Molecular genetic identification of planktonic bacteria in the Yenisei River basin and experimental study of their biogeochemical functions

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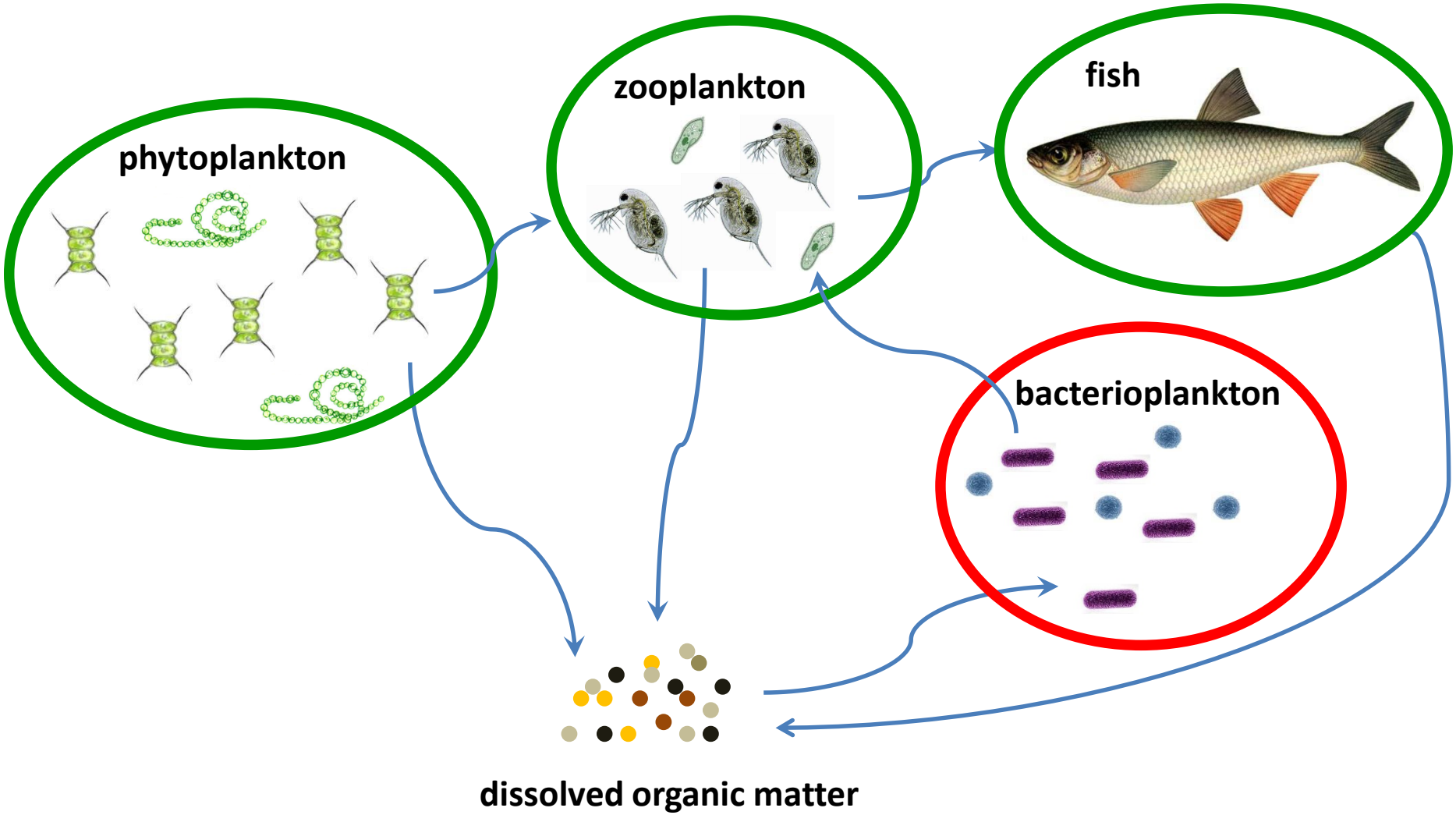
Identification of species composition

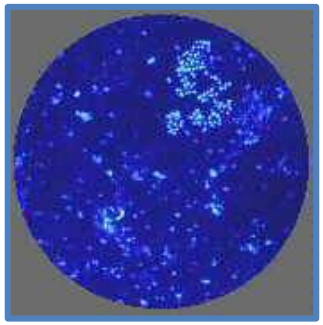
Determination of species functions



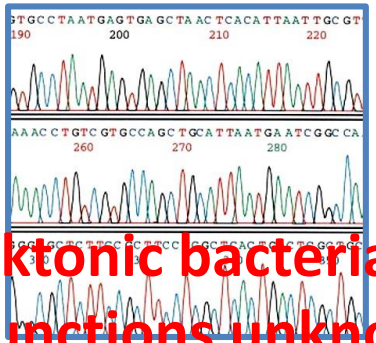
Methods of bacterioplankton species identification

Light microscopy

Selective culture media



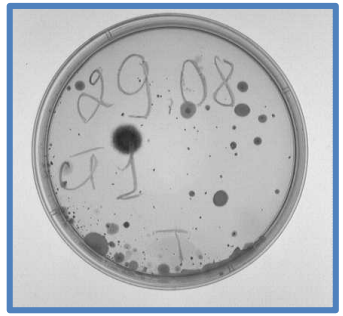
0,5-100 · 10^6 cells · mL^{-1}



>99 % planktonic bacteria unidentified

functions unknown

16S rDNA sequencing



0,125-1000 · 10^3 cells · mL^{-1}

Sequencing methods

First generation

How to determine biogeochemical functions of individual species of uncultured bacteria?

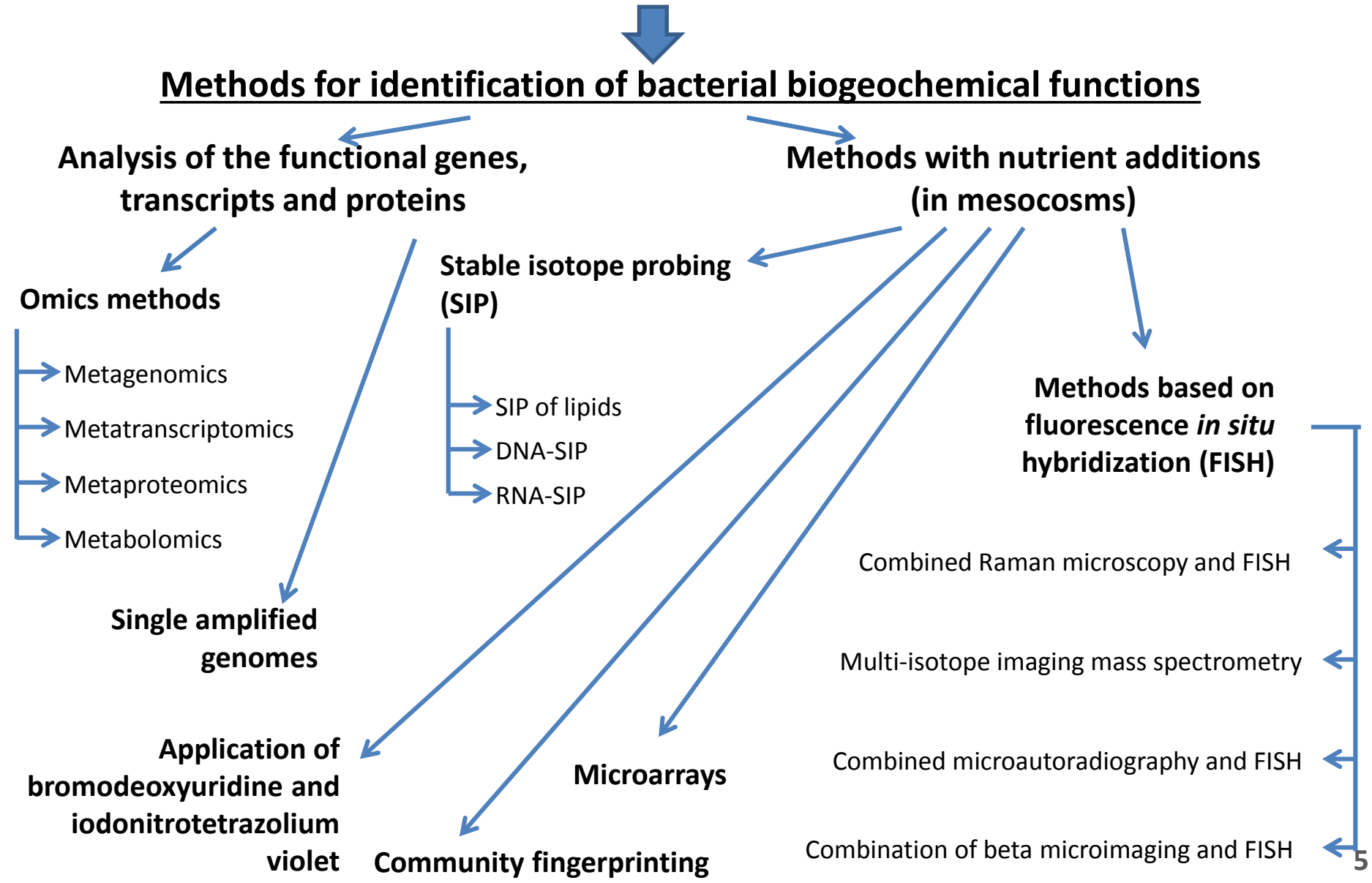
The potential is manyfold exceeded by the number of bacterial species in environmental samples

Next generation sequencing (NGS) – massive parallel sequencing

Enables identification of all bacteria in the sample

Most species (*rare biosphere*) remain unknown

Kolmakova O.V. (2013) Contemporary methods for identification of bacterioplankton species-specific biogeochemical functions. Journal of Siberian Federal University. Biology. Vol. 1. № 6. P. 73-95 (in Russian)



Cultivation of a natural pelagic community in experimental mesocosms with nutrient additions combined with molecular genetic identification of bacterial species consuming the added nutrients

water body

sampling

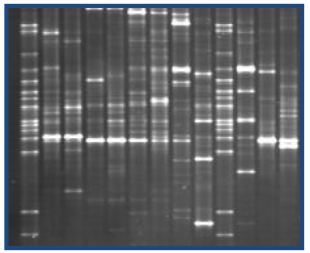
mesocosm amendment experiment

sampling

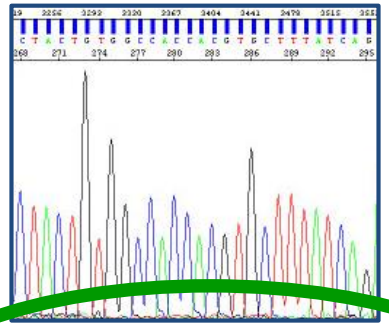
filtration

DNA extraction

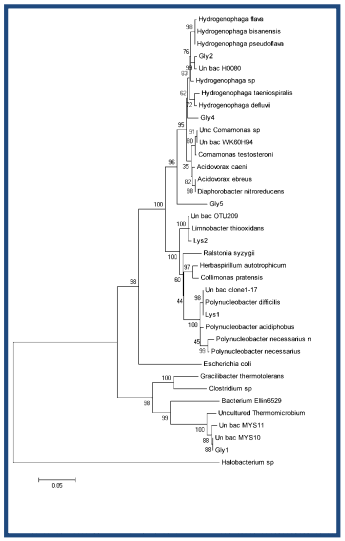
16S rDNA amplification



community fingerprinting



cloning and sequencing of the separated 16S rDNA fragments



phylogenetic analysis

Objectives

1. To study the biodiversity of bacterioplankton in the Yenisei River by next-generation sequencing

2. To identify bacteria consuming certain kinds of amino acids in a natural planktonic community

3. To study the seasonal dynamics of bacterial community response to the addition of various amino acids

Study of the Yenisei River bacterioplankton using molecular genetic techniques

Kolmakova et al. // PLoS ONE 2014

Kolmakova et al. // FEMS Microb Ecol 2014

The PARTNERS program

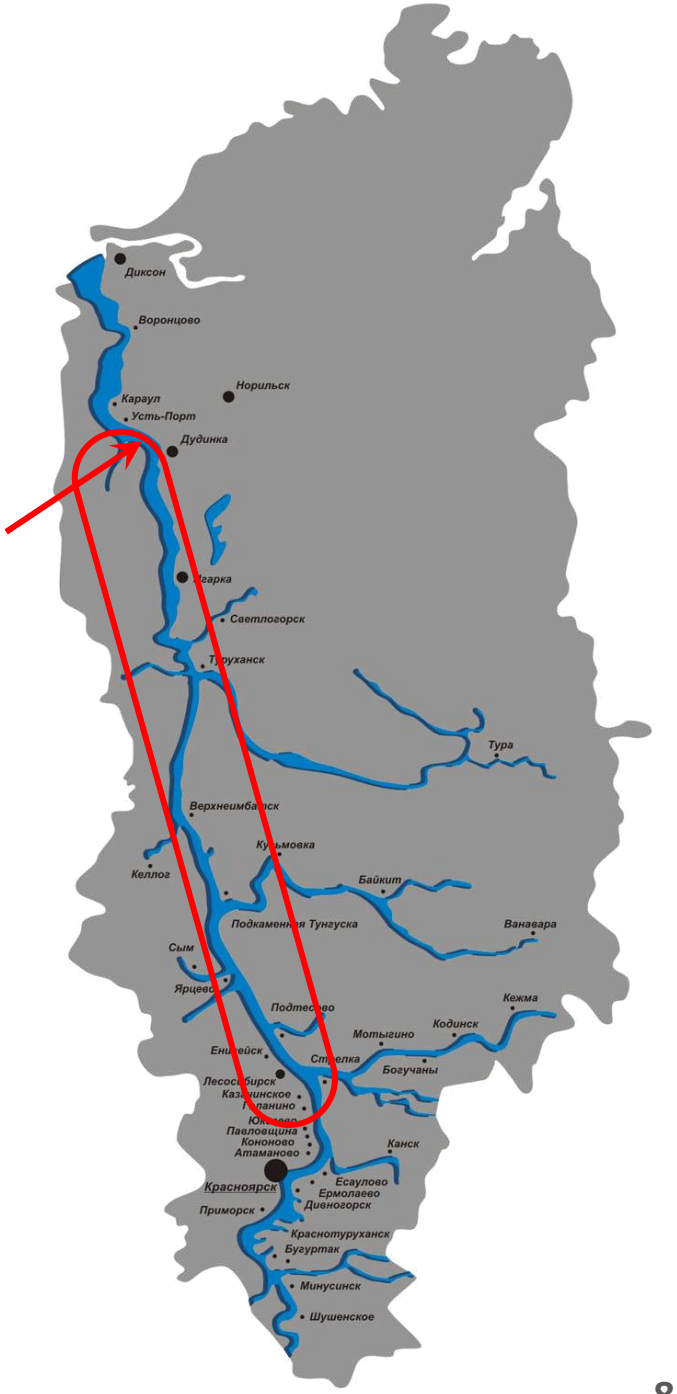
Yenisei River research cruise

11 stations, 24 June 2004

1800 km

Method: 16S rDNA clone library, Sanger sequencing

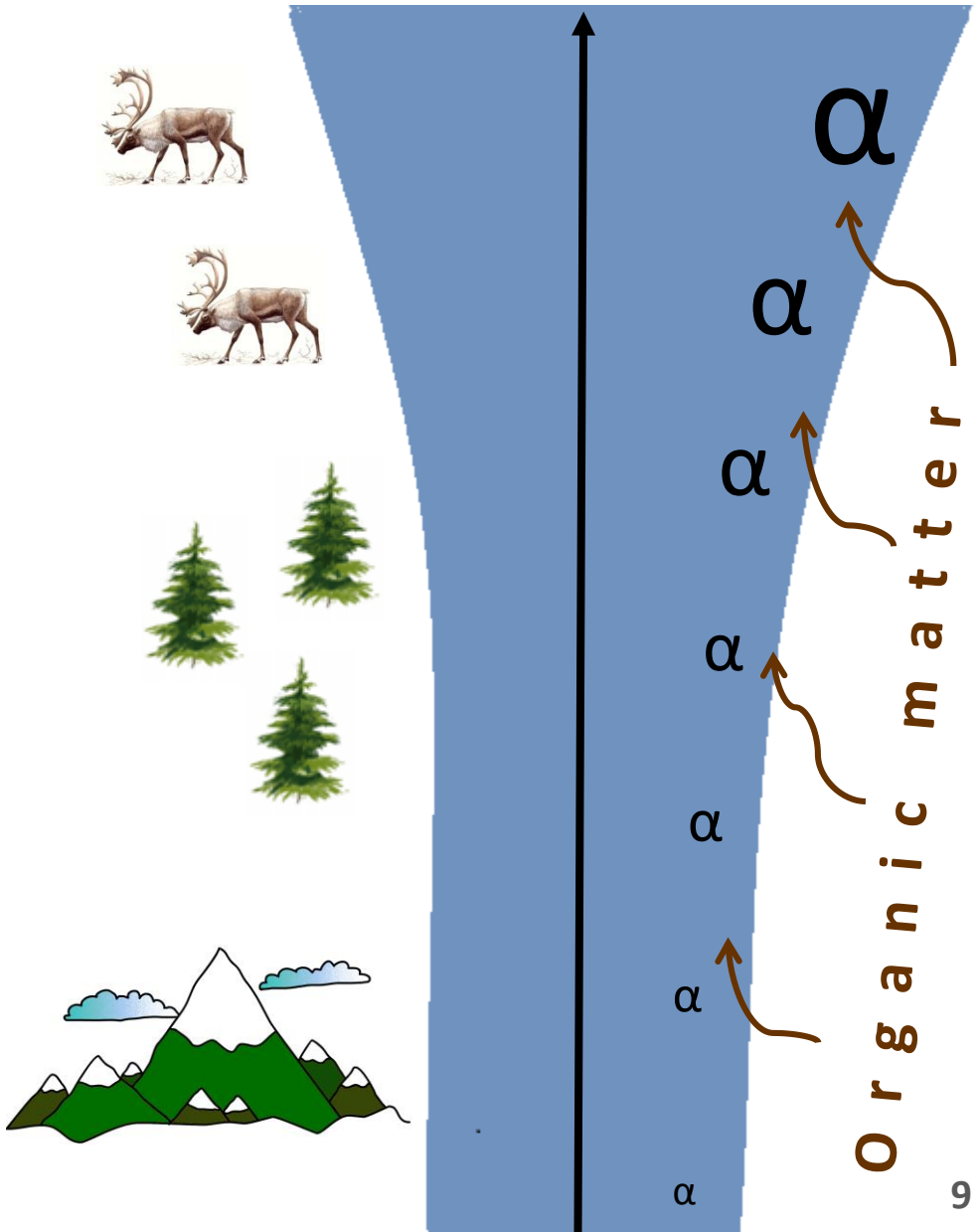
The first study of bacterial composition of a large river throughout 1800 km



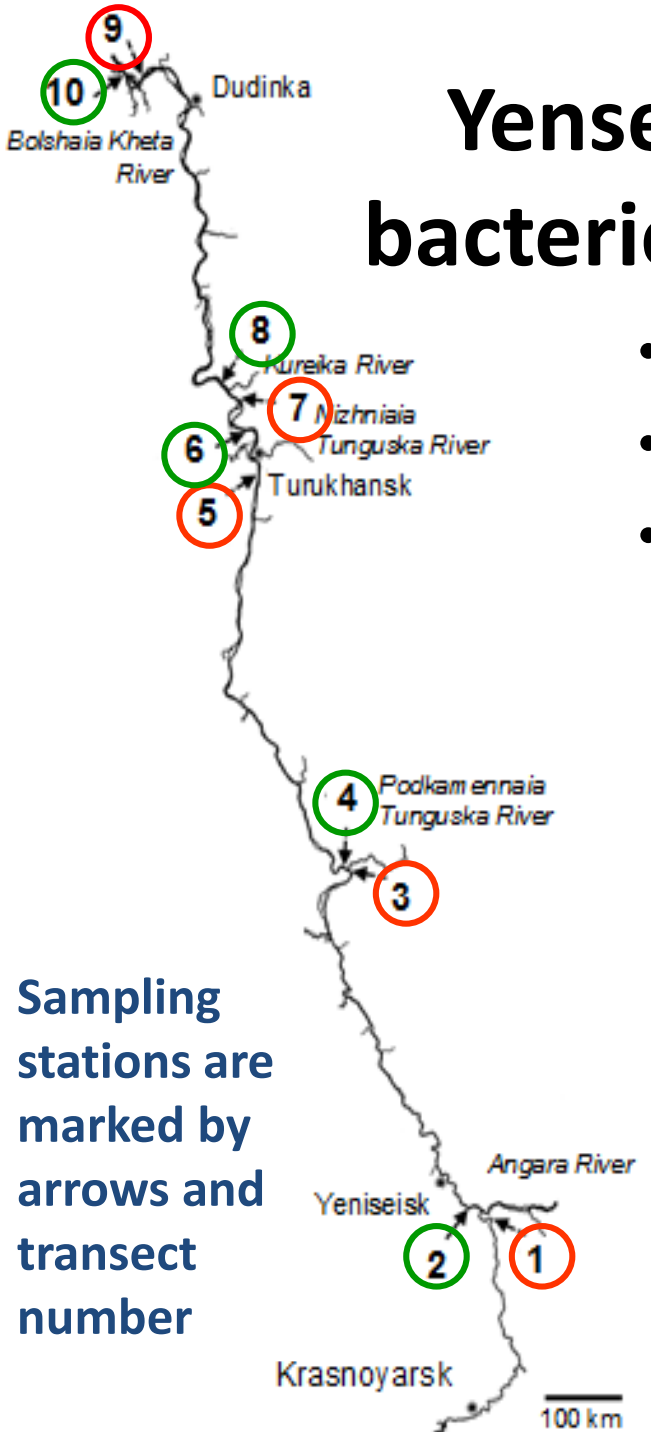
Tested hypotheses

1. Alpha diversity (diversity inside a community) of planktonic bacteria increases monotonously downstream

2. Beta diversity (diversity between communities) of planktonic bacteria in the river is shaped by the surrounding landscape (biome)



Yensei River water sampling for bacterioplankton biodiversity study



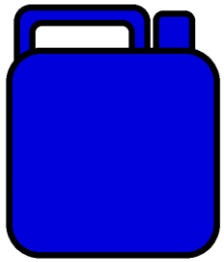
Sampling stations are marked by arrows and transect number

- 1800 km
- 12-28 June 2012
- 30 depth-integrated samples =

  10 transects × 3 sampling sites:
  - ✓ right bank
  - ✓ left bank
  - ✓ mainstream

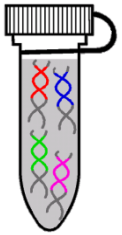
Low-pressure pump

Yenisei River bacterial community analysis by NGS

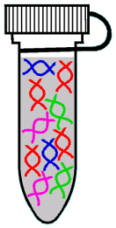


water sample

filtration



DNA extraction



16S rDNA amplification, amplicon library preparation

bioinformatic (QIIME) and statistical (STATISTICA, Community Analysis Package) analyses

>yneN
TTAATGCCTCTTCTCATTTCTTCTGCTGTCATCCGCACAGCAGAAGAATTCCTCATTGAC
TATTATTTCGCAATTTGCTCACATGGATTAAATTAAACTACATACTATAAGATATAAACT
TCTGCCTACAGCTGTAAGAAACTCCGCTCAGTACTGAAGCACCAGTCCTATTTCCTCTTT
TCTCCAGCCTGTTATATTAAGCATACTGATTAACGATTTTTAACGTTATCCGCTAAATAA
ACATATTTGAAATGCATGCGACCACAGTGAAAAACAAAATCACGCAAAGAGACAACTATA
A
>yegR
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G
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T
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ATACTTCTTGCGATAATAACTACAACTGTAAGATAACCCTTTCAAAATGACCGTTGCTCT
CTGATTTCTCATTTCATGCTCACCCAATATGATGGCGGCGTTTTCTAAAACTGTTAAAGA
ATGAGGTAAGTATGAAACGTTTAATTATGGCCACGATGGTCACAGCAATTCTGGCATCTT
C

nucleotide sequences of 16S rDNA

MiSeq (Illumina) sequencing

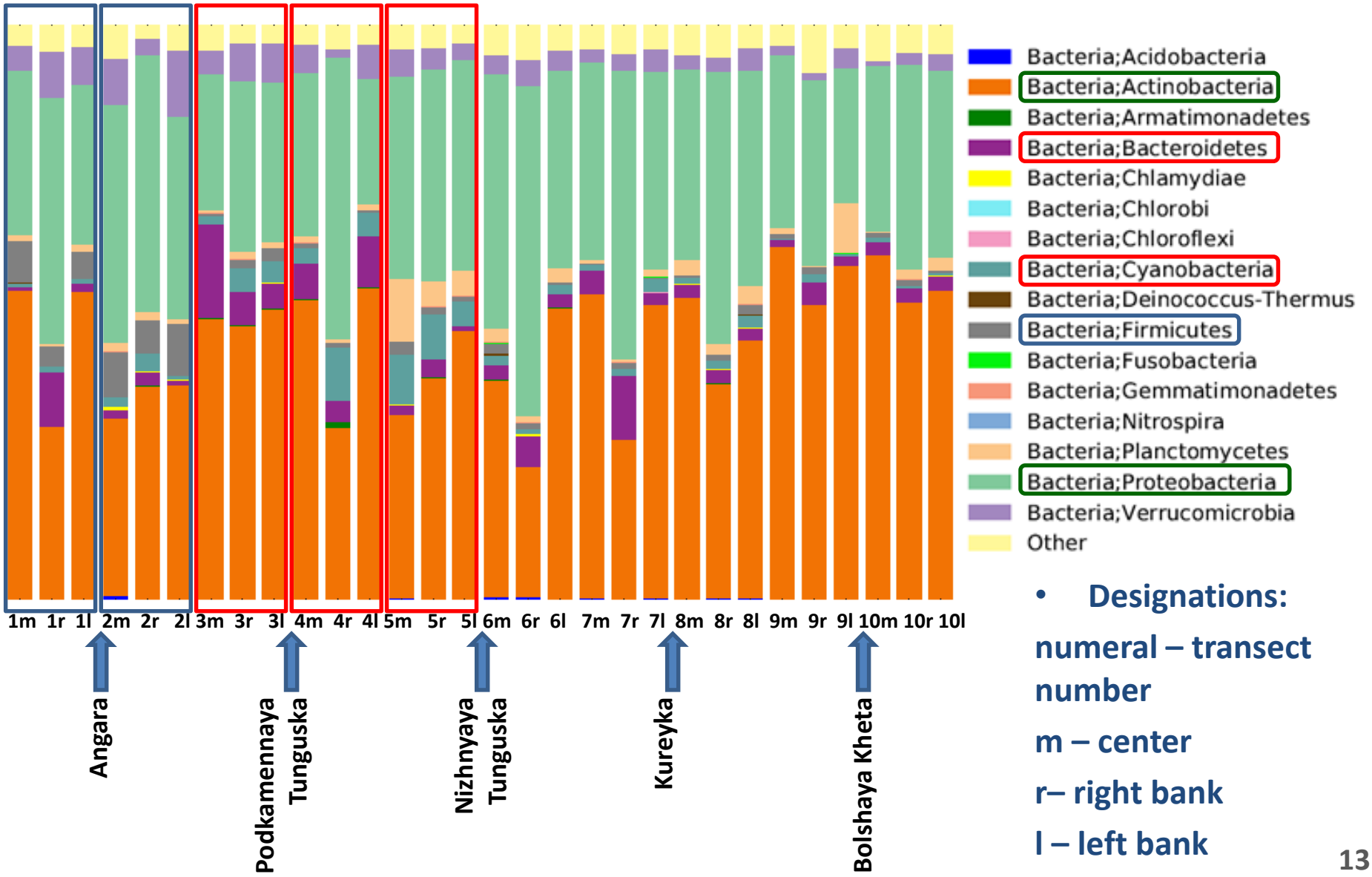
Major groups of the Yenisei River water bacteria

No	Phyla	OTU
1	Proteobacteria	914
2	Actinobacteria	837
3	Unknown bacteria	552
4	Bacteroidetes	200
5	Verrucomicrobia	162
6	Firmicutes	60
7	Planctomycetes	58
8	TM7 genera incertae sedis	58
9	Cyanobacteria	55
10	Acidobacteria	37
11	Chlamydiae	32

No	Phyla	OTU
12	OD1 genera incertae sedis	13
13	Armatimonadetes	9
14	Chloroflexi	7
15	Gemmatimonadetes	7
16	SR1 genera incertae sedis	5
17	Deinococcus-Thermus	4
18	Fusobacteria	4
19	OP11 genera incertae sedis	4
20	Euryarchaeota	2
21	Chlorobi	1
22	Nitrospirae	1
	Total	**3022**

240500 nucleotide sequences are deposited in The Sequence Read Archive
http://www.ncbi.nlm.nih.gov/sra
Accession number SRP036054

Relative abundance of bacterial phyla in the Yenisei River, June 2012



- **Designations:**

numeral – transect number

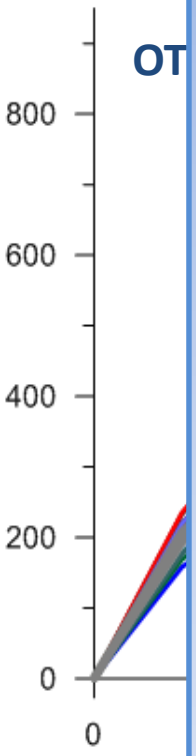
m – center

r– right bank

l – left bank

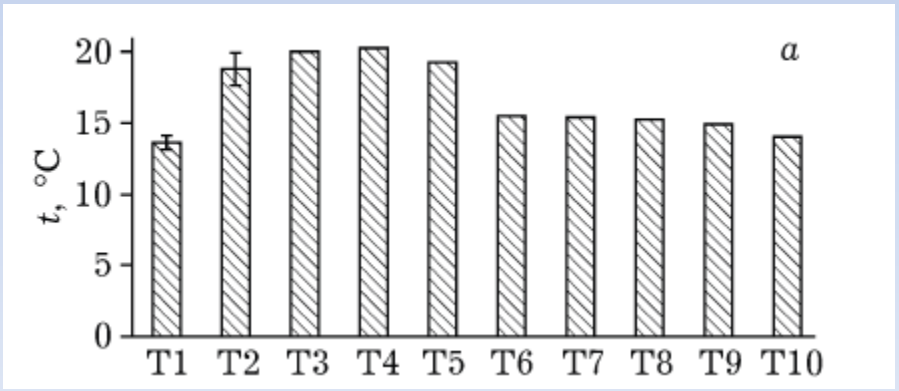
Alpha-diversity of bacterial comm

Sample	Number of reads	Number of OTUs	Shannon index
1m	7780	776	7,18
			7,55
			7,43
			7,82
			7,51
			6,89
			7,91
			7,92
			7,92
			8,04
			7,07
			7,93
			7,48
			7,66
			7,66
			8,25
			8,17
			7,89
			7,65
			7,85
			7,87
			7,65
			7,86
			7,91
			7,44
			7,16
			7,66
10m	7321	822	7,46
10r	8141	909	7,57
10l	7578	896	7,68



~~Alpha-diversity of bacterial communities increases downstream the Yenisei River~~

- Temperature influence?



Rare
composition

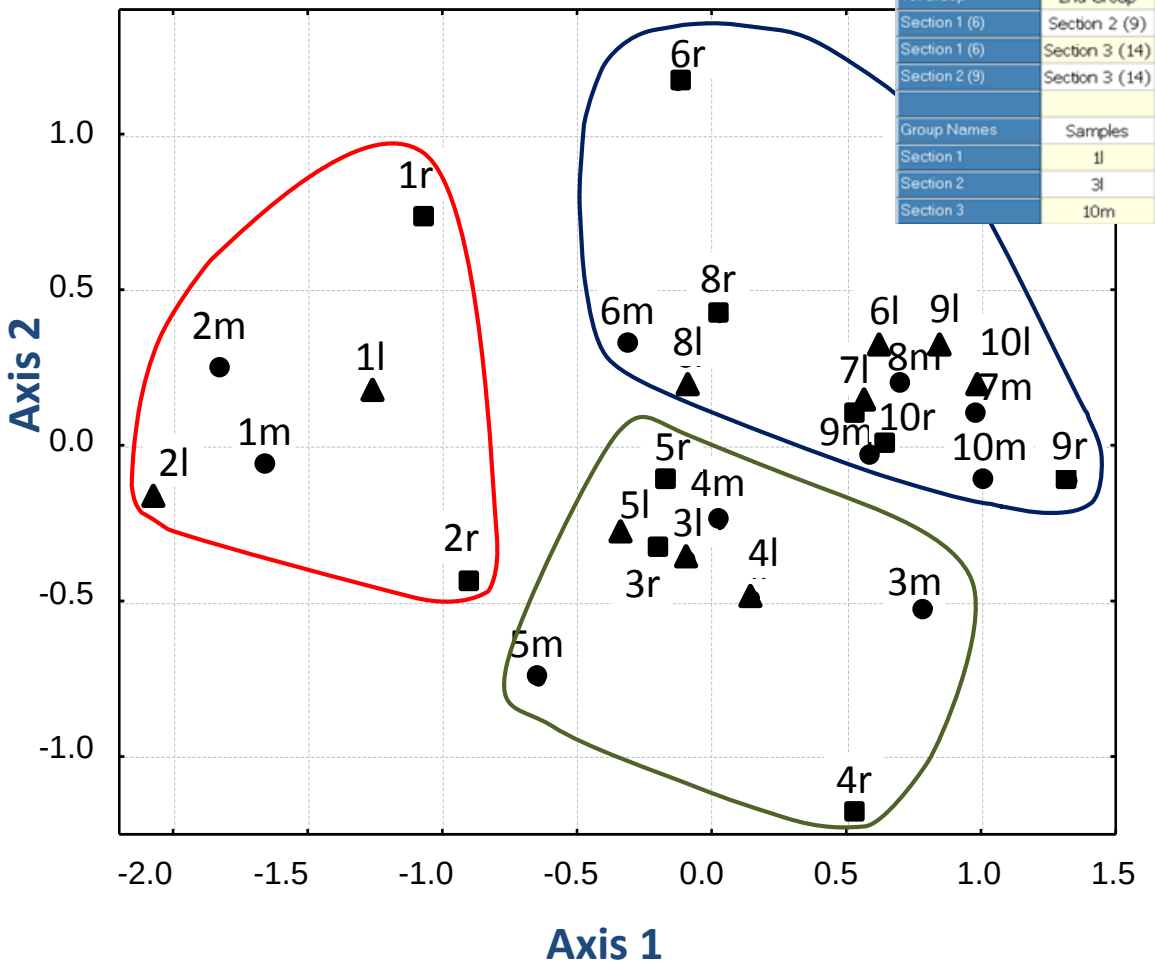
Bray-Curtis similarity coefficients of bacterial beta diversity

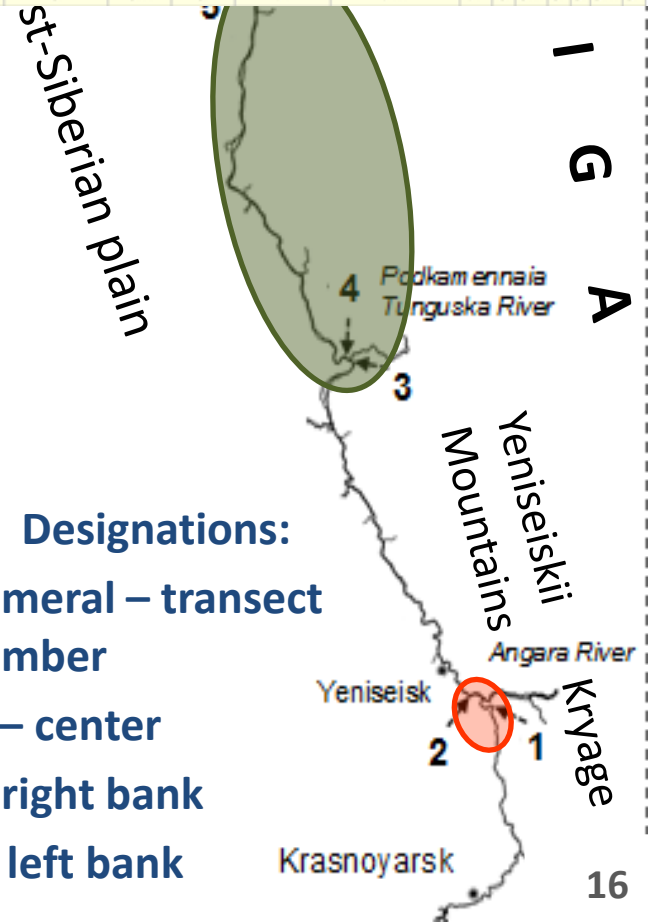
15	1m	1r	1l	2m	2r	2l	3m	3r	3l	4m	4r	4l	5m	5r	5l	6m	6r	6l	7m	7r	7l	8m	8r	8l	9m	9r	9l	10m	10r
1r	0.46	1.00																											
1l	0.70	0.56	1.00																										
2m	0.47	0.40	0.44	1.00																									
2r	0.42	0.40	0.45	0.53	1.00																								
2l	0.51	0.42	0.49	0.58	0.47	1.00																							
3m	0.24	0.40	0.33	0.21	0.34	0.20	1.00																						
3r	0.39	0.44	0.47	0.41	0.55	0.38	0.53	1.00																					
3l	0.40	0.44	0.47	0.36	0.51	0.35	0.55	0.69	1.00																				
4m	0.38	0.43	0.47	0.35	0.48	0.33	0.59	0.71	0.69	1.00																			
4r	0.23	0.33	0.28	0.24	0.36	0.18	0.42	0.44	0.47	0.44	1.00																		
4l	0.33	0.40	0.42	0.32	0.43	0.30	0.63	0.65	0.67	0.70	0.45	1.00																	
5m	0.36	0.32	0.37	0.41	0.47	0.34	0.31	0.53	0.46	0.50	0.30	0.44	1.00																
5r	0.36	0.38	0.41	0.36	0.49	0.31	0.44	0.60	0.56	0.60	0.44	0.54	0.64	1.00															
5l	0.33	0.31	0.37	0.39	0.55	0.35	0.40	0.57	0.54	0.56	0.36	0.51	0.55	0.59	1.00														
6m	0.41	0.39	0.41	0.44	0.49	0.34	0.38	0.53	0.52	0.52	0.38	0.46	0.54	0.59	0.55	1.00													
6r	0.31	0.34	0.32	0.40	0.40	0.27	0.32	0.38	0.40	0.36	0.46	0.32	0.33	0.42	0.36	0.55	1.00												
6l	0.27	0.33	0.32	0.22	0.35	0.19	0.50	0.49	0.50	0.54	0.42	0.50	0.39	0.50	0.51	0.48	0.39	1.00											
7m	0.23	0.33	0.29	0.21	0.31	0.17	0.55	0.45	0.47	0.48	0.43	0.49	0.30	0.43	0.39	0.43	0.40	0.60	1.00										
7r	0.22	0.34	0.26	0.23	0.31	0.18	0.51	0.40	0.42	0.42	0.49	0.41	0.29	0.41	0.35	0.45	0.52	0.51	0.55	1.00									
7l	0.28	0.35	0.33	0.25	0.36	0.22	0.54	0.51	0.51	0.54	0.39	0.53	0.41	0.53	0.52	0.50	0.39	0.65	0.65	0.52	1.00								
8m	0.25	0.32	0.31	0.22	0.35	0.18	0.53	0.48	0.50	0.52	0.47	0.50	0.37	0.50	0.45	0.51	0.44	0.67	0.69	0.55	0.66	1.00							
8r	0.36	0.36	0.39	0.36	0.45	0.29	0.41	0.52	0.52	0.51	0.45	0.47	0.47	0.56	0.53	0.61	0.56	0.55	0.49	0.51	0.55	0.58	1.00						
8l	0.36	0.36	0.41	0.38	0.44	0.31	0.41	0.54	0.51	0.56	0.33	0.50	0.55	0.59	0.59	0.59	0.42	0.53	0.45	0.43	0.59	0.50	0.62	1.00					
9m	0.33	0.35	0.40	0.25	0.37	0.24	0.53	0.49	0.49	0.54	0.37	0.51	0.41	0.52	0.49	0.47	0.36	0.58	0.61	0.46	0.65	0.62	0.53	0.56	1.00				
9r	0.21	0.31	0.28	0.21	0.29	0.21	0.50	0.38	0.38	0.42	0.33	0.44	0.28	0.39	0.34	0.35	0.32	0.43	0.61	0.45	0.53	0.52	0.39	0.40	0.59	1.00			
9l	0.27	0.27	0.33	0.18	0.31	0.17	0.48	0.44	0.45	0.50	0.32	0.48	0.38	0.46	0.48	0.44	0.31	0.65	0.58	0.43	0.63	0.63	0.49	0.52	0.62	0.44	1.00		
10m	0.25	0.34	0.32	0.23	0.32	0.21	0.53	0.43	0.44	0.47	0.36	0.48	0.33	0.44	0.40	0.40	0.34	0.50	0.61	0.47	0.58	0.58	0.46	0.46	0.68	0.70	0.52	1.00	
10r	0.28	0.34	0.35	0.27	0.38	0.25	0.52	0.50	0.48	0.53	0.33	0.50	0.43	0.53	0.50	0.47	0.36	0.56	0.58	0.49	0.64	0.60	0.54	0.58	0.71	0.60	0.61	0.67	1.00
10l	0.23	0.29	0.28	0.17	0.31	0.14	0.51	0.43	0.46	0.48	0.42	0.48	0.30	0.42	0.42	0.41	0.36	0.64	0.66	0.51	0.64	0.67	0.48	0.45	0.62	0.52	0.65	0.60	0.61

Multidimensional sca
similarity coefficients

Results - ANOSIM

Full Data ANOSIM																			
Sample Statistic (r)	0,583522																		
P Value	0,001																		
P as %	0,1																		
No Randomizations	1000																		
No >= Obs	1																		
Pairwise Tests																			
1st Group	2nd Group	Permutations	Perms. done	P Value	P as %	No >= Obs	Sample Stat. (r)												
Section 1 (6)	Section 2 (9)	5005	1000	0,001	0,1	1	0,729121												
Section 1 (6)	Section 3 (14)	38760	1000	0,001	0,1	1	0,938005												
Section 2 (9)	Section 3 (14)	817190	1000	0,001	0,1	1	0,39745												
Group Names	Samples																		
Section 1	1l	1m	1r	2l	2m	2r	5l	5m	5r										
Section 2	3l	3m	3r	4l	4m	4r													
Section 3	10m	10r	6l	6m	6r	7l	7m	7r	8l	8m	8r	9l	9m	9r					





- **Designations:**

numeral – transect number

m – center

r– right bank

l – left bank

Three distinct river sections with different OTU composition

Mean relative abundance of the most numerous OTUs at three sections of the Yenisei River

OTU	Class	Order	Family	Genus	Section I transects 1-2	Section II transects 3-5	Section III transects 6-10
1297	Actinobacteria		imicrobiaceae	Ilumatobacter	5.80±1.49	3.99±0.59	4.57±0.48
2	α-Proteobacteria				4.25±1.18	3.87±0.76	1.62±0.32
2179	Actinobacteria				0.34±0.16	2.69±0.46	5.42±0.73
18	Sphingobacteria	Sphingobacteriales	Chitinophagaceae				0.27
13	α-Proteobacteria	Rhizobiales	Rhizobiaceae				0.20
8	Actinobacteria	Actinomycetales	Microbacteriaceae				0.08
10			Comamonadaceae				0.29
15			Rhodobacteraceae				0.04
25			Micrococcaceae				0.09
21			Family II				0.07
2588					0.59±0.33	1.60±0.37	2.23±0.27
671			Acidimicrobiaceae	Ilumatobacter	0.18±0.09	1.59±0.32	2.32±0.25
4							2
6	α-Prote		ystaceae	Methylocysti			1
47	Actinob		teriaceae				5
12	Actinob						1
11	β-Proteobacteria	Burkholderiales	Alcaligenaceae		1.29±0.09	2.27±0.51	1.85±0.24
16	α-Proteobacteria	Sphingomonadales	Sphingomonadaceae		1.79±0.73	0.68±0.13	0.33±0.05
5	β-Proteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter	1.76±0.23	2.01±0.51	3.53±0.52
17	γ-Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.10±0.04	0.65±0.36	2.19±0.50

Plant root nodule bacteria

Abundant in waters with a high contribution of allochthonously derived dissolved organic matter

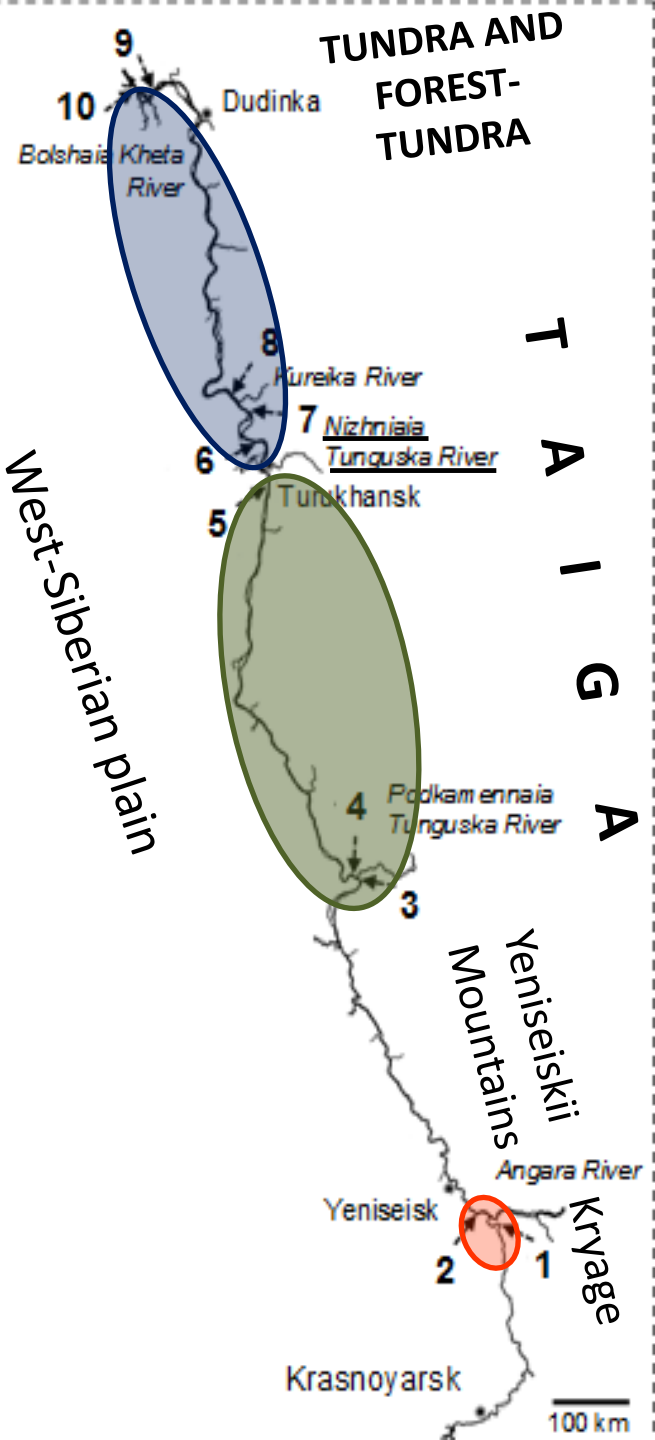
Increase abundance in river biofilms after an addition of pesticides

Copiotrophic river bacteria

Degrade aromatic pollutants

Beta diversity of bacterial communities in the river is shaped by the surrounding landscape

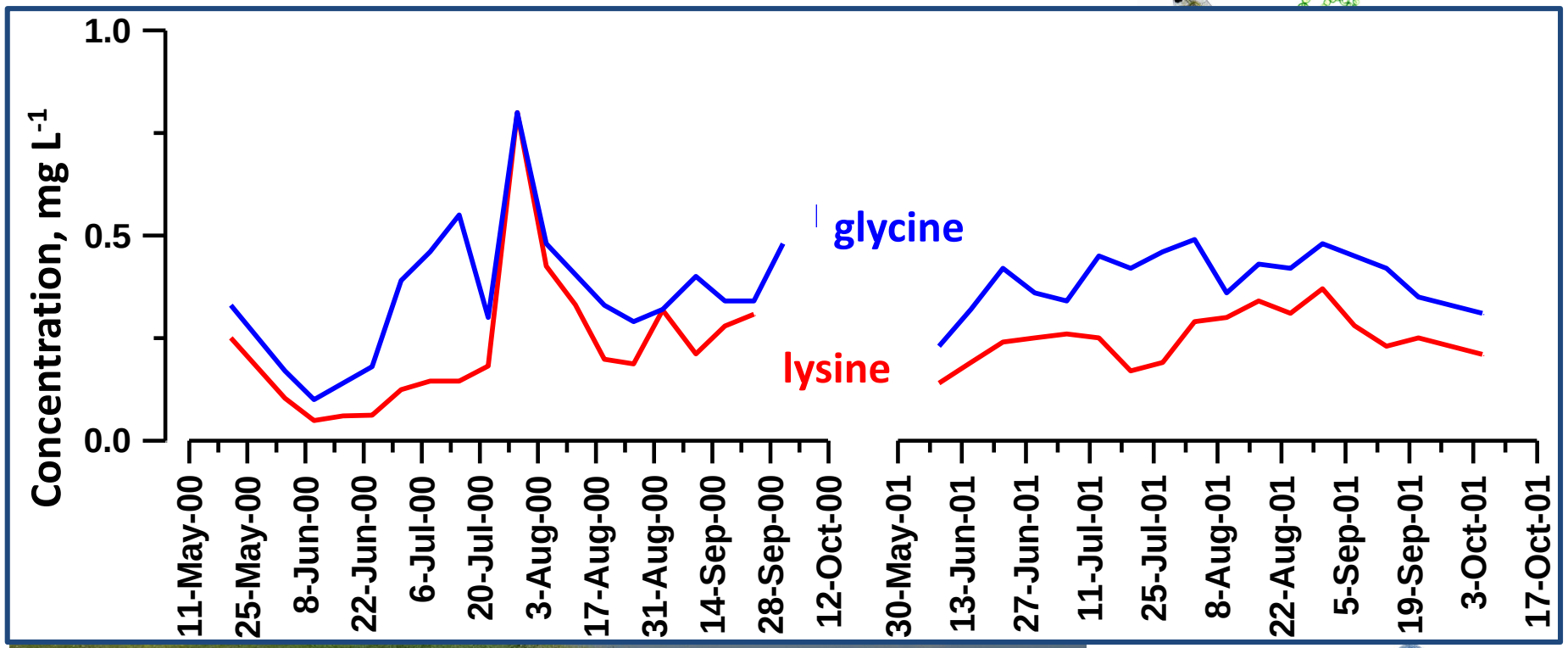
One of the mechanisms by which the landscape might influence the bacterial diversity in the Yenisei River is via dispersal of different bacterial communities by tributaries arising from the different types of landscape



The next step should be linking biodiversity of bacteria and their biogeochemical functions

There are no generally accepted methods for determination of biogeochemical functions of individual bacterial species in environmental communities

Experimental study of biogeochemical functions of water bacteria

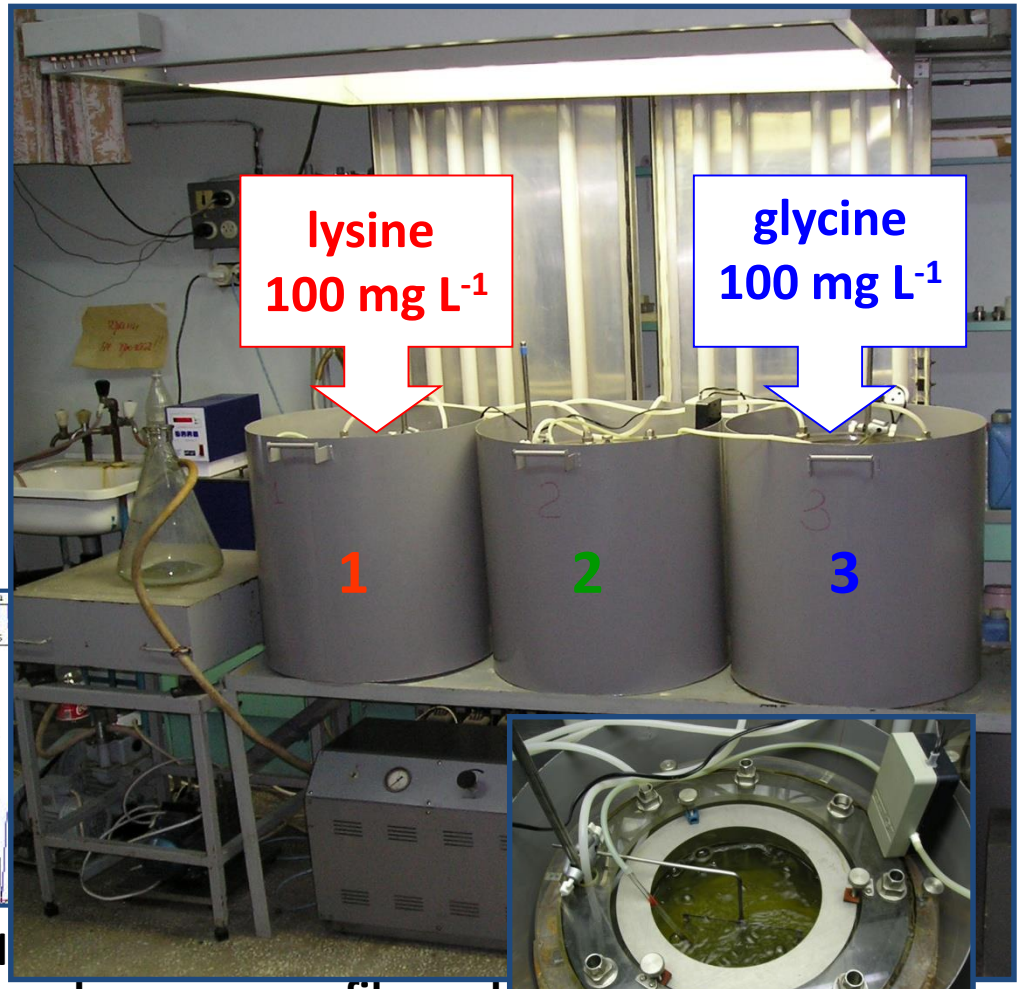


Glycine and lysine concentration in water of Bugach reservoir in 2000-2001
(Kalachova et al // Aquat Ecol 2004)

Experimental mesocosms and subsequent molecular genetic analysis of bacterial population dynamics

reservoir

reservoir sampling

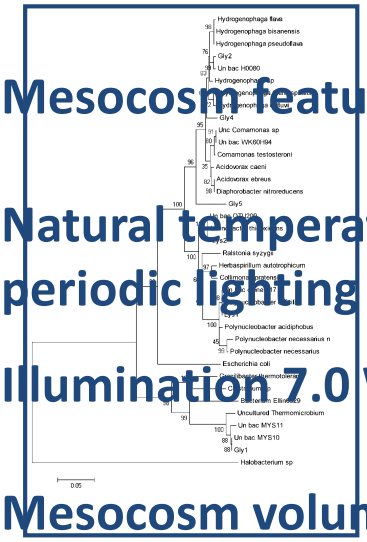


Mesocosm features:

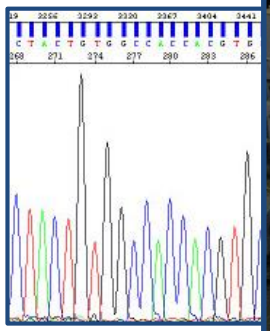
Natural temperature and periodic lighting mode

Illumination 7.0 W m^{-2}

Mesocosm volume – 10 L



phylogenetic analysis

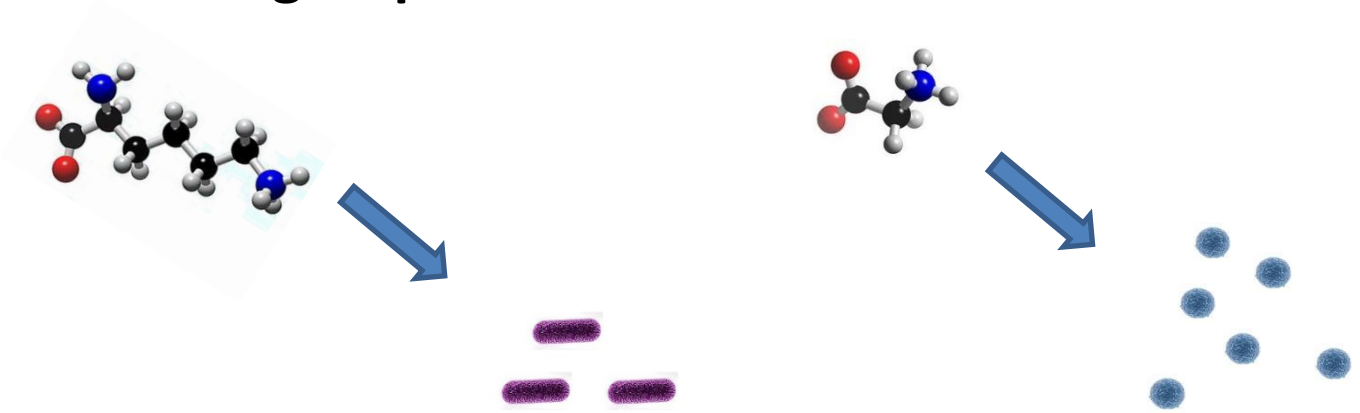


cloning and seq
selected bands

profile anal

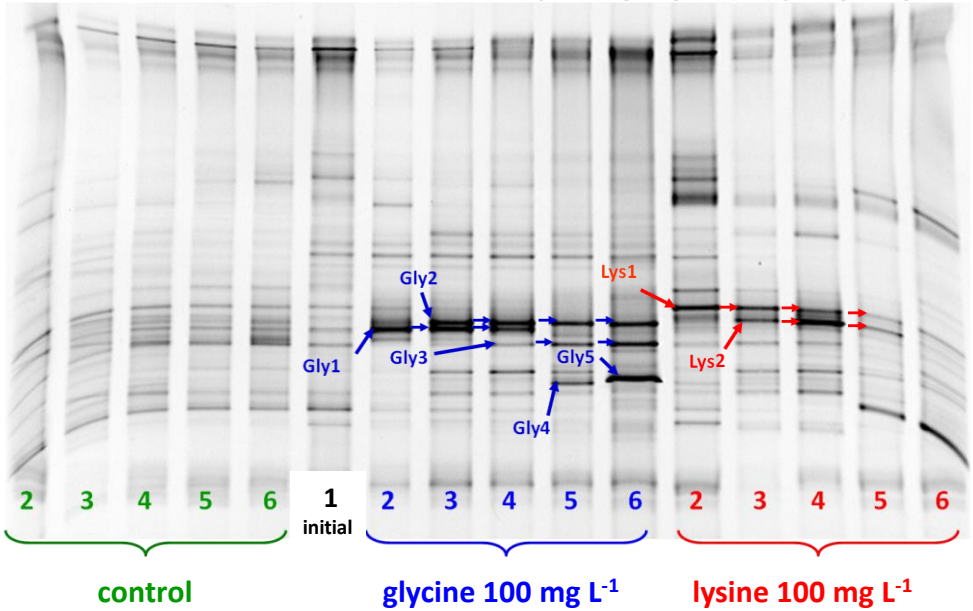
Possible experiment scenarios:

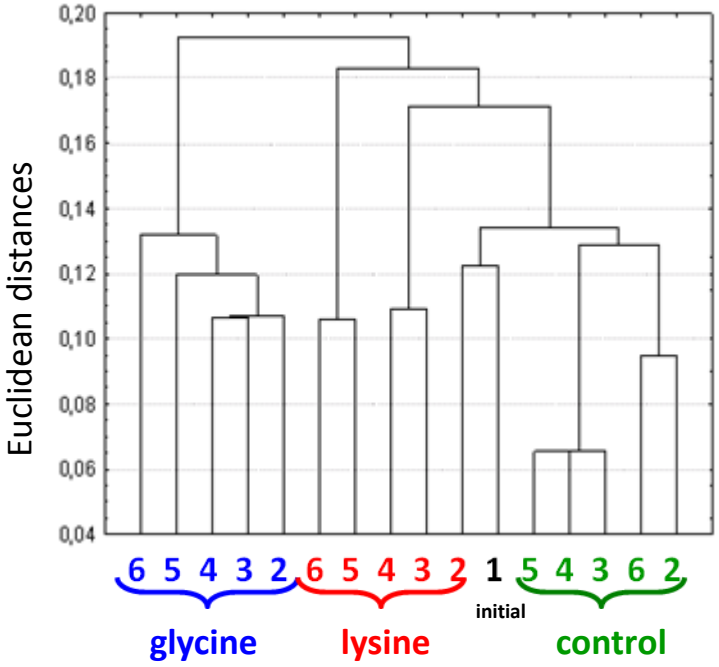
- **1) different bacterial species respond to the addition of different amino acids: lysine – one group of species; glycine – another group**

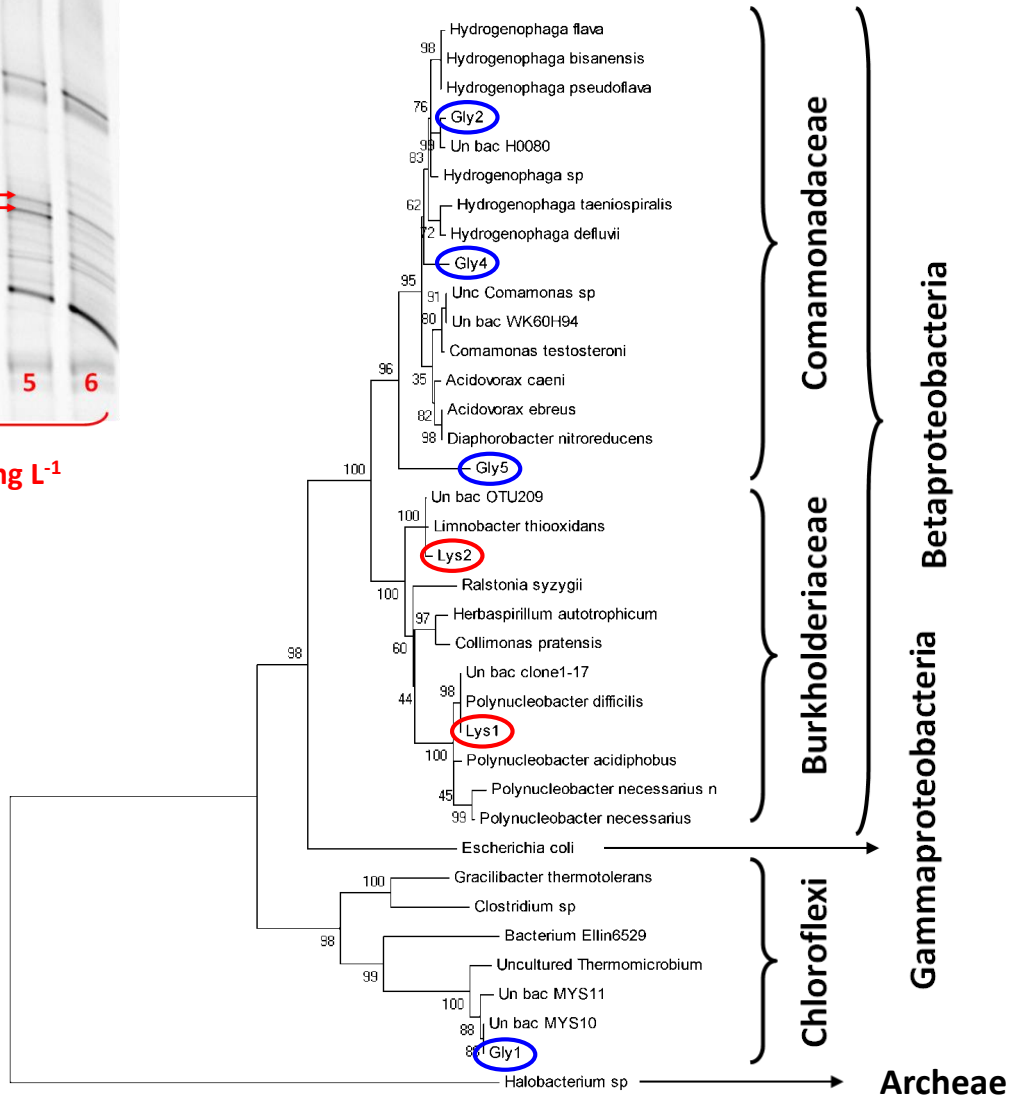


- **2) the same bacterial species will respond to amino acids additions**

Glycine and lysine additions caused the response of species belonging to different taxonomic groups in mesocosm experiment I (30 June – 6 July 2004)







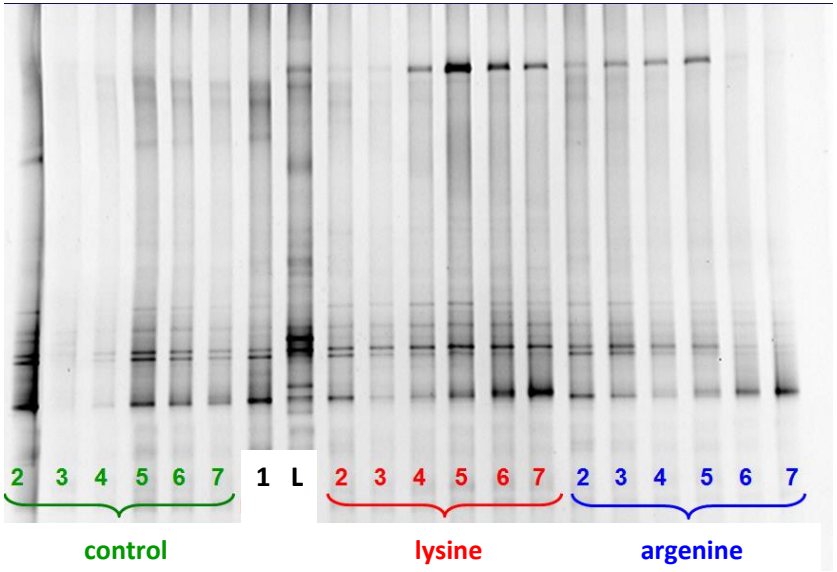
Certain species of uncultured water bacteria were specialized in consumption of certain amino acids in July 2004

Will amino acid addition provoke the same response of bacterial community in other seasons and years?

List of mesocosm experiments with amino acid additions

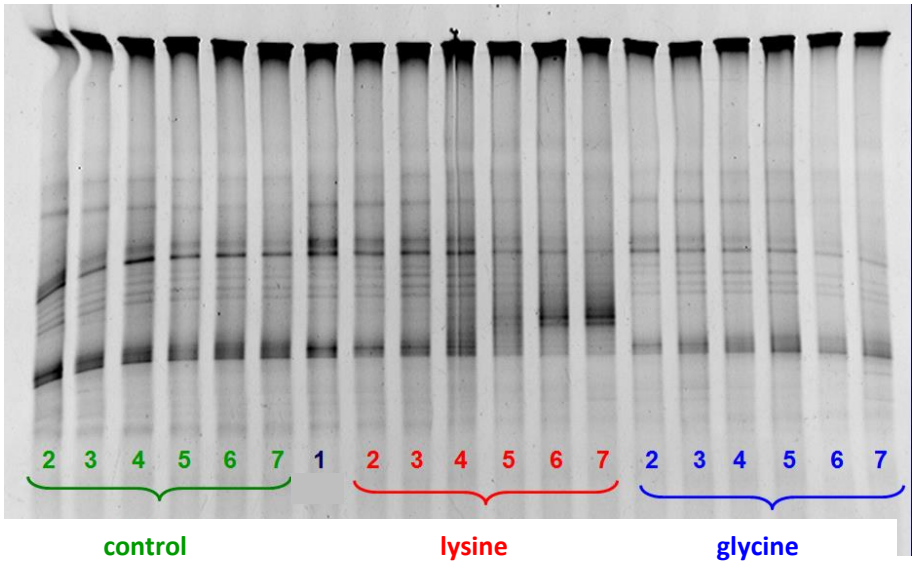
№	Experiment dates	Additions		
		Mesocosm 1	Mesocosm 2	Mesocosm 3
I	30 June – 6 July 2004	lysine 100 mg L^{-1}	control	glycine 100 mg L^{-1}
II	17 – 23 August 2005	lysine 100 mg L^{-1}	control	arginine 100 mg L^{-1}
III	17 – 23 May 2006	lysine 100 mg L^{-1}	control	glycine 100 mg L^{-1}
IV	31 July – 5 August 2009	lysine 1 mg L^{-1}	lysine 100 mg L^{-1}	lysine 10 mg L^{-1}
V	14 – 20 May 2013	lysine 1 mg L^{-1}	control	lysine 5 mg L^{-1}

DGGE of 16S rDNA amplified fragments of bacterioplankton samples from bacterial samples in experiments II, III and V

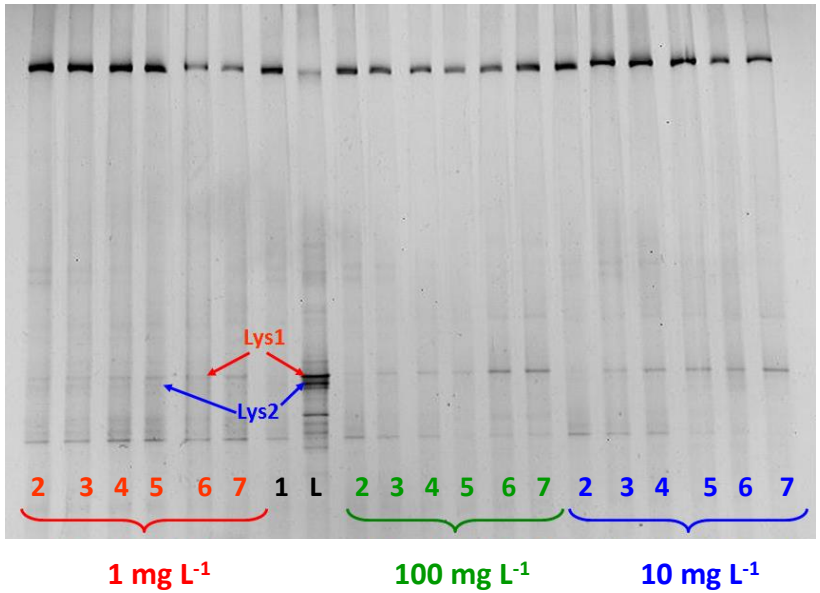


Experiment II
17 – 23 August 2005

No significant changes in bacterial community profiles in the experimental mesocosms compared to the initial sample and to the control mesocosm



Experiment III
17 – 23 May 2006



Experiment V
14 – 20 May 2009

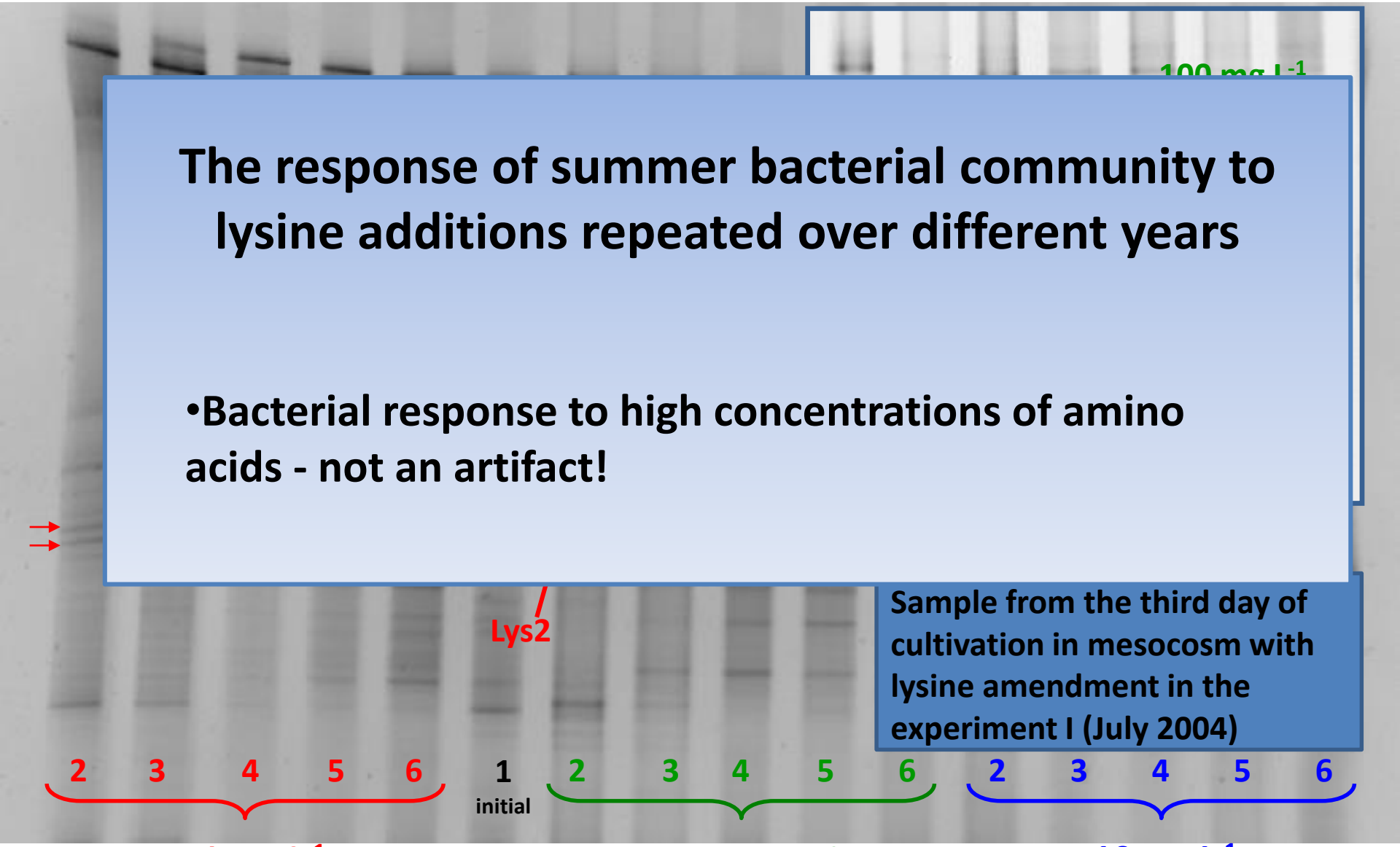
Sampling date	Water temperature, °C	Shannon index(H)
Experiment I (July 2004)		
30.06		
01.07		
02.07		
03.07		
04.07		
05.07		
SD		
Exper		
17.08		
18.08		
19.08		
20.08		
21.08		
22.08.05	20.9	4.29
23.08.05	20.5	4.21
SD	–	0.143

Sampling date	Water temperature, °C	Shannon index (H)
Experiment III (May 2006)		
		4.35
		4.3
		4.23
		4.37
		4.38
		4.11
		4.07
		0.126
		4.08
		3.87
		3.53
		3.89
04.08.09	22.7	3.61
05.08.09	22.7	4.08
SD	–	0.231

In July and early August, the bacterial community response on lysine amendments was more pronounced than in May and at the end of August

- **Low water temperature?**
- **Other adverse environmental factors?**

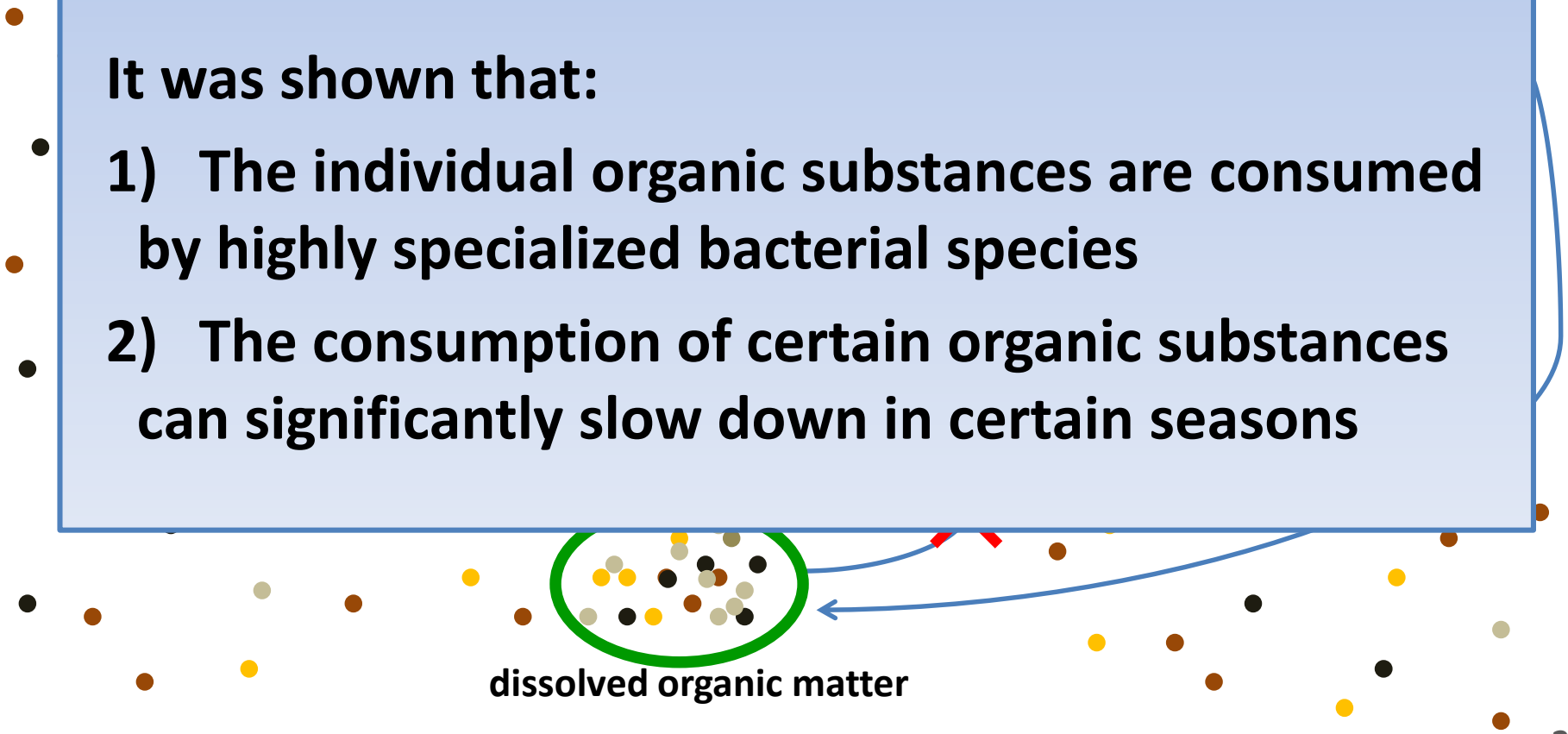
DGGE of 16S rDNA amplified fragments of bacterioplankton samples from bacterial samples in experiment IV (31 July – 5 August 2009)



Understanding the mechanisms and patterns of self-purification is of great theoretical and practical importance

It was shown that:

1) **The individual organic substances are consumed by highly specialized bacterial species**
2) **The consumption of certain organic substances can significantly slow down in certain seasons**



Conclusions

1. 3022 operational taxonomic units of bacteria were found in water of the Yenisei River. Alpha diversity of bacterioplankton reached maximum values in the middle section of the river

2. Three bacterioplankton assemblages significantly differing in species composition and structure were detected in the Yenisei River. These assemblages were probably a result of biogeochemical influence of the surrounding landscape: mountain taiga (the upper part of the river), the plain taiga (middle section) and forest-tundra and tundra (the lower section). Dominant taxa of each assemblage specialized in the consumption of various groups of organic substances

Conclusions

3. Bacteria consuming lysine and glycine were identified in the natural bacterial community in mesocosm experiments. These bacteria specialized in consuming certain amino acids

4. In mid-summer, the response of the reservoir bacterial community to lysine additions was stable and repeated in different years. In contrast, lysine additions did not cause significant changes in the quantitative and qualitative composition of the bacterial community in spring and late summer. The capability of aquatic ecosystems for self-purification of certain organic substances may vary considerably throughout a year

Thank you for your attention!

NGS studies of river bacterioplankton

- Ghai et al // PLoS ONE 2011 – Amazon, 1 sample site, metagenome
- Fortunato et al // ISME J 2012 – Columbia estuary, a few samples over 2 years
- Schultz et al // Aquat Microb Ecol 2013 – a small section of Ohio River and an tributary, 7 sample sites
- Staley et al // J Appl Microbiol 2013 – upper Mississippi and 2 tributaries, 400 km, 10 stations

- Read et al. // ISME J 2015 – 9948 km^2 Thames basin, 23 sites
- Savio et al. // Environ Microbiol 2015 (in print) – Danube and tributaries, 2600 km river continuum, 96 sites

Loss of bacterial biodiversity associated with larger organisms

- 100 µm – some of microplankton (large algae and protozoa, copepod nauplii)
- 50 µm – most of microplankton (all rotifers)
- 20 µm – all microplankton (most phytoplankton and ciliates)
- 10 µm – most small flagellates, diatoms and other phytoplankton
- 5 µm – most of nanoplankton, leaving just the free-living bacteria
- 0.2 µm – all picoplankton will be removed, including most bacterial cells. Just femtoplankton will remain, that is viruses and ultramicrobacteria