

Final report for the project CEN M424 WP6.

Development and inter-laboratory comparison to enhance the draft European Standard on water quality – Guidance on quantitative and qualitative sampling of phytoplankton from inland waters based on draft document N118 (2008/04/15)

Authors and affiliations

Andrew M. Dolman¹, Marko Järvinen², Kristiina Vuorio², José Pahissa³, Caridad de Hoyos³, Jacqueline Rücker¹, Ingo Henschke¹, Brigitte Nixdorf¹, Helmut Fischer⁴, Gemma Urrea-Clos^{5,6}, Eberhard Hoehn⁷

1. Brandenburg University of Technology Cottbus-Senftenberg (BTU), Department of Freshwater Conservation, Bad Saarow, Germany.
2. Finnish Environment Institute (SYKE), Freshwater Centre, Finland.
3. Centro de Estudios y Experimentación de Obras Públicas (CEDEX), Centre for Hydrographic Studies, Madrid, Spain.
4. Bundesanstalt für Gewässerkunde (BfG), Federal Institute of Hydrology, Koblenz, Germany.
5. Institute of Aquatic Ecology, University of Girona, E-17071 Girona, Spain.
6. Institut Català de Recerca de l'Aigua (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, Girona, Spain.
7. Limnology Bureau Hoehn (LBH), Freiburg, Germany.

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2 Introduction

This report details the results of a sampling campaign conducted in 2013 and 2014, and subsequent analysis of the data, to verify specific features of the guidelines for sampling of phytoplankton from inland waters in the draft European Standard on water quality – Guidance

on quantitative and qualitative sampling of phytoplankton from inland waters based on draft document N118 (2008/04/15).

Sampling of lakes was carried out in 2013 in three countries, Finland, Spain and Germany, with the principal aim of determining the influence of the depth range from which water is collected on the measured phytoplankton biovolume and chlorophyll *a* concentration for various lake types and conditions. In addition, in Germany the influence of the device used to collect water was also tested.

Sampling of rivers was carried out in 2013 in Spain to assess the influence of macrophyte beds on estimates of phytoplankton composition, and in 2014 in Germany to assess the influence of sampling position in relation to dams and the confluence with tributaries.

Details of the lakes sampling campaign are given first, followed by results of the lake data analysis. Then the river sampling and interpretation are given in subsequent sections.

3 Summary of results

3.1 Lakes

The four different water column sampling devices, when used to sample from the same water layer, gave comparable results with no clear bias or differences in agreement (Figure 6.1, Figure 6.2, Table 6.1 and Table 6.2). If anything, the shorter Limnos sampler gave slightly less consistent results, but this is more likely due to the fact that non-contiguous samples were taken so that certain aggregations of phytoplankton may have been missed, rather than an effect of the device itself. Nevertheless, there was still approximately 10% disagreement between the devices. This is likely to be close to the baseline level of repeatability that would be seen if the same sampler were used repeatedly on the same water layer.

Differences were larger between samples taken from different water layers. Between the euphotic zone and upper mixed water layer there was typically 23-29% disagreement for biovolume, and a small but consistently positive bias (Table 6.3, Figure 6.3). There was more biovolume found in the euphotic zone, regardless of whether Z_{eu} was deeper than Z_{mix} (indicating a DCM), or shallower than Z_{mix} (indicating a concentration of biovolume towards the surface) (Figure 6.5). In some cases this disagreement and bias was much higher, and the degree of bias was dependent on the relative depths of the euphotic and mixed layers (Figure 6.6). Z_{eu}/Z_{mix} was largest for some of the German lakes, and for these the bias was around 22% for biovolume and 64% for chlorophyll *a* – indicating a DCM comprised of phytoplankton with higher than average cellular chlorophyll contents. There was also a consistent positive bias for Spanish reservoirs with very low Z_{eu}/Z_{mix} ratios, but this bias was much smaller.

Similar conclusions can be drawn from the comparison of Finnish surface-2 m samples with samples from the euphotic and mixed layers. Disagreement was much higher in clear lakes when Z_{eu} or Z_{mix} was greater than 2 m, although there was no clear bias; sometimes phytoplankton were aggregated in the upper 2 m, sometimes below. Even in clear lakes, disagreement was only around 20% (Table 6.4).

Surface samples, when compared to euphotic zone samples from clear German lakes, had the highest bias: -27% for biovolume and -45% for chlorophyll *a* (Table 6.6).

3.1.1 Recommendations

The device used to collect water makes very little difference to biovolume and chlorophyll estimates therefore there should be no recommendation made for a specific device.

A continuous sample, either via contiguous discrete samples (e.g. Limnos), IWS or Hose is better than a discontinuous sample taken with gaps in the vertical profile.

Surface sampling gives a reasonable approximation to a sample from the upper mixed layer – and might be considered when lakes are expected to be well mixed and/or lacking deep chlorophyll maxima (DCMs). The reduction in accuracy for individual samples may be worth the increase in the total number of samples that can be taken as part of a large programme. When looking across widely differing lakes, with 2-3 orders of magnitude range in biovolume, a 20%, or even 50% bias (0.1 - 0.2 magnitude) in individual samples would not appear to be very important.

3.2 Rivers

It was concluded in the expert workshops that in fully mixed rivers the location and frequency of sampling is much more important than the precise method of water collection. Sampling campaigns therefore did not aim on the sampling device, but included case studies on the heterogeneity of phytoplankton distribution in specific river sections that were suspected to show heterogeneity in phytoplankton distribution on a relatively small scale. Each case study was underpinned with additional examples from the literature for this report.

Relatively small scale heterogeneities can occur in all rivers that were sampled for the case studies. In riverine impoundments, phytoplankton biovolume differed by a factor of two on a relatively short distance of only 12 km length. Additionally to this longitudinal effect, strong vertical differences can occur when these impoundments stratify. The impact of tributaries on phytoplankton biovolume can be strong, even if those tributaries are much lower in discharge than the studied river. Lastly, sampling between macrophytes can strongly bias the sampled phytoplankton biovolume in several ways. The following recommendations followed from the expert workshops, the case studies and the literature survey:

- Sampling must be representative for the river section under study. This means that the sampling site must be „known“ and checked for homogenous distribution of phytoplankton.
- If vertical stratification is measured or suspected, rivers must be sampled like shallow lakes taking into account the vertical inhomogeneity of phytoplankton distribution.
- If the river section is laterally fully mixed, samples from the flow centre of the river are sufficient; if not, cross sectional sampling is advised. Sampling downstream of tributaries should be avoided.
- Resuspension of benthic or epiphytic algae during sampling should be avoided.
- A bucket can be used for sampling of fast-flowing, fully mixed rivers. In slow-flowing rivers (fully mixed), other sampling devices recommended for lake sampling can also be applied. Stratified rivers should be sampled like shallow lakes.
- Sampling should cover the dynamics (phytoplankton peaks) of the time period under study and provide representative mean values (e.g. for a season). At least monthly sampling is recommended to represent an annual course; however short phytoplankton peaks might then be missed and maximum as well as mean values derived from such a sampling scheme might be somewhat erroneous.

4 Lakes: sampling campaign

This section of the report describes the sampling campaign for lakes in Finland, Spain and Germany carried out in 2013 as part of the development of the European guidance on quantitative and qualitative sampling of phytoplankton from inland waters based on draft document N118 (2008/04/15).

4.1 Sampling of Finnish lakes by SYKE

Marko Järvinen & Kristiina Vuorio
Finnish Environment Institute (SYKE), Freshwater Centre, Finland

4.1.1 Description of the studied Finnish lakes

Studied lakes are located in southern Finland within a maximum distance of ca. 50 km from each other in the Häme region (Figure 4.1). The lakes have surface areas of >30 ha with the exception of Merrasjärvi, which is slightly smaller in size. Clearwater lakes have a mean summer water colour of $\leq 30 \text{ g m}^{-3} \text{ Pt}$, comparable to criteria for clearwater lake types used in European intercalibration lake typology, whereas all humic brown-coloured lakes have a water colour of $\geq 50 \text{ g m}^{-3} \text{ Pt}$. All lakes are typically ice-covered during winter from November/December until late April/May. Characteristics of the lakes are presented in Table 4.1.

Table 4.1 Characteristics of Finnish lakes sampled in 2013. Water colour and total phosphorus (Tot-P) represent mean summer (June-August) values in the epilimnion and for polymictic lakes generally the uppermost 1 m layer (data from literature and/or from water quality data base of SYKE (Hertta)).

Lake	Stratification / Mixing	Area (ha)	Mean depth (m)	Maximum depth (m)	Colour ($\text{g m}^{-3} \text{ Pt}$)	Tot-P (mg m^{-3})	Status ¹
Clearwater lakes							
Alasenjärvi	dimictic	272	6.1	15.2	10	14	good
Arkiomaanjärvi	dimictic	208	5.1	20.2	25	17	good
Ruuhijärvi	dimictic	573	5.6	18.7	30	18	moderate
Joutjärvi	polymictic	39	3.3	5.0	25	25	moderate
Humic lakes							
Alinen Rautjärvi	dimictic	45		12.0	100		n.a.
Pääjärvi	dimictic	1342	14.8	85.0	80	12	good
Työtjärvi	dimictic	56		7.0	50	25	n.a.
Merrasjärvi	polymictic	24	1.5	2.6	50	30	moderate

1) Status= Ecological status class 2013, n.a. = not assessed.



Figure 4.1 – Location of study lakes in Häme region in Finland (source: SYKE, Centres for Economic Development, Transport and the Environment, and National Land Survey of Finland).

4.1.1.1 Clearwater lakes

4.1.1.1.1 Stratified clearwater lakes

Lake Alasenjärvi

Lake Alasenjärvi is a clearwater and rather deep lake situated in the city of Lahti (Figure 4.1, Figure 4.2, Figure 4.3). The lake is surrounded mainly by urban and forested catchments (15 km²). The lake has high importance for recreational use. The lake has a long retention time (5.3 yrs) (Keto 2006). The signs of eutrophication became evident in the lake in the 1970's and in the 1980's the lake suffered from cyanobacterial blooms and deep water anoxia. Since then the lake has been restored, and its state has recovered during the last decades, but e.g. occasional deep water oxygen depletion still occurs.

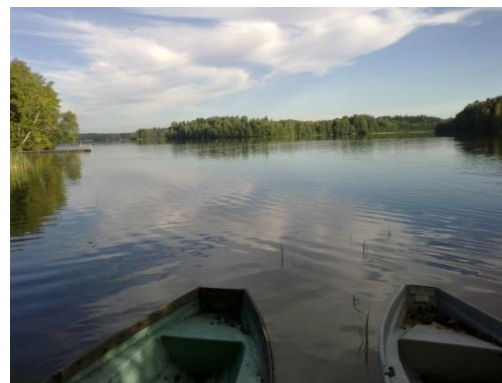


Figure 4.2 Lake Alasenjärvi (Lahti, southern Finland), a dimictic urban clearwater lake (photos © Marko Järvinen).

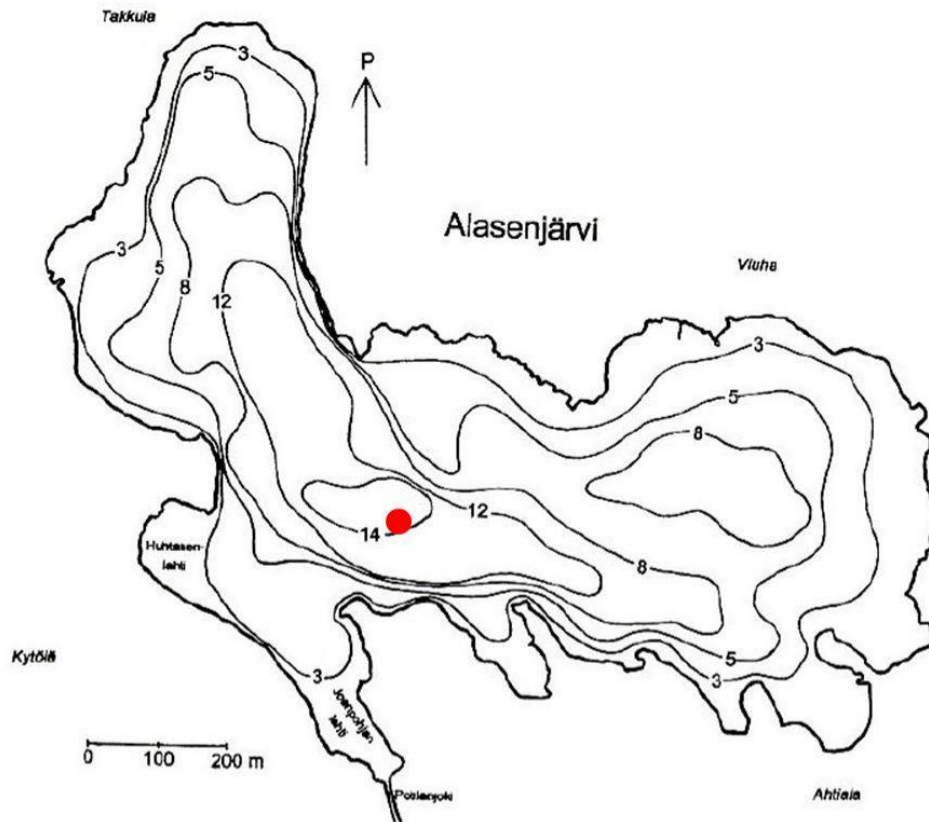


Figure 4.3 - Bathymetric map of Lake Alasenjärvi (Lahti). Red dot indicates the sampling point. Modified from Keto (2006).

Lake Arkiomaanjärvi

Lake Arkiomaanjärvi is a clearwater lake situated in the municipality of Hollola (Figure 4.1, Figure 4.4, Figure 4.5). The lake is relatively shallow with two deeper parts (20 m in depth). The shore line of the catchment (11.1 km²) is dominated by summer cottages and permanent houses. The status of the lake is good, and it has not suffered e.g. strong cyanobacterial blooms.

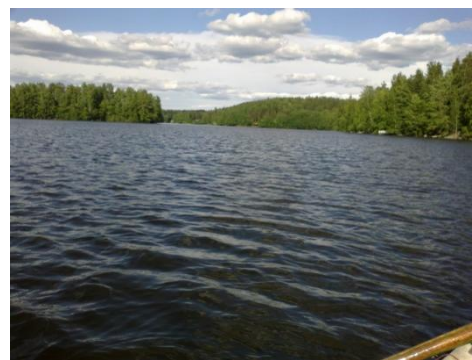


Figure 4.4 - Lake Arkiomaanjärvi (Hollola, southern Finland), a dimictic clearwater lake (photos © Marko Järvinen).

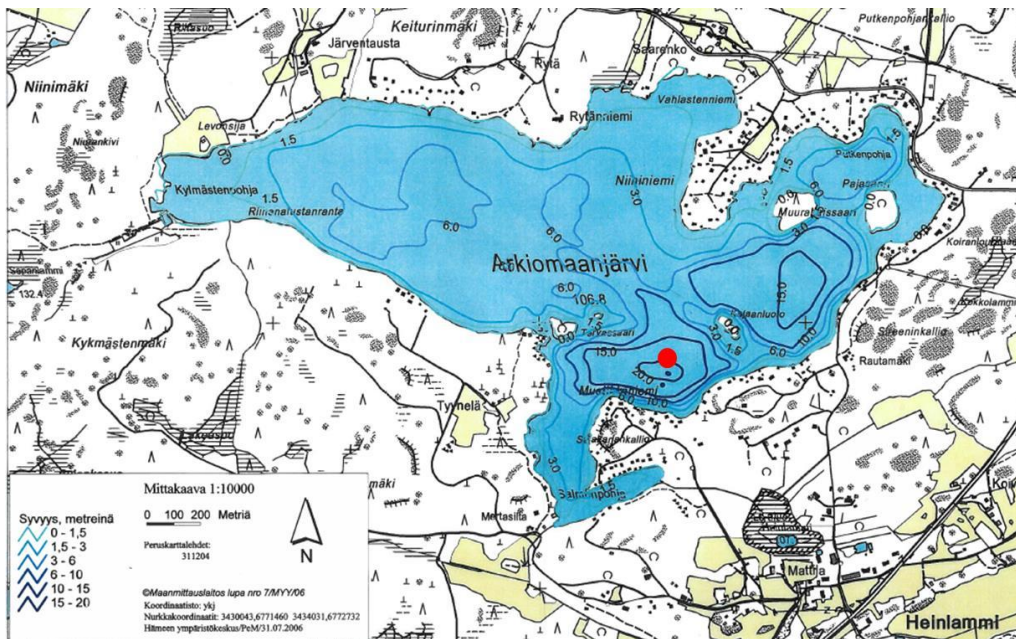


Figure 4.5 - Bathymetric map of Lake Arkiomaanjärvi (Hollola). Red dot indicates the sampling point. (source:Häme Centre for Economic Development, Transport and the Environment, and National Land Survey of Finland).

Lake Ruuhijärvi

Lake Ruuhijärvi locates in the municipality of Nastola (Figure 4.6 Figure 4.7). The catchment area of the lake is 273.2 km². The lake is slightly eutrophic (Tot-P 18 mg m⁻³; Tot-N 490 mg m⁻³) and it's ecological status is moderate. During 2000's the lake has suffered oxygen depletion of deep water layers both in summer and winter at the end of the stratification periods. The lake has high importance for recreational use (summer cottages, bathing, fishing).



Figure 4.6 - Lake Ruuhijärvi (Nastola, southern Finland), a dimictic clearwater lake (photos © Marko Järvinen).

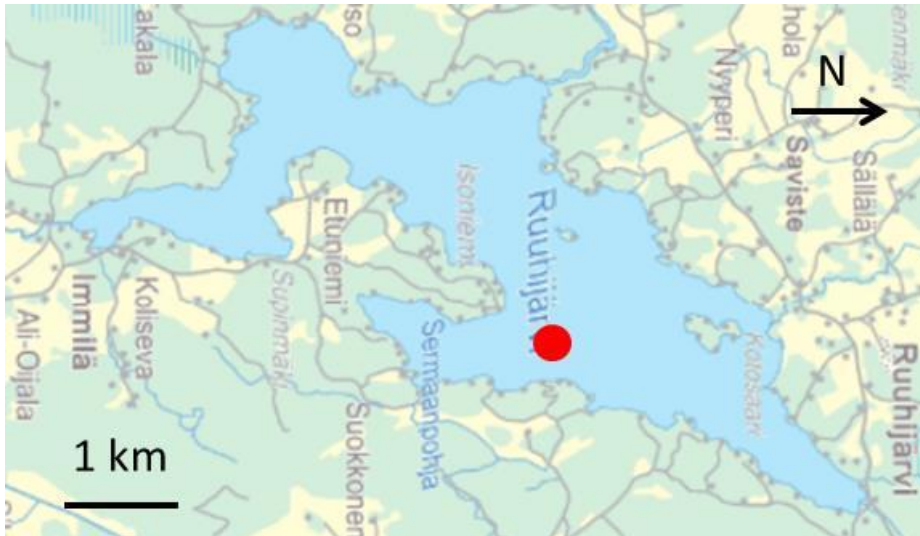


Figure 4.7 - Map of Lake Ruuhijärvi (Nastola). Red dot indicates the sampling point.

4.1.1.1.2 Polymictic clearwater lake

Lake Joutjärvi

Lake Joutjärvi is a polymictic urban lake located in the city of Lahti (Figure 4.8 Figure 4.9). The lake has high recreational value (bathing, canoeing, summer and permanent residences). The catchment area of the lake is 1.6 km². The lake is shallow and slightly eutrophic (Tot-P 25 mg m⁻³; Tot-N 540 mg m⁻³). It receives most of its water as groundwater. The ecological status is moderate. Status of the lake has varied markedly between the years, most likely due to large variation in dense young fish communities (Keto 2006). The shallower parts of bottom areas are covered by submerged macrophytes. The raphidophyte alga *Gonyostomum semen* has been observed in the lake, as well as occasional cyanobacterial blooms.



Figure 4.8 - Lake Joutjärvi (Lahti, southern Finland), a polymictic clearwater lake (photos © Marko Järvinen).

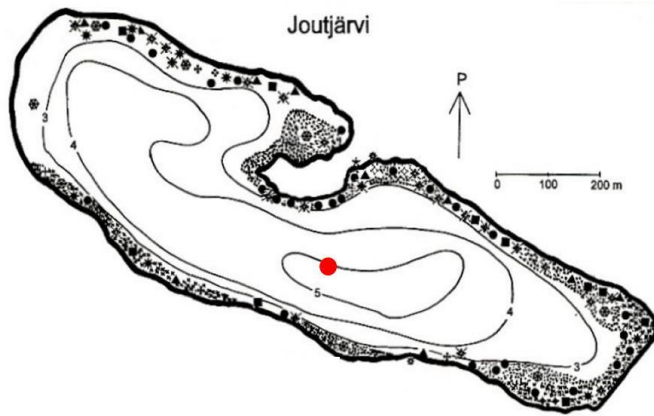


Figure 4.9 - Bathymetric map of Lake Joutjärvi (Lahti). Red dot indicates the sampling point. Modified from Keto (2006).

4.1.1.2 Humic lakes

4.1.1.2.1 Stratified humic lakes

Lake Alinen Rautjärvi

Lake Alinen Rautjärvi locates in the Evo region (Hämeenlinna) (Figure 4.10 Figure 4.11). It a relative small highly humic lake with a water colour of ca. 100 g m^{-3} Pt. The lake is mainly surrounded by the forested catchment (32 km^2).

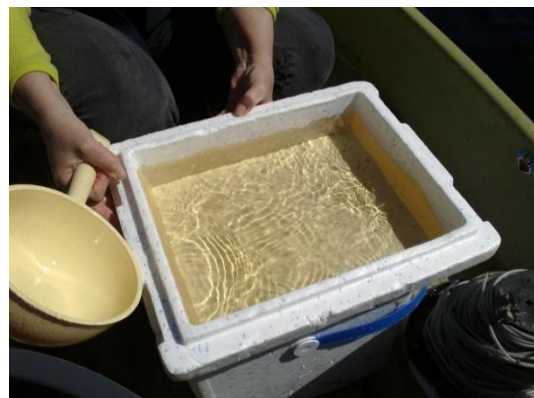


Figure 4.10 - Lake Alinen Rautjärvi (Lammi, southern Finland), a dimictic humic lake (photos © Marko Järvinen).



Figure 4.11 - Map of Alinen Rautjärvi (Evo, Hämeenlinna). Red dot indicates the sampling point.

Lake Pääjärvi

Lake Pääjärvi locates in Lammi (Hämeenlinna) and is one of the deepest lakes in Finland (Figure 4.12Figure 4.13). It is a relative large brown-water lake with a large catchment area (244 km²) dominated by coniferous and mixed forest, but also with a relative large contribution of agriculture in some sub-catchments. The lake has representative long-term physico-chemical and biological data series, collected mainly by the Lammi Biological Station of the University of Helsinki locating on the western shore of the lake. The ecological status of the lake is good and even deepest parts of the lake are well oxygenated. Due to dark water colour, euphotic zone is typically shallower than the mixed layer during stratification.



Figure 4.12 - Lake Pääjärvi (Lammi, southern Finland), a dimictic humic lake (photos © Marko Järvinen).

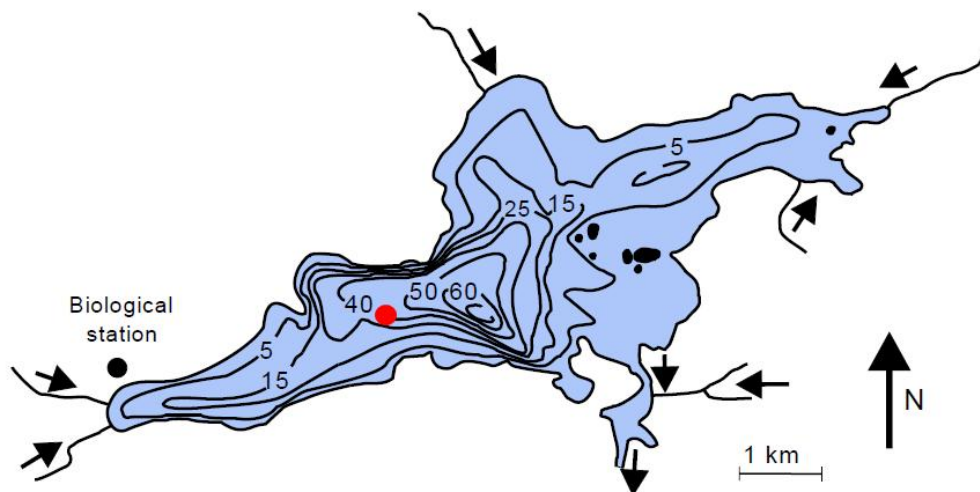


Figure 4.13 - Bathymetric map of Lake Pääjärvi (Lammi, Hämeenlinna). Red dot indicates the sampling point.

Lake Työtjärvi

Lake Työtjärvi is a relatively shallow humic lake, with one deeper part (ca. 7 m) (Figure 4.14Figure 4.15). During the study in 2013 it appeared that this “dimictic” lake may experience almost complete mixing during strong wind events during summer. The catchment of the lake is characterized by urban and peatbog areas, and the shallow parts of the lake/littoral have rather dense macrophyte vegetation.



Figure 4.14 - Lake Työtjärvi (Hollola, southern Finland), a dimictic humic lake (photos © Marko Järvinen).



Figure 4.15 - Map of Lake Työtjärvi (Hollola). Red dot indicates the sampling point.

4.1.1.2.2 Polymictic humic lake

Lake Merrasjärvi

Lake Merrasjärvi is a shallow (mean depth 1.5 m) small humic lake in the city of Lahti (Figure 4.16 Figure 4.17). The lake is surrounded by forests, peatland areas and urban areas (4.3 km²). The retention time of the lake is short (0.5 yr). The lake has high recreational value (bathing). The lake suffers anoxia during winter ice-covered period (Keto 2006).



Figure 4.16 - Lake Merrasjärvi (Lahti, southern Finland), a polymic humic lake (photos © Marko Järvinen).



Figure 4.17 - Map of Lake Merrasjärvi (Lahti, southern Finland). Red dot indicates the sampling point.

4.1.2 Methods

4.1.2.1 Sampling

The lakes were sampled four times during the summer in June-August (Table 4.2). The sampling was carried out in the deepest part of the lake using rowing or motor boats. During measurements, the boat was anchored. During anchoring sediment disturbance was avoided also in the shallower lakes Joutjärvi and Merrasjärvi.

Table 4.2 - Sampling times of lakes and the prevailing weather conditions during the measurement campaign.

Lake	18°C partly cloudy		20°C partly cloudy		26°C cloudy/rainy		14-15°C pouring		11°C cloudy		20°C sunny		18-22°C sunny		20-23°C sunny		
	Round 1	Round 2	Round 3	Round 4	Round 1	Round 2	Round 3	Round 4	Round 1	Round 2	Round 3	Round 4	Round 1	Round 2	Round 3	Round 4	
	18.6.2013	19.6.2013	26.6.2013	17.7.2013	18.7.2013	19.7.2013	23.7.2013	20.8.2013	21.8.2013	27.8.2013	28.8.2013						
Alasenjärvi	x			x				x								x	
Alinen Rautjärvi	x					x			x				x			x	
Arkiomaanjärvi		x			x					x			x			x	
Joutjärvi		x					x			x							x
Merrasjärvi			x	x				x					x				x
Pääjärvi	x						x			x			x				
Ruuhijärvi		x			x			x					x				x
Työtjärvi			x	x			winds & gusts						x				x

4.1.2.2 Weather conditions

During the sampling campaign, air temperature and precipitation represented typical June-August weather in southern Finland (Figure 4.18) with the exception of a rather cold (11-15 °C) weather period during the 2nd sampling in July 2013 (Table 4.2).

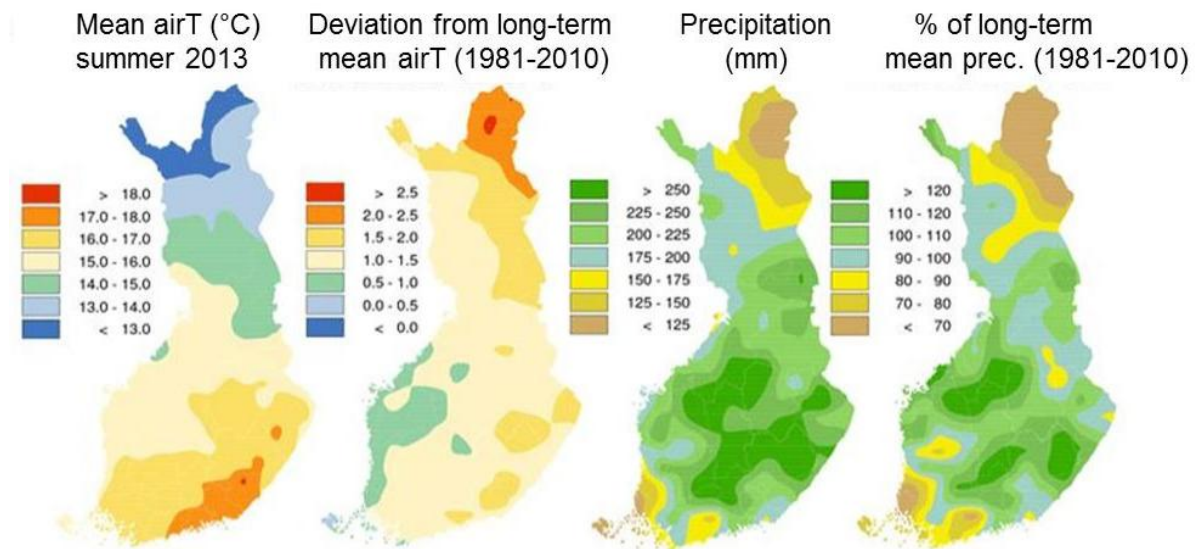


Figure 4.18 - Mean air temperature (airT) and cumulative precipitation and their deviation from long-term values (1981-2010) in Finland in summer 2013 (source: Finnish Meteorological Institute).

4.1.2.3 Transparency

Transparency was measured with a Secchi disc of 20 cm in diameter from the shadow side of the boat, but without the use of "a Secchi binocular", which may have resulted in a slight underestimation of the Secchi depth. The measurements were generally done by both sampling staff and repeated if differences in readings were observed.

4.1.2.4 Profile measurements

Profiles of water temperature (°C), dissolved oxygen (O₂, mg l⁻¹), conductivity (μS m⁻¹), turbidity (NTU), chlorophyll *a* concentration (μg l⁻¹) and the amount of cyanobacteria (cells ml⁻¹) were measured in situ with YSI 6600 V2 fluorometer (Fig. 2.19). The fluorometer was lowered at the speed of ca. 1 cm 2-4 s⁻¹ from the surface to the deep layers of the lake. After measurement the profile readings were downloaded into a laptop in the field for plotting the water temperature and oxygen profiles that were used to estimate the depth of the thermocline.

Later, final chlorophyll results were corrected for water temperature changes with depth (a temperature compensation of 1.48%/°C), and calibrated against measured chlorophyll-*a* values. Chlorophyll values were not corrected for humic substances. To reduce scatter in profiles, the measurements in each depth and for each measured variable were averaged together with ten measurement results from above and 10 results from below of the respective depth.



Figure 4.19 - Vertical profiles of studied variables were recorded in the lakes using submersible YSI 6600 VS sonde.

4.1.2.5 *Estimation of euphotic zone depth and thermocline depth*

For clearwater lakes euphotic zone was estimated by multiplying the Secchi depth readings by 2.0. For humic lakes, euphotic depth estimated by multiplying readings with 1.0. Thermocline depth was estimated from temperature and oxygen profiles both visually and by detecting the depth with highest temperature change.

4.1.2.6 *Phytoplankton and chlorophyll a sampling*

Phytoplankton composition and chlorophyll *a* samples were taken with a 0.4 m or 1.0 m long Limnos tube sampler (Figure 4.20), so that separate samples from the desired sampling depths were integrated to represent euphotic or mixed layer. From each depth 1-3 replicate samples were taken, depending on the lake and the used sampler (volume), and sample water from different depths was pooled into a 60-l clean plastic container (Figure 4.21). Sample bottles for chlorophyll *a* (1-l clean darkened plastic bottles) and phytoplankton (100-250 ml plastic bottles) were filled with the aid of a clean scoop. Phytoplankton samples were preserved with acid Lugol's solution immediately in the field. Plastic bottles with tight caps were used, instead of recommended glass bottles (CEN 15204), for phytoplankton to ease the safe sample delivery to Germany for the analysis.



Figure 4.20 - Phytoplankton samples were taken with a Limnos tube sampler to take integrated water samples that represented either euphotic or mixed layers.



Figure 4.21 - Phytoplankton samples, taken with a Limnos sampler, were pooled in a plastic container, protected from light (see Fig. 2.20), before filling in the sample bottles.

4.1.2.7 *Chlorophyll a* analysis

Chlorophyll *a* samples were filtered onto Whatman GF/F filters and stored in a freezer (-20 °C) until measurements with 1-3 weeks after sampling using a hot ethanol extraction method and spectrophotometer (SFS 5772, 1993).



Figure 4.22 - The study lakes in Finland represented a range of different water colour.

4.2 Sampling of Spanish lakes by CEDEX

José Pahissa & Caridad de Hoyos

Centro de Estudios y Experimentación de Obras Publicas (CEDEX), Centre for Hydrographic Studies, Madrid, Spain.

4.2.1 Background and objectives.

The following report intends to comply with the tasks framed within the contract signed between the Limnologie-Büro Hoehn (LBH) and the CEDEX, within the framework of the *CEN Mandate M424 WP6 project* ("Development and inter-laboratory comparison to enhance the draft European Standard on water quality – Guidance on quantitative and qualitative sampling of phytoplankton from inland waters based on draft document N118 (2008/04/15)").

In this report sampling documentation, experiences, conclusions and recommendations are included, all obtained from the sampling campaign carried out in seven Spanish reservoirs and one natural lake during the summer of 2013.

4.2.2 Introduction.

In order to compare different sampling methods, an international approach has been planned, gathering samples from different countries. The samples are taken using two approaches. To this end, the reservoirs selected in Spanish territory were intended to cover several different situations:

- 1) Polymictic sites.
 - a. Integrated sampling of the euphotic zone, considered as 2.5 times the Secchi depth measured.
 - b. Integrated sample from the top 6 m of water or until 0.25 m from the bottom.
- 2) Clear stratified sites.

- a. Integrated sampling of the euphotic zone, considered as 2.5 times the Secchi depth measured.
- b. Integrated sample from the metalimnion, assuming there is one, or DCM if this is the case.

3) Eutrophic stratified sites.

- a. Integrated sampling of the euphotic zone, considered as 2.5 times the Secchi depth measured.
- b. Integrated sample from the metalimnion, assuming there is one, or DCM if this is the case.

This approach intends to provide the data necessary to compare the two sampling methodologies, to evaluate the effectiveness in obtaining the best phytoplankton community representation, and in turn optimize the results of all the different indexes and metrics used for ecological water quality determination.

The comparison intends to produce results of general applicability in all water body types, or at least determine where the differences may arise, and what type of limitations each sampling method may imply, depending on the trophic status and hydrological characterization of each water body.

Eight representatives of these classes were selected, covering the different water body types, and sampled four times during the summer period of 2013.

4.2.3 Study area.

4.2.3.1 *Location of selected water bodies.*

The reservoirs and lakes for the present study were selected in order to fulfil the variety of water body types required based on historical knowledge and expert judgement. The different water bodies were:

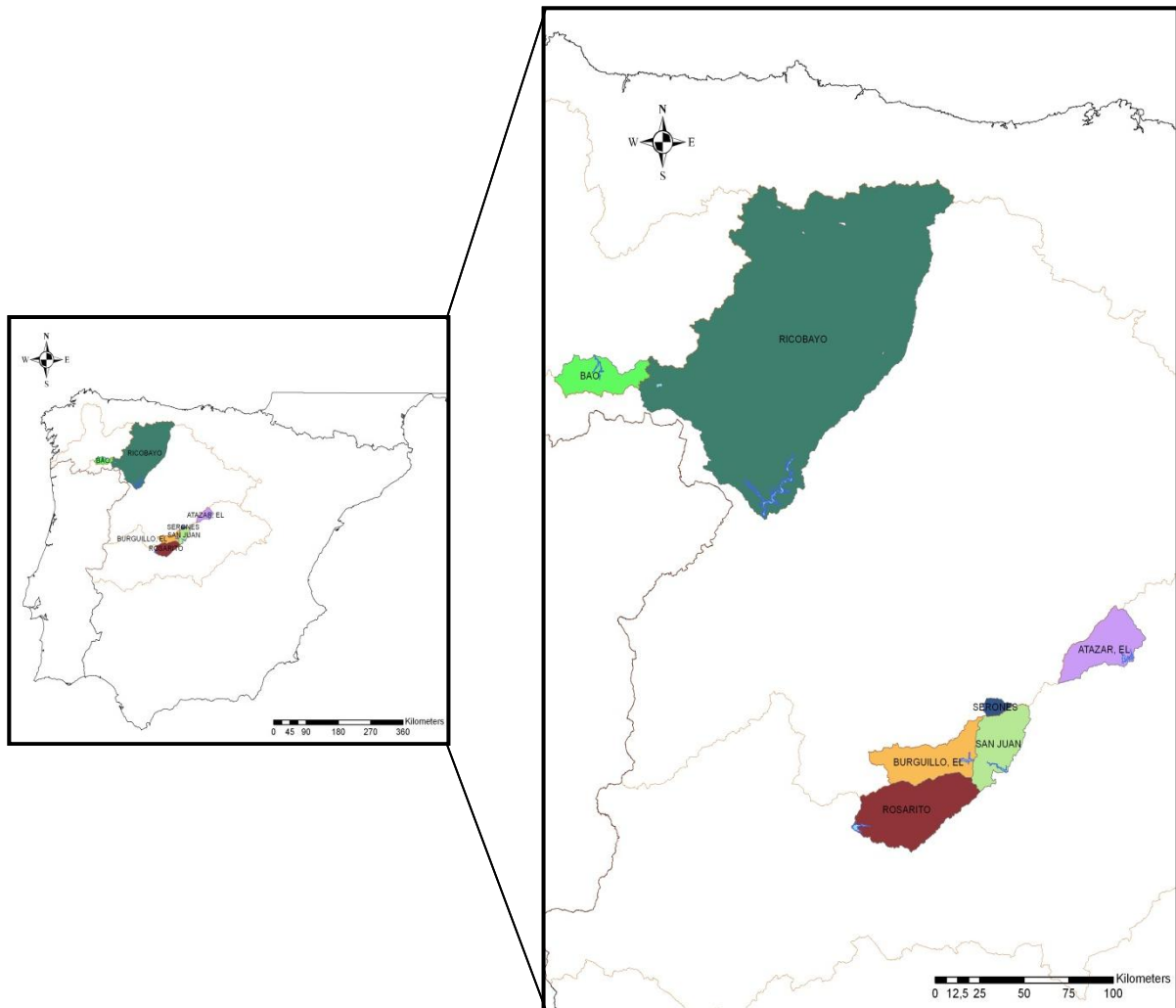


Figure 4.23 - Spanish national map showing the catchments of the selected water bodies in different colours and the water bodies in blue. The bigger map represent a close up of the region where the water bodies are located. Sanabria lake catchment is encompassed within Ricobayo's catchment, and has not been depicted. The water body is represented and labelled.

- 1) Polymictic water bodies:
 - a. Serones reservoir.
 - b. Rosarito reservoir.

- 2) Clear stratified water bodies:
 - a. Sanabria lake.
 - b. El Atazar reservoir.
 - c. Bao reservoir.

- 3) Eutrophic stratified water bodies:
 - a. Burguillo reservoir.
 - b. Ricobayo reservoir.
 - c. San Juan reservoir.

All water bodies are located either in Madrid (El Atazar and San Juan), Castilla León (Serones, Burguillo, Ricobayo and Sanabria lake), Castilla la Mancha (Rosarito) or Galicia (Bao) (Table 4.3).

Table 4.3 - UTM Coordinates of the different water bodies sampled for the CEN project. All of them are in the IMW Sheet SP 30 (International Map of the World).

WB NAME	XUTM	YUTM
SERONES	375622	4505522
ROSARITO	302104	4442712
SANABRIA	689416	4665986
ATAZAR, EL	460178	4529275
BAO	651264	4678982
BURGUILLO, EL	369982	4476228
RICOBAYO	251063	4601926
SAN JUAN	388744	4470148

4.2.3.2 Meteorological factors.

The pre-selection of reservoirs has been, as mentioned above, subedited to the three stratification types required by the contractor.

The selection of reservoirs was conducted based on previous knowledge of the reservoirs throughout the country, including, additionally, knowledge on lakes. These were selected hoping that summer patterns of stratification would follow the lines expected.

Unfortunately, 2013 has been a highly anomalous year in terms of climatic variables and temporal distribution of rain and temperature patterns.

First of all, the three first months of the year were above average in terms of precipitation, especially March, when rainfall was extremely intense throughout most of the Iberian Peninsula (Figure 4.24). This resulted in reservoirs being completely full, at a level that peaked over the mean of the last 10 years after approximately 12-18 weeks of 2013 (Figure 4.25).

The water level in 2013 was lower than the mean of the last 10 years, affecting water management aspects and inner dynamics that ultimately may affect stratification patterns and trophic status.

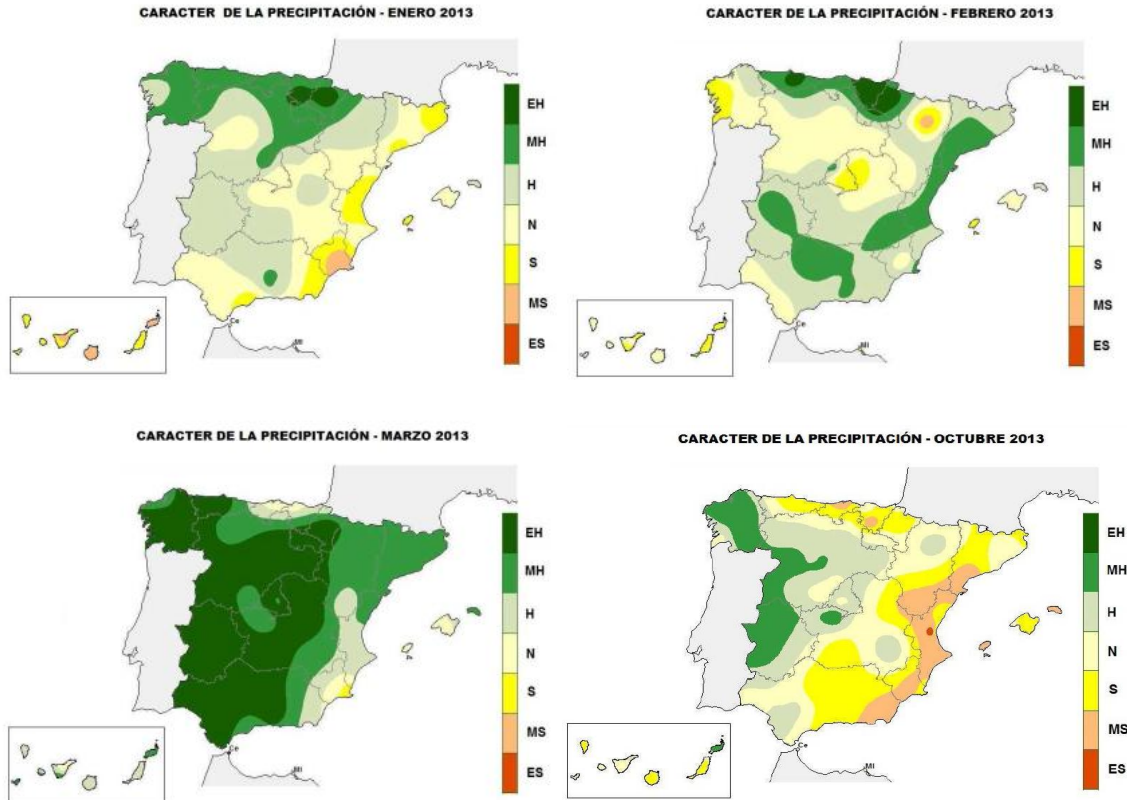


Figure 4.24 - Precipitation in Spanish territory in January, February, March and October as compared to the reference historical period (1971-2000). EH = Extremely wet: Precipitation above the maximum value registered in the reference period; MH = Very wet. Precipitation is registered within the 20% wettest months of the reference period. H = Wet: Precipitation is registered within the 40% to 20% wettest months of the reference period. N = Normal: precipitations recorded are near the mean of the reference period. S = Dry: Precipitation is registered within the 20% to 40% driest months of the reference period. MS = Very dry. Precipitation is registered within the 20% driest months of the reference period. ES = Extremely dry: Precipitation is lower than the minimum registered value for the reference period (1971–2000).

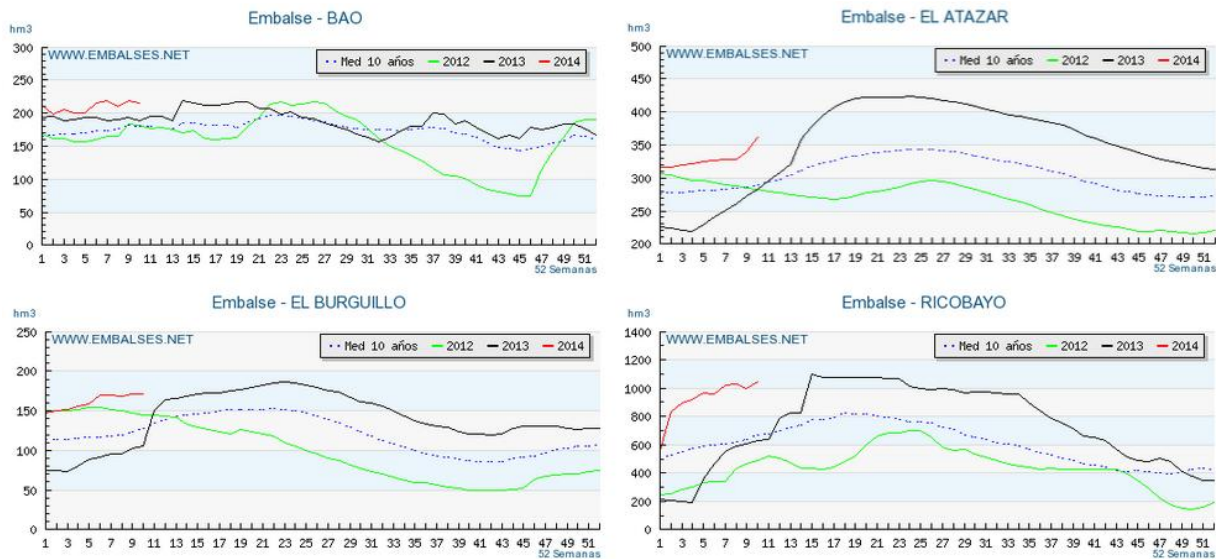


Figure 4.25 - Examples of the contained volume of four of the studied reservoirs. Dotted line represents the mean of the last 10 years (counting from 2010 backwards). In green, data from 2012; in black, data from 2013; in red, data from 2014. Time is counted in weeks after the beginning of the year (X-axis). Source: "www.embalses.net".

Another parameter considered, and essential for the development of the expected vertical profiles, which has been anomalous during the key months of 2013 is mean temperature. As can be seen in the temperature maps from the first six months of 2013, there is a consistent pattern of cold temperatures throughout the most important months for the thermoclines to develop. May and June are cold months compared to the mean of the reference period according to the Spanish climate Services (Figure 4.26).

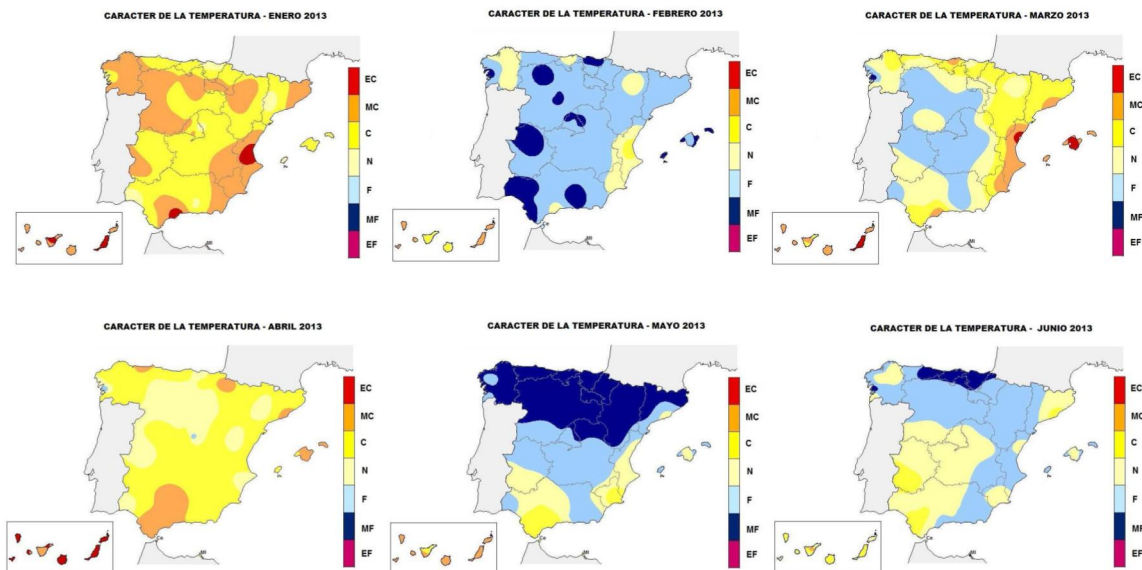


Figure 4.26 - Temperature in Spanish territory in January, February, March, April, May and June as compared to the reference historical period (1971-2000). EC = Extremely warm: Temperature above the maximum value registered in the reference period; MC = Very warm: Temperature is registered within the 20% warmest months of the reference period. C = Warm: Temperature is

registered within the 20% to 40% warmest months of the reference period. N = Normal: Temperatures recorded are near the mean of the reference period. F = Cold: Temperature is registered within the 20% to 40% coldest months of the reference period. MF = Very cold: Temperature is registered within the 20% coldest months of the reference period. EF = Extremely cold: Temperature is lower than the minimum registered value for the reference period (1971–2000).

This temperature pattern in the key months for the development of the thermocline, together with the huge amount of inflowing water, has pushed back in the year the formation of the thermoclines and neglected the basic patterns of stratification expected. Additionally, due to the singularly wet end of summer (October) in the sampling region (Figure 4.24), stratification started to weaken earlier than expected, and thermoclines were weak or inexistent in the samplings around this month.

4.2.3.3 Water body characterization.

Some general morphometric data relative to the sampled water bodies may be relevant for the results of the ongoing study (Table 4.4).

Table 4.4 - Some morphometric data related to the sampled water bodies. The first column refers to the River Basin Districts (in order: Duero, Tajo and Miño-Sil), second to the national water body type, referred to reservoir (R) or lake (L) types.

RBD	Type	Name	Mean depth (m)	Catchment SA (km ²)	WB surface (ha)
DU	R.1	Serones	3.33	108	189
TA	R.3	Rosarito	6.24	1740	1475
DU	L.6	Sanabria	27.7	127	319
TA	R.1	Atazar, El	43.78	925	1069
MS	R.1	Bao	29.02	730	820
TA	R.4	Burguillo	22.86	1049	910
DU	R.11	Ricobayo	19.61	16017	5855
TA	R.5	San Juan	24.92	1922	650

The national types involved in this study are the following:

- Reservoirs type 1: Siliceous (estimated alkalinity < 1 meq/L), wet zone (Humidity index > 0.75), headwaters and high reaches (catchment surface area < 1000 km²), mean annual temperature < 15°C.
- Reservoirs type 3: Siliceous (estimated alkalinity < 1 meq/L), wet zone (Humidity index > 0.75), main network waters (catchment surface area > 1000 km² and smaller than 20000 km²).
- Reservoirs type 4: Siliceous (estimated alkalinity < 1 meq/L), arid zone (Humidity index < 0.75), headwaters and high reaches (catchment surface area < 1000 km²).

- Reservoirs type 5: Siliceous (estimated alkalinity < 1 meq/L), arid zone (Humidity index < 0.75), main network waters (catchment surface area > 1000 km² and smaller than 20000 km²).
- Reservoirs type 11: Calcareous (estimated alkalinity > 1 meq/L), arid zone (Humidity index < 0.75), main network waters (catchment surface area > 1000 km² and smaller than 20000 km²).
- Lakes type 6: Glacial origin, mid altitude mountain (900- 1500 m), deep (> 10 m) and siliceous waters (< 0,2 meq/L)

Table 4.5 - Additional characteristics of the Spanish reservoirs. The data are extracted from the CEDEX database of reservoirs and De Hoyos & Comín, 1999.

Name	Total volume	Reservoir use	Year built	Altitude	Max depth	Mean depth	Alkalinity (derived)	Mean annual temp.	Mean CHL-a
	(hm ³)				(m)	(m)	(meq/L)	(°C)	(µg/L)
SERONES	6.3	Drinking water	1982	1247	11	3.3	0.98	9.7	11.6
ROSARITO	92	Irrigation and hydropower	1958	307	24	6.2	0.86	14.3	61.3
SANABRIA	96.3	.	.	.	51	.	0.05	.	1.9
ATAZAR, EL	468	Drinking water	1972	870	124	43.8	0.45	11.9	1.9
BAO	238	Hydropower	1960	654	98	29.0	0.56	9.5	3.4
BURGUILLO	208	Irrigation and hydropower	1913	729	77	22.9	0.31	11.8	6.2
RICOBAYO	1148	Hydropower	1934	684	92	19.6	1.61	11.9	5
SAN JUAN	162	Irrigation and hydropower	1955	580	67	24.9	0.34	13.1	11

Many of the above listed characteristics do not apply to Sanabria lake. Some other data is given regarding the natural system (Table 4.6). This lake has two distinct basins, an eastern and a western one. The deepest one is the eastern basin.

Table 4.6 - Some parameters relative to lake Sanabria (Sources: Vega et al. 2005 and De Hoyos & Comín, 1999).

Altitude	(masl)	997
Percentage of catchment area occupied by the lake (A/Ac)	(%)	2.73
Volume (V)	(hm ³)	96.3
Maximum length (Lmax)	(m)	3160
Maximum width (E basin) (Bmax)	(m)	1530
Maximum depth (E basin) (Zmax)	(m)	51
Relative depth (Zr)	(%)	2.42
Mean water residence time	(years)	0.48-0.67
Alkalinity	(meq/L)	0.045
Euphotic zone mean chlorophyll-a	(µg/L)	1.9

Serones reservoir (Figure 4.27) is typically a mesotrophic reservoir. It is a shallow water body with a polymictic mixing regime. Oxygen depletion at mid stages of the summer in the deepest water layers is typical

Rosarito reservoir (Figure 4.28) is typically a hypereutrophic reservoir with high chlorophyll-a values and low transparency. It is a shallow water body with a polymictic mixing regime. In this reservoir, water level decreases a lot during the summer.

Sanabria lake (Figure 4.29) is the biggest glacier lake in the Iberian peninsula. It is a moraine lake, oligotrophic or oligo-mesotrophic, with clear water and low chlorophyll-a content. It is a deep water body with a very high renewal rate. In this lake, phytoplankton during the stratification period is just below the thermocline, as nutrient concentration decreased in the upper layers of water.

El Atazar reservoir (Figure 4.30) is typically an oligo-mesotrophic and clear water reservoir with low chlorophyll-a values and high transparency.

Bao reservoir (Figure 4.31) is a clear water reservoir with low chlorophyll-a values and high transparency. Available historical data would classify it as mesotrophic, although the data from 2013 would classify it as oligotrophic.

Burguillo reservoir (Figure 4.32) is typically a meso-eutrophic water reservoir.

Ricobayo reservoir (Figure 4.33) is typically a deep stratified meso-eutrophic reservoir with periodical *Microcystis aeruginosa* blooms.

San Juan reservoir (Figure 4.34) is typically a deep stratified meso-eutrophic reservoir. It is known for sustaining *Microcystis aeruginosa* bloom episodes in summer.

4.2.4 Methodology.

4.2.4.1 Sampling campaign.

4.2.4.1.1 Sampling points.

The sampling points are sited at the deepest point of the water body, in reservoirs, typically around 200 m away from the dam, and in Sanabria lake, in the deepest point of the Western basin (Figure 4.27Figure 4.34). This was checked with an eco-probe that enables to determine

depth from the sampling vessel. The scale of the different maps is not comparable; therefore they are only intended to illustrate the sampling site.

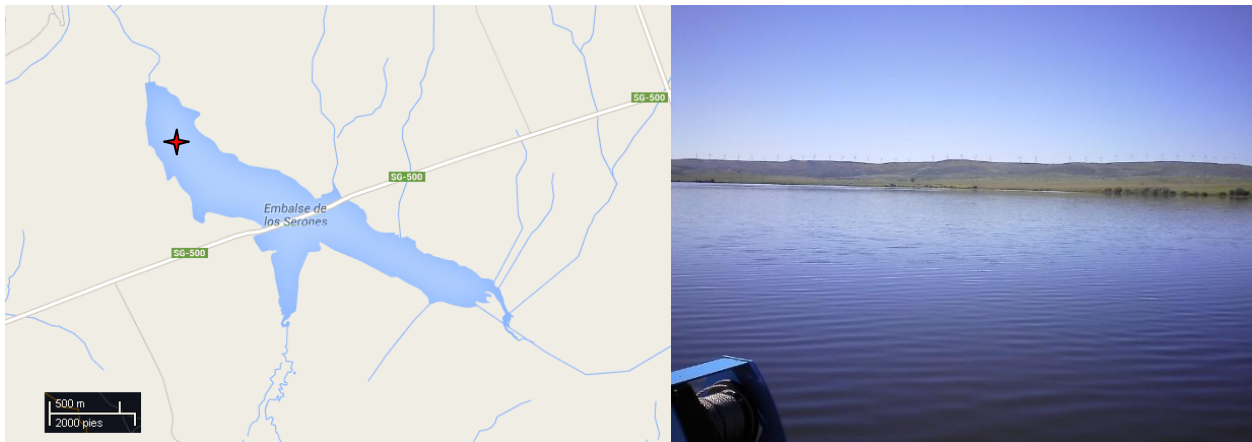


Figure 4.27 - Location of the sampling site for Serones reservoir. The sampling site is located where the red four-pointed star is.



Figure 4.28 - Location of the sampling site for Rosarito reservoir. The sampling site is located where the red four-pointed star is.

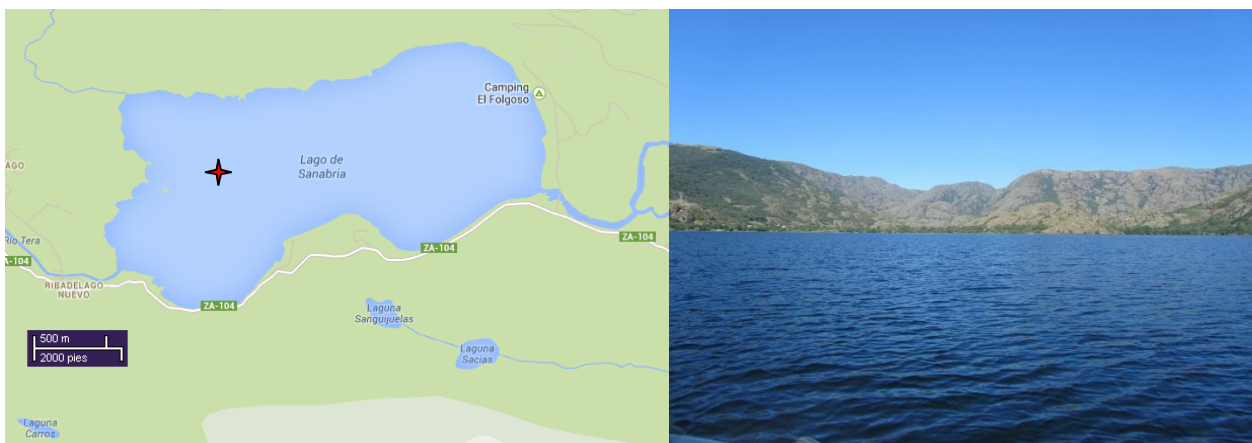


Figure 4.29 - Location of the sampling site for Sanabria lake. The sampling site is located where the red four-pointed star is.

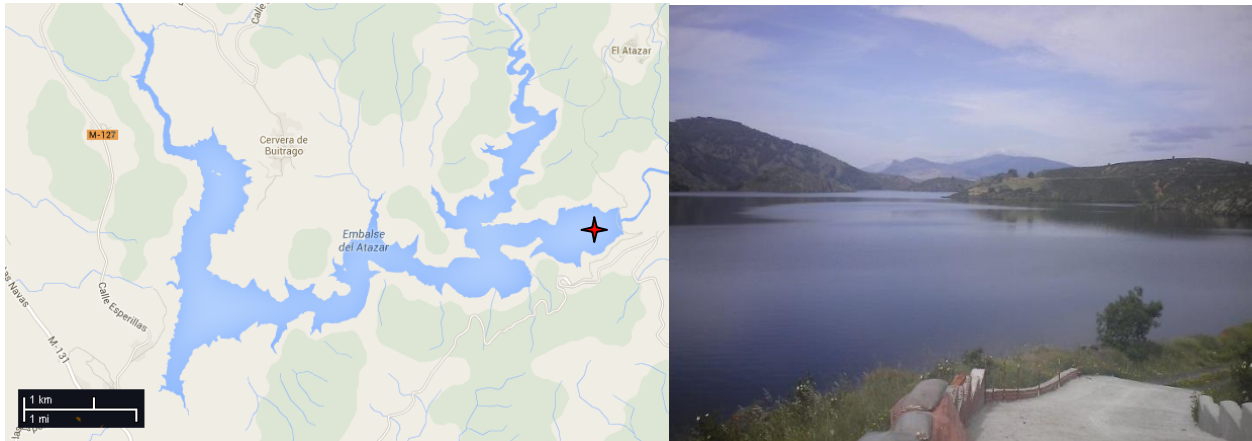


Figure 4.30 - Location of the sampling site for El Atazar reservoir. The sampling site is located where the red four-pointed star is.

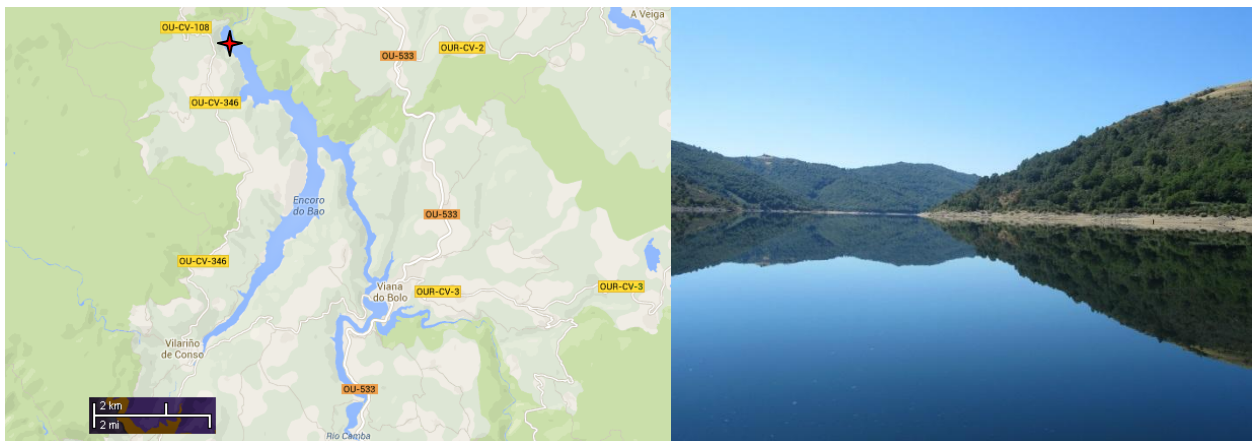


Figure 4.31 - Location of the sampling site for Bao reservoir. The sampling site is located where the red four-pointed star is.

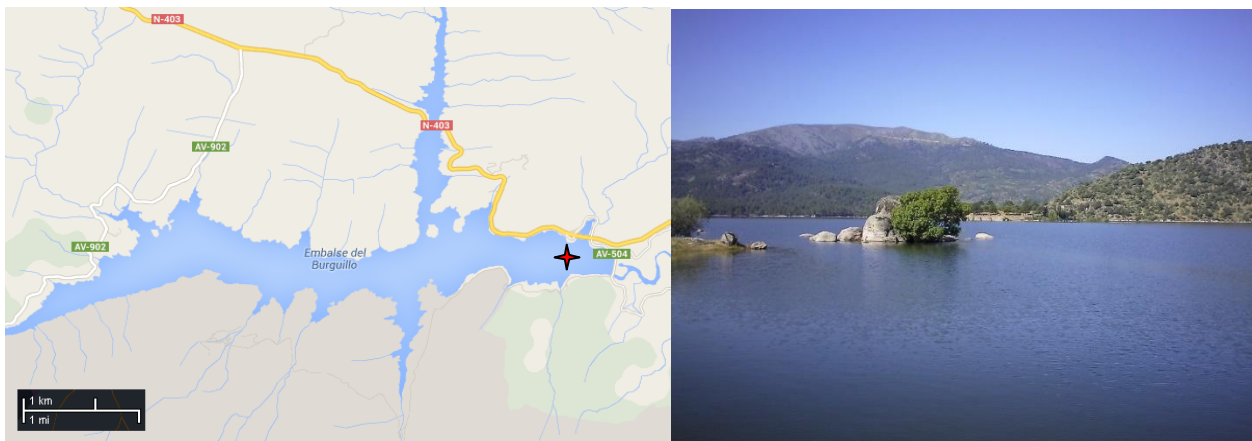


Figure 4.32 - Location of the sampling site for Burguillo reservoir. The sampling site is located where the red four-pointed star is.

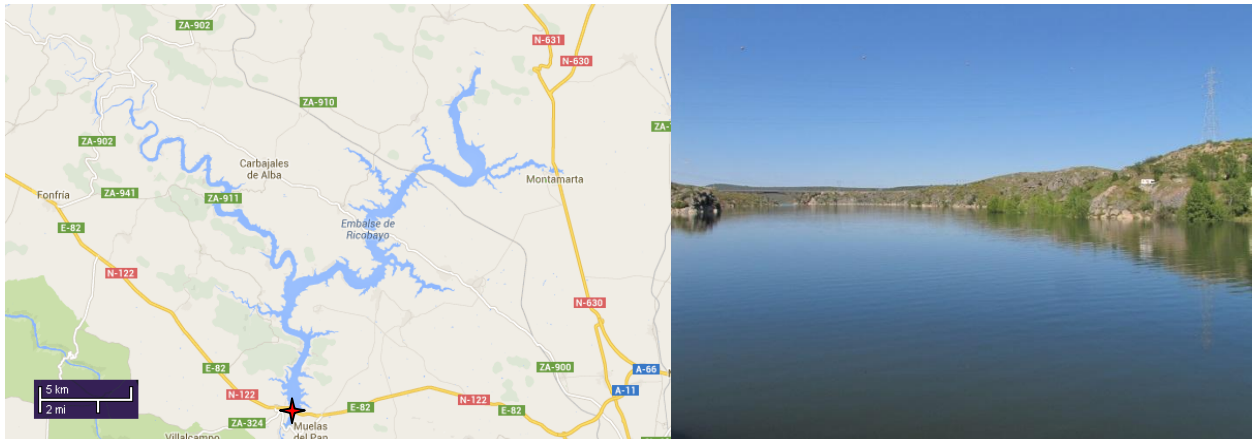


Figure 4.33 - Location of the sampling site for Ricobayo reservoir. The sampling site is located where the red four-pointed star is.

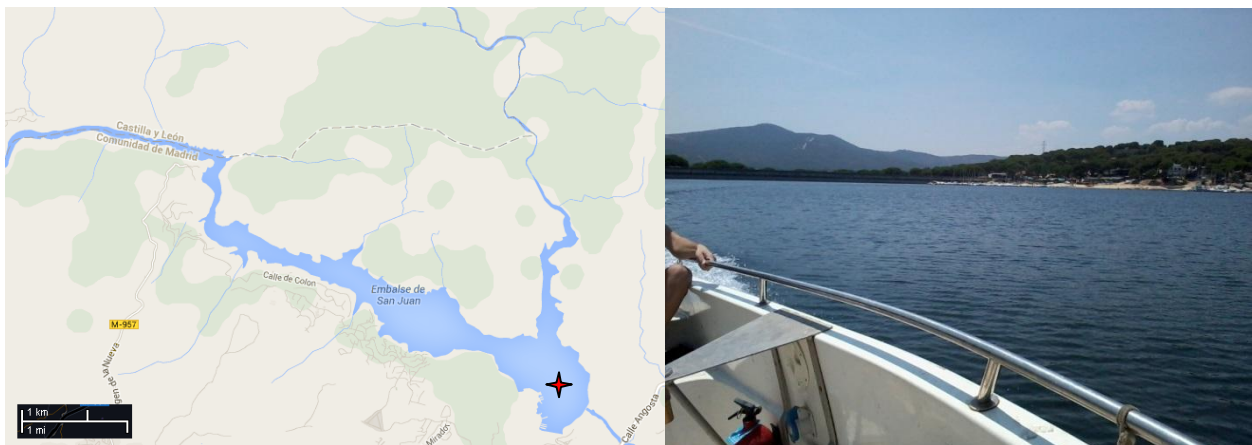
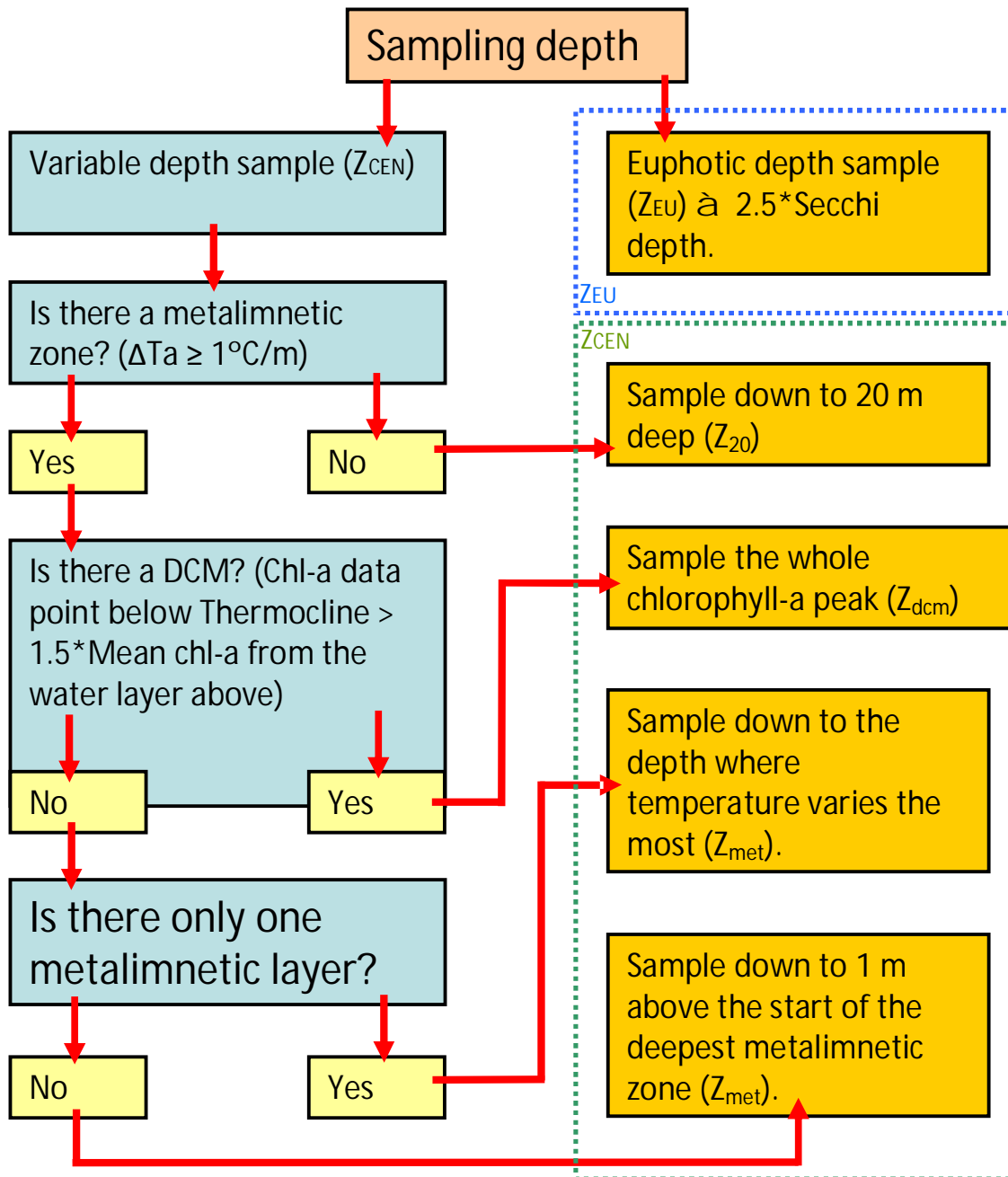


Figure 4.34 - Location of the sampling site for San Juan reservoir. The sampling site is located where the red four-pointed star is.

4.2.4.1.2 Sampling procedure.

When sampling polymictic reservoirs two depths were integrated, one encompasses the euphotic zone and the other down to 6 m deep.

The following flowchart represents the procedure to select the depth at which the water column sample must be integrated when sampling clear stratified and eutrophic stratified water bodies.



In the field, Secchi depth was established with a 20 cm diameter disk. Together with this measurement, a vertical profile was measured, taking readings of temperature, chlorophyll-a, oxygen, pH and conductivity every metre, with an YSI 6600 probe with a 60 m cable (Figure 4.35). The data was inputted in an Excel template that integrated all the above stated criteria for depth sample selection. The integrated samples were then taken with a UWITEC integrated sampler (Figure 4.35).



Figure 4.35 - Left, the YSI 6600 probe being lowered in the water, and right, the UWITEC integrated sampler before extracting the sample to the homogenizing container.

4.2.4.2 Laboratory work.

Samples for chlorophyll-a were filtered with a vacuum system and glass microfiber filters of 0.4-0.6 μm pore. The filter were frozen below - 20° C in liquid nitrogen and analyzed in laboratory according to Parson & Strickland (1963).

Phytoplankton samples (125-250 ml) were fixed with Lugol and kept in a fresh place protected from the light in amber colored glass bottles. They were sent to LBH for analysis

4.2.5 Bibliography.

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http://www.aemet.es/es/serviciosclimaticos/vigilancia_clima/resumenes

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Parson, T.R. & Strickland, J.D.H., 1963. Discussion opf spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophyll and carotenoids. *J. Mar. Res*, 21: 155-163.

Vega, J.C., De Hoyos, C., Aldasoro, J.J., De Miguel, J. & Fraile, H., 2005. Nuevos datos morfométricos para el Lago de Sanabria. *Limnetica* 24 (1-2): 115-122.

4.3 Sampling of German lakes by BTU - CS

Andrew M. Dolman, Ingo Henschke, Jacqueline Rücker, Brigitte Nixdorf
Brandenburg University of Technology Cottbus - Senftenberg (BTU), Department of Freshwater Conservation, Bad Saarow, Germany.

4.3.1 Introduction

This section of the report describes the sampling campaign for German lakes in the Central European Plains region carried out by the Department of Freshwater Conservation at the Brandenburg University of Technology Cottbus – Senftenberg (BTU).

The aim of the work was to compare two different aspects of phytoplankton sampling:

1. The device used to collect water samples
2. The depth of the layer from which water is collected

The question of depth concerns whether to sample the euphotic zone or the upper mixed water layer (epilimnion during stratification; complete water column during mixing, down to a maximum of 20 m). A difference in the phytoplankton biovolume and composition between the mixed and euphotic zones might be expected under two contrasting conditions:

- When the water is clear and the euphotic zone extends below the mixed layer into the metalimnion. Under these conditions a Deep Chlorophyll Maximum (DCM) can form consisting of (specialized) taxa who take advantage of the increased availability of nutrients at the boundary with the hypolimnion.
- When the water is especially turbid and the euphotic zone is very shallow, shallower than the mixed layer. Under these conditions the upper 30cm or so may not be representative of the mixed layer, certain taxa may regulate their buoyancy to either float higher in the water column to gain exposure to light, or sink lower to avoid high temperatures at the surface.

Accordingly, lakes were chosen so as to present these conditions: deep clear water lakes where we expect a DCM formation, and shallow eutrophic lakes where the euphotic zone was expected to be shallower than the mixed layer.

4.3.2 Overview of region and studied lakes

4.3.2.1 *Region and climate*

Ten lakes were sampled between July and October. All are located in the state of Brandenburg in the north east of Germany (Figure 4.36) and experience a similar northern Mediterranean climate with an average of around 550 mm of precipitation per year (Figure 4.38). Temperatures in March 2013 were unusually low, but had returned to normal by April-May, well before sampling started in July (Figure 4.37).



Figure 4.36 - A map of northeast Germany showing the locations of the ten German lakes sampled by BTU in 2013.

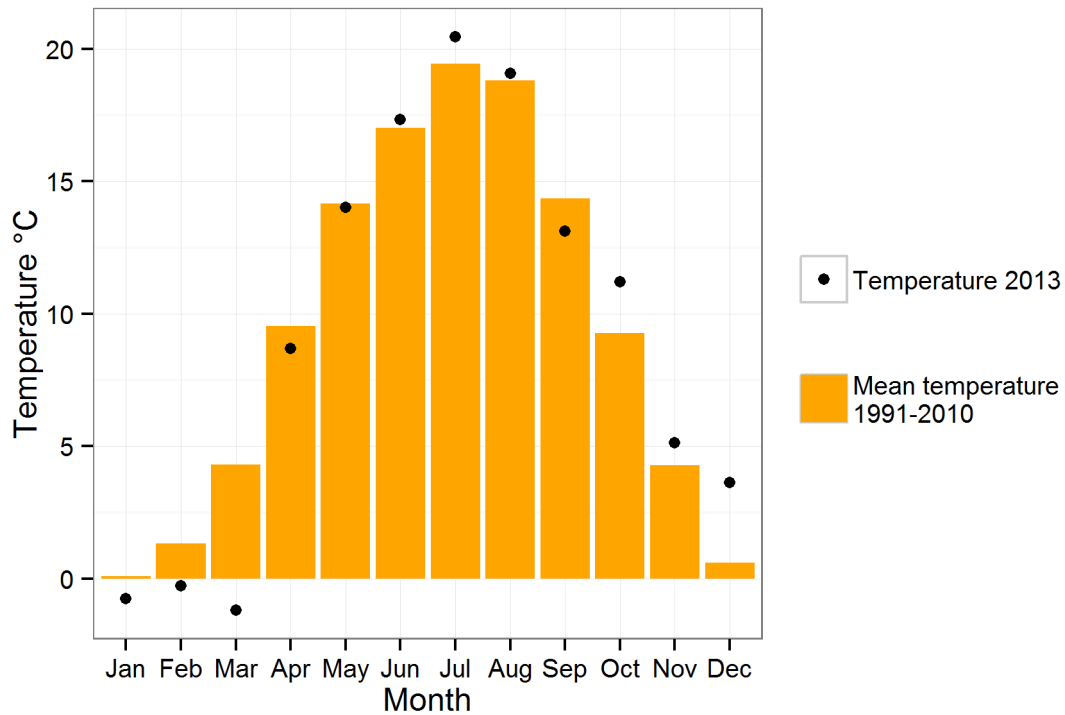


Figure 4.37 - Mean monthly temperature at the Lindenberg weather station in Brandenburg in 2013 (dots), and over the period 1991-2010 (bars). Data were obtained from the Deutscher Wetterdienst (German weather service)

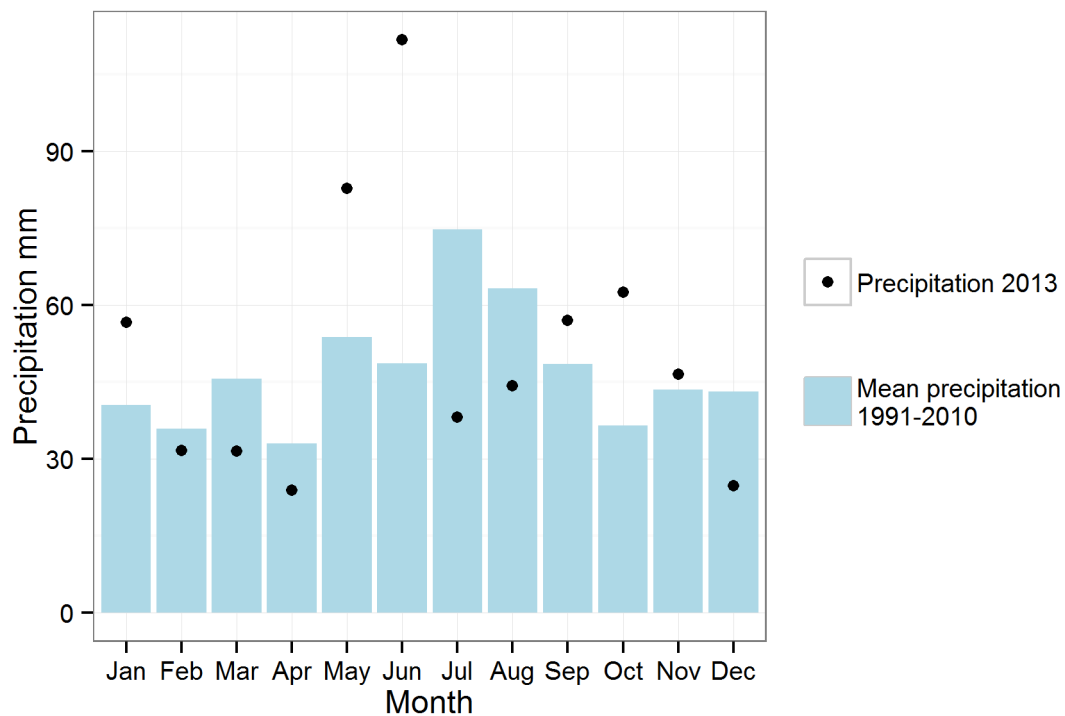


Figure 4.38 - Mean monthly precipitation at the Lindenberg weather station in Brandenburg in 2013 (dots), and over the period 1991-2010 (bars). Data were obtained from the Deutscher Wetterdienst (German weather service)

4.3.3 Description of lakes

The majority of the studied lakes (8/10) are natural lowland calcareous lakes of glacial or post glacial origin. Two lakes, Helenesee and Felixsee, are mining lakes. Felixsee is acidic and therefore rather different to the other 9 lakes, while Helenesee is calcareous and similar to the natural lakes. Table 4.7 gives the names, locations and main characteristics of the German lakes sampled. There follows a map and short description for each lake.

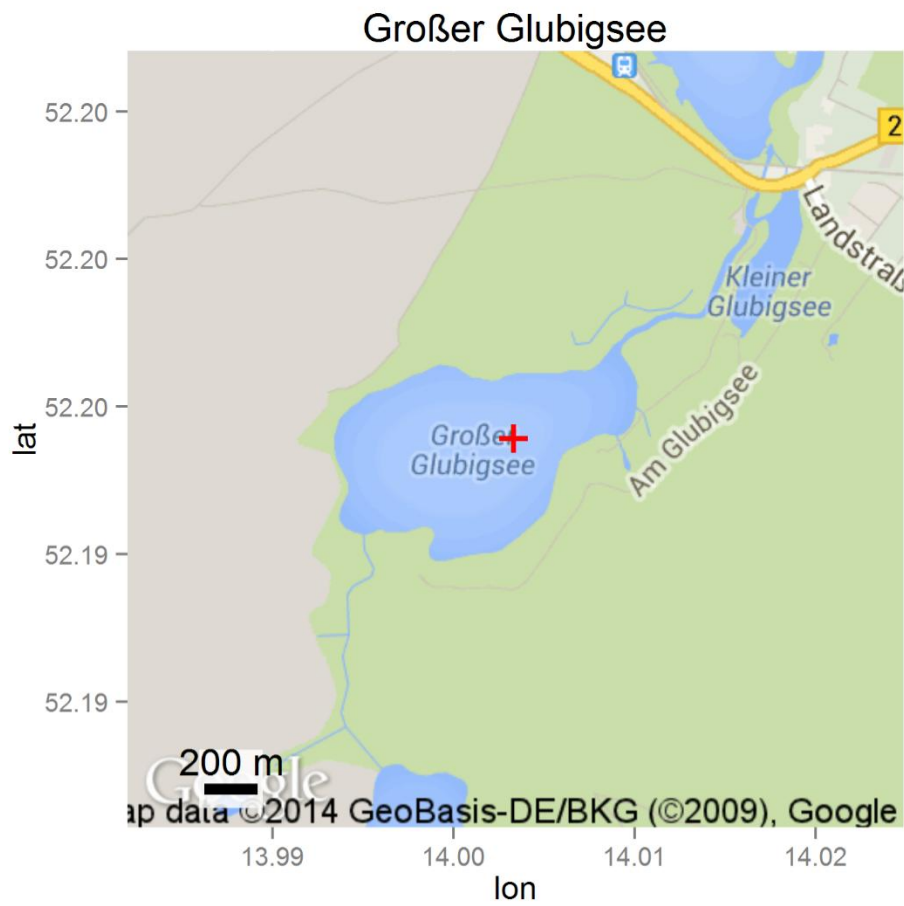
Table 4.7 - Characteristics of the German lakes sampled in 2013. Total phosphorus (TP) and Chlorophyll-a (Chla) data are vegetation period means for the period 2005-2010 (unpublished data from Nitrolimit project, BTU). For Kleiner Wentowsee TP and Chlorophyll-a values are from the summer of 2001 (Glaßer 2002). For Dobrasee they are from April and July 2008; for Felixsee the summer of 2002 (unpublished BTU Bad Saarow data).

Lake	Lake name	Type	Mixing regime	Lon.	Lat.	Mean Depth (m)	Max Depth (m)	Area (ha)	No. visits	TP μg^{-1}	Chla μg^{-1}
DOB	Dobrasee	Clear	Stratified	13.88	52.18	3.6	10	24	2	20	7
FEL	Felixsee	Clear	Stratified	14.55	51.61	13	18	13	2	5	
GGL	Großer Glubigsee	Clear	Stratified	14.00	52.19	4.6	13	56.2	2	38	16
HEL	Helenesee	Clear	Stratified	14.50	52.27	36	55	225	2	10	4.5
SCHER	Schermützelsee	Clear	Stratified	13.79	52.24	17	38	137	2	15	1.2
SPR	Springsee	Clear	Stratified	14.06	52.57	11	18	58.1	2	30	22
STECH	Stechlinsee	Clear	Stratified	13.99	52.18	24	70	412	2	13	5.6
TIE	Tiefer See	Clear	Stratified	13.02	53.14	12	23	59.9	2	20	3.9
LAN	Langer See	Turbid	Polymictic	14.00	52.15	2.2	3.8	147	6	66	86
WENT	Kleiner Wentowsee	Turbid	Polymictic	13.18	53.08	1.9	3.2	49	4	110	

4.3.3.1 Natural lowland calcareous lakes

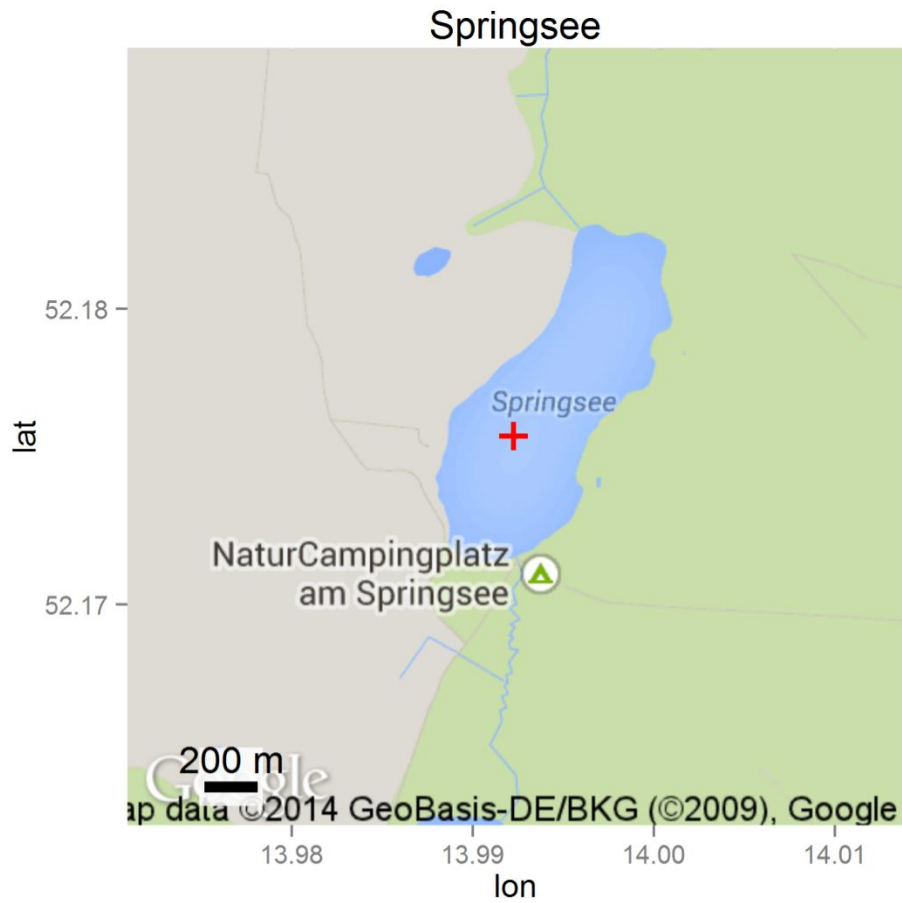
Großer Glubigsee

Großer Glubigsee is a dimictic steep sided lake, with a mean depth of 4.6 m and maximum 13 m. Its catchment comprises approximately 60% woodland & wetland, 2% human settlement, and 36% agricultural land (Nixdorf *et al.* 2004). It is part of the Glubig lake chain along with Springsee and Tiefersee, which were also sampled as part of this project. The lake chain formed during the end of the last glacial period and is a classic glacial melt water gully.



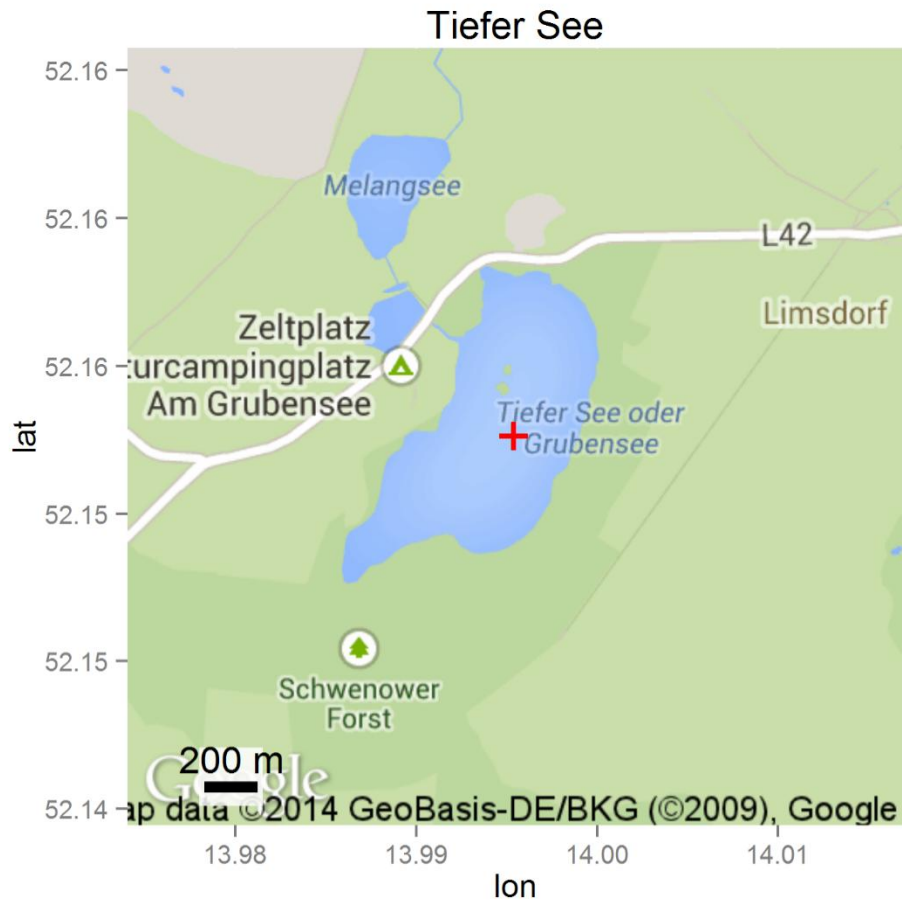
Springsee

Also part of the Glubig lake chain, Springsee is dimictic with a mean depth of 11 m and maximum 18 m. It has a catchment of approximately 88% woodland & wetland, 10% human settlement, and 2% agricultural land (Nixdorf *et al.* 2004).



Tiefer See (Grubensee)

The first lake in the Glubig lake chain, Tiefer See is dimictic with mean depth 12 m, maximum 23 m. It is mostly groundwater fed has low TP and chlorophyll a concentrations (20 and $3.9 \mu\text{g L}^{-1}$ respectively) and is popular for recreational bathing in the summer.



Schermützelsee

Schermützelsee is a deep dimictic Kettle Hole lake, mean depth 17 m, maximum 38 m. It has a 50% agricultural catchment area but is mostly groundwater fed. It has a low TP concentration ($10 \mu\text{g L}^{-1}$) and exceptionally low chlorophyll-a concentration ($1.2 \mu\text{g L}^{-1}$).



Stechlinsee

Stechlinsee is a deep dimictic lake, mean depth 24 m, maximum 70 m, formed from two glacial tunnel valleys. It has a catchment of over 80% woodland, low TP ($13 \mu\text{g L}^{-1}$) and chlorophyll-a ($5.6 \mu\text{g L}^{-1}$). It contains the only population of the Stechlin cisco (*Coregonus fontanae* 2014) a small freshwater whitefish in the family Salmonidae.



Langer See

Langer See is a very shallow polymictic lake with a mean depth of just 2.2 m. It is eutrophic with TP concentrations around $66 \mu\text{g L}^{-1}$.



Kleiner Wentowsee

Kleiner Wentowsee is a shallow (mean depth 1.9 m) polymictic lake fed by canals and influenced by irrigation channels with high nutrient loads. TP concentration in summer is $110 \mu\text{g L}^{-1}$



4.3.3.2 Artificial mining lakes

Helensee (natural like)

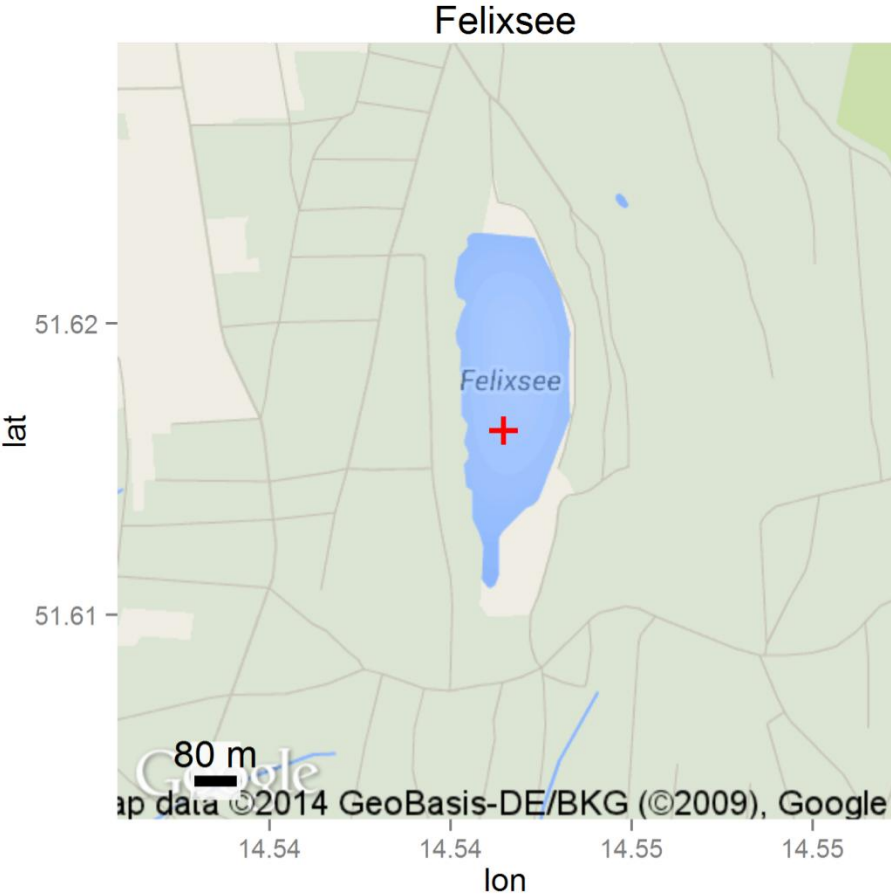
Helensee is a deep (mean depth 36 m, maximum 55 m) coal-mining lake dating from about 1970 (end of filling period), however it behaves as a natural lake with a pH of 8.09 (Nixdorf *et al.* 2004). It is almost exclusively groundwater fed with an 80% wooded catchment. TP

concentration is $10 \mu\text{g L}^{-1}$ and water clarity is good.



Felixsee

Felixsee is a small but deep (mean depth 13 m, maximum 15 m) mining lake dating from 1933. It is acidic, with a pH of 3.8. It is groundwater fed and TP is exceptionally low, less than $5 \mu\text{g L}^{-1}$.



4.3.4 Sampling

All sampling was carried out by boat by Ingo Henschke.

Prior to the collection of water samples, two probes were used to obtain depth profiles of temperature, oxygen, conductivity, and fluorescence. A multi probe (Hydrolab DS5) was used to obtain depth profiles of temperature and oxygen concentration. A FluoroProbe (bbe-Moldaenke) was used to produce a fluorescence depth profile.

The euphotic depth was calculated as 2.5 times the Secchi depth, and the depth of the epilimnion was estimated from the temperature profile with the epilimnion judged to end when the rate of temperature change exceeded 1°C m^{-1} .

4.3.4.1 *Comparison of alternative water sampling devices.*

The majority of sampling to compare devices was carried out in the clear stratified lakes. The euphotic zones of the eight deep clear lakes were each sampled on two occasions with the 5 different sampling devices detailed below. In addition, on one occasion the mixed water layer of shallow Langer See was sampled with all five devices.

4.3.4.1.1 Hose sampler

- Flexible 16 mm diameter hose, weighted at one end, with a thin cord attached to the weighted end and a clamp to close the un-weighted end.
- Weighted end of hose lowered to required depth, upper end sealed below the water with a clamp, weighted end retrieved, contents emptied into barrel, process repeated until the required volume of water was obtained.



Figure 4.39 - Sampling with a hose sampler (photos © Eberhard Hoehn).

4.3.4.1.2 Lasso surface sampler

- 5L glass flask, weighted on one side of the neck, attached to a 5m rope
- Flask thrown away from boat approximately 3m, flask fills with water from upper 30 cm of lake and is retrieved

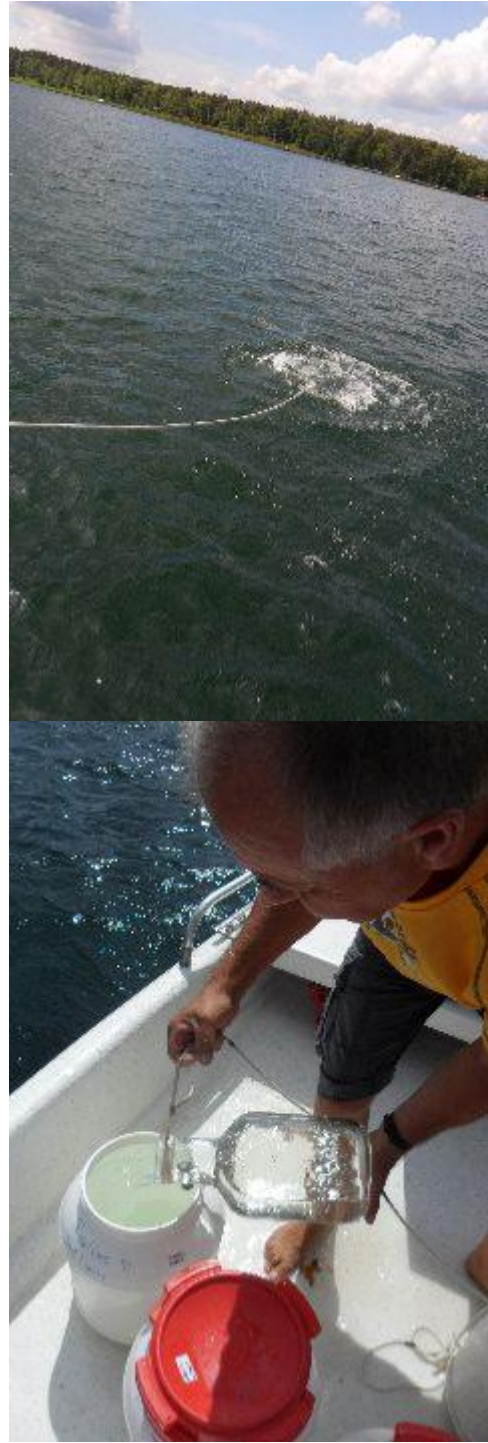


Figure 4.40 The “Lasso” surface sampler (photos © Eberhard Hoehn).

4.3.4.1.3 Limnos sampler 30 cm – discontinuous samples

- 2 liter, 30 cm long, Limnos sampler
- Samples taken every 1 m down to required depth, so 70 cm vertical gaps between samples.



Figure 4.41 A 30 cm Limnos sampler (photos © Eberhard Hoehn).

4.3.4.1.4 Limnos sampler 50 cm – contiguous samples

- 3.5 liter, 50 cm long, Limnos sampler
- Samples taken every 50 cm down to required depth, so no vertical gaps between samples.



Figure 4.42 - A 50 cm, 3.5 L, Limnos sampler.

4.3.4.1.5 IWS – integrating water sampler

- Integrating water sampler
- IWS programmed with sample depth and lowered according to speed calculated by device.



Figure 4.43 - Integrating water sampler.

4.3.4.2 *Sampling to compare different depths, Zmix with Zeu.*

4.3.4.2.1 Deep clear lakes

The eight stratified clear water lakes were each sampled on two occasions during 2013. Separate samples of the mixed water layer Zmix (epilimnion) and euphotic zone Zeu were taken with an integrating water sampler (IWS). The IWS is described in more detail in section 4.3.4.1.5.

4.3.4.2.2 Shallow turbid lakes

Langer See was sampled six times and Kleiner Wentowsee four times during 2013. Samples of the mixed water layer (epilimnion or complete water column) and euphotic zone were taken with a 50 cm long, 3.5 liter Limnos sampler, described in more detail in the section "Comparison of alternative water sampling devices". Samples were taken every 0.5 meters such that they formed a continuous column. i.e. if Zmix was 5 m deep then the Zmix sample comprised of 10 x 50 cm samples. Discrete depth samples were then mixed together on the boat.

4.3.4.3 *Preparation of samples, chlorophyll-a extraction, preservation for cell counting.*

To measure chlorophyll-a concentration, for each sample a pre-determined volume of water was filtered through a glass fibre filter. This volume was dependent on the Secchi depth so as to obtain an appropriate concentration of chlorophyll-a. The glass filters were frozen to before further measurement. Frozen filters were ground up and the ground filters were then extracted three times using 4 ml of 90% ethanol. The extraction was heated for 4 minutes in a 70°C water bath, then further treated with a 2 minute ultrasound exposure. The extraction was then stored in the dark for 1-2 hours before filtering through paper filters to remove the glass fibres. Chlorophyll-a concentrations on the paper filters were then measured photometrically.

Samples for cell counting and biovolume estimation were preserved in Lugol's solution and sent to EBH for counting.

4.3.5 Cited literature

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5 Phytoplankton analysis at LBH

All phytoplankton samples, from rivers and lakes, were analysed at Limnology Bureau Hoehn (LBH), Freiburg, Germany, according to the following methodology.

For the analysis of the phytoplankton samples a sub-volume (1-25 mL) is taken from the total sample volume and placed in a sedimentation tube chamber (Hydrobios) for 24 hours. The microscopic counting of the phytoplankton is performed according to Utermöhl (1958) with an inverted phase contrast microscope at 100x or 125x and 400x or 500x magnification (see HOEHN *et al.* 1998).

Phytoplankton taxonomy primarily follows HUBER-PESTALOZZI (1938-83) and Ettl *et al.* (1978 *et seqq.*). Identification is attempted, as far as possible, to the minimum taxonomic resolution required for the status assessment of natural lakes according to the EU water framework directive (MISCHKE & KUSBER 2009).

The calculation of phytoplankton biomass is made by estimating cell volumes. An average cell volume is established for each taxon. Cell measurements are made from several individuals from each studied sampling site. Length and width measurements are made with digital image analysis software (Intec EasyMeasure 1.7). Measurements exclude the gelatinous cell covering (see WILLEN 1976, ROTT 1981), and taxon-specific geometric shapes and volume formulae are used following (ROTT 1981 + 1983, DEISINGER 1984, phytoplankton register of Institute for Botany University Innsbruck, as well own formulae (HOEHN *et al.* 1998), see there Table 1). An average cell volume for each taxon is calculated as the median volume of the individual measured cells.

The total biovolume of a taxon is calculated by multiplying the average cell volume by the cell concentration (cells/L). Because the specific density of free-floating phytoplankton is barely distinguishable from that of water, the biovolume can be converted to biomass with the expression (1,000,000 mm³ biovolume = 1 mg biomass, LOHMANN 1908). The biomass of the individual taxa are then summed to obtain the total phytoplankton biomass.

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6 Lakes: data analysis and results

Data collected from lakes in Finland, Spain and Germany were analysed together to address the following specific questions about phytoplankton sampling in lakes.

6.1 Influence of the water collection device on the measured chlorophyll *a* concentration and/or phytoplankton biovolume?

Four different devices for sampling from a defined column of water were compared.

- Integrating water sampler (IWS)
 - IWS programmed with sample depth and lowered according to calculated speed.
- Limnos 50 cm Contiguous
 - 3.5 liter, 50 cm long, Limnos sampler
 - Samples taken every 50 cm down to required depth, so no vertical gaps between samples.
- Limnos 30cm Spaced
 - 2 liter, 30 cm long, Limnos sampler
 - Samples taken every 1 m down to required depth, so 70 cm vertical gaps between 30 cm samples.
- Hose
 - Flexible 16mm diameter hose, weighted at one end, with a thin cord attached to the weighted end and a clamp to close the un-weighted end
 - Weighted end of hose lowered to required depth, upper end sealed below the water with a clamp, weighted end retrieved, contents emptied into barrel, process repeated until the required volume of water was obtained. 200 ml per meter.

The four devices were used to collect water from the euphotic zone of eight stratified German lakes, on four occasion each (see section 4.3.4.1).

Agreement between the four different devices was examined using Tukey mean-difference plots, also known as Bland-Altman plots (Bland and Altman 1986). These show the difference between measurements taken by two or more devices on the y-axis, against the means of these measurements on the x-axis (e.g. Figure 6.1). In this case, as there were four devices, there are four subplots; in each subplot the x-axis gives the mean value measured across all four devices, and the y-axis the difference between this mean, and one specific device. Because these calculations were performed on log₁₀ transformed data, the y-axis shows the device specific value as a proportion of the mean across devices.

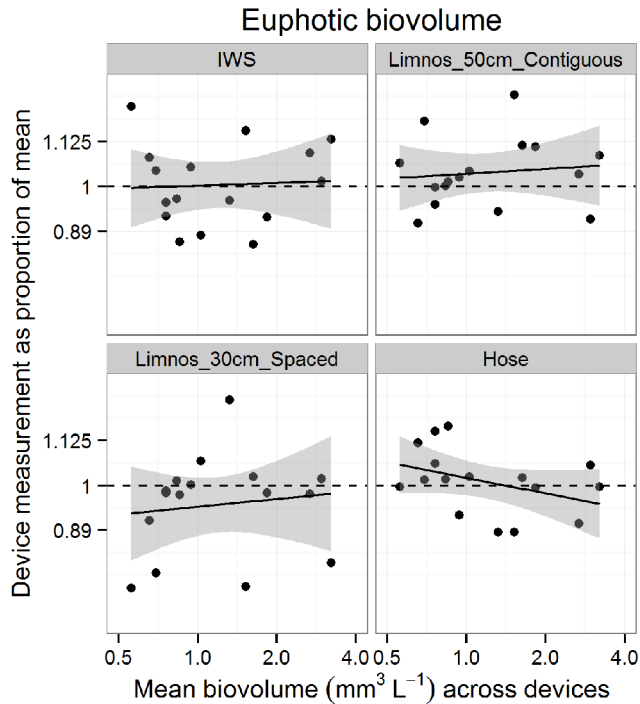


Figure 6.1 - agreement between phytoplankton biovolume estimates for euphotic zone water samples taken with four different collection devices.

The information in these mean-difference plots is summarised in tables as % Disagreement and % Bias. Table 6.1 summarises the results seen in Figure 6.1. *Disagreement* is given by the standard deviation of the positions of the points on the y-axis and is the average % difference between values for a specific device and the mean across all four devices. *Bias* is the mean of the positions of the points on the y-axis and measures whether "on average" using a particular device tends to result in an under or overestimated biovolume relative to the mean.

Table 6.1 - summary statistics for agreement between phytoplankton biovolume estimates for euphotic zone water samples taken with four different collection devices.

Variable	Device	% Bias	% Disagreement	n
Biovolume [mm ³ L ⁻¹]	IWS	0 (-2 - 3)	11 (9 - 14)	16
Biovolume [mm ³ L ⁻¹]	Limnos 50 cm Contiguous	4 (1 - 6)	10 (8 - 11)	16
Biovolume [mm ³ L ⁻¹]	Limnos 30cm Spaced	-5 (-8 - -2)	14 (11 - 17)	16
Biovolume [mm ³ L ⁻¹]	Hose	1 (-1 - 3)	9 (7 - 10)	16

The four devices showed very similar levels of agreement and bias. Agreement is indicated by the vertical scatter of the points, while bias is indicated by the position of the solid regression line relative to the dashed horizontal line. The dashed horizontal line is at height 1, indicating perfect agreement. If the regression line deviates significantly from this horizontal line, this indicates a bias in the samples taken by that particular device. None of the devices show

significant bias as the confidence region for each regression line overlapped with the horizontal line.

The scatter of the individual points shows how well individual samples taken by the different devices agree. All four devices show similar levels of scatter, with slightly more scatter for the Limnos 30cm Spaced sampling method. This was the only method that took a "non-contiguous" set of samples from the water column.

Overall, agreement and bias were similar for chlorophyll *a* measurements as for biovolume (Figure 6.2 and Table 6.2), but with two samples that were large outliers for particular devices. In both cases (Großer Glübigsee on 26.07.2013 and Dobrasee 14.08.2013) the depth of the euphotic zone corresponded closely with a large spike in their fluorescence profiles (ref to depth profile). So the variation was probably not caused the devices themselves, but rather by variation in the depth to which they were lowered.

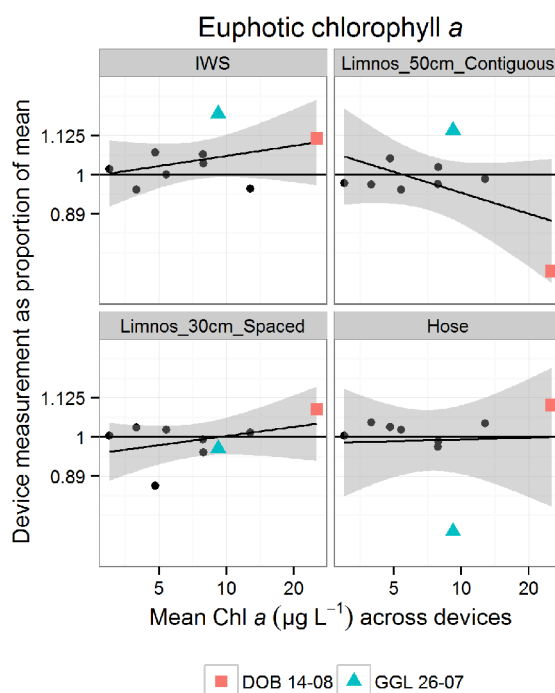


Figure 6.2 - agreement between chlorophyll *a* estimates for euphotic zone water samples taken with four different collection devices

Table 6.2 - summary statistics for agreement between chlorophyll *a* [$\mu\text{g L}^{-1}$] estimates for euphotic zone water samples taken with four different collection devices

Variable	Device	% Bias	% Disagreement	n
Chlorophyll <i>a</i> [$\mu\text{g L}^{-1}$]	IWS	4 (2 - 7)	8 (6 - 9)	9
Chlorophyll <i>a</i> [$\mu\text{g L}^{-1}$]	Limnos 50 cm Contiguous	-2 (-6 - 1)	12 (9 - 15)	9
Chlorophyll <i>a</i> [$\mu\text{g L}^{-1}$]	Limnos 30cm Spaced	-1 (-3 - 1)	7 (5 - 8)	9
Chlorophyll <i>a</i> [$\mu\text{g L}^{-1}$]	Hose	-1 (-4 - 3)	11 (9 - 14)	9

6.2 Influence of the water layer sampled

In the following series of analyses we examined the influence of the water layer sampled (or depth sampled down to) on the measured phytoplankton biovolume and chlorophyll *a* concentration. Relevant samples for comparison were taken in Finland, Germany and Spain.

6.2.1 Influence of sampling from either the euphotic zone or the upper mixed layer.

A frequent decision is whether to sample from the euphotic zone or the upper mixed layer of lakes.

Figure 6.3 gives a series of Tukey mean-difference plots showing the proportional difference between the phytoplankton biovolume measured in the euphotic zone versus the upper mixed water layer. The data are divided by region (columns) and according to whether the euphotic zone extends deeper than the mixed layer (bottom row, clearwater lakes) or is shallower than the mixed layer (turbid or humic lakes). As before, the dashed horizontal line at 1 indicates perfect agreement between the two samples. Figure 6.4 shows the same but for chlorophyll *a*. Summary statistics are given in Table 6.3.

There was a small positive bias in biovolume measured in the euphotic zone relative to the upper mixed layer for some sets of lakes. For the clearwater German (BTU) lakes this bias was quite large, especially for chlorophyll *a*.

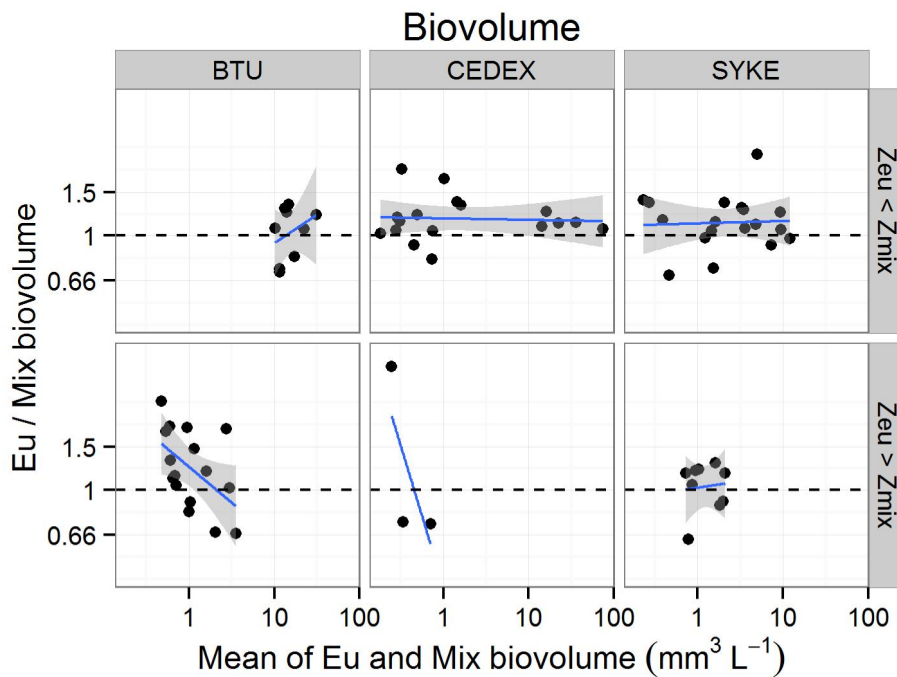


Figure 6.3 - agreement between biovolume estimates for euphotic zone (Z_{eu}) and mixed layer (Z_{mix}) samples. Upper row is for lakes where the euphotic depth was less than the depth of the mixed layer (turbid and humic lakes), the lower row is for lakes where the euphotic zone extended below the upper mixed layer (clearwater lakes).

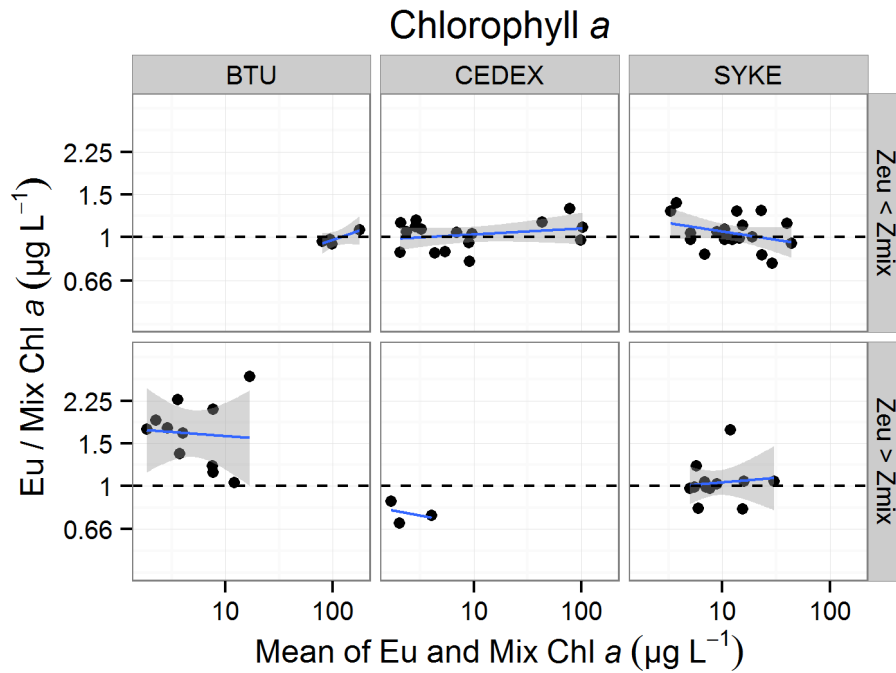


Figure 6.4 - agreement between chlorophyll a estimates for euphotic zone (Zeu) and mixed layer (Zmix) samples. Upper row is for lakes where the euphotic depth was less than the depth of the mixed layer (turbid and humic lakes), the lower row is for lakes where the euphotic zone extended below the upper mixed layer (clearwater lakes).

Table 6.3 - summary statistics for the agreement between estimates for euphotic zone (Zeu) and mixed layer (Zmix) biovolume and chlorophyll a.

Variable	Institute	Zeu_Zmix	% Bias	% Disagreement	n
Biovolume [mm^3L^{-1}]	BTU	Zeu < Zmix	3 (-5 – 12)	28 (21 - 35)	9
Biovolume [mm^3L^{-1}]	CEDEX	Zeu < Zmix	17 (11 – 23)*	23 (18 - 27)	17
Biovolume [mm^3L^{-1}]	SYKE	Zeu < Zmix	13 (6 – 20)*	29 (23 - 34)	18
Biovolume [mm^3L^{-1}]	BTU	Zeu > Zmix	22 (11 – 34)*	45 (35 - 54)	16
Biovolume [mm^3L^{-1}]	CEDEX	Zeu > Zmix	20 (-26 – 95)	132 (64 - 228)	3
Biovolume [mm^3L^{-1}]	SYKE	Zeu > Zmix	3 (-4 – 11)	26 (19 - 33)	9
Chlorophyll a [$\mu\text{g L}^{-1}$]	BTU	Zeu < Zmix	-2 (-4 – 1)	6 (4 - 8)	4
Chlorophyll a [$\mu\text{g L}^{-1}$]	CEDEX	Zeu < Zmix	2 (-1 – 6)	15 (12 - 17)	16
Chlorophyll a [$\mu\text{g L}^{-1}$]	SYKE	Zeu < Zmix	4 (0 – 8)	17 (14 - 20)	19
Chlorophyll a [$\mu\text{g L}^{-1}$]	BTU	Zeu > Zmix	64 (50 – 80)*	36 (28 - 46)	11
Chlorophyll a [$\mu\text{g L}^{-1}$]	CEDEX	Zeu > Zmix	-23 (-28 – -18)*	11 (6 - 16)	3
Chlorophyll a [$\mu\text{g L}^{-1}$]	SYKE	Zeu > Zmix	3 (-2 – 8)	20 (16 - 25)	13

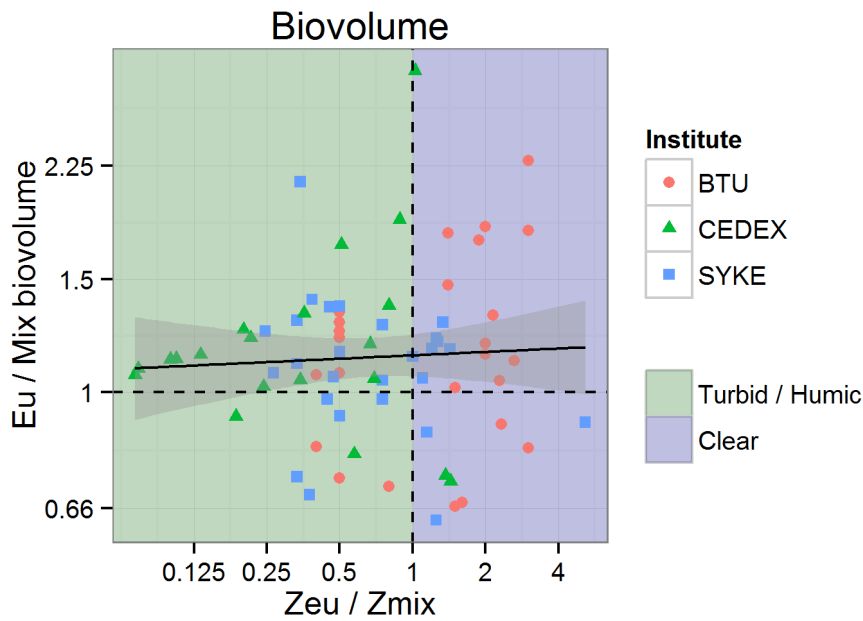


Figure 6.5 - agreement between biovolume estimates for euphotic zone (Z_{eu}) and mixed layer (Z_{mix}) samples plotted against the ratio of the depth of the euphotic zone and upper mixed layer

Figure 6.5 shows the proportional difference in phytoplankton biovolume between the euphotic zone and mixed layer as a function of the proportional difference in the depth of the euphotic vs. mixed layer. Figure 6.6 shows the same for chlorophyll *a*.

Phytoplankton biovolume tended to be greater in the euphotic zone, for both lakes where the euphotic depth was less than the mixing depth, and lakes where the euphotic zone extended below the upper mixed layer (Figure 6.5).

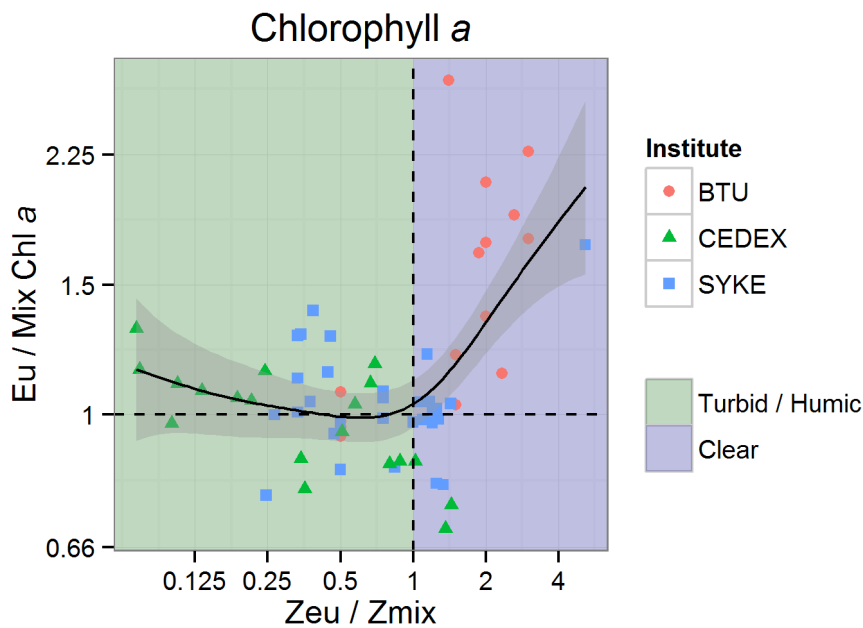


Figure 6.6 - agreement between chlorophyll *a* estimates for euphotic zone (Z_{eu}) and mixed layer (Z_{mix}) samples plotted against the ratio of the depth of the euphotic zone and upper mixed layer

The pattern for chlorophyll *a* was much stronger, chlorophyll *a* concentration was greater in the euphotic zone than the mixed layer when the euphotic zone extended more than about 1.5x deeper than the mixed layer. This occurred mostly in the German data (BTU), and once in the Finnish data (SYKE)(Figure 6.5). There was also a tendency for euphotic chlorophyll *a* to be greater than mixed layer chlorophyll *a* when the euphotic depth was much less than the mixed depth. This was seen in the Spanish data (CEDEX) but the effect was much smaller than that for $Z_{eu} > Z_{mix}$.

6.2.2 Comparison of surface-to-2m samples with euphotic and mixed layer samples

In the Finnish lakes (SYKE) an additional comparison was made between the chlorophyll *a* content of fixed depth samples (from the surface to 2m) and variable depth samples of the euphotic zone or upper mixed layer.

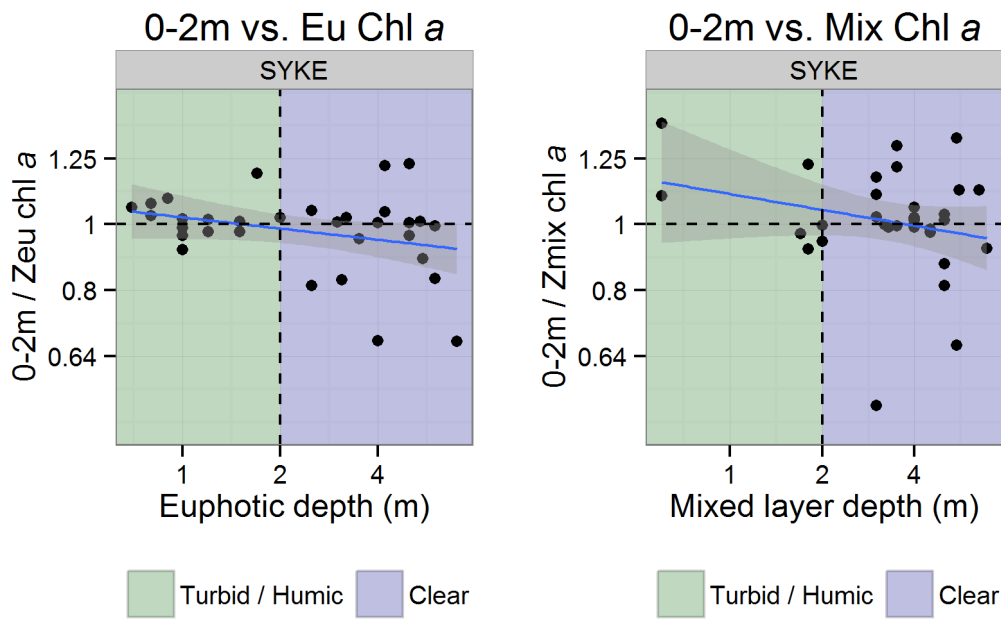


Figure 6.7 - agreement between chlorophyll a estimates for surface to 2m samples and euphotic zone (left) and mixed layer (right) samples in Finnish lakes.

The amount of disagreement depended on the whether the euphotic sample extended deeper than 2m or less than 2m, with deeper samples having greater disagreement. However, even the higher levels of disagreement were 20% or less (Table 6.4). Bias was significant (but small -6%) for 0-2 m vs. Euphotic sample in clear lakes, with more chlorophyll a in the euphotic zone, when it extended deeper than 2 m. In contrast the 0-2 m sample contained more chlorophyll than the mixed layer when the mixed layer was very shallow (< 1m).

Table 6.4 - summary statistics for comparison between surface-2m samples and euphotic zone or mixed layer samples in Finnish lakes

variable	Comparison	Depth_ratio_2	% Bias	% Disagreement	n
Chla_µg_L	Z2 vs. Zeu	Zeu < 2m	2 (0 - 4)	7 (5 - 8)	14
Chla_µg_L	Z2 vs. Zeu	Zeu > 2m	-6 (-9 - -2)*	18 (15 - 21)	18
Chla_µg_L	Z2 vs. Zmix	Zmix < 2m	7 (1 - 13)*	17 (12 - 22)	7
Chla_µg_L	Z2 vs. Zmix	Zmix > 2m	-0 (-4 - 3)	21 (18 - 24)	25

6.2.3 Comparison of surface-to-DCM with euphotic zone samples

In the Spanish reservoirs (CEDEX), when a DCM was detected, a comparison was made between sampling the euphotic zone and sampling to a depth determined from the fluorescence profile to encompass the DCM. This included both cases where the DCM ended within the euphotic zone, so DCM samples were shallower than Zeu, and cases where the DCM extended below the euphotic zone so that DCM samples went deeper than euphotic samples.

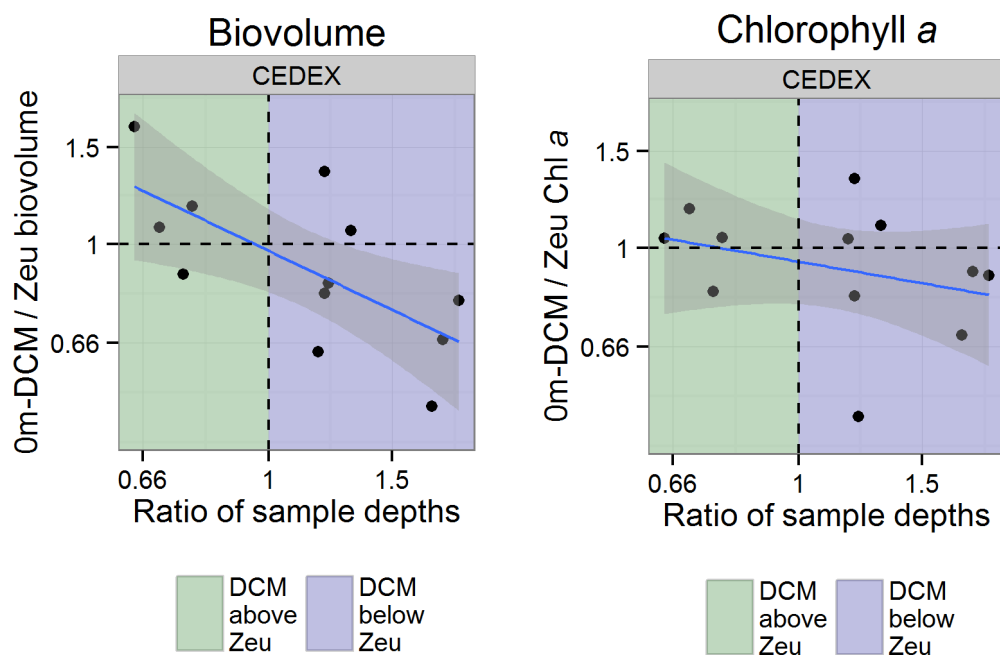


Figure 6.8 - agreement between surface-to-below-DCM samples and euphotic zone samples in Spanish reservoirs for biovolume and chlorophyll *a*.

Where DCM samples were shallower than euphotic samples there was a slight tendency for higher biovolume in the DCM sample, as might be expected as water with the highest concentration of phytoplankton was collected, and water with less phytoplankton excluded from these samples (Figure 6.8, Table 6.5). However, when the DCM was below Zeu, DCM samples had lower biovolume than Zeu samples, the reverse of what might be expected. In some cases the DCM apparent on fluorescence profiles may in fact have been bacterial, so that water samples did not then in fact contain extra chlorophyll *a* or phytoplankton biovolume.

Table 6.5 - summary statistics for surface-to-below-DCM vs. euphotic zone comparisons.

variable	DCM_location	% Bias	% Disagreement	n
Chla_μg_L	DCM above Zeu	2 (-6 - 9)	16 (10 - 22)	4
Chla_μg_L	DCM below Zeu	-13 (-22 - -3)*	36 (26 - 46)	8
Biovolume_mm3L	DCM above Zeu	16 (2 - 32)*	29 (18 - 42)	4
Biovolume_mm3L	DCM below Zeu	-20 (-28 - -11)*	36 (26 - 47)	8

6.2.4 Comparison of surface samples with samples from the euphotic zone or upper mixed layer.

This comparison is similar to those above between e.g. euphotic and mixed layer samples, but sampling just the surface (upper 30 cm) of a lake allows for a simpler sampling procedure and the possibility of sampling from the shore or outflow of a lake – removing the need for a boat.

In the German lakes, samples from the surface (upper 30 cm) of the water column were compared to samples from the upper mixed layer and euphotic zone taken with an IWS.

Surface sampler

- Lasso sampler
 - 5L glass flask on a 5m rope
 - Flask thrown away from boat approximately 3m, flask fills with water from upper 30cm of lake and is retrieved.

The surface sampler by definition cannot sample phytoplankton populations that are present below the upper 30 cm of the water column. We might therefore expect better agreement with samples from the upper mixed water layer than with samples from the euphotic zone, particularly when the euphotic zone extends significantly below the upper mixed layer.

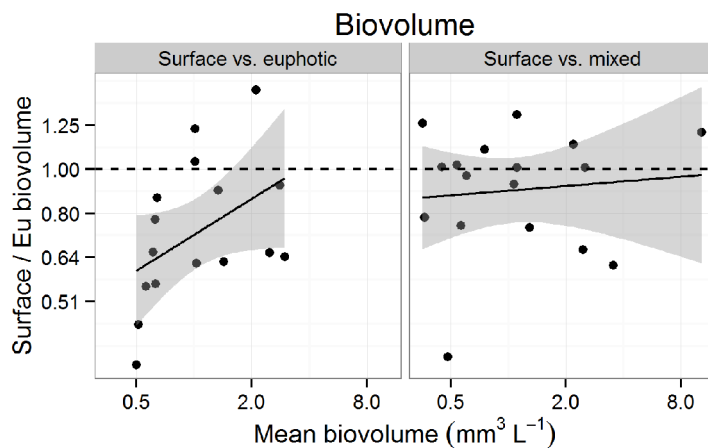


Figure 6.9 - agreement between phytoplankton biovolume estimates from a surface water sample and euphotic zone (left), and mixed layer (right) samples.

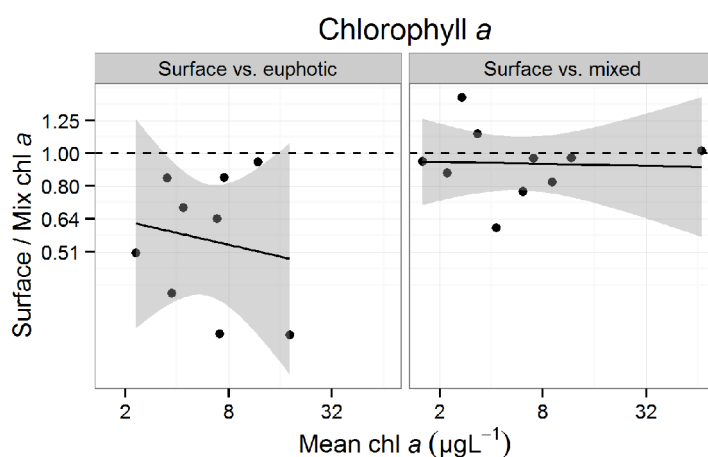


Figure 6.10 - agreement between chlorophyll a estimates from a surface water sample and euphotic zone (left), and mixed layer (right) samples.

Indeed there was a considerable difference between surface and euphotic samples, with an average -27% bias and 44% disagreement in biovolume (Figure 6.9, Table 6.6) and -45% bias and 58% disagreement in chlorophyll *a* (Figure 6.10, Table 6.6). The effect may be larger for chlorophyll *a* than for biovolume as phytoplankton near the surface are likely to have a lower cellular chlorophyll *a* content than those deeper in the water column.

However, it must be emphasised that these lakes and sampling dates were specifically chosen so as to encounter large differences between the depths of the mixed and euphotic water layers and to maximise the chances of finding deep chlorophyll maxima. The extent to which surface samples are biased will depend on the vertical distribution of the phytoplankton community.

If we compare surface samples to samples from the upper mixed water layer the agreement is much better and comparable to that found for different devices sampling from the same layer of the water column. Surface samples therefore may adequately represent the upper mixed layer, but will give misleading results when there is a distinct vertical distribution of the phytoplankton.

Table 6.6 - summary statistics for comparison between surface water and euphotic zone or upper mixed layer

variable	Comparison	% Bias	% Disagreement	n
Chla_µg_L	Surface vs. euphotic	-45 (-53 - -36)	58 (42 - 77)	9
Biovolume_mm3L	Surface vs. euphotic	-27 (-34 - -21)	44 (35 - 53)	16
Chla_µg_L	Surface vs. mixed	-7 (-14 - 0)	27 (20 - 34)	10
Biovolume_mm3L	Surface vs. mixed	-10 (-17 - -3)	37 (29 - 44)	17

6.3 Cross lake perspective

In the above analyses we focussed on the difference in biovolume or chlorophyll *a* concentration estimates obtained using different sampling methods at the same lake. The bias and/or disagreement between methods was expressed as a % of the mean estimate at the lake. For many applications it might make more sense to look at the error/bias involved with a particular method in the context of cross-lake variation in biovolume and chlorophyll.

Figure 6.11 shows the correlation between euphotic and mixed layer biovolume across all lakes for which samples were taken. When samples are taken across a set of lakes with widely differing trophic status then the correlation between samples from different water layers will always be high, and the proportion of total variance due to the sampling method will be low (approximately 3% in this case).

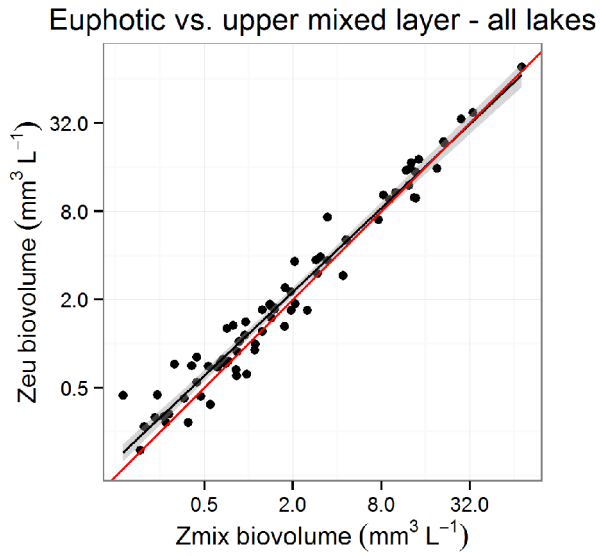


Figure 6.11 - correlation between euphotic and upper mixed layer biovolume across all lakes (where sampled)

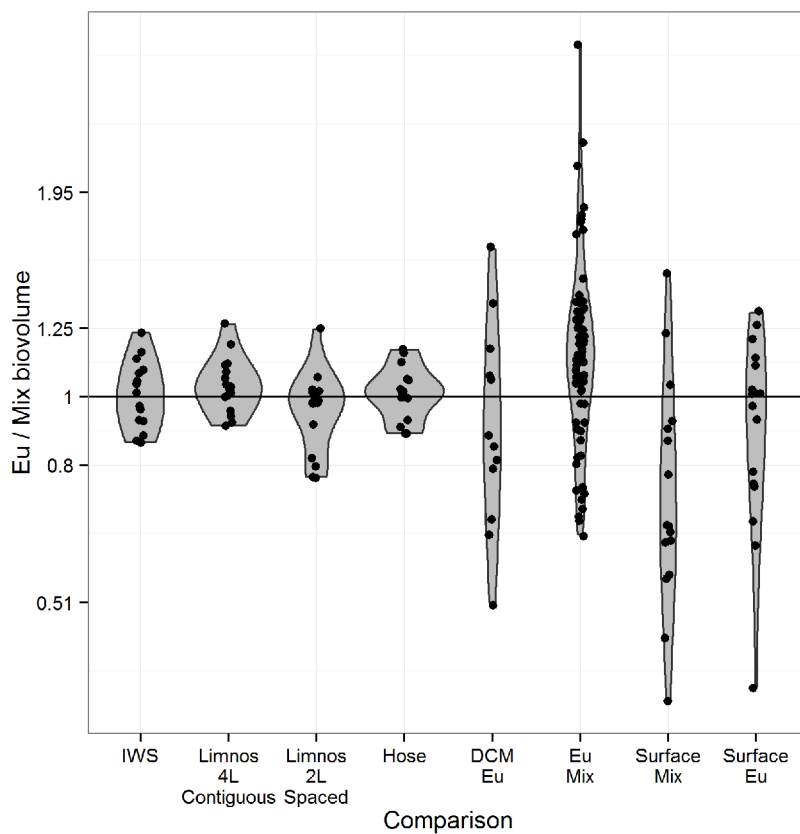


Figure 6.12 – overview of the disagreement and bias involved with sampling the same water layer with different devices and different water layers with the same device. The vertical extent of the grey shaded “violins” indicates the disagreement (tendency for a difference in the measurement obtained by different methods), while the position of the violin relative to the horizontal line at 1 indicates the bias (whether one method tends to over- or underestimate relative to the other).

7 Rivers

Authors:

Helmut Fischer, Andrew M. Dolman,

Sample analysis: Eberhard Hoehn

Samples provided by Helmut Fischer and Gemma Urrea

7.1 Background and objectives.

The following report intends to comply with the tasks framed within the contract signed between the Limnologie-Büro Hoehn (LBH) and the BfG, within the framework of the *CEN Mandate M424 WP6 project* ("Development and interlaboratory comparison to enhance the draft European Standard on water quality – Guidance on quantitative and qualitative sampling of phytoplankton from inland waters based on draft document N118 (2008/04/15)").

This report includes experiences, conclusions and recommendations on phytoplankton sampling obtained during the project workshops, literature surveys and sampling campaigns. The project did not include extensive funding for sampling campaigns and documentation and should therefore rely mostly on expert knowledge gathered at the workshops. However, sampling campaigns were also planned in order to tackle questions specific for the distribution of phytoplankton in rivers. Sampling campaigns were carried out in the impounded rivers Saar and Neckar (Germany), and in freely flowing sections of the rivers Rhine (Germany) and Ebro (Spain). The phytoplankton was analysed according to section 5.

7.2 Results from the project workshop

7.2.1 CEN-workshop April 2013, Koblenz: „Quantitative sampling of phytoplankton from inland waters: rivers“

List of workshop participants:

Jean-Pierre Descy (second day), Andrew Dolman, Helmut Fischer, Eberhard Hoehn, Maria Leitão, Ursula Riedmüller (first day), Gemma Urrea

Workshop-presentations were provided from Croatia (Igor Stanković), France (Maria Leitão), Germany (Helmut Fischer) and Spain (Gemma Urrea).

The participants concluded that "*in fully mixed rivers, the location and frequency of sampling is much more important than the precise method of water collection*". Further conclusions are listed below:

- Monthly sampling (at least) is recommended
- Sampling should start in early spring (e.g. March), depending on local phytoplankton dynamics
- The parallel sampling of phytoplankton, chlorophyll and physico-chemical parameters is strongly recommended
- Measurement of light conditions (e.g. Secchi depth) is advisable, though technically difficult

- Multiple practical problems can occur during sampling and should be addressed (e.g. suspended sediment, strong currents, difficult access to sampling points in the main flow)
- High qualification of the sampling crew would be desirable

The workshop participants further agreed that case studies on the spatial heterogeneity of phytoplankton in rivers shall be performed. Some of these case studies have been performed in the project and are described in the following sections of this report.

7.3 Case study: Vertical heterogeneity of phytoplankton distribution in two impounded rivers of western Germany

7.3.1 Saar, sampling report

The Saar, located in the catchment of the Rhine, originates in the French Vosges mountains at 785 m a.s.l. Covering a watershed of 7431 km², the Saar flows along 246 km through France and Germany. The lower 96 km of the Saar were impounded for cargo ship transport purposes between 1976 and 2000. Six dams with ship-locks and hydropower plants were installed to provide a minimum depth of 4 m within the main channel. This led to prolonged water residence times, lower flow velocities, and to increased water depths. Because of low flow velocities, diurnal stratification frequently occurs during times of low discharge and high temperature and global radiation (Becker et al. 2010).

When the impoundments were built, efforts were increased to improve water quality by the construction of wastewater treatment plants in the catchment. However, strong phytoplankton spring blooms still develop regularly in the Saar, while phytoplankton biomasses are relatively low during the rest of the year (more information in Becker et al. (2010), IKSMS (2013)).

Table 7.1 - Discharge characteristics (1981-2013) of the Saar at the gauge at Fremersdorf (Saar-km 48.5), 17 km upstream of the sampled river section.

	Winter	Summer	Year
NQ	14	9,1	9,1
MNQ	28	16,6	16,6
MQ	110	40,5	74,9
MHQ	634	276	667
HQ	1280	990	1280

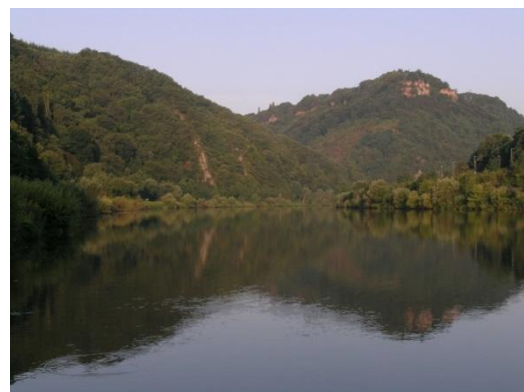


Figure 7.1 - The Saar in the impoundment of Serrig (Photo: H. Fischer)

7.3.1.1 Sampling strategy:

At the Saar it was tested whether the heavy impoundment with flow velocities below 10 cm/s leads to longitudinal and vertical inhomogeneities of the phytoplankton community. Phytoplankton was sampled in distances of 1000 – 2000 m in downstream direction along the 13 km river stretch between the weirs of Mettlach (Saar-km 31.4) and Serrig (Saar-km 18.5), in early April 2014 (fig. 1-2). This is the deepest impoundment of the Saar, with water depths of up to 12 m. Samples were taken at 50 cm water depth and at 2/3 of the total water depth. Discharge during sampling was 35 m³/s, and thus relatively low (cf Table 7.1), weather conditions were sunny.

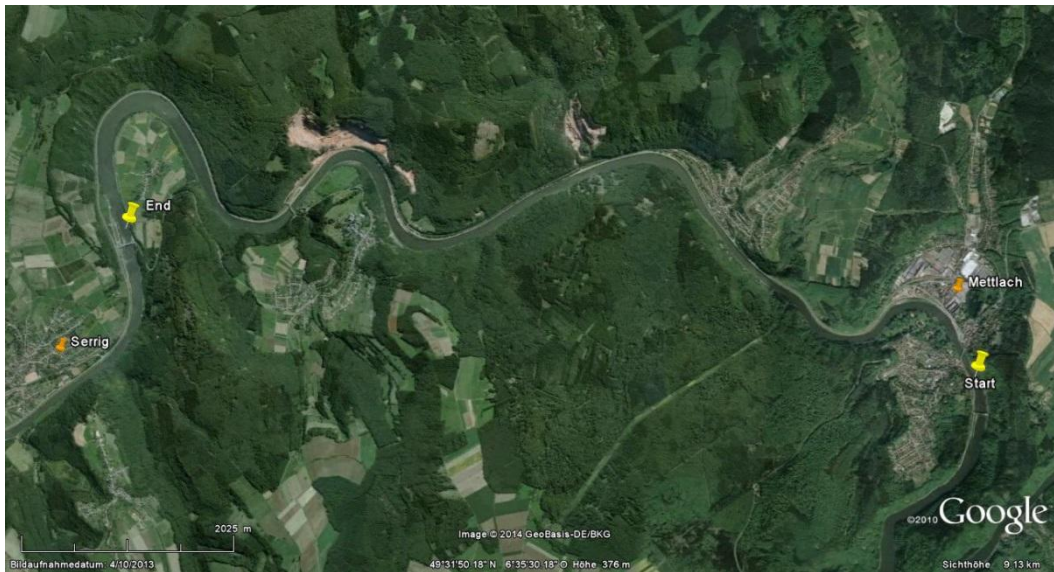


Figure 7.2 - Sampled river stretch of the Saar between Mettlach and Serrig (impoundment of Serrig). Start and end mark the stretch of the one-day sampling campaigns between Saar-km 31.4 and 18.5.

7.3.2 Neckar, sampling report

The Neckar, a major tributary of the Rhine, originates at 705 m a.s.l. at the Eastern Slope of the Black Forest and flows for 305 km through a (in most parts) densely populated and highly industrialised watershed of 14000 km² in southwest Germany. The downstream 202 km of the river were made navigable by 27 weirs with ship locks. Like the Saar, the Neckar is particularly susceptible to problems with its oxygen budget (Haag 2006). Strong phytoplankton spring blooms still develop regularly in the Neckar, while phytoplankton biomasses are relatively low during the rest of the year. More information on the Neckar is provided by Haag (2006).

7.3.2.1 Sampling strategy:

At the Neckar, similar questions as at the Saar were tested, but under higher discharge and flow velocities. Thus, it was tested whether the heavy impoundment with flow velocities below 20 cm/s leads to longitudinal and vertical in-homogeneities of the phytoplankton community. Phytoplankton was sampled in distances of 1000 – 2000 m in downstream direction along the 14 km river stretch between the weirs of Rockenau (Neckar-km 61,4) and Hirschhorn (Neckar-km 47,7) in May 2014 (fig. 3-5). Samples were taken at 50 cm water depth and at 2/3 of the total

water depth (maximum depth in this section about 10 m). Discharge during sampling was 85 m³/s (cf. table 2).

Table 7.2 - Discharge characteristics (1981-2013) of the Neckar at Rockenau (sampling site).

	Winter	Summer	Year
NQ	29,1	21,1	21,1
MNQ	56	42	40,1
MQ	189	102	146
MHQ	1190	663	1270
HQ	2690	1890	2690



Figure 7.3 - Neckar, upstream view from the weir of Hirschhorn (Photo: E. Hoehn)



Figure 7.4 - Sampling on the Neckar with a tube sampler (Photo: H. Fischer)

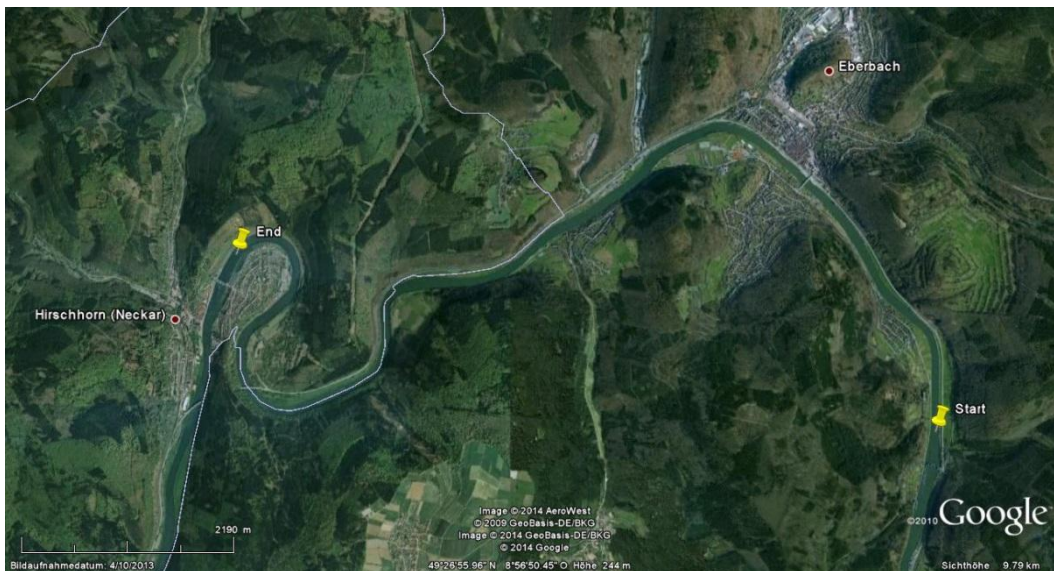


Figure 7.5 - Sampled river stretch of the Neckar between Rockenau (Neckar-km 61,4) and Hirschhorn (Neckar-km 47,7). Start and end mark the stretch of the one-day sampling campaigns.

7.3.3 Results on the vertical distribution of phytoplankton in the rivers Saar and Neckar

It is discussed that low flow velocities in impounded rivers may impact the dynamics of phytoplankton in several ways.

- River borne phytoplankton might sink out in the flow-reduced sections, e.g. upstream of weirs (Descy et al. 2012).
- Long water residence times in impounded sections might enhance phytoplankton biomass (Søballe & Kimmel 1987).
- Light availability in deep impoundments might be reduced, because phytoplankton spends relatively more time in deeper and darker layers of the water column.
- The water column might stratify so that vertical mixing is prevented. Thus phytoplankton would not be homogenously distributed in the water column and might grow excessively in the upper layer of the water column (Becker et al. 2010).

Figure 7.6 reveals phytoplankton biovolumes during the spring phytoplankton bloom at the rivers Neckar and Saar. The results show longitudinal and vertical differences in phytoplankton biovolume in both rivers. However, neither the longitudinal trend nor the vertical differences in the Neckar was significant.

In contrast, the phytoplankton biovolume decreased significantly within the impoundment sampled at the Saar. This result is difficult to interpret. It might well be that phytoplankton is sinking out during downstream transport at the Saar. It could also be that a peak in phytoplankton was sampled in the Saar at km 31, and that the boat travel downstream during sampling was faster than the transportation of this phytoplankton peak along the river. It is also possible that re-suspension by ship travel has influenced the phytoplankton biovolume in both rivers. However, the results demonstrate that considerable spatial differences can occur within one impoundment, and that great care should be taken in the choice of the sampling station and in the interpretation of results.

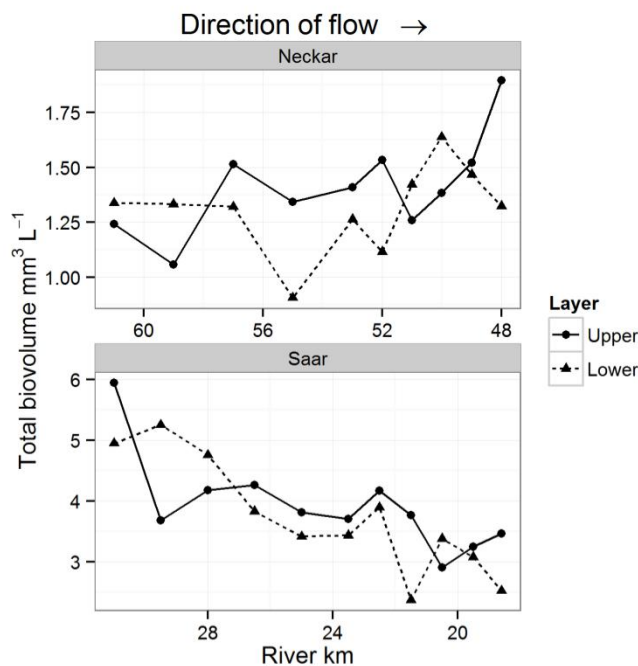


Figure 7.6 - Longitudinal and vertical distribution of phytoplankton biovolume in the rivers Neckar (top) and Saar (lower). Samples were taken from the water column at 0,5 m depth ("upper") and at 2/3 of the total water depth ("lower"). Water depth in the main channel

ranged from 4 to 10 m in the Neckar and from 4 to 12 m in the Saar. Flow direction is from left to right, both figures encompass one impoundment of the respective river.

However, it had been shown before that diurnal temperature stratification can occur at the Saar (Becker et al. 2010). During these occasions, strong vertical differences in phytoplankton biomass can occur, with high chlorophyll-*a* concentrations developing in the uppermost layer of the water column during late afternoon. The same data also demonstrate that, besides the spatial differentiation, strong temporal patterns can occur that are probably related to vertical mixing processes during the night and phytoplankton growth during daytime (Figure 7.7). Thus, representative sampling in impoundments must take these processes into account. It should follow a consistent scheme, and basic variables like sampling time of the day, sampling depth, and position in the impoundment should be held constant. If vertical stratification occurs, these impoundments should be sampled like shallow lakes.

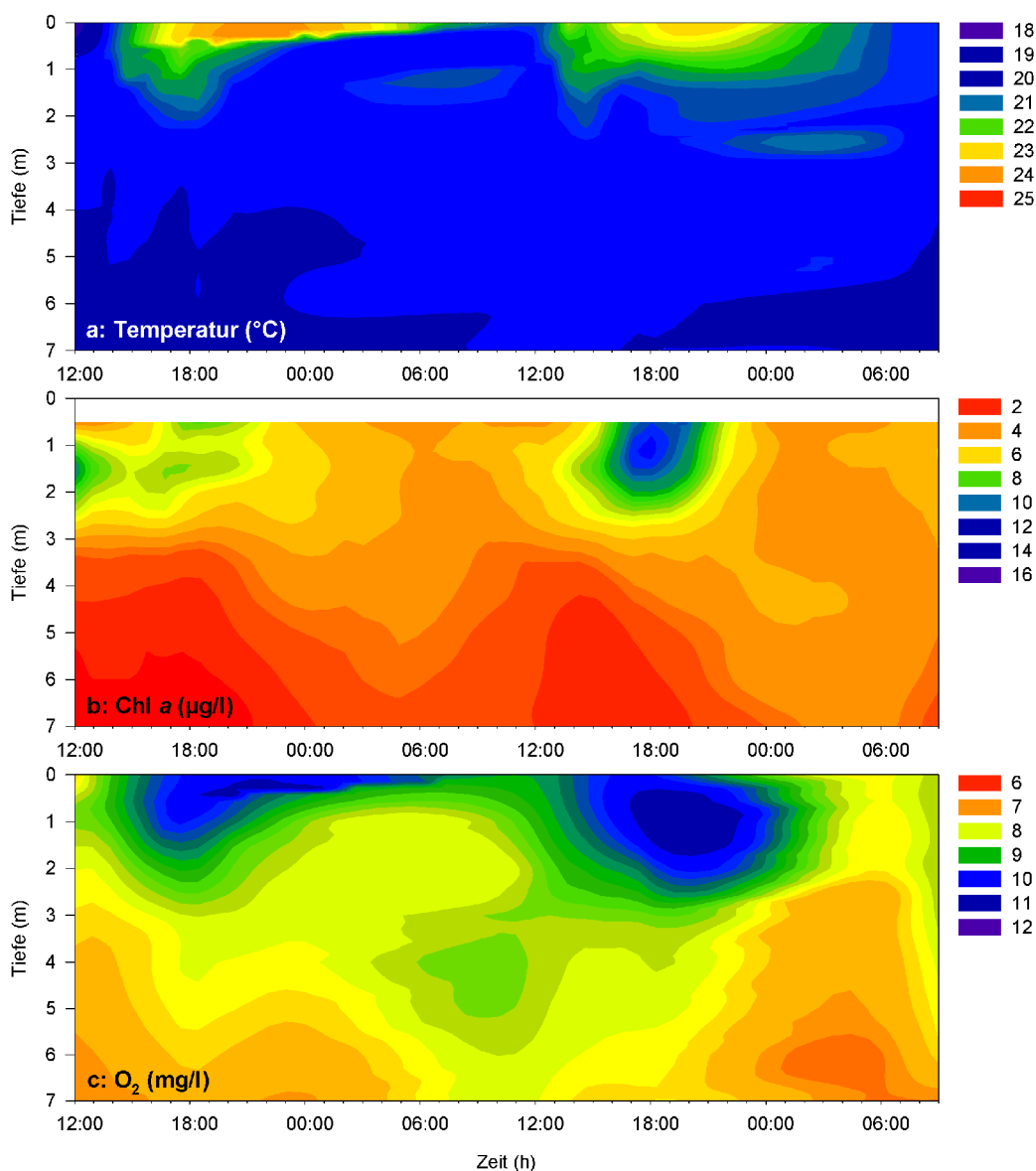


Figure 7.7 - Diurnal vertical stratification of temperature, chlorophyll-*a* and oxygen during a 48 hours sampling campaign at the Saar, August 2006. The sampling station was located at Saar-km 20.5 (c.f. figures 2 and 6) (Data from Becker et al. 2010).

7.4 Case study: Lateral heterogeneity of phytoplankton distribution in the Rhine and its tributaries

7.4.1 Rhine, sampling report

The Rhine is the largest River in Western Europe. It originates in the Swiss Alps of Graubünden at 2345 m a.s.l. The Rhine is 1239 km long and has a mean discharge of 2300 m³ at the diversion into the Delta Rhine section, and of 1655 m³ at the lower end of the study section (gauge of Kaub). The alpine section of the Rhine ends in the large, prealpine Lake Constance which shapes the plankton community of the following section. However, further downstream, in the section of the study site, the lake effect can be neglected and the phytoplankton of the Rhine is widely shaped by autochthonous production and by several plankton-rich tributaries. Phytoplankton studies in the Rhine have a long history (e.g. Lauterborn 1910, Kolkwitz 1912). However, the phytoplankton biomass has decreased dramatically during the last three decades which might be a consequence of decreasing nutrient concentrations or, more probably, increased grazing by benthic filter feeders (Friedrich & Pohlmann 2008, Hardenbicker et al. 2014). The studied section forms the transition zone between the Upper Rhine and the Middle Rhine and is characterised by low gradient and relatively high morphological diversity due to the presence of several islands and man-made structures for navigation (groynes, longitudinal dams). The upper section of the Rhine is characterized by a nival flow regime, while in the sampled section, the Rhine exhibits a mixed flow regime (nival and pluvial) which leads to relatively balanced flows during the year (Table 7.3)

Table 7.3 - Discharge characteristics (33 years, 1981-2013) of the Rhine at Worms (upstream of the sampling site).

	Winter	Summer	Year
NQ	516	487	487
MNQ	754	786	693
MQ	1430	1470	1450
MHQ	3520	2920	3690
HQ	5270	5250	5270



Figure 7.8 - "Inselrhein" between Mainz and Bingen (Photo: German Federal Water and Navigation Administration).

7.4.1.1 Sampling strategy:

At the Rhine, the mixing process with a major tributary (the Main) was exemplarily tested in June 2014. At that time, the Rhine usually carries low phytoplankton concentrations while the

concentrations in the Main are usually still high. Measurements were performed in both rivers upstream of the confluence and in several transects downstream of the confluence (fig. 8, 9). The influence of a second, smaller tributary (the Nahe) was also included in the sampling concept.



Figure 7.9 – River stretch of the Rhine sampled in June 2014. Sampling encompassed a 50 km river stretch. It started at Rhine-km 495, upstream of the confluence with the Main (Rhine-km 496.6), included the confluence with the Nahe (Rhine-km 529.1) at the city of Bingen and reached to the city of Lorch (Rhine-km 540).

7.4.2 Results on the lateral distribution of phytoplankton in the Rhine

Tributaries can strongly influence the amount and spatial distribution of phytoplankton in a river. The influence of the plankton-rich tributary, the Main, could still be observed 50 km downstream of the confluence, as phytoplankton biovolumes on the right side of the Rhine consistently exceeded those measured in the middle and on the left side (Figure 7.10). Even small tributaries might influence the phytoplankton community in a larger river. Although the total phytoplankton biovolume of the Nahe was slightly lower than in the Rhine, and the discharge of the Nahe was low, the diatom *Cocconeis* sp. could be tracked in the Rhine as a signal from the Nahe (Figure 7.11).

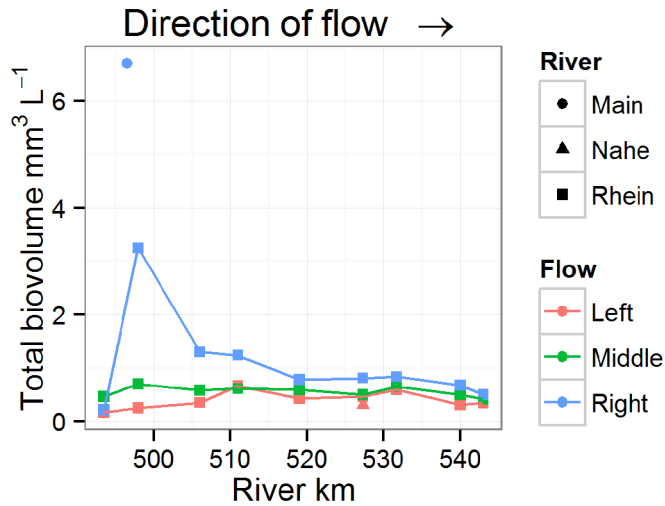


Figure 7.10 - Spatial distribution of phytoplankton biovolume in the Rhine as compared to the tributaries Main and Nahe.

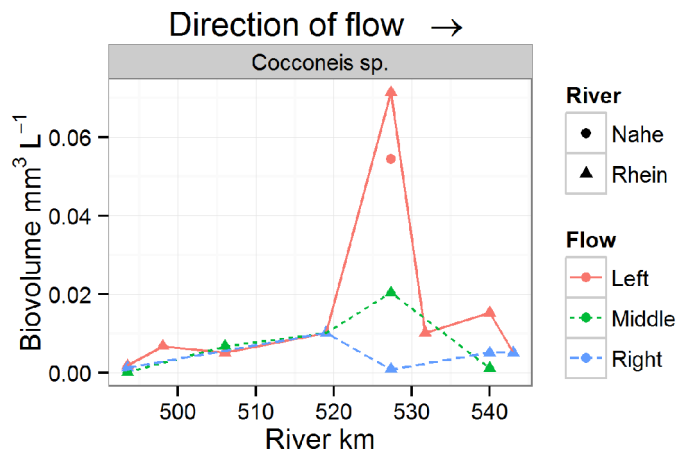


Figure 7.11 - Spatial distribution of the biovolume of *Cocconeis* sp. in the Rhine, influenced by the inflow of the small tributary Nahe.

Lateral differences in phytoplankton distribution might not only occur through tributary influences, but also from varying water residence times and retention of water close to the river banks. In the Elbe, higher primary productivity was found in the lateral areas (“groynes fields”) than in the main stem of the river, leading to slightly enhanced phytoplankton biomass at the margins of the river (Böhme 2006; Figure 7.12).

Higher sinuosity of the river banks can generally lead to enhanced retention of zooplankton and fish larvae (“inshore retention concept”, Reckendorfer et al. 1999/Schiemer et al. 2001). These lateral storage zones can maintain phytoplankton concentrations representing several times the concentration in the main channel of rivers (Lair and Reyes-Marchant 1997; Reckendorfer et al. 1999, and references therein). However, the development of such cross-sectional variability in phytoplankton concentrations is mostly connected with low water exchange between these

lateral zones and the main flow of the river. Consequently, samples must be taken from the main channel (and the main flow) of a river if they should represent the phytoplankton community of most of the discharge. For special research tasks, however, it can be advisable to sample both the lateral habitats and the main river flow, or to perform cross-sectional studies.

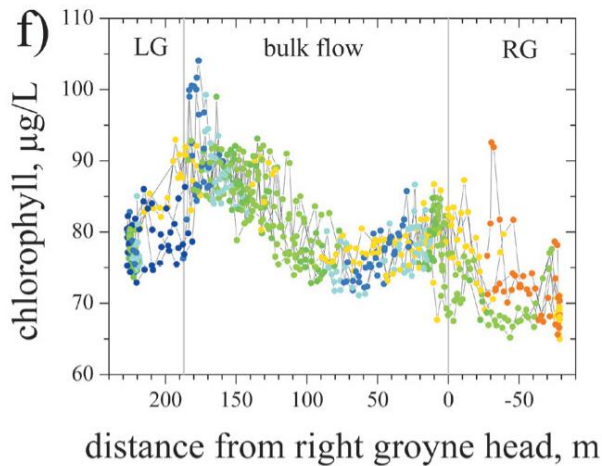


Figure 7.12 - Fig. 12: Chlorophyll concentrations during multiple crossing of the Elbe near Schnackenburg (Elbe-km 485).

7.5 Case study: Macrophytes

7.5.1 Ebro sampling report

The Ebro originates in the Cantabrian Mountains at 1880 m a.s.l. It has a catchment of 85,362 km² and flows through highly diverse regions into the Mediterranean Sea after 910 km. Climate in the watershed is mostly continental, with semi arid regions in the central part of the watershed and some oceanic climate in the high mountain areas of the Pyrenees and the Cantabrian Mountains. Water flow in the Ebro catchment is regulated by many weirs and reservoirs, with major impact of three large reservoirs (Mequinenza, Ribarroja and Flix) in the lower section of the river. Average discharge of the Ebro at Tortosa, close to the mouth of the river, is 450 m³/s. Seasonal discharge variation is high compared to the central European rivers described above, but relatively low for a Mediterranean river because of the oceanic part of the catchment (more information in Vericat and Batalla 2006, Romaní et al. 2010).

A strong decrease of PO₄-P concentrations has been observed between 1987 and 2004. Chlorophyll-a concomitantly decreased during that period, which might be attributed to decreasing nutrient concentrations as well as to the effect of the large reservoirs and, specifically, to the strong increase of macrophytes in the lower (downstream of large reservoirs) section of the Ebro (more information in Ibáñez et al. 2008, Romaní et al. 2010).

Sampling strategy:

Sampling at the Ebro mainly tackled the question whether the large beds of macrophytes would influence the phytoplankton community, and whether a small scale variation in sampling location (upstream, downstream and within macrophyte beds) would therefore impact the results found on phytoplankton biomass and community composition. The sampling campaigns took place at several locations along the Ebro, upstream and downstream of the large reservoirs, in September 2013 (figures 13-15).



Figure 7.13 - Ebro at Benifallet, upstream view (Photo: G. Urrea)



Figure 7.14 - Sampling in the fast flowing Ebro at Ascó (Photo: G. Urrea)



Figure 7.15 - Sampling sites at the Ebro. Benifallet and Mora are downstream of the large reservoir section.

7.5.2 Results on the distribution of phytoplankton in macrophyte beds in the Ebro

Sampling in between or close to macrophyte beds may lead to irregular results. Generally, phytoplankton concentrations in the macrophyte beds exceeded those outside the macrophyte beds by several times (figure 16). In some cases, this was connected with strong differences in taxonomic composition as well. Along the river, the phytoplankton in the macrophyte beds also changed remarkably. It was to almost 100% composed of *Conjugatophyceae* at the upstream stations Zaragoza and Juslibol. The proportion of *Bacillariophyceae* increased downstream at Zaida, while further downstream at Mora d'Ebre *Bacillariophyceae* formed the major component of phytoplankton. At Benifallet, a high proportion of cyanobacteria was found in the samples.

Macrophytes in rivers can influence phytoplankton in several ways. At river banks or in riverine lakes, macrophyte beds might retain water and form protected areas in which phytoplankton may grow faster (Basu et al. 2008). Additionally, meroplankton might develop from the

epiphyton of macrophytes, and feed the phytoplankton community (Reference meroplankton?). Most often, however, phytoplankton in rivers is negatively influenced by strong macrophyte developments. In fact, macrophytes can effectively retain particles (including phytoplankton) from the water column, (Hilt, S., Köhler, J., Kozerskie, H.-P., van Nes, E. & Scheffer, M. 2010. Abrupt regime shifts in space and time along rivers and connected lake systems. *Oikos* 120, 766-775.) XXX

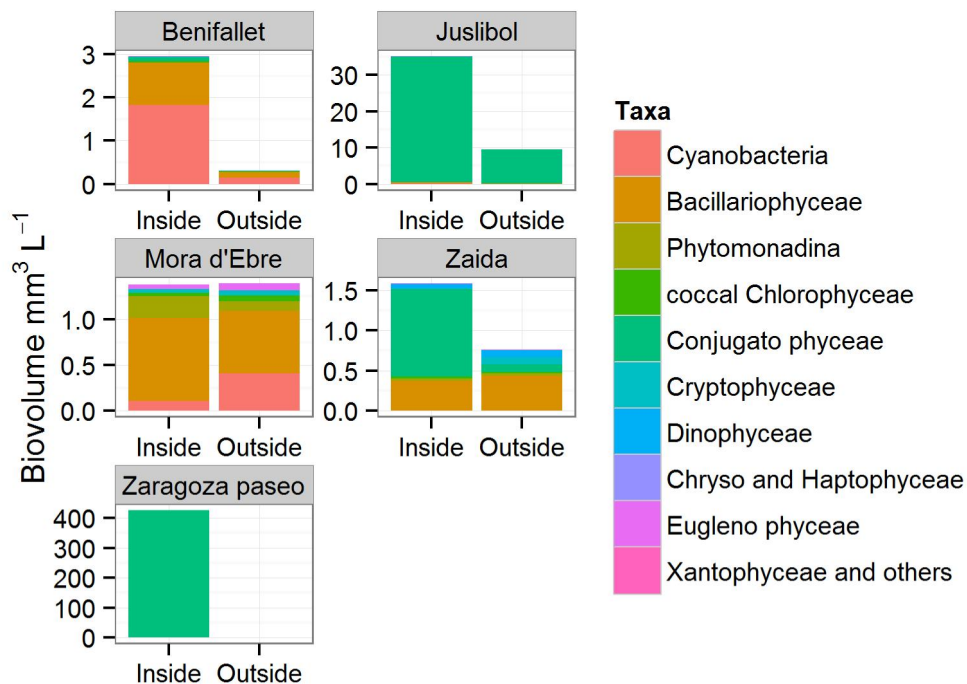


Figure 7.16 - Biovolume of phytoplankton groups inside and outside of macrophyte beds along the Ebro. Sampling stations shown in Figure 7.13.

7.6 General conclusions from the sampling campaigns and literature survey

- Sampling must be representative for the river section under study. This means that the sampling site must be „known“ and checked for homogenous distribution of phytoplankton.
- If vertical stratification is measured or suspected, rivers must be sampled like shallow lakes taking into account the vertical inhomogeneity of phytoplankton distribution.
- If the river section is laterally fully mixed, samples from the flow centre of the river are sufficient; if not, cross sectional sampling is advised. Sampling downstream of tributaries should be avoided.
- Resuspension of benthic or epiphytic algae during sampling should be avoided.

- A bucket can be used for sampling of fast-flowing, fully mixed rivers. In slow-flowing rivers (fully mixed), other sampling devices recommended for lake sampling can also be applied. Stratified rivers should be sampled like shallow lakes.
- Sampling should cover the dynamics (phytoplankton peaks) of the time period under study and provide representative mean values (e.g. for a season). At least monthly sampling is recommended to represent an annual course; however short phytoplankton peaks might then be missed and maximum as well as mean values derived from such a sampling scheme might be somewhat erroneous.

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9 Appendices

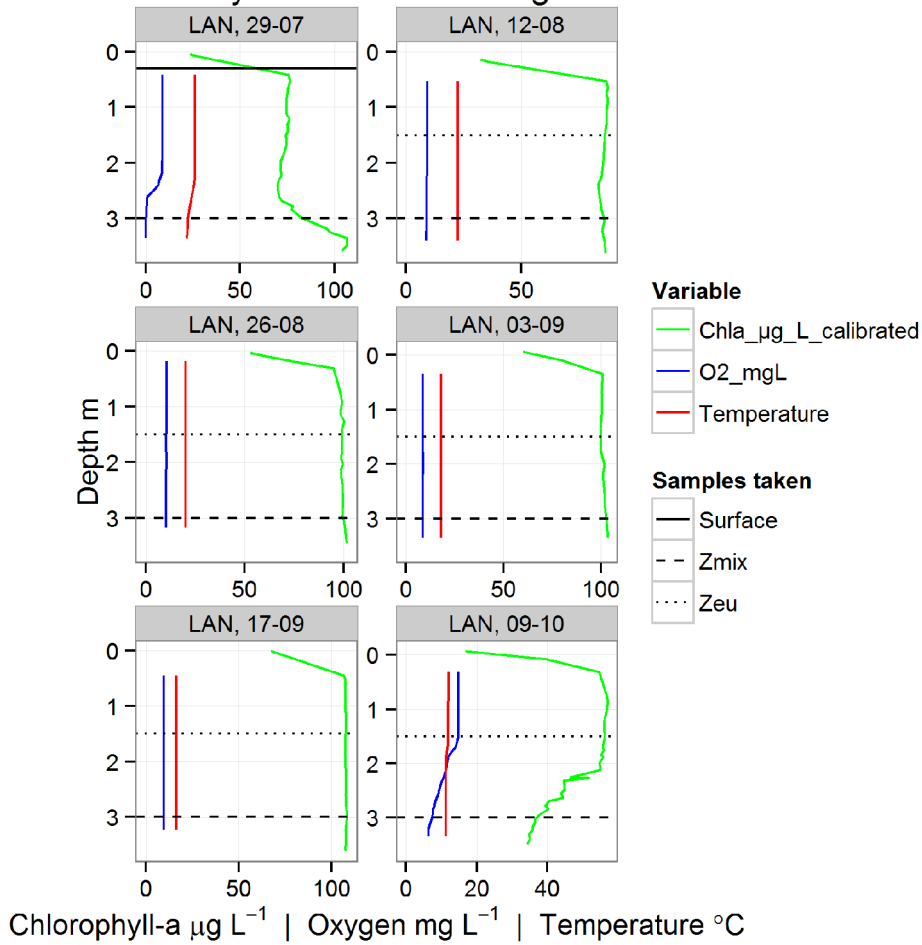
We include here two sets of figures to illustrate the data that have been collected: first, depth profiles of the chlorophyll-a concentration, oxygen concentration, and water temperature measured on each sampling occasion; second, stacked bar charts showing the total biovolume and community composition of the phytoplankton found in each sample.

9.1 Lake Depth profiles

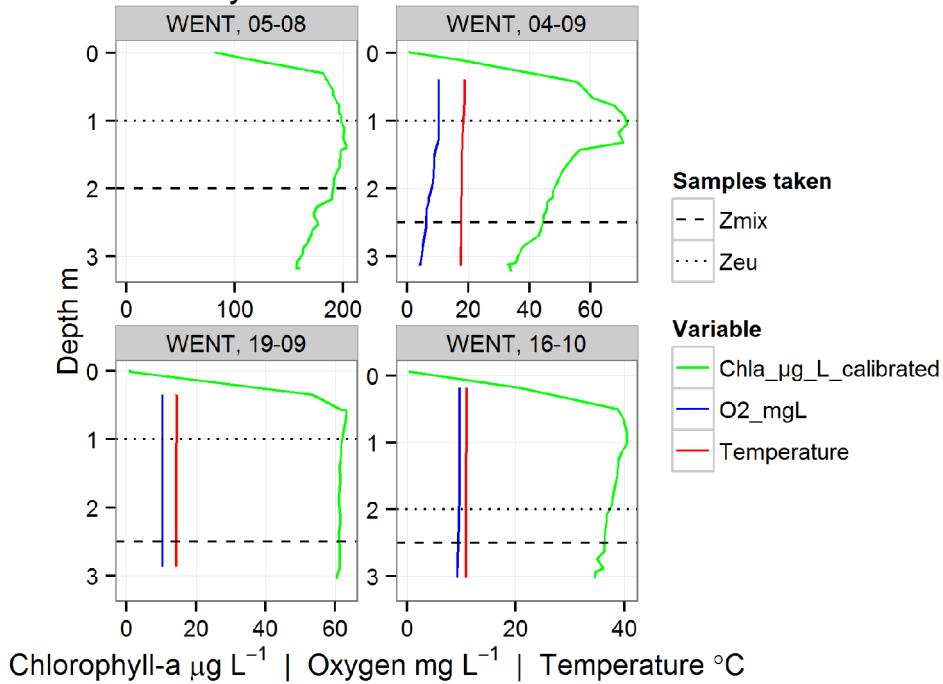
Each subplot shows the depth profiles of chlorophyll-a, temperature, and oxygen concentration measured on one visit to a lake. Subplots are grouped by lake and ordered according to the sample region and the type of lake. Within each group, subplots are arranged by sample date.

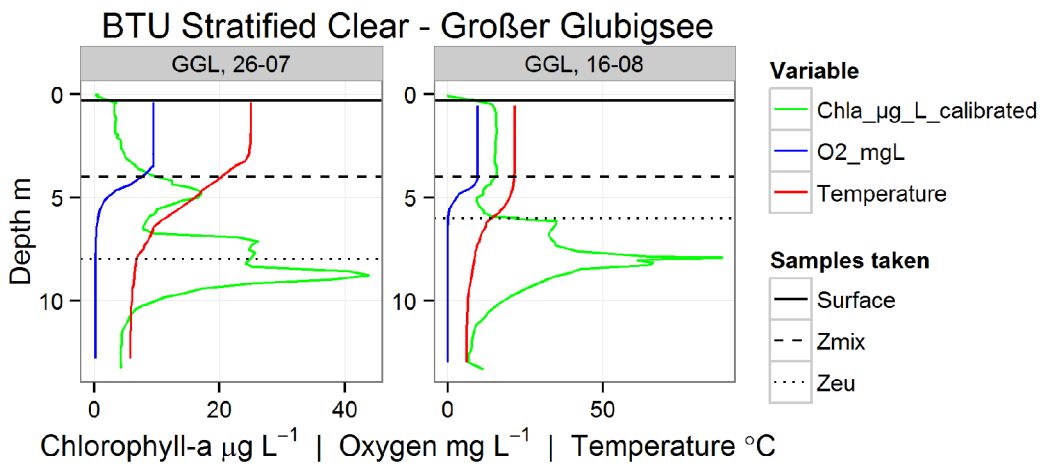
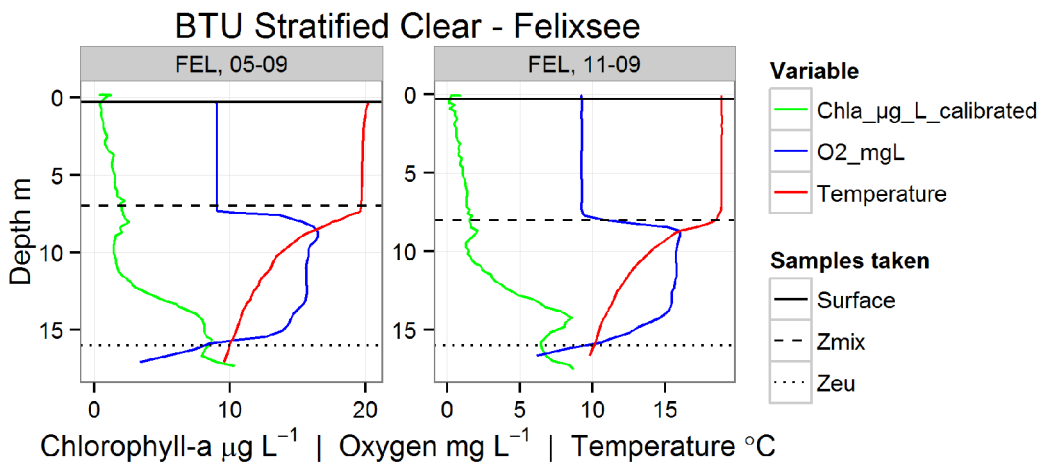
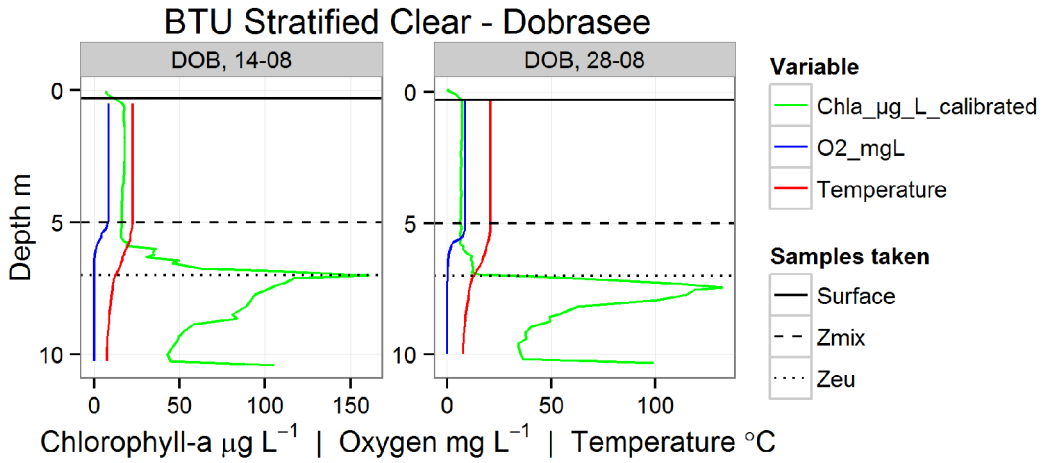
Each subplot is titled with the abbreviated name of the waterbody followed by the day and month it was sampled. Horizontal lines show the depths to which the various different water samples were collected. Chlorophyll-a was measured with fluorescence probes and has been calibrated to chlorophyll measured by extraction on each sampling date (some BTU sample could not be calibrated).

BTU Polymictic Turbid - Langer See

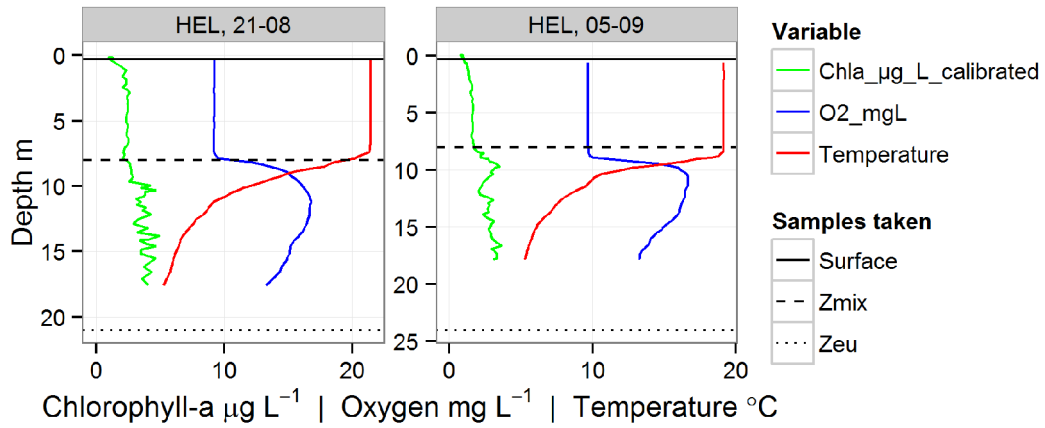


BTU Polymictic Turbid - Wentowsee

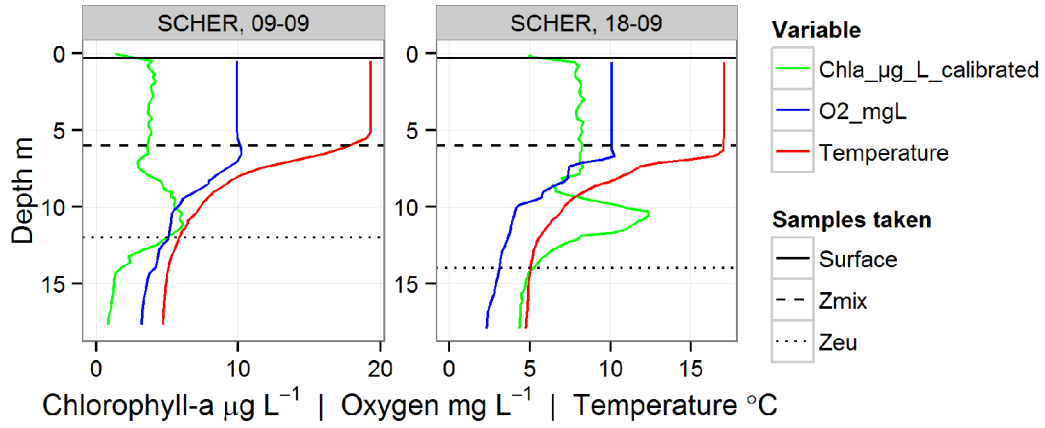




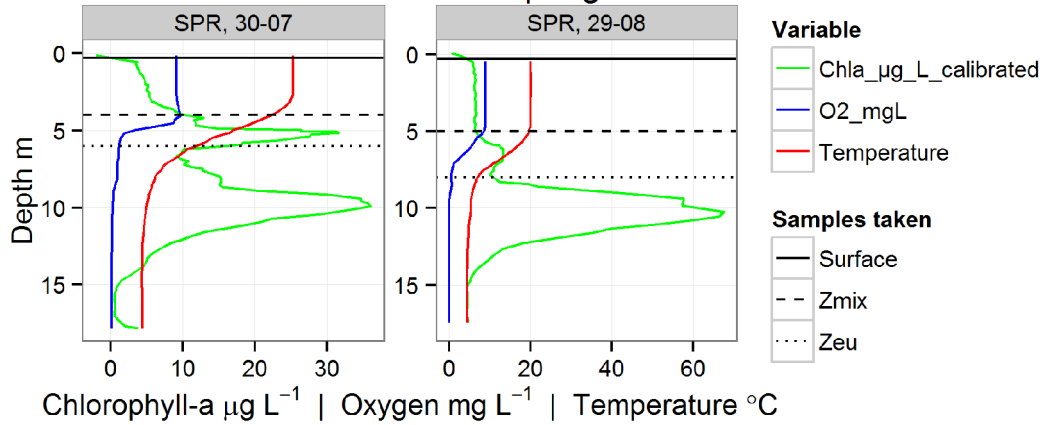
BTU Stratified Clear - Heleneesee



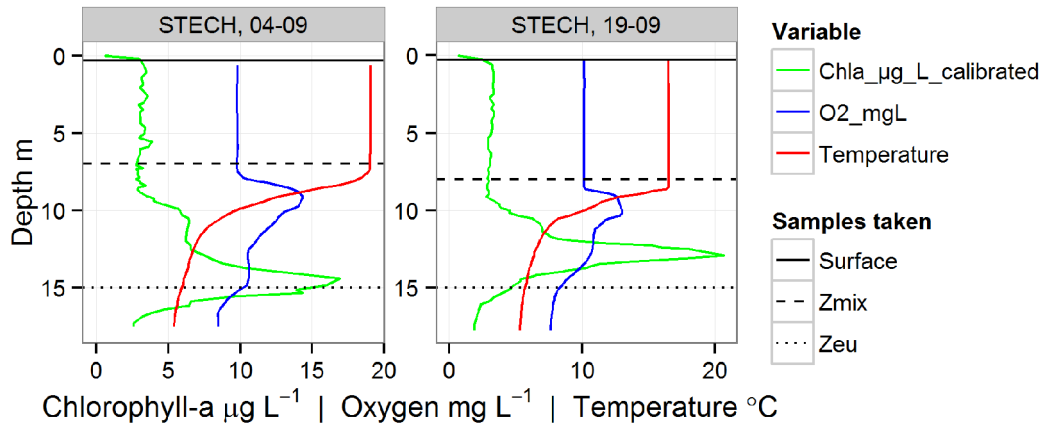
BTU Stratified Clear - Schermützelsee



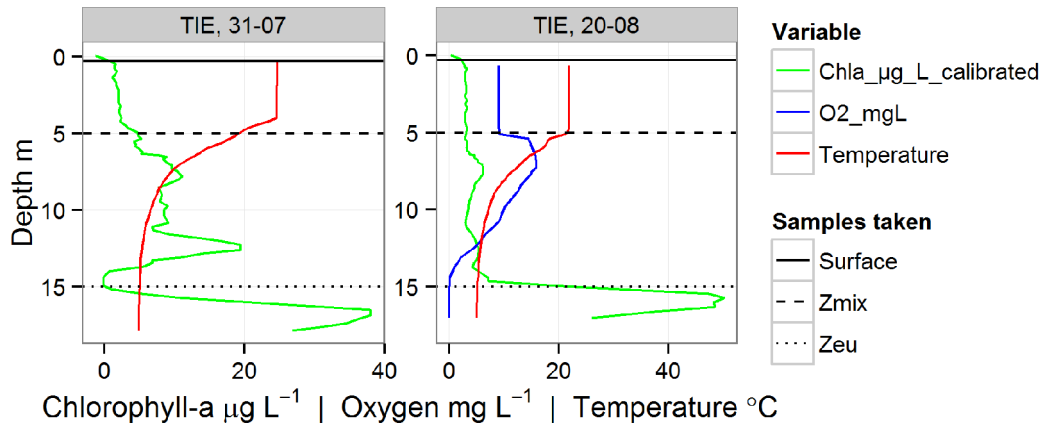
BTU Stratified Clear - Springsee



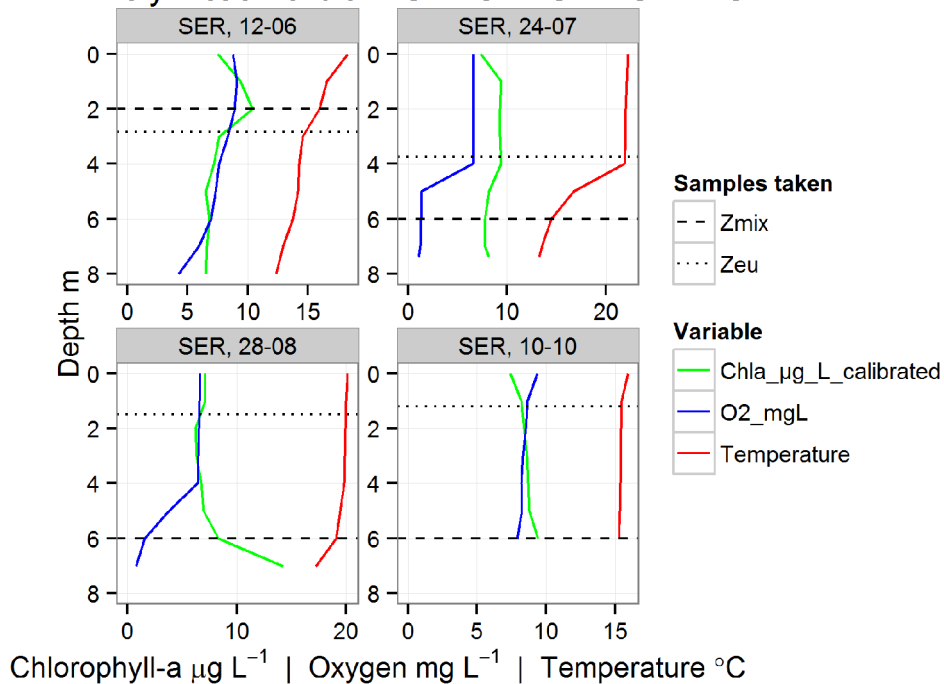
BTU Stratified Clear - Stechlinsee



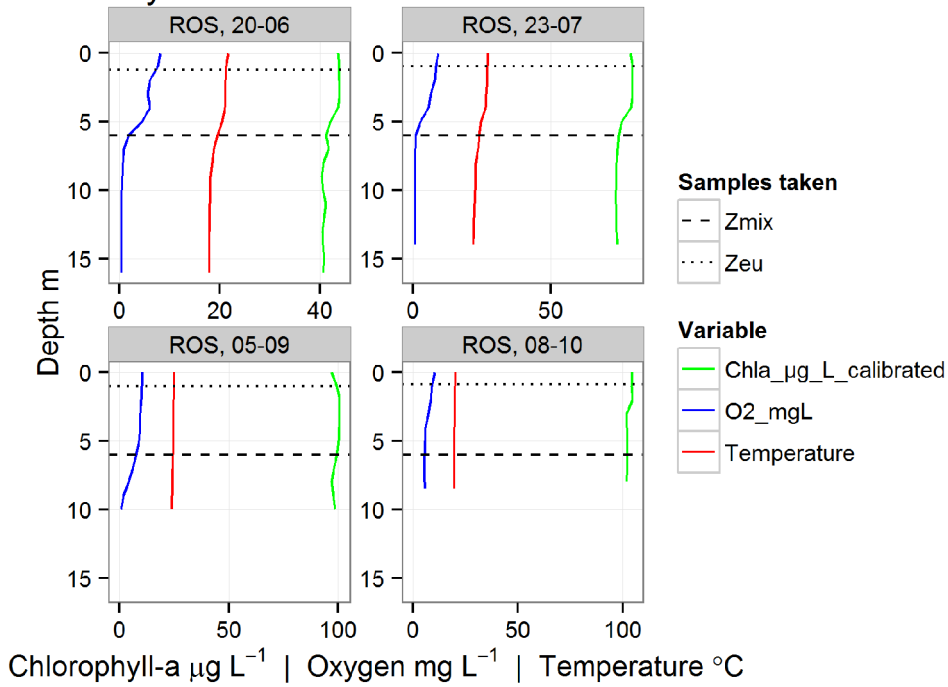
BTU Stratified Clear - Tiefer See



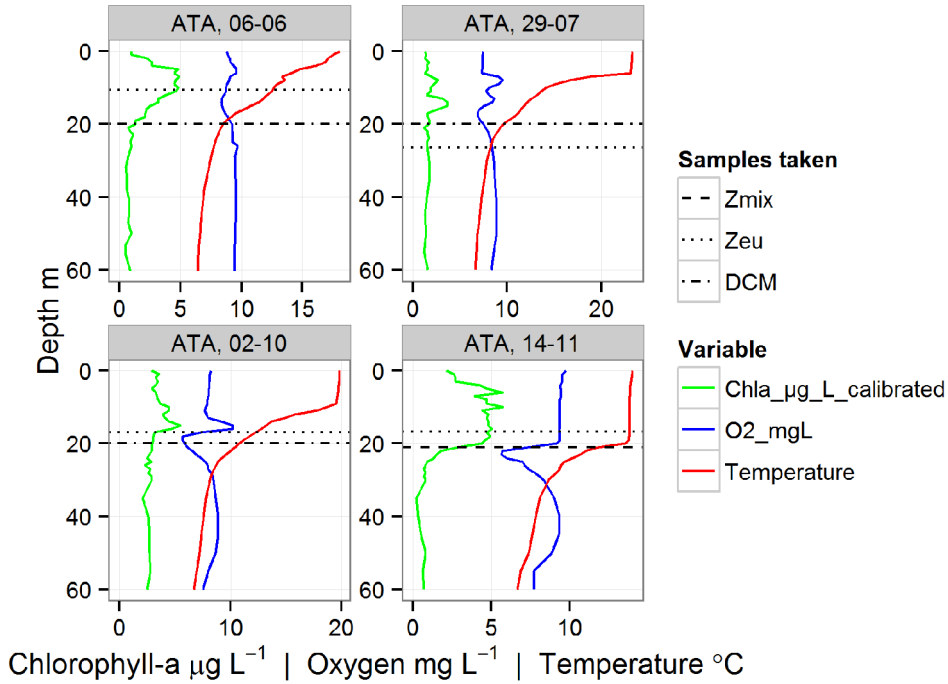
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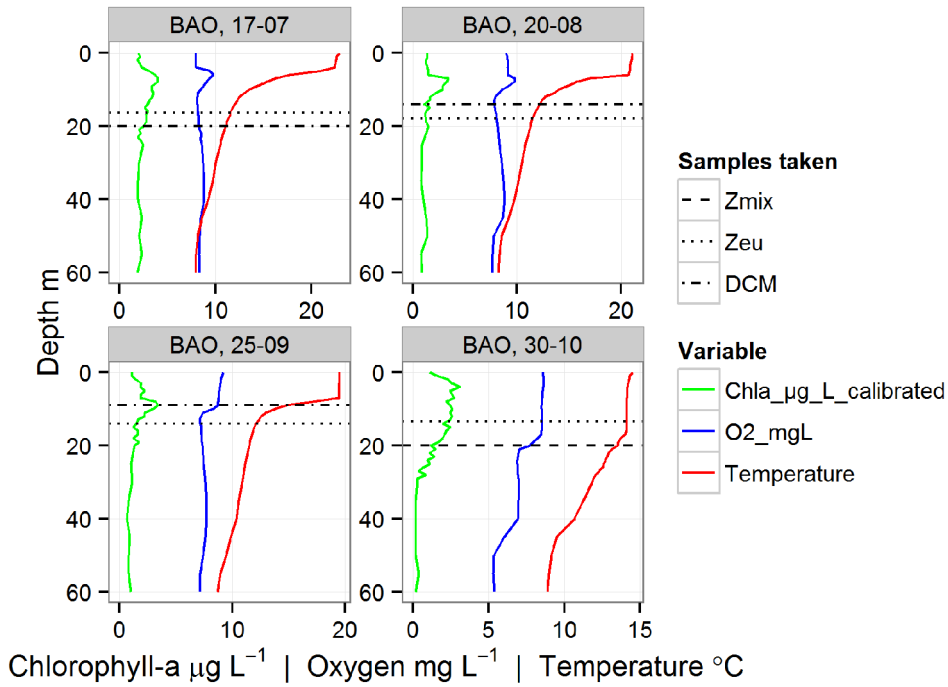
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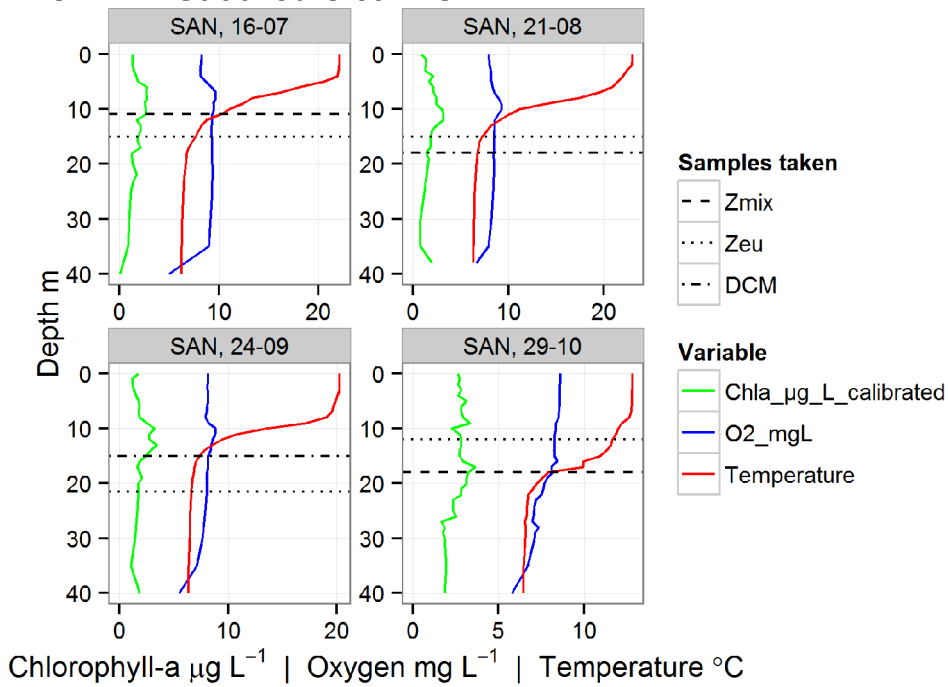
CEDEX Stratified Clear - ATAZAR RESERVOIR



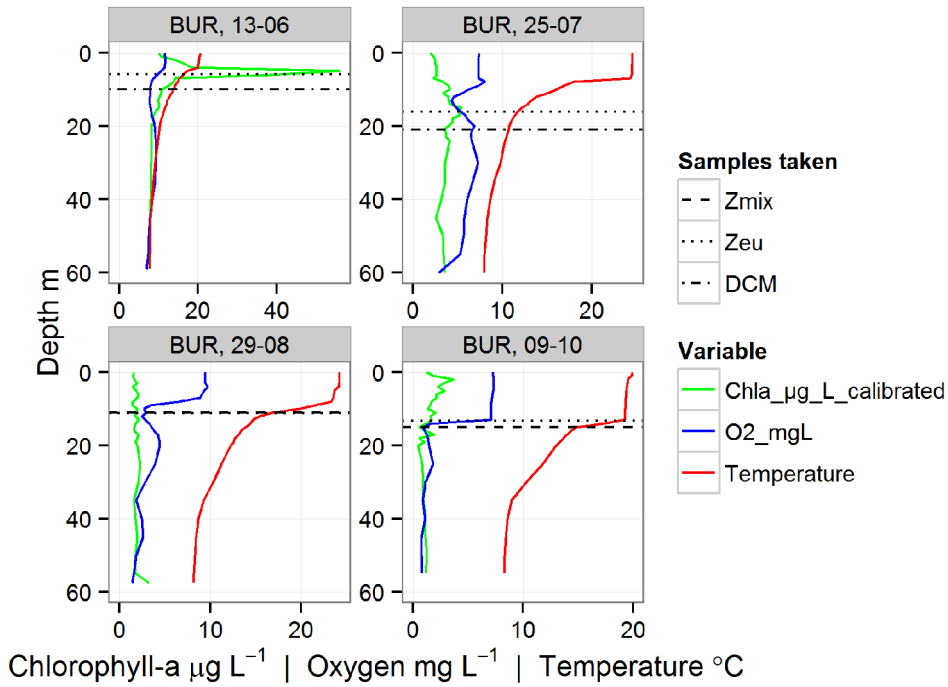
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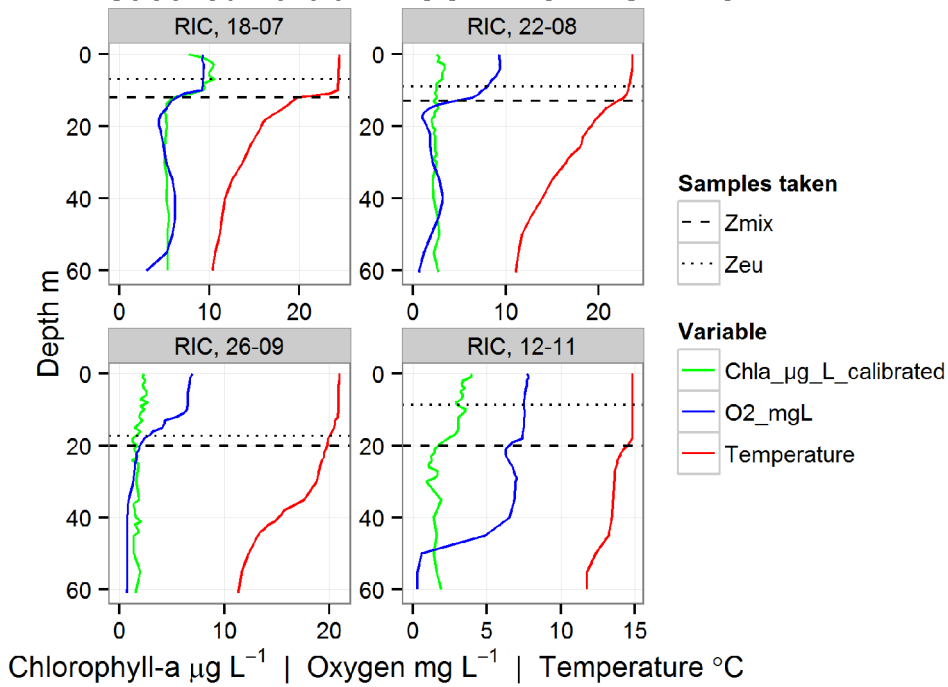
CEDEX Stratified Clear - SANABRIA LAKE



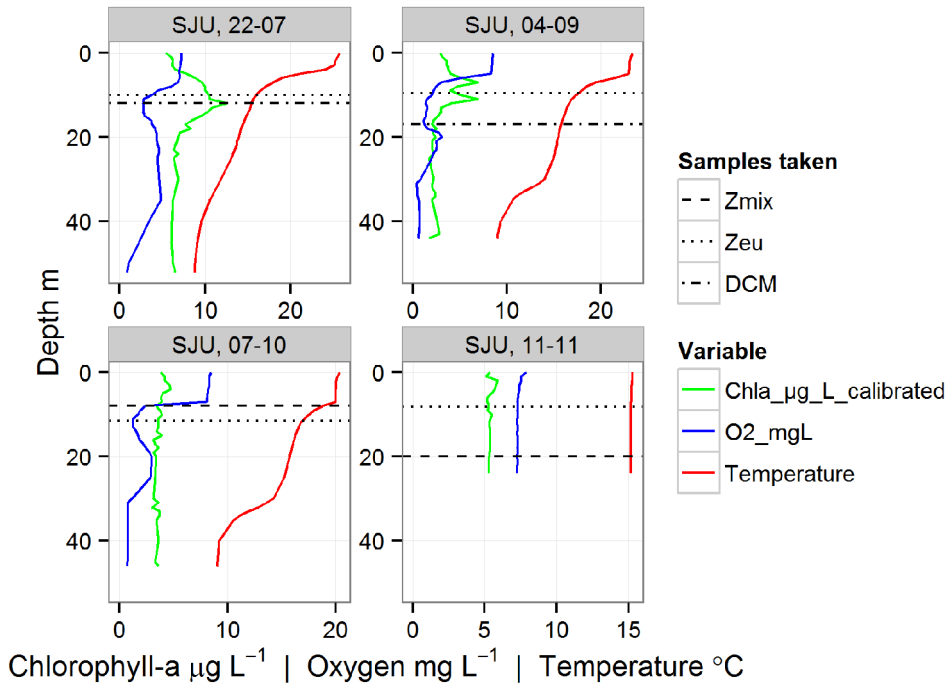
CEDEX Stratified Turbid - BURGUILLO RESERVOIR



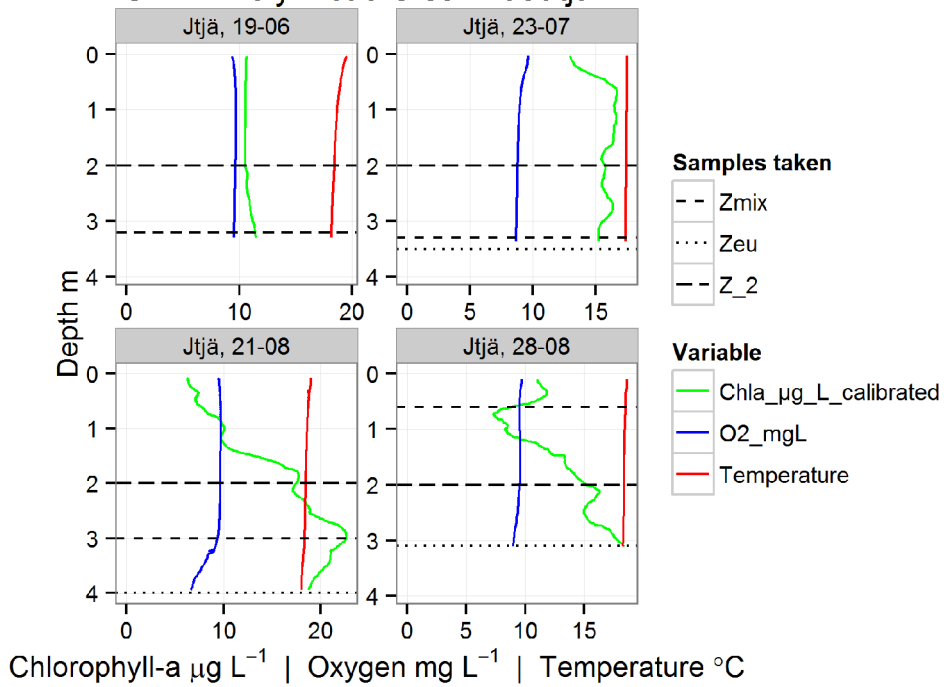
CEDEX Stratified Turbid - RICOBAYO RESERVOIR



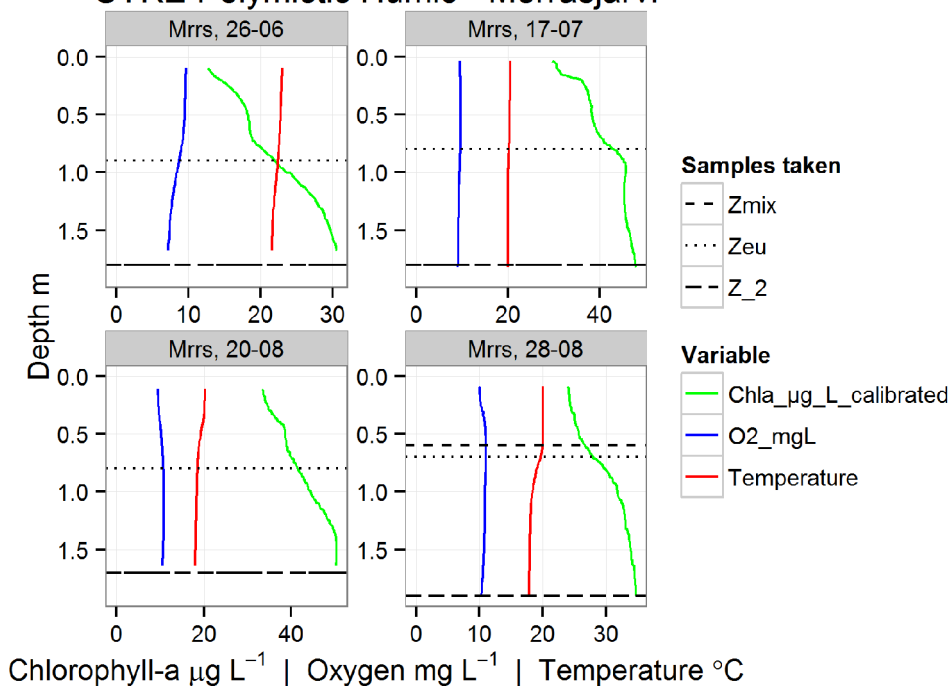
CEDEX Stratified Turbid - San Juan Reservoir



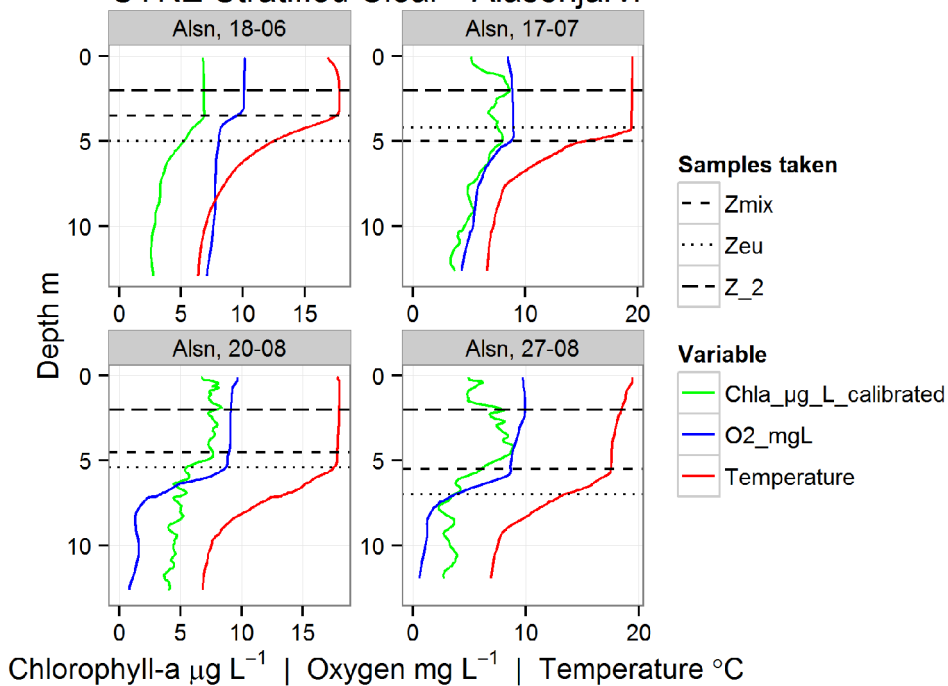
SYKE Polymictic Clear - Joutjärvi



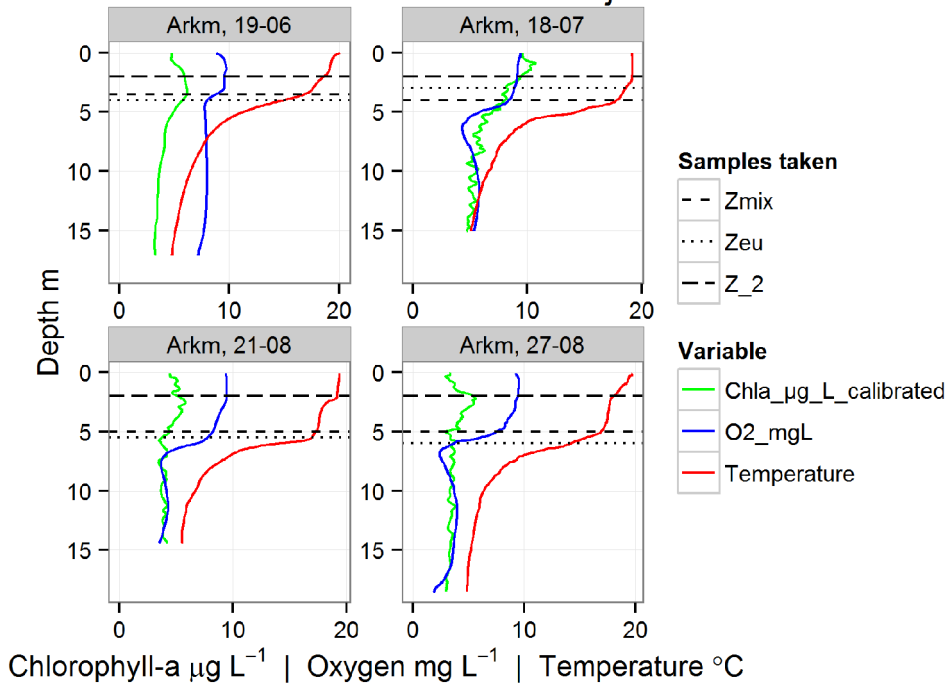
SYKE Polymictic Humic - Merrasjärvi



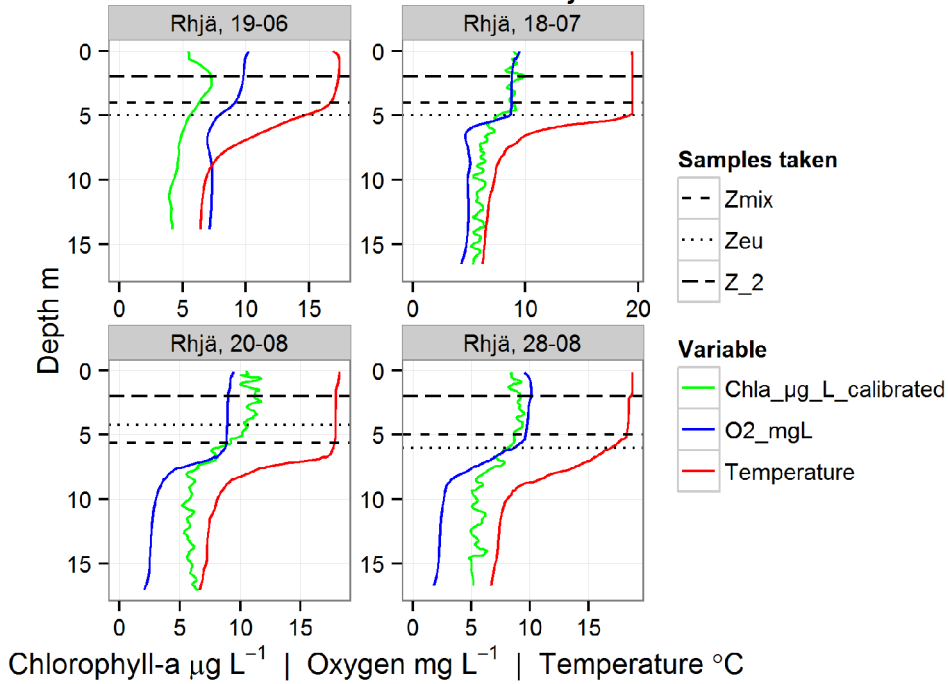
SYKE Stratified Clear - Alasenjärvi



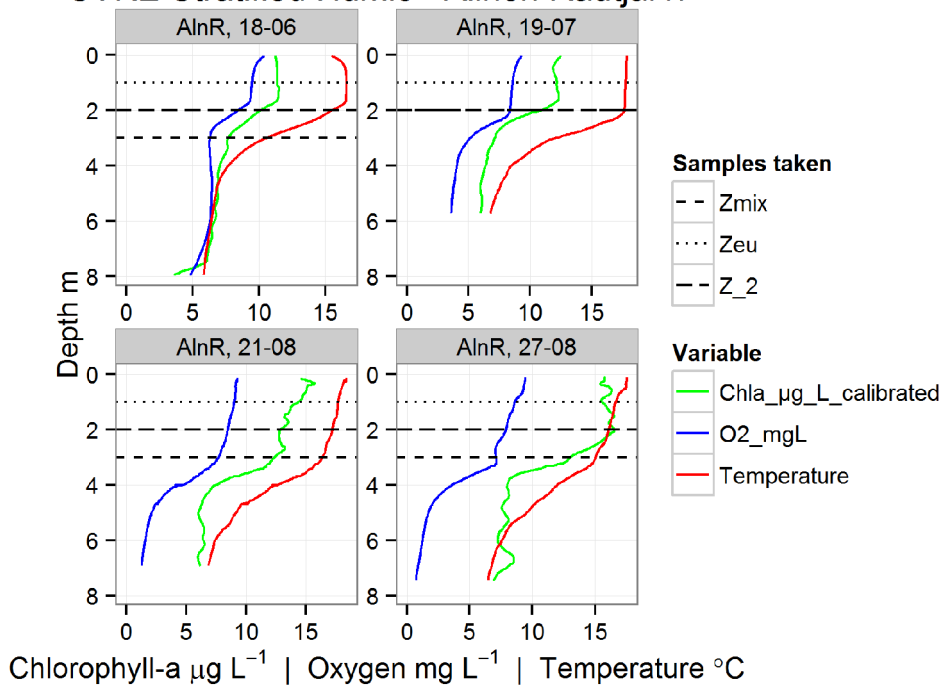
SYKE Stratified Clear - Arkiomaanjärvi



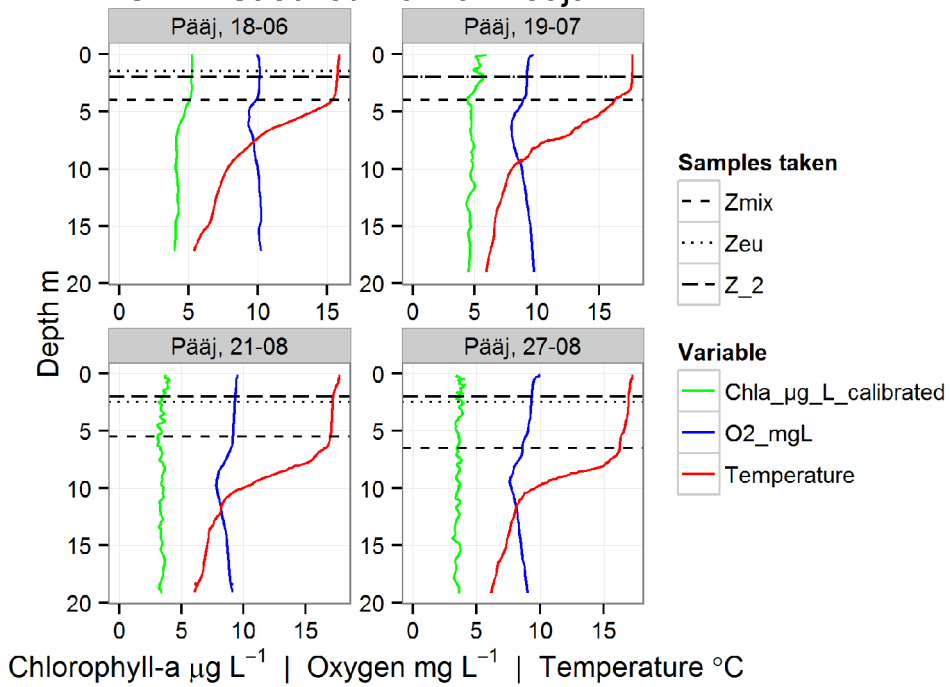
SYKE Stratified Clear - Ruuhijärvi

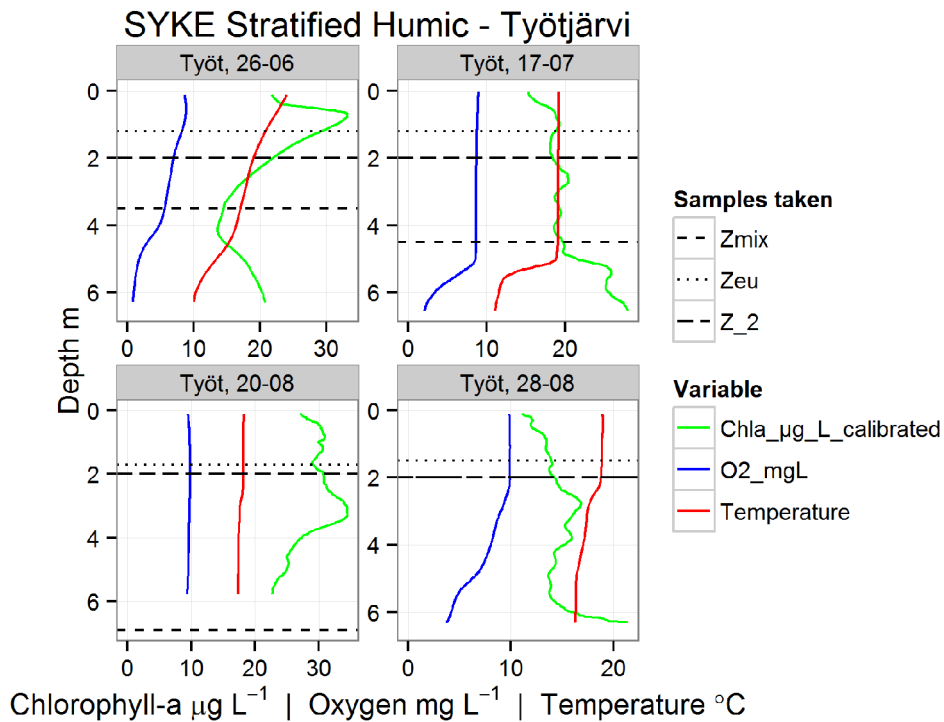


SYKE Stratified Humic - Ainen Rautjärvi



SYKE Stratified Humic - Pääjärvi

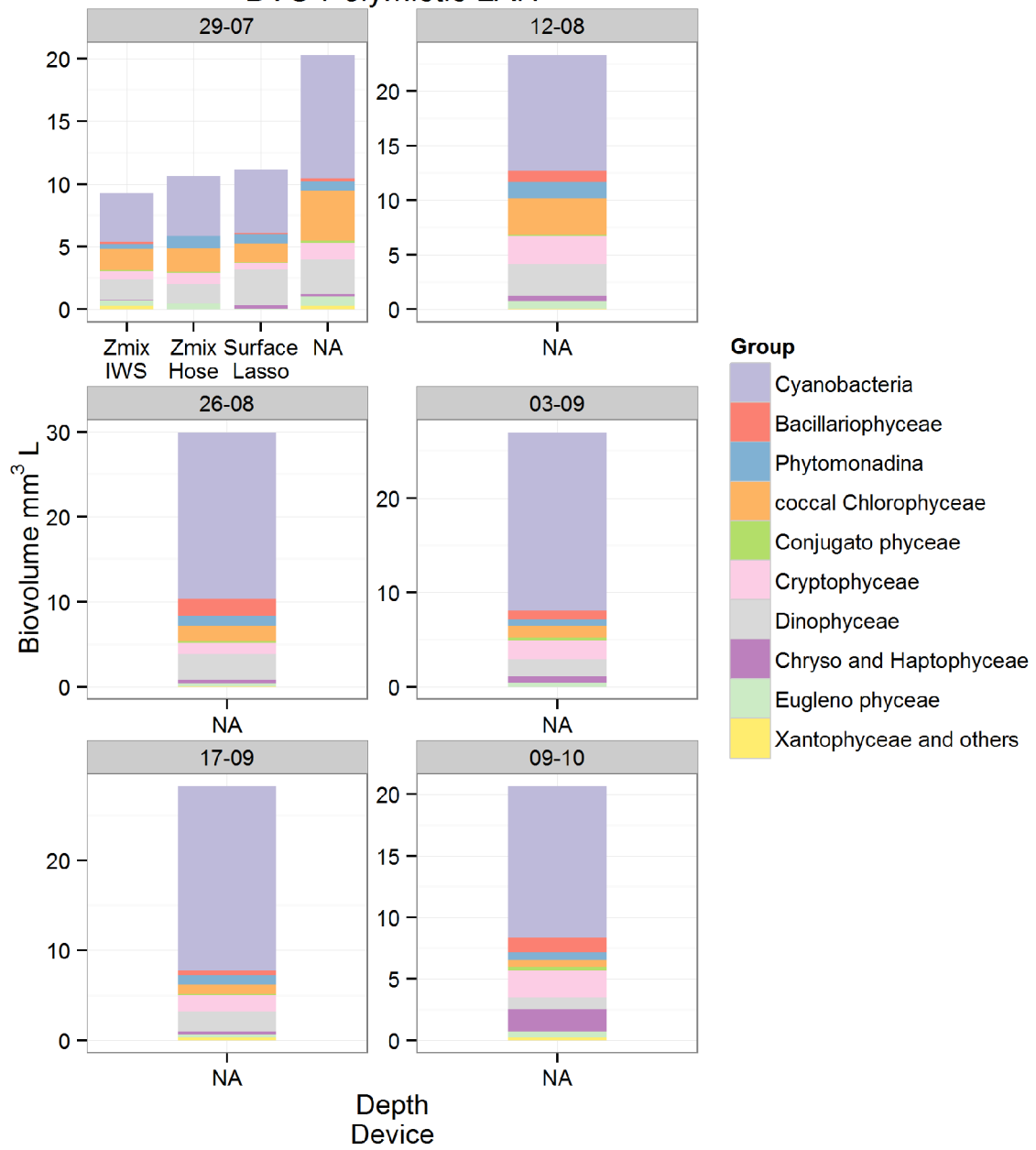




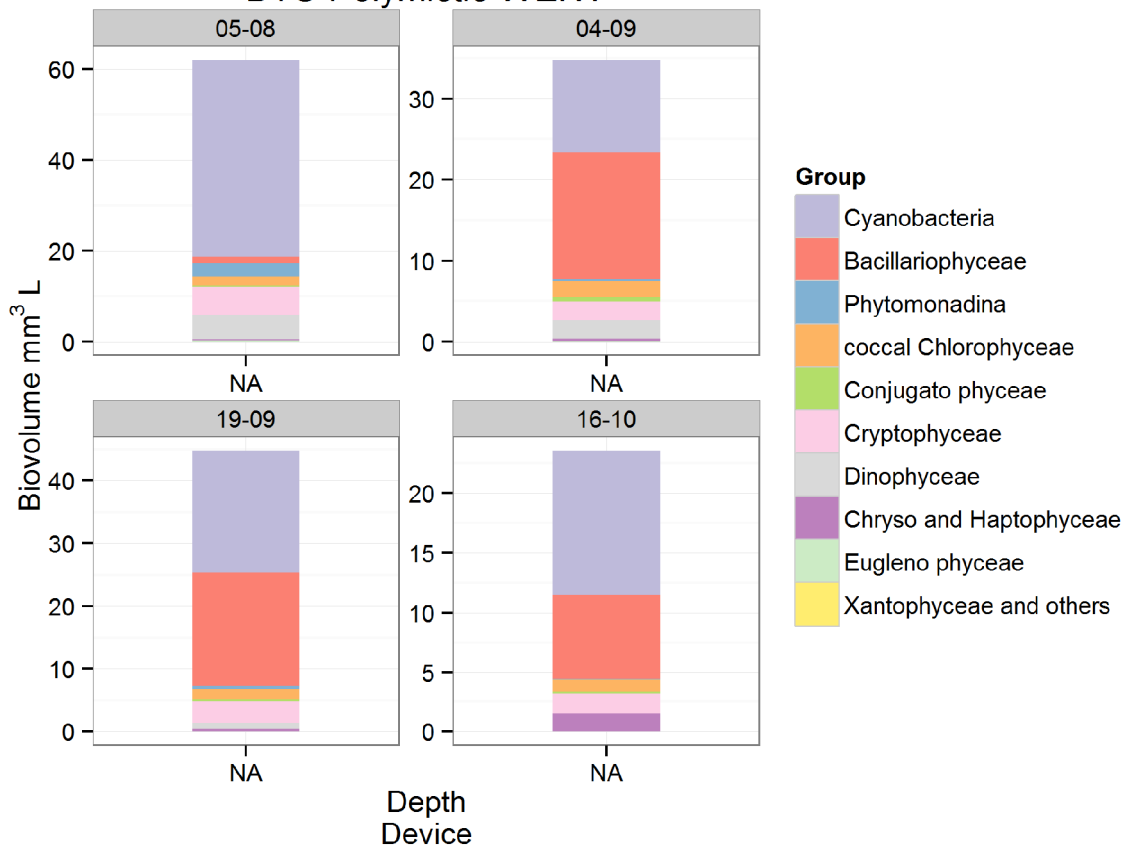
9.2 Total biovolume and community composition of lake samples

Each subplot shows the total biovolume and community composition of phytoplankton found in each sample taken from a particular lake on a particular sampling date; normally this means two samples, but there were as many as six on some occasions. Each stacked bar (column) is labelled with the water layer from which the sample was taken and the type of instrument used to take the sample. The colours used for each taxon are consistent across all subplots. Subplots are grouped by lake and ordered by sampling date.

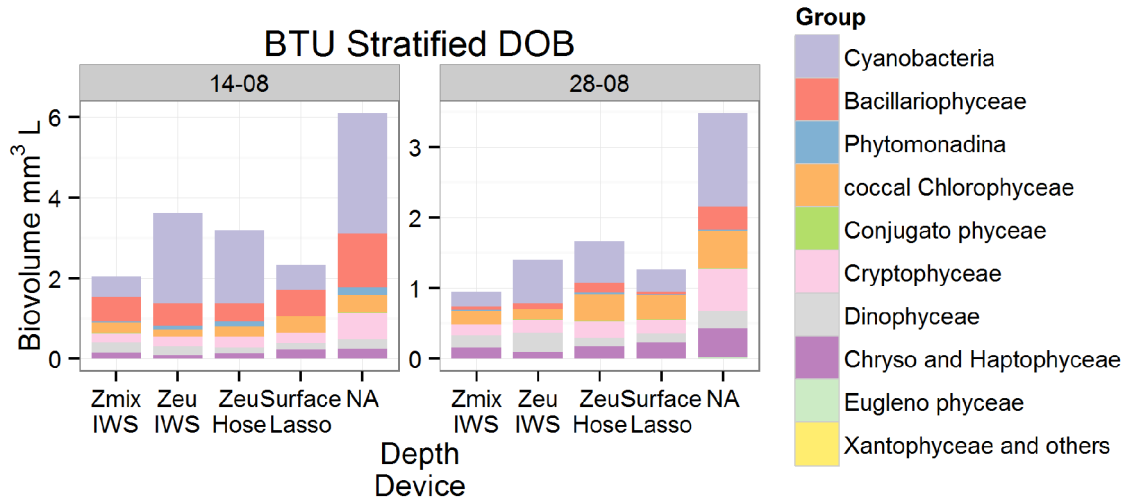
BTU Polymictic LAN

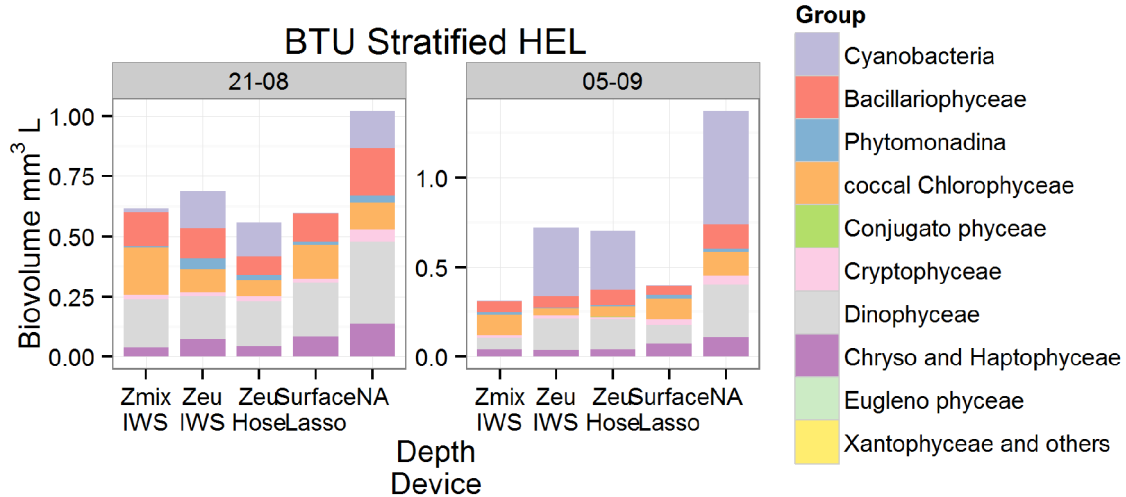
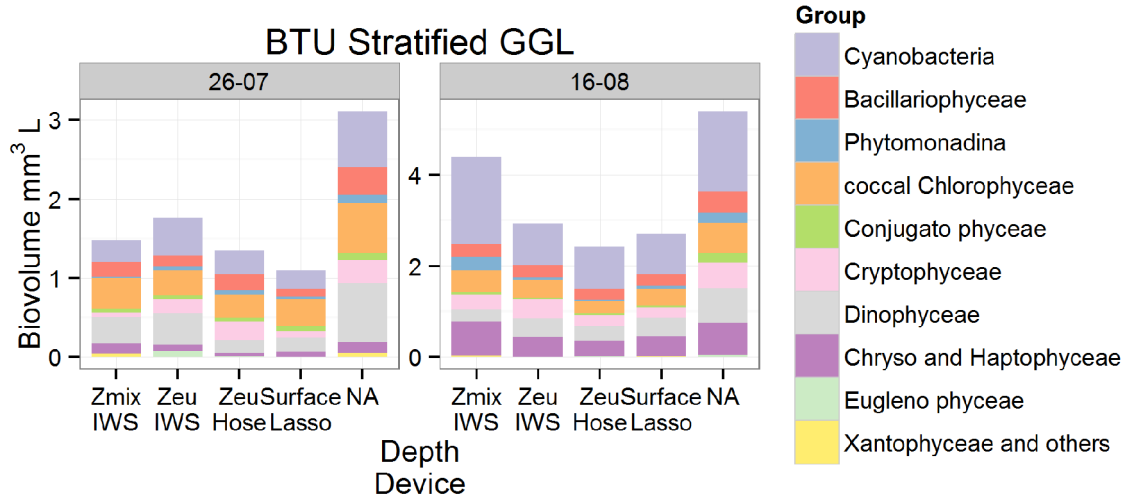
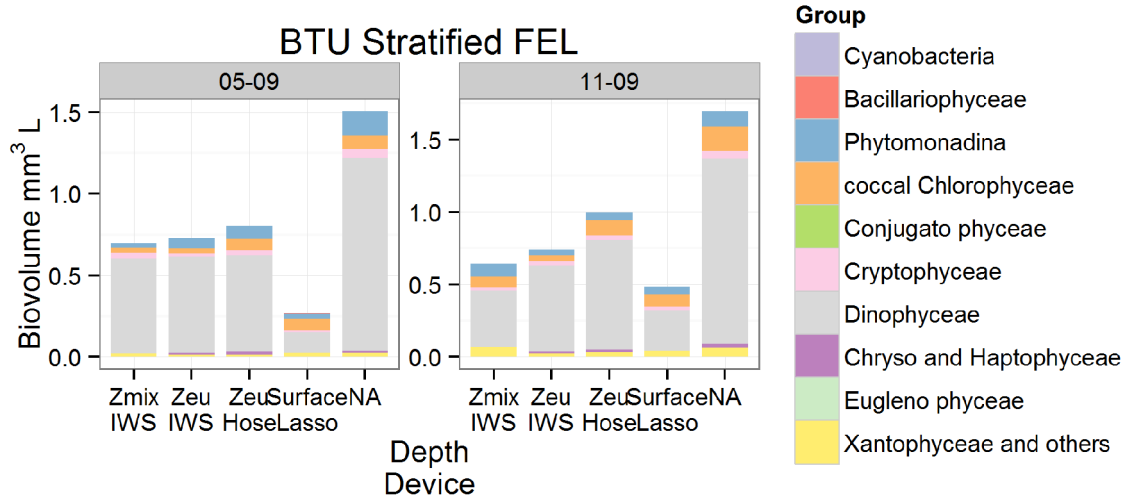


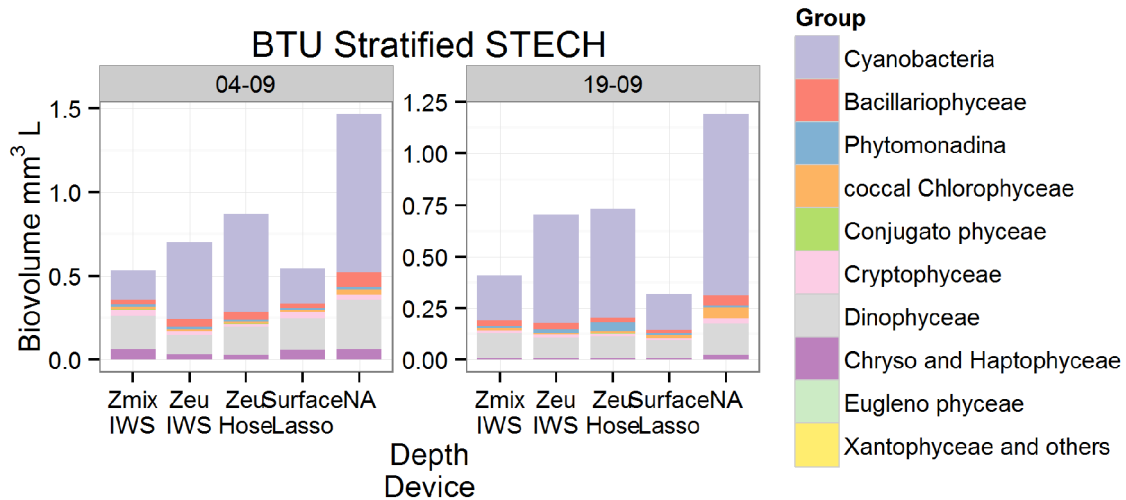
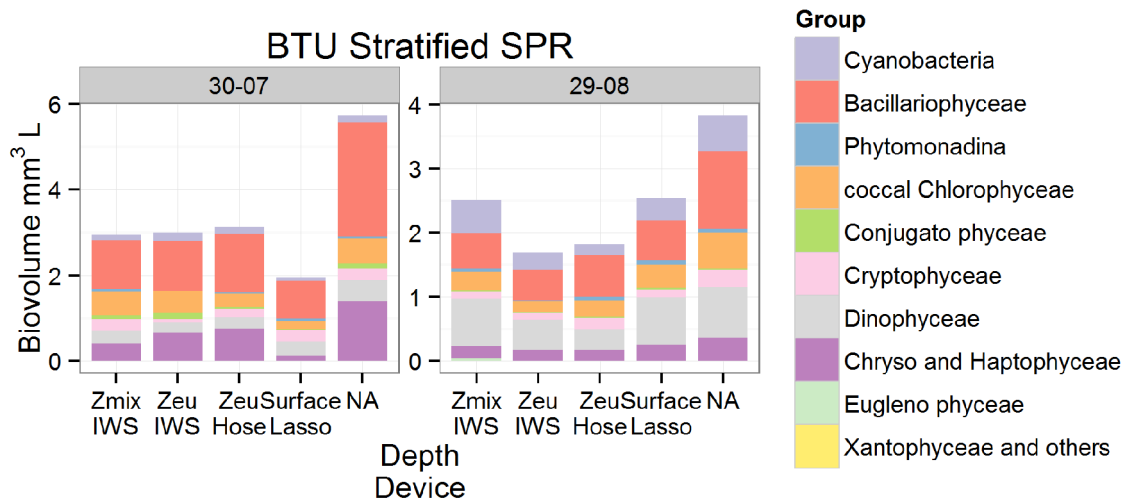
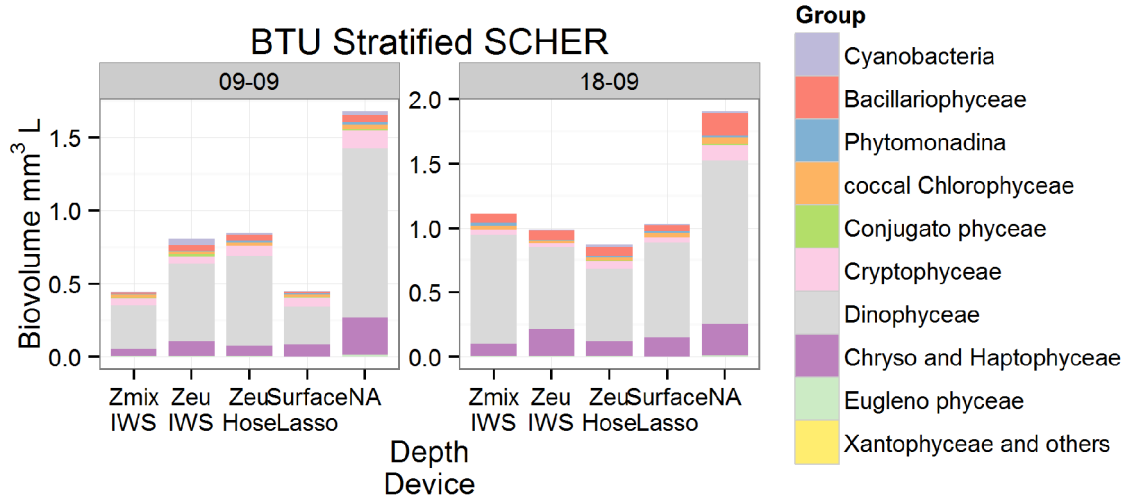
BTU Polymictic WENT

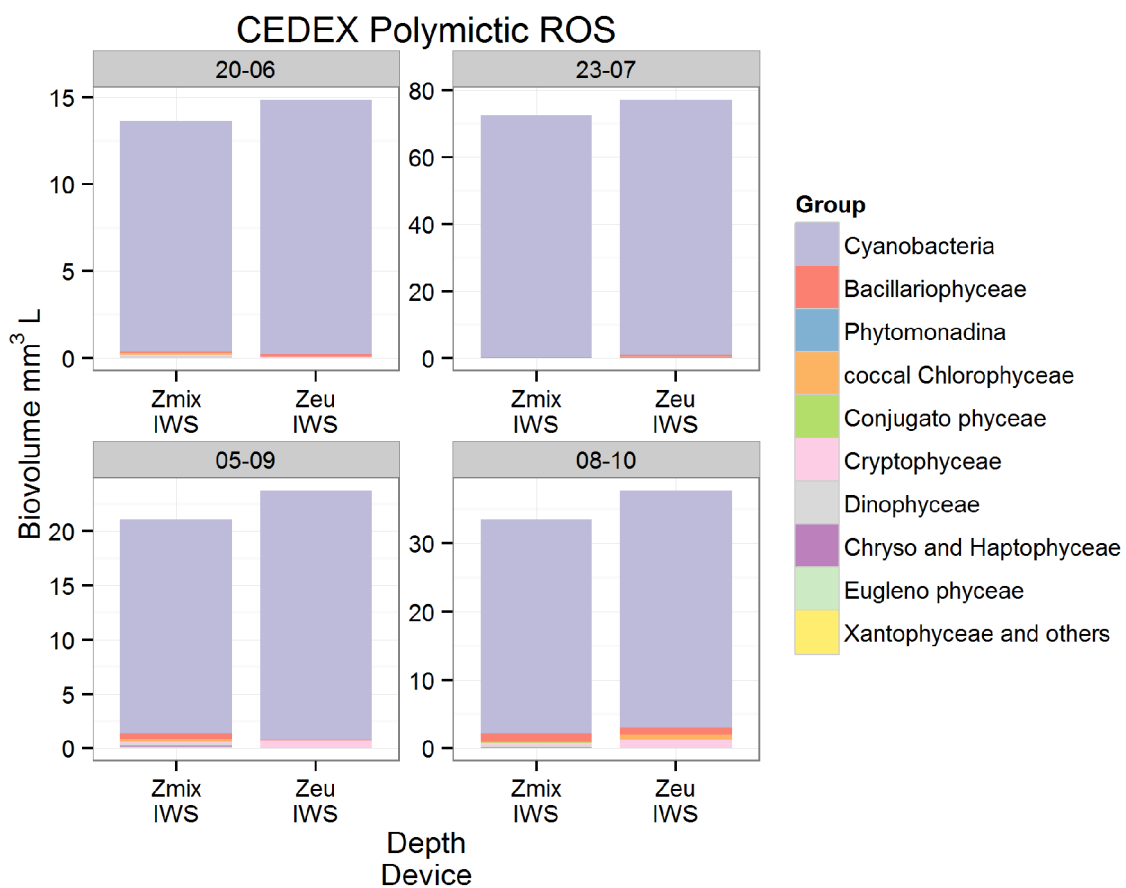
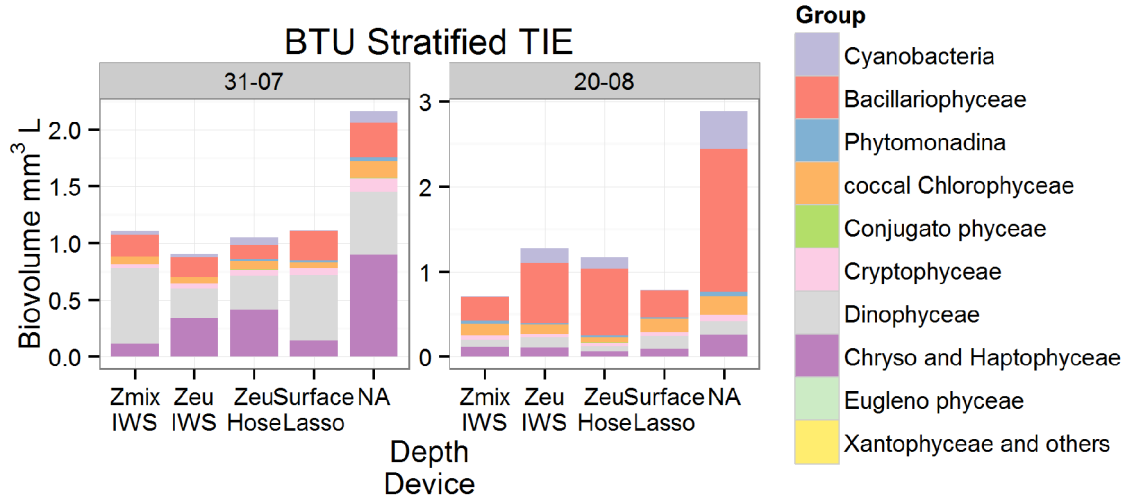


BTU Stratified DOB

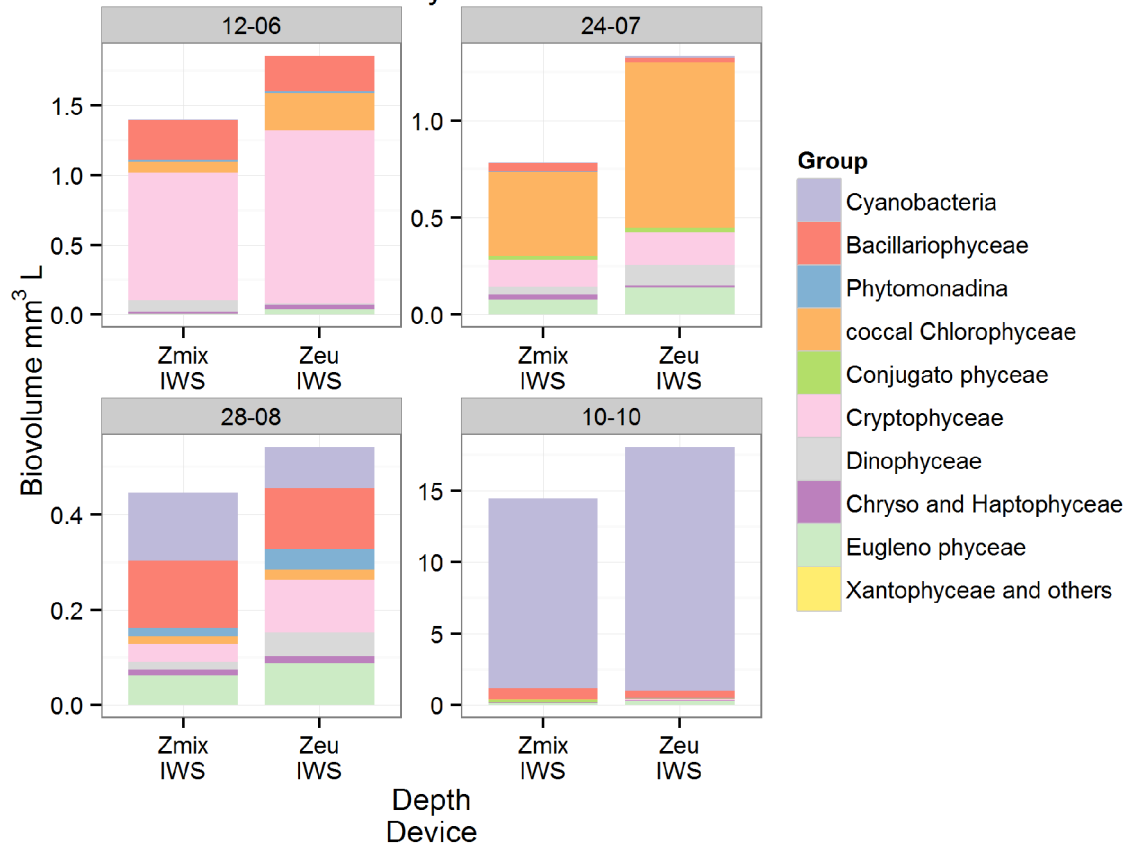




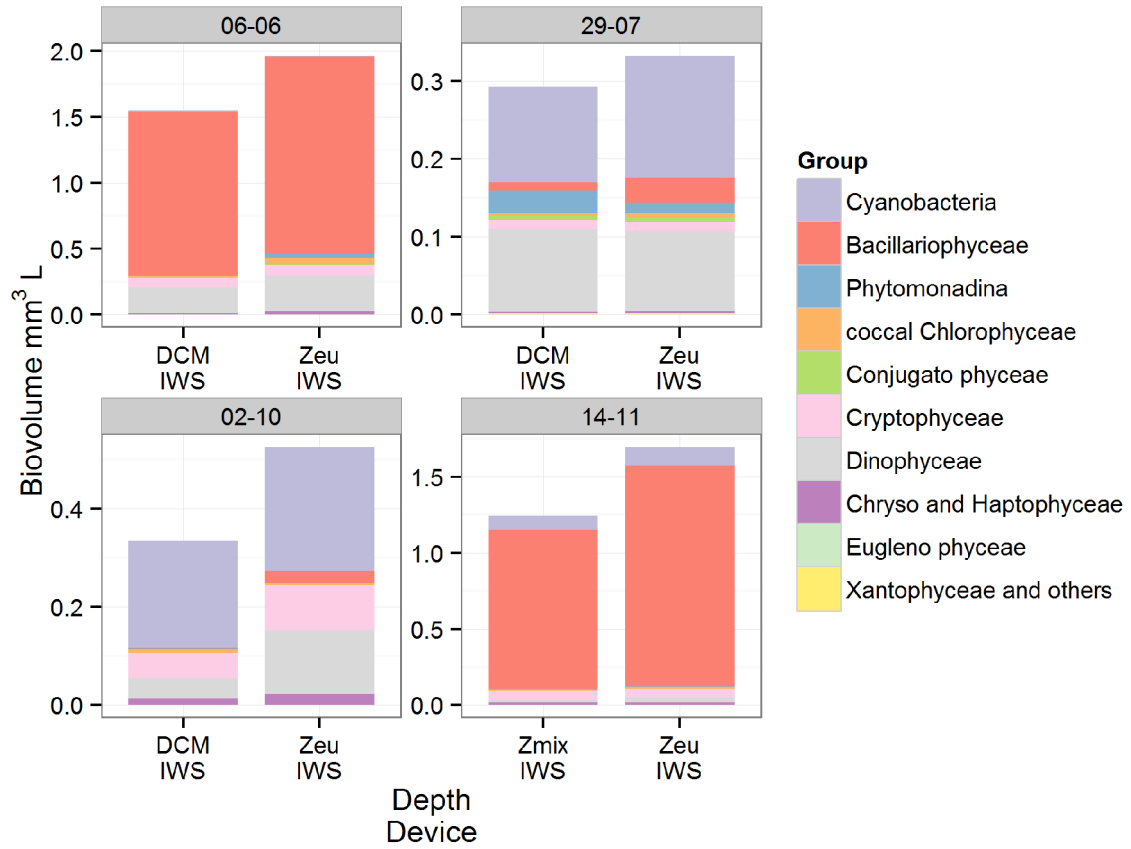




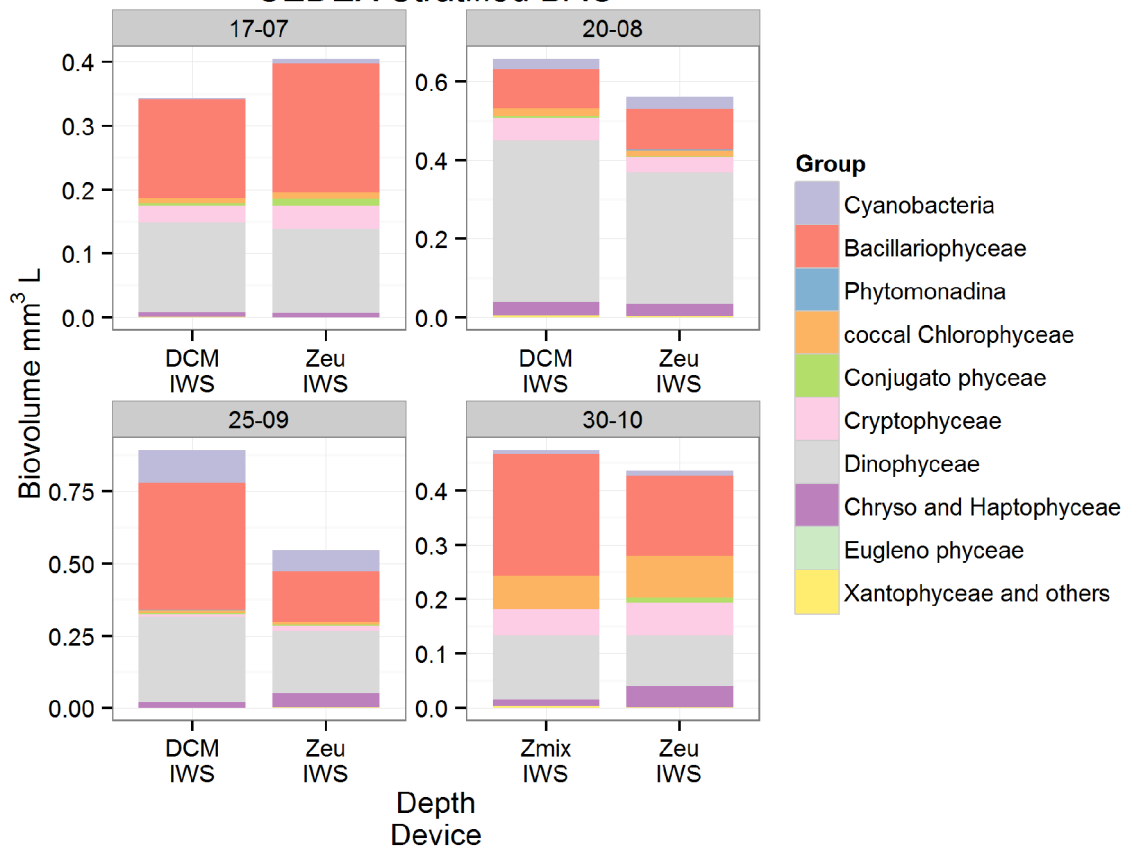
CEDEX Polymictic SER



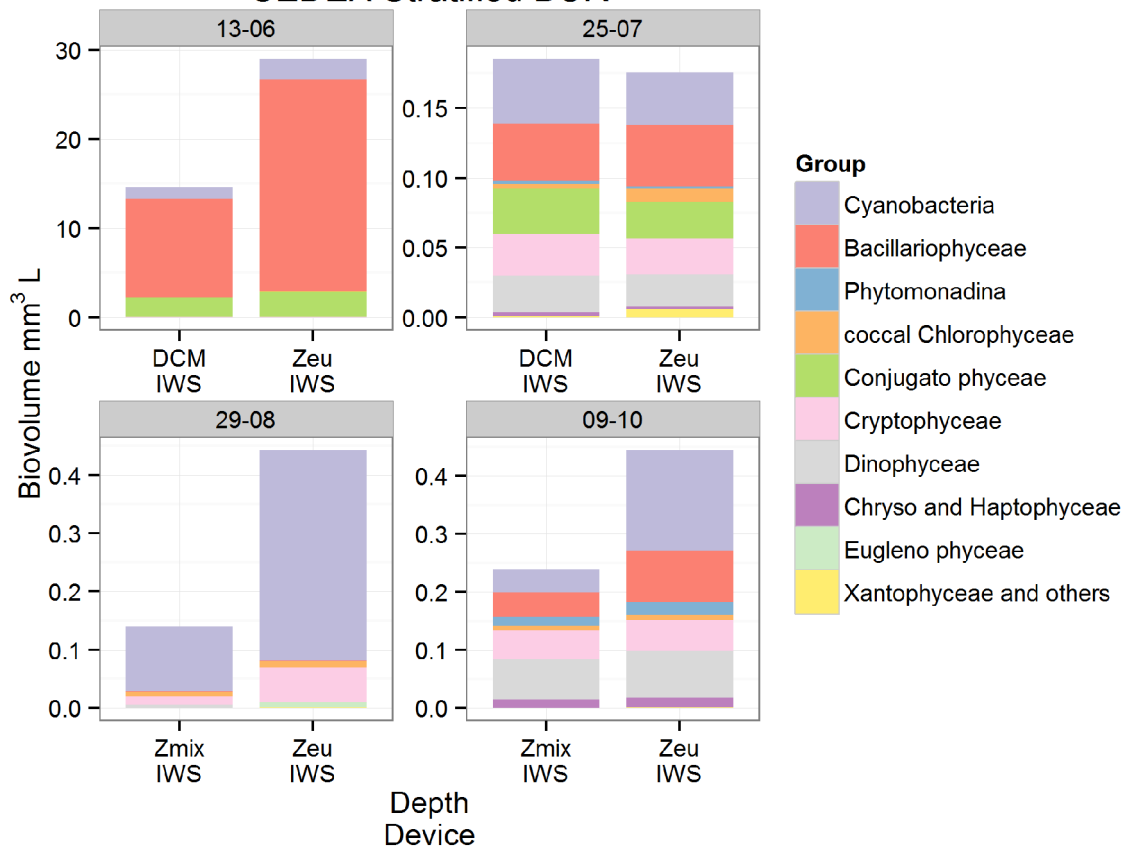
CEDEX Stratified ATA



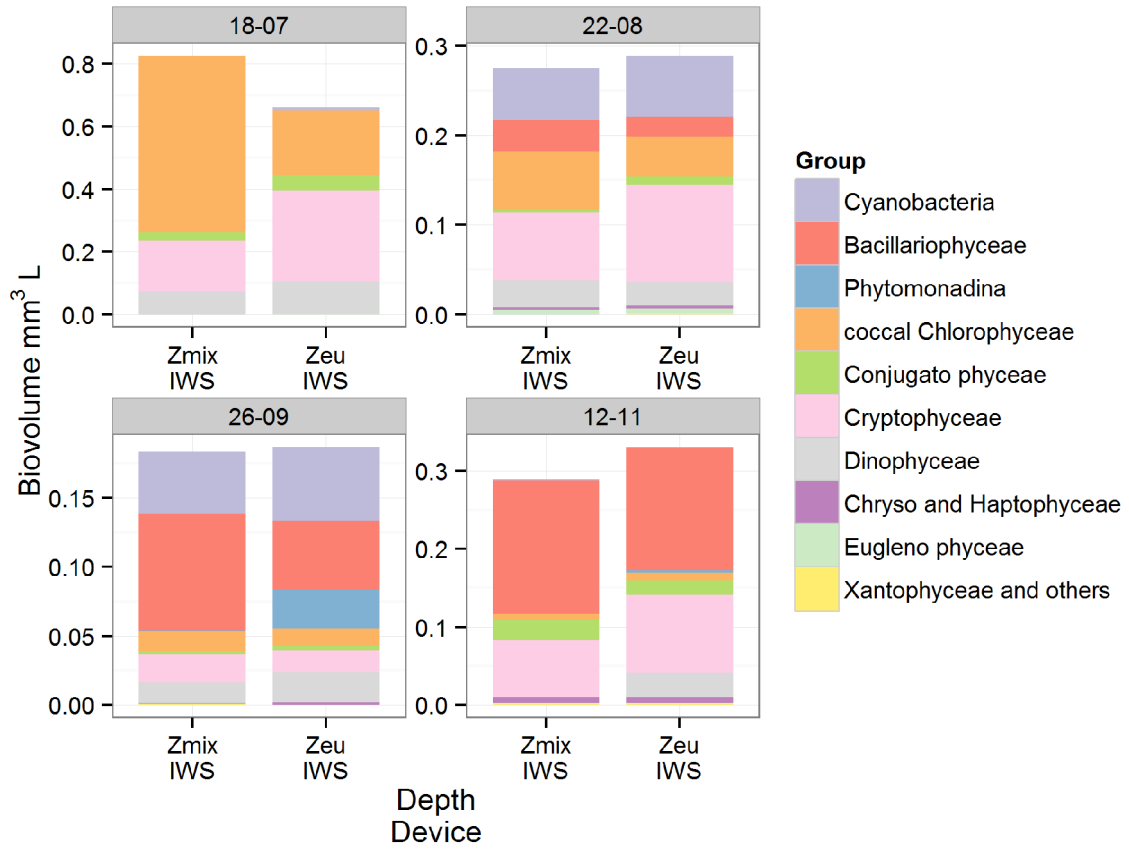
CEDEX Stratified BAO



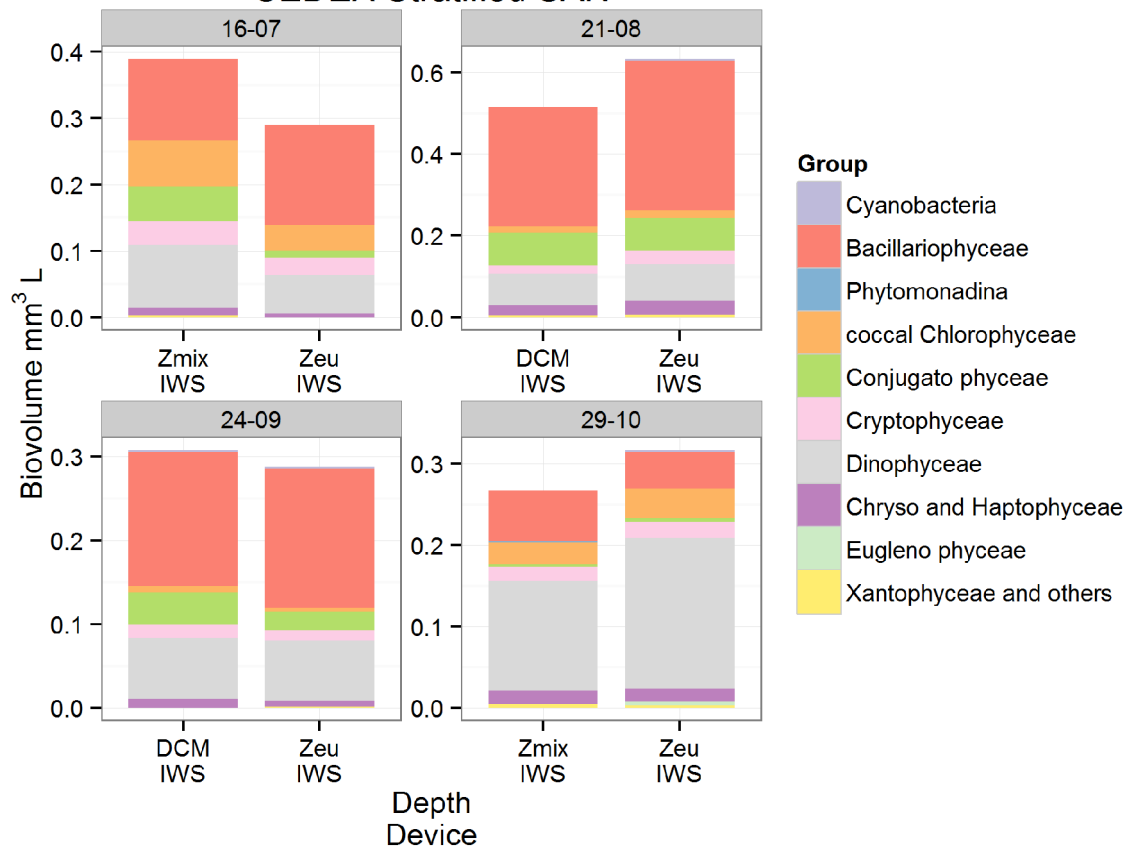
CEDEX Stratified BUR



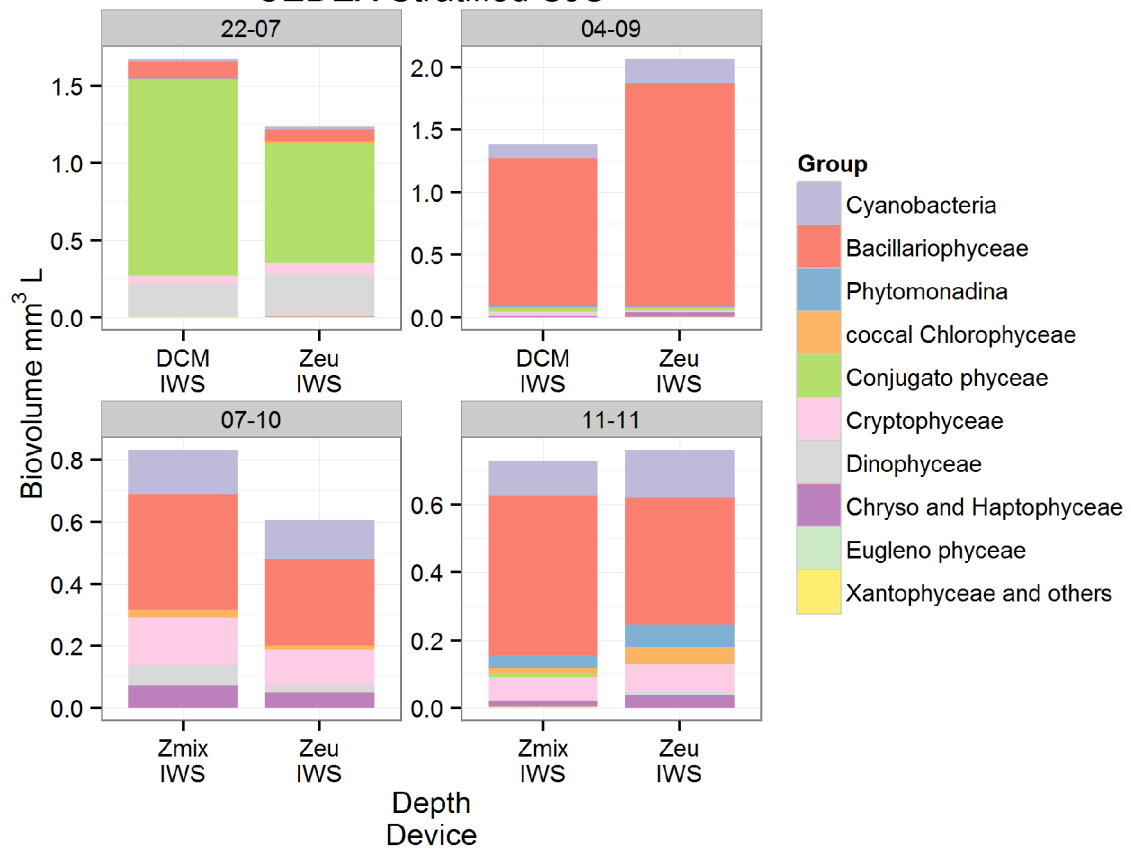
CEDEX Stratified RIC



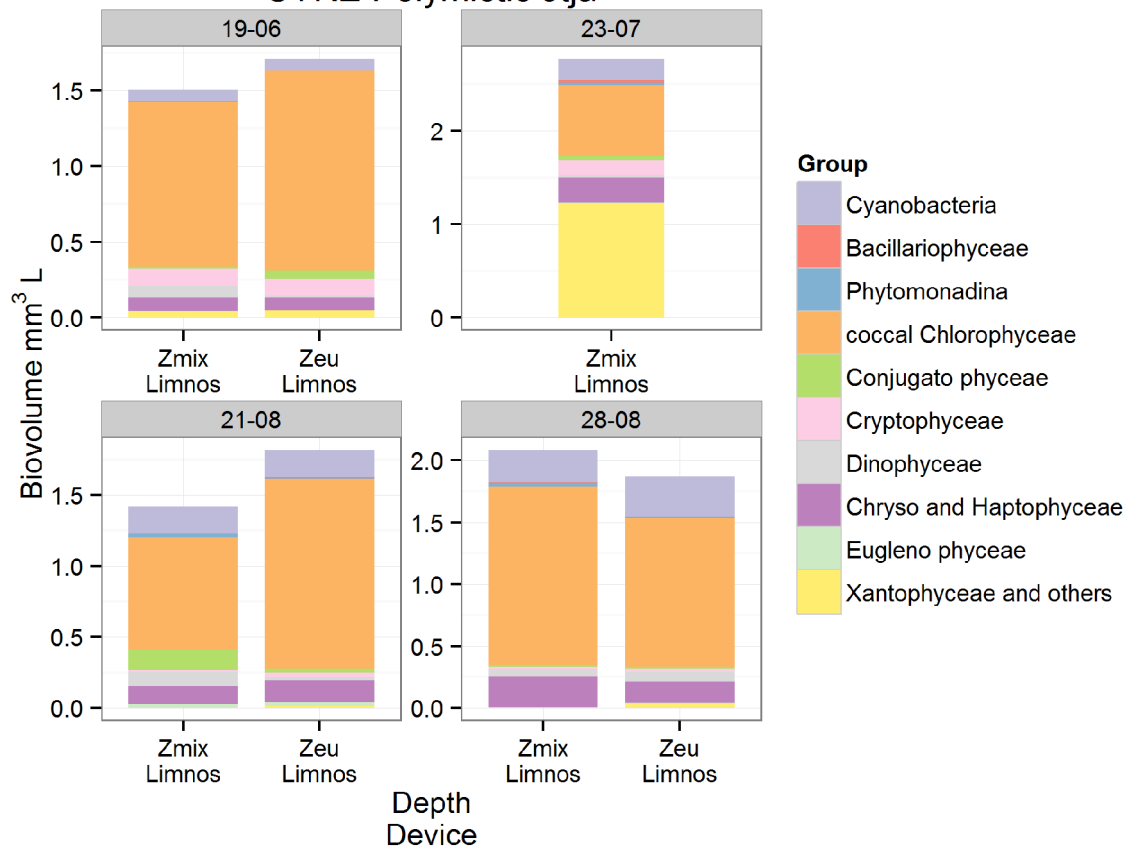
CEDEX Stratified SAN



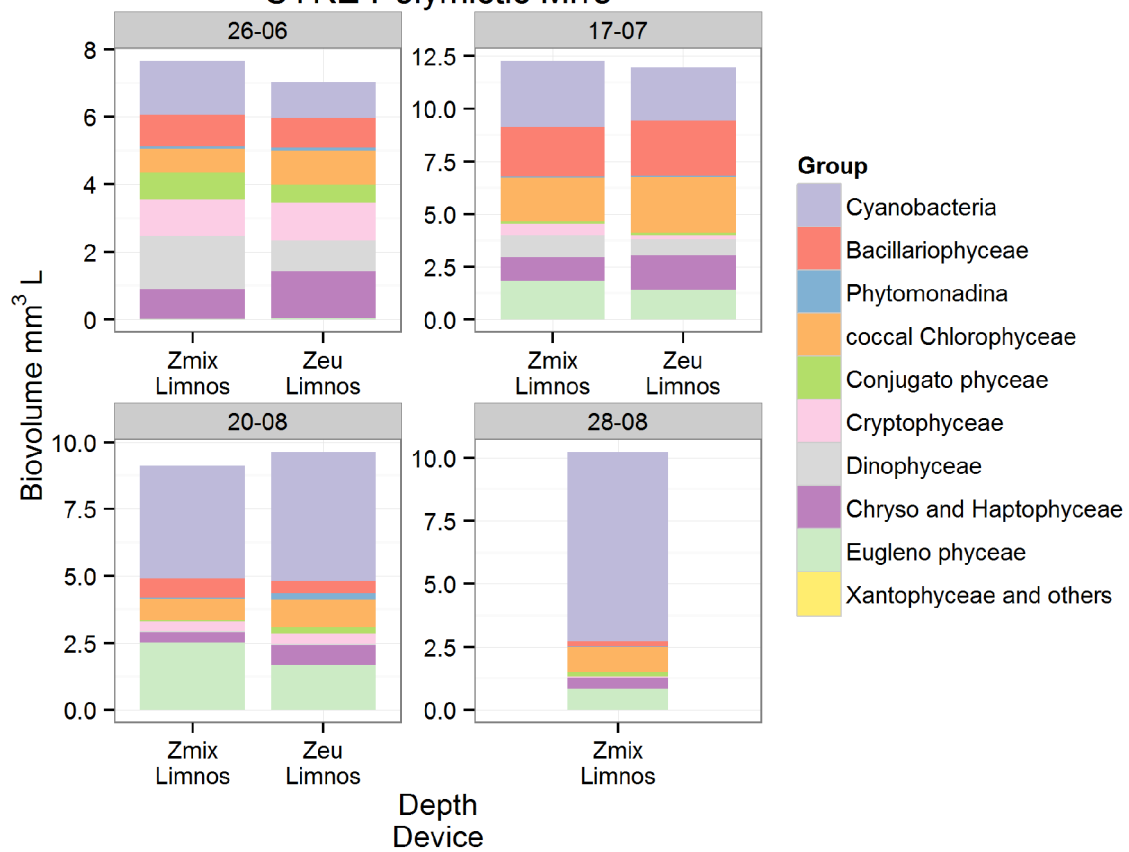
CEDEX Stratified SJU



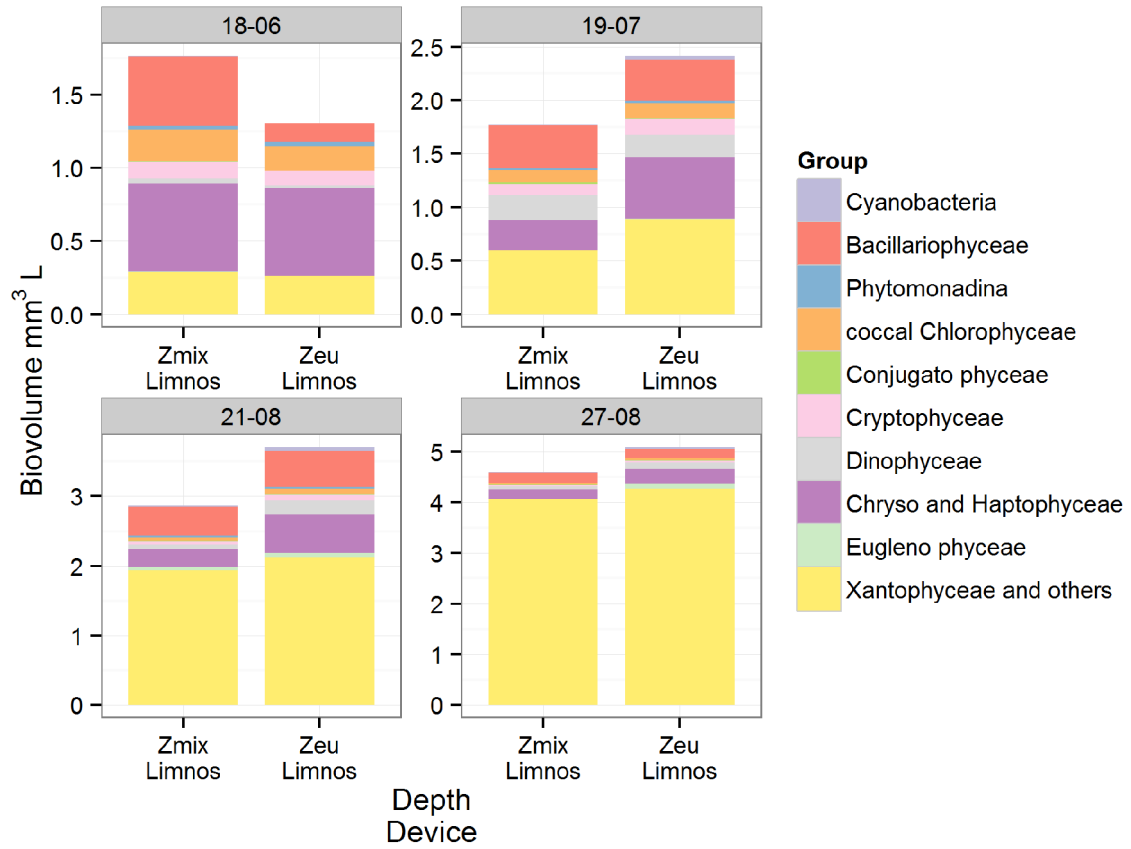
SYKE Polymictic Jtjä



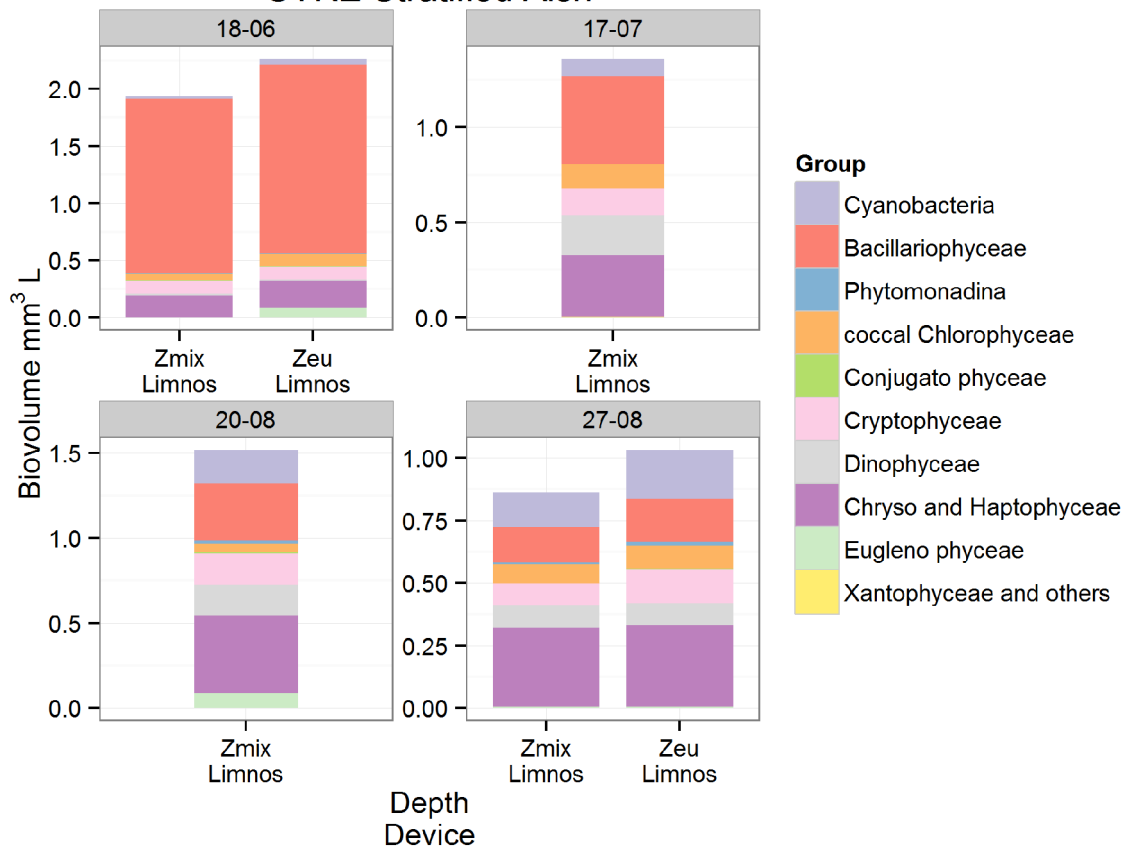
SYKE Polymictic Mrrs



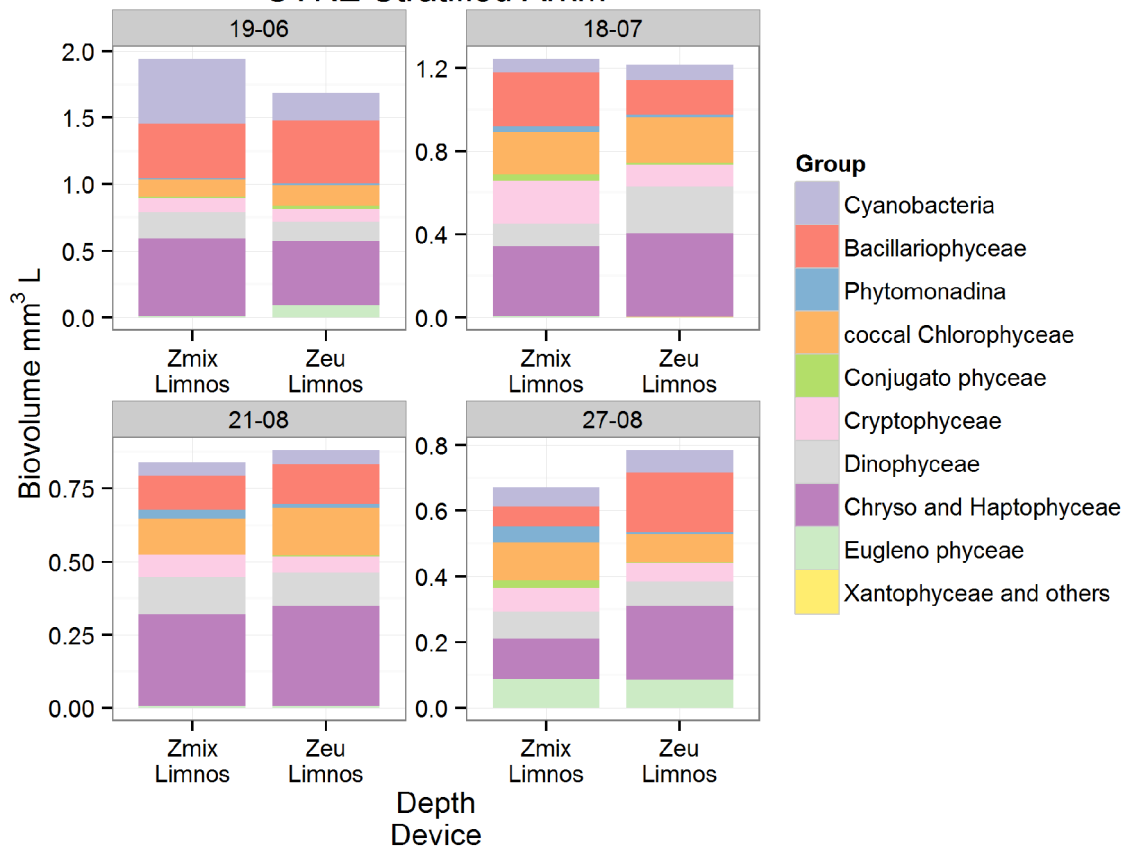
SYKE Stratified AInR



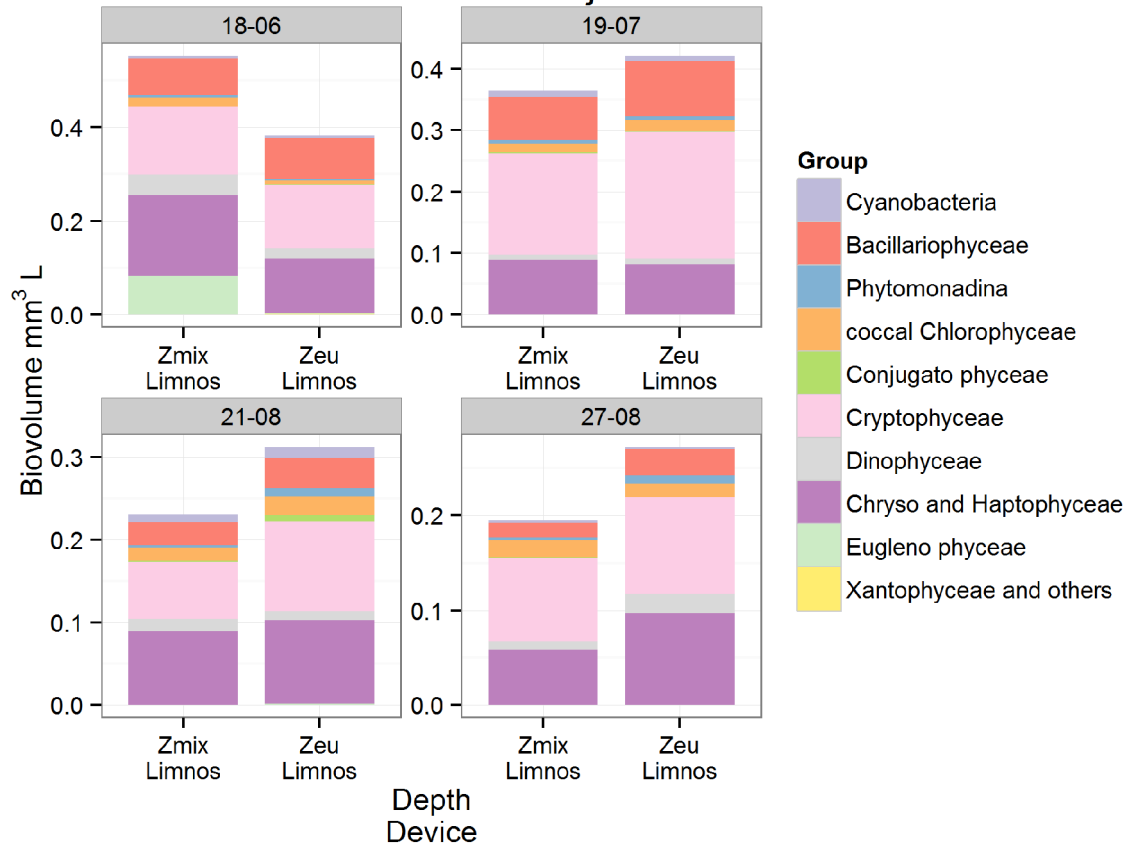
SYKE Stratified Alsn



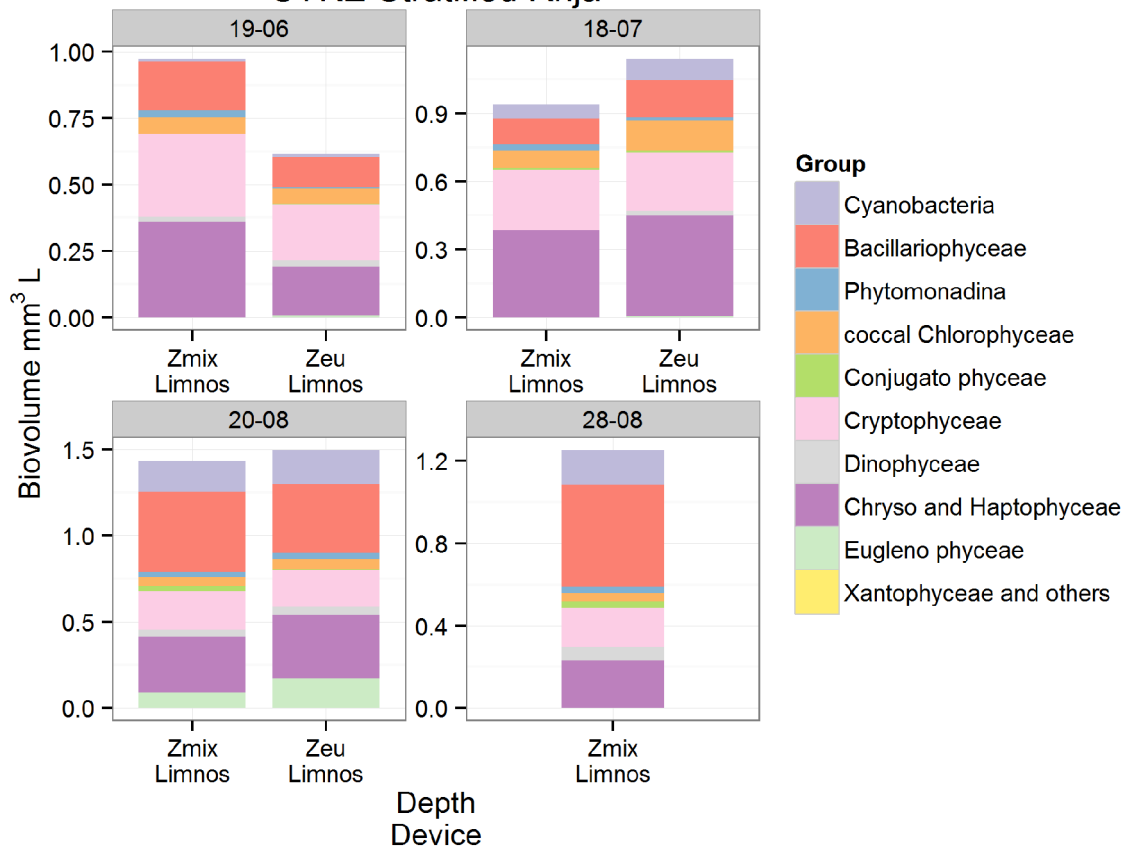
SYKE Stratified Arkm



SYKE Stratified Pääj



SYKE Stratified Rhjä



SYKE Stratified Työt

