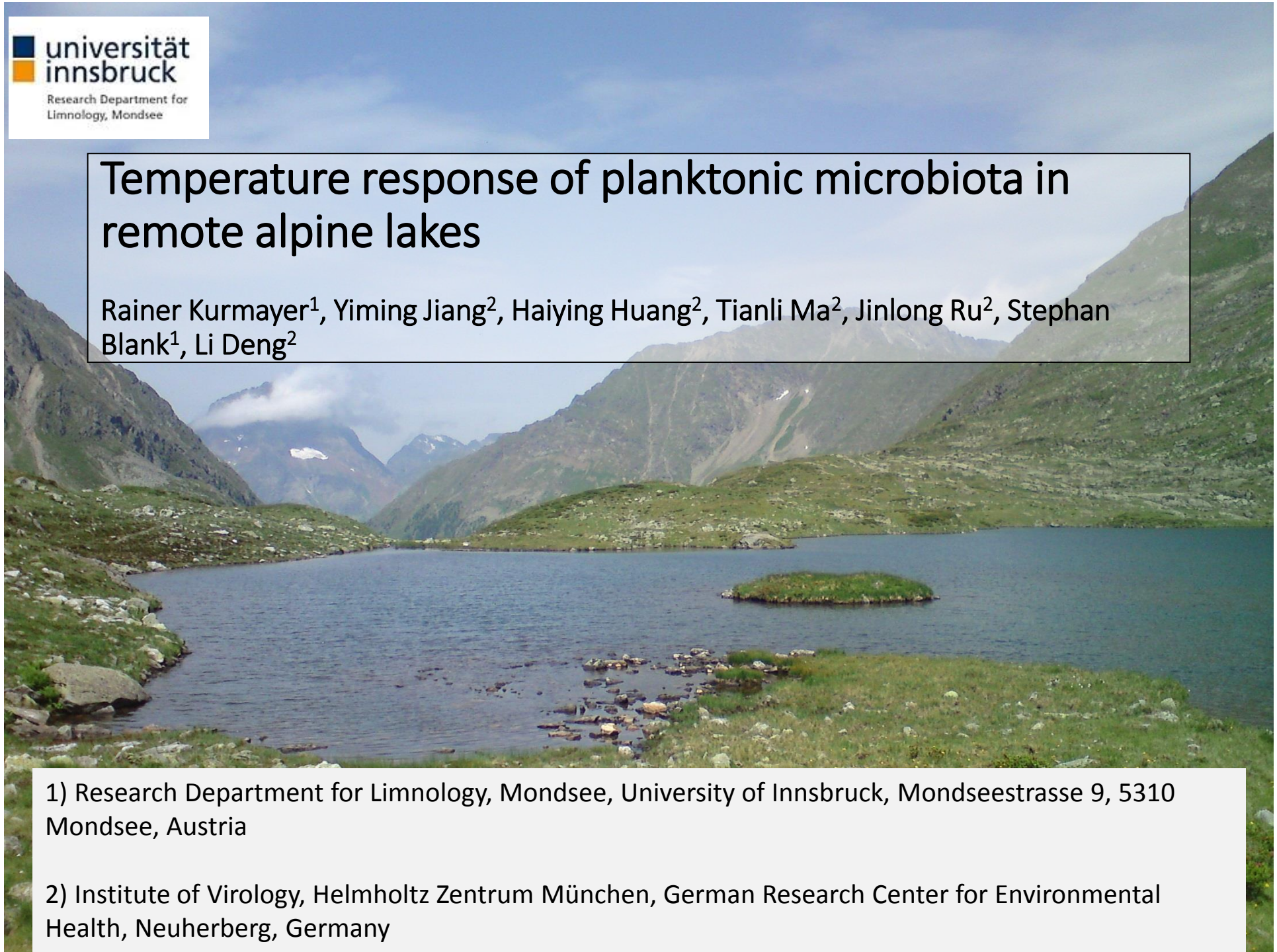

universität innsbruck
Research Department for Limnology, Mondsee

Temperature response of planktonic microbiota in remote alpine lakes

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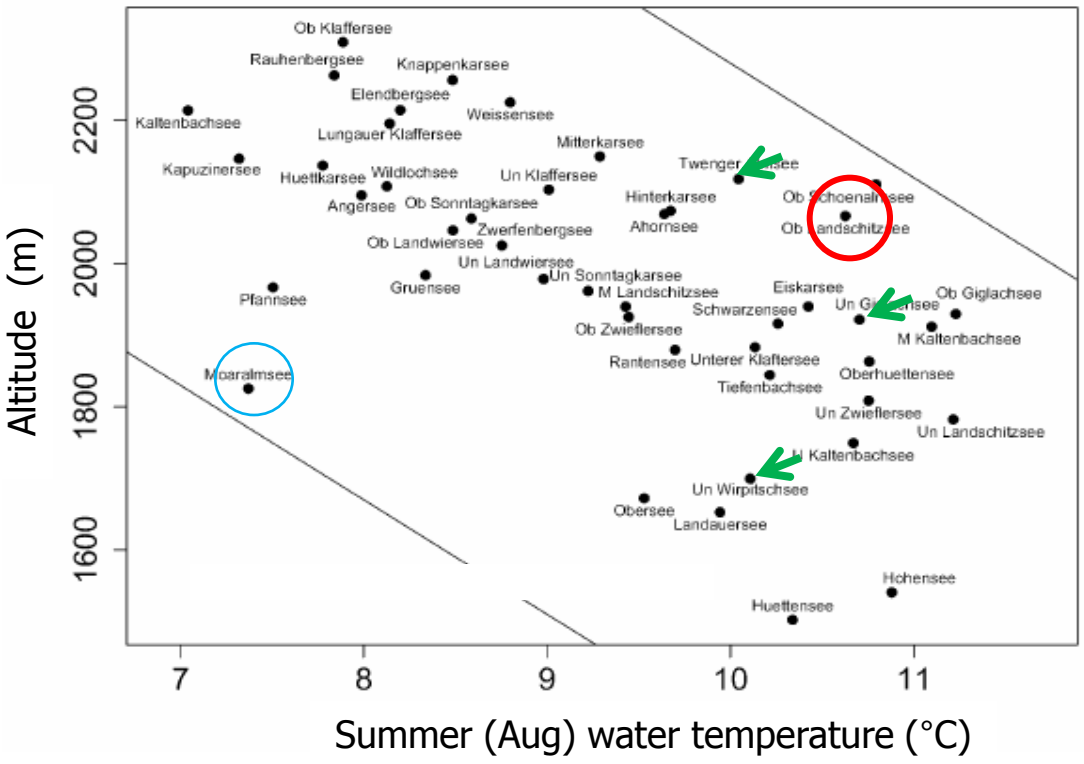
Water temperature in alpine lakes in the Niedere Tauern (Austria), 1998-1999



Thompson et al. 2005, J. Limnol.

Moaralmsee

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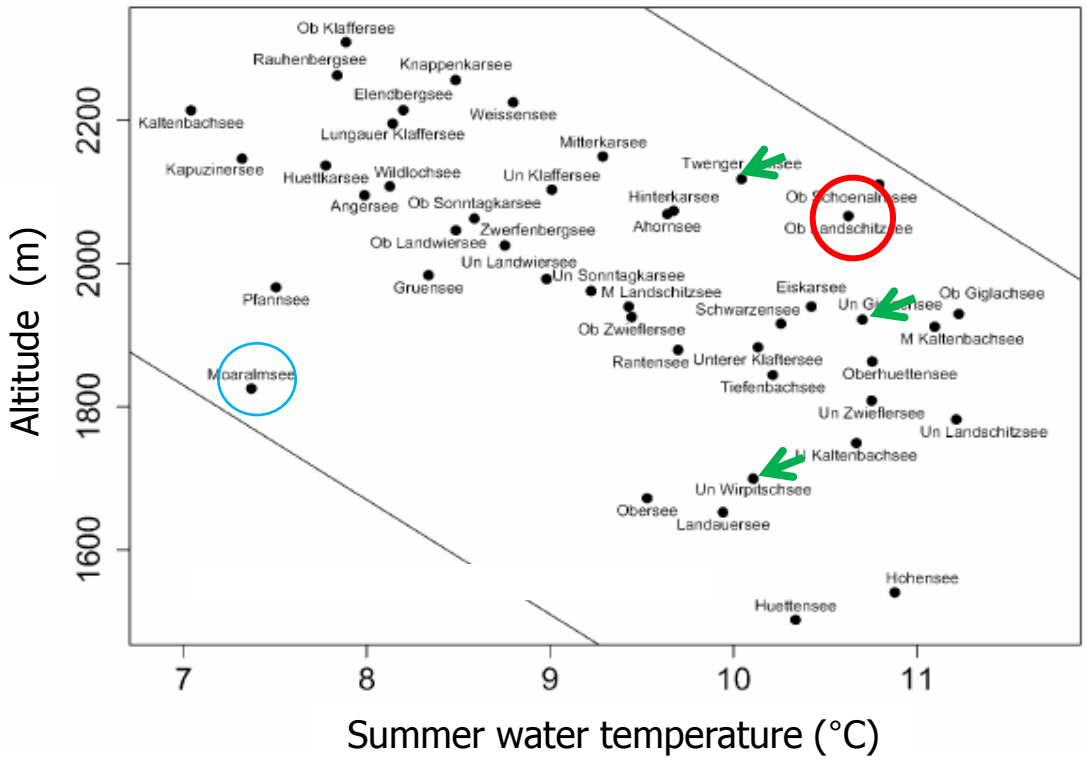
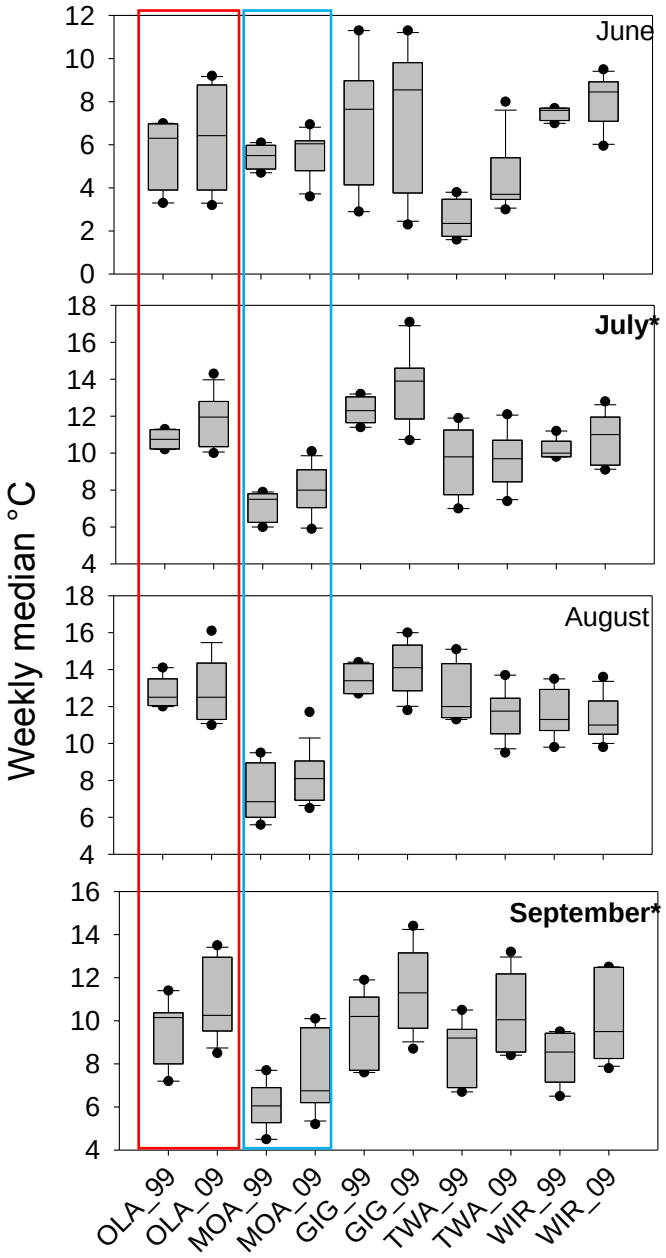
Water temperature in alpine lakes in the Niedere Tauern (Austria), (1998-1999, 2009-2012)



Hypothesis: Undercooled lakes will show most dramatic changes in plankton community as a function of regional temperature rise

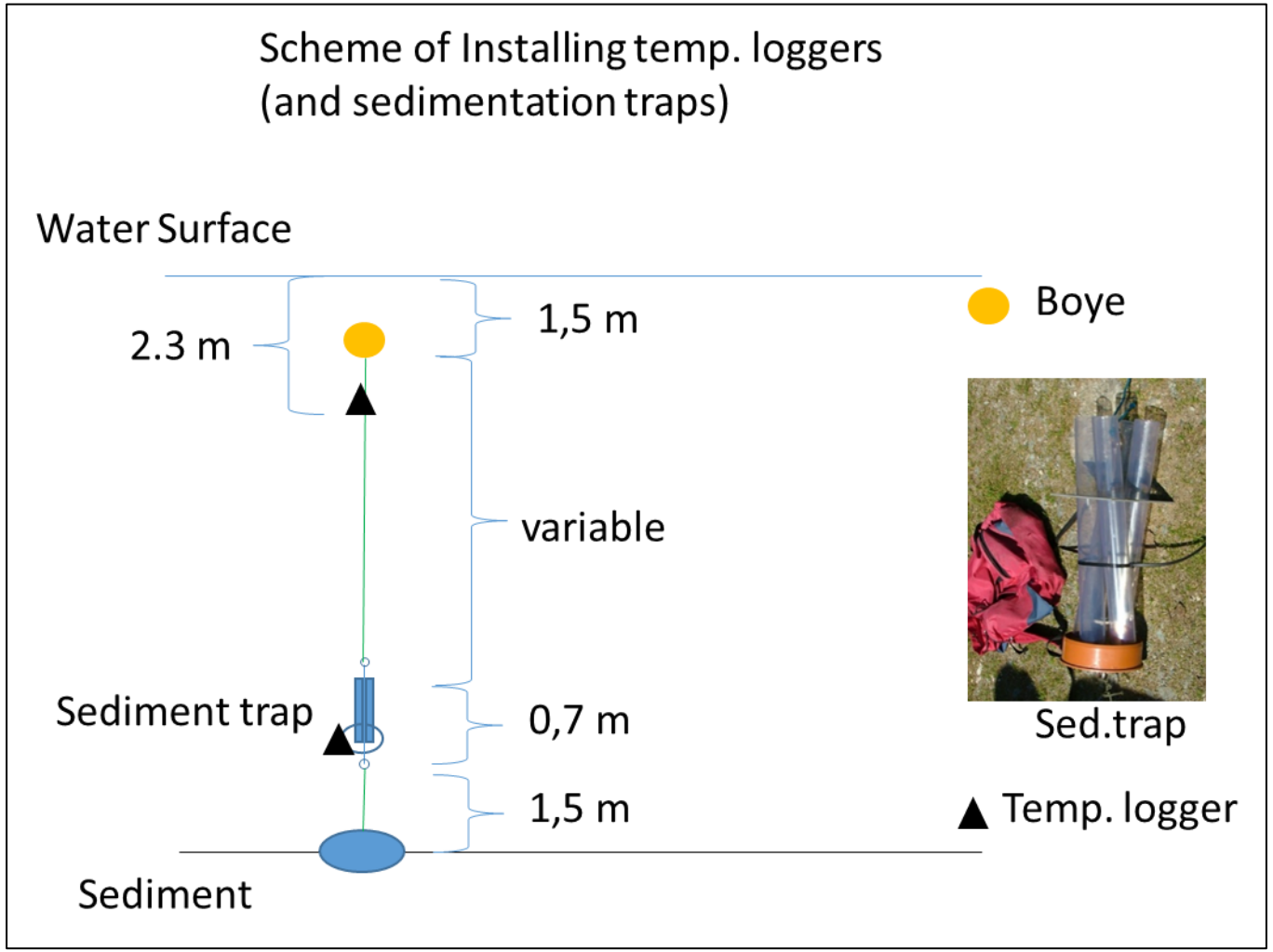
Decadal re-investigation



Estimation of ice cover duration

Ice break up in Twenger Almsee, 16 June 2012

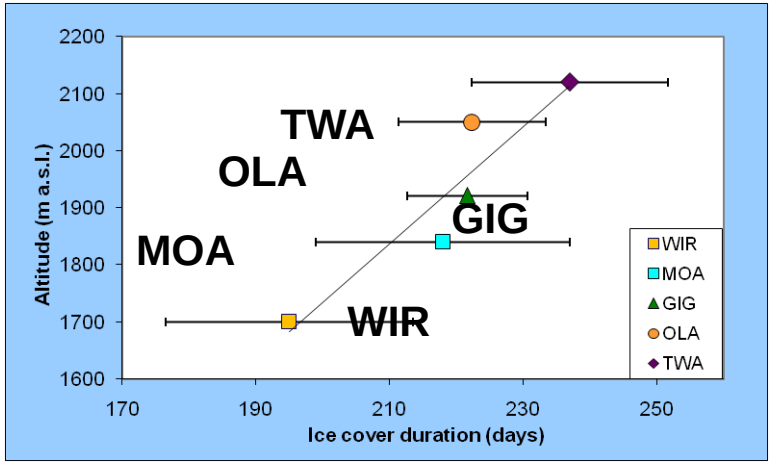
Twenger Almsee, Juli 2009



Variability in Ice cover and spring mixing

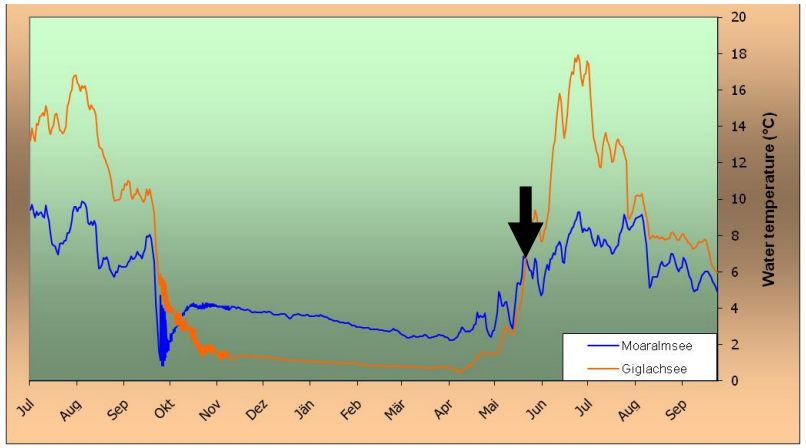
Ice cover duration (days)

Year	OLA	MOA	GIG	TWA	WIR
1998-1999	224	223	221	240	209
1999-2000	nd	nd	214	nd	nd
2009-2010	233	223	231	244	214
2010-2011	**211**	**187**	**212**	**221**	**172**
2011-2012	233	235	229	219	**155**

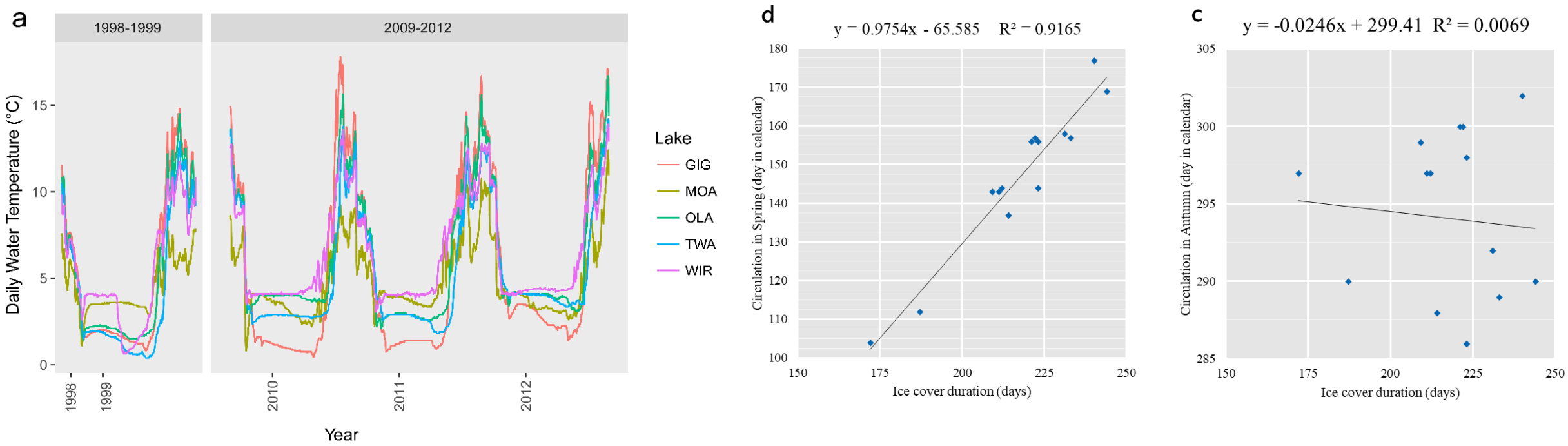


Spring mixing (Julian days)

Year	OLA	MOA	GIG	TWA	WIR
1999	157	144	156	177	143
2000	nd	nd	159	nd	nd
2010	157	144	158	169	137
2011	**143**	**112**	**144**	**156**	**104**
2012	166	157	167	170	**105**



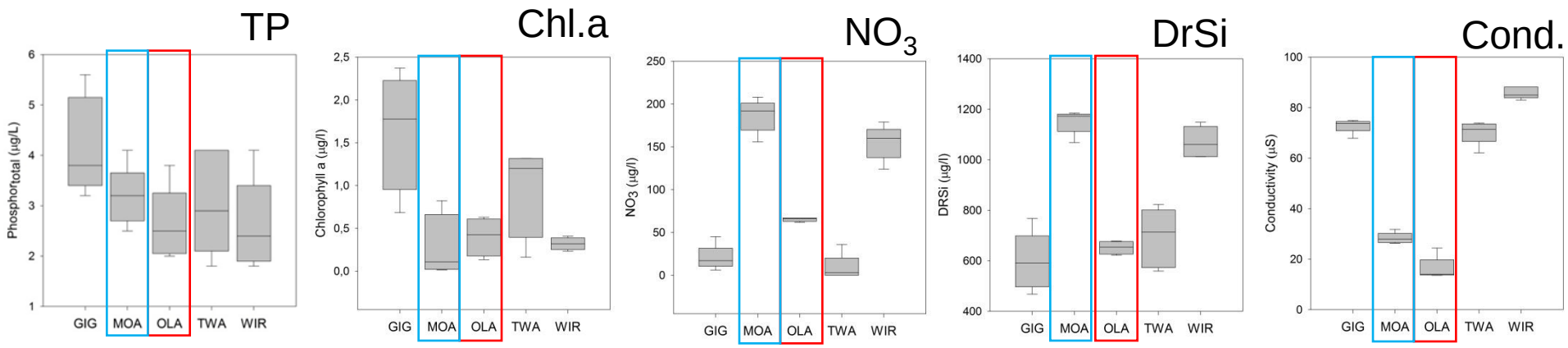
Relationship between ice cover duration and spring vs autumn mixing days

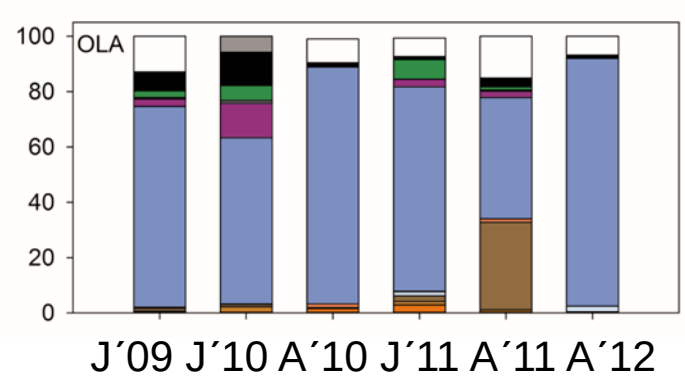


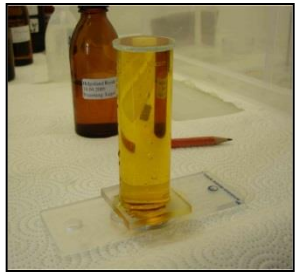
The lakes have typical ice cover duration of 200 ± 18.73 days per year and maximum water temperature < 15°C.

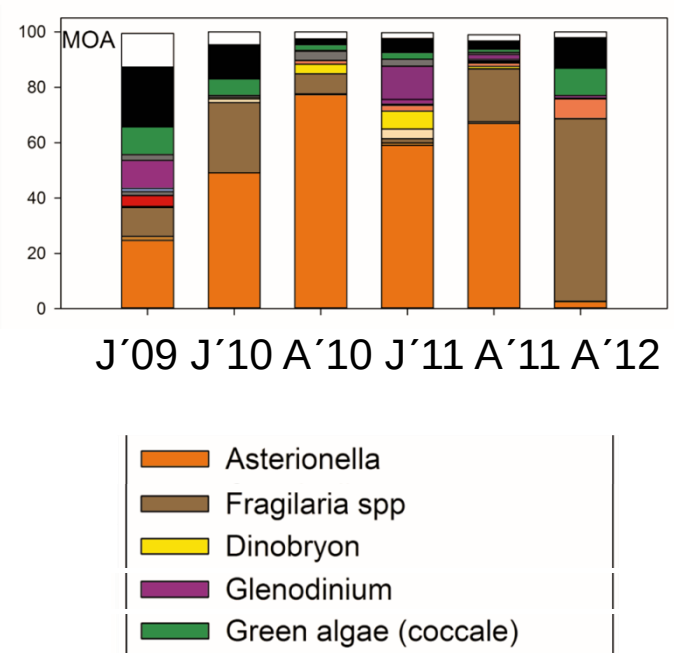
The shorter ice cover duration times correlated significantly with earlier spring mixing (R^2=0.9165) but not with the date of autumn mixing (R^2=0.0069) .

Habitat-specific chemical characteristics related to algal community composition

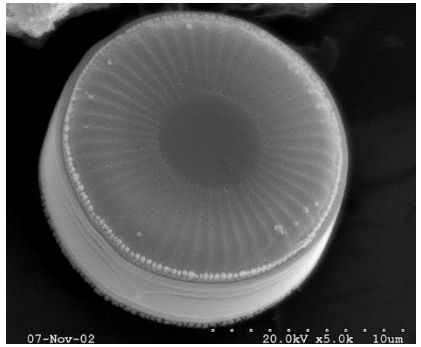






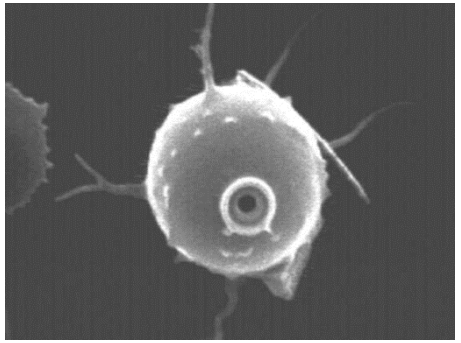


Decadal Re-investigation of diatoms, chrysophytes, chironomids (1998-1999, 2009-2012)

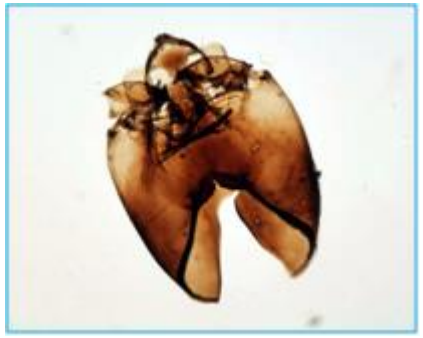


Diatom valve: *Cyclotella* sp.

Sediment traps



Resting stage (stomatocyst) from Chrysophytes



Chironomid head capsule

Diatoms

1) sudden appearance of *Asterionella formosa* (opportunistic species), not found in 45 lake samples before
2) Increase of *F. aff. Delicatissima* in several lakes (MOA, OLA, GIG)

Chrysophytes

1) D. divergens has colonized MOA during the last decade
2) general increase in different morphotype cysts number (MOA)

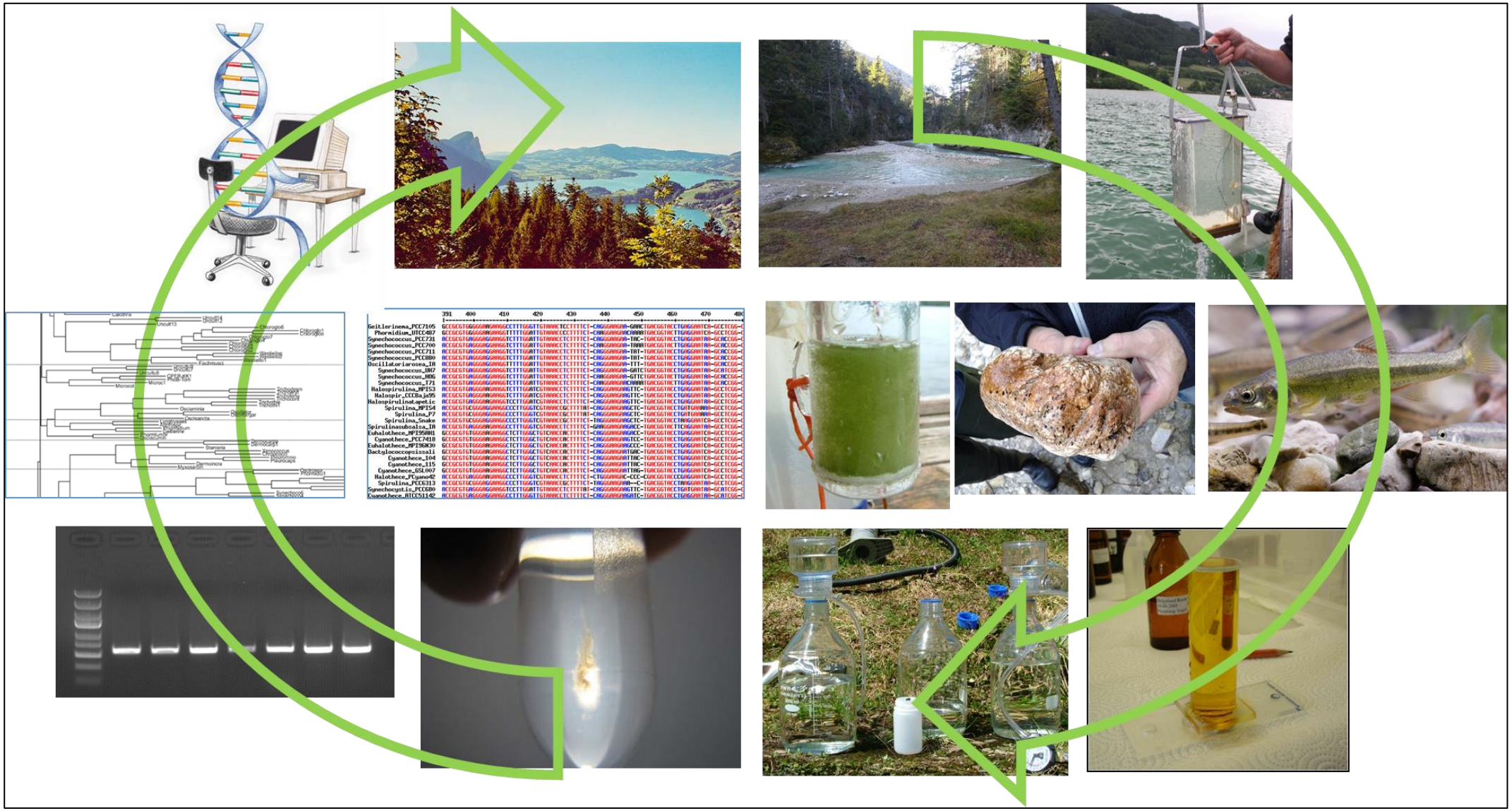
Chironomids

No siginificant changes

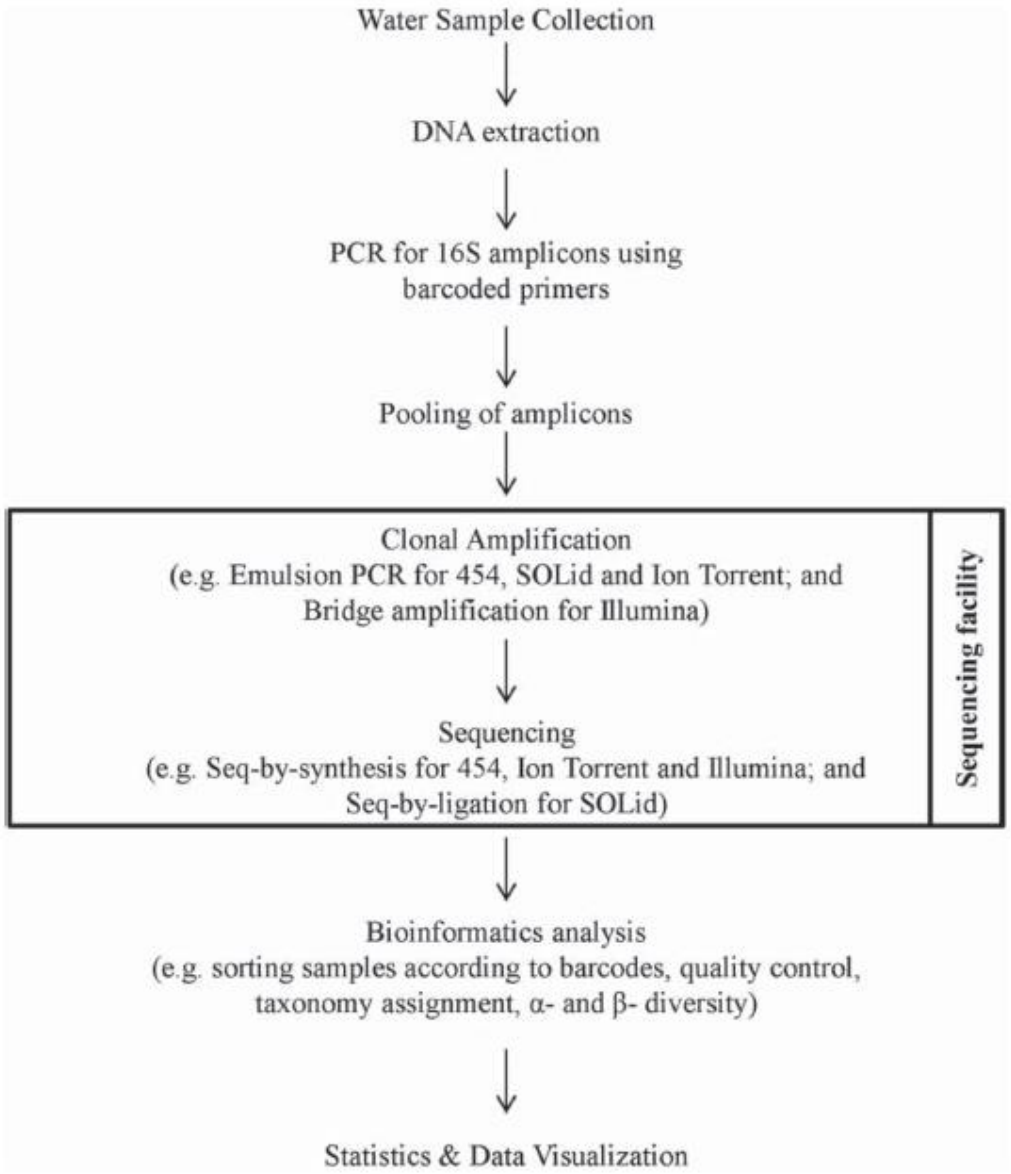
Weckström et al 2016, Arctic, Antarctic, and Alpine Research

Luoto & Nevalainen 2012, Fundam. Appl. Limnol.

Metabarcoding Workflow for community composition analysis



Standard workflow for the NGS of 16S rRNA amplicons for monitoring toxic cyanobacteria



On site filtration

Collecting depth-integrated samples
early (56 ± 16 (SD) days after ice break up) and
later (88 ± 16 days) in the growing season.

Protocoll:
+) Filtration of water samples in two fractions: >1.0µm; 0.2µm – 1.0µm
+) Amplification of 16S with adapter-primer including V3 – V6 region 338F & 1046R (726 bp of product size (*E. coli*))
+) Amplicon high throughput (454-) Sequencing (mean 534 bp) from both directions
+) Analysis with Pipeline – QIIME (Quantitative Insights Into Microbial Ecology)
+) Determining of samples by barcode-sequences; Quality check (QC: 25); Pick up of OTUs (sequence similarity > 97%); Assigning of taxa: Alignment with taxonomic databases (RDP) α – diversity and β - diversity

Deng, L. et al. (2017) in Kurmayer et al. (Eds), John Wiley & Sons Ltd

Sequencing (OTU) Output in Numbers

Forward Seq

Lake	Seq total	OTUs total	Seq/OTU	Seq avg	OTUs avg	Seq/OTU
GIG	34001	2371	14	6800	474	14
MOA	31248	1132	28	6250	226	24
OLA	16898	1321	13	4225	330	11
TWA	25879	1770	15	5176	354	12
WIR	28850	1976	15	5770	395	12
Lake	**Seq total**	**OTUs total**	**Seq/OTU**	**Seq avg**	**OTUs avg**	**Seq/OTU**
GIG	29877	447	67	5975	89	80
MOA	29287	212	138	5857	42	205
OLA	14799	347	43	3700	87	50
TWA	22659	417	54	4532	83	55
WIR	25097	289	87	5019	58	82

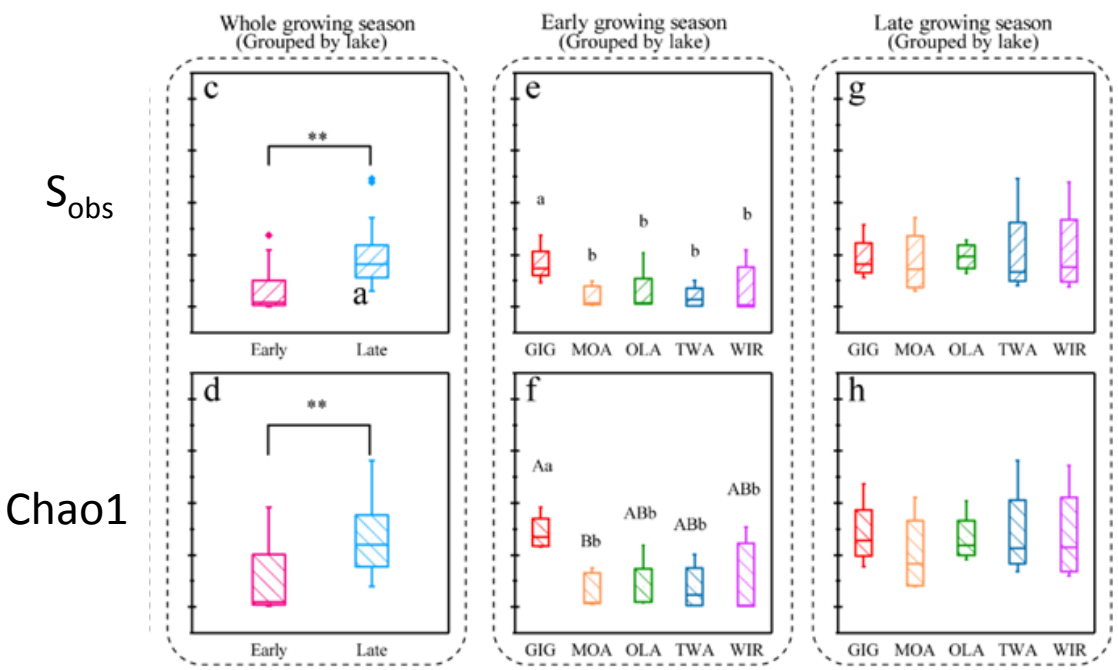
Reverse Seq

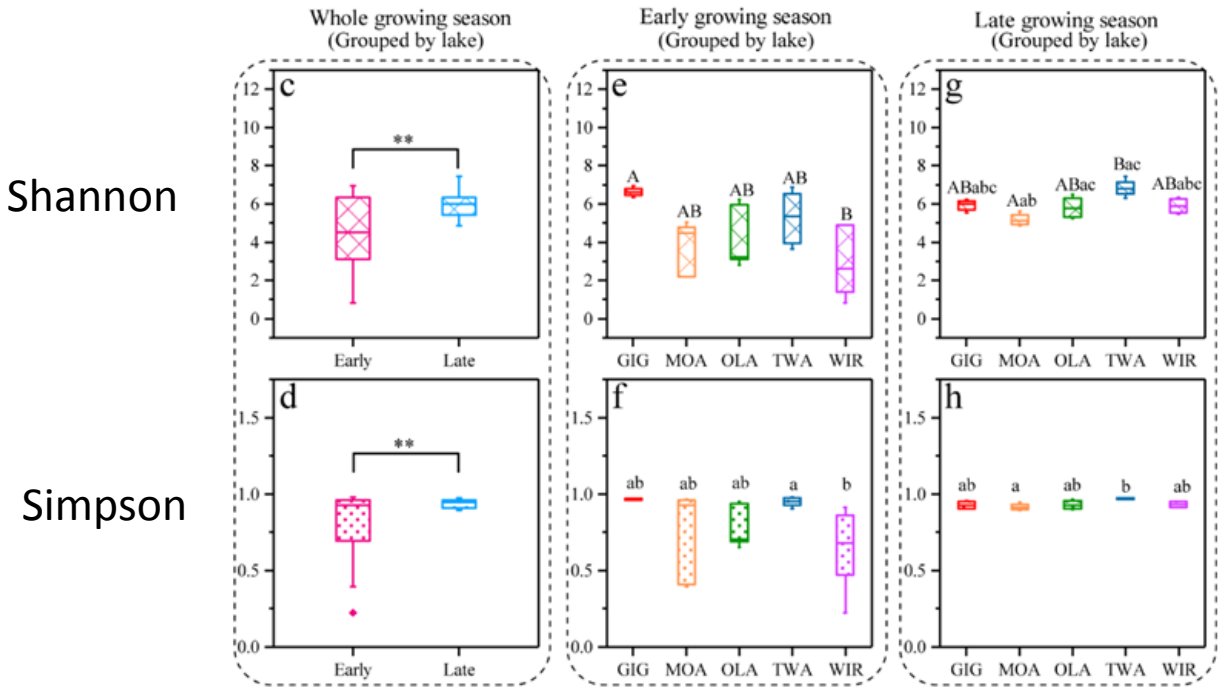
Lake	Seq total	OTUs total	Seq/OTU	Seq avg	OTUs avg	Seq/OTU
GIG	39153	1954	20	7831	604	12
MOA	34934	1117	31	6987	280	21
OLA	23572	1291	18	5893	433	12
TWA	24140	1570	15	4828	416	10
WIR	33012	1566	21	6602	464	14
Lake	**Seq total**	**OTUs total**	**Seq/OTU**	**Seq avg**	**OTUs avg**	**Seq/OTU**
GIG	35306	209	169	7061	136	54
MOA	33321	205	163	6664	70	95
OLA	21483	245	88	5371	123	39
TWA	22128	415	53	4426	157	24
WIR	30168	259	116	6034	123	49

All Seq

Seq (Proportion >0.1%)

Bacterioplankton richness and diversity estimates





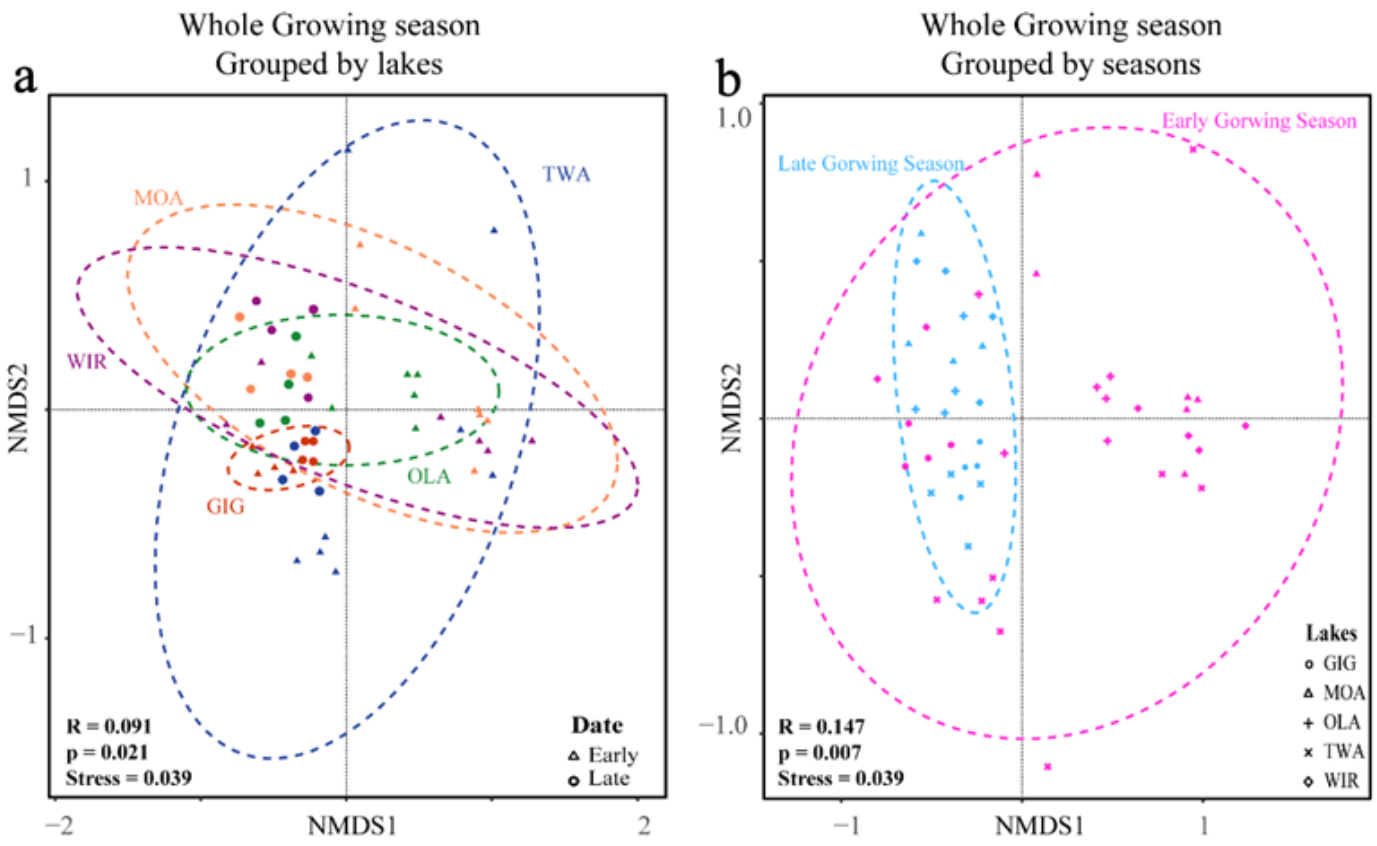
+) Both richness and diversity differed spatially more among lakes in the early growing season, but less in the late growing season

+) Seasonally α-diversity differed significantly between the two growing seasons, i.e. both richness and diversity indices were higher in the late growing season than in the early growing season

Jiang et al. 2017, Front. Microbiol.

Bacterioplankton community analysis

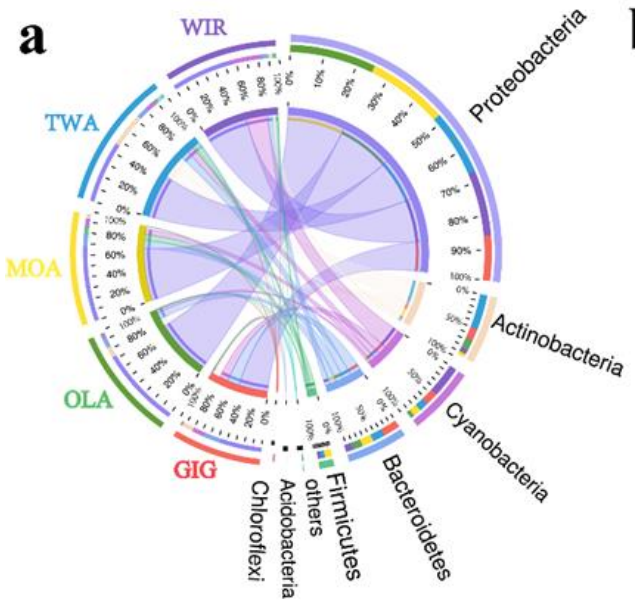
Non-metric multidimensional scaling (NMDS) ordination plot comparing the structures of different communities using the Bray-Curtis matrix



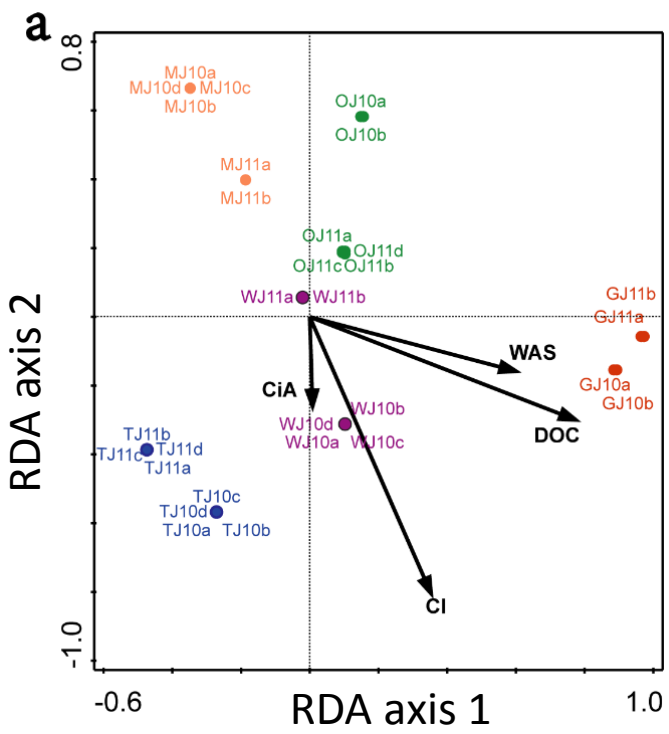
NMDS analysis revealed a higher similarity of microbiota composition among lakes, but higher variability between growing seasons (microbiota showing higher variability in the early growing season than during the late growing season)

Multivariate ordination analysis to identify relevant environmental factors

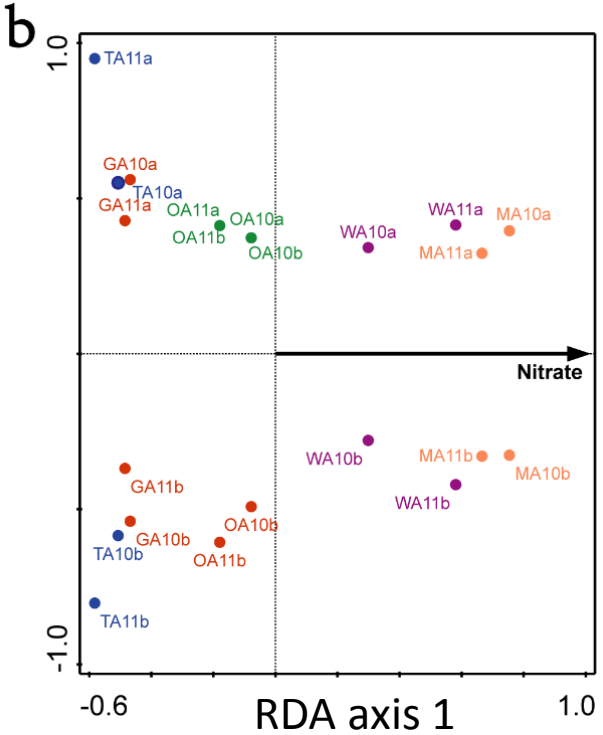
Circos plots showing bacterioplankton at phylum level



Earlier growing season



Later growing season



CiA, Calendar day of circulation in autumn; WAS, average water temperature between calendar day of circulation in spring until the sampling date; Cl^-, Chloride; DOC, dissolved organic carbon

+) in early growing season planktonic microbiota structure was found significantly related to WAS, DOC, CiA, and Cl^- (18.4% of the total inertia in OTU distribution)

+) during the later growing season, only one variable ($NO3^-$) explained 6.9% of the total OTU variation

Relationships between taxonomic richness and average water temperatures after spring circulat

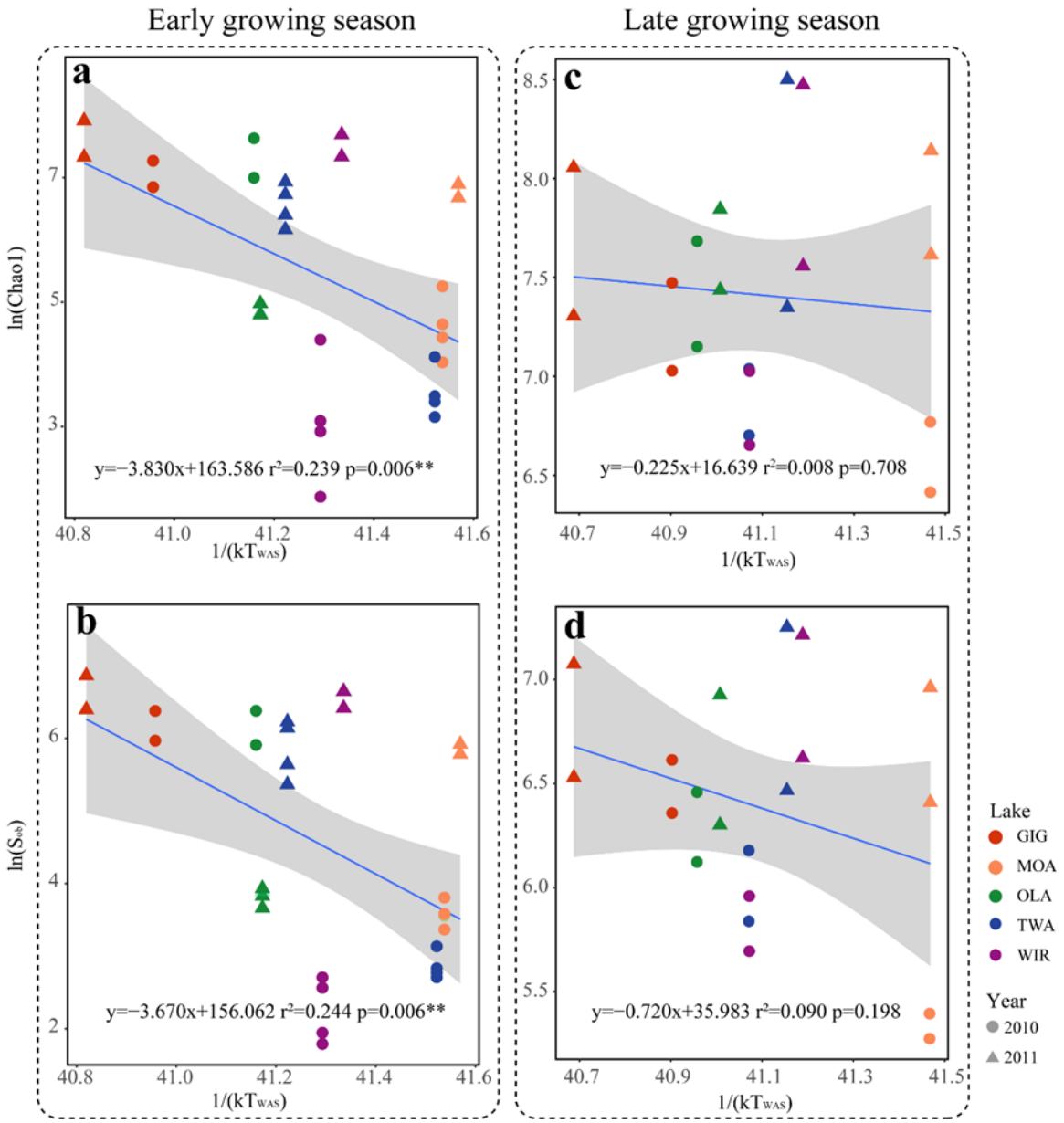
+) Use of MTE (Metabolic Theory) to explain the dependence of taxonomic richness on the bacterial metabolic activity.

$$ln(S_{chao1\ or\ obs}) = a - E_a \times \frac{1}{kT}$$

+) The dependence of species richness on temperature is based on the energetic-equivalence rule (i.e. Allen et al., 2002), assuming that the total energy flux of a population per unit area does not depend on body size.

+) The activation energy is calculated from the inverse slope of the regression curve and expected in the range of -0.65 equivalent to a Q_{10} of ~2.5.

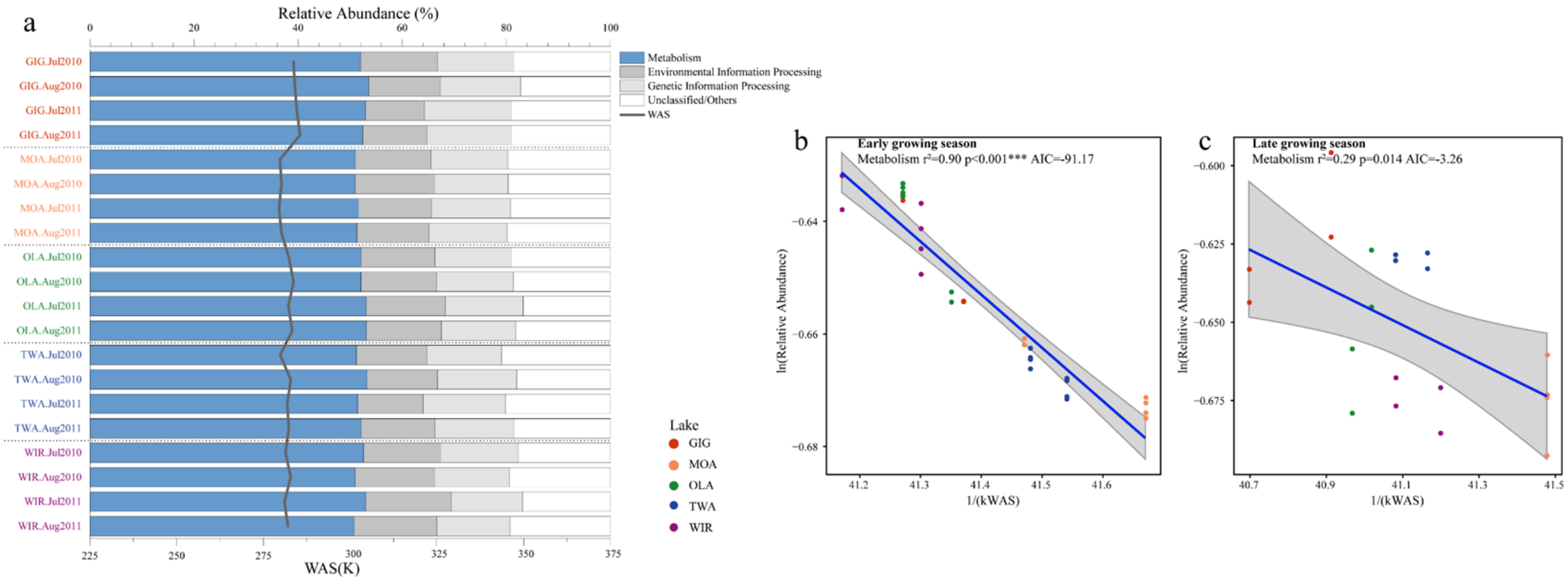
+) The activation energy varied from -3.8 eV for Chao1 and -3.7 eV for S_{obs} in the early growing season while in the later growing season the observed slopes were closer to the more frequently reported -0.65 prediction.



Jiang et al. 2017, Front. Microbiol.

Relationship between water temperature and metabolism of the bacterioplankton community

Composition and relative abundance of predicted metabolism-related genes as a function of 1/(kWAS) during early and later growing season



Metabolic genes increased proportional in response to water temperature supporting the more direct role of temperature variation in the study lakes

Jiang et al. 2017, Front. Microbiol.

Summary

+) Within the ten years period climate change was visible through reduced ice cover duration and increased average water temperature.

+) Microbial composition differed among lakes, with a greater difference among lakes in the early growing season than in the late growing season.

+) In general water temperature (Spring-Summer, CiA) as well as nutrients (Cl, DOC) were influencing the composition during early growing season, while only nutrients such as nitrate showed significant influence during later season

+) Besides the average water temperature after spring circulation (WAS), but not annual water temperature, was influencing bacterioplankton richness and diversity which fits the metabolic theory of ecology (MTE).

+) The relative abundance of metabolism-related genes increased along with WAS implying that metabolism is also directly controlled by water temperature.

Conclusions

+) strong global temperature rise effects on bacterial community in alpine lakes during early growing season. During later growth periods the limiting role of temperature rise on bacterial composition appears to be outweighed by regional and/or stochastic factors.

+) MTE could explain the dependence of taxonomic richness on bacterial metabolic activity in mathematical terms.

+) Interestingly the activation energy exceeded the MTE predicted estimate by far emphasizing the dominant role of temperature during early growing season. In contrast later in the growing season a less significant MTE dependency could be observed.

+) The dominant influence of temperature after ice break up could be explained by overall climate change effects, such as a more intense warming in spring and an overall higher amplitude of temperature variation.

+) Thus, in this study climatic change response in alpine lakes is mostly seen through reduced ice cover duration linked to earlier ice break up and increasing influence of temperature as limiting factor in the water column.

Acknowledgements

+) Johannes Pröll, Norbert Niklas and Christian Gabriel (Red Cross Transfusion Service of Upper Austria Linz) performed the sequencing.

+) Josef Franzoi and Roland Psenner (University of Innsbruck) performed the chemical analysis.

+) ZAMG (Zentralanstalt für Meteorologie & Geodynamik) provided meteorological data on temperature and precipitation from four stations.

+) Ali Hassan Ali Elbehery for bioinformatic support and Judith Feichtmayer for correcting an earlier draft of the manuscript.

+) Chinese Scholarship Council (CSC)

+) Nationalkomitee Alpenforschung of the Austrian Academy of Sciences, project DETECTIVE (DEcadal deTECTion of biodIVErsity in alpine lakes)

Calculation Metabolic Theory of Ecology

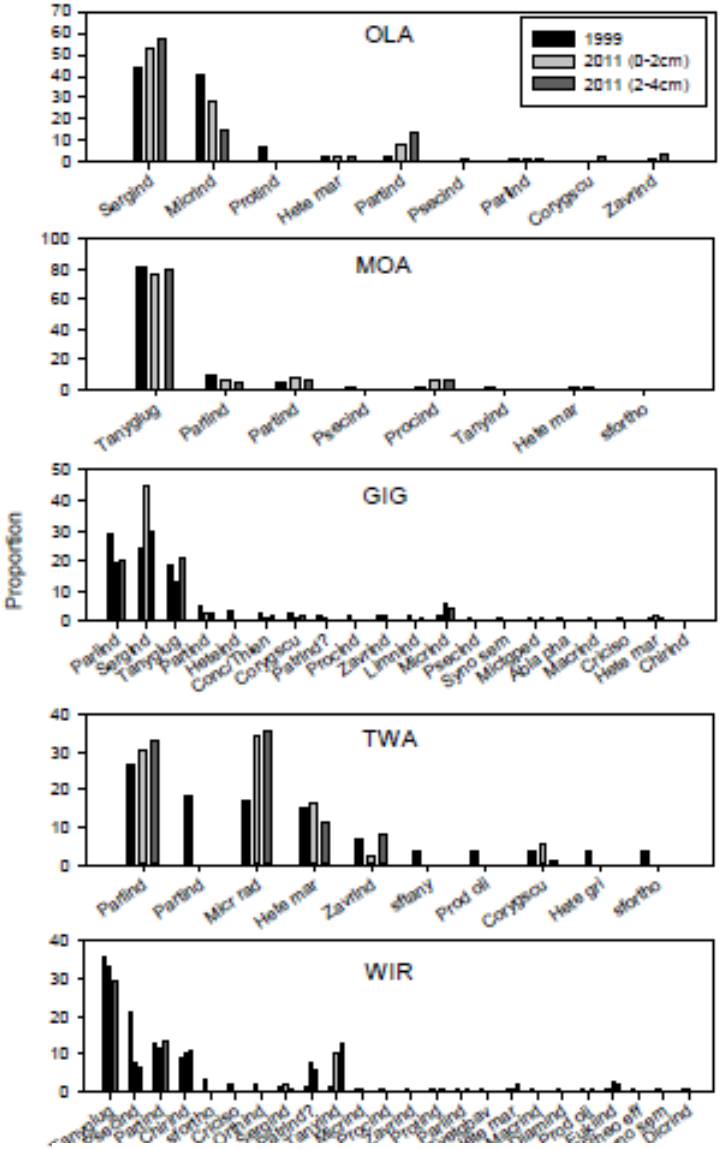
The richness indices Chao1 and S_{obs} were included into the model of metabolic theory of ecology (MTE) (Brown et al., 2004) with command *lm()*. MTE addresses the relationship between organismal metabolism and temperature using the formula (Alcaraz, 2016):

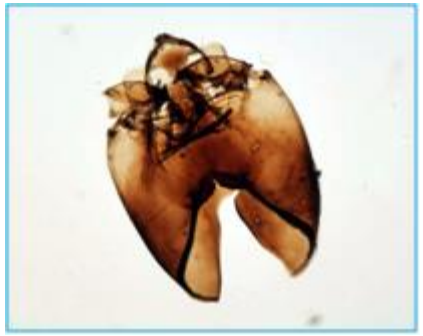
$$ln(S_{chao1\ or\ obs}) = a - E_a \times \frac{1}{kT}$$

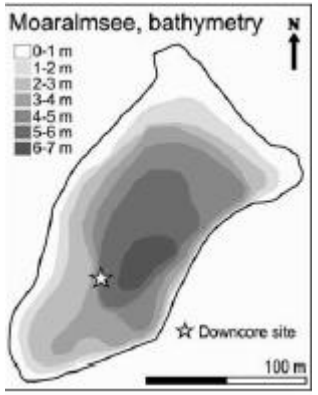
where k is Boltzmann's constant (8.62×10^{-5} eV/K), and T is the absolute temperature in Kelvin (K). E_a is the activation energy, which equals the inverse of the slope calculated from the linear regression, and a is the intercept of the same linear regression.
(1/kT means Inverse temperature is expressed using the Boltzmann–Arrhenius expression (1/kT),

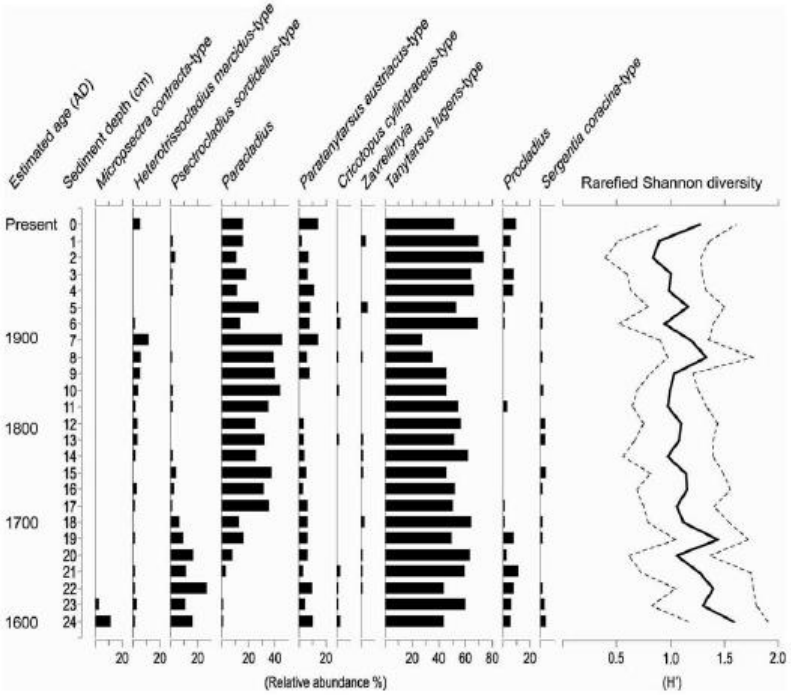
In fact the mechanistic basis of this expression remains unclear (Price et al., 2012), and indeed significant variation in the proposed size and temperature dependencies have been shown (White, Phillips & Seymour, 2006). Nonetheless, this expression provides a useful point of departure for examining the effect of temperature on richness.

Proportion of chironomid cysts in sediments









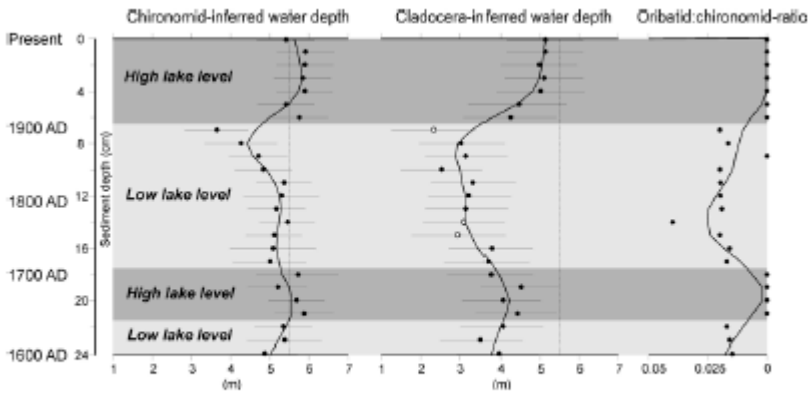


Fig. 4. Proportion of chironomid species as detected in surface sediment core samples from 1999 and 2011 (0-2 cm, 2-4cm) from the deepest part of the lake. The full list of species abbreviations is given in the appendix. The species were ordered according to their proportion in 1999.

Fig. 5. Indications for historical water level fluctuations in MOA through quantitative lake level reconstructions and a qualitative Oribatid : Chironomid ratio (data from Luoto & Nevalainen 2012).

Luoto & Nevalainen 2012, Fundam. Appl. Limnol. Vol. 181/3, 169–181