

## 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 WPT4

Activity A.T4.1

Deliverable D.T4.1.1

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### Deliverable D.T4.1.1

#### Guidelines for digital accessing of data obtained during the project by stakeholders and citizens (1)

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## Deliverable D.T4.1.1

### 1. Introduction

This delivery provides guidelines for digital accessing of data obtained during the project by stakeholders and citizens (D.T4.1.1).

Following these guidelines Eco-AlpsWater had produced a new database for the classification and distribution of aquatic species accessible by stakeholders (D.T1.2.3), which contain the site classification by national metrics of all samples or sites and indicator scores used for water assessment in the Alpine Space. Additionally this “EAW taxa analysis tool” enables the stakeholders to compare easily the traditional and metabarcoding taxa inventories while the inclusion of fish results is still in development.

This “EAW taxa analysis tool” will be completed and published with the end of the project, but is already in use for regional events.

In order to derive guidelines at first, the interests of the stakeholders working in field of freshwater monitoring were collected during regional, national and international meetings organized by the Eco-AlpsWater project, but also by projects with similar scopes such as the project DNAqua net<sup>1</sup> in which several Eco-AlpsWater partners are actively involved.

Stakeholders have raised three fields of interest to the project results:

- I) Degree of direct matches when comparing taxa inventories gained by traditional methods (EU-WFD, WHO-CH) and by metabarcoding approach
- II) Rating the applicability of metabarcoding approach to terms of cost, practical handling and processing and in terms of assessing the ecological quality of a water body
- III) Which additive and supporting information the metabarcoding approach can provide (biodiversity of all freshwater organisms; bio-geographic distribution)

Each of these fields of interest need different data and degree of aggregation of data: In case of checking the degree of matches of taxa, users want to check the proportion of taxa matches by comparing taxa list for each sample or for each water body (level “site name”). This kind of data should be arranged in tables and are ideally selectable “on demand” for sites and samples redrawn out of the whole and huge Eco-AlpsWater data set.

In case of index results for diversity or assessment metrics detailed metadata must be available and cited to understand which metric was used, and how this metric is defined. The data must be reliable and checked by scientists.

Therefore, each field of interest on metabarcoding data need an adopted format of digital access for stakeholders. The following chapters describe the strategies and formats for each field of interest.

In a second step, the complexity of the produced data must be analysed to optimize their digital access. Data by the metabarcoding approach must be prepared for the comparison with data of the traditional approaches for biomonitoring. Furthermore, countries have developed different national methods and difference for each bio-component to implement the European Water Framework Directive.

In result, the produced project data are very complex and a large number of samples were analysed.

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<sup>1</sup> EU COST Action CA15219 on “Developing new genetic tools for bioassessment of aquatic ecosystems in Europe” First DNAQUA International Conference / 9-11 March 2021 / online

## Deliverable D.T4.1.1

37 lakes and 53 river sites were assessed with traditional methods (counting lists) and with multiplex primers according the Eco-AlpsWater metabarcoding approach. For each of the applied primers large HTS (high throughput sequencing”) record tables were automatically produced by the bioinformatic pipelines. The user get the taxonomy of a sequence in case of there was a match to a gen bank.

In Figure 1 the various data are described listed in groups. The data groups are stored and analysed in different project products:

**Taxa inventories** stored in “MACH storage data base”, HTS record tables and merged in “EAW taxa analysis tool”

**Environmental data** in “MACH storage data base” and pivot Excel table

**Geographic information and sample origin** in “MACH storage data base” and maps in web side and publications (in preparation)

**Quality check and documentation data** in “MACH storage data base” with DNA sequence files and methods in protocols

**Aggregated data:** Index results for biodiversity and assessment - DT1.1.4-1 Ecological metrics Diatoms\_indicators and for biodiversity analysis by WP3.

|  |  |
|--|--|
| <b>Level 1: taxa inventories</b><br><br><i>Prerequisite: harmonisation and data check</i> <ul style="list-style-type: none"> <li>all records of one taxon are named/coded the same</li> <li>Agreed confidence level</li> <li>Contamination from connected habitats?</li> <li>proof for synonyms</li> <li>check by BLAST analysis etc.</li> </ul> | Two origins of taxa lists:<br>a) <b>Traditional method:</b> Standardized national bio-component methods with operational names and codes for taxa per sample restricted on WFD bio-components (diatom, fish etc)<br>a) <b>Metabarcoding:</b> Taxonomic assignment according curated reference data bases differs for primer and target group (12, 16, 18s, <u>rcbL</u> ) using actual nomenclature and systematics |
| <b>Level 2: environmental data</b>   | Harmonized data according units and outlier check<br>a) Trophic scores: Nutrients, chlorophyll for pressure-taxa-relationships<br>b) Identification of extreme habitats within EAW sites   |
| <b>Level 3: Geographic information and sample origin</b>   | Distribution maps of sites and taxa using the coordinates<br>Categories of sampling type (eg. plankton, biofilm etc.)  |
| <b>Level 4: Quality check and documentation data</b>   | Metadata to understand sampling, extraction and bioinformatics<br>Storage of DNA sequences, sharing with international data bases  |
| <b>Level 5: Aggregated data: Index results for biodiversity and assessment</b>   | a) Diversity indices<br>b) Application of common biological metrics on eDNA data<br>c) Suggesting new metrics based on other biological groups than EU-WFD   |

(co) Eco-AlpsWater: WP4 Deliverable -4-1-1 graph 1

Fig. 1 – Demonstration of the complexity of data

This deliverable describes the basic elements, which have to be included to enable easy access of stakeholders to the obtained data.



## Deliverable D.T4.1.1

### 2. Short description of the storage data base

The MACH institute had provide a storage data base which is not public, was created to

- List all samples with EAW sample code and bar code
- Store metadata of samples including environmental data, DNA quality check data
- Store all sequences gained by 12S rRNA, 16S rRNA, 18S rRNA, and rbcL with unique sample codes
- Taxa inventory lists gained by traditional methods (counts and taxa by light microscopy).

Each project partner filled their data into this storage data base and uploaded the DNA sequence- and counting files per each sample and inserted metadata into the agreed templates. The storage data base do not link the DNA-sequences detected by metabarcoding to any taxonomy records.

### 3. Field of interest I: Comparing taxa inventories

The most frequent interest by stakeholders was to compare the metabarcoding taxa list with the records monitored for their national metrics.

To explore the taxa inventories, a tool for easy extraction of data is necessary. For this propose the “MACH storage data base” do not provide any function, and was not addressed for.

As a product of D.T4.1.1 and D.T1.2.3 the “EAW taxa analysis tool” (see Appendix 1) was developed to execute analysis steps automatically, which are necessary to compare the taxa inventories after their listing preparation in common tables. The tool is also a feed-back to complications and user questions which were identified during the preliminary regional data analysis by the project partners in period 5 feeding into the EAW teleconference in December 2020.

#### 3.1 Data with taxa inventories detected by metabarcoding

The obtained raw data are very complex especially for the metagenomics results and are huge in terms of samples and analysis steps.

When focusing on microorganisms (phytoplankton, diatoms, bacteria and fungi) 153 plankton and 177 biofilm samples from 37 lakes and 53 river sites were assessed with multiplex primers.

To each of the samples the primers 16S and 18S were applied and for biofilm samples additionally rbcL for benthic diatom species.

For each of the three primers large HTS (high throughput sequencing”) record tables where automatically produced by the bioinformatic pipelines.

These large tables combine all samples with all detected sequences and give the detected signal (rarefied read counts) in the cross field.

**11.473 ASV sequences of 18S** by Silva 138 (all eukaryotes including phytoplankton)

**37.533 ASV sequences of 16S** by Protist Ribosomal Reference database-PR2 (all bacteria, fungi)

**1.285 ASV sequences of “chloroplast 16S”** (some eukaryotes)

**1.602 ASV sequences of rbcL** selected for diatoms by library data base Diat.barcode were detected and listed for each sample.



## Deliverable D.T4.1.1

*User question 1: How to handle a taxonomic output, which lead seldom to a species name, but mainly to higher taxonomic levels?*

The HTS record tables provide to the user also the taxonomy of a sequence in case there was a match to a gen bank (see bioinformatic deliveries D-T1.1.3 1-4). This match can be to different taxonomic levels such as order, family, genus and species. It is notable, that only a small part of all sequences in fact belongs to one specific genus or taxa. Therefore, match analysis on genus or on species level should be done separately and the tool must deliver this.

This is relevant for water assessment since indicator lists in biological WFD metrics mainly contain taxa on genus or species level (see D.T2.1.1- List of species). Therefore, the national methods for phytoplankton and benthic diatoms require analysis on species or at least genus level. In result, it is impossible to link an ASV identifying only family or order level, to match to an indicator species found by counting with light microscopy. This strongly reduce the share of direct matches between the methods approaches.

*User question 2: How to handle with a taxon, which is listed under several sequences in the metabarcoding output?*

It is an important information for users and stakeholders that one taxon can appear/ can be detected under several ASVs. Since the sequence of each ASV differ, this diversity presents “genotypes” of one species. Still, a user of the data just want to know, if a species or genus is present or not. The tool therefore should bring all sequences together, which belong to the same taxon and aggregate the result in one “present” record (additional information of interest: maximal signal, number of ASVs and first ASV found in one sample).

*User question 3: I see an output list from metabarcoding with many taxon names, which I never heard before. How to select and recognize my target taxa?*

With the aim to compare finally the taxa inventories gained by traditional methods (EU-WFD, WTO-CH) and by the metabarcoding approach the focus is on the biological target groups, the so-called bio-components of the WFD, phytoplankton including cyanobacteria and benthic diatoms.

The user needs help to separate all taxa classes relevant for its target group from all the other organisms groups, which the gen marker might also detect (e.g. trees, mammals etc.).

Furthermore, these taxa classes are named and classified according the most recent and modern taxonomy in the metabarcoding list, which are new for the user, because they are commonly not in use for the biological check lists of WFD/WTO monitoring. Common codes and taxa names for phytoplankton and benthic diatoms are necessary for comparing lists (see Chapter 3.3).

Out of all ASVs found with 16S and 18S, only a small part belong to these target groups and their classes. Therefore, a “phytoplankton” filter helps to select phytoplankton taxa within the 18S taxonomy and a “cyanobacteria filter” to select within the 16S taxonomy. These filters are part of the “EAW taxa analysis tool” and a product of the data preparation in WP4.

Applying all the selection and aggregation steps mentioned above to look at the WFD target groups finally

- the 16S ASVs extract belong to 88 cyanobacteria species or genera,
- the 18S ASVs extract to 882 phytoplankton species or genera.
- the rbcL-ASVs extract present 226 benthic diatom species or genera.





## Deliverable D.T4.1.1

These three lists are the “detectable” target taxa, which are part of the EAW taxa analysis tool. The taxonomic assignment at the species level is based on exact matching (no base pair different) between ASVs and reference strains. The preparation of library followed a standard procedure in use at the FEM, IT sequencing facility directed by M. Pindo, as described e.g. in Salmaso et al. (2018) and for diatoms according the diatom-barcode V9 taxonomy at INRA, FR.

### 3.2 Data with taxa inventories detected by counting

The produced project data comprise the results by microscopical counting and by fishing. All project partners were requested to use a common table and coding template to facilitate subsequent data analysis.

Each country had applied their national methods according the EU-WFD or WTO-CH, still there were a common understanding of the depths of biological analysis.

For phytoplankton all project partners used the Utermöhl-counting technique at light microscope (cells /ml), common determination keys and geoforms to calculate and estimate the biovolume of each taxon (mm<sup>3</sup>/l). Since the operational taxon names may include various synonyms, which hindered the comparison, all records were additionally assigned to a common code, the Rebecca-code (see [freshwaterecology.com](http://freshwaterecology.com) visited in Dec 2019).

For benthic diatoms all project partners prepared diatom slides and count at highest magnification at light microscope (about 400 valves), used common determination keys and calculate the proportion of a taxon to total (% valves). Since the operational taxon names may include various synonyms, all records were additionally assigned to a common code, the VALID-code (see [freshwaterecology.com](http://freshwaterecology.com) visited in Dec 2019).

### 3.3 Common code for taxa inventories and actuality of species names and systematics

While the harmonisation of names in fish lists is less problematic, the systematic and nomenclature of microorganisms are undergoing permanent changes in very short time.

In fact, the operational taxa lists for biomonitoring are frequently more conservative since determination keys and assessment tools adopt not very rapid to new results. In biomonitoring the taxa names are frequently synonyms of more actual taxa names and are grouped in a former systematic.

Common codes for phytoplankton and benthic diatoms were in the first step the REBECCA-code for phytoplankton and the VALID-code for benthic diatoms (see [freshwaterecology.com](http://freshwaterecology.com) visited December 2020).

Additional species and genera found in freshwaters of the Alpine Space in the Eco-AlpsWater project extended the given coding lists.

The taxa lists were checked for and linked to the most actual taxa names by the internet platform “algaebase”. This translation table is part of the final “EAW taxa analysis tool” to enable updated naming of the taxa inventory in output tables.

Partly also the NCBI code differ from these most actual names (include errors and synonyms, but systematic very actual).

## 3. Field of interest II: Rating the applicability of metabarcoding approach to terms of cost, practical handling and processing and in terms of assessing the ecological quality of a water body

Questions about the applicability will be answered in D-T.4.2.2 “Recommendations for the inclusion of innovative monitoring approaches in water quality assessment and manag. (WFD/WPO) (1)” and in the frequently asked question, FAQ catalogue.

#### Deliverable D.T4.1.1

### 3. Field of interest III: additive and supporting information provided by the metabarcoding approach

In the first step, the metabarcoding results are used within the regional stakeholder meeting to proof the reliability of phytoplankton and benthic diatom determination by light microscopy.

The main benefit by metabarcoding is the broad biodiversity survey obtained by one procedure. It is innovative that the whole micro-organisms community is recorded by one sample.

Using the taxa inventories of organisms groups, which are no target groups in the frame of the traditional water assessment according WFD, there is multiple chance to gather further information about the ecological function of freshwater.

For example, special bacteria groups such as sulphur bacteria or protist taxa (ciliates) provide supporting information to access freshwaters. Non-genetic methods to detect such taxa are not part of the monitoring and are extreme time-consuming (e.g. silver staining of ciliates).

In APPENDIX 2 is a list of heterotrophic bacteria important for sulphur processes and secondly a list for the most commonly found heterotrophic bacteria (>100 samples), which are partly important for ecosystem services (nitrate reduction, denitrification).

18S marker in the Eco-AlpsWater project detected 652 genotypes (ESVs) of ciliates in plankton samples. These genotypes belong to 54 different ciliated taxa, which were otherwise overlooked (APPENDIX 3).

A deeper biodiversity analysis is provided by WP3 delivery D.T.3.3.1 “Biodiversity check up of water resources in the Alpine Space region”.



## Deliverable D.T4.1.1

### APPENDIX 1

Short description of the “EAW taxa analysis tool”

Specified Access database with data tables and prepared customer queries.

All details are described in the database for the classification and distribution of aquatic species accessible by stakeholders (D.T1.2.3).

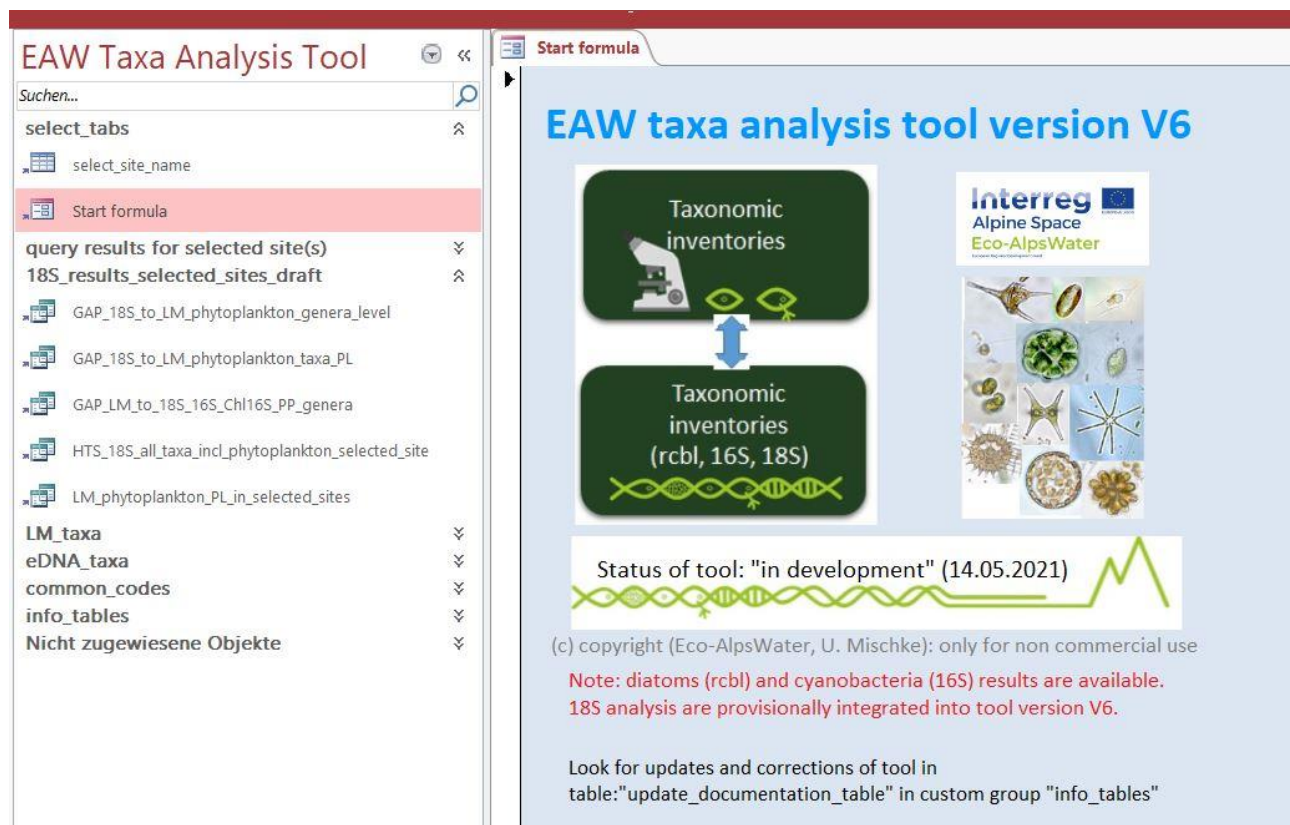


Figure A 1: Start view on the Access tool with predefined customer navigation area.

The EAW taxa analysis tool (Figure A1) is an output of the project, and linked to the project webpage.

<https://www.alpine-space.eu/projects/eco-alpswater/en/project-results/taxa-analyses-tool>

There are various possibilities to compare and visualise traditional and eDNA results for taxa inventories in the investigated lakes and rivers. To explore the data and analyses, the stakeholder should contact project partners from their countries for demonstration. Version 7 is available and some further functionalities are added until the end of the project (Oct 2021).

In the tool, all results of metabarcoding (HTS) and light microscopy (LM) are listed in tables, which can be linked to each other. Each sample and each taxon of phytoplankton and benthic diatoms had a unique code. Samples are linked to their specific site name and latter can be selected in a first



## Deliverable D.T4.1.1

step. Secondly, prepared queries help to screen specific results (=taxa lists) in all samples of the selected site. Thirdly, the GAP-queries compare taxa results by HTS to LM or visa wise.

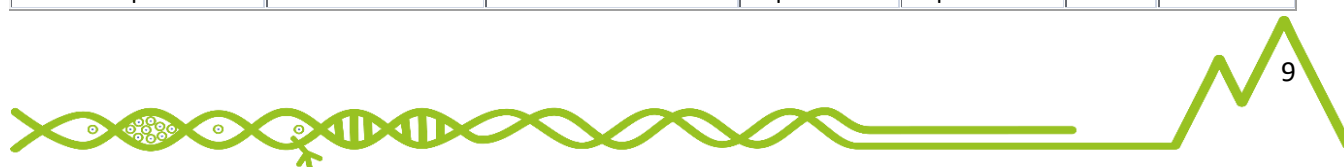
## APPENDIX 2

List of most detected sulphur bacteria in the plankton (>100 samples) excluding “Cyanobacteria”

| sulfur_bacteria_in_plankton |                   |               |         |                                |                            |     |                             |
|-----------------------------|-------------------|---------------|---------|--------------------------------|----------------------------|-----|-----------------------------|
| Class                       | Order             | Genus         | Species | 1st seq_16S<br>22Dec20_E<br>AW | 2nd seq_16S<br>22Dec20_EAW | N   | Max<br>16s_signa<br>l raref |
| Campylobacteria             | Campylobacterales | Sulfuricurvum | NA      | Seq11228                       | Seq39926                   | 7   | 3                           |
| Campylobacteria             | Campylobacterales | Sulfurimonas  | NA      | Seq24560                       | Seq43032                   | 3   | 2                           |
| Gammaproteo-<br>bacteria    | Burkholderiales   | Sulfuritalea  | NA      | Seq10799                       | Seq8804                    | 658 | 97                          |

List of most frequently detected bacteria in the plankton (>100 samples) excluding “Cyanobacteria”

| non_cyanobacteiria_aggr_PL |                    |                              |                        |                        |              |                         |
|----------------------------|--------------------|------------------------------|------------------------|------------------------|--------------|-------------------------|
| Class                      | Order              | Genus                        | 1st seq_16S<br>22Dec20 | 2nd seq_16S<br>22Dec20 | N<br>signals | Max 16s<br>signal raref |
| Acidimicrobiia             | IMCC26256          | NA                           | Seq247                 | Seq247                 | 107          | 112                     |
| Acidimicrobiia             | Microtrichales     | CL500-29 marine<br>group     | Seq29                  | Seq85                  | 129          | 594                     |
| Actinobacteria             | Frankiales         | Candidatus<br>Planktophila   | Seq39                  | Seq39                  | 139          | 328                     |
| Actinobacteria             | Frankiales         | hgcl clade                   | Seq101                 | Seq91                  | 142          | 1482                    |
| Actinobacteria             | Frankiales         | NA                           | Seq5                   | Seq80                  | 141          | 1008                    |
| Alphaproteobacteria        | Rhodobacterales    | Rhodobacter                  | Seq220                 | Seq220                 | 110          | 62                      |
| Alphaproteobacteria        | SAR11 clade        | NA                           | Seq1                   | Seq1                   | 142          | 2457                    |
| Anaerolineae               | Anaerolineales     | NA                           | Seq9                   | Seq9                   | 101          | 766                     |
| Bacteroidia                | Chitinophagales    | Candidatus Aquirestis        | Seq77                  | Seq77                  | 131          | 467                     |
| Bacteroidia                | Chitinophagales    | Sediminibacterium            | Seq24                  | Seq58                  | 131          | 210                     |
| Bacteroidia                | Cytophagales       | Algoriphagus                 | Seq44                  | Seq44                  | 131          | 205                     |
| Bacteroidia                | Cytophagales       | Pseudarcicella               | Seq48                  | Seq48                  | 131          | 186                     |
| Bacteroidia                | Flavobacteriales   | Fluviicola                   | Seq128                 | Seq43                  | 123          | 265                     |
| Bacteroidia                | Flavobacteriales   | NA                           | Seq49                  | Seq49                  | 125          | 219                     |
| Bacteroidia                | Sphingobacteriales | NA                           | Seq36                  | Seq36                  | 121          | 234                     |
| Gammaproteobacteria        | Burkholderiales    | Candidatus<br>Methylopumilus | Seq12                  | Seq12                  | 141          | 335                     |
| Gammaproteobacteria        | Burkholderiales    | Limnohabitans                | Seq18                  | Seq76                  | 141          | 274                     |
| Gammaproteobacteria        | Burkholderiales    | NA                           | Seq187                 | Seq287                 | 119          | 152                     |
| Gammaproteobacteria        | Burkholderiales    | Polynucleobacter             | Seq33                  | Seq33                  | 138          | 238                     |
| Gammaproteobacteria        | NA                 | NA                           | Seq211                 | Seq211                 | 102          | 66                      |
| Phycisphaerae              | Phycisphaerales    | CL500-3                      | Seq19                  | Seq51                  | 118          | 430                     |
| Planctomycetes             | Gemmatales         | NA                           | Seq81                  | Seq81                  | 121          | 360                     |
| SL56 marine group          | NA                 | NA                           | Seq41                  | Seq41                  | 127          | 219                     |
| Thermoleophilia            | Gaiellales         | NA                           | Seq358                 | Seq358                 | 107          | 52                      |



## Deliverable D.T4.1.1

| non_cyanobacteria_aggr_PL |                     |               |                        |                        |              |                         |
|---------------------------|---------------------|---------------|------------------------|------------------------|--------------|-------------------------|
| Class                     | Order               | Genus         | 1st seq_16S<br>22Dec20 | 2nd seq_16S<br>22Dec20 | N<br>signals | Max 16s<br>signal raref |
| Thermoleophilia           | Solirubrobacterales | NA            | Seq146                 | Seq146                 | 108          | 256                     |
| Verrucomicrobiae          | Methylacidiphilales | NA            | Seq20                  | Seq20                  | 123          | 530                     |
| Verrucomicrobiae          | NA                  | NA            | Seq13                  | Seq13                  | 117          | 489                     |
| Verrucomicrobiae          | Opitutales          | Lacunisphaera | Seq86                  | Seq86                  | 121          | 136                     |
| Verrucomicrobiae          | Opitutales          | NA            | Seq142                 | Seq142                 | 102          | 69                      |
| Verrucomicrobiae          | Pedosphaerales      | SH3-11        | Seq46                  | Seq46                  | 117          | 222                     |

## APPENDIX 3

List of ciliates at detected at least on genus level by 18S metabarcoding approach in Eco-AlpsWater plankton samples

| Family_18S          | Genus_18Sraw     | ciliats Species_18S  | example<br>ASV_18S<br>_22Dec2<br>0 | Max<br>18s_signa<br>l_raref |
|---------------------|------------------|----------------------|------------------------------------|-----------------------------|
| Cyrtolophosidida    | Apocyrtolophosis | sp.                  | Seq13295                           | 2                           |
| CONThreeP_XX        | Askenasia        | sp.                  | Seq160                             | 236                         |
| Aspidiscidae        | Aspidisca        | sp.                  | Seq14760                           | 1                           |
| Chilodonellidae     | Chilodonella     | sp.                  | Seq18856                           | 1                           |
| Climacostomidae     | Climacostomum    | sp.                  | Seq6543                            | 2                           |
| Colepidae           | Coleps           | Coleps_hirtus        | Seq4320                            | 19                          |
| Colepidae           | Coleps           | Coleps_nolandii      | Seq5035                            | 1                           |
| Colpodida           | Colpoda          | Colpoda_steinii      | Seq9057                            | 4                           |
| Conchophthiridae    | Conchophthirus   | sp.                  | Seq12278                           | 30                          |
| Pleuronematida      | Cyclidium_2      | Cyclidium_plouneouri | Seq16895                           | 1                           |
| Scuticociliatia_2_X | Cyclidium_3      | Cyclidium_glaucoma   | Seq2572                            | 105                         |
| Cyrtolophosidida    | Cyrtolophosis    | sp.                  | Seq844                             | 141                         |
| Halteriidae         | Halteria         | Halteria_grandinella | Seq5788                            | 1                           |
| Pleurostomatida     | Hemiophrys       | Hemiophrys_procera   | Seq3347                            | 2                           |
| Histiobalantiidae   | Histiobalantium  | sp.                  | Seq7                               | 1723                        |
| Holostichidae       | Holosticha       | Holosticha_diademata | Seq125                             | 4                           |
| Colepidae           | Levicolaps       | Levicolaps_biwae     | Seq1773                            | 122                         |
| Pelagostrombidiidae | Limnostrombidium | sp.                  | Seq154                             | 398                         |
| Condylostomatidae   | Linostomella     | sp.                  | Seq1665                            | 91                          |
| Loxodidae           | Loxodes          | Loxodes_striatus     | Seq2623                            | 48                          |
| Metopidae           | Metopus_1        | Metopus_es           | Seq2579                            | 56                          |
| Thigmophryidae      | Myxophyllum      | sp.                  | Seq3620                            | 7                           |
| Nassulida           | Obertruria       | Obertruria_georgiana | Seq395                             | 226                         |
| Ophryoglenida       | Ophryoglena      | Ophryoglena_catenua  | Seq433                             | 345                         |
| Parameciidae        | Paramecium       | Paramecium_caudatum  | Seq3706                            | 18                          |



## Deliverable D.T4.1.1

| Family_18S            | Genus_18Sraw        | ciliats Species_18S       | example ASV_18S_22Dec20 | Max 18s_signal_raref |
|-----------------------|---------------------|---------------------------|-------------------------|----------------------|
| Parameciidae          | Paramecium          | Paramecium_putrinum       | Seq5828                 | 8                    |
| Parameciidae          | Paramecium          | Paramecium_tetraurelia    | Seq5148                 | 18                   |
| Holophryidae          | Pelagothrix         | Pelagothrix_alveolata     | Seq10701                | 14                   |
| Holophryidae          | Prorodon            | Prorodon_teres            | Seq5538                 | 12                   |
| Scuticociliatia_2_X   | Protocyclidium      | Protocyclidium_citrullus  | Seq19121                | 13                   |
| Cyrtolophosidida      | Pseudocyrtolophosis | sp.                       | Seq6918                 | 1                    |
| Litostomatea_XX       | Pseudoholophrya     | sp.                       | Seq365                  | 119                  |
| Pseudomicrothoracidae | Pseudomicrothorax   | Pseudomicrothorax_dubius  | Seq6242                 | 2                    |
| Strobilidiidae_D      | Rimostrombidium_D   | sp.                       | Seq31                   | 358                  |
| Spirostomidae         | Spirostomum         | Spirostomum_teres         | Seq636                  | 432                  |
| Stentoridae           | Stentor             | Stentor_amethystinus      | Seq305                  | 1451                 |
| Stentoridae           | Stentor             | Stentor_coeruleus         | Seq2833                 | 25                   |
| Stentoridae           | Stentor             | Stentor_muelleri          | Seq253                  | 453                  |
| Stentoridae           | Stentor             | Stentor_roeselii          | Seq203                  | 460                  |
| Strobilidiidae_E      | Strobilidium_E      | sp.                       | Seq2098                 | 61                   |
| Tintinnidiidae        | Tintinnidium        | sp.                       | Seq33                   | 1011                 |
| Tintinnidiidae        | Tintinnidium        | Tintinnidium_balechi      | Seq3252                 | 34                   |
| Trichodinidae         | Trichodina_1        | sp.                       | Seq2763                 | 16                   |
| Chilodonellidae       | Trithigmostoma      | Trithigmostoma_steini     | Seq615                  | 6                    |
| Urocentridae          | Urocentrum          | sp.                       | Seq9915                 | 3                    |
| Urostylidae           | Uroleptus           | sp.                       | Seq274                  | 646                  |
| Urostylidae           | Urostyla            | Urostyla_grandis          | Seq3951                 | 2                    |
| Urotrichidae          | Urotricha           | sp.                       | Seq247                  | 285                  |
| Sessilida             | Vorticella          | Vorticella_aequilata      | Seq137                  | 221                  |
| Sessilida             | Vorticella          | Vorticella_convallaria    | Seq4287                 | 40                   |
| Sessilida             | Vorticella          | Vorticella_gracilis       | Seq944                  | 4                    |
| Sessilida             | Vorticella          | Vorticella_microstoma     | Seq2092                 | 533                  |
| Platyophryida         | Woodruffides        | Woodruffides_metabolicus  | Seq8113                 | 13                   |
| Orthodonellidae       | Zosterodasys        | sp. (remark: marin taxon) | Seq3960                 | 28                   |

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### 5. References

- Kurmayer, R., Sivonen, K., Wilmotte, A., and Salmaso, N. (2017). *Molecular Tools for the Detection and Quantification of Toxigenic Cyanobacteria*. Wiley, Chichester.
- Salmaso, N. (2019). Effects of habitat partitioning on the distribution of bacterioplankton in deep lakes. *Front. Microbiol.* 10, 2257. doi:10.3389/fmicb.2019.02257.
- Salmaso, N., Albanese, D., Capelli, C., Boscaini, A., Pindo, M., and Donati, C. (2018). Diversity and Cyclical Seasonal Transitions in the Bacterial Community in a Large and Deep Perialpine Lake. *Microb. Ecol.* 76, 125–143. doi:10.1007/s00248-017-1120-x.
- Water Framework Directive (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Parliam.* doi:10.1039/ap9842100196.