Eco-AlpsWater

Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers

Priority 3: Liveable Alpine Space. SO3.2 - Enhance the protection, the conservation and the ecological connectivity of Alpine Space

Project Eco-AlpsWater
Work Package WPT3
Activity A.T3.2
Deliverable D.T3.2.1.
Version 1.0
Date June 2021
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Deliverable D.T3.2.1

Report on results obtained in the six key lakes

Contents

Introduction

Compiled from EAW meta data collection by Tina Elersek, (NIB, PP4)

All key lakes (Bled, Bourget, Garda, Lugano, Mondsee, Starnberger See) are included in L-AL3 lake type of the Alpine GIG (lowland or mid-altitude, deep, moderate to high alkalinity with alpine influence, large). Mixing type of the majority of lakes is monomictic, dimictic in Bled and meromictic in Lugano. Estimated water renewal time is quite diverse; less than 5 years for Bled, 5-10 for Bourget, 10-15 for Lugano and more than 20 years for Garda and Starnberger See. The majority of lakes have catchment area in the range between 101-1000 km^2. During different limnological seasons, the temperature of water at sampling campaigns was ranging from 3 to 27°C, with conductivity from 209 to 355 µS/cm. In our key lakes we have gathered 157 lake samples composed of 78 plankton and 79 biofilm samples.

Phytoplankton in key lakes

For phytoplankton, euphotic layer was sampled in a depth-integrated manner in most of the key lakes (only Starnberger See was sampled with depth integrated point sampling). Selected key lakes were rather deep. Euphotic layers were deeper than 20m for more than 40% of samples, at 10-15m for almost 30% and at 15-20 m for the rest of samples. The volume of water filtered for eDNA analyses was 0,5-1L for half of samples, less than 0,5L for one third of samples and more than 1L for all other samples.

Trophic status

The trophic status of key lakes has been assessed by three parameters: total phosphorus, transparency and chlorophyll-*a* concentration and analysed with OECD fixed boundary trophic classification system (OECD; 1982). We have revealed/confirmed that according to **phosphorus concentrations** (Fig. 1)**,** recognised as one of the main factors for growth of primary producers in lakes, the majority of samples from key lakes are oligotrophic (68%), followed by mesotrophic state (32%). Similarly, according to **transparency**, measured as Secchi depth (Fig. 1), the majority of samples are classified as oligotrophic (49%), followed by mesotrophic conditions (42%). On the other hand, according to **chlorophyll-*a* concentrations** (Fig. 1) the majority of samples from key lakes are classified mesotrophic (60%), followed by oligotrophic state (35%). In summary the vast majority of lake plankton samples are assigned to the oligo-mesotrophic state (Fig. 1).

Biofilm in key lakes

Dry weight of biofilm samples was mostly below 4g/L (60%), but surprisingly 26% of samples had quite high dry weight (>12g/L).

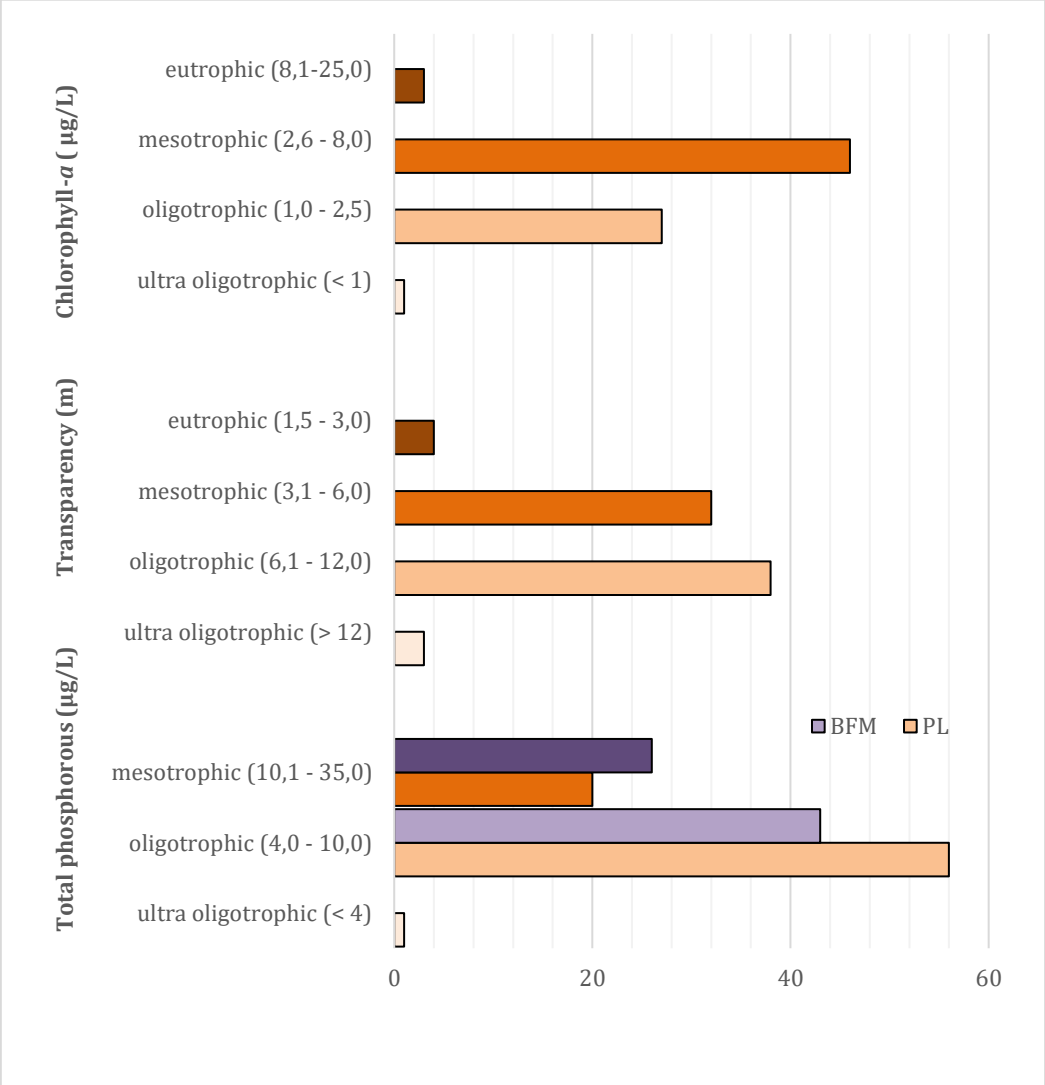


Fig. 1: Frequency distribution of samples from plankton (PL) and biofilm (BFM) according to trophic state derived from chlorophyll-a concentration (µg/L), transparency (Secchi depth) and total phosphorous concentration (µg/L), based on the trophic classification system (OECD; 1982).

1. Austria, Lake Mondsee

1.1 Phytoplankton (incl. cyanobacteria), L. Mondsee

Austria (PP2, LFUI)

Rainer Kurmayer, Hans Rund, Josef Wanzenböck

Sampling according to national legislative

Lake Mondsee in upper Austria was chosen as the pilot lake for this assessment. Samples were taken monthly starting from January in 2019 and until January 2020 given a total of 13 samples.

For the ecological assessment of the lake quality, phytoplankton samples were depth-integrated from 0-20 m corresponding to the euphotic zone at the deepest part of the lake (Fig. 1.1). Sample aliquots were used to determine the chlorophyll-a concentration as well as chemical parameters and nutrients following the Austrian legislative (GZÜV). Sample analysis according to GZÜV were provided by the Upper Austrian State Government (Linz, Austria).

The application of the Austrian method for lake assessment based on phytoplankton requires quantitative sampling from the water body. The abundance and the total biovolume of the planktonic algae were determined from a subsample under the inverted microscope (quantitative analysis).

For the year of study, the mean chlorophyll-a concentration was determined and the mean biovolume for each taxa was taken as the arithmetic mean of four or more dates. The total phytoplankton biovolume was calculated from the sum of the individual taxa. The brettum index was calculated from the relative proportions of the mean biovolumes of the individual taxa and taxa-specific trophic scores.

The ecological status assessment is a classification of the nutrient or production level of the lakes. The parameters used in the assessment included the chlorophyll-a concentration (annual mean), the total biovolume (annual mean) and the brettum index (which was calculated from the taxa list and the corresponding biovolumes in the annual mean).

In parallel water chemistry was determined according to the national legislative. Another water volume was filtered for cyanotoxin extraction according to protocol (Cyanotoxins analyses in lake and biofilm samples).

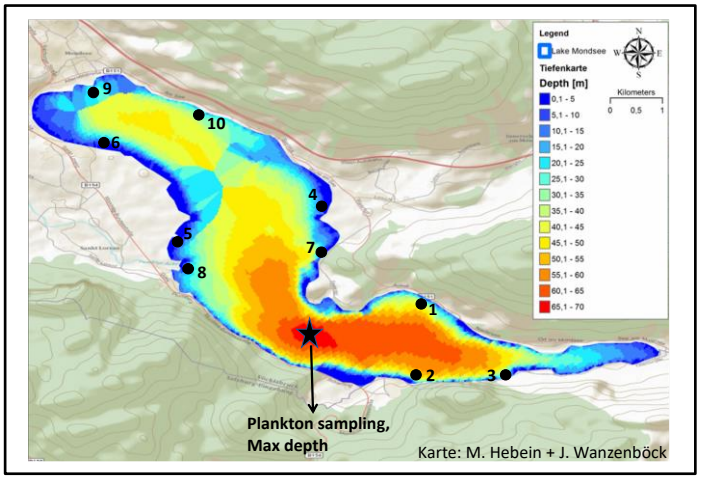


Fig. 1.1. Lake Mondsee, Upper Austria, sampling site (phytoplankton) and black circles mark sampling sites littoral (biofilm)

For DNA sequencing, the depth-integrated samples were taken in parallel and transported to the laboratory using cooling boxes. In the laboratory the planktonic samples were filtered through a Sterivex^{TM}-GP 0.22 µm filter (Millipore, Billerica, Massachusetts, USA), by pressing water manually through the filter unit with a plastic syringe following the protocol from WP1 (D.T1.1.2 -1 Lake plankton sampling). The syringe was cleaned before with MQ-Water and rinsed once with the sample itself. To estimate the filtered volume, a 1 L Duran bottle was used to capture the filtered water. The filtering was completed until the filter became clogged or when a total volume of 0.5 L was reached.

DNA extraction and sequencing

DNA was extracted using the DNeasy® PowerWater® SterivexTM Kit (Qiagen, Hilden, Germany) following the manufacturer`s protocol. (D.T1.1.2. -6 Plankton DNA extraction).

From sample DNA extracts 16S rDNA (V3-V4 region) has been amplified using primers 341Fmod CCTAYGGGRBGCASCAG and 806Rmod GGACTACNNGGGTATCTAAT under the following conditions: 95°C (5 min), 28 cycles including 95°C (30 sec), 55°C (30 sec), 72°C (30 sec), and final 72°C (5 min). For 18S rDNA (V4 region) the primers V4F-18S_ILL and CCAGCASCYGCGGTAATTCC and V4R-18S_ILL ACTTTCGTTCTTGATYRATGA were applied using the cycling conditions from above. Library preparation of purified PCR products for 16S rDNA and 18S rDNA was performed according to EAW Protocols. Bridge amplification and sequencing by synthesis were performed according to standard conditions (FEM, Miseq, Massimo Pindo). One technical replicate was sequenced (DT1.1.2. -10 Library prep 16S marker gene; DT1.1.2. -11, Library prep 18S marker gene).

Bioinformatic processing

The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2) , more details ? (Protocols DT1.1.3. - 3 Bioinf18S, Bioinformatics treatment 18S marker gene; DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene). Sequences were clustered into ASVs (no dissimilarity threshold) and assigned to the SILVA SSU reference database (or PR2 database) for taxonomic classification.

For cyanobacteria automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates (strains) and manual blastn against ASvs allowing 0-1 mismatch (405 bp alignment).

Comparison with traditional microscopy

The microscopical taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the REBECCA code for phytoplankton. To facilitate comparison an Excel Access database tool (version 6, May 2021) for all microscopical taxa and REBECCA codes assigned has been prepared (LfU, FEM, LFUI).

Mondsee overall trophic state

On the basis of the mean annual total phosphorus (TP) concentration (µg L^{-1}), mean annual chlorophyll-*a* (Chl-a) concentration (µg L^{-1}), maximum annual chlorophyll-*a* concentration (µg L^{-1}), mean annual Secchi-disk depth (m) and minimum annual Secchi-disc depth (m) the trophic state was adjusted using the OECD Fixed Boundary Trophic Classification System (OECD, 1982) (Table 1.1).

During 2019 Mondsee had an average TP concentration of 4.9 (min, max=2.4 – 7.5) µg/L, a mean Chl-a concentration of 3.5 (2.2-6) µg/L and a mean secchi depth of 4.9 (2.4-7.5) m and is thus assigned a meso-oligotrophic state.

Table 1.1. OECD Fixed Boundary Trophic Classification System (OECD, 1982)

Trophic category	Mean phosphorus concentration (μgL^{-1})	Mean chlorophyll concentration (μgL^{-1})	Maximum chlorophyll concentration (μgL^{-1})	Mean annual Secchi-disc depth (m)	Minimum annual Secchi-disc depth (m)
Ultra-oligotrophic	≤4.0	≤1.0	≤2.5	≥12.0	≥6.0
Oligotrophic	≤10.0	≤2.5	≤8.0	≥6.0	≥3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypereutrophic	≥100	≥25	≥75	≤1.5	≤0.7

Results on cyanotoxins concentrations

Microcystins (MC) were detected in lower concentration throughout the study period (5-68 ng/L). The higher share of demethylated structural variants such as MC-RR, MC-HtyR, MC-LR is likely produced by Planktothrix rubescens. During May and June 2019 low concentration of Anatoxin-a were detected (1.1-1.3 ng/L).

Results on comparison between traditional microscopy and HTS

A total of 14 algal groups were recorded under the microscope by traditional morphological analysis (Fig. 1.2). The **algal classes** with the highest biovolume were Cyanobacteria, Balliario(Medio)phyceae, Dinophyceae and Cryptophyceae, which were recorded in all 13 samples. Overall the phytoplankton composition was dominated by cyanobacteria (*Planktothrix rubescens*) representative of phytoplankton association R (sensu Reynolds et al. 2002), Fig. 1.2. The seasonal development typically started with increased growth of centric diatoms (*Cyclotella, Stephanodiscus*) in March 2019, while cyanobacteria (*P. rubescens)* became dominant during July until the end of the study period. Similarly dinoflagellates (*Peridinium, Ceratium*) and Cryptomonads became more abundant during the second half of the year 2019.

In general, ten algal classes were detected using both methods. By HTS nine algal classes were found through metabarcoding, which were not detected under the microscope (Table 1.2). However, Chlorarachniophyceae and Ulvophyceae were not found with the SILVA reference database, but only with the PR2 database. Prymnesiophyceae were only found by manual reassignment using BLASTn while Euglenophyceae were only found using the 16S rDNA. Tour algal classes taxa were not identified by metabarcoding, even though these were found under the microscope.

Table 1.2. Comparison of algal taxa at class level for Mondsee detected using the two different methods (microscopical analysis vs sequence analysis) or detected only by one method

Taxa identified by both Methods	Taxa identified only through HTS	Taxa identified only through microscope
Bacillariophyceae	Bikosea	Euglenophyceae
Chlorophyceae	Bolidophyceae	Xanthophyceae
Chrysophyceae	Chlorarachniophyceae	Zygnematophyceae
Coccolithophyceae	Dictyochophyceae	
Coscinodiscophyceae	Florideophyceae	
Cryptophyceae	Mamiellophyceae	
Cyanophyceae	Perkinsea	
Dinophyceae	Prymnesiophyceae	
Mediophyceae	Ulvophyceae	
Synurophyceae		

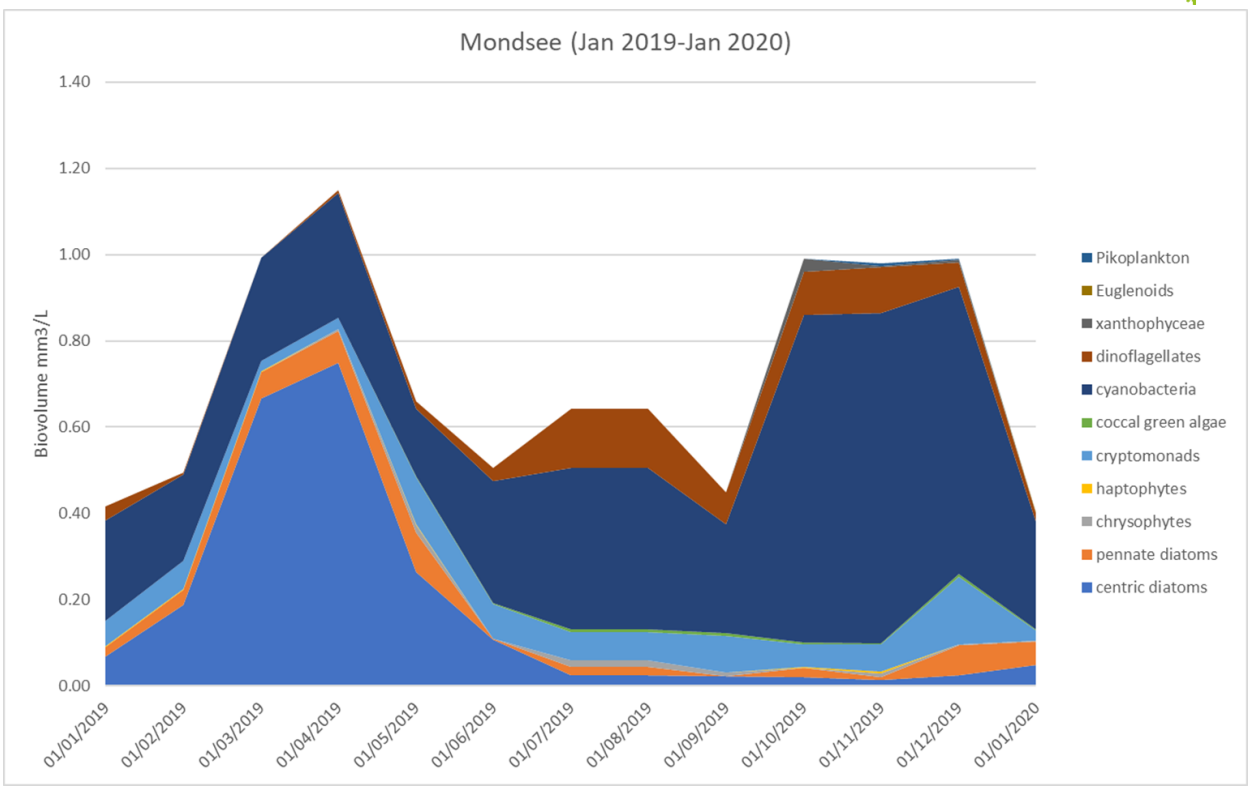


Fig. 1.2. Absolute abundance of phytoplankton biovolume composition as inferred from microscopical analysis (Mondsee Jan 2019-Jan 2020)

Since the assessment of ecological status classification is based on **phytoplankton species** an important question is, how well the resolution of the modern HTS method works on a species level. The species that could be found through morphological analysis were compared, to see which ones could be identified with the modern method of metabarcoding. Additionally, species which could not be found under the microscope, were also analyzed. For taxonomic precision the REBECCA code was used.

It can be seen from the results from Lake Mondsee (Suppl. Table 1.1-) that > 30 of the species detected under the microscope were recognized through 16S rDNA or 18S rDNA sequencing. This listed included abundant cyanobacteria (*Planktothrix rubescens*), Bacillariophyceae (*Asterionella, Ulnaria, Fragilaria*), Chrysophyceae (*Dinobryon, Mallomonas*), dinoflagellates (*Ceratium, Peridinium*), Cryptophyceae (*Cryptomonas*), and Chlorophyta (*Botryococcus, Planctonema*), Haptophyta (*Chrysochromulina*). Together this species accounted for …. Of the biovolume.

On the other hand species not recognized through HTS were mainly included among the centric diatoms, i.e. genera *Cyclotella* and *Stephanodiscus*. Together the non-corresponding species accounted for … of the biovolume.

A number of taxa which were not detected under the microscope were identified through HTS, i.e. Florideophyceae (*Batrachospermum*), Prymnesiophyceae, *Synechococcus* and *Cyanobium*. Florideophyceae were assigned to *Batrachospermum* and *Bangia*.

During the study period the cyanobacteria Prochlorophyta, and the nuisance cyanobacteria Tychonema and Microcystis (which were identified in other study lakes) were not detected in Lake Mondsee

Notably flagellates of the algal classes Chrysophyceae, Dinophyceae and Chlorophyta (Volvocales, Prasinophyta) were not detected under the microscope bur reported through HTS. Certain groups of flagellates shared a rather high number of genotypes, i.e. for Chrysophyceae (146 18S rDNA genotypes) were recorded.

Conclusion on results obtained for phytoplankton

Relevant information derived from sequencing includes

(i) overall good qualitative relationship between HTS derived genera and microscopy derived genera, i.e. sequence based confirmation of microsciopy results on genus level

(ii) additional information on certain groups of algae which have not been well recorded before, i.e. Rhodophyta, and picocyanobacterial and eukaryotic flagellates (Chrysophyceae, Dinophyta, Prasinophyta)

(iii) additional (biogeographic) information on presence/absence of nuisance algae, i.e. *Planktothrix rubescens/agardh*ii, *Tychonema bourellyi*, *Microcystis aeruginosa*

(iv) information on intraspecific genetic variation among populations, i.e. detection of novel genotypes within populations of algal species.

1.2 Biofilm composition (littoral), L. Mondsee

Austria (PP2, LFUI)

Rainer Kurmayer, Hans Rund, Josef Wanzenböck

Sampling

Phytobenthos has proven to be an indicator for ecological quality status in rivers. In Austria all phytobenthic algae groups, including Cyanobacteria, are used as biological quality elements. Exempt from this are only Charophyceae who, by tradition, are recorded within the scope of the macrophyte method. In contrast to rivers, in Austria for the littoral in lakes no biological quality element based on phytobenthos has been ever applied. No national legislative on littoral (biofilm) sampling is available. Thus for this project the guidelines from the national legislative on sampling in rivers have been applied along with the protocol developed in WP1 (DT1.1.2. -2, Lake biofilms sampling protocol).

There were ten different sampling locations chosen for Mondsee sampled at 11-12 October 2018 (Fig. 1.1). During 2018 the minimum water level recorded for Mondsee was 113 cm which was 50 cm below minimum water during previous years. Thus because of the rather dry summer conditions during 2018 the overall water level in Mondsee was found reduced. For reach site 5 stones were selected along the shoreline representing an area of 50-100 m^2 and then transported into the laboratory using cooling boxes. The shoreline of Mondsee is mostly affected through building activity, and natural shorelines are rare. The ten sampling sites were distributed along the shoreline to represent both the more eutrophic and more shallow Eastern basin, and the more oligotrophic and deep Western basin. Nevertheless the environmental gradient in TP concentration among more oligotrophic sites (No. 1,2,3) and more eutrophic sites (No. 6, 9, 10) was relatively weak. TP concentration ranged from ca. 6 µg/L (No1,2,3) to 8 µg(L (no 6, 9, 10).

In general samples were brushed off from stones from a representative surface area (> 100 cm^2) using a clean tray. Aliquots were further processed for microscopical assignment (so-called soft algae or non-diatoms) using formaldehyde-fixed samples. Diatoms were identified and counted by their silicate frustules subsequent to acid digestion and mounting in Naphrax for microscopical analysis. In traditional phytobenthic assessments, diatoms and non-diatoms are evaluated at a ratio of 1:1. This means the evaluated taxa will sum up to two hundred percent.

In parallel to sampling for microscopy, for DNA extraction from the same stones aliquots were preserved using 80% Ethanol as described in protocol (DT1.1.2. -2, Lake biofilms sampling protocol).

Finally aliquots were scratched directly onto pre-weighed GF/C Filters and the dry-weight was determined from the difference in dried filter (105°C, 24 h) weight before and after filtration. Aliquots without drying but stored at -20°C were then used for cyanotoxin extraction (protocol: Cyanotoxins analyses in lake and biofilm samples).

Results on cyanotoxins concentrations

Out of 10 sampling sites only Anatoxin- a was detected at 5 sites (no2,4, 7,8,9) in variable concentration. At sites no2, 4, 7, 8 rather low Atx-a concentrations (0.01-0.05 ng/mg DW) occurred. At site no9 the maximum of 1.9 ng Atx-a/mg DW were recorded.

DNA extraction and sequencing

DNA was extracted using the Machery and Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (DT1.1.2. -7, DNA extraction biofilms)

From sample DNA extracts 16S rDNA (V3-V4 region) has been amplified using primers 341Fmod CCTAYGGGRBGCASCAG and 806Rmod GGACTACNNGGGTATCTAAT under the following conditions: 95°C (5 min), 28 cycles including 95°C (30 sec), 55°C (30 sec), 72°C (30 sec), and final 72°C (5 min). For 18S rDNA (V4 region) the primers V4F-18S_ILL and CCAGCASCYGCGGTAATTCC and V4R-18S_ILL ACTTTCGTTCTTGATYRATGA were applied using the cycling conditions from above. One technical replicate was sequenced (DT1.1.2. -10 Library prep 16S marker gene; DT1.1.2. -11, Library prep 18S marker gene).

PCR amplification and library preparation of purified PCR products for rbcL was performed according to WP1 protocol (DT1.1.2. -9, Library prep RbcL marker gene). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions.

Bioinformatic processing

The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2), (DT1.1.3. - 1 BioinfRbcL, Bioinformatics treatment RbCL marker gene, DT1.1.3. - 3 Bioinf18S, Bioinformatics treatment 18S marker gene, DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene).

For cyanobacteria automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates (strains) and manual blastn against ASvs allowing 0-1 mismatch (405 bp alignment).

Sequences were clustered into ASVs (no dissimilarity threshold) and assigned to the SILVA SSU reference database (or PR2 database?) for taxonomic classification. For rbcL gene assignment to diatom taxa the curated database R-Syst::diatom (Rimet et al. 2016) was used (INRA).

Comparison with traditional microscopy

All microscopical taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the VALID code system for diatoms in phytobenthos (LfU) and the REBECCA code for non-diatoms (soft algae) An Excel Access database for all microscopical taxa and the VALID codes assigned has been prepared (LfU, FEM, LFUI).

In traditional phytobenthic assessments, diatoms and non-diatoms (soft algae) are evaluated at a ratio of 1:1 summing up to 200%. Microscopical countings were performed according to the national legislative by DWS HydroÖkologie GmbH (Vienna, Austria).

Results on comparison between traditional microscopy and HTS

Overall, 9 algal groups were reported through microscopical, counting, most importantly Cyanobacteria, Bacillariophyceae, Rhodophyta and Chlorophyta. Via Metabarcoding, for Mondsee littoral 19 classes were found. Therefore, 10 new taxa were detected through metabarcoding. The values of the new classes differed in percentage, but they were all less common compared to the taxa that were identified by both methods.

Within soft algae cyanobacteria and chlorophyta contributed >90% to total microscopic countings. Within cyanobacteria filamentous non-heterocyst forming species were most adundant, i.e. *Pseudanabaena catenata, Planktolyngbya limnetica, Limnothrix redekei, Schizothrix lacustris, Phormidium incrustatum*, and Oscillatoria tenuis. *Pseud. catenata, Plankto. limnetica, Limno. redekei, Schiz. lacustris* dominated at the more

oligotrophic sites (No1,2,3). At the more eutrophic site (No9, 10) *Phorm. incrustatum, Oscill. tenuis* as well as the green algae *Mougeotia* sp were more frequent.

According to the Austrian phytobenthos trophic indication system (Pfister et al. 2016) *Pseud. catenata* and *Phorm. Incrustatum* are indicating mesotrophic conditions, while *Schiz. Lacustris* is indicating (ultra)oligotrophic conditions. In contrast *O. tenuis* is indicating more eutrophic conditions.

For cyanobacteria, 26 taxa were detected through both microscopy and 16S rDNA sequencing. As for phytoplankton, the correspondence between both methods was found on the genus level, i.e. Pseudanabaena, Phormidium, Leptolyngbya.

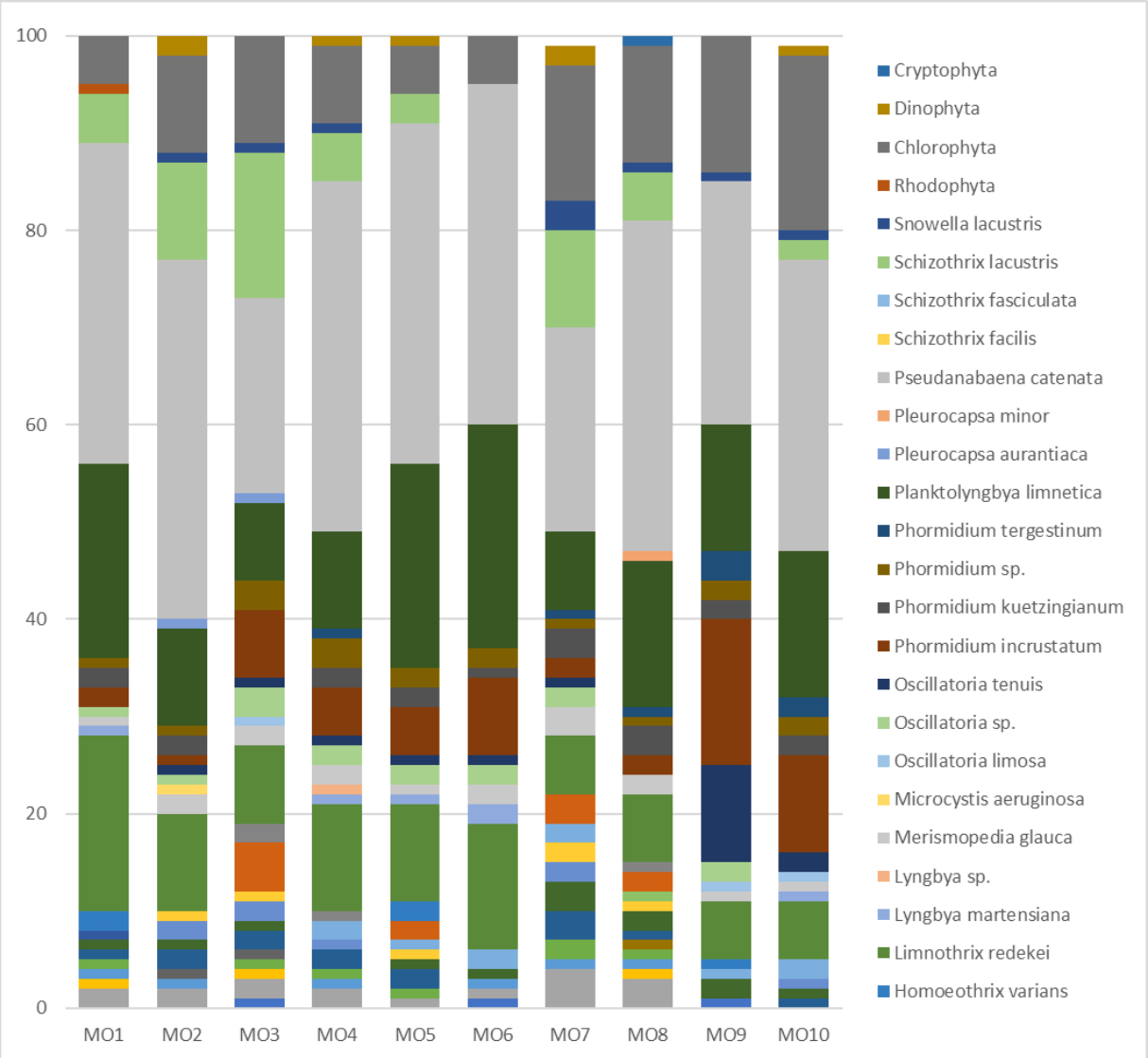


Fig. 1.3. Relative abundance of cyanobacteria and other algal groups at ten littoral sampling sites from Mondsee as revealed from microscopical counting (for location of sites see Fig. 1.1).

Table 1.3. List of algal classes from Mondsee littoral samples as identified using both sequencing and microscopical counts.

Algal classes (16S and 18S rDNA) of Mondsee littoral	Algal classes (microscopy) of Mondsee littoral
Bacillariophyceae	*Bacillariophyceae*
Charophyceae	*Chlorophyceae*
Chlorophyceae	*Coscinodiophyceae*
Chrysophyceae	*Cryptophyceae*
Coleochaetophyceae	*Cyanophyceae*
Coscinodiscophyceae	*Dinophyceae*
Cryptophyceae	*Florideophyceae*
Cyanophyceae	*Mediophyceae*
Dinophyceae	*Zygnematophyceae*
Eustigmatophyceae	
Florideophyceae	
Mediophyceae	
Pavlovophyceae	
Stylonematophyceae	
Synchromophyceae	
Synurophyceae	
Trebouxiophyceae	
Ulvophyceae	
Zygnematophyceae	

Within diatoms, representatives of the low-profile guild were mostly found, i.e. *Achnanthidium delmontii, A. lusitanicum, A. minutissimum, A. nanum, A. zhakovschikovii, Amphora inariensis, Amph. pediculus, Encyonema bonapartii, Encyonopsis minuta, Encyonopsis subminuta.* Other more frequent taxa included

Navicula cryptotenella and *Cyclotella wuethrichiana*. *Encyon. subminuta, Encyonema bonapartii, Encyon. minuta* dominated at the more oligotrophic sites (No1,2,3). At the more eutrophic site (No9, 10) *Achn. lusitanicum, Achn. Delmontii, Encyonema. bonapartii, Encyon. minuta* were more frequent.

According to the Austrian phytobenthos trophic indication system (Pfister et al. 2016) *A. minutissimum, Encyon. minuta, Encyon. subminuta* are indicating oligo(meso)trophic conditions. *Am. inariensis* and *Am. pediculus* are indicating more eutrophic conditions. *A. delmontii* is considered a so-called invasive species.

A larger number of taxa which were not detected under the microscope were identified through HTS (89 species). In particular a number of (fast) motile species assigned to *Navicula* (5) and *Nitzschia* (9) and *Sellaphora* (4) and *Surirella* (3) and *Gyrosigma sciotense* were recorded. In addition representatives of taxa assigned to the high-profile guild were more frequently detected, i.e. *Eunotia arcus*, *Gomphonema* (5), Gomphonella (2), *Melosira varians*, *Diatoma* (2), *Ellerbeckia* sp. and *Fragilaria* (3). Finally, the genera Cymbella (7) and Encyonema (5) assigned to the low-profile guild were recored with a greater variety. Only a few planktonic taxa were detected (*Aulacoseira subarctica*, *Cyclotella* spp., *Tabellaria flocculosa*).

Conclusion on results obtained for phytobenthos (cyanobacteria & diatoms)

Relevant information derived from sequencing includes the following:

(i) For cyanobacteria correspondence between microscopy and 16S rDNA sequencing is useful to confirm microscope based identification of genera

(ii) The 16S rDNA sequencing information is useful to infer the toxigenic potential of the respective biofilm community, e.g. at site No9 high contents of anatoxin-a have been reported and

candidate genera such as Tychonema can be folowode more closely to identify the respective nuisance organism

(iii) For diatoms the additional information on the occurrence of taxa assigned to the motile guild will provide relevant additional ecological information (e.g. regarding the variability of the water level).

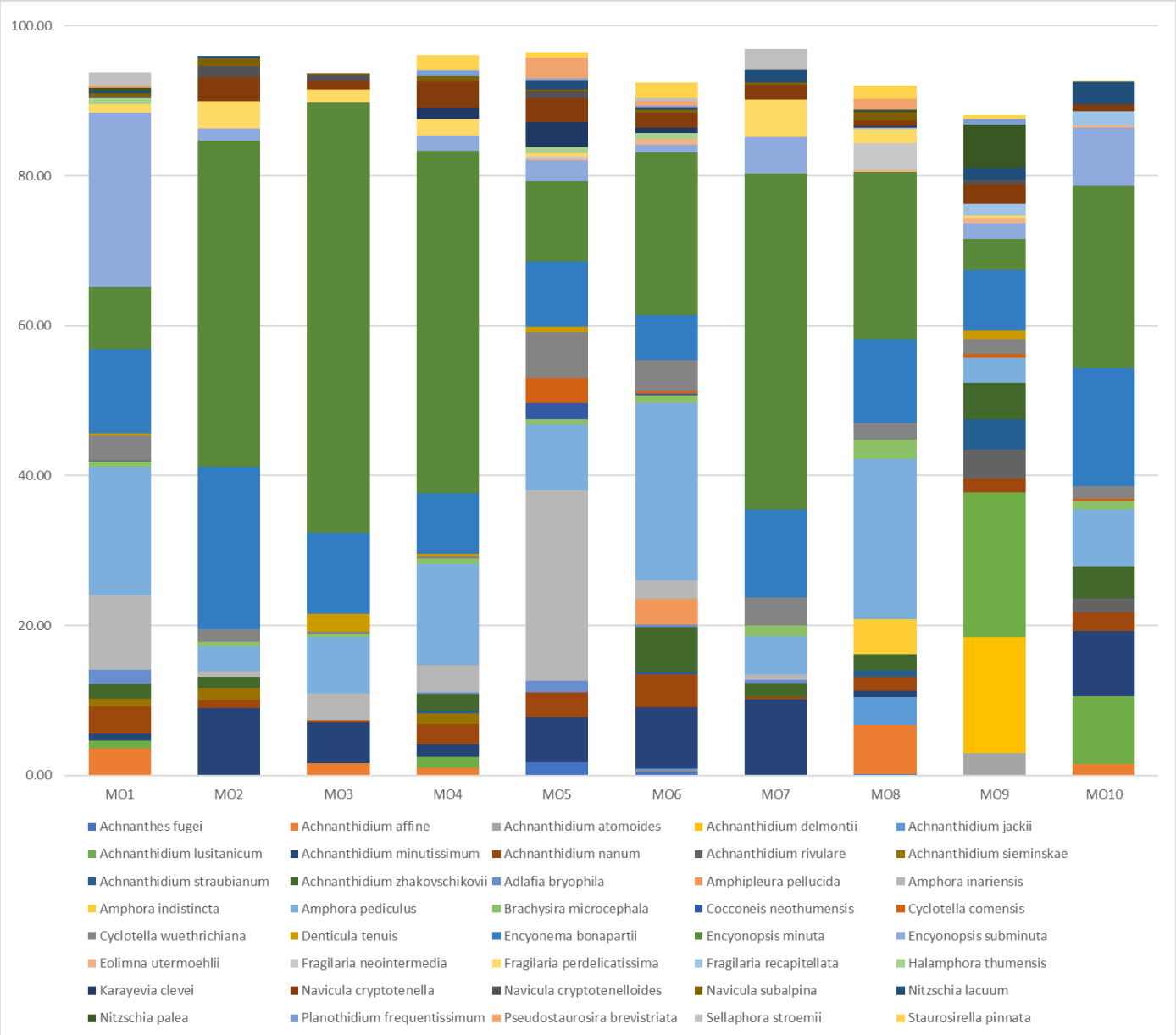


Fig. 1.4. Relative abundance of diatoms (> 2 %) at ten littoral sampling sites from Mondsee as revealed from microscopical counting (for location of sites see Fig. 1.4).

1.3 Fish composition, L. Mondsee

Hans Rund, Josef Wanzenböck, Rainer Kurmayer

Sampling

The sampling for fish eDNA at Lake Mondsee was carried out on the 19 September 2019, according to the Eco-AlpsWater protocol D.T1.3.1-4 - Lake and river eDNA Fish sample collection from the field for

downstream molecular analysis. Five 30 L samples were collected along 4 lakeshore transects (6 km each) and at three pelagic locations (Fig. 1.5 C), including the deepest point of the lake. In addition, fourteen 5 L samples were collected along shoreline stretches identical to the electrofishing sites during the last traditional sampling (Fig. 1.5 B).

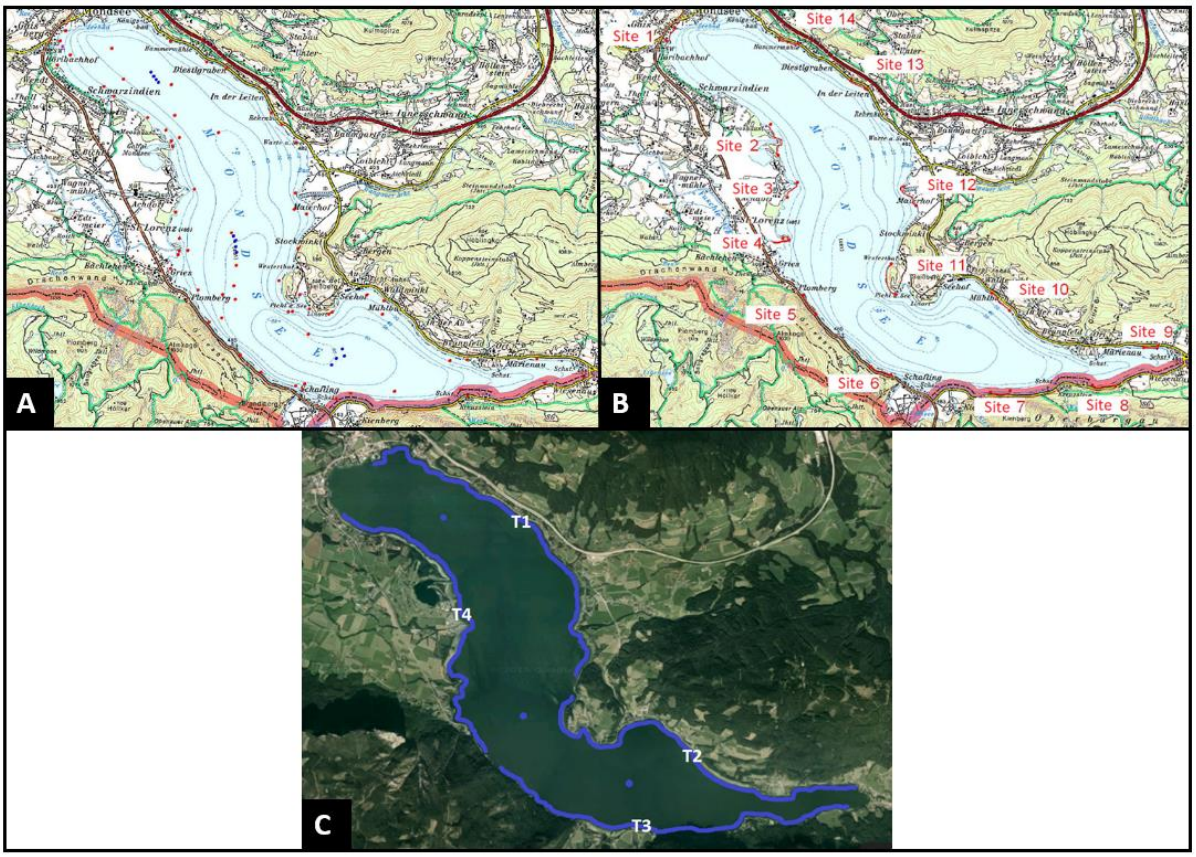


Fig. 1.5. Sampling sites at Lake Mondsee. A = pelagic (blue dots) and benthic (red dots) gillnet locations for the traditional sampling in 2010. B = electrofishing stretches (Site 1 - 14) at the shore for the traditional sampling in 2010 and additional eDNA sampling in 2019 C = sampled lakeshore transects (T1-T4) and pelagic sampling sites (blue dots) for the main eDNA sampling in 2019.

Standard sampling: By boat, 30 liters of water were collected along each transect (6 km) and filtered through the VigiDNA® 0.45 µm filter cartridges using a peristaltic pump. In addition to the shoreline transects, depth-integrated samples (from the water surface to just above the bottom) were collected (10 liters each) at three pelagic sites, including the deepest part of the lake, using an integrating water sampler (Hydrobios IWS III). The three 10-litre samples were then combined (total volume of 30 l) and filtered through a VigiDNA® 0.45-µm filter cartridge. After filtration, the cartridges were filled with a preservation buffer and stored in the fridge until extraction according to Eco-Alpswater protocol D.T1.3.1-4 Lake and river eDNA fish sampling. In the meantime, however, we would no longer recommend storing the samples in the refrigerator due to difficulties, especially with regard to DNA extraction. Therefore, it is advised to store the samples at room temperature until extraction.

Additionally and also by boat, 5 liters were collected along each electrofishing site from the previous traditional fish status assessment, carried out in 2010. An integrating water sampler (Hydrobios IWS III) was used to collect a total of 5 liter along each stretch. Back in the laboratory, the samples were filtered through glass fiber filter discs (GFC) 1.2 µm using a vertical filtration device. After filtration, the filters were stored frozen at -20° until DNA extraction.

DNA extraction and sequencing

For the fish eDNA extraction from VigiDNA® cartridges a combination of the Macherey-Nagel NucleoSpin® and the DNeasy Soil Kit® was used according to the Eco-Alpswater protocol D.T1.3.1-8.2 - Fish DNA extraction from VigiDNA® cartridges. For the fish eDNA extraction from GFC filters, the DNeasy Power Water kit (Qiagen) was used, following the manufacturer's protocol.

The PCR amplification as well as the library preparation was done by AGES (Austrian Agency for Health and Food Safety) according to the Eco-Alpswater protocol D.T1.3.1-12 - Library preparation 12S. For the sequencing, MiFish-U primers (forward: 5`- GTCGGTAAAACTCGTGCCAGC-3`, reverse: 5`- CATAGTGGGGTAT-CTAATCCCAGTTTG-3`, Miya et al. 2015) were used and for each sample. For each VigiDNA® sample nine replicates were performed, for the GFC filters only one.

Bioinformatic processing

Raw sequencing data were analyzed at the Research Department for Limnology, Mondsee. For the bioinformatics analysis, the qiime2 pipeline (Bolyen et al. 2019) was used. This pipeline was originally designed to work on microbiome data. However, previous tests showed, that the taxonomic assignment of the obitools3 pipeline, which was used by most partners in the EAW project, and the taxonomic assignment of the qiime2 pipeline delivered comparable results regarding the taxonomic assignment of fish in eDNA samples. Due to easier handling of the bioinformatics processes and a slightly finer taxonomic resolution, the German and Austrian project partners used the qiime2 approach.

Comparison with traditional fish monitoring

The taxonomic inventories obtained from the bioinformatic analysis were then compared to the dataset obtained from the latest traditional fish sampling at Lake Mondsee, which was carried out in 2010 (Gassner et al. 2013). The traditional methods consisted of pelagic and benthic gillnetting and electrofishing along the shore.

Results on comparison between traditional monitoring and HTS

VigiDNA®:
For each of the 5 VigiDNA® samples, 9 replicates were sequenced. For the analysis, the average number of reads per species (occurring in the 9 replicates) in each sample was determined and then summed up. In total 21 fish species (Table 1.4, Fig. 1.6 A) were detected during the EAW sampling campaign (2019) and the traditional sampling campaign (2010). 12 fish species (57%) were detected by both methods, 7 fish species (33%) were identified only by the traditional methods (electrofishing, pelagic & benthic gillnetting) and 2 fish species (10%) were detected only with the HTS approach.

GFC:
No replicates were used in this approach, the number of reads for each species in the 14 samples, was summed up. In total 24 fish species (Table 1, Fig. 1.6 B) were detected during the EAW sampling campaign (2019) and the traditional sampling campaign (2010). 19 fish species (79%) were detected by both methods, 0 fish species were identified only by the traditional methods (electrofishing, pelagic & benthic gillnetting) and 5 fish species (21%) were detected only with the HTS approach. Not only were all species detected with the VigiDNA® filters and the traditional methods, detected with the GFC filters, but also 5 additional species.

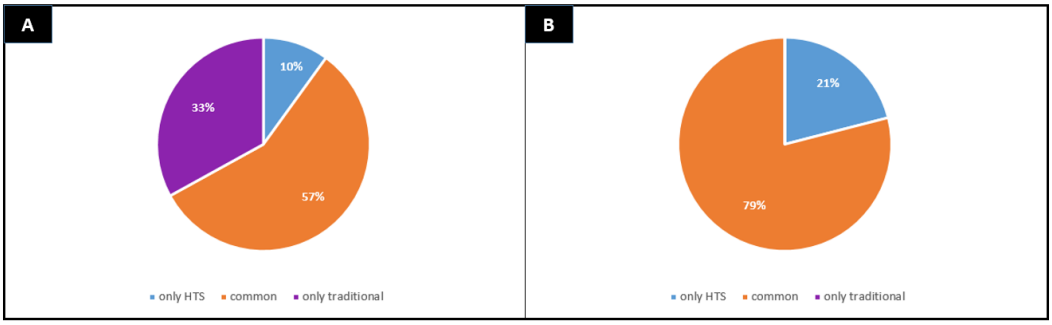


Table 1.4. Comparison of fish taxa detected with traditional and two different (VigiDNA® and GFC) eDNA assessment methods. The numbers in the molecular methods column shows the total number of reads for each species and filter type. The traditional methods columns show the number of individuals caught with different methods (gillnetting, including pelagic and benthic gillnets, and electrofishing).

Common name	Scientific name	Molecular methods			Traditional methods		
		VigiDNA	GFC	Total	Gillnetting	Electrofishing	Total
Perch	*Perca fluviatilis*	17609	741420	759029	2241	471	2712
Chub	*Squalius cephalus*	16926	463153	480079	10	62	72
Roach	*Rutilus rutilus*	23384	348881	372265	254	61	315
European pearlfish	*Rutilus meidingeri*	5864	247527	253391	45	6	51
Tench	*Tinca tinca*	5203	122415	127618	0	1	1
Danube bleak	*Alburnus mento*	20669	91330	111999	162	4	166
Bream	*Abramis brama*	5602	96908	102510	0	1	1
Pike	*Esox lucius*	1559	69801	71360	1	4	5
	Leuciscus sp.	844	54386	55230	0	9	9
European whitefish	*Coregonus lavaretus*	2304	48166	50470	30	0	30
Arctic char	*Salvelinus alpinus*	3940	34740	38680	101	0	101
Rainbow trout	*Onchorynchus mykiss*	4984	14235	19219	0	0	0
Common carp	*Cyprinus carpio*	0	17806	17806	0	0	0
Eurasian ruffe	*Gymnocephalus cernua*	0	16516	16516	186	50	236
Vimba bream	*Vimba vimba*	717	15238	15955	31	5	36
European eel	*Anguilla anguilla*	0	13161	13161	0	56	56
Brown trout	*Salmo trutta*	0	4832	4832	1	3	4
Minnow	*Phoxinus phoxinus*	0	3558	3558	0	17	17
Grayling	*Thymallus thymallus*	0	2425	2425	0	0	0
Burbot	*Lota lota*	0	1462	1462	0	2	2
Pikeperch	*Sander lucioperca*	0	1381	1381	6	0	6
Common rudd	*Scardinius erythrophtalmus*	394	948	1342	0	0	0
Bullhead	*Cottus gobio*	0	351	351	0	0	0
Wels catfish	*Silurus glanis*	0	118	118	0	1	1

Conclusion on results obtained for fish

eDNA metabarcoding for fish is a valuable tool to quickly assess the species composition of aquatic ecosystems. Depending on the method chosen, there is good (57% common) or very good (79% common) overlap with the results of the traditional methods, taking into account that 9 years have passed between both sampling events. The fact that some fish species could only be detected with GFC but not with VigiDNA® filters could be because the extraction of the VigiDNA® cartridges was not optimal due to incorrect storage conditions (fridge) and bacterial growth in the buffer which led to DNA degradation. However, both eDNA approaches were able to detect species that were not caught during the traditional sampling event (VigiDNA® = 2, GFC = 5). They are therefore well suited for studying fish communities in lakes and rivers and have proven to be sensitive, even for species that do not occur in large quantities. The eDNA approach seems to be a cost and time effective complementation to the traditional methods in order to get a more detailed insight on the fish community composition in alpine water bodies.

2. France, Lake Bourget

2.1 Phytoplankton (incl. cyanobacteria), L. Bourget

France (PP6, INRAE)

Isabelle Domaizon, Marine Vautier

Sampling according to national legislative

Lake Bourget was chosen as the pilot lake for this assessment.

Samples were taken monthly starting from January 2019 and until December 2019. A total of 12 samples were collected following the protocol developed in WP1 (D.T1.3.1-1 Lake plankton sampling). In parallel, during the first 6 months (January 2019 to June 2019), an alternative preservation strategy was tested for the same plankton samples : a preservation buffer was used instead of immediate freezing of the samples, therefore 6 additional samples were obtained, allowing to compare the alternative method of preservation to the standard protocol (D.T1.3.1-1).

For the ecological assessment of the lake quality, phytoplankton samples were depth-integrated from 0-20 m corresponding to the euphotic zone at the deepest part of the lake (Fig. 2.1). Sample aliquots were used to determine the chlorophyll-a concentration as well as chemical parameters and nutrients following the French legislative (AFNOR). Sample analysis according to AFNOR were performed at INRAE by OLA services (Observatory on LAkes).

The application of the French method for lake assessment based on phytoplankton requires quantitative data. The abundance and the total biovolume of the planktonic algae were determined from a subsample under the inverted microscope (quantitative analysis).

The brettum index was calculated from the relative proportions of the mean biovolumes of the individual taxa and taxa-specific trophic scores. The IPLAC index was calculated on the chlorophyll a content and on the specific composition of the samples.

In parallel indicators based on water chemistry were determined according to the national legislative.

Fig. 2.1. Lake Bourget, sampling site (phytoplankton and physico-chemical parameters).

For DNA sequencing, the depth-integrated samples were taken similarly as those dedicated to microscopic counts and transported to the laboratory using cooling boxes. In the laboratory the water samples were filtered through a Sterivex^{TM}-GP 0.22 µm filter (Millipore, Billerica, Massachusetts, USA) following the protocol developed in WP1 (D.T1.3.1-1 Lake plankton sampling). The filtering was completed until the filter became clogged or when a total volume of 1 L was reached. The 12 samples collected following the standard protocol were directly frozen without the addition of preservation buffer (D.T1.3.1-1 Lake plankton sampling), while for the 6 other samples the buffer was added to test the alternative preservation procedure. For the latter case, cartridges were preserved in buffer at room temperature for few hours, then in the fridge for few days and finally frozen, to mimic conditions where it is not possible to freeze rapidly the samples (remote lakes, etc), as proposed in the protocol developed in WP1 (D.T1.3.1-1 Lake plankton sampling).

DNA extraction and sequencing

DNA was extracted using the DNeasy® PowerWater® SterivexTM Kit (Qiagen, Hilden, Germany) following the manufacturer`s protocol. (D.T1.3.1-6 Plankton DNA extraction) for the samples directly frozen, and using the NucleoSpin Soil Kit (Macherey-Nagel) adapted to Sterivex cartriges and to the use of preservation buffer for the samples preserved in buffer (Vautier et al., 2021).

From obtained DNA extracts two types of PCR amplicons were produces to target prokaryotic diversity (16S) and eukaryotic diversity (18S). 16S rDNA (V3-V4 region) has been amplified using the primers 341Fmod CCTAYGGGRBGCASCAG and 806Rmod GGACTACNNGGGTATCTAAT under the following conditions: 95°C (5 min), 28 cycles including 95°C (30 sec), 55°C (30 sec), 72°C (30 sec), and final 72°C (5 min). 18S rDNA (V4 region) has been amplified using the primers V4F-18S_ILL and CCAGCASCYGCGGTAATTCC and V4R-18S_ILL ACTTTCGTTCTTGATYRATGA using the same cycling conditions as above. Library preparation of purified PCR products for 16S rDNA and 18S rDNA were performed according to EAW Protocols. Bridge amplification and sequencing by synthesis were performed according to standard conditions (FEM, Miseq, Massimo Pindo). One technical replicate was sequenced (D.T1.3.1-10 Library preparation 16S marker gene; D.T1.3.1-11, Library prep 18S marker gene).

Bioinformatic processing

The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2) (Protocols D.T1.3.2-2 Bioinformatic Protists dada2 treatment 18S marker gene; D.T1.3.2-3 Bioinformatic Prokaryotes treatment 16S marker gene). Sequences were clustered into ASVs (no dissimilarity threshold) and assigned to the SILVA SSU reference database (or PR2 database) for taxonomic classification.

For cyanobacteria automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates (strains) and manual blastn against ASvs allowing 0-1 mismatch (405 bp alignment).

Comparison with traditional microscopy

The taxa lists obtained by microscopy have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the REBECCA code for phytoplankton. To facilitate comparison an Excel Access database tool (version 6, May 2021) for all taxa identified by microscopy and REBECCA codes assigned has been prepared (LfU, FEM, LFUI).

Bourget overall trophic state

On the basis of the mean annual total phosphorus (TP) concentration (µg L^{-1}), mean annual chlorophyll-*a* (Chl-a) concentration (µg L^{-1}), maximum annual chlorophyll-*a* concentration (µg L^{-1}), mean annual Secchi-disk depth (m) and minimum annual Secchi-disc depth (m) the trophic state was adjusted using the OECD Fixed Boundary Trophic Classification System (OECD, 1982) (Table 2.1).

During 2019 lake Bourget had an average TP concentration of 8.8 (min, max=3 – 37) µg/L, a mean Chl-a concentration of 2.7 (min, max=1.2– 12.3) µg/L and a mean secchi depth of 6.8 (min, max=3.4 – 10.3) m and is thus assigned a meso-oligotrophic state.

Table 2.1. *OECD Fixed Boundary Trophic Classification System (OECD 1982)*

Trophic category	Mean phosphorus concentration (μgL^{-1})	Mean chlorophyll concentration (μgL^{-1})	Maximum chlorophyll concentration (μgL^{-1})	Mean annual Secchi-disc depth (m)	Minimum annual Secchi-disc depth (m)
Ultra-oligotrophic	≤4.0	≤1.0	≤2.5	≥12.0	≥6.0
Oligotrophic	≤10.0	≤2.5	≤8.0	≥6.0	≥3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypereutrophic	≥100	≥25	≥75	≤1.5	≤0.7

Results on comparison between freezing and buffer preservation (HTS, 18S marker gene)

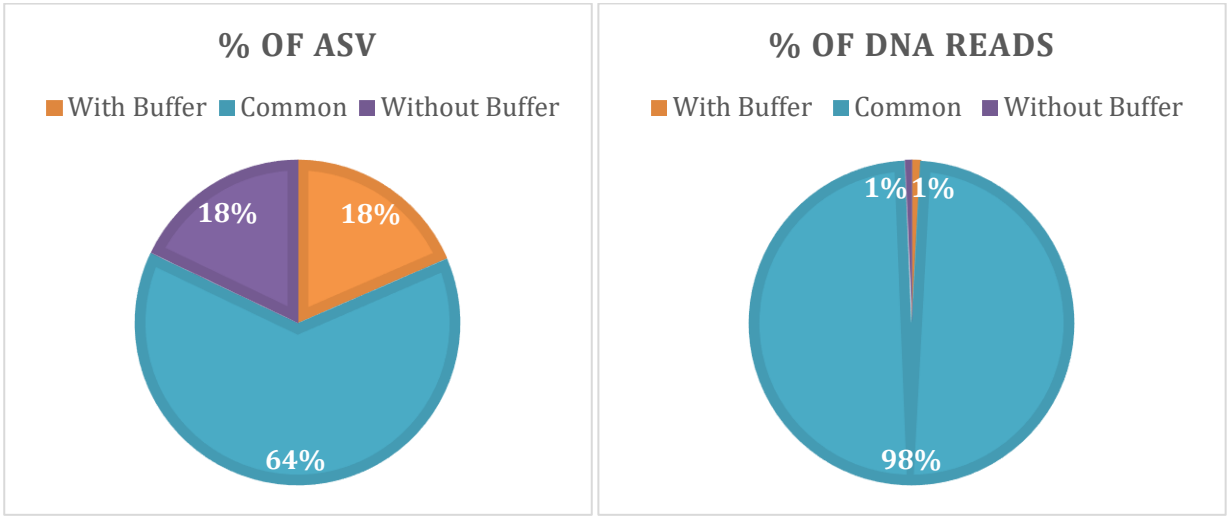


Fig. 2.2. *Percentages of ASVs and number of reads associated with microalgae identified with 18S marker gene, common to both preservation methods or specific to one of them, with or without preservation buffer. The results are presented for the 6 months of monitoring (from January to June 2019)*

64% of the 324 different ASVs are common to both preservation methods, but these ASVs are the most represented ones in terms of percentage of DNA reads, consequently the shared DNA sequences is 98% when looking at the total sequences associated with microalgae (Fig. 2.2). Specific ASVs are rare signals and all together represent a small percentage of the DNA sequences, (1.2%). Therefore the two preservation methods allow to achieve very similar results. This is particularly interesting to consider the application of this alternative protocol (use of the preservation buffer) for samples for which immediate freezing is not possible (e.g. high altitude lake).

Results on comparison between traditional microscopy and HTS

Six main algal classes were observed under the microscope using traditional morphological analyses. (Fig. 2.3). The following main phases can be observed:

- A winter phase, with low biomasses (January to March) dominated by Bacilliariophyceae.

- A spring phase (April to June) during which a rapid increase in biomass is observed, with a spring peak dominated by Chrysophyceae. During this period the maximum annual biomass is reached in May.

- A summer phase (July to August) characterized by low biomass. The compartment is dominated by Bacilliariophyceae; we note the presence of Dinophyceae and Zygophyceae.

- A late summer and autumn phase (September to November) during which the biomass increases again, first dominated by Chrysophyceae, then by Cyanobacteria.

- Finally, a winter phase (November to December), during which the biomass decreases strongly and is dominated by the Bacilliariophyceae and to a lesser extent by the Dinophyceae.

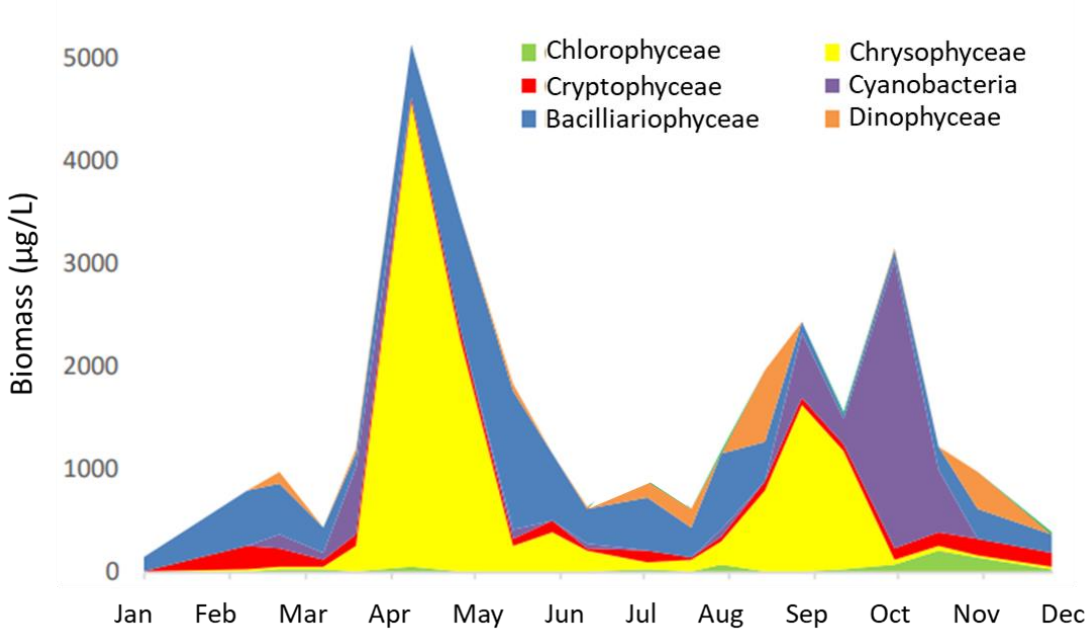


Fig. 2.3. *Seasonal variations of phytoplankton biomass by algal classes in Lake Bourget in 2019*

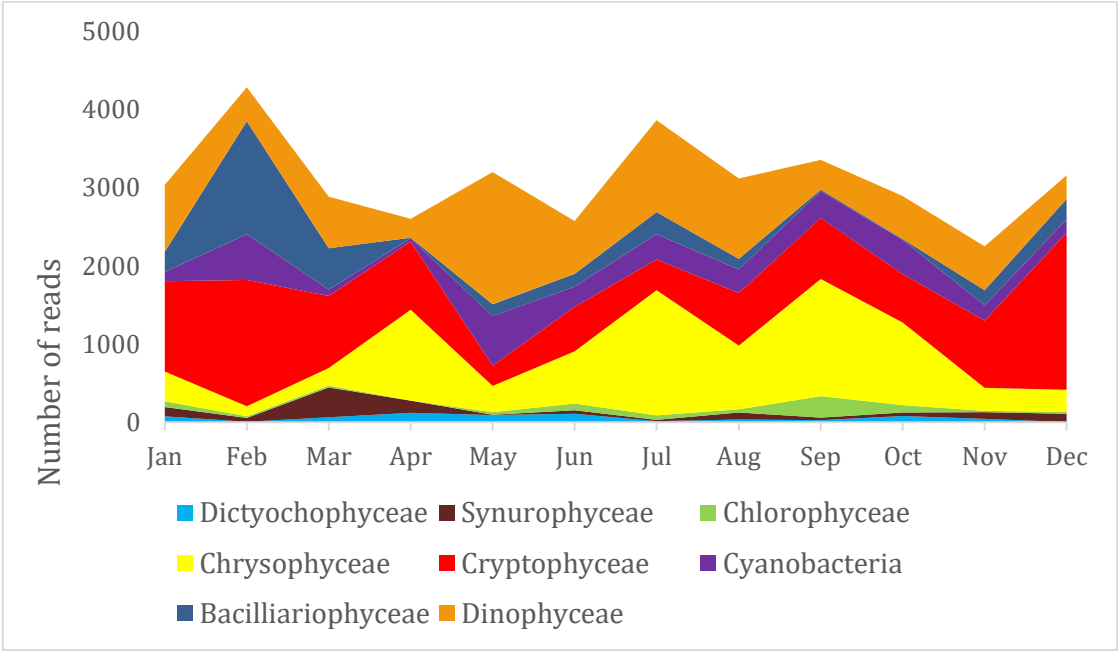


Fig. 2.4. *Seasonal variations of phytoplankton based on DNA reads number for each algal classes in lake Bourget in 2019*

With metabarcoding, the same main six algal classes are identified, but also two other classes are distinct from the minority classes, the Syrunophyceae and the Dictyochophyceae. However, the number of reads does not match with the seasonal variations of phytoplankton biomass (Fig. 2.3 and 2.4).

Table 2.2. *Comparison of algal taxa at class level for Bourget detected using the two different methods (microscopical analysis vs sequence analysis) or detected only by one method*

Taxa identified by both Methods	Taxa identified only through HTS	Taxa identified only through microscope
Chrysophyceae	Bolidophyceae	Phaeothamniophyceae
Dinophyceae	Eustigmatophyceae	Bicosoecophyceae
Bacillariophyta	Bangiophyceae	Klebsormidiophyceae
Dictyochophyceae	Prymnesiophyceae	
Cryptophyceae	Pavlovophyceae	
Trebouxiophyceae	Mamiellophyceae	
Chlorophyceae	Katablepharidaceae	
Synurophyceae	Eustigmatophyceae	
Chlorodendrophyceae	Bangiophyceae	
Cyanobacteria	Prymnesiophyceae	
Zygnemophyceae		
Conjugatophyceae		

In general, 12 algal classes were detected using both methods. 10 algal classes were found through metabarcoding, but not detected under the microscope (Table 2.2). Only 3 algal classes taxa were not identified by metabarcoding, but only under the microscope. The metabarcoding approach seems then to have a better depth of analysis than microscopy.

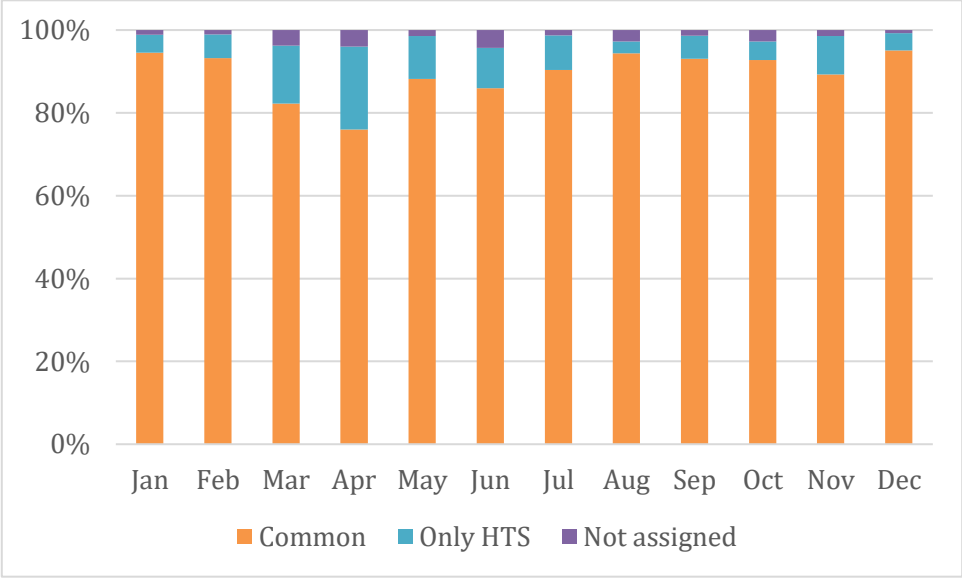


Fig. 2.5. *Percentage of ASVs associated with algal classes identified by HTS and microscopy, only HTS or not assigned to class level, for each Bourget HTS sample.*

Despite the fact that there are almost twice as many algal classes identified by HTS compared to microscopy, the common classes represent the majority of ASVs, and few ASVs are unassigned at class level for HTS data (Fig. 2.5). There is therefore a good concordance between HTS and microscopy data at the level of algal classes, and the additional classes identified in HTS are minority classes.

Since the assessment of ecological status classification is based on **phytoplankton species** an important question is, how well the resolution of the modern HTS method works on a species level. The species that

could be found through morphological analysis were compared, to see which ones could be identified with this method of metabarcoding. Additionally, species which could not be found under the microscope, were also analyzed. For taxonomic precision the REBECCA code was used.

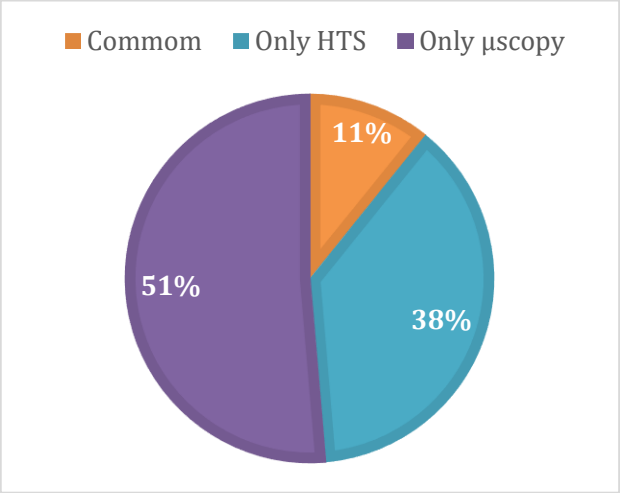


Fig. 2.6. *Percentage of algal species identified by metabarcoding and microscopy (common) (12), only microscopy (only µscopy) (57), or only by metabarcoding (only HTS) (42), for all the Bourget samples.*

The number of algal species identified by both methods, metabarcoding and microscopy, is quite low, representing only 11% of all species identified by both methods, and 12 algal species. More species were identified by microscopy compared to the metabarcoding approach, with 57 species identified by microscopy alone compared to 42 by HTS alone (Fig. 2.6). While the consistency between microscopy and metabarcoding is high at the algal class level, it appears to be lower at the species level.

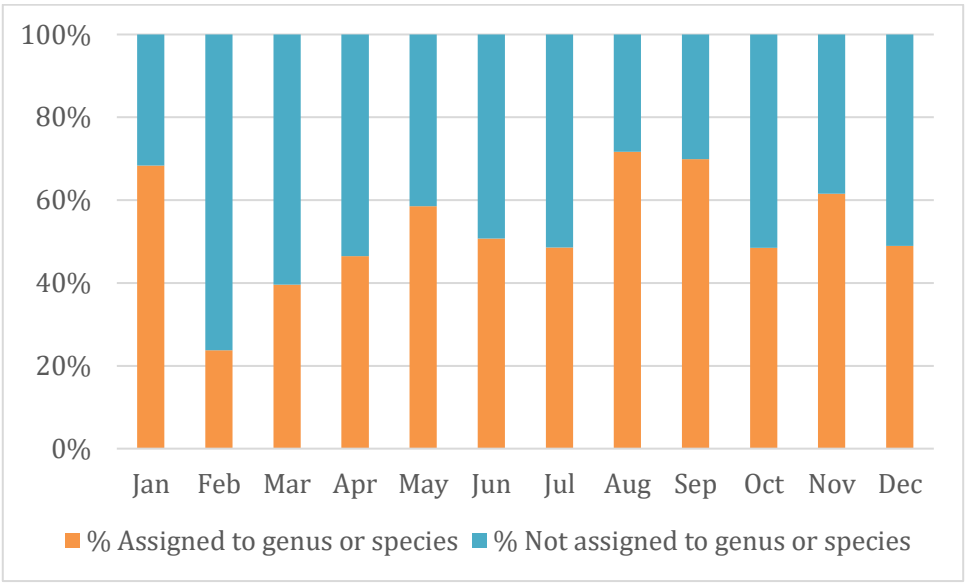


Fig. 2.7. *Percentage of ASVs associated with algal genus or species identified by HTS or not assigned to genus or species level, for each Bourget HTS sample.*

However, analyzing the HTS data, we observe that almost half of the data is not assigned at species and genus level (Fig. 2.7), and that the gap between microscopy and metabarcoding at species level is probably due to gaps in the databases, and not because of the sequencing technology.

The results (Suppl. Tables 2.1 -) show that, for Lake Bourget, dominant species that represent a high phytoplanktonic biomass, as for instance *Dinobryon divergens* and *Fragilaria crotonensis*, are well detected by both eDNA and microscopy.

On the other hand, taxa not recognized through HTS, at least at the species level, were mainly included among the centric diatoms, i.e. species within genera *Cyclotella* and *Stephanodiscus*. These taxa can also represent a significant biomass in the lake according to the seasonal period. These genera are part of both the HTS inventory and microscopical inventory but with low match at the species level; the question of relevant identification at the species level (by HTS or by microscopy) is critical.

A number of taxa which were not detected under the microscope were identified through HTS, i.e. several ASVs associated to Chrysophyceae, Prymnesiophyceae, several ASVs associated to the genus Peridinium (while only one species of Peridinium has been detected by microscopy).

Regarding flagellates of the algal classes Chrysophyceae, a rather high number of genotypes (18S rDNA genotypes) were recorded. Previous studies have reported the presence of chrysophyceae among the smallest planktonic size fraction (pico, or nano-plankton). Small phytoplanktonic cells unidentified in light microscopy are often overlooked in classical phytoplankton surveys, this is the case for eukaryotic phytoplankton, among which chrysopycean taxa as previously reported by eDNA inventories in lake Bourget (e.g. Debroas et al 2015). Our results are therefore in line with these observations.

Regarding Dinophyta a rather high number of species or genotypes was found in eDNA inventories (for peridium, for instance, and other genera). Dinoflagellates are flagellated protists belonging to the eukaryotic super-group Alveolata, and form one of the most diverse lineages of modern phytoplankton (based on genetic analyses). Light microscopy does not allow species discrimination in groups that lack clear morphological features, especially in the pico- (<2µm) and nano- (<20µm) plankton. In the last decade, the sequencing highlighted the presence of many novel dinoflagellates within pico-nano-plankton assemblages in marine and freshwater ecosystems showing that the biodiversity of pelagic dinoflagellates has been largely underestimated; our results are in line with these observations.

Conclusion on results obtained for phytoplankton

Relevant information derived from sequencing includes

(i) overall good qualitative relationship between HTS and microscopy on class level, but limited concordance between HTS and metabarcoding data at species assignment level.

(ii) overall low quantitative relationship between HTS and microscopy on class level

(ii) additional information on certain groups of algae which have not been well recorded before, i.e. picocyanobacterial and eukaryotic flagellates (Chrysophyceae, Dinophyta, Prasinophyta)

(iii) additional information on presence/absence of nuisance algae, i.e. *Planktothrix rubescens*/*agardhi*i, *Microcystis aeruginosa*

(iv) information on intraspecific genetic variation among populations, i.e. detection of novel genotypes within populations of algal species.

(v) The use of preservation buffer seems to be an effective alternative for samples for which it is not possible to freeze the sample immediately (e.g. high altitude lakes).

2.2 Biofilm composition (littoral), L. Bourget

France (PP6, INRAE)

Isabelle Domaizon, Marine Vautier, Valentin Vasselon, Frederic Rimet, Agnes Bouchez

Sampling

Diatoms has proven to be an indicator for ecological quality status in rivers. In France diatoms are used as biological quality elements for rivers and lakes. Thus for this project the guidelines from the national legislative on sampling in rivers and lakes have been adapted and applied along with the protocol developed in WP1 (D.T1.3.1-2, Lake biofilms sampling protocol). These protocols follow the European protocols for sampling diatom adapted for metabarcoding analyses (CEN 2018).

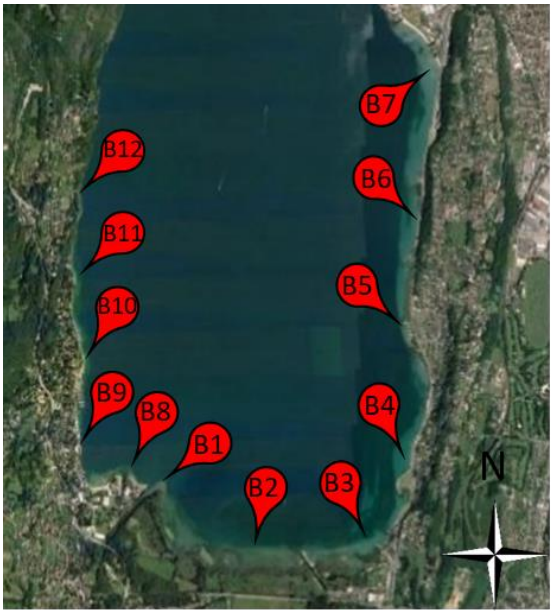


Fig. 2.8. *Sampling biofilm location on Lake Bourget.*

Twelve different sampling sites were chosen for Lake Bourget, and sampling was performed on 03 October 2018 (Fig. 2.8). The sites were selected along an urbanization gradient and on both sides of the Leysse River, a potential source of pollution. Nevertheless, the chemical data do not show a gradient between the different sampling sites.

For each site, 5 stones were selected along the shoreline representing an area of 50-100 cm^2. Biofilms were brushed off from stones from a representative surface area.

Biofilms were preserved in 80% Ethanol as described in protocol (D.T1.3.1-2, Lake biofilms sampling protocol) in two different tubes, and diatoms were identified either by microscopical analysis or by eDNA analysis.

The twelve samples were analyzed by HTS, but unfortunately only 7 could be analyzed by microscopy.

DNA extraction and sequencing

DNA was extracted using the Macherey-Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (D.T1.3.1-7, DNA extraction biofilms).

PCR amplification and library preparation of purified PCR products for rbcL (barcodes selected for Diatoms metabarcoding) was performed according to WP1 protocol (D.T1.3.1-9, Library preparation rbcL marker gene). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions.

Bioinformatic processing

The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2), (D.T1.3.2-1 BioinfRbcL, Bioinformatics treatment rbCL marker gene).

Sequences were clustered into ASVs (no dissimilarity threshold) and assigned to the curated library Diat.barcode v7 (Rimet et al. 2019) was used for diatom taxonomic classification.

Comparison with traditional microscopy

All microscopical taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the VALID code system for diatoms in phytobenthos (LfU). An Excel Access database for all taxa identified by microscopy and the VALID codes assigned has been prepared (LfU, FEM, LFUI).

Results on comparison between traditional microscopy and HTS

Results on comparison are presented in Table 2.3.

Table 2.3. Comparison of diatoms taxa at genus level for Bourget detected using the two different methods (microscopical analysis vs sequence analysis) or detected only by one method

Taxa identified by both Methods	Taxa identified only through HTS	Taxa identified only through microscope
Achnanthidium	*Caloneis*	*Adlafia*
Amphora	*Craticula*	*Cavinula*
Brachysira	*Ctenophora*	*Eucocconeis*
Cocconeis	*Cyclotella*	*Gyrosigma*
Cymbella	*Epithemia*	*Halamphora*
Cymbopleura	*Eunotia*	*Karayevia*
Denticula	*Iconella*	*Navigeia*
Diatoma	*Melosira*	*Placoneis*
Diploneis	*Neidium*	*Platessa*
Discostella	*Pinnularia*	*Punctastriata*
Encyonema	*Surirella*	*Rhopalodia*
Encyonopsis	*Ulnaria*	*Staurosirella*
Fragilaria		
Geissleria		
Gomphonema		
Navicula		
Nitzschia		
Pantocsekiella		
Planothidium		
Reimeria		
Sellaphora		
Staurosira		
Tryblionella		

Overall 23 diatoms genus were detected using both methods. 12 diatoms genus were found through metabarcoding, but were not detected under the microscope, and 12 diatoms genus were not identified by metabarcoding, but were found under the microscope (Table 2.3).

At the genus level, whether for samples analyzed by HTS or by microscopy, we observe that the vast majority of ASV or diatom abundances belong to genus identified by both methods (Fig. 2.9 and 2.10).

In microscopy, one sample (B3) has a high percentage identified only by microscopy (42%), but 30.6% (out of the 42%) is associated to the presence of a single species, *Punctastriata ovalis*, which is not identified in the HTS inventory.

The twelve genus identified only by HTS or only by microscopy represent therefore marginal species, and the main species are found by both methods. While there are few diatoms not assigned to genus under microscopy (maximum 0.5%), in HTS they can represent up to 36% of the ASVs for a sample, and with more complete databases we would expect a greater depth of analysis by HTS data.

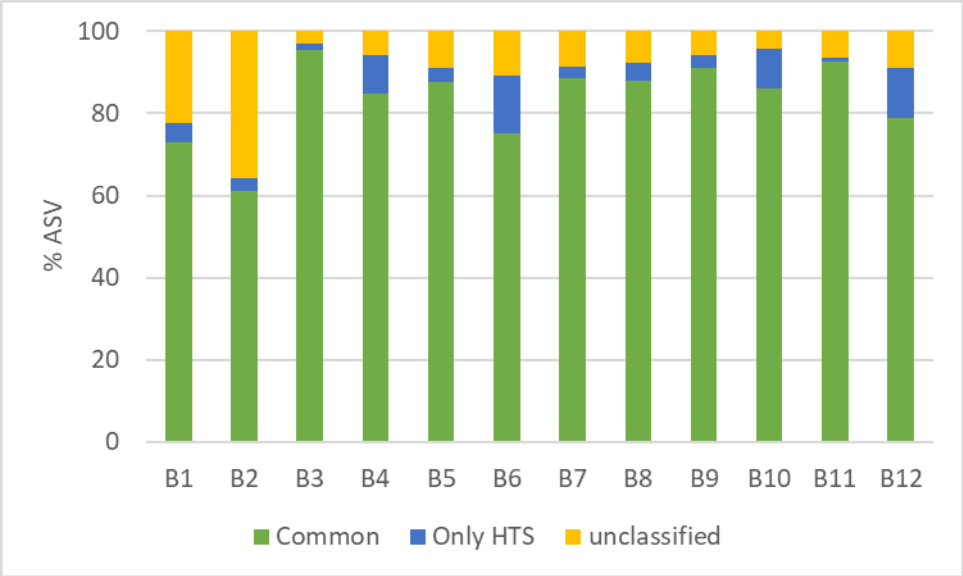


Fig. 2.9. *Percentage of ASVs numbers associated with diatom genus identified by HTS and microscopy (common), only HTS (only HTS), or not assigned to genus level, for each Bourget HTS sample.*

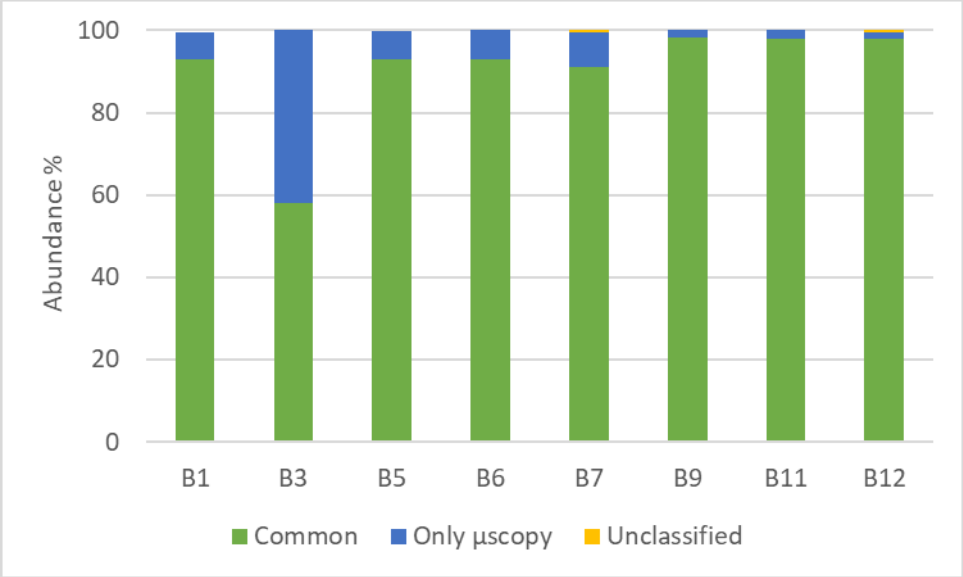


Fig. 2.10. *Abundance percentage associated with diatom genus identified by HTS and microscopy (common), only microscopy (only µscopy), or not classified to genus level, for each Bourget microscopy sample analyzed (7 samples were not analyzed).*

At the genus level, whether for samples analyzed by HTS or by microscopy, we observe that the vast majority of ASV or diatom abundances belong to genus identified by both methods (Fig. 2.9 and 2.10).

In microscopy, one sample (B3) has a high percentage identified only by microscopy (42%), but 30.6% (out of the 42%) is associated to the presence of a single species, *Punctastriata ovalis*, which is not identified in the HTS inventory.

The twelve genus identified only by HTS or only by microscopy represent therefore marginal species, and the main species are found by both methods. While there are few diatoms not assigned to genus under

microscopy (maximum 0.5%), in HTS they can represent up to 36% of the ASVs for a sample, and with more complete databases we would expect a greater depth of analysis by HTS data.

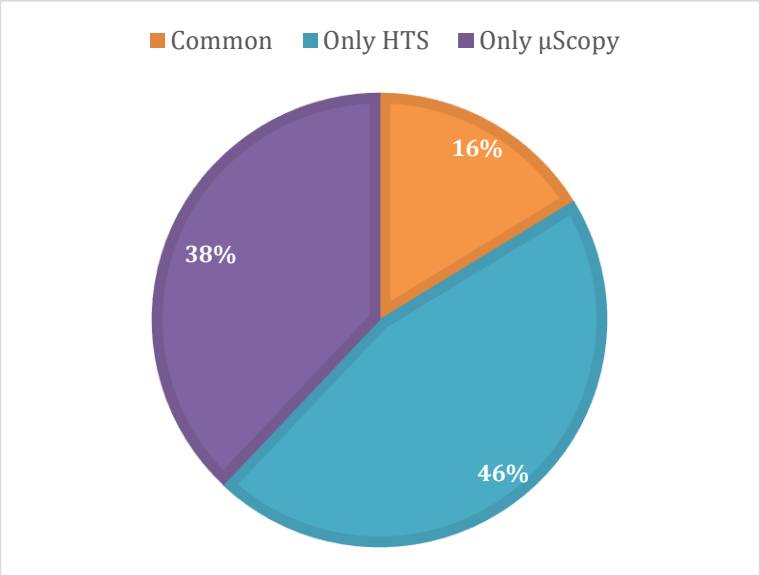


Fig. 2.11. *Percentage of diatoms species identified by HTS and microscopy (common) (27), only microscopy (only µscopy) (63), or only by HTS (only HTS) (76), for each all the Bourget samples.*

When looking at the assignment at the species level, there is less correspondence, with only 27 diatom species in common, or 16% of all species identified by both methods (Fig. 2.11). 63 species were identified only by microscopy, and 76 only by HTS. The correspondence between the two methods is therefore weaker at the species level than at the genus level.

When comparing species identified by microscopy to species identified with metabarcoding, the percentage of species identified by both methods is quite low. However, in the species lists, it appears that many species identified by one or the other method are synonyms (not updated in the database used here), sister species or identification mistakes in microscopy. Hereby a few examples:

For instance, *Achnanthidium delmontii* was identified in microscopy, this species is barcoded in Diat.barcode. However it was not detected in metabarcoding. In metabarcoding a morphologically similar species was identified, *A. pyrenaicum.* Therefore, we can conclude on an identification mistake in microscopy.

Another example, of sister species is with *Nitzschia dissipata* var. *media, N. dissipata* ssp. *oligotraphenta* which were identified in microscopy. In metabarcoding, only the nominal species was identified, *N. dissipata.*

Similar examples, could be given with: *Cymbella compacta,* identified in microscopy, and its synonym *C. helvetica* in metabarcoding; *C. lange-bertalotii* in microscopy and its sister species *C. aspera* in metabarcoding; *E. bonapartei* in microscopy (a recently described species) and *E. caespitosum* in metabarcoding (a much older described species and morphologically wide species)…etc…

Finally, there are groups of genera, in particular *Staurosira, Staurosirella, Pseudostaurosira,* which were identified in microscopy but not in DNA. They are known to be paraphyletic, and were grouped into a single genus, *Staurosira* in Diat.barcode v7. In the meantime, this group of genera has been studied by Li et al. (2018), and splitted in new genera which are now integrated in the new versions of Diat.barcode (v8, v9 and v10).

Therefore, after a careful check of synonymies, sister species, identification mistakes, the percentage of shared species identified by both methods could increase greatly.

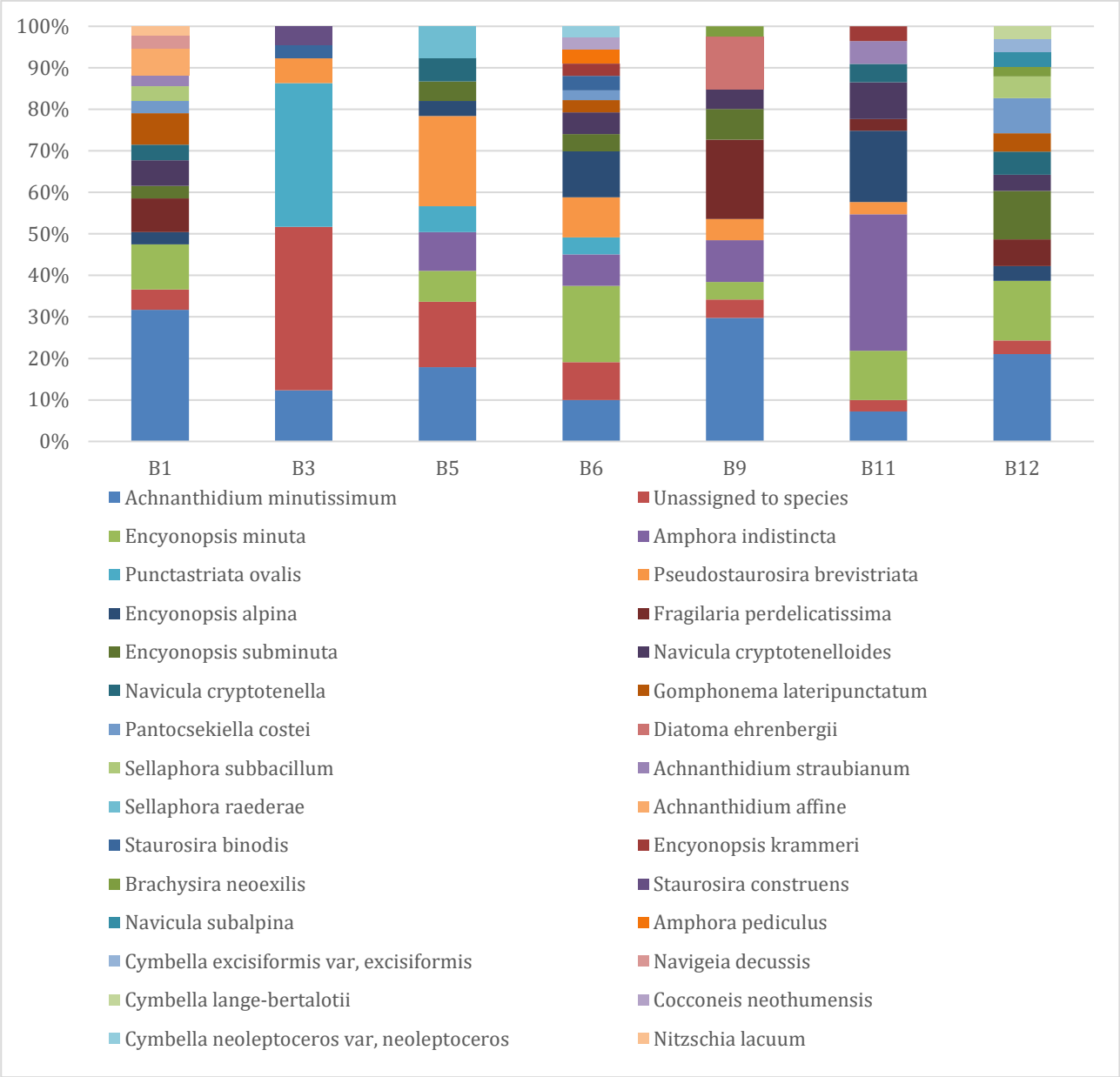


Fig. 2.12. *Relative abundance of diatoms (> 2 %) at seven littoral sampling sites from Bourget as revealed from microscopical counting (for location of sites see Fig. 2.8).*

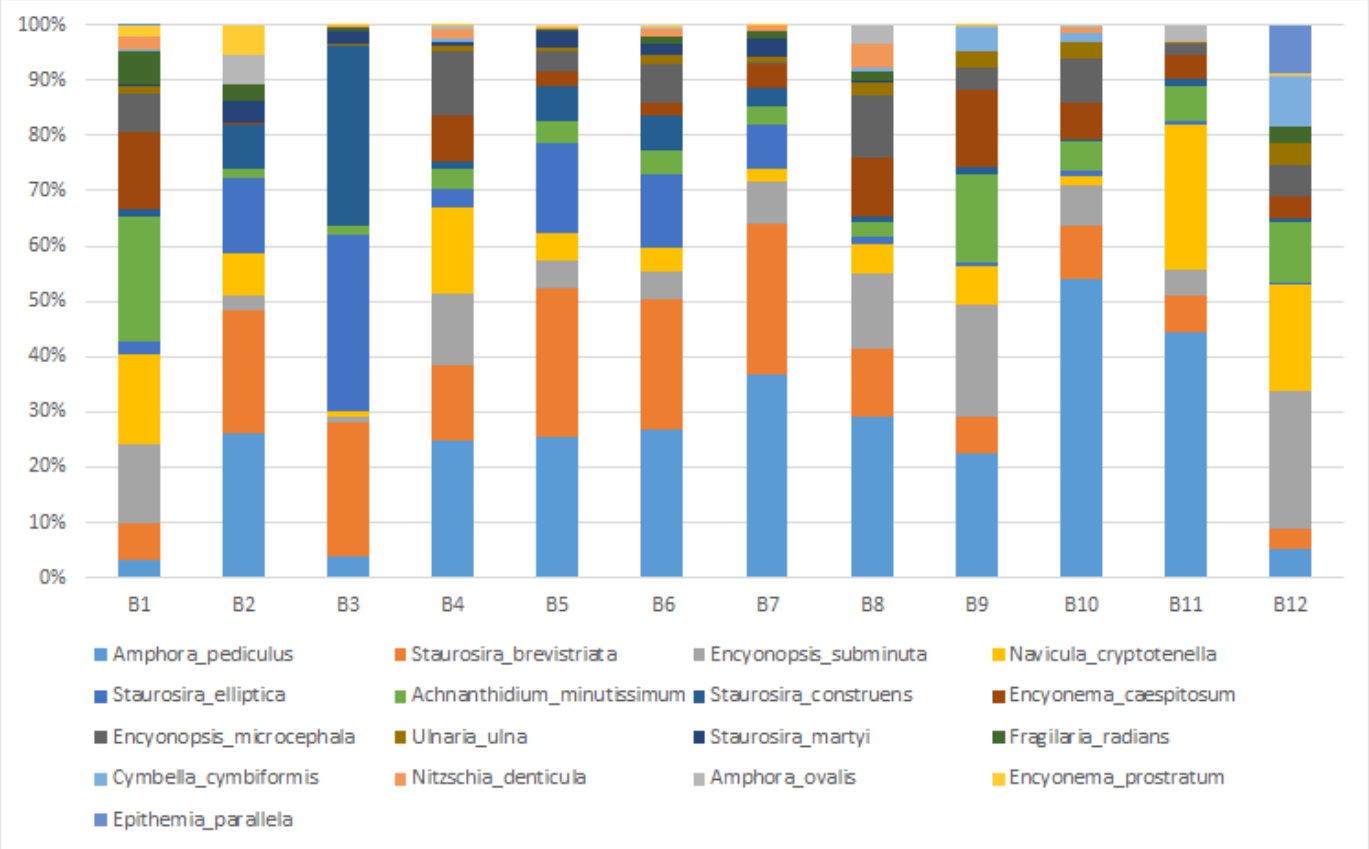


Fig. 2.13. *Relative abundance of diatoms reads number (> 2 %) at twelve littoral sampling sites from Bourget as revealed from HTS data (for location of sites see Fig. 2.8)*

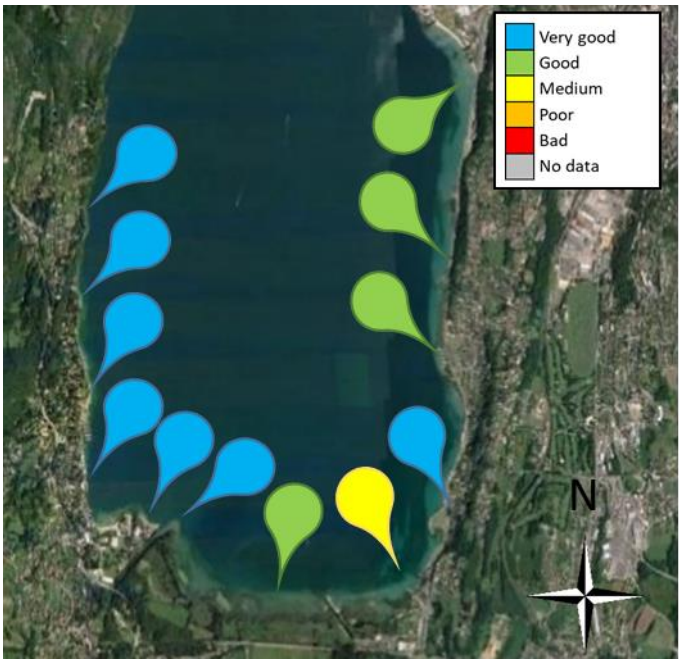


Fig. 2.14. *Map of ecologial quality indices calculated from HTS data*

Although diatoms are not a standardized indicator of ecological quality status in lakes, index calculations performed from HTS data were estimated, they show lower scores on the area of the lake (Southeast) that is directly submitted to anthropogenic pressures in comparison to the more natural shoreline (Southwest) (Fig. 2.14), although the chemistry data obtained on water do not show a marked pollution gradient.

Conclusion on results obtained for diatoms

Relevant information derived from sequencing includes the following:

(i) Good match between microscopy and HTS for assignment to genus level

(ii) The percentage of species identified by microscopy and HTS is however quite low, but many species identified by one or the other method are synonyms, sister species or identification mistakes in microscopy, and a careful verification by a specialist is needed to compare the data one by one

(iii) The diatom data in HTS allow the calculation of water quality indices that correspond to the reveal differences in the quality indices provided by diatoms (HTS data).

(iv) A better harmonization to increase the correspondence between the traditional and HTS approaches could be obtained, the calculation of indices (with HTS data) is however possible even without a perfect match between the two approaches at species level.

2.3 Fish composition, L. Bourget

France (PP6, INRAE; PP11, OFB)

Isabelle Domaizon, Marine Vautier, Maxime Logez, Jean-Marc Baudoin

Sampling

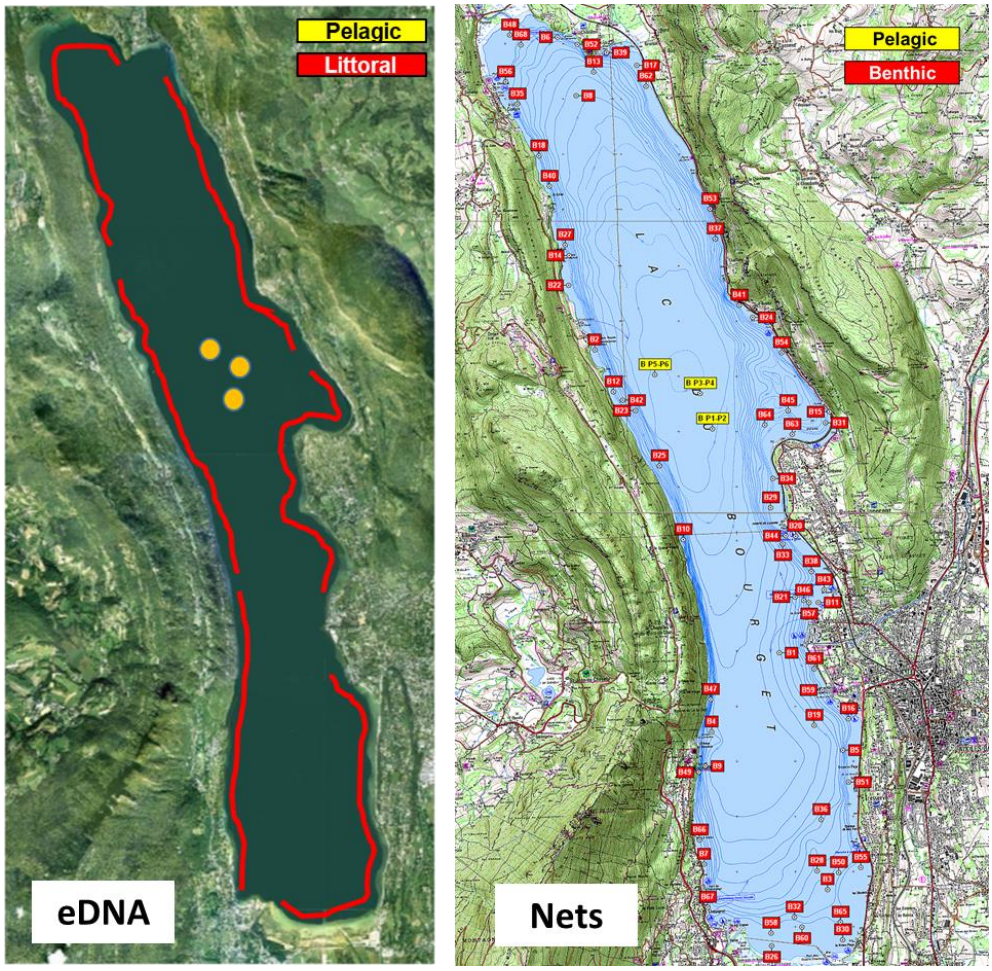


Fig. 2.15. Spatial distribution of benthic and pelagic nets (Nets) and eDNA samples (eDNA), during the October 2019 sampling campaigns.

For traditional monitoring, 68 benthic and pelagic nets were set according to CEN standards in October 2019 (week 40). Two weeks later (week 42), eDNA samples were collected. For the eDNA samples, 30L of water was collected and filtered along 6 shoreline transects (Fig. 2.15). Pelagic sampling was also carried out in the deepest part of the lake, in three different areas for a total final volume of 30L. Fish eDNA samples were then preserved in buffer according to the Eco-Alpswater protocol D.T1.3.1-4 Lake and river eDNA Fish sampling.

DNA extraction and sequencing

Fish DNA extractions were performed using the Macherey-Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (D.T1.3.1-8.2, Fish DNA extraction from VigiDNA cartridges).

PCR amplification and library preparation were performed according to WP1 protocol (D.T1.3.1-12, Library preparation 12S) and using the fish specific MiFish-U primers (Miya et al., 2015). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions. Nine PCR replicates were performed for each fish eDNA sample.

Bioinformatic processing

Fish eDNA bioinformatic processing was performed using D.T1.3.2-4 Bioinf_12S protocol from WP1. The protocol uses the OBITOOLS3 software (Boyer et al., 2016,) for the processing of raw high-throughput sequencing reads from the MiSeq platform.

Comparison with traditional methods

The final output of the eDNA analyses is a tab-delimited table with taxonomic inventories, which is comparable to the species inventories obtained during the net fisheries

Results on comparison between traditional methods and HTS

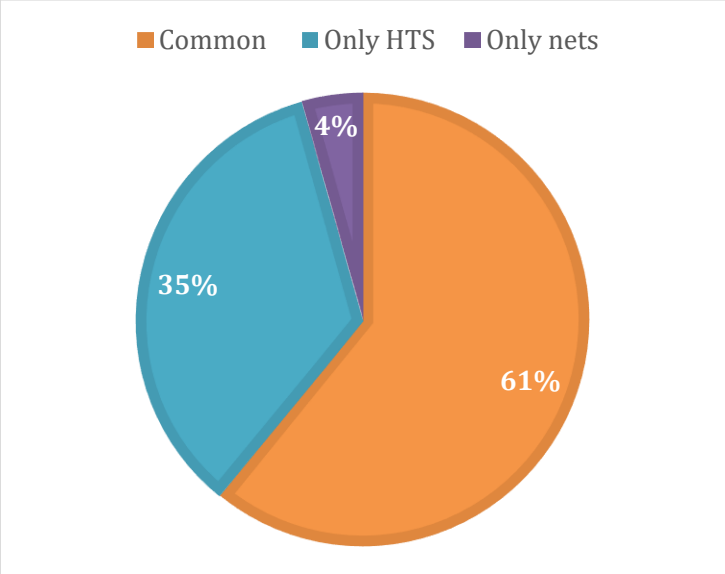


Fig. 2.16. *Percentage of fish species identified by metabarcoding and traditional methods (common) (14), only nets (only nets) (1), or only by metabarcoding (only HTS) (8), for fish monitoring in lake Bourget (68 nets and 7 eDNA samples)*

In total, 23 fish species were identified considering the cumulated results obtained from HTS and nets. 14 species (61%) were identified by both methods (shared species between nets and eDNA methods), while only one was identified only by nets and 8 were specific only to eDNA inventory (Fig. 2.16). The eDNA approach seems to be more efficient than the traditional net approach to identify fish species in lakes.

Table 2.4. *Comparison of fish taxa detected using the two different methods (nets vs eDNA sequence analysis) or detected only by one or the other method for fish monitoring in lake Bourget (68 nets and 7 eDNA samples). eDNA results are expressed in numbers of reads, and for nets results in numbers of fish.*

Both methods		
NAME	**eDNA**	**Nets**
Perca fluviatilis	2049998	1151
Rutilus rutilus	353726	335
Tinca tinca	288011	7
Esox lucius	200086	10
Coregonus lavaretus	197052	56
Abramis brama	47290	59
Scardinius erythrophthalmus	24058	38
Gymnocephalus cernua	19021	36
Lepomis gibbosus	16082	19
Lota lota	12390	2
Gobio gobio	4335	2
Squalius cephalus	3211	6
Silurus glanis	2870	9
Ameiurus melas	504	39
Only eDNA		
NAME	**eDNA**	**Nets**
Leuciscus leuciscus	28938	
Barbatula barbatula	23347	
Salmo trutta	6101	
Salvelinus alpinus	4349	
Oncorhynchus mykiss	1222	
Alburnus alburnus	776	
Cottus gobio	704	
Barbus barbus	258	
Only nets		
NAME	**eDNA**	**Nets**
Carassius carassius		3

The two species with the highest number of net catches (*P. fluviatilis* and *R. rutilus*), are also those with the highest number of reads measured in HTS, there is then some concordance between the number of reads and the number of individuals caught (Table 2.4).

However, this is not always the case, for example with *T. tinca* for which a large number of reads were measured in HTS, only 7 individuals were caught. This could be partly explained by the large biomass of *T. tinca*, which would explain why few fish release a large quantity of eDNA.

This phenomenon can also be explained by the fact that some species are less catchable with nets. We note that among the species identified only by eDNA there are three salmonid species (*salmo trutta, Salvelinus alpinus, Oncorhynchus mykiss*), which are difficult to capture with nets, and therefore underestimated during net surveys. eDNA seems therefore to be an interesting alternative to identify species that are difficult to capture with nets, and in areas where electric fishing is difficult to implement (e.g. deep lakes).

One species (*Carassius carassius*) was identified by net fishing (only 3 fish), but not by eDNA. The fact that some littoral areas were not sampled for eDNA could explain the fact that this weak signal was not captured, or it could be linked to gaps in the database used.

Conclusion on results obtained for fish

Relevant information derived from sequencing includes the following:

(i) Good match between nets and HTS for fish inventories with 61% of species in common

(ii) eDNA approach seems more effective than traditional net monitoring to perform fish inventories, especially for species that are difficult to catch with nets, such as salmonids.

(iii) A link appears between HTS reads abundance and fish abundance, but the HTS remains only semi-quantitative, with part of the variability in the DNA reads abundance that cannot be precisely explained (e.g. the difference in biomass between the species).

3. Germany, Lake Starnberger See

Germany (PP10, LfU)

Ute Mischke & Jochen Schaumburg

General introduction

The key lakes include Lake Mondsee (Austria), Lake Bourget (France), Lake LAKES Starnberg (Germany), Lake Garda (Italy), Lake Bled (Slovenia), and Lake Lugano (Switzerland). These natural and deep lakes are located in the peri-alpine area and are under a long-term monitoring programme. Despite the recovery of the trophic status (from moderate to good) due to reduced external nutrient loading, in most of the lakes the oxygenation of deep waters is still hampered by weak winter turnover owing to climate warming. Consequently, the biological communities changed considerably during the last decades.

Description which compare all of the Eco-AlpsWater pilot lakes are in the digital infographics on webpage (D C5.5.).

Within this group of lakes, Starnberger Lake is situated at highest elevation and concerning the trophic status, the lake has the lowest concentration of total phosphorus and chlorophyll a in annual mean.

The ecological classification (BQE) of the lakes is as followed: Starnberger (D) Mondsee (A) Garda (I) Bourget (F) “good” and Lugano (CH-I) and Bled (SI) “moderate”.

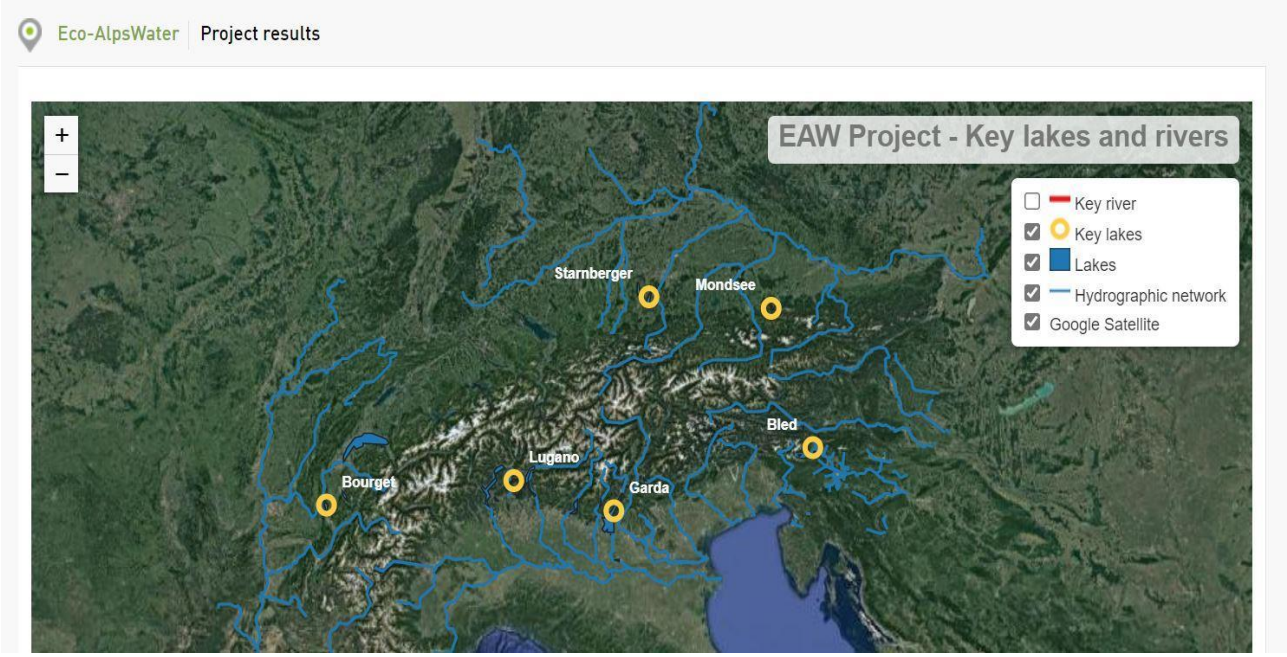


Fig. 3.1. Geographic position of the Lake Starnberger See within the Alpine Space region. Map from the project webpage (https://www.alpine-space.org/projects/eco-alpswater/en/project-results/pilots)

Starnberger See is a deep, oligotrophic and monomictic lake with 128m at the deepest point. Its water retention time is more than 20 years and its catchment area is small but drained by the Alps. See its detail description in Eco-AlpsWater WP2 Deliverable D.T2.2.1 "Identification of key lakes and rivers, and collection of previous knowledge".

Like other pre-alpine lakes, Lake Starnberg was affected by eutrophication. The peak occurred in 1980-1985 with annual means of 25-30µg/L TP and 6-9µg/l chlorophyll, resulting in a mesotrophic status. Nutrient reduction measures were implemented successfully, returning the lake to its oligotrophic state by 2001, including the recovery of deep-water oxygenation, with dissolved oxygen above 4 mg l^{-1} during all seasons. The key to reducing nutrient input was the construction of a perimeter sewage system in the years 1964-1976.

Table 3.1. Key morphological and trophic characters of Lake Starnberg

Lake elevation (m)	584
Surface area (km²)	56,2
Volume (km³)	3,00
Total N (annual mean/range, mg/L)	0,60 (0,40 - 0,80)
Total P (annual mean/range, µg/L)	6,0 (2,5 - 12,0)
Chl a (annual mean/range, µg/L)	2,4 (0,5 - 6,0)

Fig. 3.2. View on the Lake Starnberg in the northeastern direction from city Tutzing in the middle part of the lake.

3.1 Phytoplankton (incl. cyanobacteria), L. Starnberg

Germany (PP10, LfU)

Ute Mischke, Jochen Schaumburg

Sampling according to national legislative

Lake Starnberg was chosen as the Bavarian pilot lake for the implementation of the Eco-AlpsWater metabarcoding approach. Samples were taken monthly starting from February and until October in 2019 2020 given a total of 9 samples. The lake is included into regularly water monitoring since 2004.

Depth-integrated water samples (0-20 m) were taken at the deepest point of the lake, which roughly corresponding to the euphotic zone (Fig. 3.1). Depth profiles of water temperature and oxygen were measured by a multi-parameter probe. Water samples were used to determine the chlorophyll-a concentration as well as nutrients and some chemical parameters following the German standards (DEV) by the regional water laboratory (WWA-Weilheim). Lugo-fixed samples were delivered to external services for phytoplankton analysis by light microcopy.

The German method for lake assessment based on phytoplankton (PhytoSee) requires quantitative counting results. The species abundance and the total biovolume of the planktonic algae were determined from a subsample under the inverted microscope (quantitative analysis).

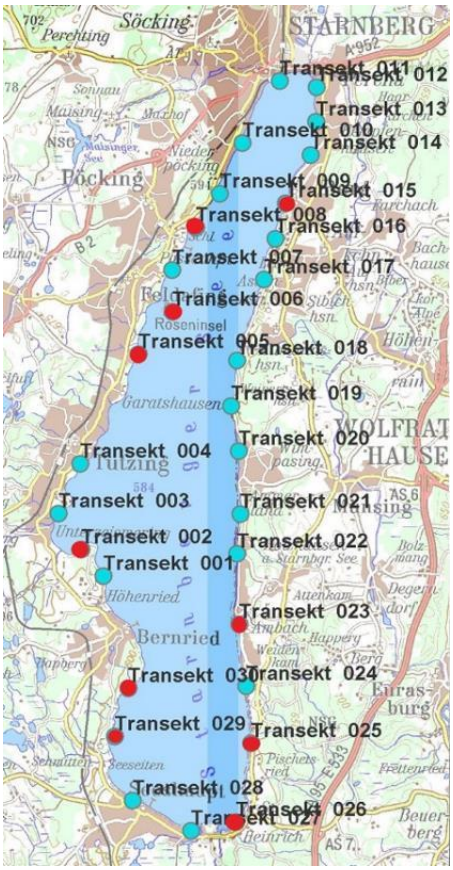
The German index PhytoSee is a multi-metric with 3 sub-metrics: (1) PTSI is calculated from the relative abundance of indicator taxa and their taxa-specific trophic scores and (2) biomass index derived from chlorophyll a and total biovolume and (3) algal class index by lake-type specific algal class proportions.

The trophic status is oligotrophic according the German trophic index based on vegetation mean of chlorophyll a, Secchi depth and total phosphorus.

Concerning cyanotoxin extraction no samples were taken in lake Starnberg.

Sampling of eDNA

eDNA samples were sub-sampled from the depth-integrated samples (0-20m) into a sterilized and DNA-free 2 liter Duran glass bottle. At board of the boot, the planktonic samples were filtered through a Sterivex^{TM}-GP 0.22 µm filter (Millipore, Billerica, Massachusetts, USA), by pressing lake water (1100 ml) manually through the same filter unit with a plastic syringe following the protocol from WP1 (D.T1.1.2 -1 Lake plankton sampling). The Sterivex filters were closed with caps, and were packed each in a sterile plastic bag and stored frozen in the laboratory until their transport to the project partner FEM for **DNA extraction and sequencing at the end of year 2019.** FEM extracted the DNA by using the DNeasy® PowerWater® SterivexTM Kit (Qiagen, Hilden, Germany) following the manufacturer`s protocol. (D.T1.1.2. -6 Plankton DNA extraction).



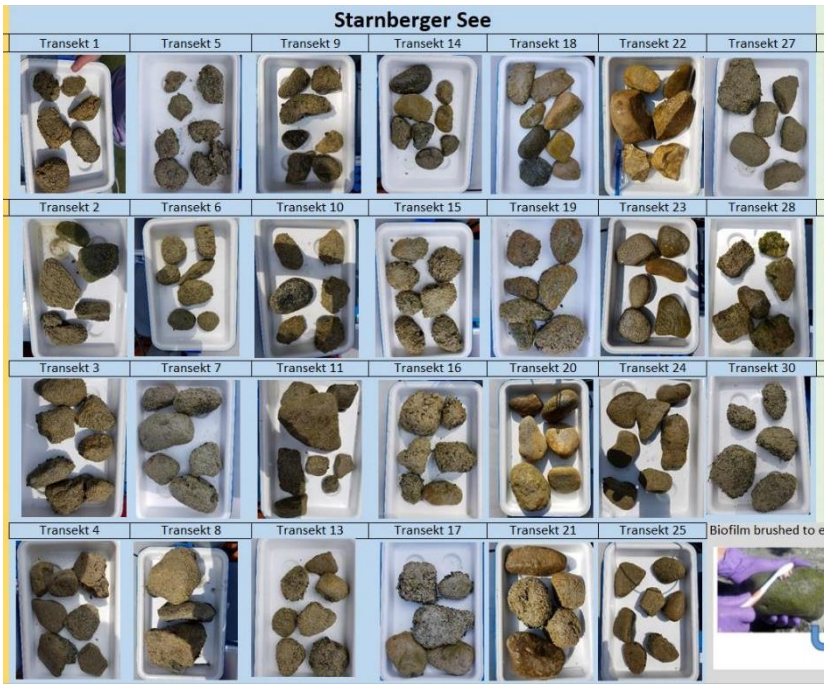


Fig. 3.3a. Map of the Lake Starnberger See with marker for littoral sampling sites (biofilm) and deepest point near Transekt 19.
Fig. 3.3b. View on the collected stones at all transects with typical calcareous brownish phytobenthos, which were brushed for eDNA samples.

Bioinformatic processing

The bioinformatics processing was done by the project partner FEM (PP1) for all Starnberger plankton samples and for the marker gens 16S and 18S. The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2) and (Protocols DT1.1.3. - 3 Bioinf18S, Bioinformatics treatment 18S marker gene; DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene). Sequences

were clustered into ASVs. 16S ASVs were assigned to the SILVA SSU reference database and 18S ASVs to PR2 database (Protist2) for taxonomic classification.

For cyanobacteria automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates (strains) and manual blastn against ASvs allowing 0-1 mismatch (405 bp alignment). These taxa are marked in column "accd_BLAST" in EAW taxa tool taxonomy table named "HTS_16S_seqs_all". By this BLAST analysis, some taxa got a different name than the original taxonomy provided by SILVA SSU.

Beside the qualitative information about the presence of a taxon, also the quantity is of relevance for interpreting the sequencing results. The counts (= reads or signal) of each ASV in each sample by the sequencer detector have been rarefied (normalized) within the whole sequencer run for signal normalisation.

In result, the bioinformatics processing produced the so-called "HTS taxa lists" based on the taxonomy detected by the high through-put sequencing (HTS).

Comparison HTS taxonomic results with traditional microscopy

To prepare the final HTS phytoplankton lists phytoplankton taxa were selected out of the total and very diverse HTS taxonomy. This HTS lists were used for the final comparison with light microscopy results.

In detail, the 16S and 18S taxa lists both contributing to the HTS results have been standardized using the established WFD (EU project WISER) taxa codes and names, i.e. the REBECCA code for phytoplankton. Additional codes were added to the EAW code list for those phytoplankton taxa, which were identified by the (HTS) but not available in the REBECCA code.

Similarly, the microscopically detected taxa were standardized using the established REBECCA code for phytoplankton.

The comparison is facilitate by a specialized Access database called "EAW taxa analysis tool" (version 6, May 2021 and updates). It includes all HTS results and all light-microscope taxa records (LM) assigned to the common REBECCA code and is product of the project partners LfU, FEM, and LFUI.

Starnberger See overall trophic state

On the basis of the mean annual total phosphorus (TP) concentration (µg L^{-1}), mean annual chlorophyll-*a* (Chl-a) concentration (µg L^{-1}), maximum annual chlorophyll-*a* concentration (µg L^{-1}), mean annual Secchi-disk depth (m) and minimum annual Secchi-disc depth (m) the trophic state was adjusted using the OECD Fixed Boundary Trophic Classification System (OECD, 1982) (Table 3.1).

Table 3.2. OECD Fixed Boundary Trophic Classification System (OECD 1982)

Trophic category	Mean phosphorus concentration (μgL^{-1})	Mean chlorophyll concentration (μgL^{-1})	Maximum chlorophyll concentration (μgL^{-1})	Mean annual Secchi-disc depth (m)	Minimum annual Secchi-disc depth (m)
Ultra-oligotrophic	≤4.0	≤1.0	≤2.5	≥12.0	≥6.0
Oligotrophic	≤10.0	≤2.5	≤8.0	≥6.0	≥3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypereutrophic	≥100	≥25	≥75	≤1.5	≤0.7

During 2019 Starnberger See had an average TP concentration of 6.8 (min, max=2.5 – 10) µg/L, a mean Chl-a concentration of 2.2 (0.5 - 3) µg/L and a mean Secchi depth of 5.7 (1.9 - 11) m and is thus assigned a oligotrophic state.

Results on cyanotoxins concentrations

There were no cyanotoxin measurements. Planktothrix rubescens is present in very low abundance.

Results on comparison between traditional microscopy and HTS

A total of ten algal groups were recorded under the microscope by traditional morphological analysis (Fig. 3.4). The **algal classes** with the highest biovolume were Bacillariophyceae, Dinophyceae and Cryptophyceae Cyanobacteria. Overall the phytoplankton composition was divers with 59 taxa. The seasonal development typically started with increased growth of centric diatoms (*Cyclotella costei, Stephanodiscus*) in March 2019, while dinoflagellates (*Ceratium hirundinella, Gymnodinium uberrimum, Peridinium cinctum and P. willei*) and cyanobacteria (*P. rubescens)* became more relevant in late summer.

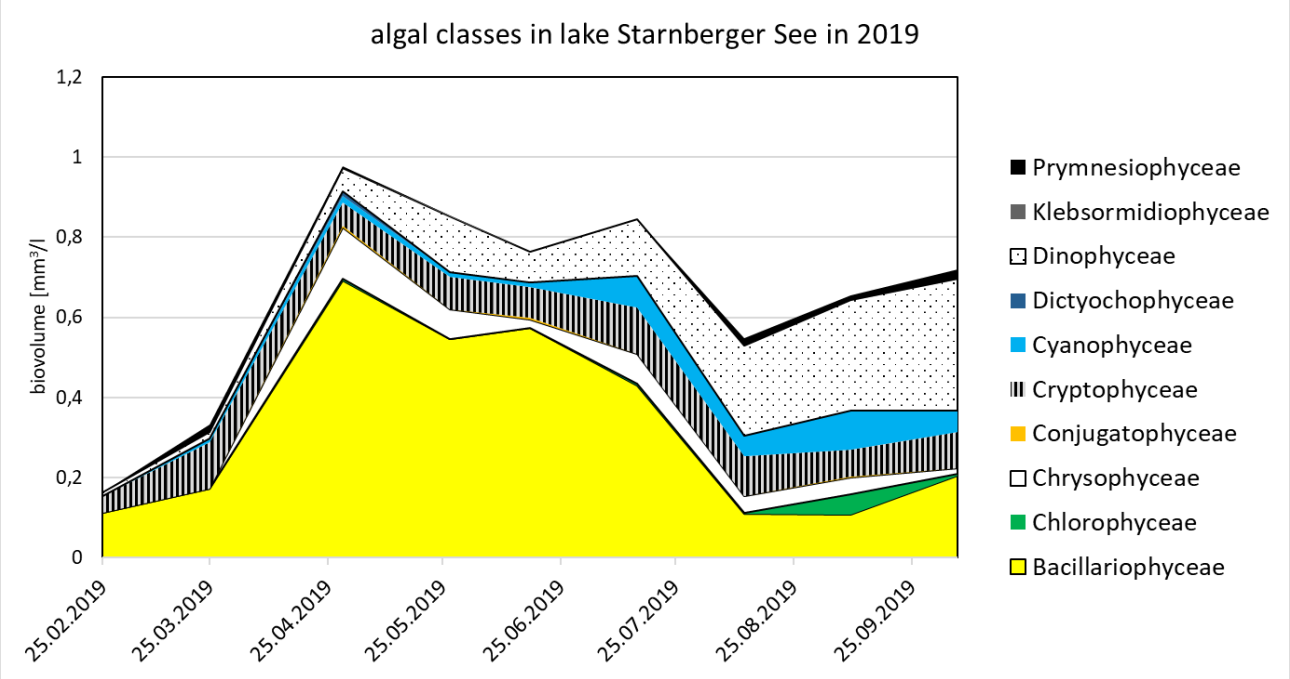


Fig. 3.4. Proportion of phytoplankton classes to total biovolume as inferred from microscopical analysis (Starnberger See 2019)

Trebouxiophyceae and Mamiellophyceae were not found by microscoy but by the PR2 database. Euglenophyceae were not present in the lake Starnberg. Two algal classes Klebsormidiophyceae and Conjugatophyceae were not identified by metabarcoding, even though these were found under the microscope.

Table 3.3. Algal classes in Starnberger See detected using the two different methods (microscopical analysis vs sequence analysis) or detected only by one method

Taxa identified by both Methods	**Taxa identified only through HTS**	**Taxa identified only through microscope**
Prymnesiophyceae	*Trebouxiophyceae*	*Klebsormidiophyceae*
Dinophyceae	*Mamiellophyceae*	*Conjugatophyceae*
Dictyochophyceae		

Cyanophyceae		
Cryptophyceae		
Chrysophyceae		
Chlorophyceae		
Bacillariophyceae		

Since the assessment of ecological status classification is based on **phytoplankton species** an important question is, how well the finding rate (or recovery rate) of species by the modern HTS method. Species detected by morphological analysis were compared to those detected by identified with the modern method of metabarcoding in the HTS result tables, and visa wise. The REBECCA code was the taxonomic nomenclature, which was extended for exotic taxa found by HTS.

To facilitate the comparison, the "EAW taxa analysis tool" (LfU, FEM, LFUI) provides prepared tables on demand (queries) for a pilot site, which is to select in the "select table" beforehand. Queries comparing the detected taxa lists are starting with Prefix "GAP_".

The results from Lake Starnberg are to find in the Appendix (Suppl. Table 3.4 - 3.6).

It can be seen that 30 species out of total 59 taxa detected under the microscope were recognized also through 16S rDNA or 18S rDNA sequencing. This listed included abundant cyanobacteria (Planktothrix rubescens), Bacillariophyceae (Asterionella, Ulnaria, Fragilaria), Chrysophyceae (Dinobryon, Mallomonas), dinoflagellates (Ceratium, Gymnodinium, Peridinium), Cryptophyceae (Cryptomonas), and Chlorophyta (Phacotus lenticularis), Haptophyta (Chrysochromulina). Together the corresponding species accounted between 20-60% of the biovolumes of the 8 samples.

On the other hand, species not recognized through HTS were included among the centric diatoms, i.e. genera Cyclotella and Stephanodiscus and also the small cryptophyte taxa (Plagioselmis, Rhodomos) were missing.

Taxa, whcih were detected only through HTS such as exotic dinophytes (Asulcocephalium miricentonis, Peridinium gatunense, Polarella glacialis (marin), Prorocentrum sp Woloszynskia tenuissima) gave an important hint for an improved morphological determination of this group. The signals of th, Synechococcus and Cyanobium were strong and distributes in different oligotypes. These nanoplankton taxa can be easily be overlooked by microscopy.

During the study period the cyanobacteria Prochlorophyta, and the nuisance cyanobacteria Tychonema and Microcystis (which were identified in other study lakes) were not detected in Lake Starnberger See.

Notably flagellates of the algal classes Chrysophyceae, Dinophyceae and Chlorophyta (Volvocales, Prasinophyta) were not detected under the microscope bur reported through HTS. Certain groups of flagellates shared a rather high number of genotypes.

Conclusion on results obtained for phytoplankton in lake Starnberg

Relevant information derived from sequencing includes

(i) overall good qualitative relationship between HTS derived genera and microscopy derived genera, i.e. sequence based confirmation of microscopical results on genus level

(ii) additional information on certain groups of algae which have not been well recorded before, picocyanobacterial and eukaryotic flagellates (Chrysophyceae, Dinophyta, Prasinophyta)

(iii) additional (biogeographic) information on presence/absence of nuisance algae, i.e. *Planktothrix rubescens*/*agardh*ii, *Tychonema bourellyi*, *Microcystis aeruginosa*

(iv) information on intraspecific genetic variation among populations, i.e. detection of novel genotypes within populations of algal species.

3.2 Biofilm composition (littoral), L. Starnberger

Germany (PP10, LFU)

Ute Mischke and Jochen Schaumburg

Sampling

Phytobenthos has proven to be an indicator for ecological quality status in lakes. In Germany, the phytobenthos with benthic diatoms is a biological quality element but not the other biofilm groups such as cyanobacteria or benthic green algae (so-called soft algae or non-diatoms). National legislative on littoral (diatoms) sampling is available by the WFD method PHYLIB. Thus for this project the guidelines from the national legislative on sampling in lakes have been applied along with the protocol developed in WP1 (DT1.1.2. -2, Lake biofilms sampling protocol).

There are 30 different sampling locations which are regularly monitored for Starnberger See and 28 were found to have stones in June 2019 (see map of the lake). The rather dry summer conditions in 2019 rarely affect the water level in Lake Starkberg because of the huge lake volume and size. At each site (called transect) 5 stones were selected along the shoreline representing an area of 50-100 m^2 (half meter depth) and then transported into the laboratory using cooling boxes.

The shoreline of Starnberger See is affected by building activity, and natural shorelines are rare. TP concentrations are not available for each littoral site, but the lake is oligotrophic and has no sewage inflow or strongly eutrophied river inflows.

In general samples were brushed off from stones from a representative surface area (> 100 cm^2) using a clean tray. Diatoms were identified and counted by their silicate frustules after mounting in Naphrax for microscopical analysis.

In parallel to sampling for microscopy, for DNA extraction from the same stones aliquots were preserved using 80% Ethanol as described in protocol (DT1.1.2. -2, Lake biofilms sampling protocol).

Finally about 10ml aliquots were filled in pre-weighed glass vessels and the dry-weight was determined from the difference in dried filter (105°C, 24 h) weight before and after filtration.

Results on cyanotoxins concentrations

No samples for cyanotoxins analyses were prepared and analyzed.

DNA extraction and sequencing

DNA was extracted using the Machery and Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (DT1.1.2. -7, DNA extraction biofilms) and done by the project partner FEM (IT).

From sample DNA extracts 16S rDNA (V3-V4 region) has been amplified using primers 341Fmod CCTAYGGGRBGCASCAG and 806Rmod GGACTACNNGGGTATCTAAT under the following conditions: 95°C (5 min), 28 cycles including 95°C (30 sec), 55°C (30 sec), 72°C (30 sec), and final 72°C (5 min). For 18S rDNA (V4 region) the primers V4F-18S_ILL and CCAGCASCYGCGGTAATTCC and V4R-18S_ILL ACTTTCGTTCTTGATYRATGA were applied using the cycling conditions from above. One technical replicate was sequenced (DT1.1.2. -10 Library prep 16S marker gene; DT1.1.2. -11, Library prep 18S marker gene).

PCR amplification and library preparation of purified PCR products for rbcL was performed according to WP1 protocol (DT1.1.2. -9, Library prep RbcL marker gene). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions.

Bioinformatic processing

The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2), (DT1.1.3. - 1 BioinfRbcL, Bioinformatics treatment RbCL marker gene, DT1.1.3. - 3 Bioinf18S, Bioinformatics treatment 18S marker gene, DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene).

For cyanobacteria automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates (strains) and manual blastn against ASvs allowing 0-1 mismatch (405 bp alignment).

Sequences were clustered into ASVs (no dissimilarity threshold) and assigned to the SILVA SSU reference database and PR2 database for taxonomic classification. For rbcL gene assignment to diatom taxa the curated database R-Syst::diatom (Rimet et al. 2016) was used (INRA).

Comparison with traditional microscopy

All microscopical taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the VALID code system for diatoms in phytobenthos (LfU) and the REBECCA code for non-diatoms (soft algae) An Excel Access database for all microscopical taxa and the VALID codes assigned has been prepared (LfU, FEM, LFUI).

Microscopical countings of diatoms were performed according to the national legislative by the external services of Cornelia Goos (Pensberg, DE).

Results on comparison between traditional microscopy and HTS

There are no soft algae counts by microscopy, so there are no results for comparing.

Anyway, the HTS cyanobacteria results are very interesting with 52 different taxa found, see in detail in Suppl. Table 3.8 in the appendix).

Table 3.4. The records of the antoxin-producing Tychonema at several locations, still all with extreme low signals, are very notable and listed in the following table:

station_DB_EAW	Taxon_REBECCA	ASV_seq	Signal 16S reref	species_16S
Starnberger_T20	Tychonema sp.	Seq16016	8	NA
Starnberger_T6	Tychonema sp.	Seq262	4	NA
Starnberger_T21	Tychonema sp.	Seq33277	1	NA
Starnberger_T19	Tychonema sp.	Seq34	13	bornetii/bourrellyi/tenue
Starnberger_T28	Tychonema sp.	Seq34	1	bornetii/bourrellyi/tenue
Starnberger_T20	Tychonema sp.	Seq5876	7	NA
Starnberger_T20	Tychonema sp.	Seq6271	21	NA
Starnberger_T22	Tychonema sp.	Seq6271	8	NA
Starnberger_T20	Tychonema sp.	Seq6515	1	NA
Starnberger_T2	Tychonema sp.	Seq920	6	NA
Starnberger_T5	Tychonema sp.	Seq920	1	NA

While there was no mass development of *Tychonema* in lake Starnberg, this gernus was recorded for a Bavarian river lake (Mandicosee) belonging to the Lech-Wertach system in Bavaria (Bauer et al. 2020). One sample of this site was transferred to the Eco-AlpsWater metabarcoding analysis and confirmed the occurrence of a benthic *Tychonema* strain with the 16S sequences 34 and 2993.

Table 3.5. List of algal classes from Starnberger See littoral samples identified using sequencing

Algal classes (16S and 18S rDNA)		
Bacillariophyceae	*Cryptophyceae*	*Stylonematophyceae*
Charophyceae	*Cyanophyceae*	*Synchromophyceae*
Chlorophyceae	*Dinophyceae*	*Synurophyceae*
Chrysophyceae	*Eustigmatophyceae*	*Trebouxiophyceae*
Coleochaetophyceae	*Florideophyceae*	*Ulvophyceae*
Coscinodiscophyceae	*Mediophyceae*	*Zygnematophyceae*
	Pavlovophyceae	

Benthic diatoms

In pilot lake Starnberger See the diatom community is very divers: In total 161 diatom taxa by light microscopy (see Fig. 3.5) and in total 95 taxa identified on genera or species level were found by rcbl with HTS in the 27 different biofilm samples. Both methods shared 44 taxa (see Suppl. Table 3.9 in Appendix).

Between the 51 taxa newly detected in Starnberger See by HTS there was *Achnanthidium delmontii* and *Achnanthidium eutrophilum*. *A. delmontii* is considered as a so-called invasive species (see Suppl, Table 3.11 in appendix).

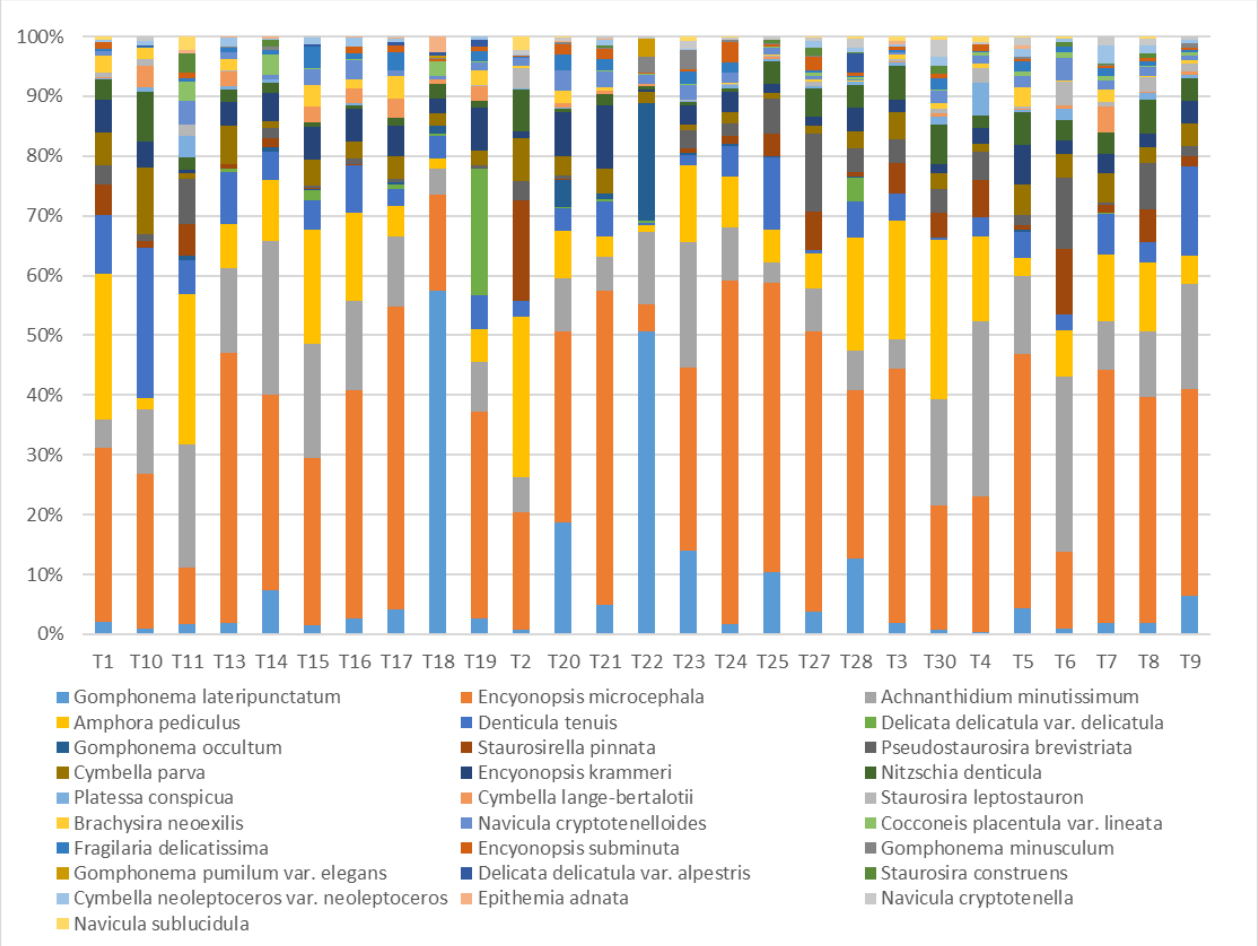


Fig. 3.5. Relative abundance of diatoms (> 2 %) at 28 littoral sampling sites from Starnberger See as revealed from microscopical counting (for location of sites see Fig. 3.2).

A larger number of taxa, which were detected under the microscope were found not by HTS (117 species; see Suppl. Table 3.10 in Appendix).

To proof the identity of non-corresponding diatom taxa, in total 45 different taxa will be checked by a scanning electronic documentation (SEM) to be compared with light microscopy (LM) pictures in an external service contract.

The first 10 taxa documentation are available in an interim report (Goos, 2021) and were able to verify specific records. For example, the presence of *Cymbella excisa* var. *excisa* in Lake Starnberg found by rcbl signals was verified by SEM and LM (see Fig. 3.6).

Similar, the scanning electronic documentation of *Achnanthidium delmontii* according characterisation by Pérès et al. (2012) and its discrimination from a very similar species *Achnanthidium pyrenaicum* detected by the light microscopy demonstrates the value of data proof by a multi-proxy approach.

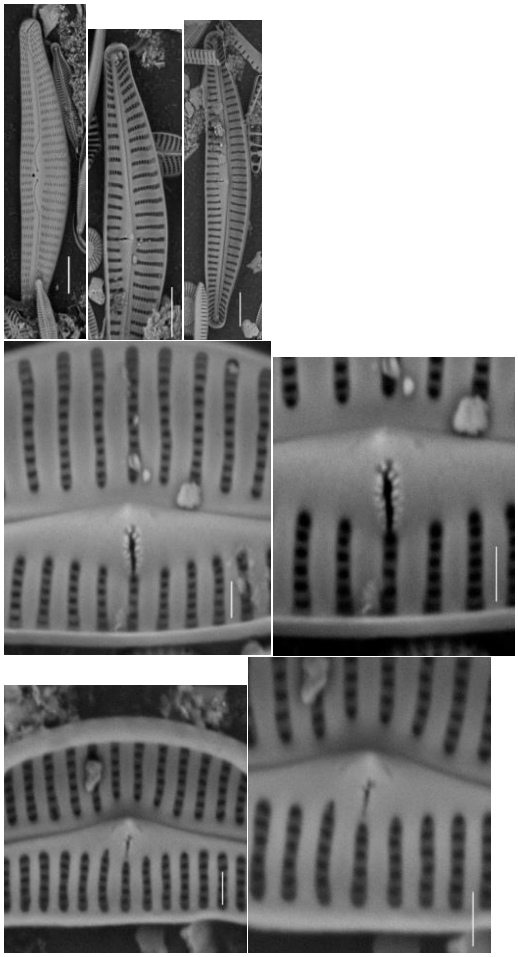


Fig. 3.6.a: Copy of Fig. 9 from SEM report (Goos 2021):: REM-Aufnahmen von Cymbella excisa var. excisa aus dem Starnberger See Transekt 21 obere Reihe: 1 außen, 2-3 innen, 3 C. excisa var. excisa cf. (Massstabsleiste 5 µm) mittlere Reihe: innen, Stigma Alveolus zahnartig (Massstabsleiste 1 µm) untere Reihe: zum Vergleich C. parva innen, Stigma Alveolus unregelmäßig (Massstabsleiste 1 µm).

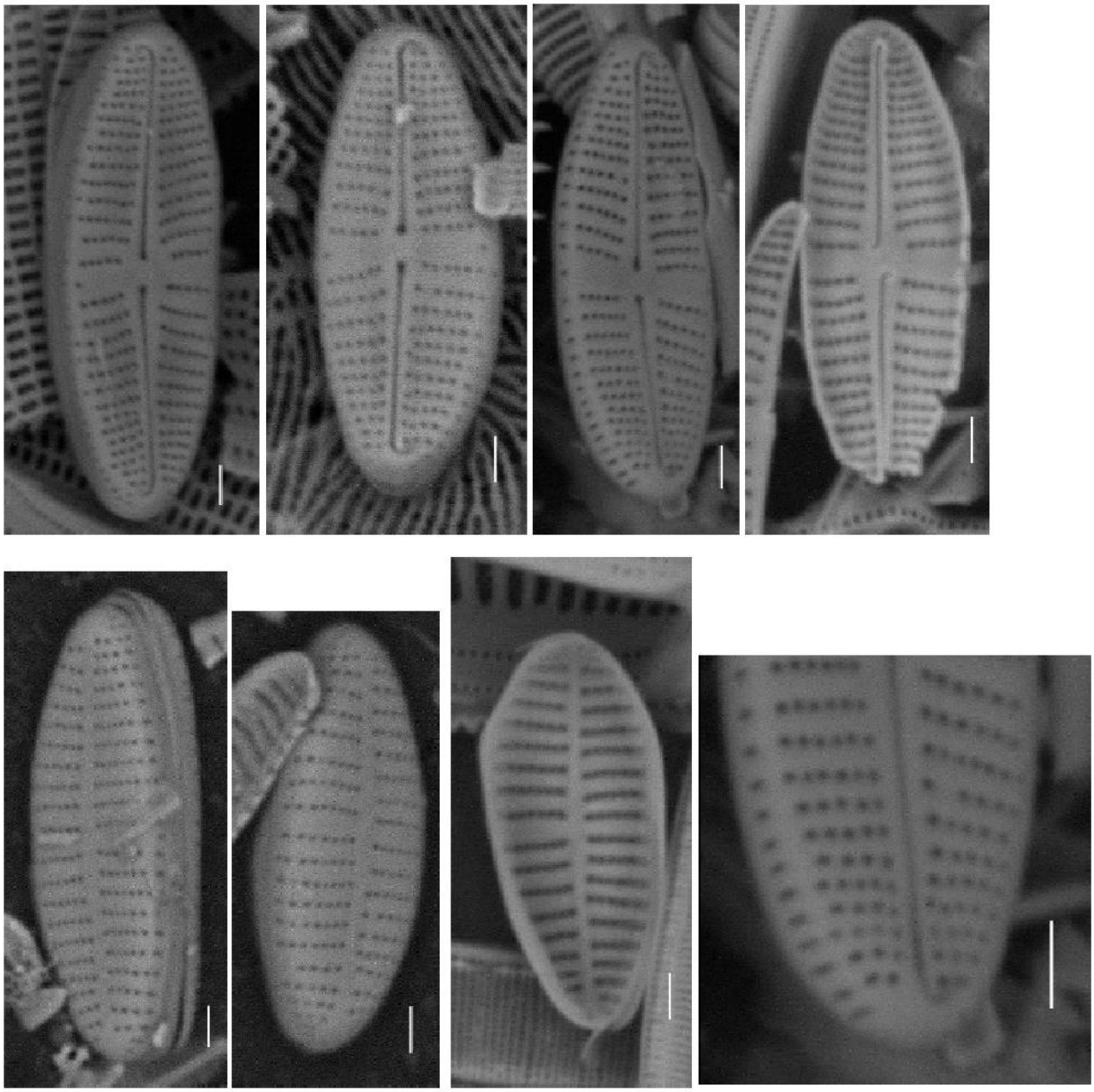


Fig. 3.6.b: Copy of Fig. 2 from SEM report (Goos 2021): REM-Aufnahmen von Achnanthidium delmontii aus der Wertach (0393_2) obere Reihe: 1-3 R-Schale außen, 4 R-Schale innen, untere Reihe: 1-1 RL-Schale außen, 3 RL-Schale innen, 4 Ausschnitt R-Schalenende (Massstabsleiste 1 µm).

Conclusion on results obtained for phytobenthos (cyanobacteria & diatoms)

Relevant information derived from sequencing includes the following:

(i) cyanobacteria taxa by 16S rDNA sequencing were very divers also for soft algae

(ii) The 16S rDNA sequencing information is useful to infer the toxigenic potential of the respective biofilm community. Candidate genera such as Tychonema can be followed more closely to identify the respective nuisance organism

(iii) For diatoms the additional information on the occurrence of invasive taxa such as Achnanthidium delmontii and Achnanthidium eutrophilum new for Bavarian region become available.

3.3 Fish composition. L. Starnberg

Christian Vogelmann, Michael Schubert (LfL)

Sampling

The sampling for fish eDNA was carried out from 13-26.September 2019 (GFC point sampling) and from 1-2.October 2019 (VigiDNA®), according to the Eco-AlpsWater protocol D.T1.3.1-4 - Lake and river eDNA fish sample collection from the field for downstream molecular analysis.

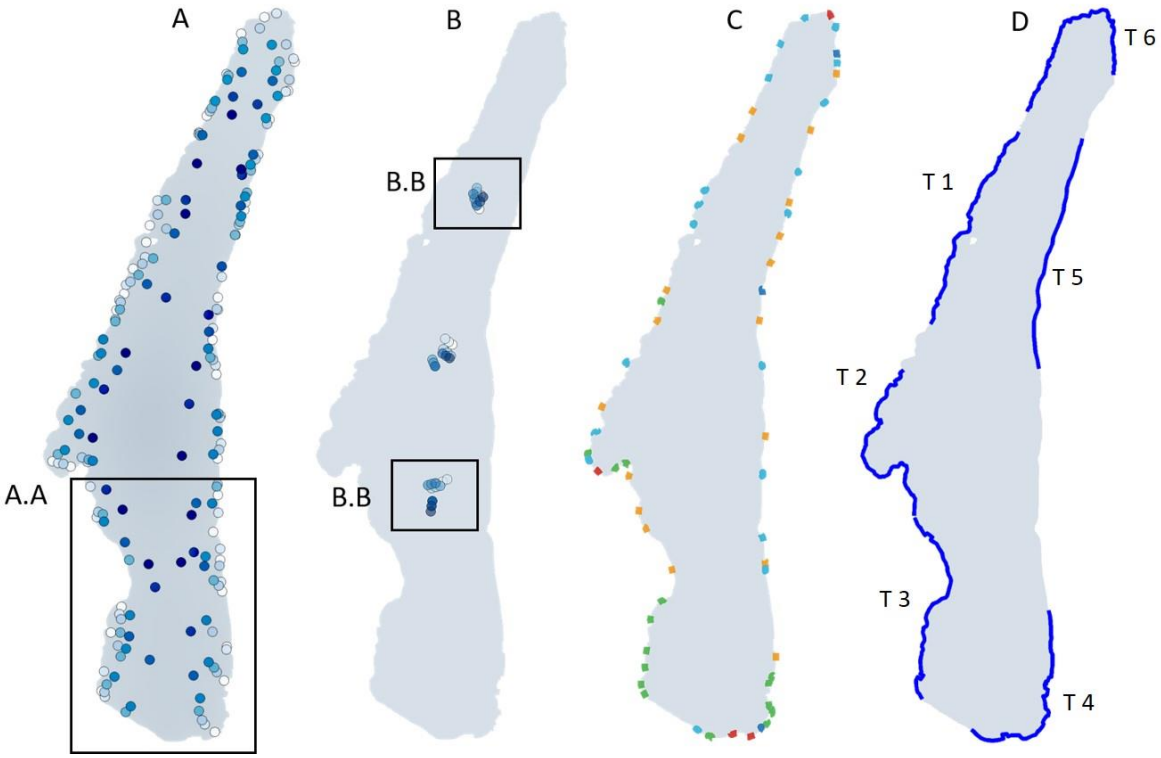


Figure 1 Sampling sites at Lake Starnberg. A = benthic and B = pelagic gillnet locations for the traditional sampling in 2019. A.A = sample sites of eDNA point samples (GFC, n = 64). B.B sample sites of eDNA VigiDNA®/point samples (vertical profile). C = electrofishing stretches (Site 1 – 61 with 200 m each track) in 2019. D = eDNA sampling (VigiDNA®) in 2019 sampled lakeshore line (transects; T1-T6).

VigiDNA®:

Standard sampling: By boat, 30 liters of water were collected along each transect (6 km; Figure 1; D) and filtered through the VigiDNA® 0.45 µm filter cartridges using a peristaltic pump (in total 6x shoreline). In addition to the shoreline transects, depth-integrated samples from the water surface to just above the bottom (three times 10 liters over the entire water column) were collected at two pelagic sites (Figure 1; B.B), including the deepest part of the lake, using an integrating water sampler (Hydrobios IWS III). The three 10-litre samples were then combined (total volume of 30 l) and also filtered through a VigiDNA® 0.45-µm filter cartridge. After filtration, the cartridges were filled with a preservation buffer and stored in the fridge until extraction according to Eco-Alpswater protocol D.T1.3.1-4 Lake and river eDNA fish sampling. In the meantime, however, we would no longer recommend storing the samples in the refrigerator due to difficulties, especially with regard to DNA extraction. Therefore, it is advised to store the samples at room temperature until extraction.

Additionally, also by boat, 5 liters were collected at each of the 64 benthic gillnet (Figure 1; A.A) and two pelagic gillnet sites (Figure 1; B.B). The eDNA samples were collected 2 hours before the nets were deployed

for traditional sampling. Using an integrating water sampler (Hydrobios IWS III), 5 litres were collected at each sampling point, at the same depths where the gillnets were placed shortly afterwards. Back in the laboratory, the samples were filtered through glass fiber filter discs (GFC) 1.2 µm using a vertical filtration device. After filtration, the filters were stored frozen at -20° until DNA extraction.

DNA extraction and sequencing

For the fish eDNA extraction from VigiDNA® cartridges a combination of the Macherey-Nagel NucleoSpin® and the DNeasy Soil Kit® was used according to the Eco-Alpswater protocol D.T1.3.1-8.2 - Fish DNA extraction from VigiDNA® cartridges. For the fish eDNA extraction from GFC filters, the DNeasy Power Water kit (Qiagen) was used, following the manufacturer's protocol.

The PCR amplification as well as the library preparation was done by AGES (Austrian Agency for Health and Food Safety) according to the the Eco-Alpswater protocol D.T1.3.1-12 - Library preparation 12S. For the sequencing, MiFish-U primers (forward: 5`- GTCGGTAAAACTCGTGCCAGC-3`, reverse: 5`- CATAGTGGGGTAT-CTAATCCCAGTTTG-3`, Miya et al. 2015) were used and for each sample. For each VigiDNA® sample nine replicates were performed, for the GFC filters only one.

Bioinformatic processing

Raw sequencing data were analyzed at the Research Department for Limnology, Mondsee. For the bioinformatics analysis, the qiime2 pipeline (Bolyen et al. 2019) was used. This pipeline was originally designed to work on microbiome data. However, previous test showed, that the taxonomic assignment of the obitools3 pipeline, which was used by most partners in the EAW project, and the taxonomic assignment of the qiime2 pipeline delivered comparable results regarding the taxonomic assignment of fish in eDNA samples. Due to easier handling of the bioinformatics processes and a slightly finer taxonomic resolution, the German and Austrian project partners used the qiime2 approach.

Comparison with traditional fish monitoring

The taxonomic inventories obtained from the bioinformatic analysis was then compared to the dataset obtained from the traditional fish sampling at Lake Starnberg, which was carried out shortly (2-3 hours later) after the eDNA approch in autumn 2019. The traditional methods consisted of pelagic and benthic gillnetting and electrofishing along the shoreline (Figure 1). Since the barcode region of *Leuciscus leuciscus* and *Leuciscus idus* is too similar, it is not possible to distinguish between these 2 species, using the eDNA approach. It is only possible to identify them up to the genus level (*Leuciscus*).

Results on comparison between traditional monitoring and HTS

VigiDNA®:
For each of the 7 VigiDNA® samples, 9 replicates were sequenced. For the analysis, the average number of reads per species (occurring in the 9 replicates) in each sample was determined and then summed up. Of the total 31 species confirmed, 26 were detected by traditional sampling (benthic and pelagic gill nets, electrofishing), 24 by the VigiDNA® system and 20 fish species by both methods. 6 fish species were identified only by traditional methods and 5 only by the HTS approach (Figure 2, A.). The results obtained from the VigiDNA® approaches were compared to the catches from all gillnet locations (Figure 1 A), since the eDNA samples were collected along the whole lakeshore.

Table 1 Comparison of fish taxa detected with traditional and eDNA (VigiDNA®) assessment method. The numbers in the molecular method column shows the total number of reads for each species. The traditional methods columns show the number of individuals caught with different methods (gillnetting, including pelagic and benthic gillnets, and electrofishing).

Common name	Scientific name	eDNA (Vigi)	Traditional methods		
			Gillnetting	Electrofishing	Total
Perch	*Perca fluviatilis*	71219	4337	787	5124
Danube bleak	*Alburnus mento*	56983	97	2	99
Roach	*Rutilus rutilus*	39297	358	1034	1392
Common dace	*Leuciscus leuciscus*	0	0	1343	1343
Bream	*Abramis brama*	25675	34	451	485
Common carp	*Cyprinus carpio*	17243	0	10	10
European whitefish	*Coregonus lavaretus*	15721	113	0	113
Bleak	*Alburnus alburnus*	14116	112	7923	8035
Chub	*Squalius cephalus*	12819	24	881	905
Pike	*Esox lucius*	11920	27	28	55
Schneider	*Alburnuides bipunctatus*	4676	0	0	0
Tench	*Tinca tinca*	3926	8	53	61
European eel	*Anguilla anguilla*	3564	0	52	52
Rainbow trout	*Onchorynchus mykiss*	2283	0	0	0
Common rudd	*Scardinius erythrophtalmus*	1693	14	0	14
Wels catfish	*Silurus glanis*	1673	0	22	22
Stone loach	*Barbatula barbatula*	1614	3	28	31
Gudgeon	*Gobio gobio*	1518	0	0	0
Grass carp	*Ctenopharyngodon idella*	1400	0	0	0
Vimba bream	*Vimba vimba*	1199	27	3	30
Eurasian ruffe	*Gymnocephalus cernua*	519	0	0	0
Barbel	*Barbus barbus*	497	0	1	1
Brook trout	*Salvelinus fontinalis*	382	10	0	10
Prussian carp	*Carassius gibelio*	354	0	4	4
Pikeperch	*Sander lucioperca*	180	10	0	10
Brown trout	*Salmo trutta*	0	0	2	2
Bullhead	*Cottus gobio*	0	0	3	3
Pumpkinseed	*Lepomis gibbosus*	0	0	2	2
Burbot	*Lota lota*	0	0	11	11
Ide	*Leuciscus idus*	0	0	384	384
	Leuciscus sp.	26005	-	-	-

GFC:

No replicates were used in this approach, the number of reads for each species in the 66 samples, was summed up. Of the total 29 species confirmed, 24 were detected by traditional sampling (benthic and pelagic gillnets, electrofishing), 23 by the GFC filter system and 18 fish species by both methods. 6 fish species were identified only by traditional methods and 5 only by the HTS approach (Figure 2, A). The results obtained from the GFC approach were compared to the catches from the gillnet locations in the southern part of the lake (Figure 1 A.A), since the samples were taken at these sites only.

Table 2 Comparison of fish taxa detected with traditional and eDNA (GFC) assessment method. The numbers in the molecular method column shows the total number of reads for each species. The traditional methods columns show the number of individuals caught with different methods (gillnetting, including pelagic and benthic gillnets, and electrofishing).

Common name	Scientific name	eDNA (GFC)	Traditional methods		
			Gillnetting	Electrofishing	Total
European whitefish	*Coregonus lavaretus*	4057562	43	0	43
Perch	*Perca fluviatilis*	1865737	1127	616	1743
Danube bleak	*Alburnus mento*	687748	37	0	37
Pike	*Esox lucius*	293644	7	17	24
Common carp	*Cyprinus carpio*	223757	0	7	7
Vimba bream	*Vimba vimba*	171060	18	1	19
Roach	*Rutilus rutilus*	168818	91	370	1281
Bream	*Abramis brama*	139141	12	326	338
Tench	*Tinca tinca*	126520	0	35	35
Chub	*Squalius cephalus*	125649	11	350	361
Common dace	*Leuciscus leuciscus*	0	0	817	817
European eel	*Anguilla anguilla*	43263	0	20	20
Bleak	*Alburnus alburnus*	27945	17	3822	3839
Bullhead	*Cottus gobio*	21900	0	0	0
Burbot	*Lota lota*	21417	0	3	3
Arctic char	*Salvelinus alpinus*	19354	3	0	3
Pikeperch	*Sander lucioperca*	17330	2	0	2
Rainbow trout	*Onchorynchus mykiss*	16627	0	0	0
Common rudd	*Scardinius erythrophtalmus*	4979	7	0	7
Grayling	*Thymallus thymallus*	1985	0	0	0
Wels catfish	*Silurus glanis*	1625	0	11	11
Brook trout	*Salvelinus fontinalis*	1040	0	0	0
Stone loach	*Barbatula barbatula*	991	2	18	20
Brown trout	*Salmo trutta*	973	0	0	0
Barbel	*Barbus barbus*	0	0	1	1
Prussian carp	*Carassius gibelio*	0	0	4	4
Pumpkinseed	*Lepomis gibbosus*	0	0	2	2
Ide	*Leuciscus idus*	0	0	359	359
White bream	*Abramis bjoerkna*	0	1	0	1
	Leuciscus sp.	65005	-	-	-

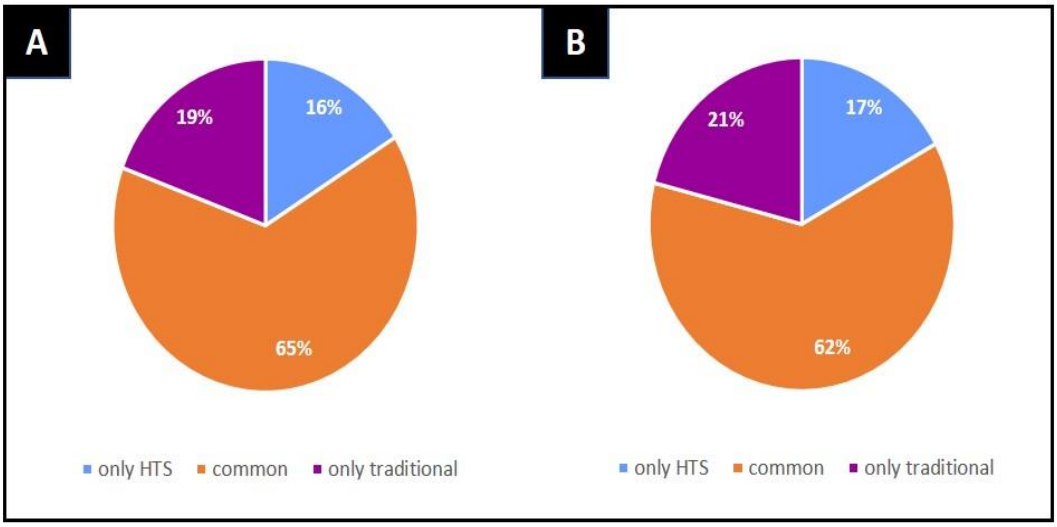


Figure 2 shows the percentage of species detected, depending on the filters used. A= VigiDNA® filter cartridges, 65% (common) were detected by both methods, 19% by the traditional methods and 16% by the HTS approach. In total 24 species were detected. B= GFC filters, 62% (common) were detected by both methods, 21% by the traditional methods and 17% only by the HTS approach. In total 23 species were detected. Leuciscus idus and Leuciscus leuciscus = Leuciscus sp..

Conclusion on results obtained for fish

eDNA metabarcoding for fish is a valuable tool to quickly assess the species composition of aquatic ecosystems. Both eDNA approaches detected species that were not caught during the traditional sampling event (VigiDNA® = 6, GFC = 5), whereby in some cases DNA entry, e.g. via fishing gear or tributaries, is to be assumed (e. G. *Thymallus thymallus* and *Ctenopharyngodon idella*). Since DNA from the genus *Leuciscus* cannot be assigned down to species level, *Leuciscus idus* and *Leuciscus leuciscus* were counted as undetected for the comparison. Molecular methods are well suited for studying fish communities in lakes and rivers and have proven to be sensitive, even for species that do not occur in large quantities. The eDNA approach seems to be a cost and time effective complementation to the traditional methods in order to get a more detailed insight on the fish community composition in alpine waterbodies.

4. Italy, Lake Garda

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[1] FEM (PP1)

[2] ARPAV (PP3)

[3] APPA TN

[4] ARPA LOMBARDIA

General introduction

The key lakes include Lake Mondsee (Austria), Lake Bourget (France), Lake Starnberg (Germany), Lake Garda (Italy), Lake Bled (Slovenia), and Lake Lugano (Switzerland). These natural and deep lakes are located in the peri-alpine area and are under a long-term monitoring programme. Despite the recovery of the trophic status (from moderate to good) due to reduced external nutrient loading, in most of the lakes the oxygenation of deep waters is still hampered by weak winter turnover owing to climate warming. Consequently, the biological communities changed considerably during the last decades.
Comparing descriptions of the lakes are in the digital infographics on webpage (D C5.5.).
Within this group of lakes, Lake Garda is situated at lowest elevation and concerning the trophic status, the lake has the lowest concentration of total phosphorus and chlorophyll *a* in annual mean.
The ecological classification (BQE) of the lakes is as followed: Starnberger (D), Mondsee (A), Garda (I), Bourget (F) “good” and Lugano (CH-I) and Bled (SI) “moderate”.
Lake Garda (Table 4.1) is a deep, oligo-mesotrophic and oligomictic lake with a 350 m at the deepest point, located in the southern edge of the Alps. Its water retention time is more than 20 years. See its detail description in Eco-AlpsWater WP2 Deliverable D.T2.2.1 “Identification of key lakes and rivers, and collection of previous knowledge”.

Table 4.1. Key morphological and trophic characters of Lake Garda

Lake elevation (m)	65
Surface area (km²)	368
Volume (km³)	49
Total N (annual mean/range, mg/L) *	0.41
Total P (annual mean/range, µg/L) *	15.0
Chl *a* (annual mean/range, µg/L) *	2.6

*data refers to 2008-2018

4.1 Phytoplankton (incl. cyanobacteria), L. Garda

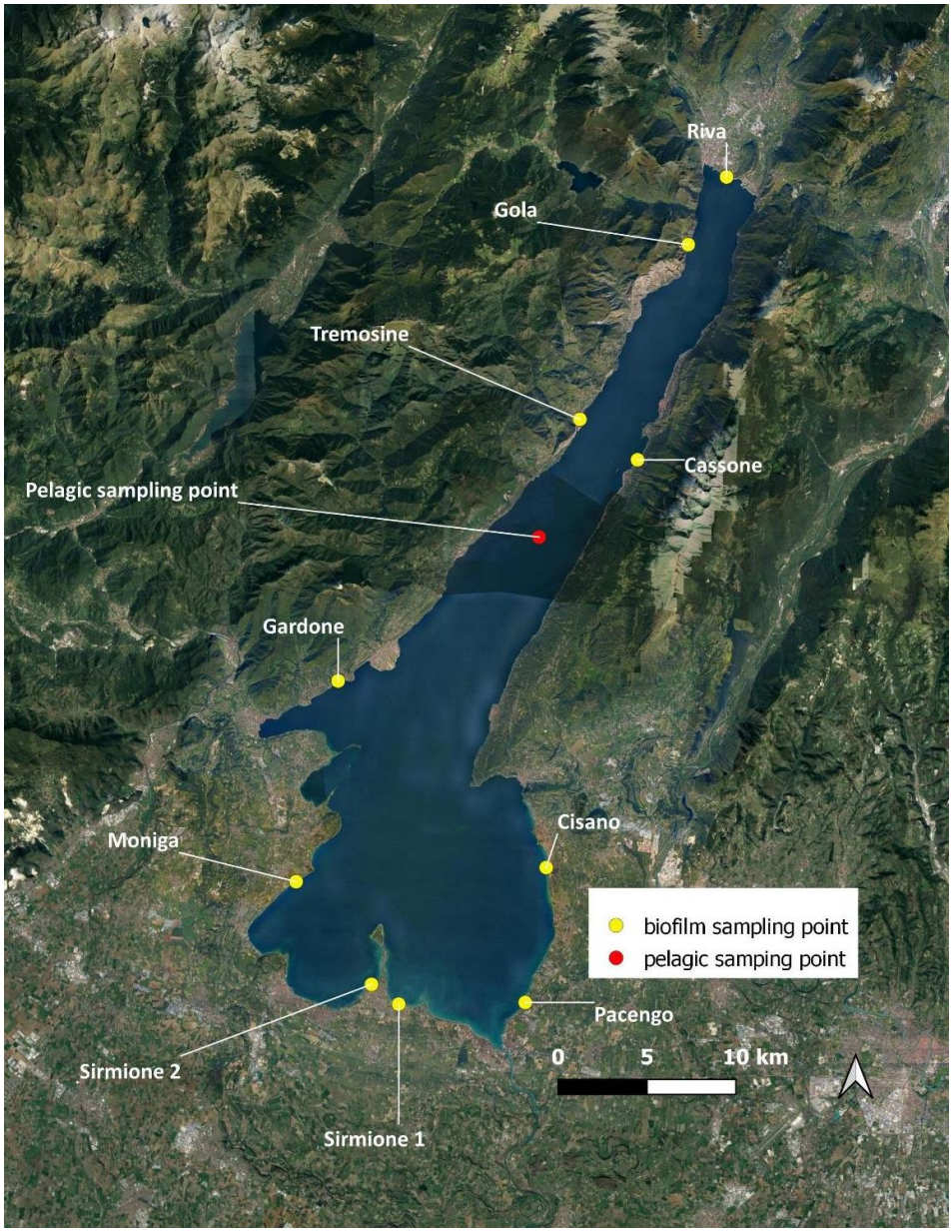

Fig. 4.1. Lake Garda - pelagic and shore biofilm sampling stations.

Sampling

Lake Garda was chosen as one of the pilot lakes for the implementation of the Eco-AlpsWater metabarcoding approach. Samples were taken monthly starting from January and until December in 2019 by PP3 (ARPAV) in the regular WFD site (Fig. 4.1), given a total of 12 samples.

According to national law, depth-integrated water samples (0-20 m), which roughly correspond to the euphotic zone, were taken at the deepest point of the lake. Depth profiles of water temperature, pH, conductivity and oxygen were measured by a multi-parameter probe. Water transparency was measured with a Secchi disk.

Additional chemical data were analyzed: alkalinity, conductivity, ammonia nitrogen, nitric nitrogen, total nitrogen, nitrous nitrogen, reactive phosphorus, total phosphorus, reactive silica, dissolved oxygen, principal anions and cations, cyanotoxins, with a total of 32 parameters analyzed (Table 4.2) and 372 data produced.

From the integrated water samples, representative of the euphotic zone, were collected 200 ml in a dark glass bottle and preserved with Lugol's solution for phytoplankton counting and two litres in a dark plastic bottle for the analysis of the chlorophyll-*a*.

The chlorophyll-*a* concentration was determined with spectrophotometric analysis (APHA Standard Methods for the Examination of Water and Wastewater ed. 23rd 2017 10200 H).

The Italian method for lake assessment is based on phytoplankton analysis following the UNI EN 15204:2006 Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). The taxa abundance and the total biovolume of the planktonic algae were determined from a subsample under the inverted microscope (quantitative analysis).

According with the Report CNR-ISE, 02.13 (updated with Alpine GIGs results developed for Alpine lakes), the metrics included in the Italian phytoplankton assessment method are the biomass metrics chlorophyll *a* and the total biovolume and the taxonomic composition metrics of the phytoplancton (PTIot - Phytoplankton Trophic Index).

For DNA sequencing, the depth-integrated samples were taken in parallel and filtered on boat on a Sterivex™ GP 0.22 µm filter (Millipore, Billerica, Massachusetts, USA), by pressing water manually through the filter unit with a sterile plastic syringe following the protocol from WP1 (D.T1.1.2 -1 Lake plankton sampling). To estimate the filtered volume, the water was collected in 1 L plastic cylinder. The filtration is completed when the filter became clogged or when a total volume of 1 L is reached.

Rules to define ecological classes and reference conditions

The classification of lakes and reservoirs from phytoplankton is based on ICF Index (Overall phytoplankton index), defined by the average of the values of two indices, the average biomass index and the composition index. The calculation of these two indices is based on: average concentration of chlorophyll-*a*, medium bio-volume, PTI (PTIot, PTIspecies, MedPTI) and percentage of cyanobacteria characteristic of eutrophic waters. The definition of reference values and class limits for the index ICF are reported in "Indici per la valutazione della qualità ecologica dei laghi" 2013. **Report CNR ISE, 02-13**: 195 pp. (versione 2018).

DNA extraction and sequencing

DNA was extracted using the DNeasy® PowerWater® SterivexTM Kit (Qiagen, Hilden, Germany) following the manufacturer`s protocol. (D.T1.1.2. -6 Plankton DNA extraction).
From the sample DNA extracts, 16S rDNA (V3-V4 region) has been amplified using primers 341Fmod CCTAYGGGRBGCASCAG and 806Rmod GGACTACNNGGGTATCTAAT under the following conditions: 95°C (5 min), 28 cycles including 95°C (30 sec), 55°C (30 sec), 72°C (30 sec), and final 72°C (5 min). For 18S rDNA (V4 region) the primers V4F-18S_ILL and CCAGCASCYGCGGTAATTCC and V4R-18S_ILL ACTTTCGTTCTTGATYRATGA were applied using the cycling conditions from above. Library preparation of purified PCR products for 16S rDNA and 18S rDNA was performed according to EAW Protocols. Bridge amplification and sequencing by synthesis were performed according to standard conditions (FEM, Miseq, Massimo Pindo). One technical replicate was sequenced (DT1.1.2. -10 Library prep 16S marker gene; DT1.1.2. -11, Library prep 18S marker gene).

Bioinformatic processing

The raw sequence data were processed using the package DADA2, (Protocols DT1.1.3. - 3 Bioinf18S, Bioinformatics treatment 18S marker gene; DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene). Sequences were assigned using the SILVA SSU reference database (bacteria/cyanobacteria) and the PR2 database (protists/microalgae).

For selected ASVs, automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates and manual BLAST against ASVs.

Elaboration of traditional microscopy data

The microscopic taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the REBECCA code for phytoplankton. To facilitate comparison an Excel Access database tool (version 7, July 2021) for all microscopical taxa and REBECCA codes assigned has been prepared (LfU, FEM, LFUI).

Chemical data

Table 4.2. Mean annual values for chemical and physical data collected in the field or analyzed in laboratory in 2019

parameters	units of measurements	annual mean (0-20m)
temperature	°C	14.13
field_ph		8.33
field_conductivity	µS/cm	235
secchi_disk_depth	m	8.9
euphotic_layer	m	20.9
oxygen_concentration	mg/l	10.98
oxygen_percentage	%	107
laboratory_ph		8.21
laboratory_conductivity	µS/cm at 25°C	236
total_alkalinity	mg CaCO3/L	104
bicarbonates	mg/L	127
nitrate_nitrogen	µgN/L	221
sulphates	mg/L	10.15
chloride	mg/L	5.98
calcium	mg/L	30.06
magnesium	mg/L	8.44
sodium	mg/L	5.15
potassium	mg/L	1.18
ammonium	µgN/L	6.00
total_nitrogen	µgN/L	390
soluble_reactive_phosphorus	µgP/L	3.27
total_phosphorus	µgP/L	10.1
reactive_silica	mgSi/L	1.0
dry_weight	mg/L	1.1
chlorophyll_a	µg/L	3.3
Atx-A	µg/L	583.8
Hatx-A	µg/L	0
MC-RRdm	µg/L	2.1
MC-HtyRdm	µg/L	0
MC-LRdm	µg/L	0
MC-RR	µg/L	0
MC-LR	µg/L	0

On the basis of the mean annual phosphorus concentration (µg L^{-1}), mean annual chlorophyll-*a* concentration (µg L^{-1}), maximum annual chlorophyll-*a* concentration (µg L^{-1}), mean annual Secchi-disk depth (m) and minimum annual Secchi-disc depth (m), with the OECD Fixed Boundary Trophic Classification System (OECD, 1982) (Table 4.3), were evaluated the trophic conditions (Table 4.4) of Lake Garda. The mean phosphorus concentration refers to the trophic zone (0-20m) showing the apparent trophic status (Salmaso et al., 2018).

Table 4.3. OECD Fixed Boundary Trophic Classification System (OECD 1982)

Trophic category	Mean phosphorus concentration ($\mu g L^{-1}$)	Mean chlorophyll concentration ($\mu g L^{-1}$)	Maximum chlorophyll concentration ($\mu g L^{-1}$)	Mean annual Secchi-disc depth (m)	Minimum annual Secchi-disc depth (m)
Ultra-oligotrophic	≤4.0	≤1.0	≤2.5	≥12.0	≥6.0
Oligotrophic	≤10.0	≤2.5	≤8.0	≥6.0	≥3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypereutrophic	≥100	≥25	≥75	≤1.5	≤0.7

Table 4.4. Trophic category calculated for Lake Garda in 2019

Parameter	Value	Trophic category
mean total phosphorus concentration (µg L^{-1})	10.1	Oligo-mesotrophic
mean chlorophyll-*a* concentration (µg L^{-1})	3.3	Mesotrophic
maximum chlorophyll-*a* concentration (µg L^{-1})	7.02	Oligotrophic
mean annual Secchi-disc depth (m)	8.9	Oligotrophic
minimum annual Secchi-disc depth (m)	5	Oligotrophic

Lake Garda was classified as oligotrophic by three parameters; for mean total phosphorus concentration is oligo-mesotrophic and for mean chlorophyll-a concentration is mesotrophic.

Sampling and Results on cyanotoxins concentrations

Two aliquots of the integrated sample were filtered onto pre-weighed GF/C Filters and the dry-weight was determined from the difference in dried filter (105°C, 24 h) weight before and after filtration. Filters without drying but stored at -20°C were then used for cyanotoxins extraction (protocol: Cyanotoxins analyses in lake and biofilm samples).

Microcystins (MC) were detected in lower concentration throughout the study period (0.1-3.4 ng L^{-1}). The presence of demethylated structural variant (MC-RRdm), is likely produced by *Planktothrix rubescens*. Anatoxin-a was detected every month (2.3-1900 ng L^{-1}) with peaks in spring (April-May) due to the presence of *Thychonema bourrellii*. Nodularins, cylindrospermopsins and saxitoxins were not detected.

Results on comparison between traditional microscopy and HTS

A total of 10 algal groups were recorded under the microscope by traditional morphological analysis (Fig. 4.2). The **algal classes** with the highest biovolume were Coniugatophyceae, Bacillariophyceae, (pennate diatoms plus centric diatoms), Cyanophyceae and Dinophyceae.

Among the Coniugatophyceae, the dominant taxon was *Mougeotia* with a peak in June (4.5 mm^3 L^{-1}).

Among the Bacillariophyceae dominates Pennate diatoms, composed principally by *Fragilaria crotonensis* with a peak in July (1.5 mm3 L^{-1}). Centric diatoms dominate in April with the species *Aulacoseira granulata v. angustissima*. Other species were *Stephanodiscus neoastraea* and *Cyclotella* sp.

Among the Cyanophyceae there was a peak of *Tychonema bourrellyi* in January (0.1 mm^3 L^{-1}), *Planktothrix rubescens* in February (0.08 mm^3 L^{-1}) and *Snowella lacustris* in October (0.08 mm^3 L^{-1}). Among the Chlorophyceae it is reported a peak of *Coenochloris fottii* (*Sphaerocystis schroeteri*) in July (0.3 mm^3 L^{-1}) and among the Chrysophyceae a peak of *Cryptomonas erosa/reflexa* in July (0.09 mm^3 L^{-1}).

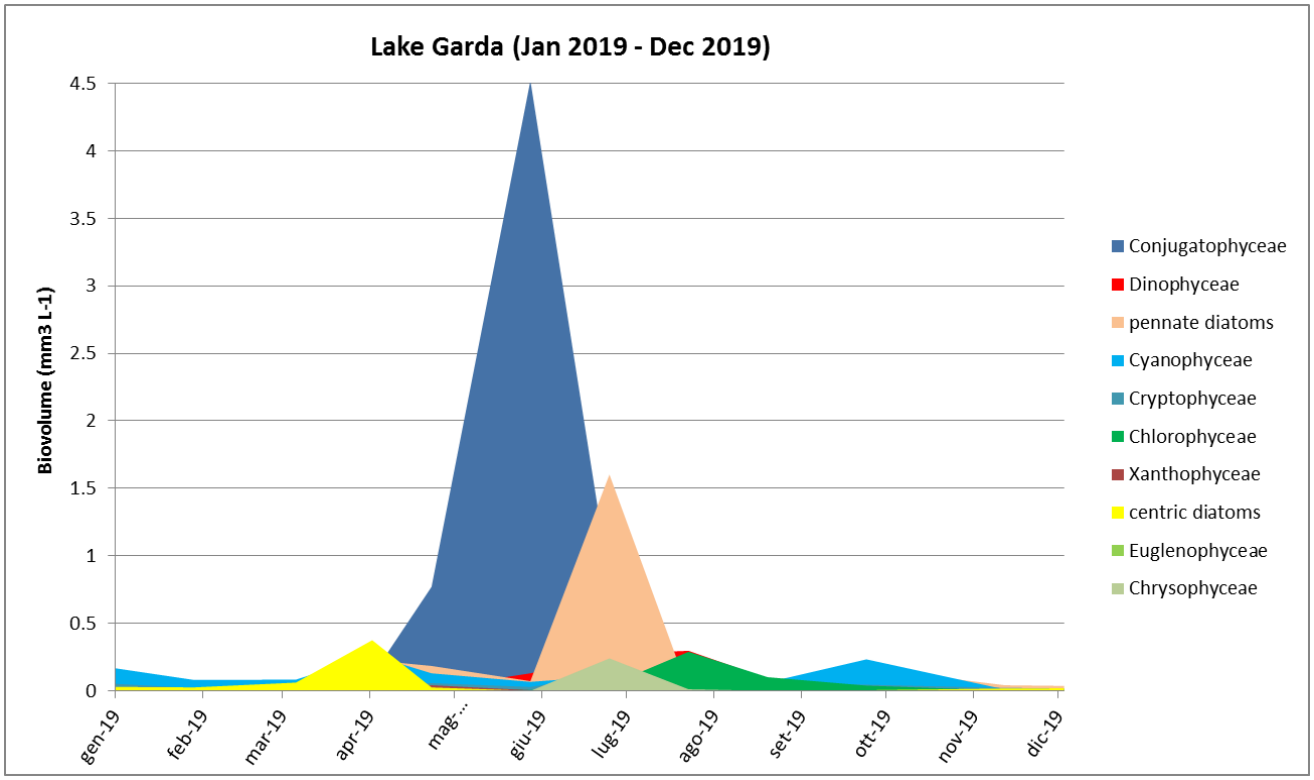

Fig. 4.2. Absolute abundance of phytoplankton biovolume composition as inferred from microscopical analysis (Lake Garda Jan 2019-Dec 2019)

Since the assessment of ecological status classification is based on **phytoplankton species** an important question is, how well the resolution of the modern HTS method works on a species level. The species that could be found through morphological analysis were compared, to see which ones could be identified with the modern method of metabarcoding. Additionally, species which could not be found under the microscope, were also analyzed. For taxonomic precision the REBECCA code was used. In general, nine algal classes were detected using both methods. No one algal classes were detected only by HTS and one algal class were detected only by microscope (Table 4.5).

Furthermore, 32 of the genera/species detected under the microscope were recognized through 16S rDNA or 18S rDNA sequencing (Suppl. Table 4.9 in appendix). This list include abundant cyanobacteria (*Planktothrix, Tychonema*), Bacillariophyceae (*Asterionella formosa, Aulacuseria granulata, Fragilaria* crotonoensis), Chrysophyceae (*Dinobryon divergens, Mallomonas tonsurata*), Dinoflagellates (*Ceratium hirudinella*), Cryptophyceae (*Cryptomonas curvata, Cryptomonas pyrenoidifera*), and Chlorophyta (*Coelastrum reticulatum, Tetraselmis cordiformis*).

On the other hand, species not recognized through HTS were mainly included among the centric diatoms, (i.e. genera *Cyclotella* and *Stephanodiscus*) and also small cryptophyte taxa (*Plagioselmis*, *Cryptomonas*), Conjugatophyceae (*Closterium*, *Staurastrum*) (Suppl. Table 4.10 in appendix).

A number of taxa which were not detected under the microscope were identified through HTS, i.e. several ASVs associated to Chrysophyceae, Prymnesiophyceae. The signals of *Synechococcus* and *Cyanobium* were marked. These nanoplankton taxa may have been recorded as other cyanobacteria such as *Cyanodiction* or *Aphanothece*, or may have been overlooked by microscopy (Suppl. Table 4.11 in appendix).

Table 4.5. Algal classes in Lake Garda detected using the two different methods (microscopic analysis vs sequence analysis) or detected only by one method.

Taxa identified by both methods	Taxa identified only through HTS	Taxa identified only through microscope
Bacillariophyceae		Klebsormidiophyceae
Chlorophyceae		
Chrysophyceae		
Conjugatophyceae		
Cryptophyceae		
Cyanophyceae		
Dinophyceae		
Euglenophyceae		
Xanthophyceae		

Conclusion on results obtained for phytoplankton

Relevant information derived from sequencing includes:

(i) Good match between microscopy and HTS for assignment to class level

(ii) Overall sufficient qualitative relationship between HTS derived genera and microscopy derived genera

(iii) Relationship between HTS derived species and microscopy derived species have to be more elaborated: some species are present at microscopy results with confidence but not in HTS results; furthermore some species are not detectable with HTS and others were recorded with different nomenclature.

(iv) Additional information on some groups of algae, which have not been well recorded before, i.e. picocyanobacterial and eukaryotic flagellates (Chrysophyceae and Dinophyta)

(v) Additional (biogeographic) information on presence/absence of nuisance algae, i.e. *Planktothrix rubescens*/*agardh*ii, *Tychonema bourellyi*, *Microcystis aeruginosa*

(vi) HTS taxa detected with low reads abundances, have a low probability to be observed under the microscope, because phytoplankton reference method for microscope analysis request the identification of algae of a sub-sample (two optical transect in 25 ml of sedimented sample), compared to the DNA that is extracted from 1 L of water.

4.2 Biofilm composition (littoral), L. Garda

Italy, (PP1, FEM, PP3, ARPAV)

Sampling

Phytobenthos has proven to be an indicator for ecological quality status in lakes.In Italy diatoms are used as biological quality elements in lakes for water quality assessment. The other biofilm groups such as cyanobacteria or benthic green algae (so-called soft algae or non-diatoms) are not used.

Thus, for this project, the guidelines from the national legislative on sampling in rivers and lakes have been adapted and applied along with the protocol developed in WP1 (D.T1.3.1-2, Lake biofilms sampling protocol).

There are 10 different sampling locations which are regularly monitored for Lake Garda (Fig. 4.1) and that were sampled for the project on 3 July and 19-20 August 2019. For each site, 5 stones were collected and the biofilm were brushed off from stones from a representative surface area (> 100 cm^2) using a clean tray. Diatoms were identified and counted by their silicate frustules after mounting in Naphrax for microscopical analysis. In parallel, samples for microscopy analysis and for DNA extraction from the same stones aliquots were collected and preserved using 80% Ethanol as described in protocol (DT1.1.2. -2, Lake biofilms sampling protocol).

Rules to define ecological classes and reference conditions

The WFD requires monitoring of macrophytes and phytobenthos (including diatoms) for the assessment of the ecological quality of lakes. The classification of lakes from diatoms is based on EPI-L index based on the recorded species and the attribution of trophic weights of the found species. The definition of reference values and class limits for the index EPI-L are reported in "Indici per la valutazione della qualità ecologica dei laghi" 2013. **Report CNR ISE, 02-13**: 195 pp. (Versione 2018)

DNA extraction and sequencing

DNA was extracted using the Machery and Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (DT1.1.2. -7, DNA extraction biofilms) and done by the project partner FEM (IT).
From sample DNA extracts 16S rDNA (V3-V4 region) has been amplified using primers 341Fmod CCTAYGGGRBGCASCAG and 806Rmod GGACTACNNGGGTATCTAAT under the following conditions: 95°C (5 min), 28 cycles including 95°C (30 sec), 55°C (30 sec), 72°C (30 sec), and final 72°C (5 min). For 18S rDNA (V4 region) the primers V4F-18S_ILL and CCAGCASCYGCGGTAATTCC and V4R-18S_ILL ACTTTCGTTCTTGATYRATGA were applied using the cycling conditions from above. One technical replicate was sequenced (DT1.1.2. -10 Library prep 16S marker gene; DT1.1.2. -11, Library prep 18S marker gene).
PCR amplification and library preparation of purified PCR products for rbcL was performed according to WP1 protocol (DT1.1.2. -9, Library prep RbcL marker gene). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions.

Bioinformatic processing

The raw sequence data were processed using the package DADA2, (Protocols DT1.1.3. - 3 Bioinf18S, Bioinformatics treatment 18S marker gene; DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene). Sequences were assigned using the SILVA SSU reference database (bacteria/cyanobacteria) and the PR2 database (protists/microalgae).
For selected ASVs, automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates and manual BLAST against ASvs.
For rbcL the raw sequence data were processed using the package DADA2, (D.T1.3.2-1 BioinfRbcL, Bioinformatics treatment rbCL marker gene).

Treatment of traditional microscopy data

Taxa lists obtained by microscope counting have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the VALID code system for diatoms in phytobenthos (LfU) and the REBECCA code for non-diatoms (soft algae) An Excel Access database tool (version 7, July 2021) for all microscopical taxa and the VALID codes assigned has been prepared (LfU, FEM, LFUI).

Sampling and Results on cyanotoxins concentrations

In the same sampling area, two aliquots were scratched from the stones and filtered onto pre-weighed GF/C Filters and the dry-weight was determined from the difference in dried filter (105°C, 24 h) weight before and after filtration. Filters without drying but stored at -20°C were then used for cyanotoxin extraction (protocol: Cyanotoxins analyses in lake and biofilm samples).

Microcystins, nodularins, cylindrospermopsins and saxitoxins were not present. HomoAnatoxin-a was detected in Gola (7.8 µg/g dw), Riva (1.2 µg/g dw) and Cisano stations (1.2 µg/g dw) and Anatoxin-a in Cisano station (0.069 µg/g dw).

Results on comparison between traditional microscopy and HTS

Soft algae

There are no soft algae counts by microscopy, due to the Italian normative, so there are no results to compare. Anyway, the HTS cyanobacteria results are very interesting with 49 different taxa found, see in detail in Suppl. Table 4.12 in the appendix. The records of *the anatoxin-producing Tychonema* at several locations, still all with extreme low signals, are very notable and listed in Table 4.6. By the modern HTS method were identified 19 algal classes (Table 4.7).

Table 4.6. List of Tychonema sp. from Lake Garda littoral samples identified using HTS

station_DB_EAW	Taxon_REBECCA	ASV_seq	signal_16S_reref	species_16S
01_PACENGO	Tychonema sp.	Seq10544	5	NA
02_CISANO	Tychonema sp.	Seq587	17	NA
03_CASSONE	Tychonema sp.	Seq6515	1	NA
04_RIVA	Tychonema sp.	Seq3470	47	NA
04_RIVA	Tychonema sp.	Seq6515	10	NA
04_RIVA	Tychonema sp.	Seq16474	9	NA
04_RIVA	Tychonema sp.	Seq2790	4	NA
05_GOLA	Tychonema sp.	Seq587	48	NA
07_GARDONE	Tychonema sp.	Seq3665	23	NA
08_MONIGA	Tychonema sp.	Seq1150	12	NA
09_SIRMIONE 2	Tychonema sp.	Seq587	13	NA
10_SIRMIONE 1	Tychonema sp.	Seq587	208	NA
10_SIRMIONE 1	Tychonema sp.	Seq262	6	NA

Table 4.7. List of algal classes from Lake Garda littoral samples identified using HTS

Algal classes (16S and 18S rDNA)		
Bacillariophyta	Cryptophyceae	Stylonematophyceae
Bangiophyceae	Cyanobacteriia	Trebouxiophyceae
Bicoecea	Dinophyceae	Ulvophyceae
Charophyceae	Eustigmatophyceae	Xanthophyceae
Chlorophyceae	Pavlovophyceae	Zygnemophyceae
Chrysophyceae	Perkinsida	
Coleochaetophyceae	Phaeophyceae	

Benthic diatoms

In the biofilm samples of Lake Garda were detected in total 121 diatom species by light microscopy and 88 taxa, identified on genera or species level, were found by rcbL with HTS. Both methods shared 37 species (see Suppl. Tables in appendix) and 84 taxa were detected only at the microscope (Suppl. Table 4.14 in appendix), whereas 93 taxa were detected only by HTS (Suppl. Table 15 in appendix).

It can be observed that the most abundant taxa in HTS are at the genus level, while at the microscope they have been identified at species level, as required by Directive, therefore the comparison is incomplete. The average percentage of taxa identified at the microscope and not detectable in HTS is 46%, due mainly to the increased taxonomy updates, which pushes up to the level of variants.

From microscopic counting, considering the relative abundance, the dominant species are *Achnanthidium minutissimum* deteected in 9 sites, *Amphora pediculus and Fragilaria capucina var. perminuta* identified in 8 sites, *Encyonopsis minuta* and *Navicula cryptotenelloides* in 6 sites. (Fig. 4.3)

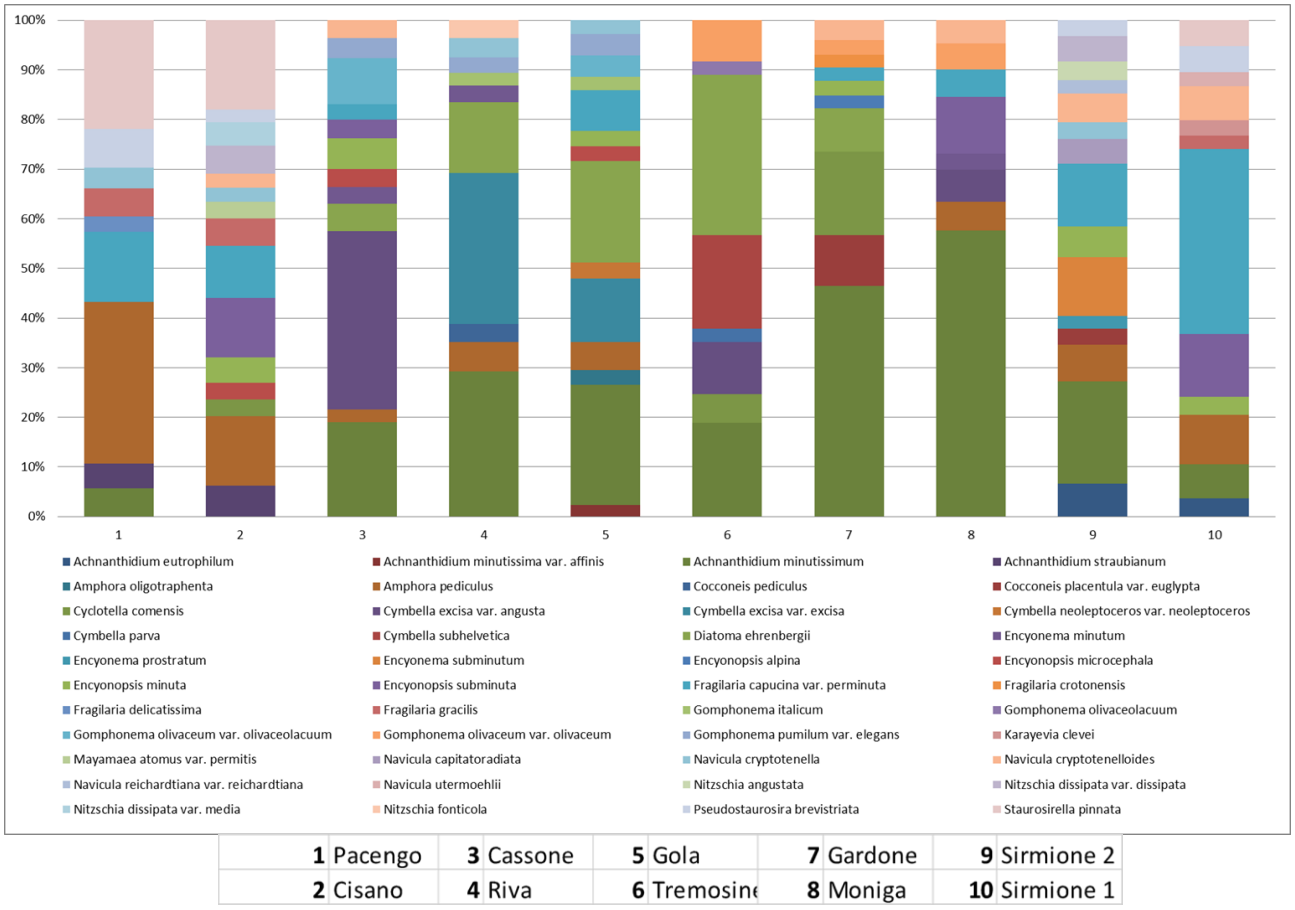

Fig. 4.3. Relative abundance of diatoms (> 2 %) at 10 littoral sampling sites from Lake Garda as revealed from microscopical counting (for location of sites see Fig. 4.1).

Conclusion on results obtained for phytobenthos (cyanobacteria & diatoms)

Relevant information derived from sequencing includes the following:
(i) Good match between microscopy and HTS for assignment to class level

(ii) Low match between microscopy and HTS at the species level though the dominant species were identified in both methods
(iii) Some species with high confidence of identification are not present in HTS results and others were recorded with more detailed nomenclature in LM
(iv) Correspondence between microscopy and rbcL or 18S rDNA sequencing is considered useful to confirm microscope based identification of genera
(v) The diatom taxonomy is constantly evolving with the subdivision of many species into subspecies on the basis of morphological characters. This detail in the classification is often not matched by the HTS.
(vi) The 16S rDNA sequencing information is useful to infer the toxigenic potential of the respective biofilm community. Presence of anatoxin-a have been reported in three sites characterized by the presence of *Tychonema*
(vii) taxa with low HTS reads abundances have a low probability to be observed under the microscope because the LM reference method is based on the identification of 400 diatoms per slide.

(viii) The absence of species determined by light microscopy in the HTS results can be due to the absence of the species in the taxonomic reference database or to microscopy misidentification or absence of corresponding sequences in HTS due to sequencing failure.

4.3 Fish composition, L. Garda

Italy (PP1, FEM; PP3, ARPAV)

Sampling

Water samples for eDNA fish identification in Lake Garda were collected on 9 October 2019 along 4 shoreline transect and in three pelagic points with integrated samples (Fig. 4.4)

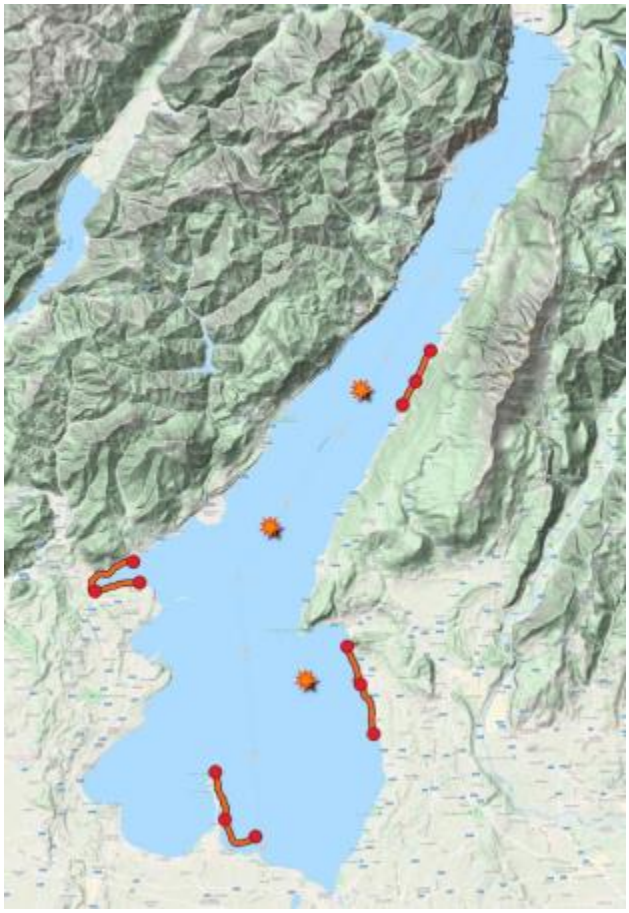

Fig. 4.4. Spatial distribution of shoreline transects and pelagic eDNA samples (eDNA), during the 9 October 2019 sampling campaigns.

Along each shoreline transect were collected 30L of water with a peristaltic pump and filtered on VigiDNA® 0.45 µm capsule. For each shoreline transect were also collected 2 L water samples at the start, the middle and the end of the transect and filtered on a Sterivex filter cartridge (0.45 µm).
Pelagic sampling was also carried out in the deepest part of the lake with integrated depth samples, in three different areas for a total final volume of 30L and filtered on VigiDNA® 0.45 µm capsule. In each location 2 L subsample were collected and filtered on a 0.45 µm Sterivex filter.
Fish eDNA samples were then preserved in buffer according to the Eco-Alpswater protocol D.T1.3.1-4 Lake and river eDNA Fish sampling.

DNA extraction and sequencing

Fish DNA extractions were performed using the Macherey-Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (D.T1.3.1-8.2, Fish DNA extraction from VigiDNA cartridges).
PCR amplification and library preparation were performed according to WP1 protocol (D.T1.3.1-12, Library preparation 12S) and using the fish specific MiFish-U primers (Miya et al., 2015). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions. Nine PCR replicates were performed for each fish eDNA sample.

Bioinformatic processing

Fish eDNA bioinformatic processing was performed using D.T1.3.2-4 Bioinf_12S protocol from WP1. The protocol uses the OBITOOLS3 software (Boyer et al., 2016,) for the processing of raw high-throughput sequencing reads from the MiSeq platform.

Comparison with fish monitoring

The final output of the eDNA analyses is a tab-delimited table with taxonomic inventories, which is comparable to the species inventories collected during the fishing campaigns with pelagic and benthic nets and electrofishing set in 2015 (Volta et al., 2018).

Results on comparison between traditional monitoring and HTS

18 genera/species were identified by HTS and 31% (9) are common with net fishing (Volta et al., 2018) and 9 genera/species (31%) were identified only by HTS and 11 (38%) by net fishing (Fig. 4.5). The eDNA approach seems to be efficient in determining fish species in lakes (Table 4.8).

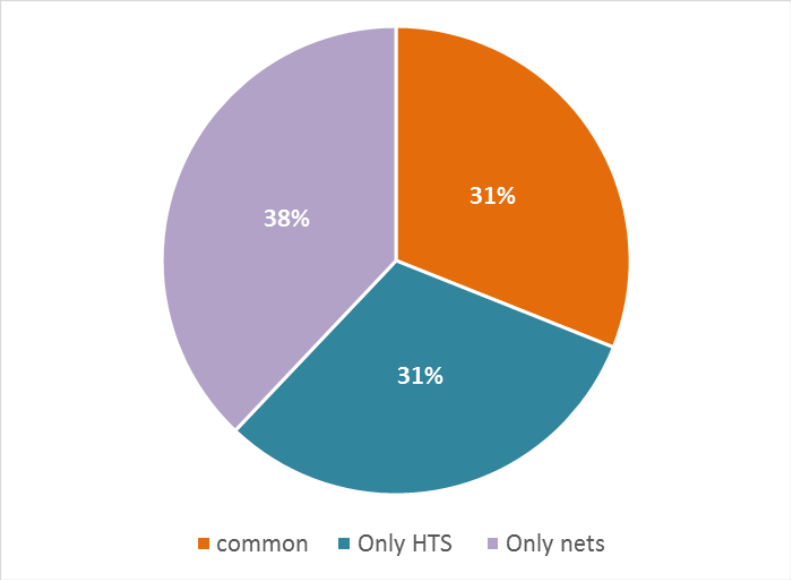

Fig. 4.5. Percentage of fish species identified by metabarcoding and nets (common) (9), only nets and electrofishing (only nets) (11), or only by metabarcoding (only HTS) (9), for eDNA fish monitoring in lake Garda

Table 4.8. Comparison of fish taxa detected using the two different methods (nets vs eDNA sequence analysis) or detected only by one or the other method for fish monitoring in lake Garda. eDNA results are expressed in numbers of reads and for nets and electrofishing results in numbers of fish.

Both methods			
Common Name	**Scientific Name**	**eDNA**	**Volta et al.,2018**
Shad	*Alosa agone* (Scopoli, 1786)	112440	423
Bullhead	*Cottus gobio* (Linnaeus, 1758)	2132	3
Burbot	*Lota lota* (Linnaeus, 1758)	1791	6
Perch	*Perca fluviatilis* (Linnaeus, 1758)	112800	1008
Chub	*Squalius squalus* (Bonaparte, 1837)	84333	84
Black bullhead	*Ameiurus melas* (Rafinesque, 1820)	20	1
European whitefish	*Coregonus lavaretus* (Linnaeus, 1758)	63096	131
Pumpkinseed	*Lepomis gibbosus* (Linnaeus, 1758)	12687	160
Largemouth bass	*Micropterus salmoides* (Lacepède, 1802)	3983	2

Only nets			
Common Name	**Scientific Name**	**eDNA**	**Volta et al.,2018**
Bleak	*Alburnus arborella* (Bonaparte, 1841)		53
Crucian carp	*Carassius carassius* (Linnaeus, 1758)		35
Carp	*Cyprinus carpio* (Linnaeus, 1758)		1
South-alpine Pike	*Esox cisalpinus* (Bianco and Delmastro, 2011)		2
Freshwater blenny	*Salaria fluviatilis* (Asso, 1801)		111
Triotto	*Rutilus aula* (Bonaparte, 1841)		654
Trout	*Salmo* spp.		3
Italian italian rudd	*Scardinius hesperidicus* (Bonaparte, 1845)		238
Riffle dace	*Telestes muticellus* (Bonaparte, 1837)		1
Topmouth gudgeon	*Pseudorasbora parva* (Nichols, 1925)		21
Bitterling	*Rhodeus amarus* (Bloch, 1782)		40

Only eDNA			
Common Name	**Scientific Name**	**eDNA**	**Volta et al.,2018**
Padanian goby	*Padogobius bonelli* (Bonaparte, 1846)	428	
Eel	*Anguilla Anguilla* Linnaeus 1758	4784	
American Eel	*Anguilla rostrata*	1822	
	Rutilus sp.	2658	
	Cyprinidae	13272	
	Leuciscus sp	214873	
	Salvelinus sp.	8712	
Tench	*Tinca tinca* (Linnaeus, 1758)	63358	
Rainbow trout	*Oncorhynchus mykiss* (Walbaum, 1792)	28170	

Conclusion on results obtained for fish

Relevant information derived from sequencing includes the following:

(i) Good match between nets and HTS for fish inventories with 46% of species in common keeping in account that net fish collection was performed in 2015.

(ii) eDNA metabarcoding data of freshwater fish is able to describe the fish community and can integrate current traditional surveys to provide a more comprehensive description of ichthyofauna diversity in the Alpine region.

5. Slovenia, Lake Bled

5.1 Phytoplankton (incl. cyanobacteria), L. Bled

Slovenia (PP5, ARSO)

Špela Remec – Rekar

Sampling according to the national legislation

In Slovenia the Lake Bled was chosen for this assessment as the pilot lake. Samples has been taken monthly from January to December 2019, given together 12 samples.

For the ecological status assessment, phytoplankton samples were depth-integrated from 0-20 m, corresponding to the euphotic zone at the deepest part of the lake (Fig. 5.1). Sample aliquots were used to determine the chlorophyll-a concentration as well as chemical parameters and nutrients following the Slovene legislation (Decree on surface water status, OG RS, 10/09, 98/10, 96/13, 24/16) and national methodologies (Methodology for the ecological status assessment with phytoplankton, Methodology for the Ecological status assessment with supporting physical and chemical quality elements). Sample analysis were conducted by the laboratories at Slovenian environment Agency (PP5).

The application of the Slovene method for lake trophic state assessment based on phytoplankton, requires qualitative and quantitative sampling from the water body for species composition determination and abundance evaluation. The abundance and the total biovolume of the planktonic algae were determined from a subsample under the inverted microscope (quantitative analysis).

For the year of study, the mean chlorophyll-a concentration was determined and the mean biovolume for each taxa was taken as the arithmetic mean of four or more dates. The total phytoplankton biovolume was calculated from the sum of the individual taxa. The Brettum index was calculated from the relative proportions of the mean biovolumes of the individual taxa and taxa-specific trophic scores.

The ecological status assessment is a classification of the nutrient or production level of the lakes. The parameters used in the assessment included the chlorophyll-a concentration (annual mean), the total biovolume (annual mean) and the brettum index (which was calculated from the taxa list and the corresponding biovolumes in the annual mean).

In parallel water chemistry was determined according to the national legislative. Another water volume was filtered for cyanotoxin extraction according to protocol (Cyanotoxins analyses in lake and biofilm samples).

Fig. 5.1. Sampling site for phytoplankton (left) and sampling sites for littoral (biofilm) (right) at Lake Bled, Slovenia

For DNA sequencing, the depth-integrated samples were taken in parallel. DNA filtration was carried out at the boat with a plastic syringe manually through the Sterile Vented Filter Unit, Sterivex^{TM}-GP 0.22 µm (Millipore, Billerica, Massachusetts, USA), following the protocol from WP1 (D.T1.1.2 -1 Lake plankton sampling). Filter units were transported using cooling boxes to the laboratory of PP4 (NIB) where further e-DNA analyses, extraction and sequencing was carried out.

DNA extraction and sequencing

DNA was extracted using the DNeasy® PowerWater® SterivexTM Kit (Qiagen, Hilden, Germany) following the manufacturer`s protocol. (D.T1.1.2. -6 Plankton DNA extraction). DNA extracts were sent to FEM, where the regions 16S rDNA (V3-V4 region) and 18S rDNA (V4 region) were sequenced.

Bioinformatic processing

The raw sequence data were processed by partners at FEM and INRA for all target organisms.

Lake Bled overall trophic state

On the basis of the mean annual total phosphorus (TP) concentration (µg L^{-1}), mean annual chlorophyll-*a* (Chl-a) concentration (µg L^{-1}), maximum annual chlorophyll-*a* concentration (µg L^{-1}), mean annual Secchi-disk depth (m) and minimum annual Secchi-disc depth (m) the trophic state was adjusted using the OECD Fixed Boundary Trophic Classification System (OECD, 1982) (Table 5.1). During 2019 Lake Bled had an average TP concentration of 11.8 (min, max = 9.,5 – 13.7) µg/L, a mean chl-a concentration of 5.7 (2.2- 9.5) µg/L and a mean secchi depth of 5.8 (3.5-9.0) m and is thus assigned a mesotrophic state. Annual EQR value was 0,58.

Table 5.1. OECD Fixed Boundary Trophic Classification System (OECD 1982)

Trophic category	Mean phosphorus concentration (μgL^{-1})	Mean chlorophyll concentration (μgL^{-1})	Maximum chlorophyll concentration (μgL^{-1})	Mean annual Secchi-disc depth (m)	Minimum annual Secchi-disc depth (m)
Ultra-oligotrophic	≤4.0	≤1.0	≤2.5	≥12.0	≥6.0
Oligotrophic	≤10.0	≤2.5	≤8.0	≥6.0	≥3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypereutrophic	≥100	≥25	≥75	≤1.5	≤0.7

Results on cyanotoxins concentrations

Different types of microcystins (MC) were detected throughout the whole study period (5.4 and 660.5ng/L). The higher share of demethylated structural variants such as MC-RRdm, MC-HtyRdm, MC-LRdm were likely produced by ***Planktothrix rubescens*** which represented 76% of the total cyanobacteral e_DNA in 2019 (Fig. 5.2).

No other cyanotoxins (anatoxin-a, cylindrospermopsins or saxitoxins) were detected in plankton.

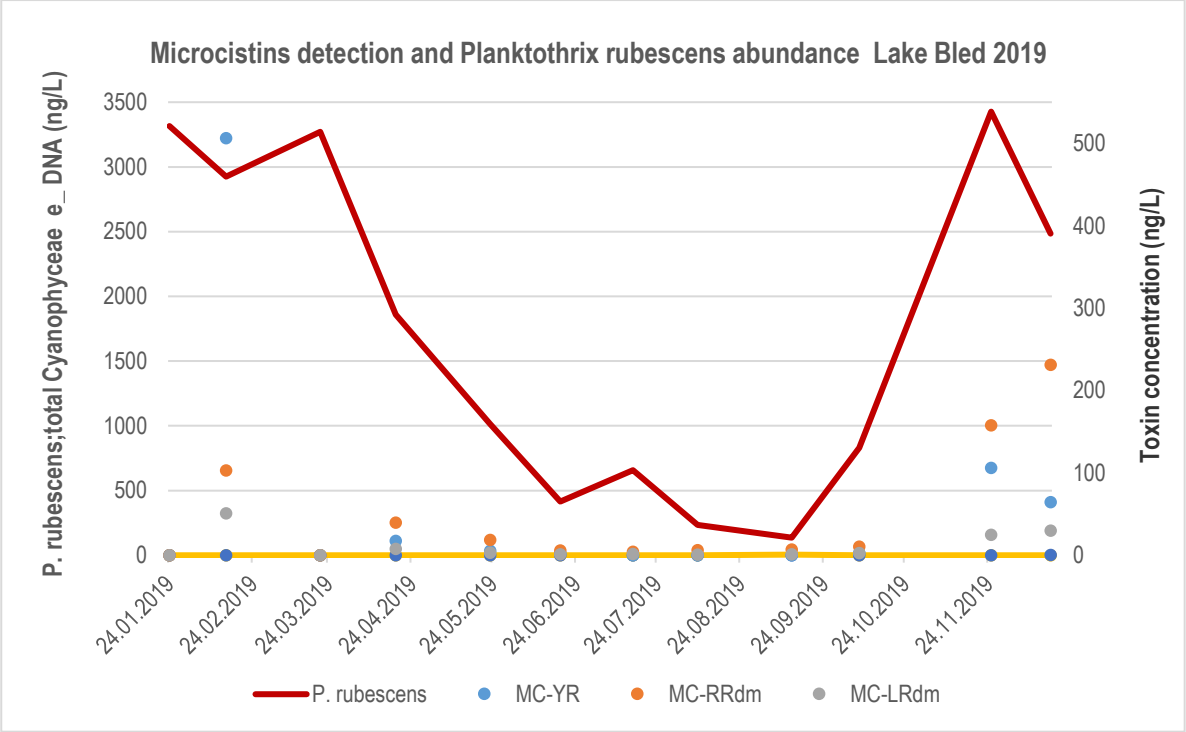

Fig. 5.2. Microcystins (MC) detection in the Lake Bled during the observed period

Comparison with traditional microscopy

The microscopical taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the REBECCA code for phytoplankton. To facilitate comparison an Excel Access database tool (version 6, May 2021) for all microscopical taxa and REBECCA codes assigned has been prepared (LfU, FEM, LFUI).

Results on comparison between traditional microscopy and HTS

In general, 12 algal classes were detected using both methods. After updating the Rebecca phytoplankton species list with the new taxonomic classification based on HTS and phylogenetics analyses from the last decade, Lake Bled phytoplankton detected by traditional microscopic analyses in 2019 ranked into 14 algal classes. Four algal classes Mamiellophycea, Bolidophyceae, Dictyochophyceae and Katablepharidaceae were not detected under the microscope but only through metabarcoding by HTS. Two algal classes Zygnematophyceae and Euglenophyceae, which were detected under the microscope were not identified by HTS (Table 5.2).

Table 5.2. Comparison of detected algal taxa at class level for the Lake Bled using two different methods (microscopical analysis and e_DNA sequence analysis)

	Taxa identified by both Methods	**Taxa identified only through HTS**	**Taxa identified only through microscope**
1	*Cyanobacteriia*	*Mamiellophyceae*	*Euglenophyceae*
2	*Chlorodendrophyceae*	*Katablepharidaceae*	*Zygnematophyceae*
3	*Chlorophyceae*	*Bolidophyceae*	
4	*Trebouxiophyceae*	*Dictyochophyceae*	
5	*Cryptophyceae*		
6	*Dinophyceae*		
7	*Chrysophyceae*		
8	*Bacillariophyta*		

9	Synurophyceae		
10	Eustigmatophyceae (Xantophyceae)		
11	Prymnesiophyceae (Haptophyta)		
12	Bicosoecophyceae		

With both HTS (38 %) and traditional (49%) method, Cyanobacteria with the dominant species *Planktothrix rubescens* prevailed in the Lake Bled phytoplankton community in 2019 especially during the spring and autumn mixing period. *Planktothrix rubescens* a typical representative of R (ruderals) phytoplankton life strategy association (sensu Reynolds et al. 2002) dominated in the Lake Bled phytoplankton community from November 2018 to April 2019 and again in November and December 2019. With both analyses the same occurrence pattern, but les massive was noticed for penate (*Asterionella, Fragillaria, Ulnaria*) and centric diatoms (*Cyclotella, Stephanodiscus*) also all R strategists.

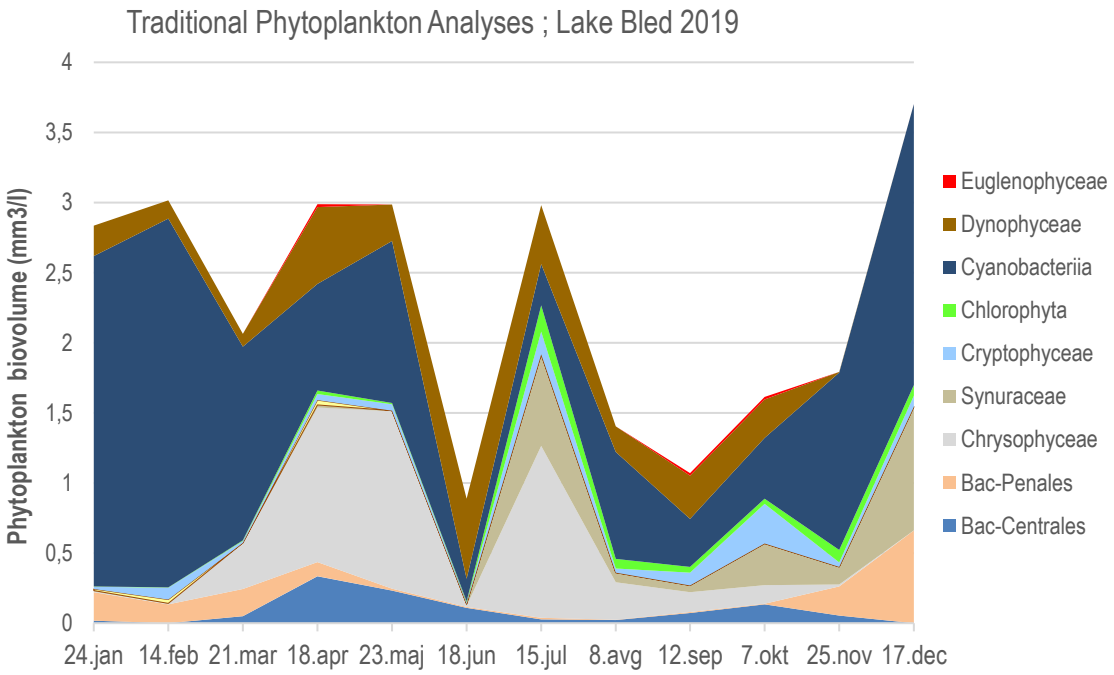

Fig. 5.3. Absolute abundance of phytoplankton biovolume composition as inferred from microscopical analysis (Lake Bled Jan 2019-Dec 2019)

Already in March quick increasing growth of competitors C life strategists (sensu Reynolds 1984) has been noticed mainly Chrysophytes (*Dinobryon, Uroglena*), and with HTS technique also Cryptophytes, wich seems to be underestimated with traditional phytoplankton biovolume determination.

With both methods the period of the lowest phytoplankton quantity in the year 2019, was noticted during June and July, after spring species declination. Specific physical and chemical conditions in the Lake Bled during the stable summer stratification after June suits more to big, relatively slow Dinoflagellates (*Peridinium, Ceratium, Gymnodinion*), Synuraceae (*Mallomonas*), Cryptophyceae and larger green algae (Chlorophyceae, Trebouxiophyceae) which became more abundant during the second half of the year 2019.

Assessment of the ecological status with phytoplankton based on phytoplankton species. Species found through LM morphological analysis were compared with species identified by the modern method of metabarcoding. Additionally, species which could not be found under the microscope, were also analyzed. For taxonomic precision the REBECCA code was used.

Results from the Lake Bled (Suppl. Table 5.1) show thatmore than 30 species detected under the microscope (90) were recognized also through 16S rDNA or 18S rDNA sequencing. This listed included abundant

Cyanobacteria (*Planktothrix, Aphanizomenon, Anabaena*), Bacillariophyceae with Araphid-pennate (Asterionella, Ulnaria, Fragilaria) and also Polar-centric-Mediophyceae (Stephanodiscus) determined only to genus, Chrysophyceae and Synuraceae with genus *Dinobryon, Ochromonas* and *Mallomonas*, dinoflagellates (*Ceratium, Gymnodinium, Peridinium*), Cryptophyceae (*Cryptomonas, Rhodomonas*), and geen algae in classes Trebouxiophyceae, Chlorophyceae and Chlorodendrophyceae (*Botryococcus, Phacotus, Chlamydomonas, Tetraselmis*) and Bicosoecophyceae (*Bicosoeca*). Together this species accounted for more than 85% of the total phytoplankton biovolume. Together the non-corresponding species accounted for less than 15 % of the total biovolume. With traditional LM analyses some very rare species i.e. Ceratium furcoides R1671, Mallomonas acocomos R1097, were detected only in quality phytoplankton samples, but not found in quantitative analyses. This species were not reported in traditional analyses, but noticed in HTS analyses.

Species not recognized through HTS were mainly included among the centric **diatoms** i.e genus *Cyclotella* and *Stephanodiscus*. Also, among **Chlorophyta** several groups were not recognized i.e. the whole class Zygnematophyceae, Ulvophycea, Oocystaceae among the class Trebouxiophyceae and especially Sphaeropleales – ex Chlorococcales in the class Chlorophyceae where many of taxonomic changes happened during the last decade on the base of phylogenetic investigations.

Among cyanobacteria several genus (*Aphanocapsa, Aphanothece, and Cyanodyctyon*) were not recognized through HTS but only through the microscope in the Lake Bled. The analysis of the cyanobacteria species quantity determined with both methods (Fig. 5.4) indicate that all species in mentioned genus belong to only one very morphological heterogeneous genus Cyanobium, which was detected through the HTS only. In the Suppl. Table 5.3 are listed species which could belong to genus Cyanobium and their photos (Suppl. Fig. 5, 6; 7; 8; 9; 10).

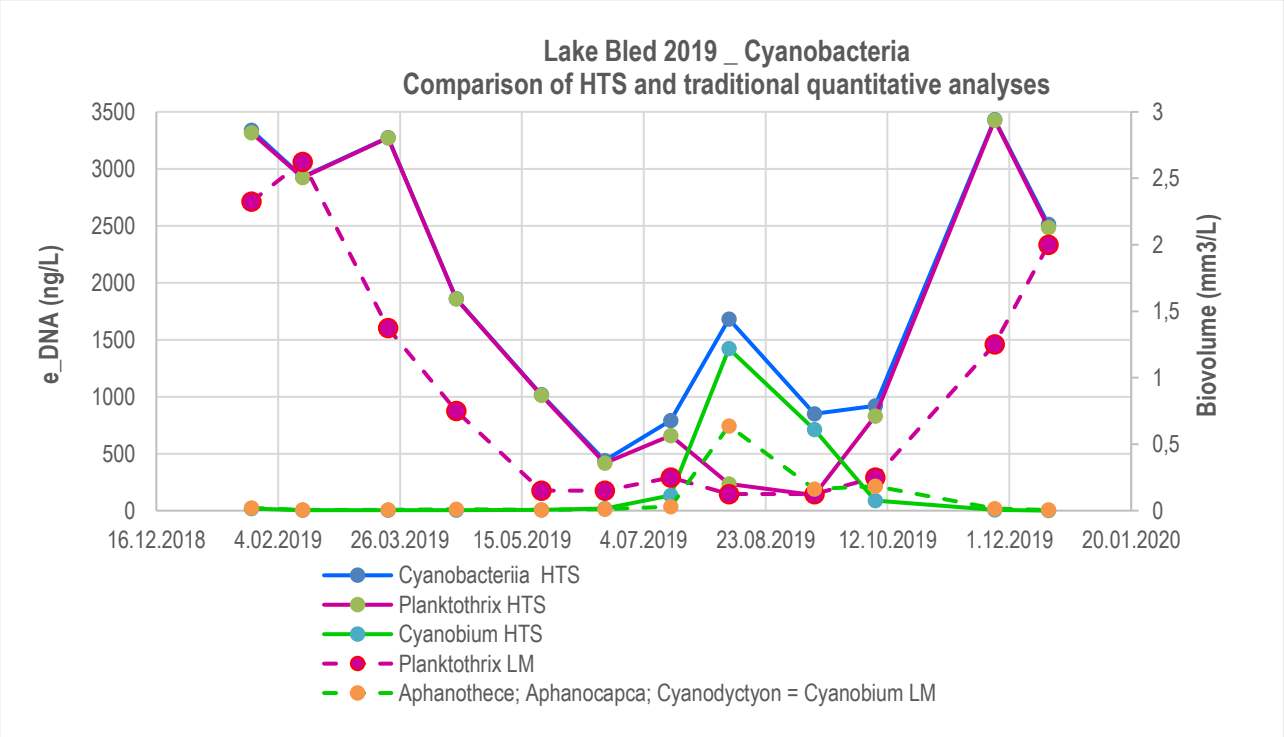

Fig. 5.4. Comparison of the cyanobacteria quantity determined with both, HTS and LM methodology.

On the other hand, some genus and several taxa were not detected under the microscope, but reported through HTS. These species mostly (10) belong to different classes of Chlorophyta i.e. Mamiellophyceae (4), Chlorodendrophyceae (1), Chlorophyceae (3), Trebouxiophyceae (2) and also dynoflagelattes are well presented with 6 new species of Dinophyceae (*Prorocentrum sp., Polarella glacialis, Asulcocephalium miricentonis*, new *Gymnodinium* species , new species in genus Thoracosphaeraceae and unknown genus of

the order Peridiniales). Only with HTS two species among Dictyochophyceae (Ochrophyta) were detected, *Pedinella hexacostata* and *Pseudopedinella* sp.

Eustigmatophyceae – Xantophyta are not very frequent in the phytoplankton community of the Lake Bled but with HTS technic 2 species were detected. One of them is quite new unknown species but another is *Pseudotetraëdriella kamillae* which is morphologically very similar to some *Tetraëdron* species, especially *Tetraëdron platyisthmum* (Chlorophyceae) with only SI code (SI3130) detected under the microscope in the Lake Bled in 2019.

Similar situation is perhaps concerned also to Chrysocapsa sp. (R2679 Chrysophyceae) species determined only with HTS metabarcoding. Under the microscope *Stichogloea globosa* (K. Starmach 1985) with only SI code (SI3235) was registered.

More improved databases would also bring an answer about the species detected under the microscope Planktosphaeria gelatinosa (R0727) and Asterarcys quadricellulare Sphaeropleaceae Gen. sp. R2456 noticed with HTS.

Conclusion on results obtained for phytoplankton

Relevant information derived from sequencing includes

(i) overall good qualitative relationship between HTS derived genera and microscopy derived genera, i.e. sequence based confirmation of microscopy and results on genus level
(ii) additional information on certain groups of algae which have not been well recorded before, i.e. picocyanobacterial and eukaryotic flagellates (Chrysophyceae, Dinophyta, Prasinophyta)
(iii) additional (biogeographic) information on presence/absence of nuisance algae, i.e. *Planktothrix rubescens*/*agardhi*i, Aphanizomenon sp.,
(iv) information on intraspecific genetic variation among populations, i.e. detection of novel genotypes within populations of algal species.

5.2 Biofilm composition (littoral), L. Bled

Slovenia (PP5, ARSO)

Katarina Novak, Urška Hren, Aleksandra Krivograd Klemenčič

Sampling

Phytobenthos has proven to be an indicator of ecological quality status in rivers. In Slovenia, only diatoms (Bacillariophyceae) are used as a biological quality element. For additional information, we also look at other phytobenthic algae groups (including cyanobacteria).

Sampling of phytobentos was performed by the Slovenian Environment Agency (ARSO) according to the standard EN 13946:2014 and national methodology [2] on 30th of August 2019 at 10 different sampling sites at Lake Bled (Fig. 5.1). Each sampling site included several different habitats representative for the water body – a multi habitat sampling. At each sampling site a field datasheet was filled out.

The sampled substarata was transferred into a tub together with little river water, where the phytobenthos was scraped with a toothbrush and poured (after mixing) into a labeled bottle with a wide neck. The sample was preserved with alcohol at a final concentration of ~30% for further lab analysis. Under laboratory conditions, the sample was purified with 65 % nitric acid (HNO_3) and heated over a fire until no more organic matter was present. The permanent slides were prepaired using Naphrax and examined according to standard EN 14407:2014 using a light microscope (Leica Leitz DMRB) equipped with a digital camera (Nikon DS-Fi3). The 500 diatoms' valves were counted in each sample. The abundance of identified taxa was expressed as a percentage. Identification was performed using the identification monographs of Lange-Bertalot et al. (2017)[3] and Krammer and Lange-Bertalot (1986[4], 1988[5], 1991a[6], 1991b[7]).

In parallel water chemistry was determined according to the national legislative. DNA from the same stones as phytobenthos was sampled was extracted and aliquots were preserved using 80% ethanol as described in protocol (DT1.1.2. -2, Lake biofilms sampling protocol).

Finally, aliquots were scratched directly onto pre-weighed GF/C filters. The dry-weight was determined from the difference in dried filter (105°C, 24 h) weight before and after filtration. Aliquots without drying but stored at -20°C were then used for cyanotoxin extraction (protocol: Cyanotoxins analyses in lake and biofilm samples).

For DNA sequencing, the depth-integrated samples were taken in parallel. DNA filtration was carried out at the boat with a plastic syringe manually through the Sterile Vented Filter Unit, Sterivex^{TM}-GP 0.22 µm (Millipore, Billerica, Massachusetts, USA), following the protocol from WP1 (D.T1.1.2 -1 Lake plankton sampling). Filter units were transported using cooling boxes to the laboratory of PP4 (NIB) where further e-DNA analyses, extraction and sequencing was carried out.

DNA extraction and sequencing

DNA was extracted using the Machery and Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (DT1.1.2. -7, DNA extraction biofilms). DNA extracts were sent to FEM and INRA, where the regions 16S rDNA (V3-V4 region), 18S rDNA (V4 region) and rbcL were sequenced.

PCR amplification and library preparation of purified PCR products for rbcL was performed according to WP1 protocol (DT1.1.2. -9, Library prep RbcL marker gene). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions.

Bioinformatic processing

The raw sequence data were processed by partners at FEM and INRA for all target organisms.

Results on cyanotoxins concentrations

Anatoxin-a was detected at 8 out of 10 sampling sites at Lake Bled (33.5 – 103.2 ng/g dry weight). In addition, microcystins were detected at one sampling site (11.0 ng/g dry weight). No other cyanotoxins were detected.

Comparison with traditional microscopy

Taxa lists (microscopy) have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the VALID code system for diatoms in phytobenthos (LfU) and the REBECCA code for non-diatoms (soft algae). An Excel Access database for all microscopical taxa and the VALID codes assigned has been prepared (LfU, FEM, LFUI).

In Slovenia only diatoms (Bacillariophyceae) are used for ecological status assessment according to standard EN 14407:2014 and national methodology. For additional information, also other phytobenthic algae groups (including cyanobacteria) are analysed according to taxa list and relative abundance is estimated using classes from 1 to 5, where 1 is very rare and 5 is dominant.

Results on comparison between traditional microscopy and HTS

Six algal classes were determined in Lake Bled littoral through microscopical observation, belonging to Cyanophyceae, Bacillariophyceae, Chrysophyceae, Chloropyceae, Trebouxiophyceae, and Zygnematophyceae. Via metabarcoding, for Lake Bled littoral 14 algal classes were identified, meaning 8 algal classes more than via microscopy (Table 5.3).

A total of 97 diatom taxa were identified in Lake Bled littoral with microscopy. Species present in all 10 samples were *Achnanthidium minutissimum*, *A. saprophilum*, *Amphora pediculus*, *Cocconeis euglypta*, *Cymbella microcephala*, *Encyonema minutum*, *Navicula cryptotenella*, *Nitzschia dissipata* ssp. *dissipata*, *N.*

fonticola, and *Psammothidium subatomoides*. Among them, *P. subatomoides* (up to 37.8%), *C. microcephala* (up to 41.8%), and *A. pediculus* (up to 41.8%) were the most dominant. As many as a third of the species appeared in only one sampling site, despite Lake Bled being considered a smaller lake. The Bray-Curtis similarity index is between 0.25 and 0.58. The sampling site T10 differs most from the other sampling sites. Ecological status of Lake Bled at all 10 sampling sites was according to biological quality element phytobenthos (diatoms) moderate (TI EQR = 0.40-0.57).

A total of 77 diatom taxa were detected by HTS analysis, of which 24 taxa are also identified by microscopy. Bray-Curtis similarity index showed even bigger differences between the species composition of the sampling sites than microscopy (0.15-0.43). *A. minutissumum*, *Amphora pediculus*, *Cyclotella costei*, *Encyonema caespitosum*, *Encyonopsis subminuta*, *Gyrosigma sciotense*, *Navicula cryptotenella*, *Nitzschia dissipata* var. *dissipata*, *N. fonticola*, *N. pusilla*, *N. palea*, *N. radiosa*, *Pseudostaurosira brevistriata*, and *Staurosira construens* were present at all sample sites. Presence of planktonic taxa such as *Cyclotella costei* and *Discostella pseudostelligera* further increases the difference between the HTS and microscopy, while in microscopy non-benthic cyclic diatoms are not counted according to the national methodology.

A. delmontii, which is considered an invasive species in some European countries, was also identified with microscopy at the T6 sampling site. HTS analyzes did not detect this species.

Because in Slovenia only diatoms (Bacillariophyceae) are used for ecological status assessment the **soft algae** are analysed just for additional information and thus taxa and relative abundance determination is not so reliable. Microscopy of soft algaeshowed the presence of representatives of Cyanophyta (50%), Charophyta (25%), Chlorophyta (17%), and 1 taxa of Chrysophyceae. The most common taxa was *Homoeothrix varians* (T1-T10) and *Oedogonium* sp., which also occurred in all samples but with a lower relative abunsdance (rare to very rare). The highest algae diversity was at sampling sites T3 (7 taxa) and T8 (6 taxa).

For cyanobacteria, 70 taxa were detected through 16S rDNA sequencing - at the level of higher taxonomic groups, 51 genera or 23 families. As for the remaining of soft algae, HTS analyses identified 89 taxa belonging to 50 genera or 36 families. Most of them belong to Chlorophyceae (26 taxa, and Chrysophyceae (17 taxa).

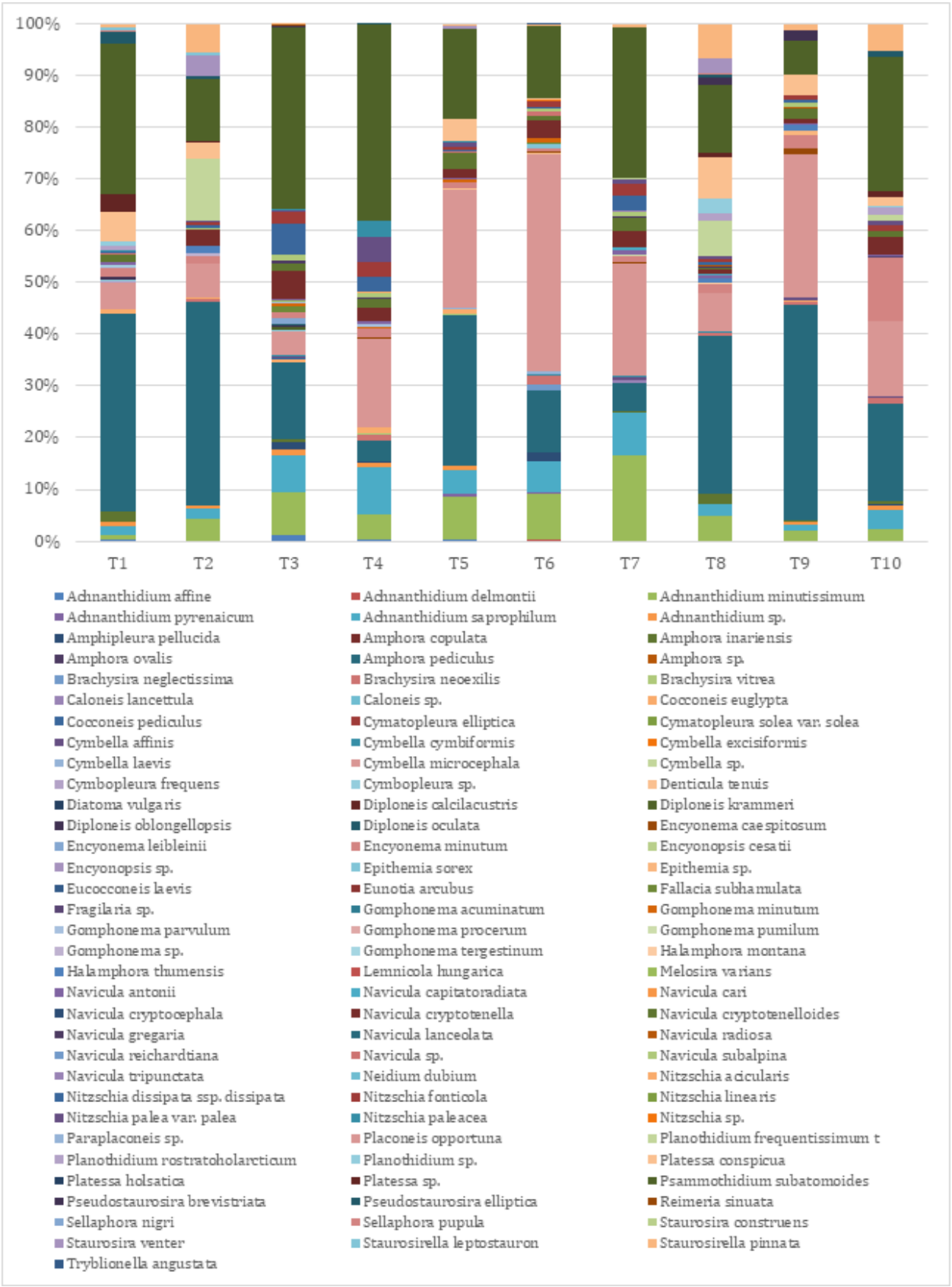

Fig. 5.5. Relative abundance of diatoms at Lake Bled sampling sites (T1-T10) as a result of counting using a light microscope.

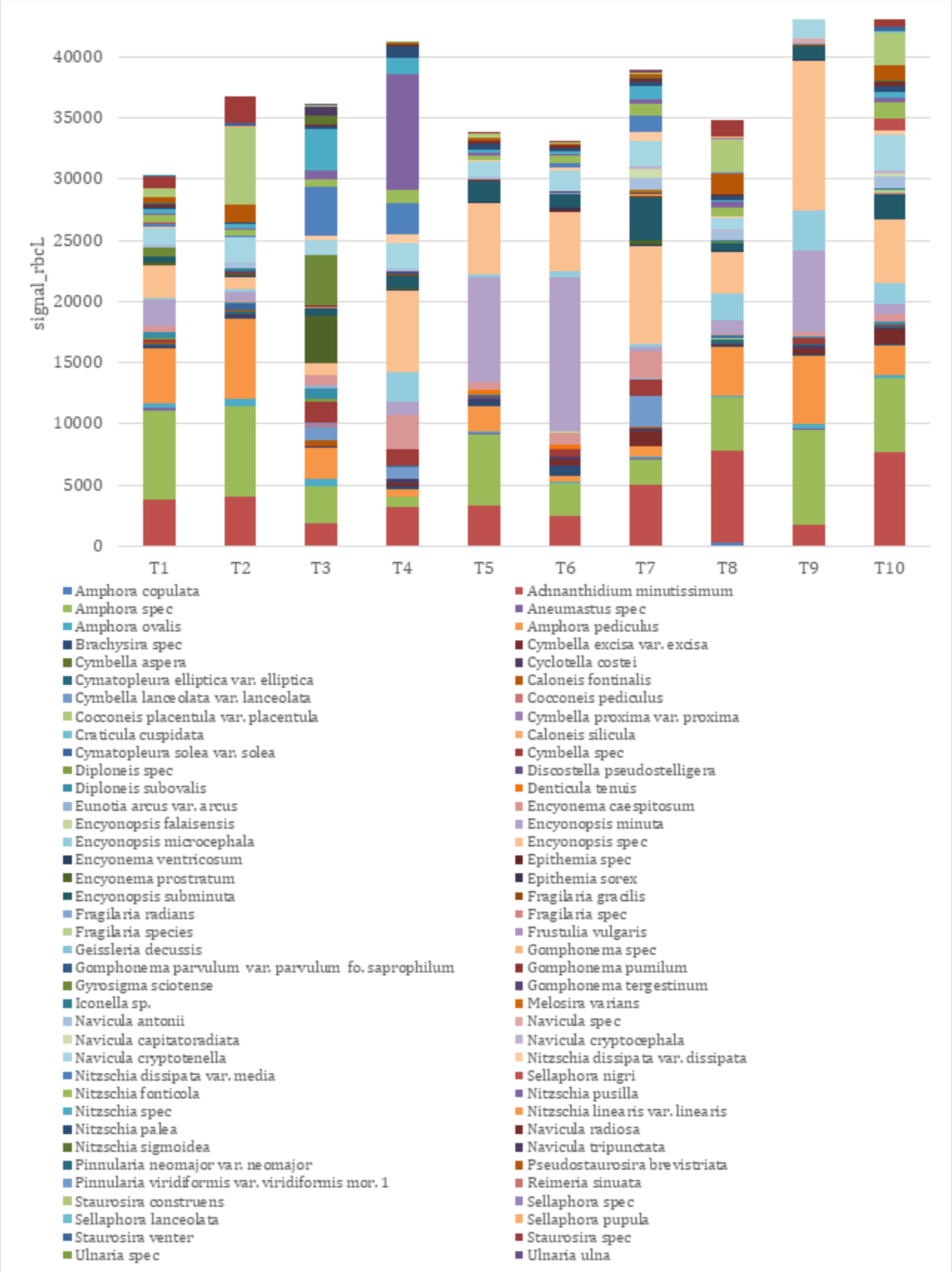

Fig. 5.6. Presence of diatoms according to rcbL signal (HTS analyses) at Lake Bled sampling sites (T1-T10)

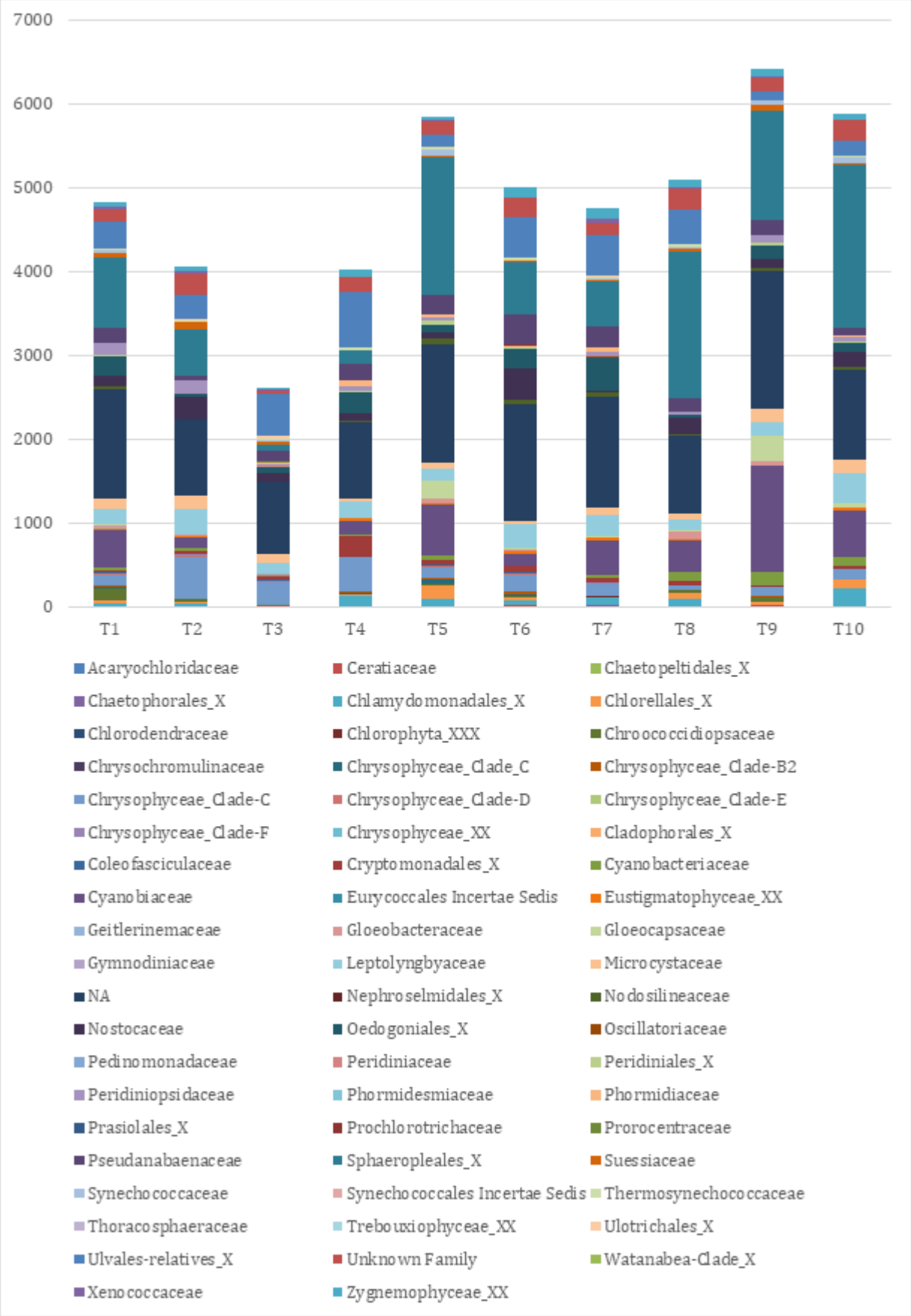

Fig. 5.7. Signal of cyanobacteria and other soft algal groups at Lake Bled sampling sites (T1-T10) as revealed from HTS analysis.

Table 5.3. List of algal classes from Lake Bled littoral samples as identified using both sequencing and microscopical counts.

Algal classes (16S and 18S rDNA) of Lake Bled littoral	Algal classes (microscopy) of Lake Bled littoral
Bacillariophyceae	*Bacillariophyceae*
Chlorodendrophyceae *Chlorophyceae*	
Chlorophyceae	*Chlorophyceae*
Chrysophyceae	*Chrysophyceae*
Cryptophyceae	
Dinophyceae *Eustigmatophyceae*	
Eustigmatophyceae	
Nephroselmidophyceae	
Pedinophyceae	
Prymnesiophyceae	
Trebouxiophyceae	*Trebouxiophyceae*
Ulvophyceae	
Zygnemophyceae	*Zygnemophyceae*

Conclusion on results obtained for phytobenthos (cyanobacteria & diatoms)

Relevant information derived from sequencing includes the following:

(i) HTS analyzes detected more taxa of cyanobacteria and soft algae compared to microscopy, as these analyzes are according to national methodology.
(ii) For diatoms, correspondence between microscopy and rbcL or 18S rDNA sequencing is considered useful to confirm microscope-based identification of species.
(iii) The results between HTS analysis and the traditional method differ significantly, so further studies are needed.

5.3 Fish composition, L. Bled

Špela Remec-Rekar, Katarina Novak (PP5, ARSO)

Sampling

Sampling for fish e_DNA at Lake Bled was carried out on the 12 September 2019, according to the Eco-AlpsWater protocol D.T1.3.1-4 - Lake and river e_DNA Fish sample collection from the field for downstream molecular analysis. Two 30 L samples were collected along two lakeshore transects – north-west (ST3 -) and south_east (ST2) (1.5 ;1,3 km each) and one pelagic sample at the deepest point of the lake (Fig. 5. 8.).

Fig 5.8: Shoreline transects (ST2 1,5 km) and (ST3 1,4 km) and pelagic location (ST1 0,5-28 m) for fish DNA sampling at the Lake Bled in September 12th 2019

Standard sampling: By boat, 30 liters of water were collected along each transect and filtered through the VigiDNA® 0.45 µm filter cartridges using a peristaltic pump. In addition to the shoreline transects, one depth-integrated water sample (30 L, from the water surface to just above the bottom) at the deepest point of the lake 28 m), using an integrating water sampler (Hydrobios IWS III) was collected. After filtration through the VigiDNA® 0.45 µm filter all three cartridges were filled with a preservation buffer and stored in the fridge. For further HTS analyses cartridges were sent to the project partner 6 INRAE, National Institute for Agricultural Research (FR).

DNA extraction and sequencing

DNA extractions performed from 3 to 4 months after sampling (preservation at 4oC in SPYGEN preservation buffer) and according to the DNA extraction protocol from Pont et al. 2018 at the National Institute for Agricultural Research (FR). The Macherey-Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (D.T1.3.1-8.2, Fish DNA extraction from VigiDNA cartridges were used.

Bioinformatic processing

PCR amplification and library preparation were performed according to WP1 protocol (D.T1.3.1-12, Library preparation 12S) and using the fish specific MiFish-U primers (Miya et al., 2015). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions.

Comparison with traditional fish monitoring

The taxonomic inventory obtained from the HTS method were compared to the dataset obtained from the last traditional fish sampling at Lake Bled, which was carried out by the Fisheries Research Instute of Slovenia during the period from the 30th August to 4th September in 2018. The traditional fish sampling in 2018 follows the standard SIST EN 14757:2015 (Water Quality – Sampling of fish with multi-mesh gillnets). The sampling in 2018 comprised pelagic and benthic gillnetting and electrofishing along the shore.

Results on comparison between traditional monitoring and HTS

With the new HTS methodology, a total of 9 species and two taxonomic groups (Cyprynidae and Salmo) were registered in Lake Bled in 2019. Majority of detected fish species (7 i.e. 62%) were the same as were catched with the traditional gillnetting and electrofishing in 2018 (Fig. 5.9.). The fish species composition in the pelagic e_DNA sample (T1) differs a lot from both e_DNA litoral samples (T2, T3). Suprisingly the signal for the roach (Rutilus rutilus), the most frequent species from the traditional net sampling missed, but the signals for pike (Esox lucius) that missed in gillnetting was strong (24%) at T1 (Table 5.4.). Signals for salmonide species prevailing (52%) at T1. Species Thymallus thymalus and Salmo labrax were detected only with HTS. The origin of these signals is most likely the River Radovna - main lake inflow and fishkeeping farm on the lake tributary Mišca. Strong e_DNA signals at all sampled locations (23% of total reads) indicate also a greater presence of species from the family Cyprinidae in the lake Bled. With traditional gillnetting 3 fish species Tinca tinca (1 fish), Scardinius erythrophtalmus (3 fishes) and Cyprynus carpio (1 fish) from the family Cyprinidae were cahaught in 2018. All these species represent only 0,1% of the total number of the caught fish in 2018, which is underestimated.

The most frequent fish caught with gillnetting and electrofishing in the Lake Bled in 2018 was perch (Perca fluviatilis), but the e-DNA signal for this species was very low (2% of the total signals). Quite good comparability with both methods except on location T1 was found for the roach (Rutilus rutilus). Better comparability for more species was found when biomas of the caught fish in parts (%) and number of e_DNA signals in parts (%) were compared (Table.5.5.)

Table 5.4. Comparison of fish taxa detected with traditional and eDNA assessment method. The numbers in the molecular method column shows the total number of reads for each species. The traditional methods columns show the number of individuals caught with different methods (gillnetting, including pelagic and benthic gillnets, and electrofishing).

Common Name	Scientific name	e_DNA (VigiDNA®)					Traditional			
		T1	T2	T3	Total	%	Gillnettin	Electrofishin	Tota	%
Cyprinida	Cyprinidae	5653	17015	75542	14909	23,1	?	?	?	0,00
Common	Cyprynus carpio	0	0	0	0	0,00	1		1	0,02
Perch	Perca fluviatilis	0	2774	11603	14377	2,23	3231	258	3489	55,3
Roach	Rutilus rutilus	0	10315	10320	20636	31,9	2551	209	2760	43,7
Pike	Esox lucius	5696	759	0	57722	8,94	0	1	1	0,02
Salmo	Salmo	5748	1127	13157	71773	11,1	?	?	?	0,00
Black sea	Salmo labrax	2168	0	14368	36052	5,59	0	0	0	0,00
Brown	Salmo trutta	2923	7003	5571	41805	6,48	3	0	3	0,05
Pikeperch	Sander	0	624	31411	32035	4,96	16	0	16	0,25
Wels	Silurus	1072	0	2443	3515	0,54	0	20	20	0,32
Chub	Squalius	0	4861	13788	18649	2,89	10	0	10	0,16
Grayling	Thymallus	1393	0	0	13938	2,16	0	0	0	0,00
Tench	Tinca tinca	0	0	0	0	0,00	1	0	1	0,02
Common	Scardinius	0	0	0	0	0,00	3	0	3	0,05

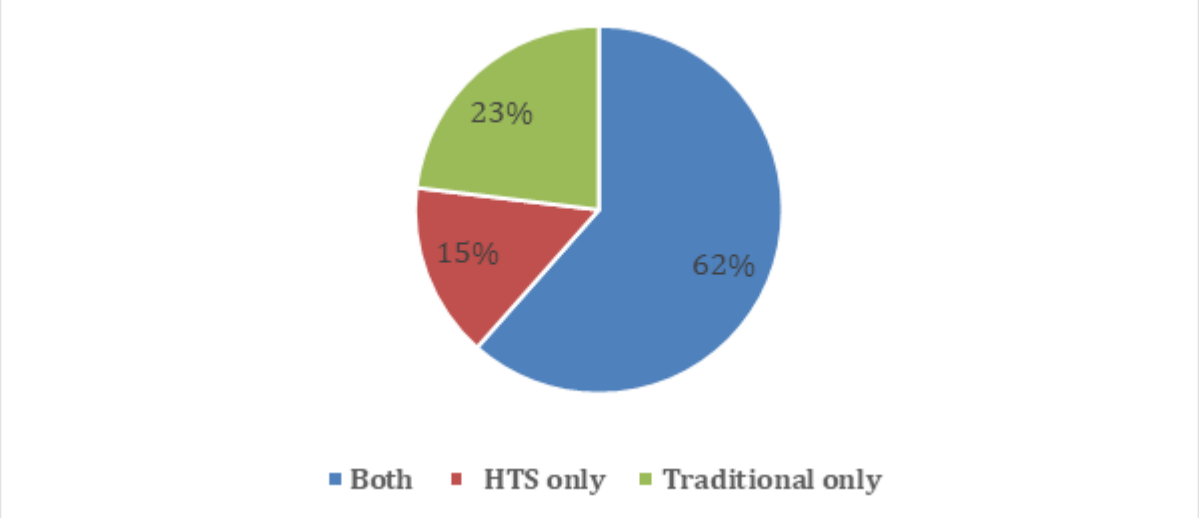

Fig 5.9. Parts of the fish species detected with traditional, molecular (HTS) and both methods in Lake Bled

Table 5.5. Comparability of traditional and e-DNA fish data from the Lake Bled. Biomass of the caught fish in parts (%) and number of e_DNA signals in parts (%) were compared

Scientific and common fish species name	% e_DNA signals	% caught fish biomass
Rutilus rutilus (roach)	32	31
Sander lucioperca (pikeperch)	5	4
Salmo trutta (brown trout)	6	5
Squalius cephalus (chub)	3	4
Silurus glanis (catfish)	1	2
Esox lucius (pike)	9	0
Salmo labrax (black sea trout)	6	0
Salmo	11	0
Thymallus thymallus (grayling)	2	0
Cyprynidae	23	0.2
Perca fluviatilis (perch)	2	53

Conclusion on results obtained for fish

eDNA metabarcoding for fish is a valuable tool to quickly assess the species composition of aquatic ecosystems. There is good (62% common) overlap with the results of the traditional methods although rare species were not detected. In the case of exact investigation of fish communities with the molecular methods in very small lakes like Bled it is necessary to also analyse fish population in the main tributaries.

We agree that the new molecular methods are well suited for studying fish communities in lakes and rivers. The eDNA approach seems to be a cost and time effective complementation to the traditional methods in order to get a more detailed insight on the fish community composition in alpine water bodies.

6. Switzerland, L. Lugano

6.1 Phytoplankton (incl. cyanobacteria), L. Lugano

Switzerland (PP12, SUPSI)

Camilla Capelli, Fabio Lepori, Federica Rotta

Sampling according to national legislative

Lake Lugano is located at the southern edge of the Alps, at the border between Switzerland and Italy, and a limnological monitoring programme, promoted by the international board managing the lake (CIPAIS), has been going on since the '80s. Lake Lugano was chosen as the Swiss pilot lake for this assessment. Samples were taken monthly starting from January in 2019 and until December 2019, given a total of 12 samples.

For the ecological assessment of the lake quality, phytoplankton samples were depth-integrated from 0-20 m corresponding to the euphotic zone at the deepest part of the lake (Fig. 6.1). Sample aliquots were used to determine the chlorophyll-a concentration as well as chemical parameters and nutrients following the CIPAIS programme. Sample analysis was jointly run by SUPSI and the administration of Canton Ticino, Switzerland. In the CIPAIS programme, the abundance and the total biovolume of the phytoplankton were determined from a subsample under the inverted microscope (quantitative analysis). The total phytoplankton biovolume was calculated from the sum of the individual taxa, and a yearly mean biovolume for each phytoplanktonic group was calculated basing on 16 sampling.

Besides phytoplankton biovolume, the ecological status assessment is based on different indicators, namely cyanobacteria percentage on total phytoplankton, and Chlorophyll concentration (annual mean). For water chemistry, nutrient (phosphorus and nitrogen), and oxygen concentration are included as indicators in the ecological status assessment of Lake Lugano.

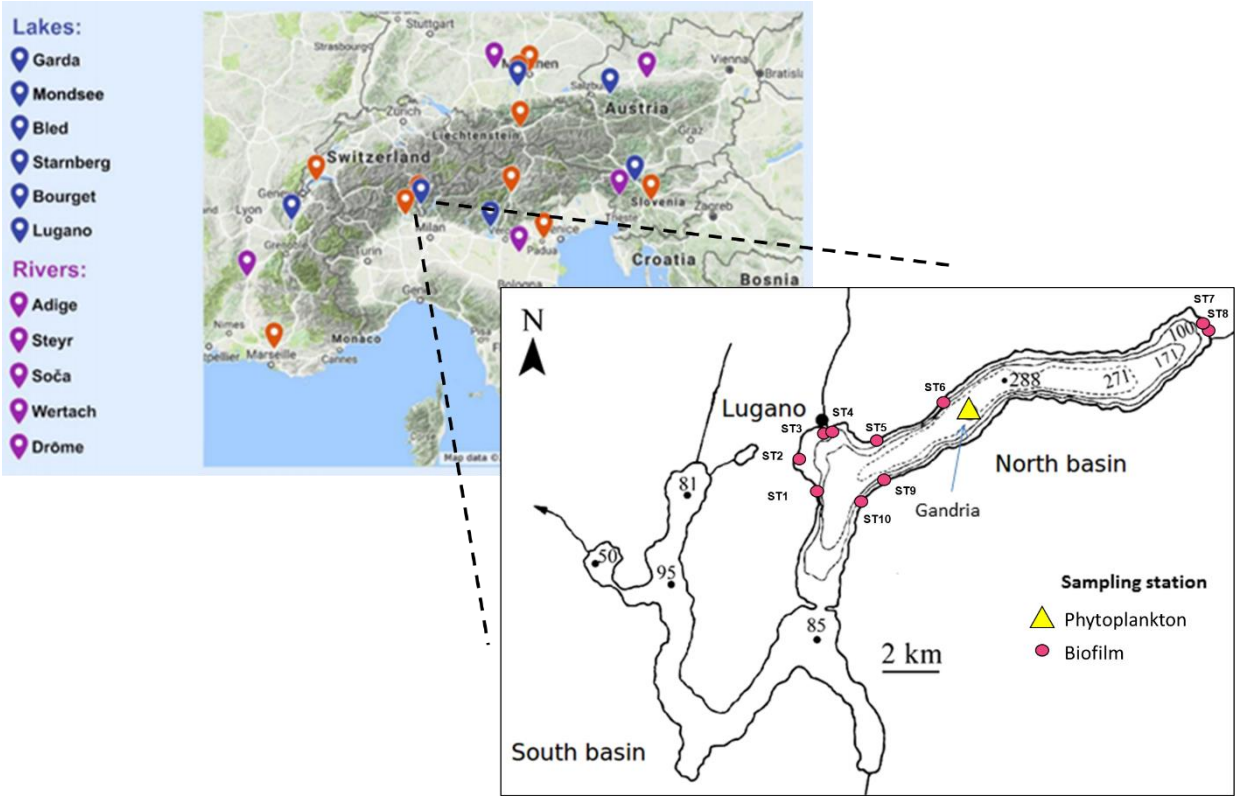

Fig. 6.1. Lake Lugano (CH-IT), sampling site (phytoplankton) and sampling sites littoral (biofilm)

For the EAW project, a subsample was filtered for cyanotoxins, according to protocol (Cyanotoxins analyses in lake and biofilm samples), and a subsample was processed for DNA sequencing following the protocol from WP1 (D.T1.1.2 -1 Lake plankton sampling). In details, in the laboratory the sample was filtered through a Sterivex^{TM}-GP 0.22 µm filter (Millipore, Billerica, Massachusetts, USA), by pressing water manually through the filter unit with a plastic syringe. The filtering was completed until the filter became clogged, and the total volume was recorded.

DNA extraction and sequencing

DNA was extracted using the DNeasy® PowerWater® SterivexTM Kit (Qiagen, Hilden, Germany) following the manufacturer`s protocol (D.T1.1.2. -6 Plankton DNA extraction).

From sample DNA extracts 16S rDNA (V3-V4 region) and 18S rDNA (V4 region) has been amplified and sequenced (Miseq) according to EAW protocols.

Bioinformatic processing

The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2), more details in protocols DT1.1.3. - 3 Bioinf18S, Bioinformatics treatment 18S marker gene; DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene. Sequences were clustered into ASVs (no dissimilarity threshold) and assigned to the SILVA SSU reference database (or PR2 database) for taxonomic classification.

For cyanobacteria automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates (strains) and manual blastn against ASvs allowing 0-1 mismatch (405 bp alignment).

Comparison with traditional microscopy

The microscopical taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the REBECCA code for phytoplankton. To facilitate comparison an Excel Access database tool (version 6, May 2021) for all microscopical taxa and REBECCA codes assigned has been prepared (LfU, FEM, LFUI).

Lake Lugano overall trophic state

On the basis of the mean annual total phosphorus (TP) concentration (µg L^{-1}), mean annual chlorophyll-*a* (Chl-a) concentration (µg L^{-1}), maximum annual chlorophyll-*a* concentration (µg L^{-1}), mean annual Secchi-disk depth (m) and minimum annual Secchi-disc depth (m) the trophic state was adjusted using the OECD Fixed Boundary Trophic Classification System (OECD, 1982) (Table 6.1).

Table 6.1. OECD Fixed Boundary Trophic Classification System (OECD 1982)

Trophic category	Mean phosphorus concentration (μgL^{-1})	Mean chlorophyll concentration (μgL^{-1})	Maximum chlorophyll concentration (μgL^{-1})	Mean annual Secchi-disc depth (m)	Minimum annual Secchi-disc depth (m)
Ultra-oligotrophic	≤4.0	≤1.0	≤2.5	≥12.0	≥6.0
Oligotrophic	≤10.0	≤2.5	≤8.0	≥6.0	≥3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypereutrophic	≥100	≥25	≥75	≤1.5	≤0.7

During 2019 Lake Lugano (North basin) had an average TP concentration (0-100 m) of 23 (min, max=16 – 35) µg/L, a mean Chl-a concentration of 6.3 (3.6-10.4) µg/L and a mean secchi depth of 7.7 (4.5-12.4) m and is thus assigned a mesotrophic state.

Results on cyanotoxins concentrations

Microcystins were detected in low concentration throughout the study period (0.3-42.5 ng/L). The higher share of demethylated structural variants such as MC-RR, MC-HtyR, MC-LR is likely produced by *Planktothrix rubescens*. Anatoxin-a was not detected.

Results on comparison between traditional microscopy and HTS

A total of 12 algal groups were recorded under the microscope by traditional morphological analysis (the most abundant were represented in Fig. 6.2). The **algal classes** with the highest biovolume were Cyanobacteria, Bacillariophyceae and Cryptophyceae. The seasonal development started early with increased growth of large colonial diatoms (*Aulacoseira islandica*), which picked between February and March and then declined due to nutrient depletion. While, cyanobacteria (especially *P. rubescens)* became dominant in the second half of the year and reached the maximum biovolume in autumn.

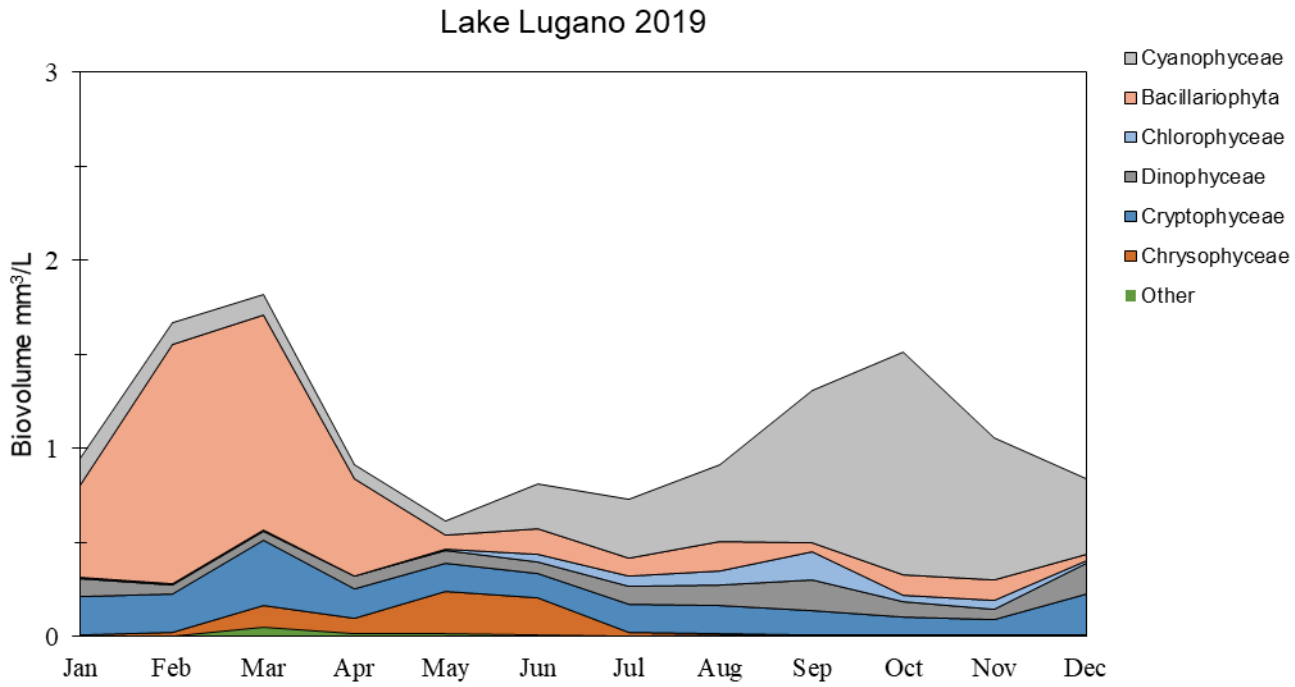

Fig. 6.2. Absolute abundance of phytoplankton biovolume composition as inferred from microscopical analysis (Lake Lugano Jan 2019-Dec 2019)

In general, 10 algal classes were detected using both methods. By HTS 5 algal classes were found through metabarcoding, which were not detected under the microscope (Table 6.2). Two algal classes taxa were not identified by metabarcoding, even though these were found under the microscope.

Table 6.2. Comparison of algal taxa at class level for Lake Lugano detected using the two different methods (microscopical analysis vs sequence analysis) or detected only by one method

Taxa identified by both Methods	Taxa identified only through HTS	Taxa identified only through microscope
Bacillariophyceae	Eustigmatophyceae	Klebsormidiophyceae
Chlorophyceae	Mamiellophyceae	Ulvophyceae
Chrysophyceae	Synurophyceae	
Conjugatophyceae	Trebouxiophyceae	
Cryptophyceae	Zygnemophyceae	
Cyanophyceae		
Dictyochophyceae		
Dinophyceae		
Prymnesiophyceae		
Xanthophyceae		

Since the assessment of ecological status classification is based on **phytoplankton species** an important question is, how well the resolution of the modern HTS method works on a species level. The species that could be found through morphological analysis were compared, to see which ones could be identified with the modern method of metabarcoding. Additionally, species which could not be found under the microscope, were also analyzed. For taxonomic precision the REBECCA code was used.

It can be seen from the results from Lake Lugano (Suppl. Table 6.1) that 44 of the taxa detected under the microscope were recognized through 16S rDNA or 18S rDNA sequencing, however in most of the cases the resolution of HTS reached only the genus level. This listed included abundant, Bacillariophyceae (e.g. *Asterionella, Fragilaria, Stephanodiscus*), Chlorophyta (e.g. *Coelastrum*), Chrysophyceae (*Dinobryon, Mallomonas*), Conjugatophyceae (e.g. *Staurastrum*), Cryptophyceae (e.g. *Cryptomonas*), Cyanobacteria (e.g. *Planktothrix, Aphanizomenon*), Dinophyceae (e.g. *Ceratium, Gymnodinium*), and Haptophyta (e.g. *Chrysochromulina*).

On the other hand, 55 species were not recognized through HTS (Suppl. Table 6.2). They were mainly included among Chlorophyta (e.g. *Pandorina, Kirchneriella*) and diatoms (e.g. *Cyclotella*)

A number of taxa which were not detected under the microscope were identified through HTS (e.g. *Synechococcus* and *Cyanobium* (Cyanobacteria) and Trebouxiophyceae, however most of them are connected to discrepancies in taxonomic attribution and to a low taxonomic resolution of HTS (Suppl. Table 6.3).

Conclusion on results obtained for phytoplankton

Relevant information derived from sequencing includes

(i) overall good qualitative relationship between HTS derived genera and microscopy derived genera, i.e. sequence based confirmation of microscopy results on genus level

(ii) additional information on certain groups of algae which have not been well recorded before, e.g. picocyanobacteria.

6.2 Biofilm composition (littoral), L. Lugano

Switzerland (PP12, SUPSI)

Camilla Capelli, Fabio Lepori, Federica Rotta

Sampling

Benthic diatoms, and phytobenthos in general, are not used as an indicator for ecological quality status in lakes in Switzerland. Therefore, for the EAW project, the identification of benthic diatoms by microscopy were specifically carried out as external service by a private company. Since no national legislative on littoral (biofilm) sampling in lakes is available, for the EAW project was applied the protocol developed in WP1 (DT1.1.2. -2, Lake biofilms sampling protocol) for both microscopy and genetic analysis.

In Lake Lugano, biofilm samples were collected in 10 stations, between 20 September and 23 October 2019 (Fig. 6.1). The sampling sites were distributed along the shoreline to represent both the more and the less polluted areas. For each site 5-8 stones were selected along the shoreline representing an area of 50-100 m^2 and the biofilm was brushed off from stones from a representative surface area (> 100 cm^2) using a clean tray. A subsample was farther fixed in formaldehyde and sent to AquaPlus (Zug, CH) for diatoms identification and counting.

In parallel to sampling for microscopy, for DNA extraction from the same stones an aliquot was preserved using 80% Ethanol as described in protocol (DT1.1.2. -2, Lake biofilms sampling protocol).

Finally, two subsamples were scratched directly onto pre-weighed GF/C Filters. The first one was used to measure the dry-weight from the difference in dried filter (105°C, 24 h) weight before and after filtration. The second subsample was stored at -20°C and then used for cyanotoxin extraction (protocol: Cyanotoxins analyses in lake and biofilm samples).

Results on cyanotoxins concentrations

Out of 10 sampling sites, the most abundant toxin identified was homoAnatoxin-a, detected at 6 sites with a range of 0.03-22.46 ng/mg DW. The highest values were measured in sites 1 and 10. Anatoxin-a was detected at 2 sites at low concentrations (< 0.1 ng/mg DW). Microcystins were detected at very low concentrations in 3 sites (< 0.01 ng/mg DW).

DNA extraction and sequencing

DNA was extracted using the Machery and Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (DT1.1.2. -7, DNA extraction biofilms)

From sample DNA extracts 16S rDNA (V3-V4 region) has been amplified using primers 341Fmod CCTAYGGGRBGCASCAG and 806Rmod GGACTACNNGGGTATCTAAT under the following conditions: 95°C (5 min), 28 cycles including 95°C (30 sec), 55°C (30 sec), 72°C (30 sec), and final 72°C (5 min). One technical replicate was sequenced (DT1.1.2. -10 Library prep 16S marker gene).

PCR amplification and library preparation of purified PCR products for rbcL was performed according to WP1 protocol (DT1.1.2. -9, Library prep RbcL marker gene). Bridge amplification and sequencing by synthesis were performed according to Miseq standard conditions.

Bioinformatic processing

The raw sequence data were processed using the package Divisive Amplicon Denoising Algorithm 2 (DADA2), (DT1.1.3. - 1 BioinfRbcL, Bioinformatics treatment RbCL marker gene; DT1.1.3. -2 Bioinf16S, Bioinformatics treatment 16S marker gene).

For cyanobacteria automated taxa assignment was improved by using reference sequences from relevant taxonomic literature, using (morphologically described) isolates (strains) and manual blastn against ASvs allowing 0-1 mismatch (405 bp alignment).

Sequences were clustered into ASVs (no dissimilarity threshold) and assigned to the SILVA SSU reference database (or PR2 database?) for taxonomic classification. For rbcL gene assignment to diatom taxa the curated database R-Syst::diatom (Rimet et al. 2016) was used (INRA).

Comparison with traditional microscopy

All microscopical taxa lists have been standardized using the established WFD (EU project WISER) taxa codes, i.e. the VALID code system for diatoms in phytobenthos (LfU) and the REBECCA code for non-diatoms (soft algae) An Excel Access database for all microscopical taxa and the VALID codes assigned has been prepared (LfU, FEM, LFUI).

Microscopical countings were performed according to the national method for rivers, developed by the the Federal Office for the Environment (Méthode d'analyse et d'appréciation des cours d'eau en Suisse, Diatomées, niveau R region, FOEN, 2007).

Results on comparison between traditional microscopy and HTS

Benthic diatom composition at the ten sites were analysed through both microscopy and metabarcoding. The most abundant diatoms, as revealed by microscopical counting, belong to the genera *Achnanthidium*, *Encyonopsis, Navicula*, and *Nitzschia* (Fig. 6.3). Two sites (4, 8), selected at the mouth of rivers Cassarate and Cuccio, differed more in compositions compared to the others.

Comparing results obtained through microscopy and HTS, 34 species were detected by both methods, while a larger number of taxa detected under the microscope were not identified through HTS (88) and vice versa (68) (Suppl. Table 6.5-7). The discrepancy between the microscopy and HTS in species identification is likely due to an incomplete coverage of species in the reference databases and a misclassification.

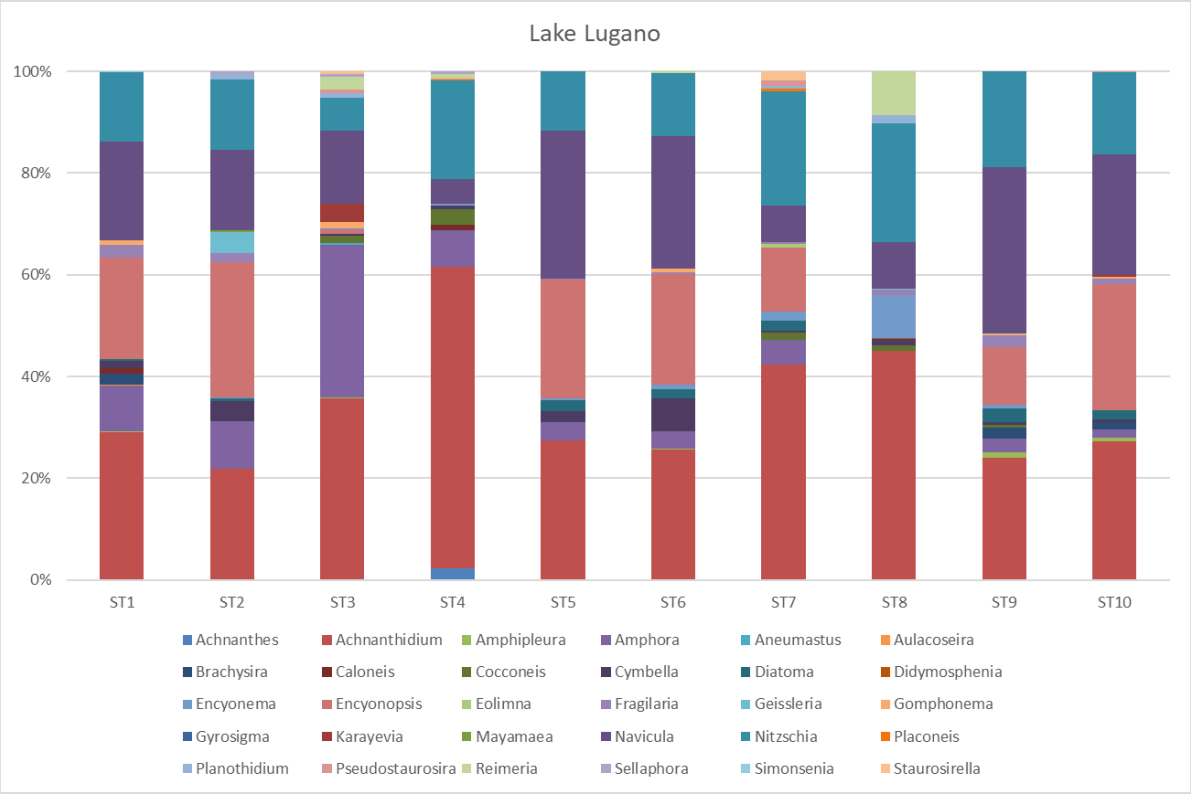

Fig. 6.3. Relative abundance of diatoms at ten littoral sampling sites from Lake Lugano as revealed from microscopical counting (for location of sites see Fig. 6.1).

The HTS approach was also used for the identification of cyanobacteria in biofilms. At the 10 sites, were identified 43 taxa, mainly represented by filamentous genera (Suppl. Table 6.4), e.g. *Geitlerinema, Leptolyngbya, Oscillatoria, Phormidium*, and *Tychonema*. Some of them are anatoxin producing species,

therefore they could be responsible for homoAnatoxin-a presence in littoral samples of Lake Lugano. Some planktonic taxa (e.g. *Aphanizomenon, Planktothrix*, and *Microcystis*) were also detected.

Conclusion on results obtained for biofilm (cyanobacteria & diatoms)

Relevant information derived from sequencing includes the following:

(i) Overall good qualitative relationship between HTS and microscopy, especially at genus level

For cyanobacteria, the HTS was useful for studying an unknown ecosystem in Lake Lugano and to infer the toxigenic potential of the biofilm community.

6.3 Fish composition, L. Lugano

Camilla Capelli, Fabio Lepori (SUPSI)

Sampling

Water samples for eDNA fish identification in Lake Lugano were collected on 26 November 2019 along 3 shoreline transect and in 3 pelagic points with integrated samples (Fig. 4.4)

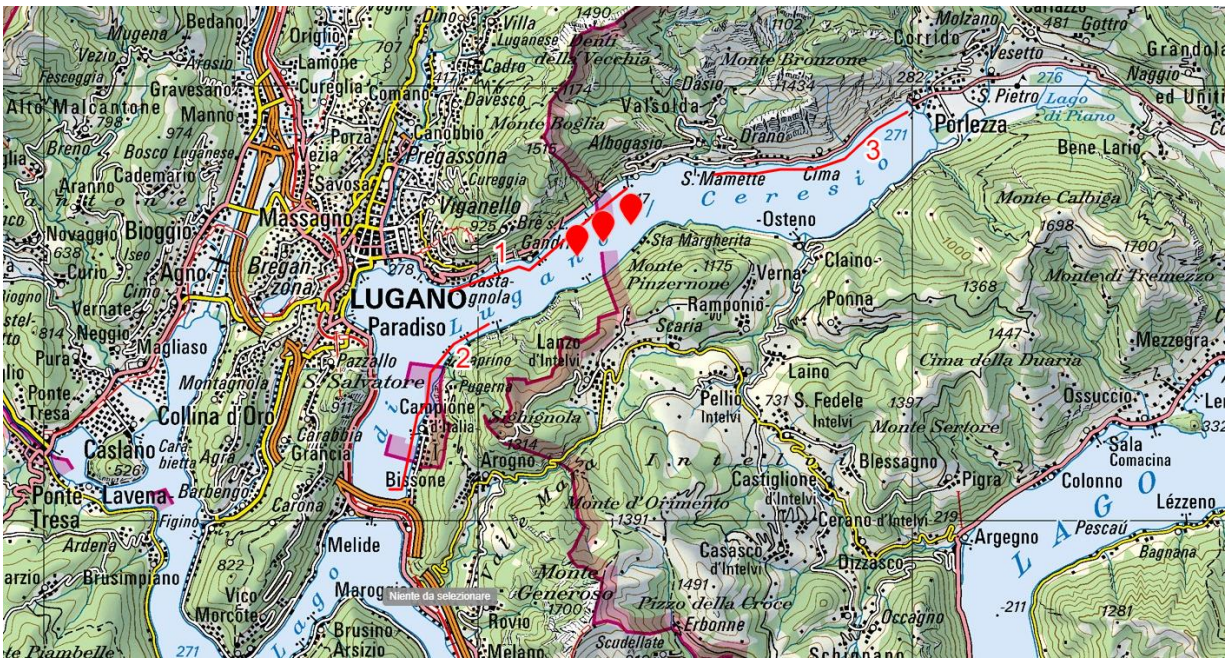

Fig. 6.4. Spatial distribution of the 3 shoreline transects and 3 pelagic eDNA samples (eDNA), during the 26 November 2019 sampling campaign.

Along each shoreline transect (6 km) were collected 32-39L of water with a peristaltic pump and filtered on VigiDNA® 0.45 µm capsule. zelagic sampling was also carried out in the deepest part of the lake with integrated depth samples (0-75 m), in three different areas for a total final volume of 32.5L and filtered on VigiDNA® 0.45 µm capsule. Fish eDNA samples were then preserved in buffer according to the Eco-Alpswater protocol D.T1.3.1-4 Lake and river eDNA Fish sampling.

DNA extraction and sequencing

Fish DNA extractions were performed using the Macherey-Nagel NucleoSpin® Soil kitDNeasy® following the WP1 protocol (D.T1.3.1-8.2, Fish DNA extraction from VigiDNA cartridges).
PCR amplification and library preparation were performed according to WP1 protocol (D.T1.3.1-12, Library preparation 12S) and using the fish specific MiFish-U primers (Miya et al., 2015). Bridge amplification and

sequencing by synthesis were performed according to Miseq standard conditions. Nine PCR replicates were performed for each fish eDNA sample.

Bioinformatic processing

Fish eDNA bioinformatic processing was performed using D.T1.3.2-4 Bioinf_12S protocol from WP1. The protocol uses the OBITOOLS3 software (Boyer et al., 2016,) for the processing of raw high-throughput sequencing reads from the MiSeq platform.

Comparison with fish monitoring

The final output of the eDNA analyses is a tab-delimited table with taxonomic inventories, which is comparable to the species inventories collected during the previous fishing survey (Project Lac) with pelagic and benthic nets and electrofishing set in 2011, which has involved more than 200 fishing activities (EAWAG 2014).

Results on comparison between traditional monitoring and HTS

In total (Table 1, Fig. 6.5) 10 fish genera/species were detected by HTS during the EAW sampling campaign. In comparison to Project Lac (2011), in which traditional methods were applied, 6 fish taxa (25%) were identified only by HTS, 14 taxa (58%) were identified only by traditional methods, and 4 taxa (17%) were detected by both methods.
The eDNA approach seems to be efficient in determining fish species in lakes (Table 4.8).

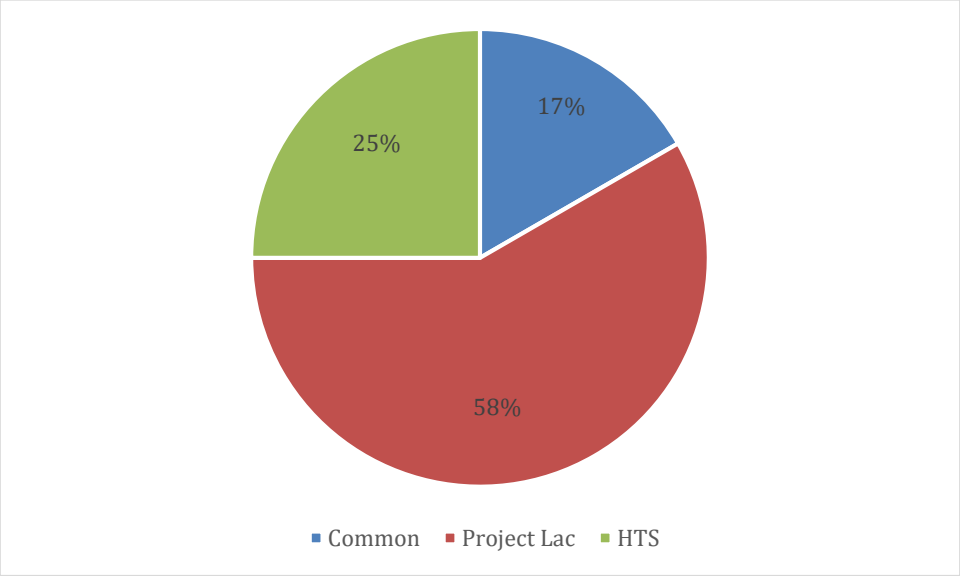

Fig. 6.5. Percentage of fish species identified only by HTS in eDNA fish monitoring in Lake Lugano, only by traditional survey in Project Lac, and by both methods (common).

Conclusion on results obtained for fish

The eDNA metabarcoding for fish is a valuable tool to quickly assess the species composition of aquatic ecosystems. Considering the different effort in eDNA sampling and traditional monitoring used in Project Lac (6 vs. 200 activities), and the time between the two samping campaigns (8 years), there is a good overlap in the taxa list. The main differences are represented by the level of resolution (species/genus) in the identification (e.g. *salmo, rutilus*), and by the detection of less common species.

Therefore, the eDNA metabarcoding is able to describe the fish community and can integrate current traditional surveys to provide a more comprehensive description of fish diversity in lakes.

Table 6.3. Comparison of fish taxa detected using the two different methods, traditional (Project Lac) vs eDNA (HTS), or detected only by one or the other method for fish monitoring in lake Garda. eDNA results are expressed in numbers of reads and for traditional method in numbers of fish.

Both methods		
Scientific name	**HTS**	**Project Lac**
Perca fluviatilis	307441	3210
Lepomis gibbosus	13441	8
Coregonus lavaretus	29262	6
Tinca tinca	5608	5
Traditional method		
Scientific name	**Project Lac**	
Rutilus sp	253	
Sander_lucioperca	50	
Lota_lota	8	
Esox_lucius	6	
Salvelinus_umbia	1	
Micropterus_salmoides	3	
Squalius_squalus	6	
Alburnus_alborella	1	
Alburnus_sp	1	
Alosa _agone	1	
Scardinius_hesperidicus	1	
Telestes_muticellus	19	
Salmo_sp	7	
Salaria_fluviatilis	3	
eDNA method		
Scientific name	**HTS**	
Oncorhynchus sp.	49411	
Padogobius martensii	7600	
Rutilus rutilus	190011	
Oncorhynchus mykiss	10416	
Salmo trutta	18494	
Squalius cephalus	32034	

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8. Appendix (Suppl. Tables)

8.1 L. Mondsee, Austria

*Suppl Table 1.1. List of **corresponding phytoplankton** species identified through microscopy and through HTS (SILVA reference database) from Mondsee **pelagic** samples (n=13).*

Locus	LM_phytoplankton	ID-REBECCA	HTS_18S+16S	class
16S	Planktothrix		Planktothrix	Cyanobacteria
16S	Aphanizomenon		Aphanizomenon	Cyanobacteria
16S	Snowella		Snowella	Cyanobacteria
18S	Asterionella formosa	R0135	Asterionella formosa	Bacillariophyta
18S	Aulacoseira islandica	R0025	Aulacoseira subarctica	Bacillariophyta
18S	Aulacoseira subarctica	R0033	Aulacoseira sp.	Bacillariophyta
18S	Fragilaria crotonensis	R0223	Fragilaria crotonensis	Bacillariophyta
18S	Stephanodiscus alpinus	R0076	Stephanodiscus sp.	Bacillariophyta
18S	Stephanodiscus minutulus	R0082	Stephanodiscus sp.	Bacillariophyta
18S	Stephanodiscus neoastraea	R0083	Stephanodiscus sp.	Bacillariophyta
18S	Ulnaria acus	R2171	Ulnaria ulna	Bacillariophyta
18S	Ulnaria delicatissima	R2173	Ulnaria ulna	Bacillariophyta
18S	Ulnaria delicatissima var. angustissima	R2174	Ulnaria ulna	Bacillariophyta
18S	Ulnaria ulna	R2175	Ulnaria ulna	Bacillariophyta
18S	Botryococcus braunii	R0493	Botryococcus sp.	Chlorophyta
18S	Dinobryon bavaricum	R1066	Dinobryon divergens	Chrysophyta
18S	Dinobryon divergens	R1073	Dinobryon sp.	Chrysophyta
18S	Dinobryon sociale	R1083	Dinobryon sp.	Chrysophyta
18S	Dinophyceae sp.	R1708	Dinophyceae sp.	Chrysophyta
18S	Mallomonas sp.	R1109	Mallomonas sp.	Chrysophyta
18S	Cryptomonas curvata	R1377	Cryptomonas curvata	Cryptophyta
18S	Cryptomonas erosa	R1378	Cryptomonas curvata	Cryptophyta
18S	Cryptomonas marssonii	R1382	Cryptomonas sp.	Cryptophyta
18S	Plagioselmis nannoplanctica	R2162	Plagioselmis nannoplanctica	Cryptophyta
18S	Ceratium cornutum	R1670	Ceratium hirundinella	Dinophyta
18S	Ceratium hirundinella	R1672	Ceratium hirundinella	Dinophyta
18S	Gymnodinium helveticum	R1647	Gymnodinium helveticum	Dinophyta
18S	Gymnodinium sp.	R1654	Gymnodinium helveticum	Dinophyta
18S	Gymnodinium uberrimum	R1660	Gymnodinium helveticum	Dinophyta
18S	Peridinium sp.	R1699	Peridinium willei	Dinophyta
18S	Peridinium umbonatum	R1903	Peridinium willei	Dinophyta
18S	Peridinium willei	R1704	Peridinium willei	Dinophyta
18S	Chrysochromulina parva	R1818	Chrysochromulina parva	Haptophyta

*Suppl Table 1.2. List of **non-corresponding phytoplankton** species from microscopy to HTS (SILVA reference database) from Mondsee **pelagic** samples (n=13).*

Locus	LM_phytoplankton	ID-REBECCA	class
16S	Chroococcus limneticus	R1438	Cyanobacteria
16S	Chroococcus minutus	R1443	Cyanobacteria
16S	Coelosphaerium kuetzingianum	R1447	Cyanobacteria
18S	Coenococcus planctonicus	R0606	Chlorophyta
18S	Cosmarium depressum	R1209	Streptophyta
18S	Cyclotella bodanica	R0040	Bacillariophyta
18S	Cyclotella comensis	R0042	Bacillariophyta
18S	Cyclotella cyclopuncta	R2195	Bacillariophyta
18S	Cyclotella distinguenda	R2196	Bacillariophyta
18S	Cyclotella kuetzingiana	R0046	Bacillariophyta
18S	Cyclotella radiosa	R0051	Bacillariophyta
18S	Cyclotella sp.	R0053	Bacillariophyta
18S	Discostella glomerata	R2058	Bacillariophyta
18S	Glenodinium sp.	R1642	Dinophyta
18S	Gloeobotrys limneticus	R1840	Chlorophyta
18S	Peridinium willei	R1704	Dinophyta
18S	Planctonema lauterbornii	R0919	Chlorophyta
18S	Rhodomonas lens	R1407	Cryptophyta
18S	Staurosira construens	R2169	Bacillariophyta
18S	Stephanocostis chantaica	R0075	Bacillariophyta
18S	Stephanodiscus alpinus	R0076	Bacillariophyta
18S	Stephanodiscus minutulus	R0082	Bacillariophyta
18S	Stephanodiscus neoastraea	R0083	Bacillariophyta

*Suppl Table 1.3. List of **non-corresponding phytoplankton** species from HTS to microscopy (SILVA reference database) from Mondsee **pelagic** samples (n=13).*

Locus	ID-REBECCA	HTS_18S + 16S	class
18S	new18R12	Asulcocephalium miricentonis	Dinophyta
18S	R0449	Bacillariophyceae sp.	Bacillariophyta
18S	R1671	Ceratium furcoides	Dinophyta
18S	R0940	Chlamydomonas reinhardtii	Chlorophyta
18S	R0832	Chlorococcales sp.	Chlorophyta
18S	R0905	Chlorophyceae sp.	Chlorophyta
18S	R1162	Chrysamoeba sp.	Chrysophyta
18S	R1819	Chrysochromulina sp.	Haptophyta
18S	R1171	Chrysophyceae sp.	Chrysophyta
18S	new18R10	Crustomastigaceae	Chlorophyta
18S	R1377	Cryptomonas curvata	Cryptophyta
18S	R1389	Cryptomonas pyrenoidifera	Cryptophyta
18S	R1394	Cryptomonas sp.	Cryptophyta
18S	R1401	Cryptomonas tetrapyrenoidosa	Cryptophyta
18S	R1412	Cryptophyceae sp.	Cryptophyta
18S	R0161	Cymatopleura elliptica	Bacillariophyta
18S	R1083	Dinobryon sociale	Chrysophyta
18S	R1086	Dinobryon sp.	Chrysophyta
18S	R1708	Dinophyceae sp.	Dinophyta
18S	new18R11	Dolichomastigaceae	Chlorophyta
18S	R0238	Fragilaria sp.	Bacillariophyta
18S	R1647	Gymnodinium helveticum	Dinophyta
18S	R1654	Gymnodinium sp.	Dinophyta
18S	new18R25	Hafniomonas reticulata	Chlorophyta
18S	R1100	Mallomonas caudata	Chrysophyta
18S	R1109	Mallomonas sp.	Chrysophyta
18S	R1111	Mallomonas tonsurata	Chrysophyta
18S	R0296	Navicula cryptotenella	Bacillariophyta
18S	R1120	Ochromonas sp.	Chrysophyta
18S	R1123	Paraphysomonas sp.	Chrysophyta
18S	new18R71	Paraphysomonas vestita	Chrysophyta
18S	R2724	Pedinella hexacostata	Dictyochophyceae
18S	R1705	Peridinales Gen. sp.	Dinophyta
18S	R0975	Phacotus lenticularis	Chlorophyta
18S	new18R76	Poterioochromonas_malhamensis	Chrysophyta
18S	new18R77	Poteriospumella_lacustris	Chrysophyta
18S	R1706	Prorocentrum sp.	Dinophyta
18S	R1154	Pseudopedinella sp.	Dictyochophyceae
18S	R1132	Spumella sp.	Chrysophyta
18S	R0086	Stephanodiscus sp.	Bacillariophyta
18S	R2862	Synedra sp.	Bacillariophyta
18S	R0098	Thalassiosira weissflogii	Bacillariophyta
18S	new18R9	Thoracosphaeraceae	Dinophyta

18S	new18R3	Trebouxiophyceae	Chlorophyta
18S	R2175	Ulnaria ulna	Bacillariophyta
18S	R0989	Volvocales sp.	Chlorophyta
16S		Cyanobium	cyanobacteria
16S		Synechococcus	cyanobacteria

*Suppl Table 1.4. List of **corresponding cyanobacteria** species from **biofilm** identified through microscopy and through HTS (16S rDNA SILVA reference database) from Mondsee littoral samples (n=10).*

ID-REBECCA	Taxon_REBECCA	genus_16S	species_16S
R1427	Aphanothece clathrata	Cyanobium PCC-6307	NA
R2710	Calothrix sp.	Calothrix UAM 374	NA
R1637	Chamaesiphon sp.	Chamaesiphon PCC-7430	NA
R1438	Chroococcus limneticus	Gleocapsa	NA
R1443	Chroococcus minutus	Gleocapsa	NA
R2302	Cyanobium sp.	Cyanobium PCC-6307	NA
R1455	Cyanodictyon sp.	Cyanobium PCC-6307	NA
R1948	Cyanothece sp.	Cyanothece PCC 7425	NA
R1961	Eucapsis sp.	Chalicogloea CCALA 975	NA
R2090	Geitlerinema sp.	Geitlerinema LD9	NA
R1576	Geitlerinema splendidum	NA	NA
R0888	Gloeocapsa sp.	Gleocapsa	NA
R0893	Gloeothece linearis	Gloeobacter PCC-7421	NA
R1580	Leptolyngbya sp.	Leptolyngbya ANT.L52.2	NA
R1478	Merismopedia sp.	Merismopedia 0BB39S01	NA
R1496	Microcystis sp.	Microcystis PCC-7914	NA
R1597	Oscillatoria sp.	Oscillatoria SAG 1459-8	NA
R1606	Phormidium sp.	Kamptonema PCC-6407	NA
R1618	Planktothrix sp.	Planktothrix NIVA-CYA 15	agardhii/rubescens
R2006	Pleurocapsa sp.	Pleurocapsa PCC-7327	NA
R1623	Pseudanabaena sp.	Pseudanabaena PCC-7429	frigida
R1500	Radiocystis geminata	NA	NA
R1513	Snowella sp.	Snowella 0TU37S04	NA
R1518	Synechococcus sp.	Synechococcus PCC-7502	NA
R1520	Synechocystis sp.	Synechocystis BDHKU-20401	NA
R2826	Tychonema sp.	Tychonema CCAP 1459-11B	NA

Not yet available from database (v6):
Lists of non-corresponding cyanobacteria from biofilm through microscopy and through HTS or vice versa

*Suppl Table 1.5. List of **corresponding diatom species** from biofilm identified through microscopy and through HTS (rbcL reference database R-Syst::diatom) from Mondsee littoral samples (n=10).*

V9 species	TAXON_R_Diatom	Validcode	Taxon_validcode
Achnanthidium delmontii	Achnanthidium delmontii	newADEL	Achnanthidium delmontii
Achnanthidium minutissimum	Achnanthidium minutissimum	ADMI	Achnanthidium minutissimum
Amphora ovalis	Amphora ovalis	AOVA	Amphora ovalis
Amphora pediculus	Amphora pediculus	APED	Amphora pediculus
Cocconeis placentula	Cocconeis placentula var. placentula	CPLA	Cocconeis placentula var. placentula
Craticula cuspidata	Craticula cuspidata	CRCU	Craticula cuspidata
Pantocsekiella costei	Cyclotella costei	CCOS	Cyclotella costei
Denticula tenuis	Denticula tenuis	DTEN	Denticula tenuis
Diatoma vulgaris	Diatoma vulgaris	DVUL	Diatoma vulgaris
Encyonema caespitosum	Encyonema caespitosum	ECAE	Encyonema caespitosum
Encyonopsis falaisensis	Encyonopsis falaisensis	ECFA	Encyonopsis falaisensis
Encyonopsis minuta	Encyonopsis minuta	ECPM	Encyonopsis minuta
Encyonopsis subminuta	Encyonopsis subminuta	ESUM	Encyonopsis subminuta
Fragilaria gracilis	Fragilaria gracilis	FGRA	Fragilaria gracilis
Navicula antonii	Navicula antonii	NANT	Navicula antonii
Navicula capitatoradiata	Navicula capitatoradiata	NCPR	Navicula capitatoradiata
Navicula radiosa	Navicula radiosa	NRAD	Navicula radiosa
Navicula tripunctata	Navicula tripunctata	NTPT	Navicula tripunctata
Navicula veneta	Navicula veneta	NVEN	Navicula veneta
Nitzschia dissipata	Nitzschia dissipata var. dissipata	NDIS	Nitzschia dissipata var. dissipata
Nitzschia dissipata var. media	Nitzschia dissipata var. media	NDME	Nitzschia dissipata var. media
Nitzschia fonticola	Nitzschia fonticola	NFON	Nitzschia fonticola
Nitzschia palea	Nitzschia palea	NPAL	Nitzschia palea
Nitzschia unclassified	Nitzschia spec	NITZ	Nitzschia spec
Pseudostaurosira brevistriata	Pseudostaurosira brevistriata	PSBR	Pseudostaurosira brevistriata
Reimeria sinuata	Reimeria sinuata	RSIN	Reimeria sinuata
Staurosira construens	Staurosira construens	SCON	Staurosira construens

*Suppl Table 1.6. List of **non-corresponding diatom species** from microscopy to HTS (rbcL reference database R-Syst::diatom) from Mondsee littoral samples (n=10).*

LM_BFM_diatoms_in_select_site.genus_VALIDCODE
Achnanthes
Adlafia
Amphipleura
Cavinula
Cocconeis
Cymbopleura
Delicata
Eolimna
Eucocconeis
Fallacia
Geissleria
Gomphonema
Karayevia
Placoneis
Planothidium
Platessa
Punctastriata
Sellaphora
Staurosirella

no VALID code?

*Suppl Table 1.7. List of **non-corresponding diatom species** from HTS to microscopy (rbcL reference database R-Syst::diatom) from Mondsee littoral samples (n=10).*

V9 species	TAXON_R_Diatom	Validcode
Achnanthidium eutrophilum	Achnanthidium eutrophilum	ADEU
Achnanthidium pyrenaicum	Achnanthidium pyrenaicum	ADPT
Adlafia minuscula	Adlafia minuscula	ADMS
Amphora copulata	Amphora copulata	ACOP
Amphora unclassified	Amphora spec	AMPH
Aneumastus pseudoapiculatus	Aneumastus pseudoapiculatus	ANEP
Aneumastus unclassified	Aneumastus spec	ANEU
Aulacoseira subarctica	Aulacoseira subarctica	AUSU
Brachysira unclassified	Brachysira spec	BRAC
Brachysira vitrea	Brachysira vitrea	BVIT
Caloneis fontinalis	Caloneis fontinalis	CFON
Caloneis silicula	Caloneis silicula	CSIL
Caloneis unclassified	Caloneis spec	CALO
Cocconeis pediculus	Cocconeis pediculus	CPED
Cyclotella distinguenda	Cyclotella distinguenda var. distinguenda	CDTG
Cyclotella meneghiniana	Cyclotella meneghiniana	CMEN
Cymbella excisa	Cymbella excisa var. excisa	CAEX
Cymbella lanceolata	Cymbella lanceolata var. lanceolata	CLAN
Cymbella neocistula	Cymbella neocistula var. neocistula	CNCI
Cymbella proxima	Cymbella proxima var. proxima	CPRX
Cymbella tumida	Cymbella tumida	CTUM
Cymbella unclassified	Cymbella spec	CYMB
Cymbopleura sp.	Cymbopleura sp.	CBPS
Diatoma tenuis	Diatoma tenuis	DITE
Diploneis subovalis	Diploneis subovalis	DSBO
Diploneis unclassified	Diploneis spec	DIPL
Ellerbeckia sp.	Ellerbeckia spec	ELLE
Encyonema minutum	Encyonema minutum	ENMI
Encyonema prostratum	Encyonema prostratum	EPRO
Encyonema silesiacum	Encyonema silesiacum	ESLE
Encyonema unclassified	Encyonema spec	ENCY
Encyonema ventricosum	Encyonema ventricosum	ENVE
Encyonopsis microcephala	Encyonopsis microcephala	ENCM
Encyonopsis sp.	Encyonopsis spec	ENCP
Epithemia gibba	Epithemia spec	EPIT
Epithemia hyndmanii	Epithemia hyndmanii	EHYN
Epithemia sorex	Epithemia sorex	ESOR
Eunotia arcus	Eunotia arcus var. arcus	EARC
Fallacia monoculata	Fallacia monoculata	FMOC
Fistulifera saprophila	Fistulifera saprophila	FSAP
Fragilaria acus/radians complex	Fragilaria radians	FRAD
Fragilaria sp.	Fragilaria species	FRAS
Fragilaria unclassified	Fragilaria spec	FRAG
Frustulia vulgaris	Frustulia vulgaris	FVUL
Gomphonella coxiae	Gomphonella spec	newGOMP
Gomphonella olivacea	Gomphonella olivacea	GLOV
Gomphonella olivaceoides	Gomphonema olivaceum var. olivaceoides	GOOL
Gomphonema minutum	Gomphonema minutum fo. minutum	GMIN
Gomphonema pumilum var. pumilum	Gomphonema pumilum	GPUM
Gomphonema saprophilum	Gomphonema parvulum var. parvulum fo.	GPAS
Gomphonema unclassified	Gomphonema spec	GOMP

Gyrosigma sciotense	Gyrosigma sciotense	GSCI
Hippodonta capitata	Hippodonta capitata	HCAP
Iconella unclassified	Iconella sp.	ICON
Mayamaea permitis	Mayamaea atomus var. permitis	MAPE
Melosira varians	Melosira varians	MVAR
Navicula cari	Navicula cari	NCAR
Navicula cryptocephala	Navicula cryptocephala	NCRY
Navicula cryptotenella	Navicula cryptotenella	NCTE
Navicula gregaria	Navicula gregaria	NGRE
Navicula oblonga	Navicula oblonga	NOBL
Nitzschia acidoclinata	Nitzschia acidoclinata	NACD
Nitzschia denticula	Nitzschia denticula	NDEN
Nitzschia draveillensis	Nitzschia draveillensis	NDRA
Nitzschia linearis	Nitzschia linearis var. linearis	NLIN
Nitzschia pusilla	Nitzschia pusilla	NIPU
Nitzschia recta	Nitzschia recta	NREC
Nitzschia sigmoidea	Nitzschia sigmoidea	NSIO
Nitzschia soratensis	Nitzschia soratensis	newNSOR
Nitzschia unclassified	Nitzschia spec	NITZ
Pantocsekiella costei	Cyclotella costei	CCOS
Pinnularia neomajor	Pinnularia neomajor var. neomajor	PNEO
Planothidium victori	Planothidium spec	PLTD
Sellaphora lanceolata	Sellaphora lanceolata	SLCL
Sellaphora nigri	Sellaphora nigri	newSNIG
Sellaphora obesa	Sellaphora obesa	SOBE
Sellaphora unclassified	Sellaphora spec	SELL
Stauroneis gracilis	Stauroneis gracilis	SGRC
Staurosira sp.	Staurosira spec	STRS
Staurosira venter	Staurosira venter	SSVE
Stephanodiscus unclassified	Stephanodiscus spec	STEP
Surirella elliptica	Cymatopleura elliptica var. elliptica	CELL
Surirella solea	Cymatopleura solea var. solea	CSOL
Surirella unclassified	Surirella spec	SURI
Tabellaria flocculosa	Tabellaria flocculosa	TFLO
Tryblionella sp.	Tryblionella spec	TRYB
Ulnaria ulna	Ulnaria ulna	UULN
Ulnaria unclassified	Ulnaria spec	ULNA

8.2 L. Bourget, France

*Suppl Table 2.1. List of **corresponding phytoplankton** species identified through microscopy and through HTS (SILVA reference database) from Bourget **pelagic** samples (n=12).*

Taxon_REBECCA	ID-REBECCA	Class
Asterionella formosa	R0135	Bacillariophyceae
Fragilaria crotonensis	R0223	Bacillariophyceae
Botryococcus braunii	R0493	Chlorophyceae
Tetraselmis cordiformis	R0996	Chlorophyceae
Dinobryon divergens	R1073	Chrysophyceae
Dinobryon sp.	R1086	Chrysophyceae
Mallomonas sp.	R1109	Chrysophyceae
Ochromonas sp.	R1120	Chrysophyceae
Aphanizomenon flos-aquae	R1558	Cyanophyceae
Pseudopedinella sp.	R1154	Dictyochophyceae
Ceratium hirundinella	R1672	Dinophyceae
Gymnodinium sp.	R1654	Dinophyceae

*Suppl Table 2.2. List of **non-corresponding phytoplankton** species identified only in microscopy but not in HTS (SILVA reference database) from Bourget **pelagic** samples (n=12).*

Taxon_REBECCA	ID-REBECCA	Class
Achnanthidium catenata	R2503	Bacillariophyceae
Cyclotella costei	R2671	Bacillariophyceae
Diatoma tenuis v. elongatum	R0190	Bacillariophyceae
Puncticulata comta	R2582	Bacillariophyceae
Stephanodiscus alpinus	R0076	Bacillariophyceae
Stephanodiscus hantzschii	R0079	Bacillariophyceae
Ulnaria acus	R2171	Bacillariophyceae
Ulnaria delicatissima var. angustissima	R2174	Bacillariophyceae
Bicosoeca ovata	R2760	Bicosoecophyceae
Bicosoeca planktonica	R0462	Bicosoecophyceae
Chlamydomonas conica	R2672	Chlorophyceae
Chlamydomonas sp.	R0941	Chlorophyceae
Coelastrum microporum	R0527	Chlorophyceae
Crucigenia quadrata	R0546	Chlorophyceae
Kirchneriella contorta var. elegans	R2888	Chlorophyceae
Monoraphidium circinale	R0664	Chlorophyceae
Monoraphidium convolutum	R0666	Chlorophyceae
Monoraphidium minutum	R0675	Chlorophyceae
Oocystis rhomboidea	R0703	Chlorophyceae
Phacotus sp.	R0976	Chlorophyceae
Sphaerocystis schroeteri	R0993	Chlorophyceae
Bitrichia chodatii	R1155	Chrysophyceae
Chrysolykos planctonicus	R1166	Chrysophyceae
Dinobryon bavaricum	R1066	Chrysophyceae
Dinobryon elegantissimum	R2198	Chrysophyceae

Dinobryon sociale v. americanum	R1084	Chrysophyceae
Epipyxis polymorpha	R1092	Chrysophyceae
Erkenia subaequiciliata	R1095	Chrysophyceae
Kephyrion littorale	R1029	Chrysophyceae
Kephyrion petasatum	R1034	Chrysophyceae
Kephyrion sp.	R1037	Chrysophyceae
Stichogloea olivacea var. sphaerica	R2821	Chrysophyceae
Cosmarium laeve	R1216	Conjugatophyceae
Cosmarium pygmaeum	R1225	Conjugatophyceae
Staurastrum pingue	R1303	Conjugatophyceae
Cryptomonas marssonii	R1382	Cryptophyceae
Cryptomonas sp.	R1394	Cryptophyceae
Plagioselmis lacustris	R2557	Cryptophyceae
Plagioselmis nannoplanctica	R2162	Cryptophyceae
Aphanocapsa delicatissima	R1413	Cyanophyceae
Aphanocapsa holsatica	R1415	Cyanophyceae
Aphanocapsa parasitica f. dinobryonis	#N/A	Cyanophyceae
Aphanocapsa planctonica	R2239	Cyanophyceae
Aphanothece clathrata var. rosea	R2757	Cyanophyceae
Chroococcus aphanocapsoides	R1434	Cyanophyceae
Chroococcus minimus	R1441	Cyanophyceae
Microcystis aeruginosa	R1482	Cyanophyceae
Planktothrix rubescens	R1617	Cyanophyceae
Pseudanabaena galeata	R2808	Cyanophyceae
Synechocystis parvula	R2822	Cyanophyceae
Synechocystis sp.	R1520	Cyanophyceae
Gymnodinium helveticum	R1647	Dinophyceae
Katodinium fungiforme	R2114	Dinophyceae
Peridinium inconspicuum	R1691	Dinophyceae
Elakatothrix gelatinosa	R0596	Klebsormidiophyceae
Chlorella vulgaris	R0504	Trebouxiophyceae
Stichococcus bacillaris	R0837	Trebouxiophyceae

*Suppl Table 2.3. List of **non-corresponding phytoplankton** species identified only in HTS but not in microscopy (SILVA reference database) from Bourget **pelagic** samples (n=12).*

Taxon_REBECCA	ID-REBECCA	Class
Cyclotella meneghiniana	R0047	Bacillariophyceae
Cymbella affinis	R2310	Bacillariophyceae
Fragilaria sp.	R0238	Bacillariophyceae
Nitzschia palea	R0382	Bacillariophyceae
Stephanodiscus sp.	R0086	Bacillariophyceae
Synedra sp.	R2862	Bacillariophyceae
Synedra ulna	#N/A	Bacillariophyceae
Chlamydomonas reinhardtii	R0940	Chlorophyceae
Phacotus lenticularis	R0975	Chlorophyceae
Chrysamoeba sp.	R1162	Chrysophyceae
Chrysophyceae Clade-B1 X sp.	#N/A	Chrysophyceae
Chrysophyceae Clade-C X sp.	#N/A	Chrysophyceae
Chrysophyceae Clade-D X sp.	#N/A	Chrysophyceae
Chrysophyceae Clade-E X sp.	#N/A	Chrysophyceae
Chrysophyceae Clade-F X sp.	#N/A	Chrysophyceae
Chrysophyceae Clade-G X sp.	#N/A	Chrysophyceae
Chrysophyceae Clade-H X sp.	#N/A	Chrysophyceae
Epipyxis sp.	R1093	Chrysophyceae
Mallomonas tonsurata	R1111	Chrysophyceae
Paraphysomonas sp.	R1123	Chrysophyceae
Paraphysomonas vestita	new18R71	Chrysophyceae
Uroglena sp.	R1151	Chrysophyceae
Closterium sp.	R1201	Conjugatophyceae
Cryptomonas curvata	R1377	Cryptophyceae
Cryptomonas pyrenoidifera	R1389	Cryptophyceae
Cryptomonas tetrapyrenoidosa	R1401	Cryptophyceae
Cyanobium sp.	R2302	Cyanophyceae
Microcystis sp.	R1496	Cyanophyceae
Planktothrix sp.	R1618	Cyanophyceae
Asulcocephalium miricentonis	new18R12	Dinophyceae
Gymnodinium sp.	R1654	Dinophyceae
Gyrodinium sp.	R1969	Dinophyceae
Peridinium inconspicuum	R1691	Dinophyceae
Peridinium cinctum	R1687	Dinophyceae
Peridinium willei	R1704	Dinophyceae
Prorocentrum sp.	R1706	Dinophyceae
Thoracosphaeraceae	new18R9	Dinophyceae
Crustomastigaceae	new18R10	Mamiellophyceae
Mamiella gilva	#N/A	Mamiellophyceae
Chrysochromulina parva	R1818	Prymnesiophyceae
Choricystis sp.	R0517	Trebouxiophyceae

*Suppl Table 2.4. List of **corresponding diatom species** from biofilm identified through microscopy and through HTS (rbcL reference database Diat.barcode v7) from Bourget littoral samples (n=12 for HTS and 7 for microscopy).*

TAXON_R_Diatom	Validcode	ID
Achnanthidium minutissimum	ADMI	2438
Amphora pediculus	APED	2890
Amphora spec	AMPH	2911
Cocconeis sp.	COCM	2570
Cymbella spec	CYMB	3677
Diatoma sp.	DIAS	101
Diploneis sp.	DIPS	4071
Encyonema spec	ENCY	4292
Encyonopsis minuta	ECPM	4417
Encyonopsis subminuta	ESUM	4453
Fragilaria gracilis	FGRA	201
Fragilaria radians	FRAD	257
Fragilaria spec	FRAG	266
Gomphonema spec	GOMP	5068
Gomphonema tergestinum	GTER	5097
Navicula capitatoradiata	NCPR	5697
Navicula cryptocephala	NCRY	5770
Navicula cryptotenella	NCTE	5774
Navicula radiosa	NRAD	6471
Navicula sp.	NASP	6621
Nitzschia palea	NPAL	8893
Nitzschia spec	NITZ	8993
Reimeria sinuata	RSIN	7965
Sellaphora spec	SELL	8043
Staurosira construens	SCON	505
Staurosira spec	STRS	519
Tryblionella spec	TRYB	9126

*Suppl Table 2.5. List of **non-corresponding diatom species** identified only in microscopy but not in HTS (rbcL reference database R-Diat.barcode v7) from Bourget littoral samples (n=12 for HTS and 7 for microscopy).*

TAXON_R_Diatom	Validcode	ID
Achnanthidium affine	#N/A	#N/A
Achnanthidium catenatum	ADCT	2412
Achnanthidium exiguum	ADEG	2422
Achnanthidium pyrenaicum	ADPT	2441
Achnanthidium straubianum	ADSB	2448
Adlafia bryophila	ABRY	2738
Amphora indistincta	newAIND	0
Brachysira neglectissima	BNEG	3070
Brachysira neoexilis	BNEO	3074
Cavinula scutelloides	CVSO	3312
Cocconeis euglyptoides	CEUO	2514
Cocconeis neothumensis	CNTH	2535
Cymbella affiniformis	CAFM	3462
Cymbella compacta	CCMP	3504
Cymbella excisiformis var. excisiformis	CEXF	3536
Cymbella lange-bertalotii	CLBE	3593
Cymbella neoleptoceros var. neoleptoceros	CNLP	3619
Cymbella parva	CPAR	3633
Cymbella subhelvetica	CSBH	3692
Denticula kuetzingii var. kuetzingii	DKUE	8324
Diatoma ehrenbergii	DEHR	88
Encyonema auerswaldii	EAUE	4104
Encyonema bonapartei	newEBNA	0
Encyonopsis alpina	ECAL	4353
Encyonopsis krammeri	ECKR	4399
Eucocconeis laevis	EULA	2589
Fragilaria austriaca	#N/A	#N/A
Fragilaria perdelicatissima	newFPEL	0
Geissleria acceptata	GACC	4693
Gomphonema elegantissimum	GELG	0
Gomphonema lateripunctatum	GLAT	4920
Gomphonema minutum fo. minutum	GMIN	4954
Gomphonema olivaceum var. calcarea	GOLC	4985
Gomphonema vibrio	GVIB	5118
Gyrosigma attenuatum	GYAT	5154
Karayevia clevei	KCLE	2597
Navicula cryptotenelloides	NCTO	5776
Navicula gottlandica	NGOT	5933
Navicula reichardtiana var. reichardtiana	NRCH	6497
Navicula subalpina	NSBN	6645
Geissleria decussis	GDEC	4699
Nitzschia dissipata var. dissipata	NDIS	8630
Nitzschia lacuum	NILA	8792
Nitzschia dissipata var. media	NDME	8632
Nitzschia dissipata ssp. oligotraphenta	NDOL	8633
Cyclotella costei	CCOS	1395

Cyclotella delicatula	CYDE	1399
Placoneis sp.	PLAS	7881
Planothidium frequentissimum	PLFR	2633
Planothidium rostratoholarcticum	newPROH	0
Platessa conspicua	PTCO	2678
Pseudostaurosira brevistriata	PSBR	449
Pseudostaurosira trainorii	PTRN	461
Punctastriata ovalis	POVA	467
Rhopalodia spec	RHOP	2172
Sellaphora chistiakovae	#N/A	#N/A
Sellaphora nigri	#N/A	#N/A
Sellaphora raederae	newSRAE	0
Sellaphora subbacillum	#N/A	#N/A
Sellaphora utermoehlii	#N/A	#N/A
Staurosira binodis	#N/A	#N/A
Staurosira venter	SSVE	522
Staurosirella spec	STRL	533

*Suppl Table 2.6. List of **non-corresponding diatom species** identified only in HTS but not in microscopy (rbcL reference database Diat.barcode v7) from Bourget littoral samples (n=12 for HTS and 7 for microscopy).*

TAXON_R_Diatom	Validcode	ID
Achnanthidium delmontii	newADEL	0
Achnanthidium digitatum	ADDI	0
Achnanthidium eutrophilum	ADEU	2419
Achnanthidium spec	ACHD	2447
Amphora copulata	ACOP	2815
Amphora ovalis	AOVA	2885
Brachysira spec	BRAC	3093
Brachysira vitrea	BVIT	3110
Caloneis silicula	CSIL	3262
Caloneis spec	CALO	3263
Cocconeis pediculus	CPED	2540
Craticula cuspidata	CRCU	3420
Ctenophora pulchella	CTPU	42
Cyclotella meneghiniana	CMEN	1427
Cymbella aspera	CASP	3474
Cymbella cymbiformis	CCYM	3514
Cymbella helvetica	CHEL	3559
Cymbella lanceolata	#N/A	#N/A
Cymbopleura inaequalis	CIQL	3795
Cymbopleura sp.	CBPS	3871
Denticula spec	DENT	8330
Denticula tenuis	DTEN	8335
Diatoma vulgaris	DVUL	111
Diploneis subovalis	DSBO	4078
Encyonema caespitosum	ECAE	4120
Encyonema prostratum	EPRO	4261
Encyonema ventricosum	ENVE	4342
Encyonopsis falaisensis	ECFA	4384
Encyonopsis microcephala	ENCM	4416
Encyonopsis spec	ENCP	4443
Rhopalodia parallela	RPAR	2167
Epithemia spec	EPIT	2130
Eunotia arcus	#N/A	#N/A
Fragilaria perminuta	FPEM	0
Geissleria sp.	GESP	4714
Geissleria decussis	GDEC	4699
Gomphonema acuminatum	GACU	4769
Gomphonema pumilum	GPUM	5033
Gomphonema saprophilum	#N/A	#N/A
Gomphonema subclavatum var. mexicanum	#N/A	#N/A
Iconella costata	#N/A	#N/A
Melosira varians	MVAR	1670
Navicula antonii	NANT	5615
Navicula gregaria	NGRE	5945
Navicula oblonga	NOBL	6263
Navicula tripunctata	NTPT	6764
Navicula trivialis	#N/A	#N/A
Navicula veneta	NVEN	6819

Neidium bisulcatum	NBIS	6986
Neidium spec	NEID	7112
Nitzschia amphibia	#N/A	#N/A
Nitzschia denticula	NDEN	8620
Nitzschia dissipata	#N/A	#N/A
Nitzschia draveillensis	NDRA	8643
Nitzschia fonticola	NFON	8679
Nitzschia linearis	#N/A	#N/A
Nitzschia pusilla	NIPU	8935
Nitzschia sigmoidea	NSIO	8980
Nitzschia supralitorea	NZSU	9025
Pantocsekiella spec	#N/A	#N/A
Pinnularia neomajor	#N/A	#N/A
Pinnularia subcommutata	#N/A	#N/A
Planothidium caputium	#N/A	#N/A
Planothidium spec	PLTD	2677
Reimeria spec	REIM	7966
Sellaphora lanceolata	SLCL	0
Sellaphora minima	#N/A	#N/A
Sellaphora pupula	SPUP	8032
Staurosira brevistriata	#N/A	#N/A
Staurosira elliptica	SELI	507
Staurosira martyi	SMAT	512
Surirella elliptica	#N/A	#N/A
Surirella minuta	SUMI	9349
Surirella solea	#N/A	#N/A
Ulnaria acus	#N/A	#N/A
Ulnaria ulna	UULN	672

8.3 L. Starnberger See, Germany

*Suppl. Table 3.5: List of **corresponding phytoplankton** species identified through light microscopy (LM) and through HTS (SILVA and PR2 reference databases) from **pelagic** samples (n=9) in Lake Starnberg.*

Locus	LM_phytoplankton	ID-REBECCA	HTS_18S+16S	class
16S	Anabaena spiroides	R1549	Anabaena	Cyanophyceae
18S	Asterionella formosa	R0135	Asterionella formosa	Bacillariophyceae
18S	Aulacoseira sp.	R0030	Aulacoseira sp.	Bacillariophyceae
18S	Pennales sp.	R0422	Bacillariophyceae sp.	Bacillariophyceae
18S	Ceratium hirundinella	R1672	Ceratium hirundinella	Dinophyceae
18S	Chrysochromulina parva	R1818	Chrysochromulina parva	Prymnesiophyceae
18S	Cryptomonas marssonii	R1382	Cryptomonas curvata	Cryptophyceae
18S	Cryptomonas ovata	R1386	Cryptomonas pyrenoidifera	Cryptophyceae
18S	Cryptomonas ovata	R1386	Cryptomonas tetrapyrenoidosa	Cryptophyceae
18S	Dinobryon divergens	R1073	Dinobryon divergens	Chrysophyceae
18S	Dinobryon sociale	R1083	Dinobryon sociale	Chrysophyceae
18S	Fragilaria crotonensis	R0223	Fragilaria sp.	Bacillariophyceae
18S	Gymnodinium helveticum	R1647	Gymnodinium helveticum	Dinophyceae
18S	Gymnodinium lantzschii	R1650	Gymnodinium sp.	Dinophyceae
18S	Gymnodinium sp.	R1654	Gymnodinium sp.	Dinophyceae
18S	Gymnodinium uberrimum	R1660	Gymnodinium sp.	Dinophyceae
18S	Mallomonas sp.	R1109	Mallomonas sp.	Chrysophyceae
16S	Microcystis aeruginosa	R1482	Microcystis	Cyanophyceae
18S	Peridinium cinctum	R1687	Peridinium cinctum	Dinophyceae
18S	Peridinium sp.	R1699	Peridinium gatunense	Dinophyceae
18S	Peridinium willei	R1704	Peridinium willei	Dinophyceae
18S	Phacotus lenticularis	R0975	Phacotus lenticularis	Chlorophyceae
18S	Plagioselmis nannoplanctica	R2162	Plagioselmis nannoplanctica	Cryptophyceae
16S	Planktothrix rubescens	R1617	Planktothrix	Cyanophyceae
18S	Pseudopedinella erkensis	R1153	Pseudopedinella sp.	Dictyochophyceae
16S	Snowella lacustris	R1510	Snowella	Cyanophyceae
18S	Stephanodiscus alpinus	R0076	Stephanodiscus sp.	Bacillariophyceae
18S	Stephanodiscus minutulus	R0082	Stephanodiscus sp.	Bacillariophyceae
18S	Stephanodiscus neoastraea	R0083	Stephanodiscus sp.	Bacillariophyceae
18S	Ulnaria ulna	R2175	Ulnaria ulna	Bacillariophyceae
18S	Uroglena sp.	R1151	Uroglena sp.	Chrysophyceae

Suppl. Table 3.6: List of species or genera detected only by light microscopy (LM) in pelagic samples (n=8) of Starnberger See.

LM_phytoplankton	ID-REBECCA	class
Aphanizomenon flos-aquae	R1558	Cyanophyceae
Aphanocapsa delicatissima	R1413	Cyanophyceae
Aphanothece clathrata	R1427	Cyanophyceae
Aulacoseira sp.	R0030	Bacillariophyceae
Bitrichia chodatii	R1155	Chrysophyceae
Chroococcus minutus	R1443	Cyanophyceae
Chrysolykos planctonicus	R1166	Chrysophyceae
Cosmarium sp.	R1233	Conjugatophyceae
Cyclotella comensis	R0042	Bacillariophyceae
Cyclotella costei	R2671	Bacillariophyceae
Cyclotella radiosa	R0051	Bacillariophyceae
Discostella stelligera	R2060	Bacillariophyceae
Elakatothrix gelatinosa	R0596	Klebsormidiophyceae
Geitlerinema sp.	R2090	Cyanophyceae
Monoraphidium contortum	R0665	Chlorophyceae
Oocystis marssonii	R0698	Chlorophyceae
Pediastrum boryanum	R0713	Chlorophyceae
Plagioselmis lacustris	R2557	Cryptophyceae
Rhodomonas lens	R1407	Cryptophyceae
Stephanocostis chantaica	R0075	Bacillariophyceae
Tetraedron minimum	R0848	Chlorophyceae
Ulnaria acus	R2171	Bacillariophyceae
Ulnaria delicatissima var. angustissima	R2174	Bacillariophyceae
Dinobryon bavaricum	R1066	Chrysophyceae
Dinobryon crenulatum	R1069	Chrysophyceae
Dinobryon faculiferum	R2062	Chrysophyceae
Dinobryon sertularia	R1081	Chrysophyceae
Dinobryon sertularia v. protuberans	R1082	Chrysophyceae

Suppl. Table 3.7 List of non-corresponding phytoplankton species from HTS to microscopy (SILVA reference database) in pelagic samples (n=8) of Lake Starnberger See

Locus	ID-REBECCA	HTS_18S + 16S	class
18S	Asulcocephalium miricentonis	new18R12	Dinophyceae
18S	Chaetophorales	new18R5	Chlorophyceae
18S	Chlamydomonas reinhardtii	R0940	Chlorophyceae
18S	Choricystis sp.	R0517	Trebouxiophyceae
18S	Chrysamoeba sp.	R1162	Chrysophyceae
18S	Chrysosphaerella sp.	R1063	Chrysophyceae
18S	Crustomastigaceae	new18R10	Mamiellophyceae
18S	Cryptomonas curvata	R1377	Cryptophyceae
18S	Cryptomonas pyrenoidifera	R1389	Cryptophyceae
18S	Cryptomonas tetrapyrenoidosa	R1401	Cryptophyceae
18S	Dolichomastigaceae	new18R11	Mamiellophyceae
18S	Epipyxis sp.	R1093	Chrysophyceae
18S	Hafniomonas reticulata	new18R25	Chlorophyceae
18S	Mallomonas tonsurata	R1111	Synurophyceae
18S	marine taxa	new18R34	Mamiellophyceae
18S	Ochromonas sp.	R1120	Chrysophyceae
18S	Paraphysomonas vestita	new18R71	Chrysophyceae
18S	Pedinella hexacostata	R2724	Dictyochophyceae
18S	Peridinium gatunense	R2588	Dinophyceae
18S	Polarella glacialis	new18R75	Dinophyceae
18S	Prorocentrum sp.	R1706	Dinophyceae
18S	Prymnesiaceae Gen. sp.	R2427	Prymnesiophyceae
18S	Pseudopedinella sp.	R1154	Dictyochophyceae
18S	Synedra sp.	R2862	Bacillariophyta
18S	Synura sp.	R1141	Synurophyceae
18S	Tetraselmis cordiformis	R0996	Chlorodendrophyceae
18S	Thoracosphaeraceae	new18R9	Dinophyceae
18S	Trebouxiophyceae	new18R3	Trebouxiophyceae
18S	Volvocales sp.	R0989	Chlorophyceae
18S	Woloszynskia tenuissima	R1666	Dinophyceae
16S	Cyanobium		Cyanobacteriia
16S	Limnothrix		Cyanobacteriia
16S	Pseudanabaena		Cyanobacteriia
16S	Radiocystis		Cyanobacteriia
16S	Synechococcus		Cyanobacteriia
16S	Woronichinia		Cyanobacteriia

*Suppl. Table 3.8: List of **cyanobacteria** species from **biofilm** identified through HTS (16S rDNA SILVA reference database) from Starnberger See littoral samples (n= 28).*

Taxon_REBECCA	ID-REBECCA	Max signal 16S	N ASV seqs	1st station taxon found	last station taxon found
Cyanobacteria genotype unidentified	NA	311	1076	T1	T9
Aliterella	marin2	45	14	T13	T7
Anabaena lemmermannii	R1539	21	20	T1	T9
Annamia sp.	fresh_new2	10	22	T1	T8
Aphanothece clathrata	R1427	133	30	T1	T9
Calothrix sp.	R2710	257	188	T1	T9
Candidatus Gloeomargarita	biofilm_new12	6	1	T16	T16
Chalicogloea sp.	aerophytic1	4	1	T14	T14
Chamaesiphon sp.	R1637	33	19	T11	T9
Chroococcidiopsaceae	new_16S cyano_family1	6	6	T2	T6
Chroococcidiopsis	aerophytic2	146	30	T1	T9
Chroococcus limneticus	R1438	113	22	T1	T9
Chroococcus minutus	R1443	3	3	T13	T7
Cyanobiaceae	new_16S cyano_family3	39	9	T10	T8
Cyanobium sp.	R2302	630	307	T1	T9
Cyanothece sp.	R1948	15	20	T11	T9
Eucapsis sp.	R1961	5	4	T14	T5
Geitlerinema sp.	R2090	3	4	T11	T19
Geitlerinema splendidum	R1576	5	3	T11	T8
Geminocystis	Picoplank1	75	46	T1	T9
Gloeocapsa sp.	R0888	43	139	T1	T9
Gloeothece linearis	R0893	240	44	T1	T9
Leptolyngbya sp.	R1580	128	124	T1	T9
Leptolyngbyaceae	new_16S cyano_family4	231	181	T1	T9
Merismopedia sp.	R1478	6	7	T17	T7
Microcoleus	biofilm_new9	165	8	T2	T3
Microcystaceae	new_16S cyano_family5	18	48	T1	T8
Microcystis sp.	R1496	26	41	T1	T9
Nodosilinea	biofilm_new5	57	76	T1	T9
Nodosilineaceae	new_16S cyano_family6	29	16	T1	T9
Nostoc sp.	R2398	8	4	T15	T30
Nostocaceae	new_16S cyano_family7	175	90	T1	T9
Oscillatoria sp.	R1597	36	15	T11	T9
Phormidesmis	biofilm_new6	5	9	T11	T4
Phormidiaceae	new 16S cyano_family9	89	6	T1	T3
Phormidium sp.	R1606	77	27	T1	T6
Pleurocapsa sp.	R2006	77	87	T1	T9
Prochlorothrix PCC-9006	fresh_new5	10	22	T1	T9

Pseudanabaena sp.	R1623	143	45	T1	T9
Pseudanabaenaceae	new_16S cyano_family10	92	165	T1	T9
Radiocystis geminata	R1500	31	11	T10	T9
Rivularia	biofilm_new3	18	5	T1	T28
Schizothrix	biofilm_new4	2	2	T14	T8
Scytolyngbya	biofilm_new10	1	1	T11	T11
Snowella litoralis	R1511	4	1	T30	T30
Snowella sp.	R1513	17	10	T15	T7
Synechococcaceae	new_16S cyano_family11	15	31	T1	T9
Synechococcus sp.	R1518	41	43	T1	T9
Synechocystis sp.	R1520	3	5	T11	T6
Tychonema sp.	R2826	21	11	T19	T6
Xenococcaceae	new_16S cyano_family12	14	15	T1	T8
Xenococcus	biofilm_new8	11	5	T23	T30

Suppl. Table 3.9 . List of 44 corresponding diatom species from biofilm identified through microscopy and through HTS (rbcL reference database R-Syst::diatom) from Starnberger See littoral samples (n=28).

V9 species	HTS TAXON_R_Diatom	LM Validcode	LM diatoms BFM
Achnanthidium minutissimum	Achnanthidium minutissimum	ADMI	Achnanthidium minutissimum
Achnanthidium pyrenaicum	Achnanthidium pyrenaicum	ADPT	Achnanthidium pyrenaicum
Achnanthidium unclassified	Achnanthidium spec	ACHD	Achnanthidium spec
Amphora copulata	Amphora copulata	ACOP	Amphora copulata
Amphora ovalis	Amphora ovalis	AOVA	Amphora ovalis
Amphora pediculus	Amphora pediculus	APED	Amphora pediculus
Amphora unclassified	Amphora spec	AMPH	Amphora spec
Brachysira vitrea	Brachysira vitrea	BVIT	Brachysira vitrea
Caloneis silicula	Caloneis silicula	CSIL	Caloneis silicula
Cocconeis pediculus	Cocconeis pediculus	CPED	Cocconeis pediculus
Cocconeis placentula	Cocconeis placentula var. placentula	CPLA	Cocconeis placentula var. placentula
Cymbella excisa	Cymbella excisa var. excisa	CAEX	Cymbella excisa var. excisa
Cymbella helvetica	Cymbella helvetica	CHEL	Cymbella helvetica
Cymbella lanceolata	Cymbella lanceolata var. lanceolata	CLAN	Cymbella lanceolata var. lanceolata
Denticula tenuis	Denticula tenuis	DTEN	Denticula tenuis
Encyonema caespitosum	Encyonema caespitosum	ECAE	Encyonema caespitosum
Encyonema minutum	Encyonema minutum	ENMI	Encyonema minutum
Encyonema silesiacum	Encyonema silesiacum	ESLE	Encyonema silesiacum
Encyonopsis falaisensis	Encyonopsis falaisensis	ECFA	Encyonopsis falaisensis
Encyonopsis microcephala	Encyonopsis microcephala	ENCM	Encyonopsis microcephala
Encyonopsis subminuta	Encyonopsis subminuta	ESUM	Encyonopsis subminuta
Epithemia sorex	Epithemia sorex	ESOR	Epithemia sorex
Fragilaria acus/radians complex	Fragilaria radians	FRAD	Fragilaria radians
Gomphonella olivaceoides	Gomphonema olivaceum var. olivaceoides	GOOL	Gomphonema olivaceum var. olivaceoides
Gomphonema tergestinum	Gomphonema tergestinum	GTER	Gomphonema tergestinum
Gomphonema unclassified	Gomphonema spec	GOMP	Gomphonema spec
Halamphora oligotraphenta	Amphora oligotraphenta	AOLG	Amphora oligotraphenta
Melosira varians	Melosira varians	MVAR	Melosira varians
Navicula antonii	Navicula antonii	NANT	Navicula antonii
Navicula cari	Navicula cari	NCAR	Navicula cari
Navicula cryptotenella	Navicula cryptotenella	NCTE	Navicula cryptotenella
Navicula radiosa	Navicula radiosa	NRAD	Navicula radiosa
Navicula tripunctata	Navicula tripunctata	NTPT	Navicula tripunctata
Nitzschia denticula	Nitzschia denticula	NDEN	Nitzschia denticula
Nitzschia dissipata	Nitzschia dissipata var. dissipata	NDIS	Nitzschia dissipata var. dissipata
Nitzschia linearis	Nitzschia linearis var. linearis	NLIN	Nitzschia linearis var. linearis
Nitzschia palea	Nitzschia palea	NPAL	Nitzschia palea
Nitzschia unclassified	Nitzschia spec	NITZ	Nitzschia spec
Pseudostaurosira brevistriata	Pseudostaurosira brevistriata	PSBR	Pseudostaurosira brevistriata

Reimeria sinuata	Reimeria sinuata	RSIN	Reimeria sinuata
Sellaphora pupula	Sellaphora pupula	SPUP	Sellaphora pupula
Staurosira construens	Staurosira construens	SCON	Staurosira construens
Surirella elliptica	Cymatopleura elliptica var. elliptica	CELL	Cymatopleura elliptica var. elliptica
Ulnaria ulna	Ulnaria ulna	UULN	Ulnaria ulna
Halamphora oligotraphenta	Amphora oligotraphenta	AOLG	Amphora oligotraphenta
Melosira varians	Melosira varians	MVAR	Melosira varians
Navicula antonii	Navicula antonii	NANT	Navicula antonii
Navicula cari	Navicula cari	NCAR	Navicula cari
Navicula cryptotenella	Navicula cryptotenella	NCTE	Navicula cryptotenella
Navicula radiosa	Navicula radiosa	NRAD	Navicula radiosa
Navicula tripunctata	Navicula tripunctata	NTPT	Navicula tripunctata
Nitzschia denticula	Nitzschia denticula	NDEN	Nitzschia denticula
Nitzschia dissipata	Nitzschia dissipata var. dissipata	NDIS	Nitzschia dissipata var. dissipata
Nitzschia linearis	Nitzschia linearis var. linearis	NLIN	Nitzschia linearis var. linearis
Nitzschia palea	Nitzschia palea	NPAL	Nitzschia palea
Nitzschia unclassified	Nitzschia spec	NITZ	Nitzschia spec
Pseudostaurosira brevistriata	Pseudostaurosira brevistriata	PSBR	Pseudostaurosira brevistriata
Reimeria sinuata	Reimeria sinuata	RSIN	Reimeria sinuata
Sellaphora pupula	Sellaphora pupula	SPUP	Sellaphora pupula
Staurosira construens	Staurosira construens	SCON	Staurosira construens
Surirella elliptica	Cymatopleura elliptica var. elliptica	CELL	Cymatopleura elliptica var. elliptica
Ulnaria ulna	Ulnaria ulna	UULN	Ulnaria ulna

Suppl. Table 3.10. List of 117 non-corresponding diatom species from microscopy to HTS (rbcL reference database R-Syst::diatom) from Starnberger See littoral samples (n=28).

LM diatoms BFM	LM Validcode
Achnanthes holsatica	AHOS
Achnanthes petersenii	APET
Achnanthes ziegleri	AZIE
Achnanthidium minutissima var. affinis	ADMF
Achnanthidium rosenstockii	newADRK
Adlafia bryophila	ABRY
Amphora inariensis	AINA
Amphora indistincta	newAIND
Amphora thumensis	ATHU
Aneumastus stroesei	ANSS
Brachysira liliana	BLIL
Brachysira neglectissima	BNEG
Brachysira neoexilis	BNEO
Caloneis lancettula	CLCT
Caloneis schumanniana	CSHU
Cavinula scutelloides	CVSO
Cocconeis neothumensis	CNTH
Cocconeis placentula var. euglypta	CPLE
Cocconeis placentula var. lineata	CPLI
Cymatopleura solea var. apiculata	CSAP
Cymbella cymbiformis	CCYM
Cymbella dorsenotata	CDNO
Cymbella hustedtii var. hustedtii	CHUS
Cymbella laevis var. laevis	CLAE
Cymbella lange-bertalotii	CLBE
Cymbella neoleptoceros var. neoleptoceros	CNLP
Cymbella parva	CPAR
Cymbella percapitata	CPCA
Cymbella subaequalis	CSAE
Cymbella subhelvetica	CSBH
Cymbella vulgata var. vulgata	CVUL
Cymbopleura amphicephala	CBAM
Cymbopleura frequens var. frequens	CBFQ
Cymbopleura lata var. lata	CYBL
Delicata delicatula var. alpestris	DDAL
Delicata delicatula var. delicatula	DDEL
Diploneis krammeri	DKRA
Encyonema lacustre	ELAC
Encyonema reichardtii	ENRE
Encyonopsis alpina	ECAL

Encyonopsis cesatii	ECES
Encyonopsis krammeri	ECKR
Eolimna minima	EOMI
Epithemia adnata	EADN
Epithemia smithii	ESMI
Eucocconeis flexella	EUFL
Eucocconeis laevis	EULA
Eunotia arcubus	EARB
Fallacia lenzi	FLEN
Fragilaria capucina var. capucina	FCAP
Fragilaria capucina var. mesolepta	FCME
Fragilaria capucina var. perminuta	FCPE
Fragilaria capucina var. rumpens	FCRP
Fragilaria delicatissima	FDEL
Fragilaria martyi	FMAR
Fragilaria nanana	FNAN
Fragilaria tenera	FTEN
Fragilaria ulna var. acus	FUAC
Geissleria cummerowi	GCUW
Gomphocymbellopsis ancyli	GPAN
Gomphonema auritum	GAUR
Gomphonema lateripunctatum	GLAT
Gomphonema lippertii	GLIP
Gomphonema micropus var. micropus	GMIC
Gomphonema minusculum	GMIS
Gomphonema minutum fo. minutum	GMIN
Gomphonema occultum	GOCU
Gomphonema olivaceum var. olivaceum	GOLI
Gomphonema procerum	GPRC
Gomphonema pumilum var. elegans	GPEL
Gomphonema sarcophagus	GSAR
Gomphonema stauroneiforme	GSTA
Gomphonema vibrio	GVIB
Karayevia clevei	KCLE
Mastogloia smithii	MSMI
Mayamaea atomus var. permitis	MAPE
Navicula associata	NXAS
Navicula capitatoradiata	NCPR
Navicula cincta	NCIN
Navicula concentrica	NCCT
Navicula cryptocephala	NCRY
Navicula cryptotenelloides	NCTO
Navicula gottlandica	NGOT

Navicula gregaria	NGRE
Navicula irmengardis	NIGD
Navicula oligotraphenta	NOLI
Navicula praeterita	NPRA
Navicula spec	NAVI
Navicula subalpina	NSBN
Navicula sublucidula	NSLU
Navicula utermoehlii	NUTE
Navicula viridulacalcis var. viridulacalcis	NVCC
Navicula vulpina	NVUL
Naviculadicta vitabunda	NDVI
Nitzschia angustata	NIAN
Nitzschia brunoi	NBNO
Nitzschia gessneri	NGES
Nitzschia gisela	newNGIS
Nitzschia lacuum	NILA
Nitzschia paleacea	NPAE
Nitzschia recta	NREC
Nitzschia tabellaria	NTAB
Pinnularia spec	PINU
Placoneis pseudanglica	PPSA
Planothidium delicatulum	PTDE
Planothidium frequentissimum	PLFR
Planothidium lanceolatum	PTLA
Planothidium rostratum	PRST
Platessa conspicua	PTCO
Platessa oblongella	newPOBL
Platessa sp.	PTSA
Pseudostaurosira parasitica	PPRS
Rhopalodia gibba var. gibba	RGIB
Staurosira construens fo. venter	SCVT
Staurosira construens var. binodis	SCBI
Staurosira leptostauron	SSLE
Staurosirella pinnata	newSTPN

Suppl Table 3.11. List of 51 diatoms detected only by HTS and not found by microscopy (rbcL reference database R-Syst::diatom) from Starnberger See littoral samples (n=28).

V9 species	validcode	HTS TAXON R Diatom
Achnanthidium delmontii	newADEL	Achnanthidium delmontii
Achnanthidium eutrophilum	ADEU	Achnanthidium eutrophilum
Adlafia minuscula	ADMS	Adlafia minuscula
Aneumastus pseudoapiculatus	ANEP	Aneumastus pseudoapiculatus
Aneumastus unclassified	ANEU	Aneumastus spec
Asterionella formosa	AFOR	Asterionella formosa
Brachysira unclassified	BRAC	Brachysira spec
Caloneis fontinalis	CFON	Caloneis fontinalis
Caloneis unclassified	CALO	Caloneis spec
Pantocsekiella costei	CCOS	Cyclotella costei
Cyclotella distinguenda	CDTG	Cyclotella distinguenda var. distinguenda
Surirella solea	CSOL	Cymatopleura solea var. solea
Cymbella neocistula	CNCI	Cymbella neocistula var. neocistula
Cymbella proxima	CPRX	Cymbella proxima var. proxima
Cymbella unclassified	CYMB	Cymbella spec
Cymbopleura sp.	CBPS	Cymbopleura sp.
Diatoma vulgaris	DVUL	Diatoma vulgaris
Diploneis subovalis	DSBO	Diploneis subovalis
Ellerbeckia sp.	ELLE	Ellerbeckia spec
Encyonema prostratum	EPRO	Encyonema prostratum
Encyonema unclassified	ENCY	Encyonema spec
Encyonema ventricosum	ENVE	Encyonema ventricosum
Encyonopsis minuta	ECPM	Encyonopsis minuta
Encyonopsis sp.	ENCP	Encyonopsis spec
Epithemia hyndmanii	EHYN	Epithemia hyndmanii
Epithemia gibba	EPIT	Epithemia spec
Eunotia arcus	EARC	Eunotia arcus var. arcus
Fragilaria gracilis	FGRA	Fragilaria gracilis
Fragilaria unclassified	FRAG	Fragilaria spec
Fragilaria sp.	FRAS	Fragilaria species
Gomphonella olivacea	GLOV	Gomphonella olivacea
Gomphonella coxiae	newGOMP	Gomphonella spec
Gomphonella olivaceolacuum	newGOMP	Gomphonella spec
Gomphonema saprophilum	GPAS	Gomphonema parvulum var. parvulum fo. saprophilum Lange-Bert. & Reichardt
Gomphonema pumilum var. pumilum	GPUM	Gomphonema pumilum
Gyrosigma sciotense	GSCI	Gyrosigma sciotense
Iconella unclassified	ICON	Iconella sp.
Navicula oblonga	NOBL	Navicula oblonga
Nitzschia acidoclinata	NACD	Nitzschia acidoclinata

Nitzschia dissipata var. media	NDME	Nitzschia dissipata var. media
Nitzschia fonticola	NFON	Nitzschia fonticola
Nitzschia pusilla	NIPU	Nitzschia pusilla
Nitzschia sigmoidea	NSIO	Nitzschia sigmoidea
Pinnularia neomajor	PNEO	Pinnularia neomajor var. neomajor
Lindavia radiosa	PRAD	Puncticulata radiosa
Sellaphora nigri	newSNIG	Sellaphora nigri
Sellaphora unclassified	SELL	Sellaphora spec
Stauroneis gracilis	SGRC	Stauroneis gracilis
Staurosira sp.	STRS	Staurosira spec
Staurosira venter	SSVE	Staurosira venter
Ulnaria unclassified	ULNA	Ulnaria spec

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Suppl. Table 4.9. List of corresponding phytoplankton species or genera identified through light microscopy (LM) and through HTS (SILVA and PR2 reference databases) from pelagic samples (n=12) in Lake Garda.

Locus	LM_phytoplankton	ID-REBECCA	HTS_16s+18s	class
16S	Anabaena lemmermannii	R1539	Anabaena	Cyanophyceae
16S	Cyanodictyon sp.	R1455	Cyanodictyon	Cyanophyceae
16S	Microcystis aeruginosa	R1482	Microcystis	Cyanophyceae
16S	Planktothrix rubescens	R1617	Planktothrix	Cyanophyceae
16S	Snowella lacustris	R1510	Snowella	Cyanophyceae
16S	Snowella sp.	R1513	Snowella	Cyanophyceae
16S	Tychonema bourrellyi	R1636	Tychonema	Cyanophyceae
18S	Asterionella formosa	R0135	Asterionella formosa	Bacillariophyceae
18S	Aulacoseira granulata v. angustissima	R0024	Aulacoseira granulata	Bacillariophyceae
18S	Ceratium hirundinella	R1672	Ceratium hirundinella	Dinophyceae
18S	Chlamydomonas sp.	R0941	Chlamydomonas reinhardtii	Chlorophyceae
18S	Chlamydomonas sp.	R0941	Chlamydomonas sp.	Chlorophyceae
18S	Closterium aciculare	R1176	Closterium sp.	Conjugatophyceae
18S	Closterium acutum	R1178	Closterium sp.	Conjugatophyceae
18S	Closterium pronum	R1199	Closterium sp.	Conjugatophyceae
18S	Coelastrum reticulatum	R0530	Coelastrum reticulatum	Chlorophyceae
18S	Cryptomonas curvata	R1377	Cryptomonas curvata	Cryptophyceae
18S	Cryptomonas erosa	R1378	Cryptomonas sp.	Cryptophyceae
18S	Cryptomonas marssonii	R1382	Cryptomonas sp.	Cryptophyceae
18S	Cryptomonas pyrenoidifera	R1389	Cryptomonas pyrenoidifera	Cryptophyceae
18S	Dinobryon divergens	R1073	Dinobryon divergens	Chrysophyceae
18S	Fragilaria crotonensis	R0223	Fragilaria crotonensis	Bacillariophyceae
18S	Gymnodinium helveticum	R1647	Gymnodinium helveticum	Dinophyceae
18S	Gymnodinium sp.	R1654	Gymnodinium sp.	Dinophyceae
18S	Mallomonas tonsurata	R1111	Mallomonas tonsurata	Chrysophyceae
18S	Melosira varians	R0062	Melosira varians	Bacillariophyceae
18S	Mougeotia sp.	R1003	Mougeotia sp.	Conjugatophyceae
18S	Stephanodiscus neoastraea	R0083	Stephanodiscus sp.	Bacillariophyceae
18S	Tetraselmis cordiformis	R0996	Tetraselmis cordiformis	Chlorophyceae
18S	Tribonema sp.	R1868	Tribonema aequale	Xanthophyceae
18S	Tribonema sp.	R1868	Tribonema sp.	Xanthophyceae
18S	Uroglena sp.	R1151	Uroglena sp.	Chrysophyceae

Suppl. Table 4.10. List of species and genera detected only by light microscopy (LM) in pelagic samples (n=12) of Lake Garda

Locus	LM_phytoplankton	ID-REBECCA	class
18S	Ankyra lanceolata	R0490	Chlorophyceae
16S	Aphanocapsa delicatissima	R1413	Cyanophyceae
16S	Aphanocapsa incerta	R1416	Cyanophyceae
16S	Aphanocapsa planctonica	R2239	Cyanophyceae
16S	Aphanocapsa sp.	R1423	Cyanophyceae
16S	Chroococcus limneticus	R1438	Cyanophyceae
16S	Chroococcus minutus	R1443	Cyanophyceae
16S	Coelosphaerium kuetzingianum	R1447	Cyanophyceae
18S	Coenochloris fottii	R0533	Chlorophyceae
18S	Cosmarium depressum	R1209	Conjugatophyceae
18S	Cosmarium depressum v. planctonicum	R1210	Conjugatophyceae
18S	Cosmarium sp.	R1233	Conjugatophyceae
18S	Cyclotella sp.	R0053	Bacillariophyceae
18S	Diatoma sp.	R0188	Bacillariophyceae
18S	Dictyosphaerium pulchellum	R0571	Chlorophyceae
18S	Elakatothrix genevensis	R0597	Klebsormidiophyceae
18S	Erkenia subaequiciliata	R1095	Chrysophyceae
18S	Eudorina elegans	R0963	Chlorophyceae
18S	Euglena sp.	R1726	Euglenophyceae
18S	Fragilaria capucina ssp. rumpens	R2520	Bacillariophyceae
18S	Glenodinium sp.	R1642	Dinophyceae
18S	Gloeocystis sp.	R0891	Chlorophyceae
16S	Limnothrix obliqueacuminata	R2369	Cyanophyceae
18S	Monoraphidium sp.	R0682	Chlorophyceae
18S	Oocystis lacustris	R0697	Chlorophyceae
18S	Oocystis sp.	R0705	Chlorophyceae
18S	Pandorina morum	R0971	Chlorophyceae
18S	Pediastrum simplex	R0722	Chlorophyceae
18S	Peridinium sp.	R1699	Dinophyceae
18S	Peridinium willei	R1704	Dinophyceae
18S	Plagioselmis lacustris	R2557	Cryptophyceae
18S	Plagioselmis nannoplanctica	R2162	Cryptophyceae
16S	Planktolyngbya limnetica	R1610	Cyanophyceae
18S	Planktosphaeria sp.	R0728	Chlorophyceae
18S	Pseudosphaerocystis lacustris	R0736	Chlorophyceae
18S	Sphaerocystis schroeteri	R0993	Chlorophyceae
18S	Staurastrum cingulum	R1283	Conjugatophyceae
18S	Staurastrum gracile	R1288	Conjugatophyceae
18S	Staurastrum sp.	R1309	Conjugatophyceae
18S	Ulnaria acus	R2171	Bacillariophyceae

Suppl. Table 4.11. List of non-corresponding phytoplankton species and genera from HTS to microscopy (SILVA reference database) in pelagic samples (n=12) of Lake Garda

Locus	ID-REBECCA	HTS 18S+16S	class
16S_chlo	R0489	Ankyra judayi	Chlorophyceae
18S	new18R12	Asulcocephalium miricentonis	Dinophyceae
18S	R0023	Aulacoseira granulata	Bacillariophyta
18S	R0030	Aulacoseira sp.	Bacillariophyta
18S	R0449	Bacillariophyceae sp.	Bacillariophyta
18S	R0493	Botryococcus braunii	Trebouxiophyceae
18S	R0940	Chlamydomonas reinhardtii	Chlorophyceae
18S	R0832	Chlorococcales sp.	Chlorophyceae
18S	R0905	Chlorophyceae sp.	Chlorophyceae
18S	R0517	Choricystis sp.	Trebouxiophyceae
18S	R1818	Chrysochromulina parva	Prymnesiophyceae
18S	R1819	Chrysochromulina sp.	Prymnesiophyceae
18S	R1171	Chrysophyceae sp.	Chrysophyceae
18S	R1171	Chrysophyceae sp.	Synurophyceae
16S_chlo	R2680	Colacium vesiculosum	Euglenophyceae
18S	new18R10	Crustomastigaceae	Mamiellophyceae
18S	R1401	Cryptomonas tetrapyrenoidosa	Cryptophyceae
18S	R1412	Cryptophyceae sp.	Cryptophyceae
18S	R1708	Dinophyceae sp.	Dinophyceae
18S	new18R11	Dolichomastigaceae	Mamiellophyceae
16S_chlo	R1093	Epipyxis sp.	Chrysophyceae
18S	new18R6	Gymnodiniaceae	Dinophyceae
18S	R1109	Mallomonas sp.	Synurophyceae
18S	new18R34	marine taxa	Mamiellophyceae
18S	R1815	Monomastix sp.	Mamiellophyceae
18S	new18R37	Mychonastes sp.	Chlorophyceae
18S	R1120	Ochromonas sp.	Chrysophyceae
18S	new18R71	Paraphysomonas vestita	Chrysophyceae
18S	R2724	Pedinella hexacostata	Dictyochophyceae
18S	R0975	Phacotus lenticularis	Chlorophyceae
18S	R1706	Prorocentrum sp.	Dinophyceae
16S_chlo	R2145	Pseudodictyosphaerium jurisii	Chlorophyceae
18S	R1154	Pseudopedinella sp.	Dictyochophyceae
18S	R2456	Sphaeropleaceae Gen. sp.	Chlorophyceae
18S	R2862	Synedra sp.	Bacillariophyta
18S	new18R9	Thoracosphaeraceae	Dinophyceae
18S	new18R3	Trebouxiophyceae	Trebouxiophyceae
18S	R1151	Uroglena sp.	Chrysophyceae
18S	R0989	Volvocales sp.	Chlorophyceae
18S	R1340	Zygnematales sp.	Zygnemophyceae
16S		Nodosilinea	cyanobacteria
16S		Pseudanabaena	cyanobacteria
16S		Synechococcus	cyanobacteria
16S		Radiocystis	cyanobacteria
16S		Cyanobium	cyanobacteria

Suppl. Table 4.12. List of cyanobacteria taxa from biofilm identified through HTS (16S rDNA SILVA reference database) from Lake Garda littoral samples (n= 10).

Taxon_REBECCA	ID-REBECCA	Max signal 16S	N ASV seqs	1st station taxon found	last station taxon found
Cyanobacteria genotype unidentified	NA	808	347	01_PACENGO	10_SIRMIONE 1
Acaryochloris (Synechococcales)	marin1	2	1	04_RIVA	04_RIVA
Aliterella	marin2	167	22	01_PACENGO	10_SIRMIONE 1
Anabaena lemmermannii	R1539	12	2	02_CISANO	05_GOLA
Annamia sp.	fresh_new2	9	7	01_PACENGO	10_SIRMIONE 1
Aphanothece clathrata	R1427	71	7	01_PACENGO	10_SIRMIONE 1
Calothrix sp.	R2710	181	94	01_PACENGO	10_SIRMIONE 1
Chalicogloea sp.	aerophytic1	1	1	05_GOLA	05_GOLA
Chamaesiphon sp.	R1637	162	30	01_PACENGO	10_SIRMIONE 1
Chroococcidiopsaceae	new_16S_cyano_family1	60	8	01_PACENGO	10_SIRMIONE 1
Chroococcidiopsis	aerophytic2	154	19	01_PACENGO	10_SIRMIONE 1
Coleofasciculaceae	new_16S_cyano_family2	12	2	01_PACENGO	02_CISANO
Cyanobiaceae	new_16S_cyano_family3	35	3	01_PACENGO	05_GOLA
Cyanobium sp.	R2302	453	90	01_PACENGO	10_SIRMIONE 1
Cyanodictyon sp.	R1455	5	2	01_PACENGO	02_CISANO
Cyanophyceae sp.	R1638	3	3	02_CISANO	04_RIVA
Cyanothece sp.	R1948	25	6	01_PACENGO	10_SIRMIONE 1
Eucapsis sp.	R1961	3	1	01_PACENGO	01_PACENGO
Geitlerinema sp.	R2090	40	6	02_CISANO	10_SIRMIONE 1
Geminocystis	Picoplank1	7	5	01_PACENGO	10_SIRMIONE 1
Gloeocapsa sp.	R0888	22	15	01_PACENGO	08_MONIGA
Gloeothece linearis	R0893	12	3	01_PACENGO	05_GOLA
Leptolyngbya sp.	R1580	259	77	01_PACENGO	10_SIRMIONE 1
Leptolyngbyaceae	new_16S_cyano_family4	452	89	01_PACENGO	10_SIRMIONE 1
Lyngbya sp.	R1570	3	1	02_CISANO	02_CISANO
Merismopedia sp.	R1478	1	1	10_SIRMIONE 1	10_SIRMIONE 1
Microcystaceae	new_16S_cyano_family5	11	14	01_PACENGO	10_SIRMIONE 1
Microcystis sp.	R1496	5	4	02_CISANO	05_GOLA
Microseira	fresh_new4	20	2	02_CISANO	02_CISANO
Nodosilinea	biofilm_new5	23	13	01_PACENGO	10_SIRMIONE 1
Nostocaceae	new_16S_cyano_family7	586	42	01_PACENGO	10_SIRMIONE 1
Oscillatoria sp.	R1597	45	7	01_PACENGO	10_SIRMIONE 1
Phormidesmis	biofilm_new6	79	34	01_PACENGO	10_SIRMIONE 1
Phormidiaceae	new_16S_cyano_family9	8	1	04_RIVA	04_RIVA
Phormidium sp.	R1606	24	28	01_PACENGO	10_SIRMIONE 1
Pleurocapsa sp.	R2006	142	44	01_PACENGO	10_SIRMIONE 1
Pseudanabaena sp.	R1623	83	39	01_PACENGO	10_SIRMIONE 1
Pseudanabaenaceae	new_16S_cyano_family10	183	16	03_CASSONE	09_SIRMIONE 2
Schizothrix	biofilm_new4	132	8	04_RIVA	10_SIRMIONE 1
Scytolyngbya	biofilm_new10	13	7	01_PACENGO	10_SIRMIONE 1
Symphothece	biofilm_new2	10	3	09_SIRMIONE 2	10_SIRMIONE 1
Synechococcaceae	new_16S_cyano_family11	71	9	01_PACENGO	10_SIRMIONE 1
Synechococcus sp.	R1518	27	8	01_PACENGO	08_MONIGA
Synechocystis sp.	R1520	16	3	01_PACENGO	02_CISANO
Tolypothrix	biofilm_new1	6	1	01_PACENGO	01_PACENGO
Tychonema sp.	R2826	208	13	01_PACENGO	10_SIRMIONE 1
Wilmottia	biofilm_new7	19	2	04_RIVA	04_RIVA
Xenococcaceae	new_16S_cyano_family12	167	6	03_CASSONE	10_SIRMIONE 1
Xenococcus	biofilm_new8	2	2	01_PACENGO	02_CISANO

Suppl. Table 4.13. List of 37 corresponding diatom species from biofilm identified through microscopy and through HTS (rbcL reference database R-Syst::diatom) from Lake Garda littoral samples (n=10).

V9 species	HTS TAXON_R_Diatom	LM Validcode	LM diatoms BFM
Achnanthidium eutrophilum	Achnanthidium eutrophilum	ADEU	Achnanthidium eutrophilum
Achnanthidium minutissimum	Achnanthidium minutissimum	ADMI	Achnanthidium minutissimum
Amphora copulata	Amphora copulata	ACOP	Amphora copulata
Halamphora oligotraphenta	Amphora oligotraphenta	AOLG	Amphora oligotraphenta
Amphora ovalis	Amphora ovalis	AOVA	Amphora ovalis
Amphora pediculus	Amphora pediculus	APED	Amphora pediculus
Cocconeis pediculus	Cocconeis pediculus	CPED	Cocconeis pediculus
Cymbella excisa	Cymbella excisa var. excisa	CAEX	Cymbella excisa var. excisa
Cymbella helvetica	Cymbella helvetica	CHEL	Cymbella helvetica
Cymbella lanceolata	Cymbella lanceolata var. lanceolata	CLAN	Cymbella lanceolata var. lanceolata
Denticula tenuis	Denticula tenuis	DTEN	Denticula tenuis
Diatoma vulgaris	Diatoma vulgaris	DVUL	Diatoma vulgaris
Encyonema caespitosum	Encyonema caespitosum	ECAE	Encyonema caespitosum
Encyonema prostratum	Encyonema prostratum	EPRO	Encyonema prostratum
Encyonopsis subminuta	Encyonopsis subminuta	ESUM	Encyonopsis subminuta
Epithemia sorex	Epithemia sorex	ESOR	Epithemia sorex
Fragilaria acus/radians complex	Fragilaria radians	FRAD	Fragilaria radians
Gomphonema minutum	Gomphonema minutum fo. minutum	GMIN	Gomphonema minutum fo. minutum
Gomphonema pumilum var. pumilum	Gomphonema pumilum	GPUM	Gomphonema pumilum
Gomphonema unclassified	Gomphonema spec	GOMP	Gomphonema spec
Gomphonema tergestinum	Gomphonema tergestinum	GTER	Gomphonema tergestinum
Mayamaea permitis	Mayamaea atomus var. permitis	MAPE	Mayamaea atomus var. permitis
Navicula antonii	Navicula antonii	NANT	Navicula antonii
Navicula capitatoradiata	Navicula capitatoradiata	NCPR	Navicula capitatoradiata
Navicula cryptotenella	Navicula cryptotenella	NCTE	Navicula cryptotenella
Navicula radiosa	Navicula radiosa	NRAD	Navicula radiosa
Navicula tripunctata	Navicula tripunctata	NTPT	Navicula tripunctata
Navicula veneta	Navicula veneta	NVEN	Navicula veneta
Nitzschia dissipata	Nitzschia dissipata var. dissipata	NDIS	Nitzschia dissipata var. dissipata
Nitzschia dissipata var. media	Nitzschia dissipata var. media	NDME	Nitzschia dissipata var. media
Nitzschia fonticola	Nitzschia fonticola	NFON	Nitzschia fonticola
Nitzschia inconspicua	Nitzschia inconspicua	NINC	Nitzschia inconspicua
Pseudostaurosira brevistriata	Pseudostaurosira brevistriata	PSBR	Pseudostaurosira brevistriata
Rhoicosphenia abbreviata	Rhoicosphenia abbreviata	RABB	Rhoicosphenia abbreviata
Sellaphora pupula	Sellaphora pupula	SPUP	Sellaphora pupula
Staurosira construens	Staurosira construens	SCON	Staurosira construens
Staurosira venter	Staurosira venter	SSVE	Staurosira venter

Suppl. Table 4.14. List of 84 non-corresponding diatom species from microscopy to HTS (rbcL reference database R-Syst::diatom) from Lake Garda littoral samples (n=10).

LM diatoms BFM	LM Validcode
Achnanthidium lineare	ACLI
Achnanthidium minutissima var. affinis	ADMF
Achnanthidium saprophila	ADSA
Achnanthidium straubianum	ADSB
Achnanthidium subatomus	ADSU
Amphora inariensis	AINA
Amphora indistincta	newAIND
Aneumastus stroesei	ANSS
Caloneis lancettula	CLCT
Cavinula scutelloides	CVSO
Cocconeis neothumensis	CNTH
Cocconeis placentula var. euglypta	CPLE
Cocconeis placentula var. lineata	CPLI
Cyclotella comensis	CCMS
Cymbella compacta	CCMP
Cymbella excisa var. angusta	CEAN
Cymbella excisiformis var. excisiformis	CEXF
Cymbella hustedtii var. hustedtii	CHUS
Cymbella neoleptoceros var. neoleptoceros	CNLP
Cymbella parva	CPAR
Cymbella perparva	CPPV
Cymbella subhelvetica	CSBH
Diatoma ehrenbergii	DEHR
Diploneis oculata	DOCU
Discostella pseudostelligera	DPST
Encyonema auerswaldii	EAUE
Encyonema lacustre	ELAC
Encyonema minutum	ENMI
Encyonema silesiacum	ESLE
Encyonema subminutum	ENSU
Encyonopsis alpina	ECAL
Encyonopsis microcephala	ENCM
Encyonopsis minuta	ECPM
Epithemia adnata	EADN
Epithemia goeppertiana	EGOE
Fallacia subhamulata	FSBH
Fragilaria capucina var. austriaca	FCAU
Fragilaria capucina var. perminuta	FCPE
Fragilaria capucina var. radians	FCRA
Fragilaria capucina var. rumpens	FCRP
Fragilaria capucina var. vaucheriae	FCVA
Fragilaria crotonensis	FCRO
Fragilaria delicatissima	FDEL
Fragilaria gracilis	FGRA

Fragilaria neointermedia	newFNIN
Fragilaria tenera	FTEN
Geissleria ignota	GINO
Gomphonema angustivalva	GAGV
Gomphonema italicum	GITA
Gomphonema minutiforme	GMNF
Gomphonema olivaceolacuum	newGOUM
Gomphonema olivaceum var. olivaceolacuum	newGOVL
Gomphonema olivaceum var. olivaceum	GOLI
Gomphonema pumilum var. elegans	GPEL
Gomphonema pumilum var. rigidum	GPRI
Karayevia clevei	KCLE
Mayamaea atomus	MAAT
Navicula cryptotenelloides	NCTO
Navicula gottlandica	NGOT
Navicula jakovljevicii	NJAK
Navicula reichardtiana var. reichardtiana	NRCH
Navicula subalpina	NSBN
Navicula utermoehlii	NUTE
Navicula viridula	NVIR
Nitzschia angustata	NIAN
Nitzschia angustatula	NZAG
Nitzschia archibaldii	NIAR
Nitzschia frustulum var. frustulum	NIFR
Nitzschia heufleriana	NHEU
Nitzschia lacuum	NILA
Nitzschia palea var. debilis	NPAD
Nitzschia sociabilis	NSOC
Placoneis pseudanglica	PPSA
Planothidium frequentissimum	PLFR
Planothidium lanceolatum	PTLA
Planothidium rostratum	PRST
Platessa conspicua	PTCO
Rhopalodia gibba var. gibba	RGIB
Sellaphora verecundiae	SVER
Simonsenia delognei	SIDE
Staurosira construens var. binodis	SCBI
Staurosira elliptica	SELI
Staurosira martyi	SMAT
Staurosirella pinnata	newSTPN

Suppl. Table 4.15. List of 93 diatoms detected only by HTS and not found by microscopy (rbcL reference database R-Syst::diatom) from Lake Garda littoral samples (n=10).

V9 species	LM Validcode	HTS TAXON_R_Diatom
Achnanthidium delmontii	newADEL	Achnanthidium delmontii
Achnanthidium eutrophilum	ADEU	Achnanthidium eutrophilum
Achnanthidium minutissimum	ADMI	Achnanthidium minutissimum
Achnanthidium pyrenaicum	ADPT	Achnanthidium pyrenaicum
Achnanthidium unclassified		Achnanthidium spec
Amphora copulata	ACOP	Amphora copulata
Amphora ovalis	AOVA	Amphora ovalis
Amphora pediculus	APED	Amphora pediculus
Amphora unclassified	AMPH	Amphora spec
Aneumastus pseudoapiculatus	ANEP	Aneumastus pseudoapiculatus
Aneumastus unclassified	ANEU	Aneumastus spec
Caloneis fontinalis	CFON	Caloneis fontinalis
Caloneis unclassified	CALO	Caloneis spec
Cocconeis pediculus	CPED	Cocconeis pediculus
Cocconeis placentula	CPLA	Cocconeis placentula var. placentula
Craticula cuspidata	CRCU	Craticula cuspidata
Cyclotella meneghiniana	CMEN	Cyclotella meneghiniana
Cymbella excisa	CAEX	Cymbella excisa var. excisa
Cymbella helvetica	CHEL	Cymbella helvetica
Cymbella lanceolata	CLAN	Cymbella lanceolata var. lanceolata
Cymbella proxima	CPRX	Cymbella proxima var. proxima
Cymbella unclassified	CYMB	Cymbella spec
Cymbopleura inaequalis	CIQL	Cymbopleura inaequalis
Denticula tenuis	DTEN	Denticula tenuis
Diatoma vulgaris	DVUL	Diatoma vulgaris
Diploneis subovalis	DSBO	Diploneis subovalis
Discostella nipponica	DNIP	Discostella nipponica
Discostella woltereckii	DWOL	Discostella woltereckii
Encyonema caespitosum	ECAE	Encyonema caespitosum
Encyonema prostratum	EPRO	Encyonema prostratum
Encyonema unclassified	ENCY	Encyonema spec
Encyonema ventricosum	ENVE	Encyonema ventricosum
Encyonopsis sp.	ENCP	Encyonopsis spec
Encyonopsis subminuta	ESUM	Encyonopsis subminuta
Encyonopsis unclassified		Encyonopsis spec
Epithemia gibba	EPIT	Epithemia spec
Epithemia hyndmanii	EHYN	Epithemia hyndmanii
Epithemia sorex	ESOR	Epithemia sorex
Epithemia unclassified	EPIT	Epithemia spec
Fragilaria acus/radians complex	FRAD	Fragilaria radians
Fragilaria sp.	FRAS	Fragilaria species
Fragilaria unclassified	FRAG	Fragilaria spec
Gomphonella olivacea	GLOV	Gomphonella olivacea
Gomphonella olivaceolacuum	newGOMP	Gomphonella spec

Gomphonema minutum	GMIN	Gomphonema minutum fo. minutum
Gomphonema pumilum var. pumilum	GPUM	Gomphonema pumilum
Gomphonema saprophilum	GPAS	Gomphonema parvulum var. parvulum fo. saprophilum Lange-Bert. & Reichardt
Gomphonema tergestinum	GTER	Gomphonema tergestinum
Gomphonema unclassified	GOMP	Gomphonema spec
Gyrosigma sciotense		Gyrosigma sciotense
Halamphora oligotraphenta	AOLG	Amphora oligotraphenta
Iconella linearis	ICON	Iconella sp.
Iconella undassified	ICON	Iconella sp.
Mayamaea permitis	MAPE	Mayamaea atomus var. permitis
Melosira varians	MVAR	Melosira varians
Navicula antonii	NANT	Navicula antonii
Navicula capitatoradiata	NCPR	Navicula capitatoradiata
Navicula cari	NCAR	Navicula cari
Navicula cryptotenella	NCTE	Navicula cryptotenella
Navicula radiosa	NRAD	Navicula radiosa
Navicula tripunctata	NTPT	Navicula tripunctata
Navicula unclassified		
Navicula veneta	NVEN	Navicula veneta
Neidium unclassified		
Nitzschia denticula	NDEN	Nitzschia denticula
Nitzschia dissipata	NDIS	Nitzschia dissipata var. dissipata
Nitzschia dissipata var. media	NDME	Nitzschia dissipata var. media
Nitzschia draveillensis	NDRA	Nitzschia draveillensis
Nitzschia fonticola	NFON	Nitzschia fonticola
Nitzschia inconspicua	NINC	Nitzschia inconspicua
Nitzschia linearis	NLIN	Nitzschia linearis var. linearis
Nitzschia palea	NPAL	Nitzschia palea
Nitzschia paleacea	NPAE	Nitzschia paleacea
Nitzschia pusilla	NIPU	Nitzschia pusilla
Nitzschia sigmoidea	NSIO	Nitzschia sigmoidea
Nitzschia unclassified	NITZ	Nitzschia spec
Pantocsekiella costei	PSBR	Cydotella costei
Pseudostaurosira brevistriata	PSBR	Pseudostaurosira brevistriata
Reimeria sinuata	RSIN	Reimeria sinuata
Rhoicosphenia abbreviata	RABB	Rhoicosphenia abbreviata
Sellaphora lanceolata	SLCL	Sellaphora lanceolata
Sellaphora nigri	newSNIG	Sellaphora nigri
Sellaphora obesa	SOBE	Sellaphora obesa
Sellaphora pupula	SPUP	Sellaphora pupula
Sellaphora undassified	SELL	Sellaphora spec
Staurosira construens	SCON	Staurosira construens
Staurosira sp.	STRS	Staurosira spec
Staurosira unclassified	STRS	Staurosira spec
Staurosira venter	SSVE	Staurosira venter
Surirella elliptica	CELL	Cymatopleura elliptica var. elliptica
Surirella solea	CSOL	Cymatopleura solea var. solea
Ulnaria ulna	UULN	Ulnaria ulna
Ulnaria unclassified	ULNA	Ulnaria spec

8.5 L. Bled, Slovenia

*Suppl Table 5.1. List of **corresponding phytoplankton** species identified through microscopy and through HTS (SILVA reference database) from the Lake Bled **pelagic** samples (n=12).*

Locus	LM_phytoplankton	ID-REBECCA	HTS_18S+16S	Class
S16	Planktothrix rubescens	R1617	Planktothrix sp.	Cyanobacteria
S16	Aphanizomenon sp.	R1562	Aphanizomenon sp.	Cyanobacteria
S16	Anabaena lemmermannii	R1539	Aphanizomenon sp.	Cyanobacteria
S18	Asterionella formosa	R0135	Asterionella formosa	Bacyllaryophyceae
S18	Fragilaria crotonensis	R0223	Fragilaria crotonensis	Bacyllaryophyceae
S18	Ulnaria delicatissima	R2173	Ulnaria ulna	Bacyllaryophyceae
S18	Ulnaria delicatissima var. *angustissima*	R2174	Ulnaria ulna	Bacyllaryophyceae
S18	Stephanodiscus neoastrea	R0086	Stephanodiscus sp.	Bacyllaryophyceae
S18	Bicosoeca eurystoma	R0464	Bicosoeca sp.	Bicosoecophyceae
S18	Dinobryon crenulatum	R1086	Dinobryon sp.	Chrysophyceae
S18	Dinobryon cylindricum	R1086	Dinobryon sp.	Chrysophyceae
S18	Dinobryon divergens v. schauinslandii	R1086	Dinobryon sp.	Chrysophyceae
S18	Dinobryon petiolatum	R1086	Dinobryon sp.	Chrysophyceae
S18	Dynobryon sertularia	R1086	Dinobryon sp.	Chrysophyceae
S18	Ochromonas sp.	new18R63	Ochromonas danica	Chrysophyceae
S18	Uroglena sp. (americana)	R1151	Uroglena sp.	Chrysophyceae
S18	Mallomonas akaroides	R1109	Mallomonas sp.	Synurophyceae
S18	Mallomonas caudata	R1109	Mallomonas sp.	Synurophyceae
S18	Mallomonas elongata	R1109	Mallomonas sp.	Synurophyceae
S18	Mallomonas sp.	R1109	Mallomonas sp.	Synurophyceae
S18	Cryptomonas marssonii	R1382	Cryptomonas sp.	Chrysophyceae
S18	Cryptomonas ovata	R1386	Cryptomonas sp.	Chrysophyceae
S18	Cryptomonas erosa	R1378	Cryptomonas sp.	Chrysophyceae
S18	Ceratium hirrundinela	R1672	Ceratium hirundinella	Dinophyceae
S18	Peridinium cinctum	R1687	Peridinium cinctum	Dinophyceae
S18	Peridinium umbonatum - complex	R1704	Peridinium willei	Dinophyceae
S18	Gymnodinium fuscum	R1647	Gymnodinium helveticum	Dinophyceae
S18	Gymnodinium uberrimum	R1654	Gymnodinium sp.	Dinophyceae
S18	Ankyra ancora	R0489	Ankyra sp.	Chlorophyceae
S18	Ankyra lanceolata	R0489	Ankyra sp.	Chlorophyceae
S18	Botryococcus braunii	R0493	Botryococcus_braunii	Treuboxiophyceae
S18	Chlamydomonas sp.	R0941	Chlamydomonas sp.	Chlorophyceae
S18	Phacotus lenticularis	R0975	Phacotus lenticularis	Chlorophyceae
S18	Tetraselmis cordiformis	R0996	Tetraselmis cordiformis	Chlorodendrophyceae

*Suppl Table 5.2. List of **non-corresponding phytoplankton** species from microscopy to HTS (SILVA reference database) from Lake Bled **pelagic** samples (n=12).*

Locus	LM_phytoplankton	ID-REBECCA	class
16S	Chroococcus planctonicus	R1444	Cyanophyceae
16S	Microcystis flos-aquae	R1487	Cyanophyceae
16S	Anabaena lemmermannii	R1539	Cyanophyceae
16S	Pseudanabaena	R1623	Cyanophyceae
18S	Achnanthes sp.	R0117	Bacillariophyceae
18S	Cocconeis placentula	R0155	Bacillariophyceae
18S	Cyclotella comensis	R0042	Bacillariophyceae
18S	Cyclotella cyclopuncta	R2195	Bacillariophyceae
18S	Cyclotella ocellata	R0048	Bacillariophyceae
18S	Cyclotella radiosa	R0051	Bacillariophyceae
18S	Nitzschia sp.	R0394	Bacillariophyceae
18S	Nitzschia acicularis	R0343	Bacillariophyceae
18S	Navicula cryptocephala	R0295	Bacillariophyceae
18S	Bitrichia chodatii	R1155	Chrysophyceae
18S	Chrysolykos planctonicus	R1166	Chrysophyceae
18S	Kephiryon sp.	R1037	Chrysophyceae
18S	Pseudokephyrion pseudospirale	R1050	Chrysophyceae
18S	Stichogloea globosa	SI3235	Chrysophyceae
18S	Chilomonas sp.	R1367	Cryptophyceae
18S	Rhodomonas lacustris	SI3300	Cryptophyceae
18S	Ankistrodesmus falcatus	R0480	Chlorophyceae
18S	Chlorococcales - pico	R0505	Chlorophyceae
18S	Closterium acutum var. variabile	R1181	Conjugatophyceae
18S	Coenococcus planctonicus	R0606	Chlorophyceae
18S	Coelastrum reticulatum	R0530	Chlorophyceae
18S	Cosmarium bioculatum var. depressum	R2278	Conjugatophyceae
18S	Chloromonas sp.	R0962	Chlorophyceae
18S	Lagerheimia genevensis	R0649	Chlorophyceae
18S	Chlamydocapsa planctonica	R0930	Chlorophyceae
18S	Elakatothrix gelatinosa	R0596	Ulvophyceae
18S	Koliella longiseta	R0635	Ulvophyceae
18S	Planktonema lauterbornii	R0919	Ulvophyceae
18S	Monoraphidium griffithii	R0670	Chlorophyceae
18S	Oocystis lacustris	R0697	Chlorophyceae
18S	Oocystis marssonii	R0698	Chlorophyceae
18S	Planktosphaeria gelatinosa	R0727	Chlorophyceae
18S	Schroederia setigera	R0820	Chlorophyceae
18S	Scenedesmus linearis	R0792	Chlorophyceae
18S	Tetrastrum komarekii	R0866	Chlorophyceae
18S	Tetraedron minimum	R0848	Chlorophyceae
18S	Tetraedron tumidulum	R0861	Chlorophyceae
18S	Euglena sp. (texta)	R1726	Euglenophyceae
18S	Phacus longicauda	R1741	Euglenophyceae

18S	Trachelomonas volvocina	R1776	Euglenophyceae

*Suppl Table 5.3. List of cyanobacteria species detected under the microscope, which could belong to the new genus Cyanobium detected by HTS (SILVA reference database) Lake Bled **pelagic** samples (n=12).*

Locus	LM_phytoplankton	ID-REBECCA	Class
16S	Aphanocapsa holsatica	R1415	Cyanophyceae
16S	Aphanothece clathrata	R1427	Cyanophyceae
16S	Aphanothece floccosa	R1428	Cyanophyceae
16S	Aphanocapsa delicatissima	R1413	Cyanophyceae
16S	Aphanocapsa planctonica	R2239	Cyanophyceae
16S	Cyanodictyon	R1455	Cyanophyceae
16S	Cyanodictyon planctonicum	R1453	Cyanophyceae
16S	Cyanodictyon reticulatum	R1454	Cyanophyceae

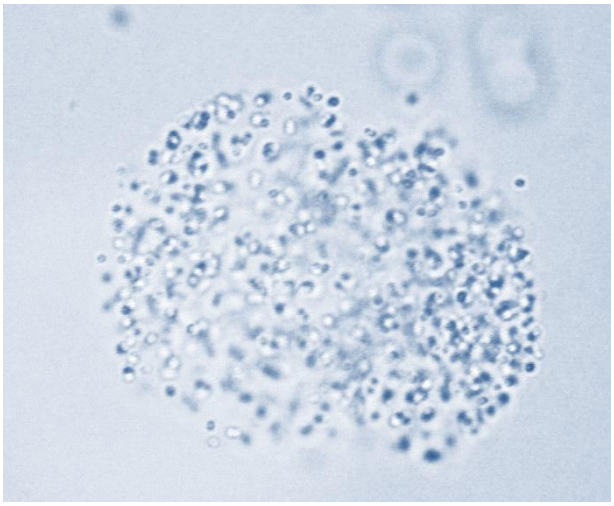

Fig 5.4: Lake Bled, 09.08.2019 Aphanocapsa sp.

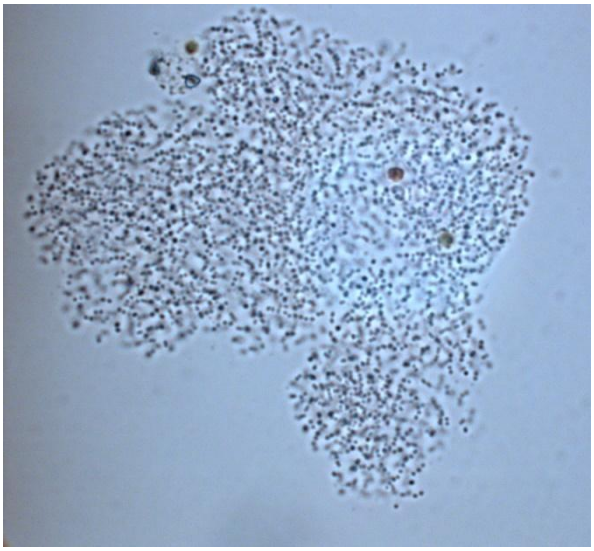

Fig 5.6: Lake Bled, 26.11.2019 Cyanodyction sp.

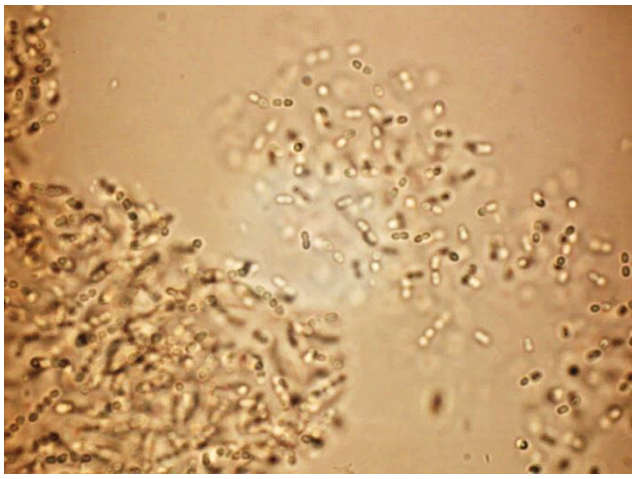

Fig 5.7: Lake Bled, 26.11.2019 Cyanodyction sp.

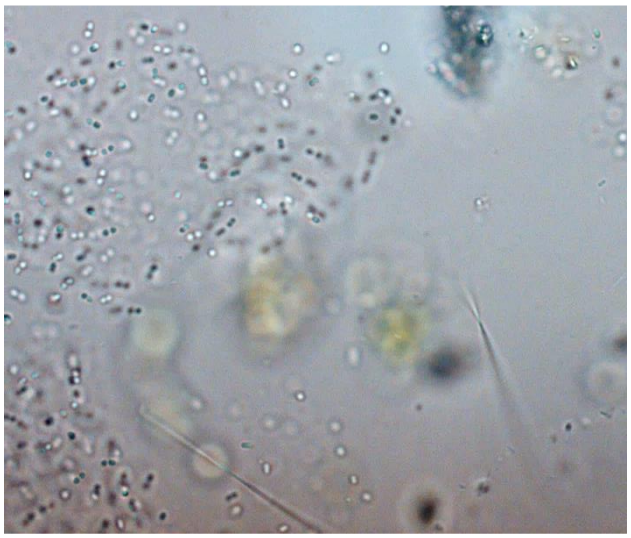

Fig 5.8: Lake Bled 09.08.2019 Cyanobium sp.

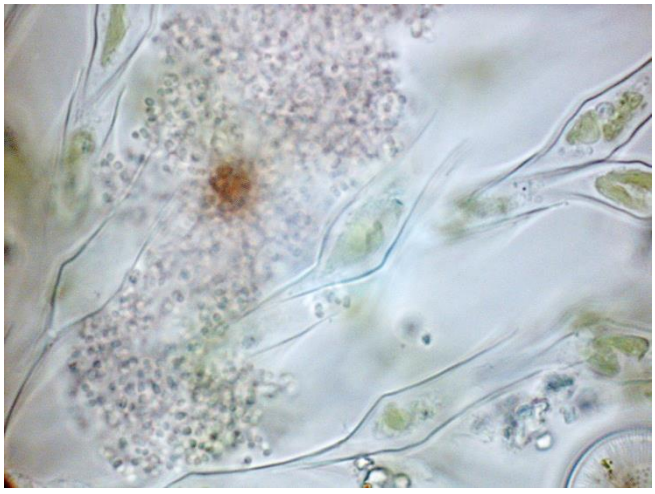

Fig 5.9: Lake Bled 08.10.2019 (Aphanothece sp.)

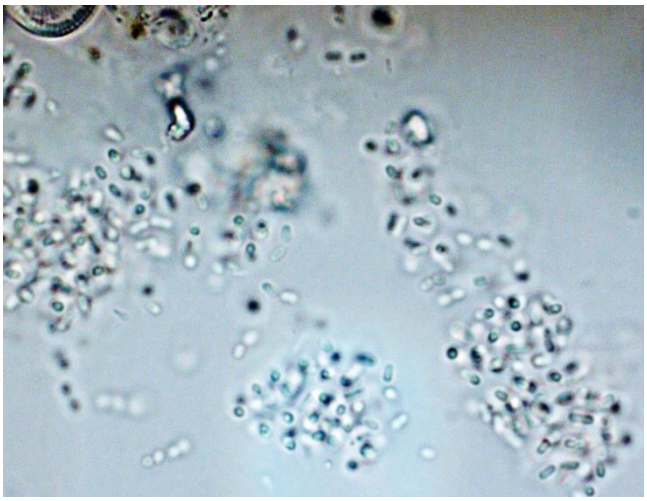

Fig 5.10: Lake Bled 08.10.2019 (Aphanothece sp.)

*Suppl Table 5.4. List of **non-corresponding phytoplankton** species from HTS to microscopy (SILVA reference database) from Lake Bled **pelagic** samples (n=12).*

Locus	ID-REBECCA	HTS_18S + 16S Taxon	Phylum/Class
16S	R2302	Cyanobium sp.	Cyanophyceae
16S	new_16S_cyano_family3	Cyanobiaceae	Cyanophyceae
16S	R1518	Synechococcus sp.	Cyanophyceae
16S	new_16S_cyano_family3	Prochlorothrix PCC-9006	Cyanophyceae
16S	new_16S_cyano_family8	Obscuribacteraceae	Cyanobacteriia
18S	R0449	Bacillariophyceae sp.	Bacillariophyceae
18S	R2862	Synedra sp.	Bacillariophyceae
18S	R2175	Ulnaria ulna	Bacillariophyceae
18S	R2784	Encyonopsis sp.	Bacillariophyceae
18S	R0238	Fragilaria sp.	Bacillariophyceae
18S	R0086	Stephanodiscus sp.	Bacillariophyceae
18S	new	Bicoecaceae_X_sp.	Bicoecea
18S	new	Pseudodendromonadales_XX_sp.	Bicoecea
18S	new	Parmales_env_2_X_sp.	Bolydophyceae
18S	new	Parmales	Bolydophyceae
18S	R0905	Chlorophyceae sp.	Chlorophyta/Chlorodendrophyceae
18S	new18R25	Hafniomonas reticulata	Chlorophyta/Chlorophyceae
18S	R0941	Chlamydomonas sp.	Chlorophyta/Chlorophyceae
18S	R0989	Volvocales sp.	Chlorophyta/Chlorophyceae
18S	R2456	Sphaeropleaceae Gen. sp.	Chlorophyta/Chlorophyceae
18S	R0832	Chlorococcales sp.	Chlorophyta/Chlorophyceae
18S	new18R3	Trebouxiophyceae	Chlorophyta/Trebouxiophyceae
18S	R0517	Choricystis_sp.	Chlorophyta/Trebouxiophyceae Trebouxiophyceae
18S	new18R10	Crustomastigaceae	Chlorophyta/Mamiellophyceae
18S	new18R11	Dolichomastigaceae	Chlorophyta/Mamiellophyceae
18S	new18R34	Mamiella_gilva	Chlorophyta/Mamiellophyceae

18S	R1815	Monomastix sp.	Chlorophyta/Mamiellophyceae
18S	new18R10	Crustomastigaceae	Chlorophyta
18S	R2724	Pedinella hexacostata	Dictyochophyceae
18S	R1154	Pseudopedinella sp.	Dictyochophyceae
18S	new18R2	Eustigmatophyceae_X	Eustigmatophyceae Xantophyceae
18S	R2809	Pseudotetraedriella kamillae	Eustigmatophyceae Xantophyceae
18S	R1097	Mallomonas akrokomos	Synurophyceae
18S	R1109	Mallomonas sp.	Synurophyceae
18S	R1171	Chrysophyceae sp.	Synurophyceae
18S	R2679	Chrysocapsa planctonica	Chrysophyceae
18S	R1083	Dinobryon sociale	Chrysophyceae
18S	R1086	Dinobryon sp.	Chrysophyceae
18S	R1123	Paraphysomonas sp.	Chrysophyceae
18S	R1377	Cryptomonas curvata	Cryptophyceae
18S	R1389	Cryptomonas pyrenoidifera	Cryptophyceae
18S	R1401	Cryptomonas tetrapyrenoidosa	Cryptophyceae
18S	R1394	Cryptomonas sp.	Cryptophyceae
18S	R1412	Cryptophyceae sp.	Cryptophyceae
18S	R1708	Dinophyceae sp.	Dinophyta
18S	R1708	Dinophyceae sp.	Dynophyta/Dynophyceae
18S	new18R6	Gymnodiniaceae	Dynophyta/Dynophyceae
18S	R1654	Gymnodinium sp.	Dynophyta/Dynophyceae
18S	R1647	Gymnodinium helveticum	Dynophyta/Dynophyceae
18S	R1704	Peridinium willei	Dynophyta/Dynophyceae
18S	new18R9	Thoracosphaeraceae	Dynophyta/Dynophyceae
18S	R1706	Prorocentrum sp.	Dynophyta/Dynophyceae
18S	new18R12	Asulcocephalium miricentonis	Dynophyta/Dynophyceae
18S	new18R75	Polarella glacialis	Dynophyta/Dynophyceae
18S	R1818	Chrysochromulina_parva	Haptophyta/Prymnesiophyceae
18S	R1819	Chrysochromulina_sp.	Haptophyta/Prymnesiophyceae
18S	R2427	Prymnesiophyceae_Clade_C1_X_sp.	Haptophyta/Prymnesiophyceae

*Suppl Table 5.5: List of **cyanobacteria and soft algae taxons** from **biofilm** identified through microscopy from Bled littoral samples (n=10). Frequency in classes 1 to 5, where 1 is equally very rare and 5 dominant.*

Sampling site Taxon	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Homoeothrix varians	3	3	2	3	3	3	2	2	3	2
Pleurocapsa minor	1			1	1	3		1	1	
***Oedogonium* sp.**	1	1	1	1	2	1	2	1	1	1
***Oscillatoria* sp.**		1								
***Phormidium* sp.**		1	1	1	1		1	1		
Dinobryon divergens		1	1	2	1	2		1		1
***Aphanothece* sp.**			1				1			
***Schizothrix* sp.**			1							
Planctonema lauterbornii			1							
***Spirogyra* sp.**							1			
***Cosmarium* sp.**								1		
***Mougeotia* sp.**										1

*Suppl Table 5.6. List of **cyanobacteria** species from **biofilm** identified through HTS (16S rDNA SILVA reference database) from Bled littoral samples (n=10).*

ID_REBECCA	Taxon_REBECCA	family_16S	genus_16S	species_16S
marin1	Acaryochloris (Synechococcales)	Acaryochloridaceae	Acaryochloris MBIC11017	NA
aerophytic2	Chroococcidiopsis	Chroococcidiopsaceae	Chroococcidiopsis PCC 7203	NA
marin2	Aliterella	Chroococcidiopsaceae	Aliterella	NA
new_16S_cyano_family1	Chroococcidiopsaceae	Chroococcidiopsaceae	NA	NA
NA	NA	Coleofasciculaceae	Potamolinea 1PC	NA
new_16S_cyano_family2	Coleofasciculaceae	Coleofasciculaceae	NA	NA
biofilm_new2	Symphothece	Cyanobacteriaceae	Symphothece PCC-7002	NA
fresh_new2	Annamia sp.	Cyanobacteriaceae	Annamia HOs24	NA
NA	(prazno)	Cyanobacteriaceae	NA	NA
Picoplank1	Geminocystis	Cyanobacteriaceae	Geminocystis PCC-6308	NA
R1478	Merismopedia sp.	Cyanobacteriaceae	Merismopedia AICB1015	NA
R2090	Geitlerinema sp.	Cyanobacteriaceae	Geitlerinema LD9	NA
R1427	Aphanothece clathrata	Cyanobiaceae	Cyanobium PCC-6307	NA
R1518	Synechococcus sp.	Cyanobiaceae	Cyanobium PCC-6307	NA
R2302	Cyanobium sp.	Cyanobiaceae	Cyanobium PCC-6307	gracile
biofilm_new12	Candidatus Gloeomargarita	Eurycoccales	Candidatus Gloeomargarita	NA
R2090	Geitlerinema sp.	Geitlerinemaceae	Geitlerinema PCC-7105	NA
R2090	Geitlerinema sp.	Geitlerinemaceae	Geitlerinema PCC-9228	NA
R0893	Gloeothece linearis	Gloeobacteraceae	Gloeobacter PCC-7421	NA
R0888	Gloeocapsa sp.	Gloeocapsaceae	Gleocapsa	NA
R1438	Chroococcus limneticus	Gloeocapsaceae	Gleocapsa	NA
biofilm_new10	Scytolyngbya	Leptolyngbyaceae	Scytolyngbya XSP1	NA
new_16S_cyano_family4	Leptolyngbyaceae	Leptolyngbyaceae	HAVOmat113	NA
new_16S_cyano_family4	Leptolyngbyaceae	Leptolyngbyaceae	LB3-76	NA
new_16S_cyano_family4	Leptolyngbyaceae	Leptolyngbyaceae	NA	NA
R1580	Leptolyngbya sp.	Leptolyngbyaceae	Leptolyngbya SAG 2411	NA
R1637	Chamaesiphon sp.	Leptolyngbyaceae	Chamaesiphon PCC-7430	NA
aerophytic1	Chalicogloea sp.	Microcystaceae	Chalicogloea CCALA 975	NA
NA	(prazno)	Microcystaceae	SU2 symbiont group	NA
new_16S_cyano_family5	Microcystaceae	Microcystaceae	NA	NA
R1496	Microcystis sp.	Microcystaceae	Microcystis PCC-7914	NA
R1500	Radiocystis geminata	Microcystaceae	NA	NA
R1513	Snowella sp.	Microcystaceae	Snowella 0TU37S04	NA
R1520	Synechocystis sp.	Microcystaceae	Synechocystis BDHKU-20401	NA
R1520	Synechocystis sp.	Microcystaceae	Synechocystis CCALA 700	NA
R1520	Synechocystis sp.	Microcystaceae	Synechocystis PCC-6803	NA
R1948	Cyanothece sp.	Microcystaceae	Cyanothece PCC-8801	NA
R1961	Eucapsis sp.	Microcystaceae	Chalicogloea CCALA 975	NA
R2006	Pleurocapsa sp.	Microcystaceae	Pleurocapsa PCC-7327	NA
R1500	Radiocystis geminata	NA	NA	NA
R1576	Geitlerinema splendidum	NA	NA	NA
biofilm_new5	Nodosilinea	Nodosilineaceae	Nodosilinea PCC-7104	NA

new_16S_cyano_family6	Nodosilineaceae	Nodosilineaceae	NA	NA
aerophytic3	Fischerella	Nostocaceae	Fischerella PCC-9339	NA
biofilm_new3	Rivularia	Nostocaceae	Rivularia PCC-7116	atra
new_16S_cyano_family7	Nostocaceae	Nostocaceae	NA	NA
R2710	Calothrix sp.	Nostocaceae	Calothrix PCC-6303	NA
R2710	Calothrix sp.	Nostocaceae	Calothrix UAM 374	NA
NA	NA	Oscillatoriaceae	Planktothricoides SR001	NA
R1606	Phormidium sp.	Oscillatoriaceae	Phormidium ETS-05	NA
biofilm_new6	Phormidesmis	Phormidesmiaceae	Phormidesmis ANT.LACV5.1	NA
new_16S_cyano_family9	Phormidiaceae	Phormidiaceae	NA	NA
R1606	Phormidium sp.	Phormidiaceae	Kamptonema PCC-6407	NA
R1634	Tychonema bornetii	Phormidiaceae	Tychonema CCAP 1459-11B	bornetii
R2826	Tychonema sp.	Phormidiaceae	Tychonema CCAP 1459-11B	NA
fresh_new5	Prochlorothrix PCC-9006	Prochlorotrichaceae	Prochlorothrix PCC-9006	NA
new_16S_cyano_family10	Pseudanabaenaceae	Pseudanabaenaceae	NA	NA
R1518	Synechococcus sp.	Pseudanabaenaceae	Synechococcus PCC-7502	NA
R1623	Pseudanabaena sp.	Pseudanabaenaceae	Pseudanabaena PCC-7429	foetida/limnetica
R1623	Pseudanabaena sp.	Pseudanabaenaceae	Pseudanabaena PCC-7429	frigida
R1623	Pseudanabaena sp.	Pseudanabaenaceae	Pseudanabaena PCC-7429	galeata
R1623	Pseudanabaena sp.	Pseudanabaenaceae	Pseudanabaena PCC-6802	NA
R1623	Pseudanabaena sp.	Pseudanabaenaceae	Pseudanabaena PCC-7403	NA
R1623	Pseudanabaena sp.	Pseudanabaenaceae	Pseudanabaena PCC-7429	NA
new_16S_cyano_family11	Synechococcaceae	Synechococcaceae	NA	NA
biofilm_new4	Schizothrix	Synechococcales Incertae Sedis	Schizothrix LEGE 07164	NA
R1948	Cyanothece sp.	Thermosynechococcaceae	Cyanothece PCC 7425	NA
NA	(prazno)	Unknown Family	NA	NA
R1580	Leptolyngbya sp.	Unknown Family	Leptolyngbya ANT.L52.2	NA
R1597	Oscillatoria sp.	Unknown Family	Oscillatoria SAG 1459-8	NA
R1606	Phormidium sp.	Unknown Family	Phormidium CYN64	NA
R2710	Calothrix sp.	Unknown Family	Calothrix KVSF5	NA
new_16S_cyano_family12	Xenococcaceae	Xenococcaceae	NA	NA
R2006	Pleurocapsa sp.	Xenococcaceae	Pleurocapsa PCC-7319	NA

*Suppl Table 5.7: List of **soft algae** from **biofilm** identified through HTS (18S rDNA SILVA reference database) from Lake Bled littoral samples (n = 10)*

ID REBECCA	Taxon_REBECCA	Family_18S	Genus_18S	Species_18Sraw
new18R4	Ulvophyceae	Ulvales-relatives_X	Acrochaete	Acrochaete_leptochaete
new18R105	Apatococcus lobatus	Watanabea-Clade_X	Apatococcus	Apatococcus_lobatus
R2456	Sphaeropleaceae Gen. sp.	Sphaeropleales_X	Asterarcys	Asterarcys_quadricellulare
new18R12	Asulcocephalium miricentonis	Suessiaceae	Asulcocephalium	Asulcocephalium_miricentonis
R1672	Ceratium hirundinella	Ceratiaceae	Ceratium	Ceratium_hirundinella
R0989	Volvocales sp.	Chlamydomonadales_X	Chlamydomonadales_XX	Chlamydomonadales_XX_sp.
R0940	Chlamydomonas reinhardtii	Chlamydomonadales_X	Chlamydomonas	Chlamydomonas_reinhardtii
R0941	Chlamydomonas sp.	Chlamydomonadales_X	Chlamydomonas	Chlamydomonas_sp.
R0517	Choricystis sp.	Trebouxiophyceae_XX	Choricystis	Choricystis_sp.

R1818	Chrysochromulina parva	Chrysochromulinaceae	Chrysochromulina	Chrysochromulina_parva
R1171	Chrysophyceae sp.	Chrysophyceae_Clade-C	Chrysophyceae_Clade-C_X	Chrysophyceae_Clade-C_X_sp.
R1171	Chrysophyceae sp.	Chrysophyceae_Clade-D	Chrysophyceae_Clade-D_X	Chrysophyceae_Clade-D_X_sp.
R1209	Cosmarium depressum	Zygnemophyceae_XX	Cosmarium	Cosmarium_depressum
R1389	Cryptomonas pyrenoidifera	Cryptomonadales_X	Cryptomonas	Cryptomonas_pyrenoidifera
R1401	Cryptomonas tetrapyrenoidosa	Cryptomonadales_X	Cryptomonas	Cryptomonas_tetrapyrenoidosa
new18R16	Desmochloris sp.	Ulvales-relatives_X	Desmochloris	Desmochloris_sp.
new18R17	Desmodesmus communis	Sphaeropleales_X	Desmodesmus	Desmodesmus_communis
new18R2	Eustigmatophyceae	Eustigmatophyceae_XX	Eustigmatophyceae_XXX	Eustigmatophyceae_XXX_sp.
R0617	Golenkinia sp.	Chlamydomonadales_X	Golenkinia	Golenkinia_sp.
R1654	Gymnodinium sp.	Gymnodiniaceae	Gymnodinium	Gymnodinium_sp.
new18R27	Hemidinium nasutum	Peridiniales_X	Hemidinium	Hemidinium_nasutum
R1171	Chrysophyceae sp.	Chrysophyceae_XX	Leukarachnion	Leukarachnion_sp.
R1003	Mougeotia sp.	Zygnemophyceae_XX	Mougeotia	Mougeotia_sp.
new18R37	Mychonastes sp.	Sphaeropleales_X	Mychonastes	Mychonastes_sp.
new18R2	Eustigmatophyceae	Eustigmatophyceae_XX	NA	NA
new18R3	Trebouxiophyceae	Chlorellales_X	NA	NA
new18R3	Trebouxiophyceae	Prasiolales_X	NA	NA
new18R3	Trebouxiophyceae	Watanabea-Clade_X	NA	NA
new18R4	Ulvophyceae	Cladophorales_X	NA	NA
new18R4	Ulvophyceae	NA	NA	NA
new18R4	Ulvophyceae	Ulotrichales_X	NA	NA
new18R4	Ulvophyceae	Ulvales-relatives_X	NA	NA
new18R40	Aphanochaete sp.	Chaetophorales_X	Aphanochaete	NA
new18R5	Chaetophorales	Chaetophorales_X	NA	NA
new18R51	Poterioochromonas sp.	Chrysophyceae_Clade-C	Poterioochromonas	NA
new18R9	Thoracosphaeraceae	Thoracosphaeraceae	NA	NA
R0506	Chlorococcum sp.	Chlamydomonadales_X	Chlorococcum	NA
R0517	Choricystis sp.	Trebouxiophyceae_XX	Choricystis	NA
R0617	Golenkinia sp.	Chlamydomonadales_X	Golenkinia	NA
R0747	Radiococcus sp.	Sphaeropleales_X	Radiococcus	NA
R0811	Scenedesmus sp.	Sphaeropleales_X	Desmodesmus	NA
R0811	Scenedesmus sp.	Sphaeropleales_X	Scenedesmus	NA
R0832	Chlorococcales sp.	Sphaeropleales_X	NA	NA
R0902	Oedogonium sp.	Oedogoniales_X	Oedogonium	NA
R0905	Chlorophyceae sp.	Chaetopeltidales_X	NA	NA
R0905	Chlorophyceae sp.	NA	NA	NA
R0905	Chlorophyceae sp.	Oedogoniales_X	NA	NA
R0941	Chlamydomonas sp.	Chlamydomonadales_X	Chlamydomonas	NA
R0989	Volvocales sp.	Chlamydomonadales_X	NA	NA
R1055	Hydrurus foetidus	Chrysophyceae_Clade-D	Hydrurus	NA
R1086	Dinobryon sp.	Chrysophyceae_Clade-C	Dinobryon	NA
R1093	Epipyxis sp.	Chrysophyceae_Clade-C	Epipyxis	NA
R1120	Ochromonas sp.	Chrysophyceae_Clade_C	Ochromonas	NA
R1162	Chrysamoeba sp.	Chrysophyceae_Clade-B2	Chrysamoeba	NA

R1171	Chrysophyceae sp.	Chrysophyceae_Clade-B2	NA	NA
R1171	Chrysophyceae sp.	Chrysophyceae_Clade-C	NA	NA
R1171	Chrysophyceae sp.	Chrysophyceae_Clade-E	NA	NA
R1171	Chrysophyceae sp.	Chrysophyceae_Clade-F	NA	NA
R1171	Chrysophyceae sp.	NA	NA	NA
R1201	Closterium sp.	Zygnemophyceae_XX	Closterium	NA
R1340	Zygnematales sp.	Zygnemophyceae_XX	NA	NA
R1343	Spirogyra sp.	Zygnemophyceae_XX	Spirogyra	NA
R1358	Monosiga sp.	Zygnemophyceae_XX	Mougeotia	NA
R1394	Cryptomonas sp.	Cryptomonadales_X	Cryptomonas	NA
R1412	Cryptophyceae sp.	Cryptomonadales_X	NA	NA
R1699	Peridinium sp.	Peridiniaceae	Peridinium	NA
R1708	Dinophyceae sp.	NA	NA	NA
R1708	Dinophyceae sp.	NA	NA	NA
R1816	Pedinomonas sp.	Pedinomonadaceae	Pedinomonas	NA
R2456	Sphaeropleaceae Gen. sp.	NA	NA	NA
R1811	Nephroselmis olivacea	Nephroselmidales_X	Nephroselmis	Nephroselmis_olivacea
R1120	Ochromonas sp.	Chrysophyceae_Clade_C	Ochromonas	Ochromonas_sp.
new18R64	Ochromonas sphaerocystis	Chrysophyceae_Clade_C	Ochromonas	Ochromonas_sphaerocystis
R0902	Oedogonium sp.	Oedogoniales_X	Oedogonium	Oedogonium_sp.
new18R101	Oocystella oogama	Chlorellales_X	Oocystella	Oocystella_oogama
new18R66	Oocystis nephrocytioides	Chlorellales_X	Oocystis	Oocystis_nephrocytioides
R1123	Paraphysomonas sp.	Chrysophyceae_Clade-F	Paraphysomonas	Paraphysomonas_sp.
R1691	Peridinium inconspicuum	Peridiniopsidaceae	Parvodinium	Parvodinium_inconspicuum
R1687	Peridinium cinctum	Peridiniaceae	Peridinium	Peridinium_cinctum
R0975	Phacotus lenticularis	Chlamydomonadales_X	Phacotus	Phacotus_lenticularis
R2005	Planctonema sp.	Chlorellales_X	Planctonema	Planctonema_sp.
R1706	Prorocentrum sp.	Prorocentraceae	Prorocentrum	Prorocentrum_sp.
R1409	Rhodomonas sp.	Cryptomonadales_X	Rhodomonas	Rhodomonas_sp.
R0762	Scenedesmus armatus	Sphaeropleales_X	Scenedesmus	Scenedesmus_armatus
new18R86	Scenedesmus obliquus	Sphaeropleales_X	Scenedesmus	Scenedesmus_obliquus
new18R88	Scotinosphaera lemnae	Chlorophyta_XXX	Scotinosphaera	Scotinosphaera_lemnae
R1343	Spirogyra sp.	Zygnemophyceae_XX	Spirogyra	Spirogyra_sp.
R2469	Staurastrum punctulatum	Zygnemophyceae_XX	Staurastrum	Staurastrum_punctulatum
R0996	Tetraselmis cordiformis	Chlorodendraceae	Tetraselmis	Tetraselmis_cordiformis

*Suppl Table 5.8. List of **corresponding diatom taxa** from biofilm identified through microscopy (LM) and through HTS (rbcL reference database R-Syst::diatom) from Bled littoral samples (n=10).*

V9 species	TAXON_HTS	Validcode	Taxon_LM
Achnanthidium minutissimum	Achnanthidium minutissimum	ADMI	Achnanthidium minutissimum
Amphora ovalis	Amphora ovalis	AOVA	Amphora ovalis
Amphora pediculus	Amphora pediculus	APED	Amphora pediculus
Cocconeis pediculus	Cocconeis pediculus	CPED	Cocconeis pediculus
Encyonema caespitosum	Encyonema caespitosum	ECAE	Encyonema caespitosum
Encyonema prostratum	Encyonema prostratum	EPRO	Encyonema prostratum
Encyonopsis microcephala	Encyonopsis microcephala	ENCM	Encyonopsis microcephala
Fragilaria unclassified	Fragilaria spec	FRAG	Fragilaria spec
Gomphonema tergestinum	Gomphonema tergestinum	GTER	Gomphonema tergestinum
Gomphonema unclassified	Gomphonema spec	GOMP	Gomphonema spec
Melosira varians	Melosira varians	MVAR	Melosira varians
Navicula antonii	Navicula antonii	NANT	Navicula antonii
Navicula cryptotenella	Navicula cryptotenella	NCTE	Navicula cryptotenella
Navicula radiosa	Navicula radiosa	NRAD	Navicula radiosa
Navicula tripunctata	Navicula tripunctata	NTPT	Navicula tripunctata
Nitzschia dissipata	Nitzschia dissipata var. dissipata	NDIS	Nitzschia dissipata var. dissipata
Nitzschia fonticola	Nitzschia fonticola	NFON	Nitzschia fonticola
Nitzschia palea	Nitzschia palea	NPAL	Nitzschia palea
Nitzschia unclassified	Nitzschia spec	NITZ	Nitzschia spec
Pseudostaurosira brevistriata	Pseudostaurosira brevistriata	PSBR	Pseudostaurosira brevistriata
Sellaphora nigri	Sellaphora nigri	newSNIG	Sellaphora nigrii
Staurosira construens	Staurosira construens	SCON	Staurosira construens
Staurosira venter	Staurosira venter	SSVE	Staurosira venter
Surirella solea	Cymatopleura solea var. solea	CSOL	Cymatopleura solea var. solea

*Suppl Table 5.9. List of **non-corresponding diatom species** from microscopy to HTS (rbcL reference database R-Syst::diatom) from Lake Bled littoral samples (n=10).*

Taxon mycroscopy	Validcode	detectable by rcbl_V9
Achnanthidium spec	ACHD	yes
Achnanthidium minutissima var. affinis	ADMF	
Achnanthidium pyrenaicum	ADPT	yes
Achnanthidium saprophilum	ADSA	yes
Achnanthes holsatica	AHOS	
Amphora inariensis	AINA	
Amphora montana	AMMO	yes
Amphipleura pellucida	APEL	
Amphora thumensis	ATHU	
Brachysira neglectissima	BNEG	
Brachysira neoexilis	BNEO	
Brachysira vitrea	BVIT	yes
Cymbella affinis var. affinis	CAFF	

Caloneis spec	CALO	yes
Cymbopleura frequens var. frequens	CBFQ	
Cymbopleura spec	CBPL	
Cymbella cymbiformis	CCYM	yes
Cymatopleura elliptica var. elliptica	CELL	yes
Cymbella excisiformis var. excisiformis	CEXF	
Cymbella laevis var. laevis	CLAE	
Caloneis lancettula	CLCT	
Cocconeis placentula var. euglypta	CPLE	
Diploneis krammeri	DKRA	
Diploneis oculata	DOCU	
Denticula tenuis	DTEN	yes
Diatoma vulgaris	DVUL	yes
Eunotia arcubus	EARB	
Encyonopsis cesatii	ECES	
Encyonema minutum	ENMI	yes
Epithemia spec	EPIT	yes
Eucocconeis laevis	EULA	
Fallacia subhamulata	FSBH	
Gomphonema acuminatum	GACU	yes
Gomphonema minutum fo. minutum	GMIN	yes
Gomphonema spec	GOMP	yes
Gomphonema parvulum var. parvulum fo. parvulum	GPAR	yes
Gomphonema procerum	GPRC	
Lemnicola hungarica	LHUN	
Nitzschia acicularis	NACI	yes
Navicula spec	NAVI	yes
Navicula cari	NCAR	yes
Navicula cryptotenelloides	NCTO	
Neidium dubium	NEDU	
Achnanthidium delmontii	newADEL	yes
Diploneis calcilacustris	newDCAL	
Diploneis oblongellopsis	newDOBL	
Paraplaconeis sp	newPARP	
Planothidium rostratoholarcticum	newPROH	
Staurosirella pinnata	newSTPN	
Navicula gregaria	NGRE	yes
Navicula lanceolata	NLAN	yes
Nitzschia linearis var. linearis	NLIN	yes
Navicula opportuna	NOPP	
Nitzschia paleacea	NPAE	yes
Navicula reichardtiana var. reichardtiana	NRCH	
Navicula subalpina	NSBN	
Planothidium frequentissimum	PLFR	yes
Planothidium spec	PLTD	yes
Psammothidium subatomoides	PSAT	

Platessa conspicua	PTCO	
Platessa sp.	PTSA	
Reimeria sinuata	RSIN	yes
Staurosira elliptica	SELI	yes
Sellaphora pupula	SPUP	yes
Staurosira leptostauron	SSLE	
Tryblionella angustata	TANG	
Cyclotella costei		

*Suppl Table 5.10. List of **non-corresponding diatom species** from HTS to microscopy (rbcL reference database R-Syst::diatom) from Lake Bled littoral samples (n=10).*

V9 species	TAXON_R_Diatom	Validcode
Amphora copulata	Amphora copulata	ACOP
Amphora unclassified	Amphora spec	AMPH
Aneumastus unclassified	Aneumastus spec	ANEU
Brachysira unclassified	Brachysira spec	BRAC
Caloneis fontinalis	Caloneis fontinalis	CFON
Caloneis silicula	Caloneis silicula	CSIL
Cocconeis placentula	Cocconeis placentula var. placentula	CPLA
Craticula cuspidata	Craticula cuspidata	CRCU
Cymbella aspera	Cymbella aspera	CASP
Cymbella excisa	Cymbella excisa var. excisa	CAEX
Cymbella lanceolata	Cymbella lanceolata var. lanceolata	CLAN
Cymbella proxima	Cymbella proxima var. proxima	CPRX
Cymbella unclassified	Cymbella spec	CYMB
Denticula tenuis	Denticula tenuis	DTEN
Diploneis subovalis	Diploneis subovalis	DSBO
Diploneis unclassified	Diploneis spec	DIPL
Discostella pseudostelligera	Discostella pseudostelligera	DPST
Encyonema ventricosum	Encyonema ventricosum	ENVE
Encyonopsis falaisensis	Encyonopsis falaisensis	ECFA
Encyonopsis minuta	Encyonopsis minuta	ECPM
Encyonopsis sp.	Encyonopsis spec	ENCP
Encyonopsis subminuta	Encyonopsis subminuta	ESUM
Epithemia gibba	Epithemia spec	EPIT
Epithemia sorex	Epithemia sorex	ESOR
Eunotia arcus	Eunotia arcus var. arcus	EARC
Fragilaria acus/radians complex	Fragilaria radians	FRAD
Fragilaria gracilis	Fragilaria gracilis	FGRA
Fragilaria sp.	Fragilaria species	FRAS
Frustulia vulgaris	Frustulia vulgaris	FVUL
Geissleria decussis	Geissleria decussis	GDEC
Gomphonema pumilum var. pumilum	Gomphonema pumilum	GPUM
Gomphonema saprophilum	Gomphonema parvulum var. parvulum fo. saprophilum	GPAS
Gyrosigma sciotense	Gyrosigma sciotense	GSCI
Iconella unclassified	Iconella sp.	ICON

Navicula capitatoradiata	Navicula capitatoradiata	NCPR
Navicula cryptocephala	Navicula cryptocephala	NCRY
Navicula unclassified	Navicula spec	NAVI
Nitzschia dissipata var. media	Nitzschia dissipata var. media	NDME
Nitzschia linearis	Nitzschia linearis var. linearis	NLIN
Nitzschia pusilla	Nitzschia pusilla	NIPU
Nitzschia sigmoidea	Nitzschia sigmoidea	NSIO
Pantocsekiella costei	Cyclotella costei	CCOS
Pinnularia neomajor	Pinnularia neomajor var. neomajor	PNEO
Pinnularia viridiformis	Pinnularia viridiformis var. viridiformis mor. 1	PVIF
Reimeria sinuata	Reimeria sinuata	RSIN
Sellaphora lanceolata	Sellaphora lanceolata	SLCL
Sellaphora pupula	Sellaphora pupula	SPUP
Sellaphora unclassified	Sellaphora spec	SELL
Staurosira sp.	Staurosira spec	STRS
Staurosira unclassified	Staurosira spec	STRS
Surirella elliptica	Cymatopleura elliptica var. elliptica	CELL
Ulnaria ulna	Ulnaria ulna	UULN
Ulnaria unclassified	Ulnaria spec	ULNA

8.6 L. Lugano, Switzerland-Italy

*Suppl Table 6.1. List of **corresponding phytoplankton** species identified through microscopy and through HTS (SILVA reference database) from Lake Lugano **pelagic** samples (n=12).*

Locus	LM_phytoplankton	ID-REBECCA	HTS_18S+16S	class
18S	Asterionella formosa	R0135	Asterionella formosa	Bacillariophyceae
18S	Aulacoseira granulata v. angustissima	R0024	Aulacoseira sp.	Bacillariophyceae
18S	Aulacoseira islandica v. helvetica	R0027	Aulacoseira sp.	Bacillariophyceae
18S	Diatoma ehrenbergii	R0184	Diatoma sp.	Bacillariophyceae
18S	Diatoma vulgaris	R0191	Diatoma sp.	Bacillariophyceae
18S	Fragilaria capucina ssp. rumpens	R2520	Fragilaria sp.	Bacillariophyceae
18S	Fragilaria crotonensis	R0223	Fragilaria crotonensis	Bacillariophyceae
18S	Fragilaria cyclopum	R0224	Fragilaria sp.	Bacillariophyceae
18S	Fragilaria radians	R0235	Fragilaria sp.	Bacillariophyceae
18S	Fragilaria sp.	R0238	Fragilaria sp.	Bacillariophyceae
18S	Stephanodiscus alpinus	R0076	Stephanodiscus sp.	Bacillariophyceae
18S	Stephanodiscus minutulus	R0082	Stephanodiscus sp.	Bacillariophyceae
18S	Stephanodiscus neoastraea	R0083	Stephanodiscus sp.	Bacillariophyceae
18S	Coelastrum astroideum	R0523	Coelastrum reticulatum	Chlorophyceae
18S	Coelastrum polychordum	R2269	Coelastrum reticulatum	Chlorophyceae
18S	Phacotus lenticularis	R0975	Phacotus lenticularis	Chlorophyceae
18S	Volvocales sp.	R0989	Volvocales sp.	Chlorophyceae
18S	Dinobryon divergens	R1073	Dinobryon divergens	Chrysophyceae
18S	Dinobryon sociale	R1083	Dinobryon sp.	Chrysophyceae
18S	Mallomonas akrokomos	R1097	Mallomonas akrokomos	Chrysophyceae
18S	Mallomonas caudata	R1100	Mallomonas caudata	Chrysophyceae
18S	Mallomonas sp.	R1109	Mallomonas sp.	Chrysophyceae
18S	Uroglena sp.	R1151	Uroglena sp.	Chrysophyceae
18S	Closterium aciculare	R1176	Closterium sp.	Conjugatophyceae
18S	Closterium acutum v. variabile	R1181	Closterium sp.	Conjugatophyceae
18S	Staurastrum pingue	R1303	Staurastrum sp.	Conjugatophyceae
18S	Cryptomonas sp.	R1394	Cryptomonas sp.	Cryptophyceae
16S	Anabaena lemmermannii	R1539	Anabaena	Cyanophyceae
16S	Anabaena planctonica	R1544	Anabaena	Cyanophyceae
16S	Aphanizomenon flos-aquae	R1558	Aphanizomenon	Cyanophyceae
16S	Microcystis sp.	R1496	Microcystis	Cyanophyceae
16S	Planktothrix rubescens	R1617	Planktothrix	Cyanophyceae
16S	Pseudanabaena catenata	R1620	Pseudanabaena	Cyanophyceae
16S	Pseudanabaena limnetica	R1621	Pseudanabaena	Cyanophyceae
16S	Snowella lacustris	R1510	Snowella	Cyanophyceae
18S	Pseudopedinella erkensis	R1153	Pseudopedinella sp.	Dictyochophyceae
18S	Ceratium hirundinella	R1672	Ceratium hirundinella	Dinophyceae
18S	Gymnodinium helveticum	R1647	Gymnodinium helveticum	Dinophyceae
18S	Gymnodinium lantzschii	R1650	Gymnodinium helveticum	Dinophyceae
18S	Gymnodinium sp.	R1654	Gymnodinium helveticum	Dinophyceae
18S	Peridinium cinctum	R1687	Peridinium gatunense	Dinophyceae
18S	Peridinium sp.	R1699	Peridinium gatunense	Dinophyceae
18S	Chrysochromulina sp.	R1819	Chrysochromulina sp.	Prymnesiophyceae
18S	Tribonema sp.	R1868	Tribonema aequale	Xanthophyceae

*Suppl Table 6.2. List of **non-corresponding phytoplankton** species from microscopy to HTS (SILVA reference database) from Lake Lugano **pelagic** samples (n=12).*

Locus	LM_phytoplankton	ID-REBECCA	class
18S	Achnanthes sp.	R0117	Bacillariophyceae
18S	Cyclotella catenata	R0014	Bacillariophyceae
18S	Cyclotella ocellata	R0048	Bacillariophyceae
18S	Cyclotella radiosa	R0051	Bacillariophyceae
18S	Cyclotella sp.	R0053	Bacillariophyceae
18S	Nitzschia acicularis	R0343	Bacillariophyceae
18S	Tabellaria fenestrata	R0440	Bacillariophyceae
18S	Ulnaria acus	R2171	Bacillariophyceae
18S	Ulnaria delicatissima var. angustissima	R2174	Bacillariophyceae
18S	Ulnaria ulna	R2175	Bacillariophyceae
18S	Ankyra judayi	R0489	Chlorophyceae
18S	Carteria sp.	R0923	Chlorophyceae
18S	Coenocystis sp.	R0537	Chlorophyceae
18S	Crucigeniella sp.	R0556	Chlorophyceae
18S	Dictyosphaerium pulchellum	R0571	Chlorophyceae
18S	Didymocystis sp.	R0582	Chlorophyceae
18S	Eutetramorus sp.	R0607	Chlorophyceae
18S	Kirchneriella arcuata	R0625	Chlorophyceae
18S	Kirchneriella irregularis	R0628	Chlorophyceae
18S	Lagerheimia genevensis	R0649	Chlorophyceae
18S	Lobocystis sp.	R0656	Chlorophyceae
18S	Micractinium pusillum	R0660	Chlorophyceae
18S	Monoraphidium contortum	R0665	Chlorophyceae
18S	Oocystis sp.	R0705	Chlorophyceae
18S	Pandorina morum	R0971	Chlorophyceae
18S	Pseudosphaerocystis sp.	R0738	Chlorophyceae
18S	Radiococcus sp.	R0747	Chlorophyceae
18S	Scenedesmus ecornis	R0781	Chlorophyceae
18S	Scenedesmus obtusus	R0760	Chlorophyceae
18S	Scenedesmus sp.	R0811	Chlorophyceae
18S	Tetrachlorella alternans	R0840	Chlorophyceae
18S	Tetraedron minimum	R0848	Chlorophyceae
18S	Westella sp.	R2047	Chlorophyceae
18S	Willea sp.	R0884	Chlorophyceae
18S	Bitrichia sp.	R1161	Chrysophyceae
18S	Kephyrion sp.	R1037	Chrysophyceae
18S	Synura uvella	R1145	Chrysophyceae
18S	Cosmarium depressum v. planctonicum	R1210	Conjugatophyceae
18S	Cosmarium laeve	R1216	Conjugatophyceae
18S	Katablepharis ovalis	R1404	Cryptophyceae
18S	Rhodomonas sp.	R1409	Cryptophyceae
16S	Aphanocapsa sp.	R1423	Cyanophyceae
16S	Aphanothece sp.	R1432	Cyanophyceae
16S	Chroococcus dispersus	R1436	Cyanophyceae
16S	Chroococcus limneticus	R1438	Cyanophyceae
16S	Cyanodictyon sp.	R1455	Cyanophyceae
16S	Gomphosphaeria aponina	R1462	Cyanophyceae
16S	Limnothrix redekei	R1582	Cyanophyceae
16S	Limnothrix sp.	R1583	Cyanophyceae
18S	Glenodinium sp.	R1642	Dinophyceae
18S	Peridiniopsis cunningtonii	R2116	Dinophyceae
18S	Peridiniopsis elpatiewskyi	R1679	Dinophyceae
18S	Peridiniopsis polonicum	R1682	Dinophyceae

18S	Elakatothrix gelatinosa	R0596	Klebsormidiophyceae
18S	Planctonema lauterbornii	R0919	Ulvophyceae

*Suppl Table 6.3. List of **non-corresponding phytoplankton** species from HTS to microscopy (SILVA reference database) from Lake Lugano **pelagic** samples (n=12).*

Locus	ID-REBECCA	HTS_18S + 16S	class
18S	R0030	Aulacoseira sp.	Bacillariophyta
18S	R0449	Bacillariophyceae sp.	Bacillariophyta
18S	R0188	Diatoma sp.	Bacillariophyta
18S	R0062	Melosira varians	Bacillariophyta
18S	R0086	Stephanodiscus sp.	Bacillariophyta
18S	R2862	Synedra sp.	Bacillariophyta
18S	R0940	Chlamydomonas reinhardtii	Chlorophyceae
18S	R0832	Chlorococcales sp.	Chlorophyceae
18S	R0905	Chlorophyceae sp.	Chlorophyceae
18S	R0530	Coelastrum reticulatum	Chlorophyceae
18S	new18R37	Mychonastes sp.	Chlorophyceae
18S	R2456	Sphaeropleaceae Gen. sp.	Chlorophyceae
18S	R0989	Volvocales sp.	Chlorophyceae
18S	R1162	Chrysamoeba sp.	Chrysophyceae
18S	R1171	Chrysophyceae sp.	Chrysophyceae
18S	R1086	Dinobryon sp.	Chrysophyceae
18S	R1120	Ochromonas sp.	Chrysophyceae
18S	new18R70	Paraphysomonas butcheri	Chrysophyceae
18S	R1123	Paraphysomonas sp.	Chrysophyceae
18S	R1377	Cryptomonas curvata	Cryptophyceae
18S	R1389	Cryptomonas pyrenoidifera	Cryptophyceae
18S	R1401	Cryptomonas tetrapyrenoidosa	Cryptophyceae
18S	R1412	Cryptophyceae sp.	Cryptophyceae
16S	R2302	Cyanobium	Cyanophyceae
16S	R1518	Synechococcus	Cyanophyceae
16S	R1526	Woronichinia	Cyanophyceae
18S	R2724	Pedinella hexacostata	Dictyochophyceae
18S	R1154	Pseudopedinella sp.	Dictyochophyceae
18S	new18R12	Asulcocephalium miricentonis	Dinophyceae
18S	R1708	Dinophyceae sp.	Dinophyceae
18S	R2588	Peridinium gatunense	Dinophyceae
18S	R1706	Prorocentrum sp.	Dinophyceae
18S	new18R9	Thoracosphaeraceae	Dinophyceae
18S	new18R2	Eustigmatophyceae	Eustigmatophyceae
18S	new18R49	Nannochloropsis sp.	Eustigmatophyceae
18S	new18R10	Crustomastigaceae	Mamiellophyceae
18S	new18R11	Dolichomastigaceae	Mamiellophyceae
18S	R1815	Monomastix sp.	Mamiellophyceae
18S	R1818	Chrysochromulina parva	Prymnesiophyceae
18S	R1171	Chrysophyceae sp.	Synurophyceae
18S	R1111	Mallomonas tonsurata	Synurophyceae
18S	R0493	Botryococcus braunii	Trebouxiophyceae
18S	R0517	Choricystis sp.	Trebouxiophyceae
18S	new18R3	Trebouxiophyceae	Trebouxiophyceae

18S	R1865	Tribonema aequale	Xanthophyceae
18S	R1201	Closterium sp.	Zygnemophyceae
18S	R1309	Staurastrum sp.	Zygnemophyceae

*Suppl Table 6.4. List of **cyanobacteria** species from **biofilm** identified through HTS (16S rDNA SILVA reference database) from Lake Lugano littoral samples (n=10).*

ID-REBECCA	Taxon_REBECCA	genus_16S	species_16S
NA		Potamolinea 1PC	NA
R1539	Anabaena lemmermannii	Aphanizomenon NIES81	NA
fresh_new1	Ancylothrix sp.	Ancylothrix 8PC	NA
fresh_new2	Annamia sp.	Annamia HOs24	NA
R1562	Aphanizomenon sp.	Aphanizomenon NIES81	NA
R1427	Aphanothece clathrata	Cyanobium PCC-6307	NA
R2710	Calothrix sp.	Calothrix KVSF5	NA
R1637	Chamaesiphon sp.	Chamaesiphon PCC-6605	minutus
R1637	Chamaesiphon sp.	Chamaesiphon PCC-7430	investiens
R1637	Chamaesiphon sp.	Chamaesiphon PCC-7430	subglobosus
R2302	Cyanobium sp.	Cyanobium PCC-6307	gracile
R1948	Cyanothece sp.	Cyanothece PCC 7425	NA
R1961	Eucapsis sp.	Chalicogloea CCALA 975	NA
R2090	Geitlerinema sp.	Geitlerinema LD9	NA
R1576	Geitlerinema splendidum	NA	NA
Picoplank1	Geminocystis	Geminocystis PCC-6308	NA
R0888	Gloeocapsa sp.	Gleocapsa	NA
R0893	Gloeothece linearis	Gloeobacter PCC-7421	NA
R1580	Leptolyngbya sp.	Leptolyngbya ANT.L52.2	NA
new_16S_cyano_family4	Leptolyngbyaceae	Arthronema SAG 12.89	NA
R1583	Limnothrix sp.	Limnothrix	NA
R1478	Merismopedia sp.	Merismopedia 0BB39S01	NA
R1496	Microcystis sp.	Microcystis PCC-7914	NA
fresh_new4	Microseira	Microseira Carmichael-Alabama	NA
biofilm_new5	Nodosilinea	Nodosilinea PCC-7104	NA
R1597	Oscillatoria sp.	Oscillatoria PCC-10802	duplisecta
biofilm_new6	Phormidesmis	Phormidesmis ANT.L52.6	NA
R1606	Phormidium sp.	Kamptonema PCC-6407	NA
R1606	Phormidium sp.	Phormidium CYN64	NA
R1618	Planktothrix sp.	Planktothrix NIVA-CYA 15	agardhii/prolifica/pseudagardhii/rubescens/suspensa
R2006	Pleurocapsa sp.	Pleurocapsa PCC-7319	minor
R2006	Pleurocapsa sp.	Pleurocapsa PCC-7327	concharum
fresh_new5	Prochlorothrix PCC-9006	Prochlorothrix PCC-9006	NA
R1623	Pseudanabaena sp.	Pseudanabaena PCC-7429	foetida/limnetica
R1623	Pseudanabaena sp.	Pseudanabaena PCC-7429	frigida
R1623	Pseudanabaena sp.	Pseudanabaena PCC-7429	galeata
biofilm_new10	Scytolyngbya	Scytolyngbya XSP1	NA
biofilm_new11	Scytonema	Scytonema UCFS19	cf.
R1511	Snowella litoralis	Snowella 0TU37S04	litoralis

R1518	Synechococcus sp.	Synechococcus PCC-7502	NA
R1520	Synechocystis sp.	Synechocystis CCALA 700	NA
R2044	Trichodesmium sp.	Trichodesmium IMS101	NA
R2826	Tychonema sp.	Tychonema CCAP 1459-11B	NA

*Suppl Table 6.5. List of **corresponding diatom species** from **biofilm** identified through microscopy and through HTS (rbcL reference database R-Syst::diatom) from Lake Lugano littoral samples (n=10).*

V9 species	TAXON_R_Diatom	Validcode	Taxon_validcode
Achnanthidium delmontii	Achnanthidium delmontii	newADEL	Achnanthidium delmontii
Achnanthidium minutissimum	Achnanthidium minutissimum	ADMI	Achnanthidium minutissimum
Achnanthidium pyrenaicum	Achnanthidium pyrenaicum	ADPT	Achnanthidium pyrenaicum
Achnanthidium subatomus	Achnanthidium subatomus	ADSU	Achnanthidium subatomus
Amphora pediculus	Amphora pediculus	APED	Amphora pediculus
Cocconeis pediculus	Cocconeis pediculus	CPED	Cocconeis pediculus
Cocconeis placentula	Cocconeis placentula var. placentula	CPLA	Cocconeis placentula var. placentula
Cymbella excisa	Cymbella excisa var. excisa	CAEX	Cymbella excisa var. excisa
Diatoma vulgaris	Diatoma vulgaris	DVUL	Diatoma vulgaris
Didymosphenia geminata	Didymosphenia geminata mor. geminata	DGEM	Didymosphenia geminata mor. geminata
Encyonema caespitosum	Encyonema caespitosum	ECAE	Encyonema caespitosum
Encyonema minutum	Encyonema minutum	ENMI	Encyonema minutum
Encyonema silesiacum	Encyonema silesiacum	ESLE	Encyonema silesiacum
Encyonema ventricosum	Encyonema ventricosum	ENVE	Encyonema ventricosum
Encyonopsis microcephala	Encyonopsis microcephala	ENCM	Encyonopsis microcephala
Encyonopsis subminuta	Encyonopsis subminuta	ESUM	Encyonopsis subminuta
Fragilaria gracilis	Fragilaria gracilis	FGRA	Fragilaria gracilis
Halamphora montana	Amphora montana	AMMO	Amphora montana
Mayamaea permitis	Mayamaea atomus var. permitis	MAPE	Mayamaea atomus var. permitis
Navicula antonii	Navicula antonii	NANT	Navicula antonii
Navicula capitatoradiata	Navicula capitatoradiata	NCPR	Navicula capitatoradiata
Navicula cryptotenella	Navicula cryptotenella	NCTE	Navicula cryptotenella
Navicula gregaria	Navicula gregaria	NGRE	Navicula gregaria
Navicula radiosa	Navicula radiosa	NRAD	Navicula radiosa
Navicula tripunctata	Navicula tripunctata	NTPT	Navicula tripunctata
Navicula veneta	Navicula veneta	NVEN	Navicula veneta
Nitzschia costei	Nitzschia costei	newNCOS	Nitzschia costei
Nitzschia dissipata	Nitzschia dissipata var. dissipata	NDIS	Nitzschia dissipata var. dissipata
Nitzschia fonticola	Nitzschia fonticola	NFON	Nitzschia fonticola
Nitzschia palea	Nitzschia palea	NPAL	Nitzschia palea
Nitzschia soratensis	Nitzschia soratensis	newNSOR	Nitzschia soratensis
Planothidium lanceolatum	Planothidium lanceolatum	PTLA	Planothidium lanceolatum
Pseudostaurosira brevistriata	Pseudostaurosira brevistriata	PSBR	Pseudostaurosira brevistriata
Reimeria sinuata	Reimeria sinuata	RSIN	Reimeria sinuata

*Suppl Table 6.6. List of **non-corresponding diatom species** from microscopy to HTS (rbcL reference database R-Syst: diatom) from Lake Lugano littoral samples (n=10).*

LM diatoms	Validcode
Achnanthes minutissima var. jackii	AMJA
Achnanthidium atomoides	ADAM
Achnanthidium caledonicum	ADCA
Achnanthidium delmontii	newADEL
Achnanthidium lineare	ACLI
Achnanthidium minutissima var. affinis	ADMF
Achnanthidium minutissimum	ADMI
Achnanthidium pfisteri	newAPFI
Achnanthidium pyrenaicum	ADPT
Achnanthidium straubianum	ADSB
Achnanthidium subatomus	ADSU
Amphipleura pellucida	APEL
Amphora inariensis	AINA
Amphora indistincta	newAIND
Amphora montana	AMMO
Amphora pediculus	APED
Aneumastus stroesei	ANSS
Aulacoseira granulata	AUGR
Brachysira neoexilis	BNEO
Caloneis lancettula	CLCT
Cocconeis pediculus	CPED
Cocconeis placentula var. euglypta	CPLE
Cocconeis placentula var. placentula	CPLA
Cymbella compacta	CCMP
Cymbella excisa var. excisa	CAEX
Diatoma ehrenbergii	DEHR
Diatoma vulgaris	DVUL
Didymosphenia geminata mor. geminata	DGEM
Encyonema caespitosum	ECAE
Encyonema minutum	ENMI
Encyonema silesiacum	ESLE
Encyonema ventricosum	ENVE
Encyonopsis microcephala	ENCM
Encyonopsis minuta	ECPM
Encyonopsis subminuta	ESUM
Eolimna subminuscula	ESBM
Fragilaria capucina var. austriaca	FCAU
Fragilaria capucina var. vaucheriae	FCVA
Fragilaria delicatissima	FDEL
Fragilaria gracilis	FGRA
Fragilaria tenera	FTEN
Geissleria acceptata	GACC
Geissleria cummerowi	GCUW
Gomphonema olivaceolacuum	newGOUM
Gomphonema tergestinum	GTER

Gyrosigma attenuatum	GYAT
Karayevia clevei	KCLE
Mayamaea atomus var. permitis	MAPE
Navicula antonii	NANT
Navicula capitatoradiata	NCPR
Navicula cryptotenella	NCTE
Navicula cryptotenelloides	NCTO
Navicula difficillimoides	NDFO
Navicula gregaria	NGRE
Navicula hofmanniae	NHOF
Navicula notha	NNOT
Navicula radiosa	NRAD
Navicula reichardtiana var. reichardtiana	NRCH
Navicula splendicula	NSPD
Navicula submuralis	NSMU
Navicula tenelloides	NTEN
Navicula tripunctata	NTPT
Navicula trophicatrix	NTCX
Navicula utermoehlii	NUTE
Navicula veneta	NVEN
Navicula vilaplanii	NVIP
Nitzschia archibaldii	NIAR
Nitzschia costei	newNCOS
Nitzschia dissipata var. dissipata	NDIS
Nitzschia fonticola	NFON
Nitzschia hantzschiana	NHAN
Nitzschia incognita	NICN
Nitzschia lacuum	NILA
Nitzschia palea	NPAL
Nitzschia recta	NREC
Nitzschia sociabilis	NSOC
Nitzschia soratensis	newNSOR
Nitzschia sublinearis	NSBL
Nitzschia tabellaria	NTAB
Placoneis clementis	PCLT
Planothidium dubium	PTDU
Planothidium frequentissimum	PLFR
Planothidium lanceolatum	PTLA
Pseudostaurosira brevistriata	PSBR
Reimeria sinuata	RSIN
Sellaphora nigrii	newSNIG
Simonsenia delognei	SIDE
Staurosirella pinnata	newSTPN

*Suppl Table 6.7. List of **non-corresponding diatom species** from HTS to microscopy (rbcL reference database R-Syst::diatom) from Lake Lugano littoral samples (n=10).*

V9 species	TAXON_R_Diatom	Validcode
Achnanthidium eutrophilum	Achnanthidium eutrophilum	ADEU
Achnanthidium saprophilum	Achnanthidium saprophila	ADSA
Achnanthidium unclassified	Achnanthidium spec	
Amphora copulata	Amphora copulata	ACOP
Amphora ovalis	Amphora ovalis	AOVA
Amphora unclassified	Amphora spec	AMPH
Aneumastus unclassified	Aneumastus spec	ANEU
Asterionella formosa	Asterionella formosa	AFOR
Brachysira unclassified	Brachysira spec	BRAC
Caloneis fontinalis	Caloneis fontinalis	CFON
Caloneis silicula	Caloneis silicula	CSIL
Caloneis unclassified	Caloneis spec	CALO
Cymbella aspera	Cymbella aspera	CASP
Cymbella lanceolata	Cymbella lanceolata var. lanceolata	CLAN
Cymbella neocistula	Cymbella neocistula var. neocistula	CNCI
Cymbella unclassified	Cymbella spec	CYMB
Denticula tenuis	Denticula tenuis	
Diatoma tenuis	Diatoma tenuis	DITE
Diploneis subovalis	Diploneis subovalis	DSBO
Ellerbeckia sp.	Ellerbeckia spec	ELLE
Encyonema prostratum	Encyonema prostratum	EPRO
Encyonema unclassified	Encyonema spec	ENCY
Encyonopsis sp.	Encyonopsis spec	ENCP
Epithemia sorex	Epithemia sorex	ESOR
Epithemia unclassified	Epithemia spec	EPIT
Fistulifera saprophila	Fistulifera saprophila	FSAP
Fragilaria acus/radians complex	Fragilaria radians	
Fragilaria sp.	Fragilaria species	FRAS
Frustulia vulgaris	Frustulia vulgaris	FVUL
Geissleria decussis	Geissleria decussis	GDEC
Gomphonella olivacea	Gomphonella olivacea	GLOV
Gomphonella olivaceoides	Gomphonema olivaceum var. olivaceoides	GOOL
Gomphonella olivaceolacuum	Gomphonella spec	newGOMP
Gomphonema affine	Gomphonema affine	GAFF
Gomphonema minutum	Gomphonema minutum fo. minutum	GMIN
Gomphonema pumilum var. pumilum	Gomphonema pumilum	GPUM
Gomphonema saprophilum	Gomphonema parvulum var. parvulum fo. saprophilum Lange-Bert. & Reichardt	GPAS
Gomphonema unclassified	Gomphonema spec	GOMP
Gyrosigma sciotense	Gyrosigma sciotense	GSCI
Halamphora oligotraphenta	Amphora oligotraphenta	AOLG
Hannaea arcus	Fragilaria arcus var. arcus	FARC
Iconella linearis	Iconella sp.	ICON
Melosira varians	Melosira varians	MVAR
Navicula cari	Navicula cari	NCAR
Navicula cryptocephala	Navicula cryptocephala	NCRY

Navicula rostellata	Navicula rostellata var. elongata	NRSE
Nitzschia acidoclinata	Nitzschia acidoclinata	NACD
Nitzschia dissipata var. media	Nitzschia dissipata var. media	NDME
Nitzschia inconspicua	Nitzschia inconspicua	NINC
Nitzschia linearis	Nitzschia linearis var. linearis	NINC
Nitzschia paleacea	Nitzschia paleacea	NPAE
Nitzschia pusilla	Nitzschia pusilla	NIPU
Nitzschia sigmoidea	Nitzschia sigmoidea	NSIO
Nitzschia unclassified	Nitzschia spec	NITZ
Pinnularia neomajor	Pinnularia neomajor var. neomajor	PNEO
Planothidium cryptolanceolatum	Planothidium spec	PLTD
Planothidium victori	Planothidium spec	PLTD
Psammothidium helveticum	Psammothidium helveticum	PHEL
Sellaphora lanceolata	Sellaphora lanceolata	SLCL
Sellaphora nigri	Sellaphora nigri	newSNIG
Sellaphora pupula	Sellaphora pupula	SPUP
Staurosira construens	Staurosira construens	SCON
Staurosira sp.	Staurosira spec	STRS
Surirella elliptica	Cymatopleura elliptica var. elliptica	CELL
Surirella solea	Cymatopleura solea var. solea	CSOL
Surirella unclassified	Surirella spec	SURI
Ulnaria ulna	Ulnaria ulna	UULN
Ulnaria unclassified	Ulnaria spec	ULNA