antibiotic resistance genes. The antibiotic resistance genes can be transferred between bacteria in the environment through plasmids, integrons and transposons. Fish farmers generally know that OTC-resistant (OTC<sup>r</sup>) bacteria easily occur when OTC is administered, and the OTC resistance genes can be retained in the environment. Resistance genes for ampicillin (ABPC) and erythromycin (EM), as well as OTC resistance genes, are known to be continuously present in the aquaculture sites and can even persist during untreated period.

The objective of this study is to know the prevalence of OTC<sup>r</sup> bacteria in coastal seawater and to estimate the diverse distribution patterns of multi-drug resistance in OTC<sup>r</sup> bacteria. Seawater samples were collected from coastal aquaculture sites in Japan in 2004 and Korea in 2010. The percentage of OTC<sup>r</sup> was 0.2 to 0.35% in Korea, which was considerably lower than Japan (4.7 to 64.8%). The isolated OTC<sup>r</sup> strains from the Korean coast showed nine patterns of resistance. The major resistance pattern was OTC-ABPC-SMX·TE-EM (9 strains/36), followed by OTC-EM (6 strains) and OTC-ABPC (6 strains). In OTC<sup>r</sup> strains from Japan, OTC-ABPC-mecillinam (MPC) (26%) and OTC-ABPC-EM-MPC (22%) were high. These results suggest various patterns of resistance among OTC<sup>r</sup> strains. Although various cross-resistances were found, the linking of OTC-, ABPC- and EM-resistance genes seems to be general patterns from both Korea and Japan. The location of the genes on the chromosome or plasmid is of particular interest.

**SALINITY AS A MAJOR FACTOR SHAPING BACTERIAL PHYLOGENETIC COMPOSITION IN THE BALTIC SEA**

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The Baltic Sea is characterized by a large horizontal salinity gradient stretching from saline North Sea water to almost limnic conditions in the Northeastern bays, and by an extended brackish zone (salinity 5–8) in central parts. In contrast to multicellular planktonic and benthic organism, not much is known on shifts in the phylogenetic composition of bacteria along the salinity gradient. To investigate this issue, we sampled in high resolution the salinity gradient of the Baltic Sea for a summer and a winter situation and used 454 pyrosequencing of partial (400 bp) 16S rRNA genes to analyse bacterial community composition. The analysis of the midsummer situation showed a gradually replacement of typical marine and freshwater lineages by phylogenetically related operational taxonomic units (OTUs), indicating also finer-scale phylogenetic shifts. Additionally, we identified a brackish water bacterial community comprising a diverse combination of freshwater and marine groups, along with populations unique to this environment, for example an abundant OTUs affiliated with the phylum *Verrucomicrobia*. In contrast to multicellular organisms, bacterial richness and diversity does not seem to be constrained by brackish water conditions. Since water residence times in the Baltic Sea exceed three years, the observed brackish bacterial community cannot be the result of conservative mixing of freshwater and saltwater, but points to a diverse autochthonous brackish microbiome. Currently we are examining the winter bacterioplankton situation in order to reveal more insights into the core bacterial communities at the different salinity zones. Very little is known about the ecology of the brackish bacterial community, but the mapping of the brackish microbiome provides the foundation of functional and genomic investigations of the brackish environment.

**PHYTOPLANKTON DIVERSITY AND NUTRIENTS IN MESOCOSM**

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Fluctuating parameters like temperature, salinity and light are known as some of the most important influences on the yearly periodic of the phytoplankton in the Darss - Zingst bodden chain, an estuary of the Baltic Sea. Irregular dynamics in the species system seems also to be a driving force for biodiversity and interactions between the organisms. Therefore four mesocosms were used in a long-time experiment to examine for the development and diversity of planktical photo-trophical organisms under constant laboratory conditions. With no additional input of nutrients over all months, nutrient flow is only possible by internal microbial food webs between zooplankton, bacteria and phytoplankton. Colony forming, cell-size and nitrogen fixation are known to play a role in the competition for food and space. Advantages for temperature- and light specialists could be excluded in these experiments. We could find out that the four parallel mesocosms were similar in nutrient content and species diversity during the time of experiment. Cyanobacteria dominated the phytoplankton nearly all month. The species composition of the whole plankton in detail showed some differences to the natural ecosystem. After the breakdown of the mass development of phytoplankton a high nutrient release was measured. Possible predator-prey relationships and connections between biomass and nutrient development could be seen in different periods of time.

**BACTERIAL ABUNDANCES AND PIGMENTS ON SEDIMENTS FROM  
SOUTHWEST ATLANTIC**

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Continental margins of the oceans play a key role in global biogeochemical cycles, and the microbiology of the sediments is poorly studied. Bacterial abundance and phytopigments were investigated in sediments from the Southwest Atlantic Ocean up to 3,000 m, along the Campos Basin at Brazilian coast, and we study whether variables as water depth, sediment characteristics and productivity from upper layers could explain deep-sea sediment trophic conditions. The work was on the frame of Habitats Project – Campos Basin Environmental Heterogeneity by CENPES/PETROBRAS. Sediment samples were obtained in triplicates using a box-corer device, and sub-samples for bacterial and phytopigments were transferred to sterile tubes, and flash frozen in liquid nitrogen. At laboratory samples were thawed and (1) bacteria were extracted by pyrophosphate/sonication and quantified by flow cytometry, while (2) pigments were extracted with acetone and quantified by fluorimetry. Bacterial abundances decrease towards deep waters, ranging from  $1,300 \times 10^7$  at 25 m to  $3.6 \times 10^7$  cells.g<sup>-1</sup> at 3,000 m, without differences between dry and rainy seasons. Chlorophyll a also was distributed with a clear vertical trend, with median values from 1.5 µg.g<sup>-1</sup> at 25 m to 0.02 µg.g<sup>-1</sup> deeper than 1,300 m. The values from rainy season on Plataforma (150 m) were higher than those obtained during dry season, reflecting the continental contribution. At depths higher than 400 m very low chlorophyll a values were observed, despite the season. Degradation products seem to have a different pattern, and higher values were obtained during dry season. Bacteria and phytopigments seem to be coupled the organic matter supply from surface waters and their productive cycles. The canyons were regions where high variability was observed, with higher values inside canyons than those obtained in its adjacent areas, indicating they are important for organic matter transport towards deep provinces.

**DOES TERRESTRIAL PARTICULATE ORGANIC MATTER AFFECT BENTHIC MICROBIAL METABOLISM AND CARBON FLOW?**

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Organic carbon in lakes is to a large extent of terrestrial origin which has been shown to support bacterial production in various aquatic environments. However, much less is known about the influence of terrestrial particulate organic carbon (t-POC) on microbial metabolism and carbon flow in littoral sediments. Here, we hypothesize that input of t-POC increases sediment microbial metabolism, whereas leached DOC from added t-POC have a negligible effect. Consequently, increasing concentrations of t-POC may lead to a changing ratio of respired vs. assimilated carbon and hence growth efficiency of sediment bacteria.

Sediment cores with the natural benthic community were taken in March 2011, homogenized in the lab and incubated at 15°C. Incubations comprised i) a short term approach of 48 hours and ii) a long term approach of 4 weeks to study microbial t-POC utilization at different temporal scales. Input of t-POC consisted of maize leaves to better trace the fate of t-POC via stable isotope analysis (SIA). Different quantity and quality of t-POC were solely added to the long term approach to test for different effects of input of leached vs. non-leached t-POC over time. Microbial metabolism was measured by bacterial protein production (BPP) and respiration in all sediment cores. SIA was used to determine carbon flow (respired CO<sub>2</sub> and assimilated carbon).

Our primarily results suggest that BPP in both, short and long term and t-POC addition treatments, change little over time. In contrast, respiration in t-POC addition treatments increases significantly in comparison to the respective controls. These results suggest that growth efficiency of sediment bacteria changes after t-POC addition and that a substantial fraction of t-POC is respired as CO<sub>2</sub>.

**DETERMINING BACTERIAL CARBON CONTRIBUTION TO SEDIMENT ORGANIC MATTER IN THE SETO INLAND SEA OF JAPAN**

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In shallow or coastal waters, the productivity of pelagic and benthic communities is both closely related to the sources and pathways of organic matter. Information on the productivity of benthic ecosystems is particularly essential for understanding their spatial and temporal dynamics. Quantifying the flow of organic matter and energy through benthic microorganisms requires measurement of microbial biomass, and their growth efficiency and production rate. Microalgae and bacteria play important roles in grazing and detritus-based food chains, respectively. However, accurate measurement of their respective biomass in sediments has proven to be problematic. One goal of our study was to estimate the bacterial carbon content in sediments. Sediment microbial biomass was measured as the microbial quinone content by HPLC. Microbial quinones, which are one of the coenzymes in the electron transport chain of microbial cells, are divided into two groups, respiratory quinone (including ubiquinone and menaquinone) and photosynthetic quinone. In general, one species or genus of bacteria has only one dominant species of respiratory quinone, and microalgae and cyanobacteria has the photosynthetic quinones. Thus, we calculated the bacterial carbon content of the sediment using the respiratory quinone content of the sediment. The sediment samples (up to 2 cm in depth) were collected from bays and embayments (nadas) in the Seto Inland Sea from September to October, 2008. A significant positive correlation was found between organic matter content and total quinone content of the sediments. The bacterial carbon content in the sediment accounted for  $6.2 \pm 3.6\%$  (mean  $\pm$  SD) of the total organic matter content of the sediment. The sediment bacterial carbon content was approximately 2.2 times higher than its respective algal carbon content. Thus, this result suggests that the bacterial biomass in the sediment contributed a significant fraction to the benthic production.

## ACTIVITY OF METHANE OXIDIZING BACTERIA IN DIFFERENT DEPTHS OF WATER COLUMN ALONG THE RIVER ELBE DOWNSTREAM TO ITS ESTUARY

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Methane (CH<sub>4</sub>) is the second most important greenhouse gas and so it is required to determine its sources and sinks for process studies and global budgets. Contribution of rivers and estuarine systems to the global budget is still not clear yet and for improving the estimations on the CH<sub>4</sub> emission it is essential to cover the whole natural system in large-scale studies. In this context we intend to quantify the activity of methane oxidizing bacteria (MOB), investigate the factors influencing the process rate and compare the MOB population structures along a large European river system, the river Elbe, from its source in the Czech Republic towards to its estuary in the North Sea.

The range of CH<sub>4</sub> concentrations and related microbial oxidation activities displays an increasing trend from the upper river parts (upstream Pardubice, which includes partly natural river) to higher CH<sub>4</sub> concentrations and microbial activities in the polluted downstream canalized parts of the river, where anaerobic sediments occur. In the water column of the estuarine zone (salinity about 0,36 ‰) CH<sub>4</sub> oxidation rates were mainly influenced by the higher CH<sub>4</sub> concentrations (~ 900 nM/l) and nutrient content. In contrast a decreasing CH<sub>4</sub> concentrations and MOB activities were observed from the estuarine margin (659. km downstream Elbe) to the open coastal sea.

In additional experiments MOB activities were compared in water taken from the estuarine Elbe and North Sea with varying temperature (2° - 25°C) and CH<sub>4</sub> concentration (20 nM/l – 30.000 nM/l). MOB from the Elbe water oxidize CH<sub>4</sub> faster than MOB from the North Sea especially at lower CH<sub>4</sub> concentrations. On the other hand MOB from the North Sea can still oxidize CH<sub>4</sub> even at high concentrations and temperature. Further investigations will compare the population structure of MOB at the different sites and also compare their response to other varying environmental factors.

**POLYPHOSPHATE STORAGE IN BEGGIATOA ALBA**Havemeyer, S.<sup>1</sup> and Schulz-Vogt, H.N.<sup>1</sup><sup>1</sup>Max Planck Institute for Marine Microbiology, Bremen, Germany; MARUM, Center for Marine Environmental Sciences, Bremen, Germany

Polyphosphate storage is a common feature in many microorganisms. Although it is a wide spread phenomenon, the trigger for polyphosphate storage and its function is often unknown. In this study *Beggiatoa alba* was cultivated in two different organic-rich media and we observed that in medium A (according to Strohl and Larkin, 1978) they store polyphosphate whereas in medium B (according to Schmidt *et al.*, 1987) they do not. The two media differ in the quality and quantity of organic substrate and in the composition of trace elements and basal salts. In order to find out which difference between the two media is responsible for absence or presence of polyphosphate inclusions, each difference between medium A and B was tested for its effect on polyphosphate storage. Polyphosphate was visualized in the filaments by staining with 4',6-Diamidin-2'-phenylindole (DAPI), resulting in a yellow fluorescent signal. The organic substrate and trace element composition had no effect on polyphosphate storage. However, we observed that high chloride concentrations or the ratio of chloride to calcium and magnesium prevented polyphosphate storage. A similar effect was observed for two lithotrophic, freshwater *Beggiatoa* strains. Polyphosphate storage requires a luxurious phosphate uptake. The uptake of the phosphate anions is coupled to a concurrent uptake of cations like calcium and magnesium. This uptake mechanism seems to be disturbed in the presence of high concentrations of chloride anions compared to calcium and magnesium concentrations. Our results demonstrate that polyphosphate storage is not always a feature which is present or absent in a certain microorganism, but can largely depend on yet unknown factors introduced by the composition of the medium.



**SPATIAL AND TEMPORAL VARIATION OF METHANE PRODUCTION AND EMISSION FROM A TROPICAL LAKE (PANTANAL, BRAZIL)**

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Methane is an important greenhouse gas that is produced as the end product of anaerobic degradation of organic matter. Tropical lakes are an important environment for methane production and also an important source of methane emitted to the atmosphere. The aim of this research was to evaluate the spatial and temporal variation of sediment methane production and methane emissions from a small lake close to the Paraguay River in the Pantanal (Brazil). Sediment was collected and transported to the laboratory where it was incubated to follow methane and carbon dioxide concentrations over time. In some of the vials, CH<sub>3</sub>F was used to specifically inhibit acetotrophic methanogenesis. Methane fluxes were calculated using diffusion chambers for 24 hours. Hydrogenotrophic methanogenesis was the dominated process. Methane production was higher in when the water level was higher, indicating a temporal variation, and it was also higher in the lake margin, indicating a spatial variation in methanogenesis production. Methane emission was also higher at the margin than in the center of the lake indicating spatial variation.

**LINKING BIOGEOCHEMICAL ZONATION IN HYDROCARBON CONTAMINATED  
AQUIFERS TO MICROBIAL ACTIVITY**

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Groundwater reservoirs (aquifers) are important drinking water sources. We study the natural microbial hydrocarbon degradation in a petroleum-contaminated aquifer at an industrial mega-site in Leuna (Germany). Within the mostly anoxic contaminant plume anaerobic metabolic processes are dominant. We have the hypotheses that fluctuations of the water table, introduced into the aquifer, increases the microbial degradation potential due to an enlargement of the aerated plume fringe zone, increased transport of nutrients and electron acceptors and spreading of the contaminants for enhanced availability for the microbes. In a first experiment we studied the influences of water table fluctuation using a constructed wetland as model system. Microsensors applied in situ and single cell approaches such as MAR-CARD-FISH were used to identify hotspots of contaminant degradation and active key players in the aquifer sediments. Data will be presented which show in situ analytical measurements on a high spatial resolution. We could show that the introduction of fluctuation into the groundwater saturated sediment leads to oxygen input along the area of water table fluctuation. Analytics of in- and outflow show in comparison to the control compartment contaminant degradation at higher rate. Preliminary MAR-CARD-FISH data to identify the active pollutant biodegraders will be presented.

**IDENTIFICATION OF ACETATE INCORPORATING ARCOBACTER SPP. AS POTENTIAL MANGANESE REDUCERS IN PELAGIC REDOXCLINES OF THE CENTRAL BALTIC SEA VIA 16S rRNA BASED <sup>13</sup>C STABLE ISOTOPE PROBING**

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Pelagic redoxclines in the central Baltic Sea are recognized as environments with elevated microbial activities comprising both, heterotrophic and autotrophic prokaryotes, involved in important biogeochemical cycles. Aim of our study was to reveal first insights into the identity and function of heterotrophic bacteria in this habitat which is well-studied with respect to autotrophic activities. Therefore, pelagic redoxclines of the Gotland basin were sampled in 2005 and 2009, respectively, and subjected to stimulation experiments with different organic substrates and electron acceptors, followed by the identification of stimulated bacteria using 16S rRNA gene single strand conformation polymorphism (SSCP) analyses. In addition, RNA stable isotope probing (RNA-SIP) followed by subsequent 16S rRNA based quantitative RT-PCR and fingerprinting served to identify acetate incorporating organisms. In 2005, in water from the sulfidic zone, 17.3  $\mu\text{Mol Mn}^{4+}$  were reduced after 48 h and bacteria affiliated with the epsilonproteobacterial *Arcobacter* sp. dominated the incubation. In 2009, bulk incorporation of <sup>3</sup>H labeled acetate was highest in the oxic-anoxic interface layer and still high in the sulfidic zone. After 72 hours, bacteria affiliated with *Arcobacter* sp. incorporated the <sup>13</sup>C-labeled acetate in the oxic-anoxic interface layer and the sulfidic zone while the gammaproteobacterial genera *Neptunomonas* sp. and *Colwellia* sp. incorporated acetate in the oxic-anoxic interface layer only. Together, in both experiments two phylogenetically distinct clusters within the genus *Arcobacter* sp. were identified related to previously recovered *Arcobacter* sp. from manganese-oxide rich shelf sediments in the Black Sea by Thamdrup et al. 2000. Thus, we identified acetate utilizing *Arcobacter* spp. as potential heterotrophic manganese reducers in pelagic Baltic Sea redoxclines.

**DISTRIBUTION OF SYNECHOCOCCUS AND PROCHLOROCOCCUS IN THE CENTRAL AND SOUTHERN ADRIATIC SEA**

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The abundance of Synechococcus and Prochlorococcus were determined at twenty one stations located along the eastern coast of central and southern Adriatic and three stations located in the open central Adriatic Sea. Synechococcus abundance in the coastal area ranged from  $10^2$  cell mL<sup>-1</sup> to  $10^5$  cell mL<sup>-1</sup>, while in the open sea from  $10^3$  cell mL<sup>-1</sup> to  $10^4$  cell mL<sup>-1</sup>. Prochlorococcus abundance in the coastal area ranged from 0 cell to  $10^3$  cell mL<sup>-1</sup>, while in the open sea from  $10^3$  cell mL<sup>-1</sup> to  $10^4$  cell mL<sup>-1</sup>. The seasonal distribution of Synechococcus and Prochlorococcus mostly showed an increase in density during warmer period and decrease during winter. The highest abundance of Synechococcus was found in the Kaštela Bay and amounted to  $4.57 \cdot 10^5$  cell mL<sup>-1</sup>, while the highest abundance of Prochlorococcus was determined in the Šibenik area and amounted to  $7.1 \cdot 10^4$  cell mL<sup>-1</sup>. It was mostly found the prevalence of Synechococcus abundance over Prochlorococcus abundance. The abundance of Synechococcus was influenced by HNF more than by nutrient availability, while abundance of Prochlorococcus was influenced by nutrient availability, respectively by the movement of water mass more than by HNF.

**MACROPHYTE DRIVE THE VARIATION OF BACTERIOPLANKTON COMMUNITY COMPOSITION IN SHALLOW LAKES**Wu, Q.L.<sup>1</sup>, Zeng, J.<sup>1</sup>, Bian, Y.<sup>1</sup>, Xing, P.<sup>1</sup><sup>1</sup>*Institute of Geography & Limnology, Chinese Academy of Sciences*

Macrophytes play an important role in structuring freshwater ecosystems, including controlling the composition of bacterial communities. We explored the idea that macrophyte species from freshwater bodies may be involved in determining the bacterioplankton community composition (BCC). We investigated the BCC in several areas dominated by different macrophyte species including *Nymphaea peltata*, *Zizania caduciflora*, *Vallisneria spirulosa*, and *Ceratophyllum demersum* in Taihu Lake, a large and shallow lake, over a one-year period. Following the field investigation, microcosm experiments were conducted to determine if a single species of macrophyte in an isolated environment would alter the BCC. The BCC was analyzed by denaturing gradient gel electrophoresis (DGGE) of the bacterial 16S rRNA genes and by cloning and sequence analysis of selected samples. The field investigation indicated that the BCC changed significantly from season to season, and the presence of different macrophyte species resulted in a lower BCC similarity during the summer and fall. The microcosm studies indicated that the BCCs differed significantly when associated with different species of macrophyte. The LIBSHUFF analysis of selected clone libraries confirmed the DGGE results obtained from field investigation and microcosm experiments. Our study demonstrated that macrophyte species are an important factor in shaping the BCC in freshwater ecosystems, but the influence is variable within large seasonal and spatial scale.

**OCCURRENCE OF ROSEOBACTER SUBCLUSTERS IN THE GERMAN BIGHT OF THE NORTH SEA**

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The Roseobacter clade of Alphaproteobacteria comprises a large fraction of heterotrophic marine bacteria. Members of this clade, for the most part represented by 16S rRNA gene sequences in clone libraries, were detected globally in diverse marine ecosystems. They can constitute large proportions of total Bacteria which can vary seasonally and as a function of environmental factors. However, most of the Roseobacter clusters which appear to be relevant in biogeochemical processes in surface waters, e.g. clusters RCA, NAC11-6, NAC11-7, CHAB-I-5 and SH6-1, consist predominantly of uncultured phylotypes and only scarce information exists on the simultaneous occurrence of distinct subclusters.

In order to elucidate the occurrence of the major pelagic subclusters of the Roseobacter clade in coastal waters we studied the occurrence of these clusters in the German Bight of the North Sea during a phytoplankton spring bloom in May and October 2010 and in March 2011. Due to the fact that members of the Roseobacter clade are often found in association with algae we differentiated between the particle-associated (PA, 5µm) and the free-living (FL, 0.2-5µm) bacterial fraction and analysed the DNA extracted from polycarbonate filters by PCR with cluster-specific primers. Further, we applied DGGE of 16S rRNA gene fragments to access the diversity of the Roseobacter clade and real time quantitative (q) PCR to quantify the abundance of the clusters of interest. All five subclusters were detected in the investigated area but predominantly in the FL bacterial fraction. The Roseobacter-specific DGGE showed rather diverse banding patterns and pronounced differences between the PA and FL bacterial fractions.

**CONTRIBUTION OF MAJOR PROKARYOTIC GROUPS IN THE ROMANCHE FRACTURE ZONE OF THE TROPICAL ATLANTIC**Lekunberri, I.<sup>1</sup>, Sintés, E.<sup>1</sup>, Yokokawa, T.<sup>2</sup>, and Herndl, G.J.<sup>1</sup><sup>1</sup>*Department of Marine Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria,*<sup>2</sup>*Center for Marine Environmental Studies (CMES) Ehime University, Bunkyo 3, Matsuyama, Ehime 790-8577 Japan*

The distribution of major groups of Bacteria and Archaea was determined in the meso- and bathypelagic waters along a transect through the Romanche Fracture Zone (RFZ, 2°7'S 31°79'W to 0°6'N 14°33'W) in the tropical Atlantic. The relative abundance of Crenarchaeota, assessed by catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH), was significantly higher in the deeper zones (30% of 4',6'-diamidino-2phenylindole (DAPI)-stained cells) than at the lower end of the euphotic zone (16%). Bacteria showed contrasting distribution patterns among different groups. The SAR202 clade increased slightly in abundance with depth while the SAR11 cluster decreased in abundance from 11% at the lower euphotic layer to 7% in bathypelagic waters. Less abundant groups such as Planctomycetes and SAR406 were fairly stable in abundance throughout the water column down to abyssopelagic regions. Throughout the RFZ, a distinct west-east trend in cell-specific prokaryotic heterotrophic production, with higher values in the eastern stations ( $7.6 \times 10^{-13}$  leucine incorporation  $\text{cell}^{-1} \text{h}^{-1}$ ) compare to the western stations ( $2.8 \times 10^{-13}$  leu  $\text{cell}^{-1} \text{h}^{-1}$ ). These differences in the leucine incorporation rates might be related to the dominant groups along the transect. In the western stations, the contribution of SAR202 to total prokaryotic abundance was higher than in the eastern stations (decreasing from 25% to 14%). Also, Planctomycetes was more abundant in the western stations (18% of total prokaryotes) than in the eastern stations (8%). The distribution pattern obtained by single-cell analysis using CARD-FISH was compared with that by clone libraries of Bacteria and Archaea in one of the stations (St. 23). Overall, both methods revealed similar distribution patterns with some exceptions. Generally, it was found that the distribution of specific bacterial clusters is rather similar in the bathy- and abyssopelagic waters, with major changes in some bacterial groups within the mesopelagic zone.

**HETEROTROPHIC ACTIVITY OF ARCHAEA AND BACTERIA THROUGHOUT WATER COLUMN OF THE EASTERN ATLANTIC**

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The abundance and leucine incorporation rate of Archaea and Bacteria were determined throughout the water column along a transect in the eastern Atlantic. Archaeal and bacterial abundances were determined by Catalyzed Reporter Deposition - Fluorescence In Situ Hybridization (CARD-FISH). Bacteria dominated throughout the water column, although their contribution to total prokaryotic abundance in the bathypelagic layer (1000 - 4000 m) was lower than in the surface and mesopelagic layers (0 - 1000 m). Crenarchaeota contributed about  $30 \pm 12\%$  to the total prokaryotic abundance with a generally higher contribution in the bathypelagic layer than in the surface and mesopelagic layers. Euryarchaeota contributed less than 5% to the total prokaryotic abundance throughout the water column. Leucine incorporation rates were determined for the total prokaryotic community as well as for Bacteria and Archaea separately using selective inhibitors. The bacterial inhibitor erythromycin and the archaeal inhibitor diphtheria toxin were used to determine the contribution of Bacteria and Archaea, respectively, to total heterotrophic activity. The contribution of Bacteria to heterotrophic prokaryotic activity in the Atlantic amounted to  $72 \pm 13\%$  in the surface and mesopelagic layers (0 - 1000m), decreasing to  $56 \pm 14\%$  in the bathypelagic layer (1000 - 4000m). In contrast, there was no significant depth-related trend in the archaeal contribution to heterotrophic prokaryotic activity from the surface and mesopelagic layer ( $24 \pm 9\%$  of heterotrophic prokaryotic activity) to the bathypelagic layer ( $29 \pm 14\%$ ). Our results show that heterotrophic archaeal activity is remarkably high throughout the Atlantic's water column.



**DESIGN AND OPTIMIZATION OF SPECIFIC PRIMERS FOR THE FRESHWATER THAUMARCHAEOTAL GROUP SAGMAGC-1: POPULATION DYNAMICS AND LINKS BETWEEN THE CARBON AND NITROGEN CYCLES**

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The study of the biology and ecology of uncultured Archaea has attracted much attention in aquatic microbial ecology lately. Freshwater environments contain a large archaeal phylogenetic richness, and offer great research opportunities to explore the ecology of these uncultured organisms. SAGMAGC-1 (Thaumarchaeota) is a characteristic group present in oligotrophic lakes closely related to the marine archaea nitrifiers. To gain knowledge on the biology, ecology, and biogeochemical links of SAGMAGC-1, we have analyzed a large physicochemical gradient in a high mountain lakes area (Limnological Observatory of the Pyrenees). Specific primers for the 16S rRNA gene were designed and tested to examine the distribution, composition and dynamics of this group, by cloning and quantitative PCR. Links to the nitrogen and carbon cycle (chemoautotrophy) were explored targeting the ammonia monooxygenase (*amoA*) and the 4-hydroxybutyryl-CoA dehydratase (*4-Hbd*) genes, respectively, following the same experimental design. Preliminary data show positive results for all three datasets. Further steps will explore correlational analysis among gene abundances and specific probe design for single cell analysis by MICROFISH.

**DOMINANCE OF CURVE-SHAPED BACTERIA UNDETECTABLE WITH CONVENTIONAL FISH PROBES IN HYPOLIMNION OF LAKE BIWA, JAPAN**

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A high abundance of visibly identical (cell length = 1.2-1.5  $\mu\text{m}$ , width = 0.3-0.4  $\mu\text{m}$ ) curve-shaped bacteria (CSB) was found in the aerobic hypolimnion of a monomictic lake (Lake Biwa, Japan). In the present study, the vertical distribution of CSB was monitored monthly from January 2010 to March 2011 at a pelagic station in the lake. Throughout the stratification period (April-December), CSB abundance increased from  $2.4 \times 10^4$  cells  $\text{ml}^{-1}$  (0.9% of the total bacteria) to  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$  (17% of the total bacteria) in the hypolimnion (below 15-40 m), accounting for 7 to 33% of the total bacterial biovolume. Meanwhile, in the epilimnion, CSB abundance remained low ( $0.9$ - $4.7 \times 10^4$  cells  $\text{ml}^{-1}$ ). During the mixing period, CSB abundance decreased to the level observed in April of last year (2010). CSB abundance thus showed an annually cyclic changing pattern. Similar results were found in samples taken at another station located 20 km north from the monthly monitoring station, suggesting that CSB dominates in the hypolimnion of whole the lake. Abundance of neither phytoplankton nor heterotrophic nanoflagellate correlated with abundance of CSB. CARD-FISH with the probe EUB338 successfully detected 99% of CSB. However, 80% of CSB could not be labeled with conventional specific probes (ALF968, BET42a, GAM42a, CF319a and HGC69a), suggesting that most of CSB are affiliated to one or more rare phylogenetic groups. Sequencing of 16S rRNA clone library of hypolimnetic bacterial community together with that of hypolimnion-specific DGGE bands suggested that the phylum *Chroloflexi* and the phylum *Nitrospira* were possible candidates for the phylogenetic affiliation of CSB. Probes targeting these candidate phyla may detect CSB, which will be useful for the elucidation of biochemical cycling in the lake.

**OCCURENCE OF AUTOTROPHIC BACTERIA AND ARCHAEA IN THE MESOAND BATHYPELAGIC WATERS OF THE ATLANTIC**

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Meso- and bathypelagic waters in the subtropical North Atlantic were sampled to investigate the distribution, abundance and potential biogeochemical role of planktonic prokaryotes. Thus far, autotrophic growth of mesophilic Crenarchaea using ammonia as energy source has been demonstrated. Their metabolic pathways of carbon processing remain controversial, however, with apparent hetero- and autotrophic and possibly, also mixotrophic traits. Hence, to elucidate the significance and the key players of carbon fixation in dark realm of the tropical Atlantic, we targeted bacterial and archaeal genes indicative for carbon fixation, i.e., encoding for the conserved acetyl-CoA carboxylase (*accA* and *accC* subunits) and the 4-hydroxybutyryl-CoA dehydratase (*4-hbd*), both key enzymes powering inter alia the 3-hydroxypropionate/4-hydroxybutyrate cycle. Quantification of *accA* and *accC* like genes revealed a consistent depth profile of increasing copy numbers from subsurface layers and to the oxygen minimum zone, coinciding with an increase in archaeal *amoA* gene abundance. These gene abundance profiles were related to crenarchaeal 16S rRNA (MCGI) and archaeal *amoA* gene numbers as well as inorganic carbon uptake rates to link a genetic potential to actual carbon fixation rates. We found the median crenarchaeal 16S rRNA to *amoA* gene ratio close to unity in mesopelagic waters suggesting, that virtually all MCGI have the capacity of ammonia oxidation. Dark CO<sub>2</sub> fixation rates normalized to *accA* gene abundance varied by three to five orders of magnitude at any given depth, indicating only a rather weak link between autotrophic gene abundance and CO<sub>2</sub> fixation. However, our results indicate that deep ocean prokaryotes might have a larger potential of autotrophy than assumed hitherto.

**CHANGES IN BACTERIAL DIVERSITY DURING INCUBATIONS OF SURFACE AND MESOPELAGIC ATLANTIC WATERS**

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Changes in the structure of the bacterioplankton community subjected to confinement were studied in 15 seawater culture experiments with surface and mesopelagic waters collected at 6 stations in the (sub)tropical North Atlantic. We analyzed the composition of bacterial assemblages at the beginning and the end of the incubations by terminal restriction fragment length polymorphism (T-RFLP). Generally, changes in the community composition during the course of the incubations for 5-7 days led to a clustering of the bacterial communities at the end of the incubations while the initial bacterial communities were clearly differed from each other. At the end of the incubations, the bacterial communities of the lower mesopelagic waters (~1000m depth) clustered separately from the surface communities (100m depth), while no clear depth-related pattern was found in the original communities. 2 out of the 15 experiments were further analyzed by cloning and sequencing of 16S rDNA genes. Sequence analysis confirmed the T-RFLP data revealing a significant decrease in the diversity of bacterial communities during the incubations. Sequences obtained at the beginning of the experiments were affiliated to different classes of Proteo-, Acido-, Actino-, Cyanobacteria, Bacteroidetes, Firmicutes and Verrucomicrobia. Particularly, most of the sequences were affiliated to Alphaproteobacteria (always 55%), dominated by bacteria closely affiliated to the genus *Pelagibacter*. After 5-7 days of incubation at in situ temperature, the majority of bacterial sequences belonged to 3 or 4 different classes dominated by Alpha- and Gammaproteobacteria (56 and 43%, respectively) in surface and by Gammaproteobacteria (95%) in mesopelagic (406m depth) waters. Additionally, T-RFLP of selected clones allowed us to link the T-RFLP OTUs from the experiments to specific phylogenetic groups, enabling to track the occurrence of specific phylotypes in the T-RFLP fingerprints throughout the different experiments.

**MICRODIVERSITY AND HABITAT PREFERENCES OF FRESHWATER BACTERIA OF THE GENUS *LIMNOHABITANS* (BETAPROTEOBACTERIA)**

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Currently 36 bacterial strains affiliated with the recently described *Limnohabitans* genus, mostly of its RBT lineage, were isolated from a broad spectrum of non-acidic European freshwater habitats. Phylogenetic analysis of the ribosomal SSU and ITS1 gene suggests the possibility to divide the *Limnohabitans* genus into five tribes - LimA to LimE. The isolated strains from the RBT lineage (LimB to LimE) possess diverse morphology ranging from small cocci (0.02 - 0.05  $\mu\text{m}^3$ ), rods (0.04 - 0.10  $\mu\text{m}^3$ ), thin curved rods (0.12 - 0.20  $\mu\text{m}^3$ ), to large solenoids with cell volumes of 0.28 - 1.0  $\mu\text{m}^3$ . All these morphotypes correspond to bacterial cells typically targeted with the R-BT065 FISH probe in more than 100 freshwater lakes, reservoirs, and various ponds located in central Europe on an altitudinal gradient from 290 to 2375 m a.s.l. On average, the RBT lineage accounted for 9.4% of the total bacterioplankton (range, 0 to 29%). Due to their generally larger mean cell volume compared to typical bacterioplankton cells, they contribute over-proportionally (up to 40 %) to the total bacterioplankton biomass. The relative abundance and absolute abundance of these bacteria were significantly and positively related to higher pH, conductivity, and the proportion of low-molecular-weight compounds in dissolved organic carbon (DOC) and negatively related to the total DOC and dissolved aromatic carbon contents. Surprisingly, no clear relationship of the RBT bacteria to factors indicating the trophic status of habitats (i.e., different forms of phosphorus and chlorophyll a content) was found. In addition, the occurrence of different morphologies of these bacteria in selected representative habitats will be discussed.

**ROS FORMATION BY PHOTOCHEMICAL REACTIONS AFFECT BCC IN A HUMIC LAKE AND INDUCE ADAPTIVE RESPONSES IN ABUNDANT BACTERIA**

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Sunlight-mediated photochemical reactions of colored dissolved organic matter (CDOM) is an important process in humic lakes enhancing substrate availability for heterotrophic bacterioplankton. Although bacterioplankton species benefit from generated carbon substrates they have to cope with toxic reactive oxygen species (ROS) generated simultaneously. We investigated effects of artificially increased singlet oxygen (<sup>1</sup>O<sub>2</sub>) formation and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentrations on bacterioplankton community composition (BCC) in the subsurface water layer of the humic Lake Grosse Fuchskuhle. BCC changes of abundant and metabolically active bacteria were investigated by the generation of 16S rRNA gene clone libraries and 16S rRNA targeting RT-PCR DGGE analysis using Bacteria and group-specific primer-systems. Major bacterioplankton groups respond differently to <sup>1</sup>O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> exposure. Alphaproteobacteria (*Novosphingobium acidiphilum*) and Betaproteobacteria (*Polynucleobacter necessarius* and *Limnohabitans*-related species) increased in relative abundance after <sup>1</sup>O<sub>2</sub> but not after H<sub>2</sub>O<sub>2</sub> exposure. In contrast freshwater Actinobacteria were not detected after <sup>1</sup>O<sub>2</sub> exposure but increased in relative abundance after H<sub>2</sub>O<sub>2</sub> exposure. We were able to isolate strains representing the above-mentioned Alpha- and Betaproteobacteria and used those for laboratory and in situ studies to investigate the response to ROS exposure. First experiments showed that those strains were capable to withstand increased <sup>1</sup>O<sub>2</sub> exposure after pre-incubation with moderate <sup>1</sup>O<sub>2</sub> concentrations occurring regularly in the investigated ecosystem. Our results indicate that ROS generation by CDOM photolysis is an important factor for BCC in humic lakes and favor species with adaptive response mechanisms to ROS exposure.

**BACTERIAL COMMUNITY STRUCTURE FROM THE SEDIMENTS OF  
JIULONG RIVER ESTUARY IN CHINA**

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Jiulong river estuary is located at Fujian Province in South China. With the increasing of population and the development of economics along the river, wastewater containing a variety of chemical compounds was released into the river and generated environmental problems. Those chemicals can reside in sediments of estuary where microbes play an important role in the remediation.

In order to figure out the bacterial communities, samples from the sediments of Jiulong river estuary were collected and bacterial 16S rRNA gene library was constructed. Around 60 clones were randomly selected and sequenced. RFLP and phylogenetic analysis indicated that bacteria in the Jiulong river estuary were extremely diverse, mainly including Proteobacteria, Firmicutes, Acidobacteria, Bacteroidetes, Chloroflexi, and Planctomycetes. However, most of the 16S rRNA genes were uncultivated bacteria. Proteobacteria was a dominant group in the library, in which  $\delta$ -Proteobacteria had the highest proportion. Desulfobacteraceae and Desulfobulbaceae, affiliated with the  $\delta$ -Proteobacteria, were also detected in the library and mainly related to those putatively involved in sulfur metabolism and contaminant degradation. In addition, several clones had high similarities to the bacteria with the function of degradation of heavy metals and polycyclic aromatic hydrocarbons. These results demonstrated that the sulfate reduction was a dominant metabolism process and industrial pollution had an influence on the habitat of microbes in the sediment of Jiulong river estuary.

**QUANTITATIVE SINGLE-CELL ANALYSIS AND PHYLOGENETIC CHARACTERIZATION OF THE ACTIVE BACTERIAL CLADES IN AN OLIGOTROPHIC MARINE SYSTEM (CILICIAN BASIN; NORTH-EASTERN MEDITERRANEAN SEA).**

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The identification and proper quantification of the active bacterial clades constitute the first steps to better interpret the key role of microbes in marine systems. In the framework of the EU Project SESAME, two oceanographic cruises were performed in the Cilician Basin, considered one of the most oligotrophic areas of the Mediterranean Sea. Along a transect located between the Turkish coastline and Cyprus, we selected mid-shelf and off-shore water columns, under unstratified conditions (March 2008 and February 2009). Molecular techniques in combination with epifluorescence microscopy (CARD-FISH) and image analysis allowed a detailed study on the phylogenetic composition and structuring of marine microbial communities. The analysis of epifluorescent images allowed the estimation of the size class distribution of each analyzed bacterial group, revealing that the per-cell biomass ranged on average from 10 to 20 fg C. Moreover, the frequency of cells with DNA de novo synthesis was determined counterstaining the thymidine analogue 5-bromo-2-deoxyuridine incorporating cells (BrdU-FISH). Following the specific optimization of each applied protocol, the results showed a high abundance of Alpha-proteobacteria (with a percentage ranging between 44-60%), and Cytophaga-Flavobacteria (17-27%), in comparison with the percentage of total bacterial cells (70-90%). Interestingly, the percentages of DNA synthesizing cells (2-20% of total cells) showed a strong correlation between the specific activity and abundance of the two dominant groups.



**SEASONAL CHANGES IN THE GROWTH POTENTIAL OF BACTERIAL POPULATIONS  
IN LAKE ZURICH**

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Although the growth rates of individual members of bacterioplankton can differ drastically, mixed bacterial assemblages are nevertheless maintained in pelagic environments. One factor that can prevent the numerical dominance of the fastest growing bacteria is selective predation, e.g. phagotrophic flagellates tend to preferably feed on large and fast growing bacteria. Rather small populations of highly active bacteria can, therefore, contribute considerably to total bacterial biomass production. We aimed to identify such highly productive bacteria in grazer free dilution cultures and to monitor their persistence or succession in different seasons. Highly competitive "growth specialists" were distributed among four bacterial classes and their growth rates showed contrasting seasonal patterns. Members of several species-like lineages of *Flavobacteria* were highly competitive during diatom blooms in spring and in summer, but none of them was successful at both time points.  $\gamma$ -*Proteobacteria* showed very high growth rates in periods between algal blooms, whereas the growth rates of  $\alpha$ -*Proteobacteria* were tightly correlated with temperature.  $\beta$ -*Proteobacteria*, in particular from the R-BT lineage, were highly competitive in winter and in early spring only. Our results indicate that there are opportunistically growing bacteria from different bacterial classes in Lake Zurich that experienced a growth advantage only at particular times of the year and that might encompass several closely related specialized populations. For example, the fast growing *Flavobacteria* apparently comprised several highly specialized lineages that responded to specific sets of bottom-up factors (such as algal exudates or temperature).

**PECULIAR SPACE-TIME DYNAMICS OF PROKARYOTIC PICOPLANKTON IN A VOLCANIC TROPICAL LAKE**

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We evaluated the abundance and biomass of autotrophic (APP) and heterotrophic (HPP) picoplankton in a hyposaline (6.2 g/L) maar lake in Mexico throughout a year. APP was mainly formed by small picocyanobacteria (mean size:  $1.25 \times 0.71 \mu\text{m}$ ; mean volume:  $0.46 \mu\text{m}^3$ ). APP number reached  $7 \times 10^5$  cells  $\text{ml}^{-1}$  and HPP  $10 \times 10^6$  cells  $\text{ml}^{-1}$ . Picoplankton dynamics followed the warm-monomictic hydrodynamics of the lake. The maximum APP and HPP abundance and biomass was found during mixing, in winter with minimum temperature of  $14^\circ\text{C}$ , in association with the highest nutrient availability. During stratification, picoplankton variability was related to thermal and chemical gradients in water column. HPP showed high temporal fluctuations related with nutrient availability and oxic-anoxic conditions. APP varied spatially more than temporally with a peak at thermocline depth with low irradiance and high nutrients. The whitening event observed in summer likely could be triggered by APP high number.

**SPACE-TIME DISTRIBUTION OF THE BACTERIAL COMMUNITY IN THE BRACKISH MEROMICTIC LAKE FARO (MESSINA, ITALY)**

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Due to the vertical gradients of dissolved substances, meromictic lakes have a vertical zonation of microbial community. These features produce suitable conditions for the study of key steps in the biogeochemical cycling of elements. Despite considerable attention in recent years, the composition and dynamics of lake bacterial communities over annual time scales are poorly understood.

The aim of this work was to study the bacterial vertical distribution and the patterns of change in relation to environmental variables and mixing conditions in the Lake Faro (Messina, Italy), a brackish meromictic coastal lake characterized by a permanent stratification.

During 2010, samples were collected monthly along the water column of a station located at the centre of the lake (maximum depth ~30 m). Oxic, transitional (redoxcline) and anoxic waters were taken into consideration.

Spatial variations in the abundance and structure of community composition was monitored by using the Automated Ribosomal Intergenic Spacer Analysis (ARISA). The major bacterial groups were enumerated by using the Fluorescence In Situ Hybridization - Catalyzed Reporter Deposition (CARD)-FISH with oligonucleotidic probes targeting specifically 16S rRNA gene of major bacterial groups. The population abundance in each sample was determined by epifluorescence microscope after 4,6-diamidino-2-phenylindole (DAPI)-staining.

The patterns of change in bacterial communities indicated that physical-chemical parameters are the forces that regulate the structuring of the bacterial communities of Lake Faro. It was found a clear stratification in the three different layers of water in terms of OTU and phylogenetic compositions.

**BACTERIAL COMMUNITIES ASSOCIATED WITH THE MEDITERRANEAN SEA  
PENS *PENNATULA PHOSPHOREA* AND *PTEROEIDES SPINOSUM***

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Microbes have been reported earlier in association with corals. However, bacterial communities associated with soft corals and sea pens, remain poorly characterized, even if many bioactive metabolites have been isolated from these organisms and symbiotic bacteria are thought to be the true source of these compounds. Research on sea pens is limited due to difficulties during sampling which is often complicated by the high depths where these organisms live.

The aim of this work is to provide a contribution to the knowledge of the bacterial communities associated with Mediterranean sea pens *Pennatula phosphorea* Linnaeus, 1758 and *Pteroeides spinosum* (Ellis, 1764).

Spacer Analysis (ARISA). It has been hypothesized that microbial communities found in association with corals can play an important role in the defence throughout production of antimicrobial substances. In this context, isolates from animal mucus and tissues were screened for antimicrobial activity against indicator microorganisms by applying overlay and drop techniques. Active isolates were identified by 16S rDNA sequencing.

Molecular investigations revealed differences in the bacterial community structure between mucus, animal tissue and environmental samples (i.e., seawater and sediment), thus suggesting a species-specific association. The 15.4% of bacterial isolates, which mainly derived from *P. phosphorea*, demonstrated bioactivity. Among these, isolates were predominantly affiliated to the genus *Vibrio* among the *Gammaproteobacteria* (11 isolates), whereas only one isolate belonged to the *Firmicutes* (*Bacillus* sp.). It could be noted that the presence of vibrios in healthy corals was probably due to the ability of these bacteria to establish mutual relationships with their guests by providing nutrients and secondary metabolites.

**DIEL AND TIDAL INFLUENCE ON PLANKTONIC MICROBIAL COMMUNITIES IN A COASTAL BRACKISH LAKE (LAKE GANZIRRI, ITALY)**

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Short-term temporal changes in both abundance and structure of the planktonic microbial community (i.e. virioplankton, bacterioplankton, and picophytoplankton) in relation to environmental factors, hydrography, and tidal dynamics were investigated in the highly eutrophic brackish Lake Ganzirri (Messina, Italy). Sampling was carried out synoptically at four stations (one at the external marine coast, one in the lake inlet channel and two inside the innermost part of the lake) every 3 hours for a period of 24 h in July 2010 following tidal current dynamics. Significant differences in environmental parameters were found among stations; e.g. mean temperature, salinity and chlorophyll a concentration values were in the range 22.7-30.3°C, 33.1-36.7 psu and 0.60-4.16 mg l<sup>-1</sup>, respectively. Microbial abundances and composition showed also significant changes among stations. Virioplankton abundance ranged from 2.53 x 10<sup>3</sup> to 3.40 x 10<sup>7</sup> VLP ml<sup>-1</sup> whereas bacterioplankton abundance ranged from 1.41 x 10<sup>4</sup> to 7.72 x 10<sup>6</sup> cells ml<sup>-1</sup>. Moreover, autotrophic prokaryotes, heterotrophic bacteria, and viruses displayed distinct diel patterns which were either related to water masses distribution or to close coupling with cell host, and/or to organic matter sources from anthropogenic inputs. Finally, the evolution of the bacterioplankton community structure over the 24 h sampling period was analyzed by means of Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting technique and results were summarized by diversity indices computation and ANOSIM analysis.

**PLASTID 16S rRNA GENE DIVERSITY AMONG EUKARYOTIC PICOPHYTOPLANKTON FROM THE SOUTH PACIFIC OCEAN**

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The genetic diversity of photosynthetic picoeukaryotes (3 µm in cell size) was investigated in the South East Pacific Ocean. Genetic libraries of the plastid 16S rRNA gene were constructed on picoeukaryote populations sorted by flow cytometry, using two different primer sets, OXY107F/OXY1313R commonly used to amplify oxygenic organisms, and PLA491F/OXY1313R, biased towards plastids of marine algae. Surprisingly, the two sets revealed quite different photosynthetic picoeukaryote diversity patterns, which were moreover different from what we previously reported using the 18S rRNA nuclear gene as a marker. The first 16S set revealed many sequences related to Pelagophyceae and Dictyochophyceae, the second 16S set was heavily biased toward Prymnesiophyceae, while 18S sequences were dominated by Prasinophyceae, Chrysophyceae and Haptophyta. Primer mismatches with major algal lineages is probably one reason behind this discrepancy. However, other reasons, such as DNA accessibility or gene copy numbers, may be also critical. Based on plastid 16S rRNA gene sequences, the structure of photosynthetic picoeukaryotes varied along the BIO-SOPE transect vertically and horizontally. In oligotrophic regions, Pelagophyceae, Chrysophyceae, Prymnesiophyceae dominated. Pelagophyceae were prevalent at the DCM depth and Chrysophyceae at the surface. Phylogenetic analysis revealed a new clade of Prasinophyceae (clade 16S-IX), which seems to be restricted to hyper-oligotrophic stations. Our data suggest that a single gene marker, even as widely used as 18S rRNA, provides a biased view of eukaryotic communities and that the use of several markers is necessary to obtain a complete image.

**MESOSCALE DISTRIBUTION OF NANOFLAGELLATES IN THE FIRST-YEAR SEA-ICE OF THE EASTERN CANADIAN ARCTIC ARCHIPELAGO, IN SPRING**

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Sea ice plays a significant role in the biology and ecology of polar marine ecosystems. Ice algal communities are estimated to contribute up to 25 % of the total Arctic primary production. Diverse heterotrophic organisms, ranging from bacteria to metazoans, also significantly contribute to carbon cycling in sea ice. However, global warming impacts the unique sea ice habitat in various ways. Therefore, it is crucial to gather substantial knowledge about the diversity and functioning of sea ice communities.

Historically, taxonomic studies have focused on sea ice diatoms, neglecting small flagellated cells which are particularly challenging to identify and enumerate. Fortunately, the advent of molecular methods now makes it possible to reliably identify and enumerate nano-sized flagellates. Here, we present mesoscale distribution of nanoflagellates in first-year sea-ice in the Canadian Arctic Archipelago, assessed by Catalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH) technique. We used 17 published probes for plastidic and aplastidic groups of flagellates. The total numbers of flagellates (hybridized with Euk 516 probe) ranged over two orders of magnitude (approx.  $10^6$ - $10^8$  cell l<sup>-1</sup>). We identified from 10 to 90 % of flagellates with the probes available. The most important contributors were cryptophytes and chlorophytes. From the aplastidic groups we only found representatives of the MAST-2 stramenopiles. Our results show that the distribution of nano-sized flagellates in the sea ice was very patchy, and depended on environmental factors.

**DISTRIBUTION PATTERNS OF KEY COMPONENTS OF MICROBIAL FOOD WEB IN MEROMICTIC SALINE LAKE**

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Interactions among heterotrophic bacteria, heterotrophic (HNF) and autotrophic (ANF) flagellates and nonloricate ciliates were studied in water column samples collected from small meromictic saline lake (Rogoznica Lake, Croatia). Special emphasis was paid on relationships between investigated parameters and water column oxygen saturation.

Bacterial abundances did not exhibit considerable changes in respect to water column hypoxia retaining at the similar values during both periods. However bacterial biomass and cell specific volume were much higher in hypoxic environment compared to oxic conditions. Low-DNA bacteria dominated the bacterial community in hypoxic environment accounting from 76 to 80% of total number of bacteria.

The abundances of HNF and ANF decreased 5-7 times in the presence of low O<sub>2</sub> concentrations implying their strong relation to oxygen saturation. No relationship between bacteria and their predators could be observed during hypoxia. However in oxic environment HNF exerted strong influence on bacterial population ( $R=0.45$   $n=14$ ,  $p<0.05$ ). Also the activity of bacterial populations shifted toward the domination of high-DNA cells in oxic environment.

Ciliate assemblages were dominated by oligotrichs. The abundance and biomass of nonloricate ciliates maintained at low values through the whole period of hypoxia. One month after establishing oxic conditions the ciliate populations showed the first sign of regeneration by increasing the number of small size organisms (20-40µm). In that period a strong impact on HNF population could be observed. As the result of this cascade effect, the top down control of HNF on bacteria diminished.



**ALLELOPATHY AND MIXOTROPHY IN PRYMNESIUM PARVUM DO NOT  
LIKE IT SALTY**

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The flagellate *Prymnesium parvum* is a toxin producer as well as mixotrophic, and forms large blooms in brackish waters. *P. parvum* produces toxins with hemolytic, ichthyotoxic and cytotoxic properties. Some of these toxins are also allelopathic, affecting the physiological status and growth patterns of co – occurring planktonic protists. It has been hypothesized that both allelopathy and mixotrophy confers a competitive advantage to *P. parvum*, and plays a role on the dynamics of microbial food webs. To test this, we cultivated the same strain of *P. parvum* at low (7) and high (26) salinity and measured allelopathic and mixotrophic activities using standard assays. In monocultures, *P. parvum* growth rates were similar at low and high salinities. However, both allelopathy and mixotrophy were higher at low salinity. Results indicate that both allelopathy and mixotrophy are highly salinity dependent, and may have a positive feedback on the competitive ability of *P. parvum* in brackish water microbial communities.

**PROTIST DIVERSITY IN SUBOXIC AND SULFIDIC WATERS OF THE BLACK SEA**

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The oxic-anoxic transition zone of the Black Sea comprises a large suboxic zone as well as anoxic and sulfidic waters. While prokaryotes and biogeochemical cycles that characterize this zone have been frequently studied, little is known about the diversity or ecology of its microbial eukaryotes. This study presents the first qualitative report of the protist species composition in the Black Sea redoxcline. Fingerprint analysis from the whole redoxcline revealed a complex community structure of metabolically active protists with distinct shifts along the redox gradient. Additionally, 18S rRNA gene clone libraries were used to compare protist species composition of suboxic and sulfidic water layers. Among the ciliates, sequences related to *Pleuronema* and *Strombidium* were dominant in both water layers whereas sequences affiliated with anaerobic plagiopylids and *Cyclidium* were detected only in the sulfidic zone. Among the flagellates, mainly stramenopiles (mostly bicosoecids and chrysophytes) occurred throughout the redoxcline. In the sulfidic zone we found stramenopile sequences but also euglenids, jakobids and choanoflagellates which were related to clonal sequences from other anoxic marine habitats, thus indicating the existence of globally distributed groups of anoxic flagellates. Higher species richness in the sulfidic zone and about twice as many novel sequence types of ciliates and stramenopiles compared to the suboxic layer emphasizes the importance of anoxic, sulfidic waters as habitat for high protist diversity although unknown functions.

**COMMUNITY STRUCTURE OF AUTOTROPHIC FLAGELLATES IN WATERS OF THE WEST SPITSBERGEN (SVALBARD, NORWAY)**

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Autotrophic nanoflagellates are ubiquitous in aquatic environments from the Equator to the Polar regions. They contribute substantially to primary production in oligotrophic waters thorough the year, while in coastal, polar regions they may dominate phytoplankton communities in summer. Although the diversity of microbial eukaryotes has been studied for almost 20 years, knowledge about mesoscale distribution of particular groups is scarce.

The Spitsbergen island (79° N) is characterized by highly dynamic hydrological conditions. The West Spitsbergen Current carries warm, Atlantic-derived water masses to the North that may enter the fjords, where they mix with waters of Arctic origin and from glacier run-off. This mixing creates unique conditions for planktonic organisms, resulting in changes in distribution of Arctic and Atlantic species along a fjord's axis. This phenomenon is well described for zooplankton and microphytoplankton, but nanoflagellates, the major component of summer phytoplankton, have been neglected.

The aim of this study was to describe distribution of planktonic nanoflagellates in the Kongsfjorden fjord. Catalyzed Reported Deposition – Fluorescence in situ Hybridization (CARD-FISH) technique was used for detection and enumeration of selected algae groups [Chlorophyceae (probe Chlo02), Cryptophyceae (CryptB), Pelagophyceae (Pela01), Bolidophyceae (Boli02), and Pedinellales (Ped675)] in the environmental samples. The total numbers of nanoflagellates (detected by probe Euk516) ranged from 12720 to 466 cells per litre, and we observed gradual decrease towards the inner reaches of the fjord. The maxima in the water column were at 1 to 100 % of PAR intensity. There were also substantial differences in distribution of particular groups, which were linked to environmental factors.

**TESTATE AMOEBIA COMMUNITY STRUCTURE AND DISTRIBUTION IN LAKE  
SEDIMENTS**

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Testate amoebae are unicellular organisms (Protozoa) enclosed in a solid shell, or test. Considered as worldwide distributed, they are commonly found in a wide range of moist and freshwater environments and habitats, i.e. peatlands, lakes, soils. In terrestrial and aquatic ecosystems testate amoebae are studied for their bioindicator potential, mostly in peatlands. In lakes, they are still poorly described even if some studies characterized lacustrine testate amoebae as good environmental tools for environment quality. However, their determination is mostly based on their shell morphology, and the good preservation of these shells in anoxic conditions like lake sediments and peat, provide the opportunity to proceed to paleoenvironmental studies. My presentation is focused on testate amoeba communities living at the water-sediment interface in lakes. The main objective of my research is to improve the knowledge on present day testate amoeba communities in lakes in order to better understand the response of past assemblages to climatic oscillations. The results of testate amoeba communities in several French lakes and one Japanese lake are compared. Samples from different depth water-sediment interfaces of 4 lakes with different trophic levels (France: Lake Pavin: oligo-mesotrophic; Lake Clairvaux: mesotrophic; Lake Bonlieu: eutrophic; Japan: Lake Biwa: North Basin mesotrophic- South Basin eutrophic) were analyzed. The major results confirm the indirect or direct effects of some environmental factors on testate amoeba communities (variations in abundance, diversity, structure), i.e. nutritive resources (quantity, diversity), dissolved oxygen concentration, temperature (thermocline position) and trophic status. Testate amoeba communities show a zonation with the depth, controlled by physical-chemical parameters. Moreover, common species were observed in France and Japan and the trends are similar.

**LIFE IN A BIOFILM: UNRAVELING THE EUKARYOTIC BIODIVERSITY AT S. DOMINGOS AMD IN THE SOUTH OF PORTUGAL**Valente, T.<sup>1</sup>, Esteves, F.<sup>1</sup>, Reis, M.P.<sup>1</sup>, and Fonseca, F.<sup>1</sup><sup>1</sup>*Molecular Microbial Ecology Laboratory, University of Algarve-CIMA, Portugal*

Fundamental biological research on acid mine drainage (AMD) systems show that some eukaryotic organisms, along with archaeal and bacterial species, can survive in low pH, metal-rich environments, that are toxic to more complex life forms. The Iberian Pyrite Belt (IPB), one of the world's greatest concentrations of massive sulphide deposits, harbors AMD derived acidic ecosystems like Rio Tinto (Spain), an open system created by the river, and S. Domingos (Portugal), featuring a series of closed reservoirs in the form of several ponds left from the mining activities. In common with other AMD systems worldwide, solution-air interface biofilms dominated by photosynthetic eukaryotic microorganisms have been described as typical microbial assemblages. Organisms that thrive in these environments are becoming increasingly important to biological research.

With the aim of contributing to the advance of knowledge on those systems we have been targeting the eukaryotic communities in the AMD system of S. Domingos, at three levels, incorporating molecular, physiological and functional genomics. The characterization work so far developed has confirmed the low pH (3) even during rain season, and the presence of the typical biofilms on the water-air interface, as well as areas with extensive substrate colonization. Here we present our preliminary data on diversity barcoding of eukaryotic taxa, obtained by combining information from microscopy and molecular identification based on nuclear and chloroplastidial molecular markers (18S and 23S rDNA). So far we have recorded contributions from the Rotifera, Bacillariophyceae, Chrysophyceae, Chlorophyceae and Choanoflagellida as well as the alpha-proteobacteria *Acidiphilium*, some with high homology to sequences recovered from Rio Tinto.

**TAXONOMIC NOVELTY OF HETEROTROPHIC PROTISTS REVEALED BY UNAMENDED BRACKISH WATER INCUBATIONS FROM THE BALTIC SEA**

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Our knowledge on phylogeny and diversity of aquatic protists is rapidly increasing due to molecular surveys and next generation sequencing approaches. This has led to a considerable discrepancy between the taxa known from cultures and 18S rRNA sequences gathered from environmental studies. Further it is generally difficult to assign ecological functions to new taxa detected by culture-independent molecular approaches. The aim of our study was to link molecular data on the basis of 18S rRNA sequences with a heterotrophic life style. Therefore we performed microcosm experiments at a coastal site in the South-western Baltic Sea in which the incubation conditions promoted the growth of heterotrophic protists while preserving their community structure. A consistent succession occurred which was characterized by a decline in phototrophic organisms, a peak in bacteria after 3 days and a tenfold enrichment of heterotrophic flagellates (HF) after 5 to 6 days. Clone libraries constructed at the start and end of the incubations revealed a shift from 18S rRNA sequences related to phototrophs towards those related to heterotrophic flagellates. The phylogenetic composition of enriched HF samples consisted of both, sequences which were related to cultured representatives like *Paraphysomonas* sp. and a larger proportion of yet uncultured protists, some only rarely found so far in environmental surveys. Most of our sequences affiliated to chrysophytes and choanoflagellates, with a surprisingly high degree of novelty. Moreover, we found many cercozoans, putatively mixotrophic “novel photosynthetic stramenopiles” and picobiliphytes. We assume most of the found taxa to be heterotrophic or mixotrophic protists.

**SEASONAL CHANGES IN PROTIST PICOPLANKTON PREY-SELECTION ON A PHYLOTYPE LEVEL: WHAT DOES IT IMPLY?**Bautista-Reyes, F.<sup>1</sup> and Macek, M.<sup>1</sup>*<sup>1</sup>Posgrado en Ciencias del Mar y Limnología & Proyecto de Investigación en Limnología Tropical, Universidad Nacional Autónoma de México, FES Iztacala, Tlalnepantla, Estado de México, Mexico*

Trophic interactions of protozoa with autotrophic (APP) and heterotrophic (HPP) picoplankton were studied throughout an annual cycle in a warm-monomictic lake with anoxic hypolimnion, Alchichica (México). We examined the in situ bacterial community composition (BCC) and the vacuole content of protozoa, either for water column or enclosure experiment samples at specific depths. Highest picoplankton densities and hybridization percentages were observed during mixing and the transitions mixing-stratification-mixing respectively, important changes in BCC were noticed in enclosure experiments. Density of flagellates was high during mixing (heterotrophic), late stratification and transition to mixing (pigmented) in the photic layer; peritrichs and stichotrichs dominated during mixing and fed primarily on APP, scuticociliates dominated during stratification at the suboxic and anoxic layers. Total hybridized bacteria percentages were apparently controlled by different processes at distinct layers: at the upper metalimnion, resource depletion explains the observed trends, at the suboxic / anoxic boundary, predation by scuticociliates seems important. According to vacuole contents, flagellates and ciliates often ingested preferentially just one bacterial phylotype, which differed throughout depths and time in the annual cycle. It is suggested that different protists control abundances of metabolically active bacteria in the differing spatial structure of the water column and that the composition of phlotypes found inside protists reflects the in situ growing bacteria; therefore, the protist selection might not be apparently related to the bacterial phylotype, but to the optimum prey quality (size, nutritional value) within the BCC, reflecting a “kill the winner” dynamic.

**MECHANISMS IMPLIED IN ESCHERICHIA COLI REMOVAL DURING  
WASTEWATER TREATMENT**

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The wastewater treatment reduces the assimilable organic fraction and the number of microorganisms of the effluents due to biological treatment and to the concentration of bacteria in sludge after settling. Recycling of sludge as an organic fertilizer is environment friendly but some pathogens could be concentrated in it. To make an integral tracing of *E. coli* during the activated sludge treatment, the fate of gfp-tagged cells were analysed in batch and pilot plant experiments.

The exposure of *E. coli* to wastewater in absence of microbial population did not induce the entry into the viable but nonculturable state. The wastewater microbial populations showed a different relation with *E. coli* survival process. The presence of bacteriophages did not affect the survival while decrease in population was related with the presence of protozoa. Moreover, the wastewater bacteria behaved as predation-escaping prey and maintained their population density, while the *E. coli* were predated.

Wastewater pilot plants prove an accurate model of a large scale plant. In our experiments, when pilot plant reached equilibrium, *E. coli* counts in aqueous fractions were stabilised about  $10^4$  cells ml<sup>-1</sup> and in flocs or in sludge about  $10^8$  cells g<sup>-1</sup>. When addition of inoculated wastewater was stopped, the plant continued working with non-inoculated influent. Number of gfp-tagged *E. coli* in aqueous fraction diminished progressively and, after 2 d, it was below the detection limit. However, for the same period,  $10^6$  cells g<sup>-1</sup> remained adhered to flocs and sludge.

In conclusion, despite the efficacy of the protozoa removing *E. coli* from wastewater, this bacterium is not totally eliminated by treatment but mainly concentrated in sludge.



**GRAZING ON THE CYANOBACTERIUM *MICROCYSTIS AERUGINOSA* BY  
THE HETEROTROPHIC FLAGELLATE *COLLODICTYON TRICILIATUM* IN AN  
EXPERIMENTAL POND**

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Grazing on the harmful, bloom-forming cyanobacterium *Microcystis aeruginosa* by the heterotrophic flagellate *Collodictyon triciliatum* was examined in an experimental pond with *Microcystis* bloom in the summer of 2010. The grazing rates were estimated from digestion rate and food vacuole content of *C. triciliatum*. *M. aeruginosa* had one blooming with the maximum density of  $1.4 \times 10^5$  cells ml<sup>-1</sup>. Cell density of *C. triciliatum* fluctuated between 1 to 290 cells ml<sup>-1</sup>. The number of *M. aeruginosa* cells ingested into food vacuoles of *C. triciliatum* ranged between 0.4 and 10.8 cells flagellate<sup>-1</sup>, and the digestion rate of *C. triciliatum* at 20 °C was estimated as 0.73% cell contents min<sup>-1</sup>. We finally estimated grazing rates on *M. aeruginosa* prey by *C. triciliatum* as 0.2 – 6.9 cells flagellate<sup>-1</sup> h<sup>-1</sup> and grazing impact on the *M. aeruginosa* standing stock as 0.001– 0.699 % standing stock h<sup>-1</sup>, and the grazing rate and impact of the present study agreed well with those determined in previous study (Nishibe et al. 2002, AME). So, the grazing impact by *C. triciliatum* may be of minor importance for controlling *M. aeruginosa* blooms. The functional response of *C. triciliatum* on *M. aeruginosa* prey was also examined with Michaelis-Menten model in experimental systems where the prey concentration varied from  $6.4 \times 10^3$  to  $2.1 \times 10^6$  cells ml<sup>-1</sup>. Our application of the model was successful ( $r^2 = 0.873$ ,  $p < 0.001$ ). The maximum grazing rate was estimated as 6.8 prey cells grazer<sup>-1</sup> h<sup>-1</sup>, and half-saturation constant as  $1.2 \times 10^5$  cells ml<sup>-1</sup>. The half-saturation constant suggested that grazing impact on *M. aeruginosa* by *C. triciliatum* was active when the cyanobacterial bloom attained almost to the maximum. Multiple grazing by *C. triciliatum*, some amoebae species and the rotifer *Brachionus calyciflorus* may be important for controlling *M. aeruginosa* blooms, though each grazer has small impact on *Microcystis* abundance.

**IN VIVO EXPRESSION SCREENING FOR GENES OF *MARINOBACTER ADHAERENS* HP15 ESSENTIAL FOR ITS INTERACTION WITH THE MARINE DIATOM *THALASSOSIRA WEISSFLOGII*.**

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Aggregate formation by living cells and organic matter in the ocean is an important mechanism that mediates sinking of organic carbon. Diatom-bacteria interactions play an important role during this process by inducing the secretion of different extra-cellular polysaccharides, which increase the size of marine aggregates. To study cell-to-cell diatom-bacteria interactions, a bilateral in vitro model system has been established consisting of the diatom *T. weissflogii* and the marine Gamma-proteobacterium *M. adhaerens* HP15. The bacterium was previously shown to specifically attach to *T. weissflogii* cells, to induce transparent exopolymeric particle formation, and to increase aggregation of phytoplankton cells. In addition, it has been shown that *M. adhaerens* HP15 is genetically accessible, its genome has been sequenced, and several bacterial genes potentially important during the interaction are currently being investigated. However, genes specifically expressed in vivo are still unknown. The aim of this work was to establish an In Vivo Expression Technology (IVET) screening to identify bacterial genes specifically induced when *M. adhaerens* HP15 interacts with *T. weissflogii*. The use of the  $\beta$ -galactosidase-encoding *lacZ* gene as a reporter gene for the IVET vector construction was successfully tested in *M. adhaerens* HP15. The IVET vector was constructed by cloning the full-size promoterless *lacZ* gene downstream of a promoterless *purA* gene, which encodes an essential growth factor fundamental for purine metabolism. A site-directed mutagenesis approach was used to generate a *purA*-deficient mutant in *M. adhaerens* HP15. The suitability of the IVET vector and the auxotrophic mutant for the IVET screening is being tested. Promising genes obtained will be cloned, mutagenized, and characterized in terms of their role in diatom-bacteria interaction. Results of this study will contribute to a better understanding of the molecular mechanisms of diatom-bacteria interactions.

**THE ENDOSYMBIONTS OF THE DEEP-SEA VENT TUBEWORMS 'RIFTIA PACHYPTILA'  
AND 'TEVNIA JERICHONANA' SHARE AN IDENTICAL PHYSIOLOGY**

Gardebrecht, A.<sup>1</sup>, Gardebrecht, A.<sup>2</sup>, Markert, S.<sup>3</sup>, Markert, S.<sup>2</sup>, Sievert, S. M.<sup>4</sup>, Felbeck, H.<sup>5</sup>, Thürmer, A.<sup>6</sup>, Albrecht, D.<sup>2</sup>, Albrecht, D.<sup>3</sup>, Albrecht, D.<sup>7</sup>, Wollherr, A.<sup>6</sup>, Kabisch, J.<sup>3</sup>, Kabisch, J.<sup>2</sup>, Le Bris, N.<sup>8</sup>, Le Bris, N.<sup>9</sup>, Lehmann, R.<sup>6</sup>, Daniel, R.<sup>6</sup>, Liesegang, H.<sup>6</sup>, Hecker, M.<sup>2</sup>, Hecker, M.<sup>3</sup>, Hecker, M.<sup>7</sup>, Schweder, T.<sup>1</sup>, Schweder, T.<sup>3</sup>, and Schweder, T.<sup>7</sup>

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Symbiotic invertebrate-bacteria associations are a common feature in deep-sea hydrothermal vent ecosystems. The two siboglinid tubeworms *Riftia pachyptila* and *Tevnia jerichonana* host the same single prokaryotic species in a highly complex symbiosis, which is still not fully understood. In this study, we compared the bacterial communities from both deep-sea tubeworms proteogenomically, i. e. on the metagenomic and proteomic level. 454 pyrosequencing of the bacterial metagenomes revealed perfect congruency between both symbionts regarding significant characteristics like 16S rDNA, ITS region and main metabolic gene components. This finding, which was furthermore supported by whole-genome alignments, confirmed the high homology of the prokaryotic symbionts from the different hosts even on the subspecies level. In addition to the examination of the symbionts' genetic potential, proteomics were applied, which can provide crucial indicators for metabolic regulations in response to varying environmental parameters, as expected for symbionts populating different host systems. However, our results show a high degree of physiological consistency between the bacterial communities from the two different hosts: Only minor variations were detected between the protein patterns from both symbionts. These variations supposedly reflect the slightly different geochemical conditions the tubeworm hosts were exposed to at the sampling site (as measured prior to the collection). We therefore propose very similar internal conditions inside the *Riftia* and the *Tevnia* tubeworm, which provide a buffered microhabitat for the bacterial symbiont that is largely shielded from environmental fluctuations.

**SUCCESSION OF N-ACETYL-GLUCOSAMINE CONSUMING BACTERIOPLANKTON TAXA WITH DISTINCT LIFE STRATEGIES IN A FRESHWATER LAKE**

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Strong grazing pressure by heterotrophic flagellates on freshwater bacterioplankton typically results in a shift of the dominant morphotypes, from medium-sized fast-growing bacteria towards grazing resistant forms such as very small or large filamentous cells. Protistan foraging may moreover release incompletely digested remainders of the prey cells into the water column, thereby. This can supply pelagic bacteria with additional carbon sources, such as the bacterial cell wall component peptidoglycan and its subunit N-acetyl-glucosamine (NAG). Since both, bacterial growth and predation mortality are typically high during phytoplankton spring blooms, this period provides a unique opportunity to examine the fate of such compounds.

We followed the uptake of NAG into bacterial cells by micro-autoradiography and fluorescence in situ hybridisation during an algal bloom in Lake Zurich. Only a surprisingly small fraction of the pelagic bacterial community incorporated the substrate (4%), yet the NAG-active bacteria exhibited a clear successional pattern: Fast-growing bacteria (e.g. members of the Cytophaga-Flavobacteria and Beta-Proteobacteria) dominated NAG uptake concomitant with increasing algal abundances. With rising numbers of flagellates, however, the largest part of NAG incorporation could be assigned to cells with grazing-resistant morphologies. Particularly high percentages of filamentous NAG-active bacteria were observed that were affiliated with candidatus *Aquirestis calciphila* of Saprospiraceae. During periods of high protistan predation these bacteria thus appear to profit from both, their grazing-resistant morphology and their specific substrate niche, i.e. the effective consumption of peptidoglycan-derived organic carbon released during flagellate grazing.

**BACTERIAL COLONIZATION OF THE GREEN ALGAE *DESMODESMUS ARMATUS*  
UNDER CHANGING ENVIRONMENTAL CONDITIONS**Eigemann, F.<sup>1</sup>, Hilt, S.<sup>1</sup>, Grossart, H.-P.<sup>1</sup><sup>1</sup>*Leibniz-Institute of Freshwater ecology and Inland Fisheries Berlin*

Algae cells are colonized by species-specific bacterial communities, which might play a role for differences in sensitivities of algae, e.g. towards allelochemicals. So far, however, little is known how this species-specific bacterial colonization is developing under different environmental conditions.

In the present study, the change of the bacterial colonization of the green algae *Desmodesmus armatus* was analysed after exposing xenic and axenic cultures 1) in flasks with a membrane open for bacterial passage in a humic lake containing allelopathically active macrophytes and 2) in laboratory conditions with xenic and axenic water from the same lake and the addition of the allelochemical tannic acid (TA). After exposure, the algae-cultures were filtered and the bacterial community divided into free (ambient water) and attached (to the algae cell). Bacterial community compositions were analysed by RNA extraction and subsequent denaturing gradient gel electrophoresis (DGGE) and sequencing.

All xenic cultures kept their initial (laboratory) bacterial community regardless of the external circumstances, except of cultures exposed to tannic acid. By comparing bacterial DGGE patterns of xenic laboratory cultures with formerly axenic cultures recolonized in a humic lake no marked differences were found.

The obtained results indicate that algae keep their bacterial community regardless of changing environmental conditions and species-specific colonization may thus indeed contribute to algae sensitivities towards allelochemicals.

**RAPID EVOLUTIONARY ADAPTATION OF A FRESHWATER BACTERIUM TO INTENSE  
GRAZING PRESSURE**

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The size-selective nature of protistan grazing is an important factor that not only shifts the microbial community structure towards protected species but also might select for specific adaptations in bacteria. Under strong grazing pressure bacterial traits that increase the ability to escape predation should be favored by natural selection. Therefore, new bacterial prey genotypes with phenotypes of increased antipredator fitness must continuously evolve. We established a model system consisting of the bacterivorous protist *Poteroochromonas* and a bacterial isolate affiliated with *Sphingobium*. This strain tends to form a subpopulation of grazing-resistant cell aggregates in pure bacterial culture, and the aggregated phenotype is stimulated by the presence of predators. To assess evolutionary changes of the *Sphingobium* strain in response to predation we set up long-term experiments with pure bacterial cultures and co-cultures consisting of the bacterium and the flagellate predator. Bacterial populations were allowed to evolve for several months by serial propagation in oligotrophic medium. Growth and phenotypic shifts of evolved *Sphingobium* strains from both conditions were compared every 150 generations. Surprisingly, these relatively short intervals already resulted in visible differences of growth and aggregation behavior between strains from the two cultivation conditions, pointing at the importance of predation as a driver of evolutionary changes in aquatic bacteria.

**GROWTH AND RELEASE OF EXTRACELLULAR ORGANIC COMPOUNDS BY BENTHIC DIATOMS DEPEND ON INTERACTIONS WITH BACTERIA**

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Surfaces in the littoral zone of aquatic systems are often covered by biofilms consisting mainly of diatoms and bacteria. Diatom growth and secretion of extracellular polymeric substances (EPS) are important factors for the development of such biofilms. Systematic purification of diatoms from biofilms in our laboratory resulted in axenic strains that no longer produce biofilms - obviously diatom/bacteria interactions are important for biofilm formation. By co-cultivating representative diatoms and bacteria and adding spent bacterial medium to diatoms, we found diatom growth and EPS secretion to be influenced, including induction or inhibition of diatom protein secretion. In diatom/bacteria co-cultures in addition an extracellular metaproteome accumulates, containing proteins from both organisms. Using the model organisms *Phaeodactylum tricornutum* and *Escherichia coli*, extracellular proteins of these organisms were identified, resulting in a hypothetical model for their interactions, including (1) attachment, either to the substratum or to form cell/cell aggregates; (2) secretion, modification and uptake of EPS, amino acids and carbohydrates; (3) other interactions between diatoms and bacteria that can range from processes like signaling to mucus degradation. Searching for putative bacterial signal substances involved in such diatom/bacteria interactions we found that the concentration of various dissolved free amino acids (DFAA) within the diatom cultures changed drastically during co-cultivation with bacteria. Adding amino acids to axenic diatoms resulted in changed diatom growth patterns. We postulate that diatom/bacteria biofilms are regulated by a complex network of chemical factors involving EPS and DFAA beside other substances. Temporal and spatial variability in such diatom/bacteria interactions in nature may also affect algal growth, EPS secretion and biofilm formation with pronounced consequences for organic matter and energy cycling in aquatic systems.

**FEEDING OF CILIATES ON TOXIC FILAMENTOUS CYANOBACTERIA IN LAKE ZURICH  
(SWITZERLAND)**

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Besides its recreational uses Lake Zurich (Switzerland) serves as the major source of drinking water for 1.5 million people. In the past, the harmful cyanobacterium *Planktothrix rubescens* showed recurrent mass developments and it gets increasing dominance due to ongoing global warming. The cyanobacterium is generally considered toxic for eukaryotes. Intact cyanobacterial cells contain cyclic heptapeptides (microcystins) and various other cyclic peptides. Although coccoid and filamentous cyanobacteria are often regarded as inappropriate or even toxic food for most consumers, protists and especially ciliates can be efficient predators of cyanobacterial blooms. During the last 3 years, we repeatedly observed pelagic and benthic ciliate species feeding on *P. rubescens*, and we isolated the pelagic *Obertrumia aurea* and the benthic *Trithigmotoma cucullulus*. We present a first characterization of the autecology and of population dynamics of these species. Especially *T. cucullulus* shows a fascinating handling of prey, by taking up intact filaments (up to 15 times longer than the ciliate) or fragments only from distal ends. There are at minimum four ways how the ciliate incorporates cyanobacteria: to roll up filaments resulting in maximal 4 coils, to fold the prey inside the cell with an undefined number of fractures, to ingest a major part of the filament and to break this part mechanically or enzymatically from the remaining rest, and to ingest fragments which are mostly the same length as the ciliate. Although several authors found evidence for the uptake of *Planktothrix* spp. by ciliate species, surprisingly the basic principle of this interaction is not well understood: Why do ciliates feed on toxic filamentous cyanobacteria, and, as eukaryotic organisms, why are they not poisoned by their toxic diet? There is need to study the aut- and synecology of these species for discussing their potential usage as biological control of cyanobacteria.



**TROPHIC COUPLING BETWEEN HETEROTROPHIC FLAGELLATES AND BACTERIA IN HYPERTROPHIC SHALLOW LAKES**Fermani, P.<sup>1</sup>, Diovisalvi, N.<sup>1</sup>, Torremorell, A.<sup>1</sup>, Zagarese, H.<sup>1</sup>, and Unrein, F.<sup>1</sup><sup>1</sup>*Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús, IIB-INTECH (UNSAM-CONICET), Argentina*

Gasol (1994) proposed a model to describe the maximum attainable abundance (MAA) of heterotrophic flagellates (HF) as a function of the heterotrophic bacteria (HB) abundance. He also defined the degree of uncoupling (D) as the difference between the MAA and the realized HF abundance. Several authors had suggested that top-down control of HF should prevail as the trophic state of lakes increases, consequently, D should also increase with trophic level. Here, we investigate the degree of HF-HB coupling / uncoupling in a hypertrophic shallow (Laguna Chascomús, Argentina) and compare the results with Gasol's model and additional records from other hypertrophic lakes. The HB abundance in Chascomús (up to  $1.1 \times 10^8$  cells mL<sup>-1</sup>) often exceeded the highest values included in Gasol's model. In addition, autotrophic picoplankton abundances were also high (max. =  $2.7 \times 10^7$  cells mL<sup>-1</sup>). A shift in the dominant zooplankton was observed during two consecutive summers: small cladocerans dominated during 2007-2008 and rotifers during 2008-2009. Plotting our data of HF-HB in the model, two distinct clusters were identified, corresponding to both periods. Higher D values were estimated towards the end of the study. Changes in picoplanktonic preys were unrelated to the contrasting degrees of HF-HB coupling, whereas changes in the abundance and composition of zooplankters seemed to determine a differential top-down control of HF observed in both years. The high rotifer abundance recorded in 2008-2009 (up to 5000 ind. L<sup>-1</sup>) exerted a stronger top-down control than small cladocerans. Moreover, the abundance of rotifers correlated positively with D. As a rule, the hypertrophic lakes included in this revision show that most datapoints occur above the mean realized abundance (except lakes dominated by *Daphnia* or characterized by extremely high rotifer abundances). Thus, the available evidence does not provide support for the prediction of increasing of D towards hypertrophic lakes.

**BIOSURFACTANT-PRODUCING BACTERIA FROM *SABELLA SPALLANZANII*  
(POLYCHAETA, SABELLIDAE)**

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The polychaete *Sabella spallanzanii* is known for its ability to accumulate bacteria from the marine environment. Studies dealing with the interactions between filter feeders and marine bacteria have been carried out with special attention to molluscs; by contrast, at present data relative to bacteria retention by Polychaetes are scant. Currently, there is scarce knowledge about the possible isolation and characterization of biosurfactant-producing bacteria from biotic matrices. In this work, 16 strains were isolated from enrichment culture obtained by using *S. spallanzanii* homogenate to inoculate Bushnell Haas broth supplemented with crude oil, and incubated at 28 °C for one month. Isolates were screened for the production of biosurfactants (BSs) after two and five days of incubation at 28°C on a rich medium by using standard tests: E<sub>24</sub> index detection, surface tension measure, C-TAB and Blood Agar assays. Isolates that showed an E<sub>24</sub> index ≥50% were further analyzed for the preliminary characterization of BSs, and identified by 16S rDNA sequencing. Isolates A45 and A46, which were closely related respectively to *Pseudomonas* sp. and *Thalassospira* sp., showed interesting spots on TLC plate after staining with anisaldehyde reagent. Biosurfactant presence was confirmed by yellow spots, suggesting the possible glycolipidic nature of the compounds. Both compounds have an identical R<sub>f</sub> value of 0.81 and hence they are likely to have a very similar structure. *Pseudomonas* spp. are well known producers of rhamnolipids, but they were isolated exclusively from sediment and water samples. Otherwise, for the first time members of the genus *Thalassospira* are here reported in relation to biosurfactant production. These results demonstrate that is possible isolating biosurfactant producers from filter-feeding Polychaetes.

**RESISTANCE TO HEAVY METALS AND ANTIBIOTICS OF BACTERIA ASSOCIATED WITH THE ANTARCTIC SPONGE *HALICLONA PILOSA* (KIRKPATRICK, 1907)**

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Marine sponges, as filter feeders, are capable of turning over a large volume of seawater and potentially accumulate heavy metals and other contaminants from the environment. Sponges harbour a large number of microorganisms within their tissues where they can reside in the extra- and intra-cellular spaces. Bacteria associated with sponges can be used as indicators of contamination in marine ecosystems. Numerous toxicological studies have examined the heavy metal sensitivity or resistance of bacteria isolated from different habitats. Furthermore, the increasing and indiscriminate use of antibiotics in medical, veterinary and agricultural practices resulted in release of large amounts of pharmaceutical drugs into the marine environment. The presence of antibiotics in seawater can lead to the development of antibiotic resistant strains and such increase is seen as an ecological problem.

The present study was intended to screen 72 bacterial strains isolated from the Antarctic sponge *Haliclona pilosa* for heavy metal and antibiotic resistance patterns.

Disc diffusion susceptibility test was used to investigate the antibiotic resistance profile, while the heavy metal resistance was determined by the plate diffusion method.

All the isolates were strongly resistant to the vibriostatic agent O/129 (90% of tested strains) and tetracycline (76%), while they were susceptible to ampicillin (100%), gentamicin (100%), chloramphenicol (98%) and kanamycin (97%). Sponge-associated bacteria showed resistance against tested heavy metals. Mercury (Hg) and cadmium (Cd) emerged as the highly toxic heavy metal pollutants, while all the isolates showed highest resistance against zinc (Zn). Particularly, the 18% of tested strains showed 100% growth in the presence of Zn concentrations of 10000 ppm. Finally, plasmid molecules were harboured by the 40% of isolates, suggesting that they may carry resistance factors.

**LINKS BETWEEN VIRAL AND PROKARYOTIC ABUNDANCE AND PRODUCTION  
THROUGHOUT THE WATER COLUMN ALONG A LATITUDINAL TRANSECT  
IN THE NORTH ATLANTIC**

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Viruses are an abundant, diverse and dynamic component of the marine ecosystems and play a key role in the biogeochemical processes of the ocean by controlling prokaryotic and phytoplankton abundance and diversity. Viral and prokaryotic abundance and production were determined throughout the water column (from the surface, ~10 m, to the abyssopelagic layers, ~6000 m) along a latitudinal transect in the North Atlantic to assess potential variations in the relation between viruses and prokaryotes in different oceanographic provinces. Depth-related trends in prokaryotic and viral abundance, and prokaryotic production were observed along the latitudinal transect, with the decrease by one order and three orders of magnitude over the depth profile, respectively. The lytic viral production decreased significantly with depth whereas the lysogenic viral production did not vary with depth. The percentage of lysogeny was significantly correlated with latitude, exhibiting higher values in the oligotrophic regions. Although the prokaryotic and viral communities showed similar trends with depth, the viral-to-prokaryote ratio increased from ~19 in surface waters to ~53 in the bathy- and abyssopelagic waters, due to the lower decrease of viral abundance with depth compared to prokaryotic abundance. Overall, the relationships between viral abundance and biotic and abiotic parameters differed among oceanographic provinces and depth layers, as assessed by distance-based multivariate analysis for a linear model using forward selection (DISTLM forward), suggesting that the controlling factors for the distribution of viruses are varying among different oceanic regions and depths.

**IMPORTANCE OF PROTIST GRAZING ON A CHEMOLITOAUTOTROPHIC KEY PLAYER ('SULFURIMONAS' GD17) OF BALTIC SEA REDOXCLINES**

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Barrier zones between oxic and anoxic water masses (pelagic redoxclines) host highly active prokaryotic communities that mediate important biogeochemical transformations. In central Baltic Sea redoxclines, the *Sulfurimonas* subgroup GD17 (Epsilonproteobacteria) has been shown to dominate chemoautotrophic production and be responsible for denitrification via sulfide oxidation. However, how the high chemoautotrophic production is matched by loss processes, such as mortality due to protist grazing or viral lysis, remains unknown. In the present study, we employed a combination of predator exclusion and bacterial addition experiments (using *Sulfurimonas* sp. GD1, a cultured representative of the subgroup GD17) to determine the impact of grazing and viral lysis on the *Sulfurimonas* subgroup GD17 at two depths of a Baltic Sea redoxcline (suboxic and oxygen / hydrogen sulphide interface). Additionally, RNA-Stable Isotope Probing (RNA-SIP) was used to identify specific protist grazers. Our results demonstrate a very strong bacterivorous pressure on the *Sulfurimonas* subgroup GD17, being in the same range as bacterial biomass production, and mediated by a diverse ensemble of protists. In the absence of grazers, *Sulfurimonas* subgroup GD17 doubled every 1 to 2 days, in accordance with previously recorded in situ estimates. Viruses, on the other hand, did not react to changes in *Sulfurimonas* subgroup GD17 abundance, potentially indicating a limited impact at these depths. Overall, in the present study we demonstrate that protist grazers can exert a strong top-down control on a chemolithoautotrophic redoxcline key-player, and thus potentially have a strong, till now ignored, impact on biogeochemical cycles in Baltic Sea redoxclines.

**THE IMPACT OF JELLYFISH CARCASSES ON THE BACTERIAL COMMUNITY**

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In the northern Adriatic *Aurelia* sp 8., *Rhizostoma pulmo* and *Pelagia noctiluca* are seasonally abundant and may form large blooms. When appearing in large numbers jellyfish have an impact on the marine ecosystem releasing organic and inorganic nutrients either through their metabolism or as they decompose. The focus of this study was to examine whether the addition of the jellyfish-derived substrate has a significant impact on bacterial community dynamics and phylotype selection. Preliminary results showed a difference in the protein concentrations, the carbon and the nitrogen content of the added substrate between jellyfish species. After 72 hours of incubation, protein concentrations were reduced in all experimental bottles with the addition of jellyfish substrate, and significant bacterial growth was recorded with biomass accumulation. The results of DGGE and clustering analysis confirmed that the community changed over time in the bottles with jellyfish and the analysis of the 16S rDNA clone libraries gave us further insights into the impact of jellyfish homogenate on the composition of the bacterial community. At the beginning of the experiment the bacterial community was dominated by Alphaproteobacteria (76%), Flavobacteria (11%) and Gammaproteobacteria (8%). A small percentage of clones belonged to Betaproteobacteria (3%) and Cyanobacteria (3%). After 6 days of decomposition, Gammaproteobacteria dominated in the bottles, representing from 63% to 100% of the bacterial community. There were differences in the percentages of different family representatives within Gammaproteobacteria (*Vibrionaceae*, *Pseudoalteromonadaceae*, *Shewanellaceae*, and *Oceanospirillaceae*) among bottles with different jellyfish homogenates.

**INTERACTIONS OF MARINE NITRIFIERS AND ANAMMOX BACTERIA UNDER OXYGEN LIMITATION**

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In marine oxygen minimum zones (OMZs), it is possible that AOA rather than marine ammonia-oxidizing bacteria (AOB) may provide nitrite to marine anammox bacteria. The present study explored the possibility of cooperation between marine anammox bacteria and nitrifiers as well as the competition between AOA and AOB under oxygen limitation and varying ammonium concentrations in a lab-scale model system. A bioreactor containing marine anammox bacteria was supplemented with AOA (*Nitrosopumilus maritimus*) cells. Oxygen was carefully introduced to ensure growth of both the introduced and indigenous nitrifiers as well as oxygen limited conditions. A stable culture of AOA, AOB and anammox bacteria was established. Due to rapid oxygen consumption by AOA and AOB, anammox activity was not inhibited by the introduction of oxygen. Induced expression of ammonium uptake genes was observed for all community members which may be accounted for by the increased competition for ammonium under oxygen limitation. A C18[3] ladderane fatty acid became highly dominant in the *Scalindua* anammox bacteria-dominated culture, which was never observed before and may be caused by oxygen exposure. The competition of AOA and AOB was strongly related to residual ammonium concentration, the *amoA* copy numbers of AOA remarkable increased when the residual ammonium concentration was less than 30  $\mu\text{M}$ . At the end of this study, the community composition of the culture remained quite stable with almost equal numbers of AOA and AOB *amoA* copy numbers. This observation was further confirmed by sequencing mRNA, inhibition activity analyses and lipid analyses. As far as we know, this study is the first direct proof that AOA can provide nitrite to anammox, and this AOA-associated CANON (Completely Autotrophic Nitrogen Removal Over Nitrite) system might be a new candidate in future treatment of polluted marine waters.

ISOTOPIC COMPOSITION OF NITRATE IN THE REDOXCLINE OF THE BALTIC SEA

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The central Baltic Sea is naturally prone to hypoxia due to its strong halocline separating brackish surface waters from more saline bottom waters. Therefore, it has been facing hypoxia which is further enhanced by eutrophication over the past decades. Hypoxia has a significant influence on the concentration of nitrogen compounds because several transformation processes of the nitrogen cycle are strongly coupled to oxygen concentrations. Suboxic zones of the water column are known as large sinks for nitrogen because nitrate, produced by nitrification, can be reduced to N<sub>2</sub> via denitrification or anammox. The biogeochemical processes involved alter the isotopic signature of nitrate which can be used to unravel the different processes. Here we use natural abundance profiles of concentrations and stable isotopes in nitrate and ammonium sampled during a cruise to the central Baltic Sea in July 2010. Fine resolution profiles through the redoxcline of 9 stations in the eastern, western and northern Gotland Basin were compared. The peak of the nitrate concentration was around 5 μM in the western part and 8 μM in the eastern part of the Gotland Basin. Within the peak area δ<sup>15</sup>N- NO<sub>3</sub><sup>-</sup> values were between 2-8 ‰. The δ<sup>15</sup>N of nitrate increases up to 11- 19 ‰ with decreasing nitrate concentrations depending on the station - a typical pattern found in other oxygen minimum zones in the oceans. The δ<sup>18</sup>O values in nitrate seem negatively correlated and range from -2 ‰ up to 10 ‰. These patterns are presumably the result from high denitrification activity; however, the role of nitrification for the generation of the signals will also be discussed.



**NITRATE TURNOVER DURING SPRING OUTFLOW FROM THE NITRATE-RICH CURONIAN AND SZCZECIN LAGOON**Korth, F.<sup>1</sup>, Liskow, I.<sup>1</sup>, and Voss, M.<sup>1</sup><sup>1</sup>*Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Germany*

The main sources of nitrogen compounds to the Baltic Sea are river discharges. During our study we focused on the highly eutrophied Oder and Nemunas Rivers, the second and third largest nitrogen contributors to the Baltic Sea delivering roughly 70 and 45 kt N yr<sup>-1</sup>, respectively. Run-off and annual nitrate concentrations from the rivers are highest in early spring. Both rivers flow through a lagoon before they enter the Baltic Sea. During peak outflow, in March 2009 nutrient concentrations, nitrate uptake rates and nitrate stable isotopes ( $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$ ) were measured in the outflows of the lagoons to characterize nitrate turnover processes and its fate in the coastal zone of the Baltic Sea. Salinity gradients ranging from 2 to 8 were covered. Nitrate concentrations ranged from 4-75 and 3-75  $\mu\text{mol l}^{-1}$  in the Szczecin and Curonian lagoon outflows, respectively. Nitrate uptake rates (up to 235  $\text{nmol l}^{-1} \text{h}^{-1}$ ) and chlorophyll a (25  $\text{mg m}^{-3}$ ) values were 6 and 4 times higher in the Szczecin lagoon compared to the Curonian lagoon outflow, respectively and are in the range of nitrate uptake rates reported for other European river outflows (0.25-250  $\text{nmol l}^{-1} \text{h}^{-1}$ ). Additionally, first budget calculations show high nitrate and much lower phosphate losses during spring. These may be caused by unbalanced growth but more realistic are other N-losses. Isotope values were measured to help identifying the active processes. The  $\delta^{15}\text{N-NO}_3^-$  were between 7-28 and 4-9 ‰ and  $\delta^{18}\text{O-NO}_3^-$  between 2-27 and 1-6‰ in the Szczecin and Curonian lagoon outflows, respectively. The values suggest predominant nitrification in the Curonian lagoon and assimilation in the Szczecin lagoon. The enrichment ratio of  $\delta^{18}\text{O-NO}_3^-$  to  $\delta^{15}\text{N-NO}_3^-$  of 1.3:1 in the Szczecin lagoon differs from the ratio of 1:1 associated with uptake by marine phytoplankton. This supports previous findings that denitrification acts as significant sink in the Szczecin lagoon.

**MICROBIAL COMPOSITION OF VARIOUS COMPONENTS OF CLOSED AQUACULTURE SYSTEMS: ANALYSIS OF BIOFILTERS AND INDOGENOUS MICROBIOTA OF FISH INTESTINES.**

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and Op den Camp, H.J.M.<sup>1</sup>

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Aquaculture is becoming increasingly important for the production of fish but maintenance of an optimal water quality poses challenges for aquaculture worldwide. Especially the accumulation of nitrogen compounds, which are produced by fish, is a major problem in aquaculture. Strict environmental rules for concentrations of these compounds in the effluent water and the high toxicity of these compounds force aquaculture operators to reduce nitrogen in the effluent water. This can be accomplished in different manners: 1) An efficient removal of the nitrogenous compounds from the water or 2) Reduction of the nitrogen input. Nowadays, biofiltration is often used to remove nitrogen compounds from the water. However, currently we know very little about the contribution of various groups of nitrogen cycle bacteria to the removal of nitrogenous compounds in aquaculture systems. We therefore investigated the composition of a biofilter using 16S rRNA gene analysis, fluorescence in situ hybridization (FISH) and activity assays. This revealed the presence of various members of nitrogen-cycling bacteria, including ammonium- and nitrite-oxidizing and a new type of anammox bacteria. Reducing nitrogen accumulation in the system by reducing the nitrogen input can be accomplished by decreasing the protein concentration in food. Most fish are fed high protein content food to maximize growth. Part of this protein can be exchanged for plant material, however it is important to know if cultured fish can convert the plant components to usable metabolites. We therefore investigated the intestinal microbiota of common carp (*Cyprinus carpio*) using culture-independent methods (Roche 454 pyrosequencing of the 16S rRNA gene). This revealed the presence of a large population of microorganisms able to ferment plant components. Furthermore, members of the nitrogen cycle bacteria were found to reside in the fish' gut pointing to new in situ possibilities to reduce nitrogen emissions in aquaculture.

**ANAEROBIC AMMONIUM OXIDATION IN THE OCEAN'S OXYGEN MINIMUM ZONES IS DOMINATED BY A SINGLE GENUS - 'CANDIDATUS SCALINDUA SP.'**

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Bacteria capable of anaerobic ammonium oxidation (anammox) derive their energy for growth from the conversion of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  into dinitrogen gas and constitute a significant sink for fixed nitrogen under anoxic conditions. Whereas freshwater ecosystems harbor four different anammox genera, the diversity of marine anammox bacteria is limited to a single genus: 'Candidatus Scalindua'. Representatives of this genus are responsible for the bulk of the nitrogen loss from marine oxygen minimum zones (OMZs). The underlying characteristics enabling them to prevail in marine environments remain unknown, but unique physiological adaptations might play an important role.

As their activity depends on other nitrogen transformations for supply of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , anammox bacteria thrive at the oceanic oxic/anoxic interface where they potentially interact with ammonifiers, nitrifiers, denitrifiers, etc., but also encounter several stress factors. These may include nutrient limitation or exposure to the potential inhibitory compounds such as oxygen and sulfide due to a common reaction front with steep gradients throughout the OMZ. Here we report on physiological and metabolic adaptations of a Scalindua enrichment culture towards different stresses, possibly providing a selective advantage over the freshwater genera leading to the elucidation of their predominance in marine systems.

**MOLECULAR CHARACTERIZATION OF NITROGEN-FIXING BACTERIA  
AND THEIR COLONIZATION PATTERN IN MANGROVE ROOTS**

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Nitrogen-fixing bacteria play a major role in remineralization processes in mangrove ecosystems. Anaerobic processes like denitrification take place in the anoxic layers of mangrove sediments. Consequently, most of the nitrogen is lost and thus no longer available for metabolic processes in plants. Previous studies had shown that nitrogen-fixing bacteria interact with mangrove roots making nitrogen available for plants. Although, nitrogen fixation is a very important process in mangrove ecosystems, very little is known about bacterial colonization strategies and physiological impacts on mangrove roots. Nothing is known about bacterial genes particularly required and expressed during the potential interactions with mangrove plants. The establishment of a nitrogen-fixing bacteria-mangrove interaction model system is necessary to study the molecular mechanisms of this interaction. The aim of the current investigation was to isolate, characterize, and test for genetic accessibility of nitrogen-fixing bacteria isolated from *Avicennia* sp. and *Rhizophora* mangrove roots. Nitrogen-free medium was used for the isolation of 24 bacterial strains belonging to 8 different phylogenetic taxa. The isolates were characterized in terms of their nitrogenase activity, the phylogenetic affiliation using 16S rRNA gene sequencing, and colonization pattern in mangrove roots followed by confocal laser scanning microscopy. Transformation efficiency using electroporation was tested for the most interesting isolates. Herein, it was demonstrated that some of the diazotrophs were genetically accessible and were colonizing mangrove plants. These isolates are promising candidates to establish a cell-to-cell bacteria-mangrove model system to continue our investigation of the molecular mechanisms determining bacteria-mangrove interactions.

## **AMMONIA-OXIDISING BACTERIA AND ARCHAEA IN DEEP WATER OF AN OLIGOTROPHIC SUBALPINE LAKE**

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Despite human activities are altering the tendency of the processes of the nitrogen cycle to balance each other in freshwaters, important knowledge is still missing, in particular regarding the role of microorganisms involved in this cycle. In this study, abundance of ammonia-oxidising Bacteria and Archaea have been investigated along the pelagic water column of a deep subalpine lake (Lake Maggiore, Northern Italy). Based on sequence analysis of 16S rRNA genes and the functional genes *amo*, codifying for the ammonia-monooxygenase enzymes, the relative presence and importance of both ammonia-oxidising Bacteria and Archaea in deep waters have been assessed, confirming the emerging hypothesis of their spatial niche differentiation. We found a different vertical distribution of Bacteria and Archaea and here we will discuss the interaction with chemical and other limnological parameters.

**COMMUNITY COMPOSITION AND ABUNDANCE OF AEROBIC AND ANAEROBIC AMMONIA OXIDIZERS IN AN AGRICULTURALLY IMPACTED SANDY AQUIFER**

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Groundwater in agricultural landscapes is often affected by elevated inputs of nitrogen compounds, especially nitrate, resulting from fertilizer application. However, only little is known about the microbial communities involved in nitrogen transformation processes in aquifers. The goals of this study were (i) to investigate the abundance and community composition of microorganisms involved in aerobic ammonia oxidation and anaerobic ammonia oxidation (anammox) in an agriculturally impacted sandy aquifer, and (ii) to analyze if the vertical distribution of these microbial groups could be linked to changes in groundwater geochemistry with increasing depth. Groundwater samples were obtained from ten distinct depths between 2 m and 25.5 m using a multilevel well. Physicochemical analysis revealed mostly anoxic conditions with nitrate concentrations ranging from less than 1 to 1000  $\mu\text{M}$  across the different groundwater depths. Molecular approaches targeting the *amoA* gene encoding ammonia mono-oxygenase showed that both ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were present in the groundwater with a numerical predominance and a higher diversity of AOA. Abundances of *amoA* genes decreased strongly with depth and did not correlate with nitrate concentrations. Anammox-related 16S rRNA genes as well as genes encoding hydrazine oxidoreductase (*hzo*), a key enzyme of the anammox process, were present down to 25.5 m depth. Brocadia-related 16S rRNA genes were also detected on the RNA level, suggesting the presence of active anammox-bacteria involved in nitrogen transformation processes in the aquifer. Nitrification and anammox activities still need to be determined.

**BIOLOGICAL FILTERS IN RECIRCULATION AQUACULTURE SYSTEMS:  
ROLE OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PARAMETERS**

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Blacheton, J.P.<sup>2</sup>, Triplet, S.<sup>2</sup>, and Michaud, L.<sup>1</sup>

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Nitrification efficiency of biological filters used for water purification in aquaculture facilities involves physical, chemical and biological processes that are governed by a variety of parameters such as substrate and dissolved oxygen and organic matters concentration, ORP level, temperature, pH, alkalinity and water turbulence. Slow-growing chemolithotrophic nitrifying bacteria actively compete for space and oxygen with the fast-growing heterotrophic bacteria that dominate the external layers of the biofilm.

This work was aimed to investigate the bacterial communities and the nitrification efficiency into a lab scale biological filters used in Recirculating Aquaculture System. The experiment was carried out in a facility consisting of two identical units (A and B), each one equipped with three different typology of biofilters (Moving plastic bed, MB; Static Plastic Bed, SPB; Static Bed, SB). The two Units were run with two different hydrodynamic and ORP level conditions and the bacterial community was studied by ARISA analysis. The abundances of nitrifying bacteria were determined by Flow Cytometer and the relative abundance of major phyla was studied by FISH analysis.

The nitrification efficiency was evaluated by carrying out Total ammonia removal rate dynamics and put in relation with community structure. Multivariate analysis were computed on the entire data set in order to determine the “best” condition allowing a stable and suitable ammonia removal.

**MICROBIAL DIVERSITY IN GLACIAL STREAMS OF THE AUSTRIAN ALPS**

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Glaciers are heavily affected by the global climate change, leading to increased glacier melting and subsequent changes of the connected environment. In the European Alps, glaciers give birth to streams, which eventually influence the downstream fluvial network. Due to the ongoing glacial retreat, the interaction between melt waters and moraines becomes more significant and supports the colonization of microbes at newly exposed soils. Within the glacial stream, microorganisms can either attach to surfaces and form biofilms or occur suspended as free-living microbes. Though it is known that microbes are able to survive and even grow in these habitats, little is known about their biodiversity and functioning in these glacial ecosystems.

We conducted a systematic and thorough survey of 26 glaciers in the Austrian Alps to better understand microbial community structure and biodiversity in these extreme microbial ecosystems. Massive pyrosequencing of the 16S amplicons of both rDNA and rRNA allows the separation between potential and active components of the microbial community in glacial habitats. Eventually, this approach will provide information of acclimation at community level to the extreme conditions in glacial ecosystems.



**CAN WE TRUST ON PCR-DEPENDENT MICROBIAL COMMUNITY ANALYSES?**

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Cultivation-independent but PCR-dependent technologies have revolutionized our understanding of diverse microbial communities. However, PCR primers have been claimed to discriminate certain sequences, making molecular inventories skewed. One of the most extreme estimations predicted that half of microbial richness is missed and unknown number of species and higher taxa undetected due to primer bias, even after unlimited sampling and sequencing effort. To avoid the possible bias caused by PCR primers, we established a new adapter-ligation technique to analyse the 16S rRNA diversity without gene-specific PCR amplification. This method was compared with PCR-dependent techniques in which the V1-V3 region was amplified using universal bacterial primers from DNA and reverse-transcribed cDNA.

Having acidic and neutral soil communities as model systems our study proved that with PCR all the major and minor bacterial phyla were amplified, excluding none of the existing microbial orders. However, PCR protocol slightly favoured Proteobacteria at the expense of certain Actinobacteria and Acidobacteria groups. Several of the rare orders were characteristically more abundant in neutral samples, but acidic environment (pH5.5) favoured Acidobacteria groups 1 and 2. The dormant microbes with significantly low RNA/DNA relationship were classified to several incertae sedis orders of Proteobacteria, Spartobacteria and TM7, but Myxococcales belonging to Deltaproteobacteria had especially high dominance and specific activity in both acidic and neutral environments. PCR cycles decreased the dominance of major OTUs and increased the number of rare OTUs, which was attributed to PCR artifacts. In conclusion, in spite of the slight bias towards Proteobacteria and artificial increase in the microbial richness, PCR proved to retain a fundamentally correct picture of the phylum and order-level diversity.

**PROTEOMICS OF 'GRAMELLA FORSETII' SHOW ITS VERSATILITY IN DEGRADATION OF ALGAE-DERIVED BIOPOLYMERS**

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The Bacteroidetes taxon consists of heterotrophic bacterioplankton specialised in degradation of high molecular dissolved and particular organic matter and is therefore of central importance for the marine carbon cycle. Likewise, *Gramella forsetii* KT0803, a Bacteroidetes isolate of the North Sea (Helgoland), emerged after an algae bloom that provides a wide range of polymeric organic substrates. Following cultivation on algae-based carbon sources, the physiological potential and adaptability of *G. forsetii* was determined in a subproteomic approach. This strategy implemented an enrichment of the cytoplasmic, periplasmic and membrane protein fraction of the Gram-negative bacterium as well as purification of extracellular proteins. High expression of biopolymer-specific TonB transporters, glycosyl hydrolases and hybrid signal transduction systems emphasized the relevance of polymeric carbon sources for *G. forsetii* and supported its cell proliferation under simulated, so-called feast conditions that typically occur after algae blooms.

**IRON AND CARBON LIMITATION: EFFECTS ON HETEROTROPHIC MICROBIAL METABOLISM**

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Iron is a physiological requirement for life, but its bioavailability for microorganisms is low in large regions of the ocean. Consequences of the scarcity of iron on phytoplankton, metabolism and related biogeochemical processes have been extensively studied. However, comparatively little attention has been paid to heterotrophic bacteria, the major drivers of DOM flux in the ocean. The aim of our study was to investigate the effect of iron limitation on heterotrophic bacterial metabolism using an experimental approach and two strains of *Alteromonas macleodii* (Gammaproteobacteria) isolated in contrasted marine environments. The metabolic response of a coastal and an oceanic *A. macleodii* strain was investigated in batch cultures conducted under trace metal clean conditions. Growth and respiration rates, cellular properties and the comparative analysis of the total proteomes of both strains each grown under low and high iron concentrations provide new insights into their acclimatization to low iron concentrations. These results shed new light on the role of heterotrophic bacteria in the coupling of iron and carbon cycling in the ocean

**LIMNOBOTICS: UNDERSTANDING BLOOMS OF TOXIC FRESHWATER  
CYANOBACTERIA USING AN AUTONOMOUS SAMPLING PLATFORM AND  
MOLECULAR STRAIN TYPING**

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The filamentous cyanobacterium *Planktothrix rubescens* produces toxins called microcystins (mcy). Regular blooms of *P. rubescens* are observed in European Lakes, including Lake Zürich, where it represents the dominant organism of the phytoplankton community. While the basic patterns of the annual development of *P. rubescens* in Lake Zürich are well established, the basin-wide horizontal variability within the overall seasonal dynamics of are poorly understood. Moreover, there is currently no conclusive information on the abundances and successions of mcy-producing and non-producing strains of *P. rubescens* and their relationship with environmental parameters. The Limnobotics project brings together limnology and robotics towards the autonomous acquisition of ecological data with an unprecedented spatial and temporal resolution of Lake Zürich. Our hypothesis is that mcy-producing and non-producing strains exhibit subtle differences in growth properties that might affect their in situ distribution. Our newly developed Autonomous Surface Vessel (ASV) is equipped with a variety of sensors (temperature, pH, light, oxygen, nutrients, and algal pigments) and allow for a high-frequency monitoring of cyanobacterial spatiotemporal distribution together with a comprehensive overview of environmental changes within the lake. This is complemented with cutting-edge molecular techniques based on sequences of microcystin synthetase gene cluster for *P. rubescens*. We are now working on quantitative real-time polymerase chain reaction (qPCR) approaches to quantify non-mcy producing *P. rubescens* filaments and to estimate their share of the total population. Our ASV sampling will provide an exhaustive collection of relevant field data to identify environmental parameters that control blooms of toxigenic strains of *P. rubescens*, which will be ultimately be used to develop a regional predictive model for Lake Zürich and other similar pre-alpine lakes.

**RECONSTRUCTION AND ECOLOGICAL SIGNATURES OF AQUATIC  
FILAMENTOUS CYANOBACTERIA USING RIBOSOMAL AND FUNCTIONAL  
GENE MARKERS**

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Analyses of closely related genomes of specific bacterial populations are basic to investigate the phylogenetic structure, population genetics and to perform comparative genomics. We are using the filamentous cyanobacteria of the *Pseudanabaena*/*Limnothrix* group as model microorganism to dig into fine-scale phylogenetic reconstruction of these aquatic cyanobacterial taxa and to explore ecological signatures by Unifrac statistical analyses using ribosomal and functional gene markers. We built on a culture collection of 50 *Pseudanabaena* spp. from several contrasted environments: Baltic Sea, Albufera de Valencia and Lake Konstanz. The isolates show considerable variation in cell size depending on the isolation place. Analysis of photosynthetic accessory pigment composition reveals two major phenotypes: phycocyanin-rich (PC-rich) strains and strains containing both PC and phycoerythrin. Strains of the latter phenotype are potentially capable of complementary chromatic adaptation. About 130 kb of the isolated strains genomes were sequenced including the complete 16S rRNA gene, internal transcribed spacer 1 (ITS-1), the *cpcBA* operon encoding for the PC with their corresponding intergenic spacer and the *nifH* for the structural nitrogenase gene. In addition, we investigated the presence of the *nifH* transcripts. Our results on single gene tree phylogenies comparison versus the concatenated genes trees shown an increasing phylogenetic resolution and congruence as we add more loci into the phylogenetic analyses. In addition, we performed Unifrac statistical tests based on phylogenetic distance analyses and we were able to extract ecological patterns related to: the presence/absence of functional genes, cell size distribution and the isolation source, related at the same time with salinity. Finally, the cell size showed a phylogenetic signal on the ITS which is a valuable trait to improve the taxonomical assignation within this poorly and understudied bloom-forming cyanobacterial group.

**DIVERSITY PATTERNS OF CULTURED MARINE BACTERIA USING DIFFERENT ORGANIC MATTER SOURCES AS COMPARED TO SINGLE AMPLIFIED GENOMES**

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Despite the recent spread of massive sequencing technologies into marine microbial ecology, environmental culture isolation and more recently, single amplified genomes (SAGs) are necessary alternatives to conduct in-depth phylogenetic analyses as well as physiological studies.

Our main goal was to develop new isolation approaches to enrich marine bacterial populations and screening SAGs by sequencing their 16S rRNA genes to investigate: (i) diversity patterns of both, culture (usually considered as rare taxa) and uncultured abundant genomes, (ii) to correlate the presence of specific bacterial clusters with the enrichment source and (iii) to explore distinct diversification mechanisms inherent in these bacterial populations. In an effort to enrich and culture specific bacterial phylotypes, two isolation strategies were developed and compared to standard plate isolation: (i) serial filtration by size fractionation and regrowth in standard Zobell medium and (ii) the use of organic matter derived from 7 different phytoplankton species as culture medium. Environmental SAGs retrieved from the HDNA fractions by cell sorting were compared to the whole culture isolate collection.

We have obtained 627 isolates including 87 SAGs from the Mediterranean that show that both enrichment strategies resulted in statistically different (by Unifrac phylogenetic distance analysis) cultured bacterial communities compared to the control. Bacteroidetes were preferentially isolated with algae-derived media while Gammaproteobacteria were preferentially isolated with the size fractionation strategy. In addition, within each approach, specific phylotypes were statistically associated with different algal-derived medium and different size fractions. We hardly found overlap between SAGs and cultured isolates confirming that many SAGs represent novel uncultured bacterial taxa. Finally, we observed different diversification patterns between main bacterial taxa and between cultured and SAGs.

**MUTUAL INTERCONNECTEDNESS OF CHEMOSYNTHETIC AND HETEROTROPHIC BACTERIA AND ARCHAEA IN PELAGIC MARINE OXYGEN DEPLETION ZONES**

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In recent years the scientific community became aware that pelagic marine oxygen depletion zones spread around the globe. These areas are characterized by a redox gradient, caused by hydrogen sulfide diffusing upwards in oxygenated water layers. A distinct community of chemosynthetic microorganisms inhabits such redoxclines, especially epsilon- and gammaproteobacteria thought to oxidize sulfide in parallel to nitrate or oxygen reduction, but also ammonia oxidizing thaumarchaeota. These key organisms dominate the microbial community and have a fundamental impact on biogeochemical cycles, and further provide biomass that may serve as substrate for heterotrophic microorganisms. To investigate chemolithoautotrophic denitrification, one of the central biogeochemical processes in such redoxclines catalyzed by epsilonproteobacteria, we cultivated the representative strain *Sulfurimonas* sp. GD1. We identified its soluble proteins and quantified its dark carbon dioxide fixation in parallel to its substrate turnovers during denitrification. By coupling both processes stoichiometrically and comparing it with dark carbon dioxide fixation and nutrient concentrations measured in Baltic Sea hypoxia, we estimated the role of such chemolitho-auto-trophic denitrification in the environment. Further we combined metagenomics, metaproteomic mass spectrometric peptide-mapping, catalyzed reporter deposition fluorescence in situ hybridization and chemical profiling, to resolve and interconnect the metabolic potential, activity and abundance of all relevant chemoautotrophs and associated heterotrophs during redox conditioned niching processes, including also nitrifying or sulfate reducing proteobacteria and highly abundant bacteroidetes. We clearly show their role in carbon, nitrogen and sulfur fluxes and the interconnectedness of these microbes by their ability to pass metabolic products to each other, thus catalyzing an internal biogeochemical feedback system shaping their habitat.

**PYROSEQUENCING UNRAVELS ASSEMBLY AND COMPOSITION OF COMPLEX BIOFILM COMMUNITIES IN STREAMS**

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Benthic biofilms are an important form of microbial life in streams where they control key ecosystem functions and greatly contribute to large-scale biogeochemical fluxes. Unraveling biofilm community assembly is a first crucial step toward a mechanistic understanding of biofilm functioning. We used 454 pyrosequencing of the 16S rRNA gene to study the role of the suspended microbial community for biofilm community composition. Artificial substrates were applied to three streams as substratum for biofilm formation. The suspended microbial community was repeatedly sampled during biofilm growth and biofilms were harvested after three weeks. We found clear differences between biofilm and suspended communities, while no resemblance between biofilm and suspended communities from the same stream could be observed. The biofilm OTUs were classified as members of 29 classes from 14 phyla; OTUs of the suspended community fell into 48 classes of 24 phyla. Both biofilm and suspended communities were dominated by the class *Beta-Proteobacteria*, accounting for more than one third and one fourth of the individuals in the biofilm and the suspended community, respectively. *Flavobacteria*, *Gamma-Proteobacteria* and *Bacilli* were relatively more abundant in biofilms, while *Chlamydiae*, *Delta-Proteobacteria* and members of the *ODI* group were relatively more abundant in the suspended communities. *Actinobacteria*, *Spingobacteria* and *Alpha-Proteobacteria* constituted a similar percentage in both life forms. Our findings indicate that biofilm community assembly is not a sole result of different source communities in the streamwater. Mechanisms shaping the microbial communities in biofilms might include competition or the process of niche construction through increased resource diversity in biofilms.



**PROTEOME BASED COMPARATIVE ANALYSIS OF EXPONENTIALLY GROWING AND STATIONARY PHASE MARINE BACTERIA**Muthusamy, S.<sup>1</sup>, Baltar, F.<sup>1</sup> and Pinhassi, J.<sup>1</sup>*<sup>1</sup>Marine microbiology, School of Natural sciences, Linnaeus University, Kalmar, Sweden*

Marine bacteria play principal roles in biogeochemical processes. Modern proteomics techniques and availability of genomic sequence data provide an opportunity to compare proteome profiles of individual microorganisms. To understand the cellular response and physiology of marine bacteria, we have developed protocols to generate growth phase dependent proteome maps for three representative model organisms of marine bacteria [i.e., alphaproteobacteria (MED193), gammaproteobacteria (MED92) and flavobacterium (MED134)] using two-dimensional difference gel electrophoresis (DIGE). DIGE is a powerful tool to identify and quantify differentially expressed proteins under various environmental conditions. In order to compare the proteome profiles of the strains in different growth stages, bacteria were grown in seawater media at room temperature. 2D Gel electrophoresis of extracted proteins for various samples was performed using IPG strip pH 3-10 NL. Initial analysis showed that the total proteins expressed in the model bacteria changed more than 60% from exponential to stationary phase. Identification and quantification of major expressed proteins by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF-MS) provided insights into similarities and differences in basic metabolic processes and how they differ among key marine bacteria. The techniques developed and proteome maps generated in this study will be used to study chemolithoheterotrophs under different growth and stress conditions.

**IDENTIFICATION OF ACTIVE BACTERIAL ASSEMBLAGES BY A NEW PCR INDEPENDENT 16S rRNA SINGLE STRAND CONFORMATION POLYMORPHISM (SSCP) APPROACH**

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The analysis of 16S rRNA is a powerful tool to identify potentially active microorganisms cultivation-independently. In combination with fingerprinting methods as SSCP it allows (a) to discover and screen prokaryotes in large amounts of samples, and (b) to give indications on most active prokaryotes at the time of sampling by determining relative 16S rRNA band abundances. As a disadvantage, these analyses usually need an amplification step of DNA-free 16S rRNA using reverse transcriptase PCR, which can be biased by the DNase treatment, the primers selected, and the PCR itself. Moreover, DNA digestion and PCR are time consuming, constraining analyses of high number of samples. Thus, our aim was to develop a 16S rRNA fingerprinting system which is insusceptible to DNA contamination, in general of short duration, PCR independent, and increases the reliability of the quantification procedure for dominant taxa. We successfully developed a novel 16S rRNA SSCP fingerprinting method based on a heterotrophic enrichment culture. Due to the elimination of DNase treatment and PCR this technique included only two principal steps: (1) Directly after RNA extraction the 16S rRNA was reverse transcribed into partial complementary DNA (cDNA) using the biotinylated bacterial primer 534rb, resulting in partial 16S rRNA/rcDNA hybrids. Eventually, (2) the hybrid was denaturated and by this a specific biotin labelled 16S rcDNA single strand generated. After purification and concentration via Streptavidin coated magnetic particles, 16S rcDNA molecules could be separated by SSCP fingerprinting. In comparison with analogously done PCR-dependent SSCP fingerprinting, relative quantification of bands revealed no bias in the display of single species 16S rRNA quantity in a range from 10 to 200 ng of total RNA. Thus, we conclude that this new PCR-independent 16S rRNA SSCP is a useful approach especially for the identification and also relative quantification of abundant bacterial 16S rRNAs.

**CHARACTERISTICS AND BIOREACTIVITY OF GLACIAL DISSOLVED ORGANIC MATTER**

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Recent studies increasingly show that inland waters are active components of the global carbon cycle, causing a reconsideration of the conventional perception of inland waters as “pipes” merely transporting organic carbon to the oceans. On the way from the continents to the ocean, terrestrially derived carbon is oxidized, mostly by microbial heterotrophs.

In alpine regions, glaciers are at the origin of the fluvial continuum, and their accelerated retreat due to global warming affects glacial mass balance, mobilizes formerly ice-bound organic carbon, and implies possible consequences for ecosystem processes in glacial streams by changing the quality and quantity of fluvial dissolved organic matter (DOM).

DOM concentration, composition and bioreactivity were investigated in 25 different glaciers and glacial streams using bioassays and high-resolution optical (absorbance and fluorescence) and mass-spectrometric (Fourier transform ion cyclotron resonance MS) methods. Results point to the importance of melting glaciers as sources of dissolved organic matter of low concentration but high bioreactivity.

**NOVEL CLOSTRIDIUM POPULATIONS INVOLVED IN THE ANAEROBIC DEGRADATION OF MICROCYSTIS BLOOMS**

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Understanding the microbial degradation of *Microcystis* biomass is crucial for determining the ecological consequences of *Microcystis* blooms in freshwater lakes. The purpose of this study was to identify bacteria involved in the anaerobic degradation of *Microcystis* blooms. *Microcystis* scum was anaerobically incubated for 90 days at three temperatures (15C, 25 C and 35 C). We used terminal restriction fragment length polymorphism (T-RFLP) analysis of bacterial 16S rRNA genes, followed by cloning and sequencing of selected samples, to reveal the community composition of bacteria and their dynamics during decomposition. *Clostridium* spp. were found to be the most dominant bacteria in the incubations, accounting for 72% of the sequenced clones. Eight new clusters or subclusters (designated CLOS.1–8) were identified in the *Clostridium* phylogenetic tree. The bacterial populations displayed distinct successions during *Microcystis* decomposition. Temperature had a strong effect on the dynamics of the bacterial populations. At 15 C, the initial dominance of a 207-bp T-RF (*Betaproteobacteria*) was largely substituted by a 227-bp T-RF (*Clostridium*, new cluster CLOS.2) at 30 days. In contrast, at 25 C and 35 C, we observed an alternating succession of the 227-bp T-RF and a 231-bp T-RF (*Clostridium*, new cluster CLOS.1) that occurred more than four times; no one species dominated the flora for the entire experiment. Our study shows that novel *Clostridium* clusters and their diverse consortiums dominate the bacterial communities during anaerobic degradation of *Microcystis*, suggesting that these microbes' function in the degradation process.

**PARTICULATE ORGANIC CARBON AND ITS IMPLICATIONS FOR THE OXYGEN BUDGET OF THE EMS-ESTUARY**Schöl, A.<sup>1</sup><sup>1</sup>*Federal Institute of Hydrology, Germany*

Particulate organic carbon (POC) content in the Ems-Estuary has increased ten-fold from about 0.1 to 1 g/l during the last 20 years. Thereby, the heterotrophic bacterial activity was stimulated, causing low oxygen conditions in summer. The Ems Estuary is a partially mixed mesotidal estuary located at the southern coast of the North Sea. Its total length is about 100 km from its upper boundary at the weir of Herbrum down to the Wadden Sea island of Borkum. The tidal ranges increase from 2.3 m at the inlet to about 3.5 m in the river. The annual mean river run off of the Ems is 81 m<sup>3</sup>/s. Due to strong alteration of the bathymetry, nowadays the estuary shows a strong tidal asymmetry leading to an upstream transport of sediments, especially during low flow conditions in summer. These hydrodynamic processes cause a high accumulation of suspended sediment and POC in the inner part of the Ems-Estuary. We surveyed the distribution of POC along the salinity gradient, and concomitantly measured at fixed station the vertical gradient of POC in the water column during the tidal cycle. Although the suspended sediments originate from the Wadden Sea, they showed a high organic content resulting in a mean POC content in the water column of about 4 % per suspended particulate matter (dry weight). Respiration measurements revealed strong differences in the quality of the POC, depending on the origin of the POC. While sample from both end members of the salinity gradient showed high respiration rates due to their comparably high algal content, samples from the turbidity maximum showed lower rates due to low degradability of the POC. A simple model approach is presented for calculation of the oxygen content in the water depending on the POC content. Besides the calculation of the heterotrophic respiration, this approach takes water temperature and re-aeration of the water column into account.

**OXYGEN DEMAND IN SURFICIAL SEDIMENTS DURING RESUSPENSION EVENTS**

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Organic matter degradation in aquatic surficial sediments (at sea and in rivers) is a key controlling factor of potential organic carbon sequestration in deep-sea sediments. In shallow environments, surficial sediments are frequently resuspended by intermittent physical drivers such as tide, waves or unsteady currents. Resuspension is a vertical transfer process that occurs when bed shear stress exceeds the sediment erodibility threshold at the water-sediment interface. This process not only control fluxes of sediment and interstitial water to the water column but also diffusive fluxes amongst which oxygen to the sediment. As a result, bacterial respiration and chemical oxygen demand is expected to be stimulated both in the water column and in the sediment.

In order to investigate the dynamics of oxygen demand during and after resuspension, an experiment of purely diffusive turbulence causing vertical resuspension was designed. Cores were reconstructed from sieved (2mm) river sediments and oxygen profiles dynamics across the water-sediment interface were monitored with microelectrodes for varying frequency and duration of resuspension. Strong water column and sediment oxygenation was observed after resuspension. Consumption fluxes in oxygen were calculated over time and space and related to oxygen concentration.

In the sediment, oxygen demand increased during resuspension and remains high after stirring was stopped while decreasing. Moreover, acclimation of bacteria communities to alternate oxic/anoxic conditions is observed when frequency and duration of resuspension varied.

**ALUMINIUM, pH, OR PHOSPHORUS: WHAT REALLY LIMITS MICROBIAL LEAF LITTER DECOMPOSITION IN ACIDIFIED HEADWATER STREAMS?**

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Acidic deposition, mainly related to human activities, represents a critical environmental stress and induces deleterious effects on structure and function of headwater streams. In particular, several authors reported alterations of leaf-litter breakdown, a key process in these heterotrophic ecosystems. Microbial communities, often dominated by aquatic hyphomycetes, are essential intermediaries of energy flow from allochthonous organic matter to higher trophic levels. These fungi produce several classes of exoenzymes which degrade the structural polysaccharides of the leaves and induce increased palatability and quality of leaf detritus for invertebrate shredders.

Our main objective was to determine experimentally the relative contribution of the three major stresses associated with acidification of waters, i.e. decreased pH, elevated aluminium (Al) concentrations, and decreased phosphorus (P) availability due to its complexation with Al, in the impairment of litter processing. We investigated leaf litter decomposition in stream-simulating laboratory microcosms during 3 weeks. Seven species of aquatic hyphomycetes were inoculated on sterile *Alnus glutinosa* leaf disks in 18 different conditions: 2 pH (acidic vs. neutral) x 3 P x 3 Al concentrations, each treatment being replicated 4 times.

Decomposition rates were higher in neutral, high phosphorus conditions. High Al concentrations strongly reduced leaf litter decomposition, but in lower proportions under high P conditions. Aquatic hyphomycetes inorganic nutrient acquisition was measured by investigating leaves C:N:P ratios. Leaves P content was affected by P inputs, but strongly depended on pH and Al levels. Respective effects of Al, pH and P on the structure of hyphomycetes communities were investigated using PCR-DGGE, while ergosterol was used as an indicator of fungal biomass. Enzymatic activities, mainly phosphatase activities, were also investigated during the experiment.

**ECOLOGICAL SIGNIFICANCE OF ASSIMILABLE ORGANIC CARBON MEASUREMENTS IN ALPINE KARST AQUIFERS**Wilhartitz, I.C.<sup>1</sup>, Kirschner, A.K.T.<sup>2</sup>, Stadler, H.<sup>3</sup>, Farnleitner, A.H.<sup>4</sup>, and Egli, T.<sup>1</sup><sup>1</sup>*Eawag, Swiss Federal Institute of Environmental Science and Technology Switzerland,*<sup>2</sup>*Medical University of Vienna, Austria,*<sup>3</sup>*Joanneum Research, Austria,*<sup>4</sup>*Vienna University of Technology, Austria*

Little is known about the microbial life in karst aquifers, although it is an downright important water resource for about 20 % of the global population. During two years in situ heterotrophic production (HP) was studied in two hydrographical different alpine karst springs, namely a dolomitic karst aquifer (DKAS1) and a limestone spring type (LKAS2). HP as determined by <sup>3</sup>H-leucine incorporation, revealed strong seasonal variations in the water column, giving ranges of 0.06 – 6.83 pmol C l<sup>-1</sup>h<sup>-1</sup> (DKAS1) and 0.50 – 75.58 pmol C l<sup>-1</sup>h<sup>-1</sup> (LKAS2). However, measurements in aquifer sediments revealed at least 10<sup>6</sup> fold higher HP-rates than in the planktonic fraction. Furthermore, the amount of AOC (assimilable organic carbon) was measured and varied between 10 and 130 µg C L<sup>-1</sup>, representing between 2 and 15 % of the DOC (dissolved organic carbon). The correlation between heterotrophic production and AOC was significantly higher than with DOC in LKAS2. Moreover, seasonal studies showed that the fraction of AOC available for the autochthonous community was not necessarily identical with the overall AOC pool, but depended on the availability of trace elements. AOC values were highest after rainfall events, however, analysis of the quality of the organic compounds with SEC (size exclusion chromatography) suggest that this was not solely due to surface runoff but possibly because of the “activation” of organic compounds deriving from the previously desiccated biofilms. These results would contradict previous assumptions, that autochthonous communities in alpine karstic aquifers almost exclusively depend on seasonal allochthonous nutrient input and would emphasise the importance of surface associated autochthonous microbial endokarst communities for these ecosystems, including biogeochemical- (e.g. karstification) and self-purification processes within the aquifer.



**ISOLATION OF FACULTATIVELY OLIGOTROPHIC BACTERIA FROM DIFFERENT MARINE SAMPLES**

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The dissolved organic carbon (DOC) in the ocean is several orders of magnitude lower than in other environments. Moreover, bacteria apparently use only a fraction of this organic matter. For these reasons, the majority of marine bacteria are living under oligotrophic condition. In this project we used the CANgrowth-method (changing availability of nutrients growth–method) as a strategy to isolate facultatively oligotrophic strains (Schwedt *et al.*, submitted). In this approach the medium is only composed of mineral salts without addition of a fixed nitrogen and carbon source and especially treated to avoid possible contaminations with organic matter. All glassware and the NaCl are combusted and the medium is prepared under an atmosphere of synthetic air. This results in an average DOC content of only 0.2 mg C l<sup>-1</sup>, compared to 0.4 - 1.0 mg C l<sup>-1</sup> in natural oligotrophic seawater (Schut *et al.*, 1997; Hansell *et al.*, 2009). With this medium we managed to isolate several strains belonging to *Alpha*- and *Gammaproteobacteria*. These strains were isolated from three geographical locations, one in the Mediterranean Sea (Adriatic basin), and two in the Atlantic Ocean (Azores; North Pond). Most of the isolates, which are able to grow under these oligotrophic conditions, are closely related to *Marinobacter spp.* and *Alteromonas macleodii*, which are very common marine heterotrophic bacteria, but we could show for the first time that they can actively grow under extreme nutrient limitation (8 transfers with 1:500 dilution). Interestingly, *Alteromonas* isolates are even releasing dens mucus, in spite of the very low carbon content of our medium. Currently, we are conducting experiments to compare the growth of our isolates under eutrophic and oligotrophic conditions.

**LINKING PHYTOPLANKTON AND BACTERIAL DIVERSITY THROUGH DISSOLVED ORGANIC CARBON: INSIGHTS FROM CONTINUOUS CULTURE EXPERIMENTS**

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The biological availability of marine dissolved organic carbon (DOC) to heterotrophic bacteria is tightly linked to its source and diagenetic age. How strong the cycling of different sources of marine DOC is linked to the activity of specific bacterial populations is presently an unresolved question. We tested the hypothesis that DOC originating from different phytoplankton species shapes the bacterial community composition through the selection of bacterial groups that are metabolically adapted to these different DOC compounds. To address this issue, we used continuous cultures to study the response of a natural community of heterotrophic bacteria to DOC originating from cultures of *Phaeodactylum spp.* or *Synechococcus spp.*. Continuous cultures were used to follow the growth of bacterial communities on aged seawater alone or aged seawater amended with 15  $\mu$ M of the DOC produced by *Phaeodactylum spp.* and *Synechococcus spp.*. The cultures were monitored for five generation times. In the cultures amended with phytoplankton-derived DOC, heterotrophic bacteria responded markedly in terms of bulk biomass production and enzymatic activities, compared to the not-amended cultures. By contrast, no differences in these parameters were detectable between the two sources of phytoplankton-derived DOC. We will discuss these results in view of the effect of the DOC-sources on the bacterial diversity as revealed by 454 Tag pyrosequencing.

**CO<sub>2</sub> INDUCED pH DECREASE: EFFECT ON PLANKTONIC MICROBES**

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Carbon dioxide Capture and Storage (CCS) sites are considered as a valid option to ‘permanently’ store CO<sub>2</sub> from large anthropogenic point sources. Although significant leakage from CCS sites is not expected, if it did occur, it would potentially result in local high concentrations of CO<sub>2</sub>, which could have a significant impact on organisms.

To test the pH decrease effect 2 mesocosm experiments have been carried on microbial plankton communities collected from the Gulf of Trieste (Northern Adriatic Sea) in dpring-summer (18°C) and for autumn-winter (10°C) conditions. During each experiment 3 mesocosms with different pH values were set up (6.5, 7 and an aerated control ~ 8). Experiments on plankton were conducted on 200 L initial volume. Every experiment was made up of 3 stages: short term effect (6 samplings within the first 2 days), long term effect (5 additional samplings; 21 days ), and a recovery stage (21 days during which each mesocosm was treated with air bubbling). The analyses focussed on prokaryotic abundance and metabolism (degradation processes, carbon production, respiration).

Prokaryotic abundance, which have been previously reported as weakly sensitive to CO<sub>2</sub> induced pH decrease, have confirmed previous literature findings only at 18°C. Contrarily, at 10°C prokaryotes seemed to benefit from CO<sub>2</sub> addition after long term exposure (10- and 6-fold higher at pH 6.5 and 7, respectively). Some prokaryotic activities displayed pronounced differences when subjected to CO<sub>2</sub> injection. Heterotrophic C Production rates were faster at lower pH in both experiments. The degradation of lipids was quickly enhanced at the beginning of CO<sub>2</sub> supply both at 10°C and 18°C. Very different trends characterised phosphatase activity measured at pH 6.5 and 7 from the ones measured in the control. At lower pH values, in fact, it seemed that the activity of this enzyme was pronouncedly inhibited at both temperatures.

**CLIMATIC DRIVERS BEHIND THE DYNAMICS OF AUTOTROPHIC PICOPLANKTON  
IN A BOREAL BROWN-WATER LAKE**

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In the boreal area, majority of lakes have high concentrations of allochthonous organic carbon (OC), which inputs are mainly controlled by precipitation. According to reasonable projections, climate change can increase precipitation in the area up to 15%. This can be crucial, since brownish OC absorbs solar radiation effectively and is thus the culprit for the steep stratification resulting in epilimnetic nutrient depletion. OC also deteriorates lake light climate, binds nutrients and lowers water pH. Altogether, allochthonous OC makes the lakes harsh environments for photosynthetic organisms. However, the tiny autotrophic picoplankton (APP) is effective in nutrient uptake and light harvesting, and thus it should flourish in brown-water lakes.

We studied APP in the brown-colored Lake Valkea-Kotinen in Finland during five open-water periods in 2002–2006 to reveal the importance of climate-driven abiotic factors, such as precipitation, water temperature and the stability of water column as well as water color and pH on APP dynamics. In addition, we studied the importance of major nutrients.

In general, APP thrived in high temperature and high water column stability and was most abundant in or above metalimnion. However, the timing and vertical location of the maxima as well as the cell numbers and even the dominant taxa varied among the years: in 2002 the APP composed almost entirely of colonial cyanobacteria, but in 2003–2006 of solitary eukaryotic cells. The colonial APP thrived in conditions that prevailed in metalimnion during stratification, i.e. in high temperature and low nitrogen concentrations, whereas the solitary APP preferred high water color and low pH, which both occurred after heavy rains in 2004. The increase in water color probably hindered the growth of colonial APP, however, it could not be the only reason for the expansion of solitary APP. Most likely, the incomplete lake spring overturns in 2003–2006 also disfavored the growth of picocyanobacteria.

**OCEAN ACIDIFICATION AND ITS IMPACT ON GROWTH AND ACTIVITY OF HETEROTROPHIC BACTERIA - FIRST RESULTS FROM A COUPLED PHYTOPLANKTON GROWTH AND BACTERIAL DEGRADATION EXPERIMENT.**

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Next to global ocean warming another trend in climate change is likely affecting the world's oceans in the future- the rising in CO<sub>2</sub> concentration leading to increasing ocean acidification. Changes in ocean water chemistry and species composition are likely to take place and might also change biogeochemical cycles. Whether it will have a direct impact on bacterial communities is still under debate. In this study, the effects of increasing atmospheric CO<sub>2</sub> concentrations (180, 380, and 780 ppm) on growth and activity of a natural bacterial community from the North Sea was studied using a bi-party phytoplankton growth and bacterial degradation approach. The first part examined the effect of pCO<sub>2</sub> on growth and production of the marine diatom *Thalassiosira weissflogii*, as well as bacteria associated with the algae. The non-axenic diatoms were grown in chemostats at the three pCO<sub>2</sub>, until a steady state growth was achieved and were then transferred to a batch mode for three days. After the batch incubation the first part was terminated by separating the algae from the dissolved fraction (DOM and TEP) using filtration. The second proximate part used DOM and TEP produced by *T. weissflogii* to investigate growth and activity of a natural bacterial community under the three pCO<sub>2</sub> conditions. Bacterial multiplication rate (BCM) did not show any difference between the pCO<sub>2</sub> set-ups of the two experimental parts. In contrast, bacterial protein production significantly increased at higher pCO<sub>2</sub> for bacteria present in the *T. weissflogii* cultures. This trend was not mirrored for bacteria in the subsequent degradation part using *T. weissflogii* derived DOM. Possible differences between the bacterial communities present in the two experimental parts will be determined using DGGE and might provide further explanation regarding the trends observed. On the whole, bacteria will rather directly adapt towards changing phytoplankton production, rather than being effected by ocean acidification itself.

**TEMPERATURE-MEDIATED VIRUS-INDUCED BACTERIAL MORTALITY**

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The mesocosm experiment on summer bacterioplankton was carried out in 2010 to evaluate the impact of temperature on virus production and virus-induced bacterial mortality. Triplicated in-door enclosures (1.4 m<sup>3</sup>) filled with seawater collected from Kiel (Germany) Fjord (Baltic Sea) were incubated at three temperature regimes (in situ and in situ  $\pm 4^{\circ}\text{C}$ ) followed natural conditions for 19 days. Virus production (VP) was measured by the dilution approach (Wilhelm et al., 2002) as modified for tangential flow filtration (Winget et al., 2005) using epifluorescence microscopy (Noble and Fuhrman, 1998) and by the whole cell approach using the transmission electron microscopy to assess the frequency of visibly infected cells (FVIC) and burst size (BS) (Weinbauer and Peduzzi, 1994). Frequency of infected cells (FIC) and prokaryotes mortality was calculated using conversion factors (Weinbauer et al., 2002) and, in addition, data provided by both methods were compared.

The total number of viruses and bacteria, virus to bacteria ratio, bacterial size and morphology, burst size, virus production, bacterial mortality rate, phage capsid size, virus contact rate and success as well as virus turnover time was used in the model in order to observe simultaneous response of selected variables to different temperature regimes. Non-parametric, permutation test- based, multivariate analysis of variance (Anderson, 2001; 2005) was applied for the analysis and interpretation of the experimental data. The various patterns of different variables will be presented as a function of temperature mediated processes as well as threshold for ecologically significant virus production and bacterial mortality in terms of predator-prey interactions will be proposed.

**PHOSPHORUS TRANSFORMATION BY THE CYANOBACTERIUM  
NODULARIA SPUMIGENA UNDER DIFFERENT pCO<sub>2</sub> LEVELS**

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Primary production by phytoplankton is expected to be enhanced by elevated CO<sub>2</sub> concentrations in the future. This will have consequences for the availability of dissolved organic matter (DOM) as well as for ecosystem functioning. Under this aspect we conducted a joint laboratory experiment within the BIOACID project. To investigate the phytoplankton related DOM variations under elevated pCO<sub>2</sub> levels we set up batch culture experiments applying different pCO<sub>2</sub> scenarios: 180 ppm, 380 ppm and 780 ppm representing preindustrial, present and future conditions, respectively. In the experiments, growth and DOM (DOC, DON, DOP) production by the diazotrophic cyanobacterium *Nodularia spumigena* were studied. The focus of our studies was the transformation of phosphorus components (DOP, DIP, POP) as well as the composition of DOP (ATP, phospholipids, DNA, RNA).

We detected an enhanced growth of *Nodularia spumigena* at elevated pCO<sub>2</sub> conditions accompanied by faster DIP depletion. In all experimental approaches the preferred phosphorus source was DIP, but in the course of the experiment the proportion of utilized DOP increased, with strongest effects at 780 ppm. The proportion of dissolved ATP (D-ATP) to total DOP increased until the highest biomass peak, suggesting that D-ATP was not used as a P-source by *Nodularia spumigena*. In contrast phospholipids were used to satisfy the P demand in a small proportion. This leads to the assumption that either DNA and RNA or the unidentified moiety of DOP is utilized to meet the P demand.

Our results strongly suggest that the strengthened P demand at elevated CO<sub>2</sub> concentrations leads to an enhanced DOP consumption by *Nodularia spumigena* during the bloom phase. After the collapse of the algal bloom DOP will be released again. Hence, under future conditions the competition for DOP between autotrophic and heterotrophic bacteria will be strengthened during the cyanobacterial bloom.

**DISTRIBUTION AND SEASONALITY OF POTENTIALLY PATHOGENIC VIBRIO SPECIES  
IN GERMAN COASTAL BATHING WATERS**

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*Vibrio* spp. are ubiquitous bacteria naturally found in marine and estuarine waters, including 12 human pathogens. High temperatures promote *Vibrio* spp. proliferation and records of *Vibrio*-related non-Cholera infections associated with sea food consumption or bathing water contact have been increasing worldwide in recent years. With regard to climate change, there is growing concern that *Vibrio*-related sea food poisoning and wound or ear infections may become an emerging disease even in the temperate seas of North-Western Europe. Outbreaks of *Vibrio*-related wound infections among bathers have frequently been reported for the Baltic Sea coast following heat waves since the mid Nineties. For a shorter time, sporadic cases of *Vibrio* infections have also been recorded for the more saline North Sea waters.

Within the framework of the research program KLIWAS (Impacts of Climate Change on Waterways and Navigation), the Federal Institute of Hydrology (BfG) and the Governmental Institute of Public Health of Lower Saxony (NLGA) initiated a 2,5-year-long *Vibrio* monitoring program at 11 selected bathing sites along the North Sea coast of lower Saxony and within the estuaries of the rivers Ems and Weser. Monthly water and sediment samples are screened for *Vibrio* spp. using a culturing approach accompanied by biochemical and molecular verification. Water temperatures, conductivity and grain sizes of the sediments are recorded. Aim of this project is to answer the following research questions: 1) Do potentially pathogenic *Vibrio* spp. occur at bathing sites along the East Frisian coast and within the Ems and Weser estuaries, 2) How does the occurrence and abundance of potentially pathogenic *Vibrio* spp. relate to temperature, salinity and sediment type and 3) Are there “hot spots” of *Vibrio* spp. occurrence? The results of this study will be integrated in a future-oriented health risk assessment. Preliminary data for October 2009 until July 2011 will be presented at the SAME-12.



**STRUCTURAL AND FUNCTIONAL RESPONSES OF MICROBIAL COMMUNITIES TO TEMPERATURE VARIATIONS IN AQUATIC SYSTEMS: EXAMPLE OF PHOTOTROPHIC BIOFILMS IN SOUTHWESTERN FRENCH RIVERS**

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Freshwater ecosystems are particularly vulnerable to global change. Climate projections lead to give special attention to effects of warming on aquatic communities. River biofilms are microbial consortia mainly composed of algae, bacteria and micro- and macrofauna embedded in a mucilage matrix that develop on solid substrata in rivers, in areas especially exposed to solar radiations and temperature changes. They are involved in primary production, nutrient cycling and can be considered as the base of river food webs. These biofilms can accumulate toxic metals and contaminants, and can be considered as good biological integrators of changes in water quality. This microbial consortium can then be a good model to understand biological responses to temperature variations. Biological response to temperature can be direct, beyond a metabolic effect or, indirect through changes in community structure. The aim of the present study was to characterize direct and indirect effects of warming (from 2.5°C to 23.8°C) on architectural (thickness, elasticity, covering and biomass), structural (algal and bacterial diversity) and functional (primary production and respiration, nitrification and denitrification) responses of river biofilms in two Southwestern French rivers (the Garonne and the Douctouyre rivers). Our results obtained in situ showed an acceleration of the maturation of the biofilm (thickness, biomass and biological activities) when submitted to a warming of 2.5°C, but non-significant differences in the algal and bacterial diversities. Resilience was also observed after the colonisation phase of these biofilms. When they were submitted to warmings in incubators (up to 23.8°C), biofilms showed increases of primary production, respiration and denitrification, when their structural diversity was dependant on the season.

**WAVELENGTH DEPENDENCE OF THE MOLECULAR TARGETS INVOLVED IN  
UV-INDUCED DAMAGE TO AQUATIC BACTERIA**

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The combined effects of climate change factors are expected to result in enhanced exposure of bacteria, the basis of the aquatic trophic net, to UV radiation (UVR) with unknown ecosystem-level repercussions. However, information on the molecular events that follow UV exposure, how they affect microbial function and ultimately impact biogeochemical cycles is scarce.

The molecular and physiological effects of UVR of different wavelengths (UV-A: 320-400 nm, UV-B: 290-320 nm, UV-C: 290 nm) were studied in 9 environmental bacterial isolates, used as biological models. Physiological effects (culturability and activity), overall biochemical changes (infrared spectroscopy), stress markers (DNA strand breakage, reactive oxygen species generation and oxidative damage to proteins and lipids) and the efficiency of enzymatic antioxidant systems (catalase/CAT and superoxide dismutase/SOD) induced by different UV wavelengths were assessed.

Decrease in culturability (16.8-99.9%) and activity (4.6-99.9%) caused by different UV wavelengths varied among strains, but was stronger for shorter wavelengths. DNA strand breakage (1.2-42.0%) followed the same pattern. ROS generation (1.7-53.7%), protein (-6.3-112.6%) and lipid oxidation (7.9-49.7%) varied according to the inverse trend. A wavelength-dependent variation of CAT (-94.8-90.5%) and SOD (-59.1-63.5%) activities was not observed.

Regression analysis revealed that loss of culturability induced by UV-A was determined by the extent of DNA damage while modifications at the membrane level explained cell inactivation under UV-B and UV-C. UV-A exposed cultures were differentiated by enhanced levels of oxidative stress markers while the level of DNA damage differentiated the UV-C treatment, as revealed by multivariate analysis.

These results provide new insights into the cellular targets of different UV wavelengths that can contribute to an integrative model of the interactions between UVR and bacteria.

**SEASONAL AND INTERANNUAL DYNAMICS OF PHYTOPLANKTON IN A MESOTIDAL COASTAL LAGOON: UNRAVELLING THE EFFECTS OF CLIMATE CHANGE AND LOCAL-HUMAN INFLUENCES.**

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This study aimed to evaluate seasonal and interannual dynamics of phytoplankton in a mesotidal coastal lagoon (Ria Formosa, SE Portugal), and to understand their driving forces and the relative contribution of climate and local-human influences. This ecosystem, subjected to multiple human influences, is situated in a region classified as very vulnerable to climate change. Changes in human activities and climatic processes affecting the Ria Formosa during the period 1967-2011 were synthesized and linked to current knowledge on lagoon abiotic variables and phytoplankton. Phytoplankton at inner lagoon locations exhibited unimodal annual cycles with summer peaks, linked to temperature and light availability, whereas inlet areas displayed a bimodal annual cycle. The effects of increased anthropogenic pressure were evident only at sites close to major urban centers, probably due to reduced lagoon water residence times and the impact of physical variables as phytoplankton growth limiting factors. Long-term climatic variability apparently reverberated into the lagoon's water column, leading to a generalized and significant winter warming trend, and strong interannual changes in the concentration of inorganic nutrients, particularly during the autumn-winter period. Interannual freshwater flow variability apparently exerted a relevant control on phytoplankton, particularly during periods of high flow rates and low light limitation. Phytoplankton biomass showed a generalized interannual winter declining trend that could be indirectly related to the winter warming tendency, through its effect upon phytoplankton grazers. Overall, data suggest that climate variability is an important driver of phytoplankton in the lagoon, both on short-term, seasonal, and interannual time scales. Local-human impacts are apparently spatially restricted.

**EFFECTS OF TEMPERATURE, LIGHT AND NUTRIENTS ON PHYTOPLANKTON  
COMPOSITION AND GROWTH IN A MESOTIDAL COASTAL LAGOON**

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Phytoplankton is a key component in aquatic ecosystems, regulating organic matter available for metazoans, global biogeochemical fluxes, and atmospheric CO<sub>2</sub> content. Due to its sensitivity, phytoplankton is also an indicator of ecological status and change. Recent studies show that phytoplankton in coastal ecosystems is undergoing marked changes in terms of biomass, composition and phenology, which can be linked to local anthropogenic influences and climate change. However, the exact underlying causal links between environmental and phytoplankton variability are difficult to establish, thereby hampering our capacity to forecast ecological changes in aquatic ecosystems. This study aims to: 1) quantify the effects of simultaneous changes in different phytoplankton growth, specifically temperature, light and nutrients, on the composition and growth of phytoplankton in the Ria Formosa coastal lagoon, and 2) identify growth limiting factors. The Ria Formosa is a multi-inlet mesotidal coastal lagoon (SE Portugal), a highly vulnerable region to climate change, and subjected to strong anthropogenic pressures and coastal upwelling events. To accomplish these goals, microcosm experiments were used during summer in two different stations in the Ria Formosa (seaward and landward boundary sites). Different temperature and light scenarios were simulated, with the addition of inorganic nutrients (N, P and Si, alone and in combinations). All the experiments were performed in triplicate and phytoplankton composition, abundance, and biomass were analysed throughout the experiments. Our results on the response of phytoplankton to environmental pressures are not only relevant for water management authorities, but they will also provide valuable information for existing ecological models and increase our capacity to forecast ecological changes in coastal lagoons.

**IMPACT OF WARMING ON THE COMPOSITION AND ACTIVITY OF THE BACTERIAL COMMUNITY ACCOMPANYING THE EARLY PHYTOPLANKTON SPRING BLOOM**

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The impact of raising water temperature on the algae-bacteria coupling and bacterial community composition was investigated during an indoor mesocosm experiment with natural spring plankton communities from the western Baltic Sea. Heterotrophic processes are known to be highly temperature controlled and therefore warming results in an acceleration of bacterial degradation relative to autotrophic production. Less is known how the specific activity and diversity of the dominating bacterial taxa during an early phytoplankton bloom are affected by increasing water temperatures. Here we show that an increase in water temperature of 6°C leads to the expected tighter coupling between the phytoplankton bloom and the bacterial community, and to a higher bacterial production and abundance, approximately doubling the overall carbon flow into the microbial food web. The free-living bacterial community during the phytoplankton bloom was analysed by 16S rRNA clone libraries, DGGE and CARD-FISH. The bacterial community composition during the phytoplankton peak was dominated by Gammaproteo-bacteria in the cold treatment (93% of total clones), while in the warm treatment this dominance was reduced (51%), with an increased appearance of Bacteroidetes (20%) and Betaproteobacteria (26%). Among the Gammaproteobacteria clones, the Glaciecola group was clearly dominant (55% and 44% of clones respectively in the cold and warm treatments). A newly designed specific CARD-FISH probe (GC1205) verified this dominance, showing that up to 35% of the total cell-counts corresponded to the Glaciecola group in all treatments; and demonstrating the synchronous development with the phytoplankton bloom. It shows that phytoplankton blooms are accompanied highly adapted bacterial key players which dominate the carbon flow from phyto- to bacterioplankton and which show only weak responses to changes in temperature.

**CHANGES IN ORGANIC MATTER CYCLING IN A WARMER OCEAN: RESULTS FROM A MESOCOSM STUDY WITH DIFFERENT TEMPERATURE AND LIGHT SETTINGS**

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To investigate the combined effect of temperature and light availability on organic matter production and degradation during a winter/spring plankton-bloom in Kiel Bight, we have conducted a mesocosm study applying a range of temperature regimes and light intensities. Rising temperature accelerated the onset and termination of the phytoplankton bloom, while light intensity played only a minor role. Although, dissolved inorganic carbon, nitrogen and phosphorus drawdown showed no differences among treatments, particulate organic matter build-up was less pronounced at elevated temperature. Particulate organic carbon maxima were ~30 % lower at elevated compared to ambient temperature, suggesting either a competition of phytoplankton with bacteria for nutrients or enhanced sedimentation during the bloom. The latter possibility is supported by increased transparent exopolymer particle (TEP) concentrations during the phytoplankton bloom which may promote cell aggregation and sinking. The onset of dissolved organic matter accumulation was advanced by warming as was the onset of heterotrophic decomposition processes. For a warmer future ocean we can hence expect two opposite mechanisms during phytoplankton blooms: one in which carbon will be processed more quickly in the microbial loop resulting in a faster recycling of CO<sub>2</sub> and a second that counteracts intensified heterotrophic processes with a stronger export of carbon and nutrients due to enhanced TEP formation.

**FIVE MONTHS IN DARKNESS – HOW DO BENTHIC DIATOMS COPE WITH IT?**

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Polar benthic diatoms have to cope with extreme environmental conditions, e.g. with long periods of darkness. They are also adapted to low temperatures, consequently the suspected Arctic warming may have negative effects on growth and reproduction of single diatom cells and populations. For up to five months two benthic diatoms belonging to the genus *Surirella*, which were isolated from the Arctic Adventsfjorden (Svalbard), were incubated in the dark at 0 °C, the common temperature of the polar winter, and at 5 °C, which represent Arctic warming conditions. Two fluorescence dyes were applied to analyse their physiological status. With these microscopically dyes the membrane integrity could be visualized as a marker for viability as well as the integration of silicate as a marker for growth. It was shown that the smaller *Surirella* (ca. 10 µm in length) seems to be more robust towards raising temperatures than the bigger one (ca. 20 µm in length) after a long term incubation of 20 weeks. The fluorescence dyes are easy to handle, thus they are well applicable for the investigation of individual diatom cells.

**INFLUENCE OF LIGHT AND ORGANIC SUBSTRATES ON GROWTH  
IN ERYTHROBACTER**Hauruseu D. V.<sup>1</sup><sup>1</sup>*Institute of Microbiology CAS, Třebon*

Aerobic anoxygenic phototrophs (AAPs) are prokaryotic microorganisms which harvest light energy using bacteriochlorophyll-based reaction center. They are obligate aerobes requiring oxygen for both growth and synthesis of photosynthetic pigments. AAPs are photoheterotrophs, they require presence of organic carbon substrate as they do not contain Calvin cycle to fix inorganic carbon. The influence of light and organic substrates on the growth of *Erythrobacter* sp. strain NAP1 was studied in continuous cultures. The cells were grown in carbon limited chemostat regime on defined carbon sources (glutamate, pyruvate, acetate, glucose) and illuminated with different light intensities. The amount of carbon incorporated in the biomass was determined by elemental C-N analyser. In general, the light exposure led to enhanced carbon accumulation when compared to cultures grown in dark. The bacterial growth efficiency for culture grown on glutamate was  $33.2 \pm 2.6\%$  in the dark, whereas in the light  $150 \mu\text{E m}^{-2}\text{s}^{-1}$  it was  $47.1 \pm 4.5\%$ . The growth efficiency in pyruvate-grown culture was  $14.8 \pm 0.9\%$  in the dark and  $30.4 \pm 2.4\%$  in the light. Culture growth on glutamate had its optimum around  $150 \mu\text{E m}^{-2}\text{s}^{-1}$ , but the culture was inhibited at higher light intensities. In case of pyruvate we did not find any inhibition of growth by high irradiance. These data were confirmed by cultivation culture under different dilution rates (0.3; 0.5;  $1.0 \text{ day}^{-1}$ ). In addition an incorporation of radiolabeled bicarbonate was studied with culture grown under continuous dark or light:dark regime. In the culture grown on pyruvate there was a strong stimulation of carbon incorporation by light. In contrast in glutamate grown culture there was almost no effect of light bicarbonate incorporation.



**AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA IN MOUNTAIN LAKES**

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Aerobic anoxygenic phototrophic (AAP) bacteria are bacteriochlorophyll a-containing prokaryotes that use organic substrates for growth but derive a significant portion of their energy requirements from light. Although AAP bacteria are intensively studied in marine environments, only little is known about their freshwater counterparts.

We employed infrared epifluorescence microscopy, infrared kinetic fluorometry and *pufM* gene analysis to examine the distribution and diversity of AAP bacteria in nine alpine and subalpine lakes across an altitudinal gradient above and under the treeline (913 – 2799 m a.s.l.) in the Central Alps, Austria in August 2009. AAP abundances displayed a clear vertical gradient and varied from 1.9 to  $31 \times 10^4$  cells mL<sup>-1</sup> (6 – 25 % of the total bacterial community) with altitude, DOC concentration and partially temperature as the main controlling factors. The AAP population above the treeline was composed by heavily pigmented rods (5-7 µm) and of several other smaller morphotypes in lakes below it. The analysis of *pufM* diversity revealed that over 95 % of AAP community in alpine lake Gossenkölle was composed of *Sphingomonas* species belonging to Alpha 4 subgroup of Proteobacteria, whereas the AAP communities inhabiting lakes located below the treeline had higher diversity being composed of various members both Alpha and Betaproteobacteria.

**TOP-DOWN AND BOTTOM-UP CONTROLS ON GROWTH OF  
AEROBIC ANOXYGENIC PHOTOTROPHS**

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Bacterioplankton community represents a wide spectrum of organisms with different cellular activity. Individual bacterial cells could be inactive, dormant or active and rapidly growing. The aerobic anoxygenic phototrophs (AAPs) are photoheterotrophic organisms which use bacteriochlorophyll-based reaction centers to harvest light energy. AAPs were shown to have fast grow rates. However in spite of that AAPs represent only a relatively small fraction of natural microbial communities. To address this paradox and to determine the main control factors on AAP growth rates and abundance, two manipulation experiments were performed in Blanes Bay Microbial Observatory (NW Mediterranean Sea) and on Hawaii Ocean Time-series, Station ALOHA (NW Pacific) in 2010. The percentage of AAPs ranged between 3–8% in the Blanes Bay and between 2–3% in the station ALOHA. The increase in AAP abundance and BChl a diel decays were used to determine AAP growth rates. The observed rates ranged from 1.15 to 1.29 day<sup>-1</sup> in the Blanes Bay and from 0.7 to 0.9 day<sup>-1</sup> in Pacific. Individual experimental treatments influenced all bacterial groups however to a different extend. The most significant influence had the nutrient addition. In the Blanes Bay AAP bacteria increased 26 times after phosphorus addition (from 3x10<sup>4</sup> to 80x10<sup>4</sup> cells mL<sup>-1</sup>). In the Pacific almost 6 times (from 3x10<sup>4</sup> to 17x10<sup>4</sup> cells mL<sup>-1</sup>) after nitrogen addition. The impact of presence of grazes was only minimal.

**OCCURENCE OF INTACT LIPIDS IN ANOXYGENIC PHOTOTROPHIC SPECIES**

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Lipid composition was analyzed in 26 strains of anoxygenic phototrophic bacteria (alfaproteobacteria) using high-performance liquid chromatography and electrospray ionization ion-trap mass spectrometry (HPLC/ESI-IT-MSn). The phospholipids (PLs) were found to be a major membrane component. Phosphatidylglycerol (PG) and phosphatidylcholine (PC) were present in all investigated strains; phosphatidylethanolamine (PE) was found in 81% of these strains. Analyzed strains contained also glycolipids (MGDG, SQDG except DGDG), betaine lipids (DGTS) and ornithine lipids (OLs). OLs distribution was particularly interesting. While they were present in all *Roseobacter* species, they were absent in all the species belonging to  $\alpha$ -4 subgroup of Proteobacteria. Molecular distribution of membrane lipids can differ by nutrient conditions. We cultivated phototrophic bacteria under P-, C-limiting conditions to test whether phospholipids are substituted to other non-phosphorus lipids. The results suggest their ability to conserve phosphorus by substitution of PLs for OLs like a response to low nutrient level.

**A BENEFICIAL EFFECT OF CYANOBACTERIUM *PLANKTOTHRIX RUBESCENS* GROWN UNDER SUBOPTIMAL LIGHT CONDITIONS ON *LIMNOHABITANS* SPP. (BETAPROTEOBACTERIA)**

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We examined the effect of light on the heterotrophic activity of the filamentous cyanobacterium *Planktothrix rubescens* and on the relationship between this cyanobacterium and accompanying bacteria in the subalpine mesotrophic Lake Zürich. Leucine incorporation by bacteria and cyanobacteria in natural microbial assemblages adapted to microphotic conditions from the zone of maximal *P. rubescens* densities were determined in situ and after incubation at contrasting light regimes (ambient, 100× increased, dark). *P. rubescens* from the photic zone of the lake incorporated substantially more leucine, but some heterotrophic activity was maintained in filaments from the hypolimnion. Exposure of cyanobacteria to suboptimal levels of irradiance or to darkness resulted in significantly lower leucine incorporation than at ambient light conditions. In addition, abundances and leucine incorporation rates of Betaproteobacteria from the genus *Limnohabitans* increased most within the accompanying microbial assemblage at suboptimal irradiance levels for *P. rubescens* or in dark incubations. Subsequently, two *Limnohabitans* strains (representing different species) were co-cultured with axenic *P. rubescens* at different light conditions. Cell densities and leucine incorporation rates of both strains most strongly increased at elevated irradiance levels, in parallel to a decrease of photosynthetic pigment fluorescence and the fragmentation of cyanobacterial filaments. Our results suggest that physiologically stressed *P. rubescens* growing under suboptimal light conditions may have a beneficial effect on *Limnohabitans* spp. both in mixed assemblages and in pure co-culture.

**FIRST HINTS FOR CONDUCTING AEROBIC ANOXYGENIC PHOTOSYNTHESIS BY BACTERIA OF THE ROSEOBACTER CLADE AFFILIATED (RCA) CLUSTER**

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Recent studies showed that among the main bacterioplankton groups photoheterotrophy is a surprisingly common feature. Within Alphaproteobacteria quite a few representatives of the *Roseobacter* clade are known to carry genes encoding bacteriochlorophyll *a* (BChl*a*) and thus are capable of aerobic anoxygenic photosynthesis (AAnP). However, still little is known about the genomic organisation and expression of these genes in the major bacterioplankton groups including the *Roseobacter* Clade Affiliated (RCA) cluster.

Genomic analyses of RCA isolates revealed the presence of the entire BChl*a* operon. The operon organisation and structure of these isolates differ from that of several type strains of the *Roseobacter* clade and are more related to those of the *Rhodobacter* clade. Main sections of the photosynthetic operon of strain RCA23 are analog to that of the genera of *Loktanella* and *Thallassobium* of the *Roseobacter* clade but differ in the *pufX* gene relative to *Rhodobacter* species. Physiological growth tests of RCA isolates failed to express the photosynthetic operon such that no BChl*a* containing RCA cells have been detected. Signatures of BChl*a* genes of the RCA cluster were identified in samples of the Global Ocean Sampling (GOS) data set in temperate regions complying with the known biogeographic distribution of this cluster. Expression of the photosynthetic operon of the RCA cluster in environmental samples was detected in the German Bight (North Sea) during a metatranscriptomic study. The regulation of AAnP of the RCA bacteria is still unknown, but a new specific transcriptional qPCR targeting the RCA photosynthetic genes will gain new insights into the significance of AAnP. Because RCA clones constitute the largest cluster within the *Roseobacter* clade and because of the high abundance of these organisms in several oceanic regions it is of utmost importance to understand the stimulation factors and regulation of the RCA BChl*a* expression in bacterioplankton communities.

**UNDERSTANDING THE ROLE OF GENOMIC CONTEXT ON PROTEORHODOPSIN  
FUNCTION THROUGH ANALYSIS OF THE DOKDONIA DONGHAENSIS  
PRO95 GENOME**

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Light is by definition an abundant energy source for bacterioplankton in the euphotic zone of the oligotrophic ocean. To take advantage of this source, many bacterial perform photoheterotrophic lifestyles using bacteriochlorophyll or proteorhodopsin (PR). A high percentage of whole genome sequenced PR-containing bacteria belong to the family Flavobacteriaceae. However, and despite the genomic similarities, the consequences of light exposure on the different flavo-bacteria appear to vary. A good example of this involves the responses of the two strains of *Dokdonia donghaensis* MED134 and PRO95. Previous experimental work on PRO95 showed, in contrast to MED134, no stimulation of growth or PR expression in the light. We have now sequenced and analyzed the whole genome of strain PRO95. Our aim is to compare this genome to the already annotated MED134 genome in order to understand the key features that may cause the different behaviors between these two strains.

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# Notes

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