



**ACTIVITY 6.07 - BIOLOGICAL  
DIVERSITY AND ECOSYSTEM  
FUNCTION INDICATORS (EBV, ECV)  
DATA COLLECTION AND PROVISION  
WITH A FOCUS ON FRESHWATER  
ECOSYSTEMS (LAKES).**

**Final report at 30/04/2025**



<b>Deliverable number:</b>	D6.15
<b>Work package:</b>	WP6 - Terrestrial Biosphere
<b>Intermediate Objective:</b>	WP6-IO6
<b>Deliverable type:</b>	<input checked="" type="checkbox"/> Document, report
	<input type="checkbox"/> Websites, patent filings, videos, etc.
	<input type="checkbox"/> Other: please specify .....
<b>Dissemination level:</b>	<input checked="" type="checkbox"/> Public
	<input type="checkbox"/> Restricted
<b>Estimated delivery (bimester):</b>	B15
<b>Actual delivery date:</b>	30/04/2025
<b>Author(s) (Partner-OU):</b>	Antonella PETROCELLI, Giuseppe DENTI (CNR-IRSA) with the collaboration of: Bachisio Mario PADEDDA, Antonella LUGLIE', Silvia PULINA, Paola CASIDDU, Cecilia SATTA, Cristina PITTALIS, Marco CHERCHI (UniSS, eLTER) Andrea BUFFAGNI, Simone CARDONI, Ester CECERE, Stefania ERBA, Diego FONTANETO, Lyudmila KAMBURSKA, Loredana PAPA, Roberta PISCIA, Lucia SPADA, Silvia ZAUPA (CNR-IRSA, DiSSCo)
<b>Reviewed by:</b>	ITINERIS Executive Board
<b>Note:</b>	

IR0000032 – ITINERIS, Italian Integrated Environmental Research Infrastructures System - CUP B53C22002150006 (D.D. n. 130/2022)  
Funded by EU - Next Generation EU  
Mission 4 “Education and Research” - Component 2: “From research to business” -  
Investment 3.1: “Fund for the realisation of an integrated system of research and innovation infrastructures”

## Table of contents

<b>1</b>	<b>INTRODUCTION.....</b>	<b>7</b>
<b>1.1</b>	<b>eLTER.....</b>	<b>7</b>
1.1.1	Lake of Bidighinzu.....	7
<b>1.2</b>	<b>DiSSCo.....</b>	<b>8</b>
1.2.1	Tardigrada from historical Ramazzotti collection.....	8
1.2.2	Inhabit Oligochaeta collection.....	8
1.2.3	River invertebrates collection.....	9
1.2.4	Andrea Buffagni personal collection.....	9
1.2.5	Herbarium TAR.....	9
1.2.6	Herbarium “Irma Pierpaoli” - MBMP.....	9
<b>2</b>	<b>ACTIVITIES.....</b>	<b>9</b>
<b>2.1</b>	<b>eLTER.....</b>	<b>9</b>
2.1.1	Lake of Bidighinzu.....	10
<b>2.2</b>	<b>DiSSCo.....</b>	<b>12</b>
2.2.1	Tardigrada from historical Ramazzotti collection.....	12
2.2.2	Inhabit Oligochaeta collection.....	13
2.2.3	River invertebrates collection.....	14
2.2.4	Andrea Buffagni personal collection.....	14
2.2.5	Herbarium TAR.....	15
2.2.6	Herbarium “Irma Pierpaoli” - MBMP.....	16
<b>3</b>	<b>DATA AND RESULTS.....</b>	<b>16</b>
<b>3.1</b>	<b>eLTER.....</b>	<b>16</b>
3.1.1	Lake of Bidighinzu.....	16
3.1.1.1	Abiotic parameters.....	16
3.1.1.2	Biotic parameters.....	18
3.1.1.3	Trophic state assessment.....	18
3.1.1.4	Long-Term Data Comparison.....	18
3.1.1.5	Estimation of nutritional load and modeling evaluation of expected concentrations.....	20
<b>3.2</b>	<b>DiSSCo.....</b>	<b>21</b>
3.2.1	Tardigrada from historical Ramazzotti collection.....	21
3.2.2	Inhabit Oligochaeta collection.....	22
3.2.3	River invertebrates collection and Andrea Buffagni personal collection.....	22
3.2.4	Herbarium TAR.....	25
3.2.5	Herbarium “Irma Pierpaoli” - MBMP.....	25
<b>4</b>	<b>INTERACTIONS WITH OTHER WPS OF ITINERIS OR OTHER PNRR PROJECTS.....</b>	<b>26</b>
<b>4.1</b>	<b>eLTER.....</b>	<b>26</b>
4.1.1	Lake of Bidighinzu.....	26
<b>4.2</b>	<b>DiSSCo.....</b>	<b>26</b>
4.2.1	Tardigrada from historical Ramazzotti collection and Inhabit Oligochaeta collection	26
4.2.2	River invertebrates collection and Andrea Buffagni personal collection.....	27
4.2.3	Herbarium TAR.....	27
<b>5</b>	<b>NEW EQUIPMENT.....</b>	<b>27</b>

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

<b>5.1</b>	<b>eLTER</b> .....	<b>27</b>
5.1.1	Lake of Bidighinzu.....	27
5.1.2	Other eLTER sites.....	27
<b>5.2</b>	<b>DiSSCo</b> .....	<b>27</b>
5.2.1	Tardigrada from historical Ramazzotti collection and Inhabit Oligochaeta collection 27	
5.2.2	River invertebrates collection and Andrea Buffagni personal collection .....	28
5.2.3	Herbarium TAR and Herbarium “Irma Pierpaoli” - MBMP .....	28
<b>6</b>	<b>COMMUNICATION AND DISSEMINATION</b> .....	<b>28</b>
<b>6.1</b>	<b>eLTER</b> .....	<b>28</b>
<b>6.2</b>	<b>DiSSCo</b> .....	<b>28</b>
<b>7</b>	<b>REFERENCES</b> .....	<b>29</b>

## Index of tables

Table 1 - Subdivision of slides in the boxes of the Ramazzotti collection. ....	8
Table 2 - List of collections provided for ITINERIS project from CNR-IRSA Brugherio Unit (River invertebrate collection and Andrea Buffagni personal collection). ....	23

## Index of figures

Fig. 1- Real-time remote monitoring platform in Lake Bidighinzu equipped with a multiparameter probe.....	7
Fig. 2- Monitoring activities in Lake Bidighinzu: sample collection using a Niskin bottle. ....	10
Fig. 3- Monitoring activities in Lake Bidighinzu: in situ measurements using a multiparameter probe.....	11
Fig. 4 – Monitoring activities in Lake Bidighinzu, collection of phyto- and zooplankton samples. .....	11
Fig. 5 - Monitoring activities in Lake Bidighinzu: determination of phytoplankton using inverted light microscopy.....	12
Fig. 6 – Ramazzotti Collection: Echiniscus spinuloides (RamVB-I-54). ....	13
Fig. 7 – Ramazzotti Collection: slide 54 Box I (RamVB-I-54_S).....	13
Fig. 8 – Digitalization of Inhabit Oligochaeta collection.....	13
Fig. 9 – Inhabit Oligochaeta collection: Potamothrix hammoniensis. a) Pectinate chaetae, b) spermathecal chaetae.....	14
Fig. 10 - Image acquisition from CAMPNP river invertebrate collection using a stereomicroscope. .....	14
Fig. 11 – Image acquisition of Ephemeroptera specimens using a digital microscope. ....	15
Fig. 12 – Digitization of a herbarium sheet through a planetarium scanner. ....	15
Fig. 13 – Labelling of a herbarium sheet. ....	16
Fig. 14 - Comparative summary of the investigated parameters during the activities of the ITINERIS project and the LTER series. ....	19
Fig. 15 – Ramazzotti Collection: slide 8 Box II.....	21
Fig. 16 – Ramazzotti Collection: egg of Macrobiotus hufelandii.....	21
Fig. 17 – Inhabit Oligochaeta collection: a) Slide InO-AvSL1A_S, b) specimen number 10 of the slide (InO-AvSL1A_10).....	22
Fig. 18- Limnephilidae image from Corongiu river (Sardinian slightly altered site). Image acquired with Stereomicroscope (8X). ....	22

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

Fig. 19 - Hydroptilidae image from Sardinian nearly pristine river site (catalogue number 00318_). Image acquired with Stereomicroscope (32.5X).....	23
Fig. 20 - Baetis image from Campania slightly altered river site (catalogue number 00060_). Image acquired with Stereomicroscope (8X).....	24
Fig. 21 - Ephemera image from Campania nearly pristine river site (catalogue number 00130_). Image acquired with Stereomicroscope (7X).....	24
Fig. 22 - Rhyacophylidae image from Piedmont (catalogue number 00022A). Image acquired with Stereomicroscope. a) 6.3X; b) 20X). ....	24
Fig. 23 - Image (slide) of Epeorus alpicola (catalog number EpheAB_MSH001).....	25
Fig. 24 - Two digitalized specimens of the herbarium TAR: a) Ulva laetevirens (Ulvales, Chlorophyta), b) Cystoseira compressa (Heterokontophyta, Fucales). ....	25
Fig. 25 - Two digitalized specimens of the herbarium MBMP: a) Alsidium corallinum (Rhodophyta, Ceramiales), b) Ericaria crinita (Heterokontophyta, Fucales).....	26

## 1 INTRODUCTION

Activity 6.7 “Improvement of monitoring in Italian southern freshwater and terrestrial ecosystem: structural, functional, biodiversity indicators of ecological and physical processes and integration of multiple data flow into a national system”, is in charge to the OU CNR-IRSA Taranto. It includes two different infrastructures, that is eLTER and DiSSCo, with different paths to reach the common ITINERIS main objective of creating a data hub, which will integrate the data systems of all the involved infrastructures.

### 1.1 eLTER

The Integrated European Long-Term Ecosystem, critical zone and socio-ecological Research (eLTER) is a European Research Infrastructure, still in the preparatory phase, which brings together national LTER networks from 26 European countries, including Italy. Each national network consists of several different type of territorial ecosystems, named “sites”, where long term observations are carried out to investigate their structure and functions and understand their response to environmental, societal and economic drivers. eLTER will become an operational European Infrastructure within 2027. In the context of the ITINERIS Project, CNR-IRSA will mainly contribute to the study of the Italian LTER site Lake of Bidighinzu. Another part of activities will be carried out at “Collelongo-Selva Piana”, “Foce Trigno-Marina di Petacciato”, “Piano Limina”, “Central Italy coastal dunes”, but they will be reported within the task 6.08.

#### 1.1.1 Lake of Bidighinzu

Bidighinzu Lake (<https://deims.org/3707cf71-7e04-41e3-8afc-518b293f6c07>) is a warm, monomictic, and hypereutrophic reservoir (dam) situated in northwestern Sardinia at an elevation of 334 meters above sea level. Constructed in 1956, its primary purpose is to provide drinking water for a population of approximately 160,000 users. The site features a real-time remote monitoring station equipped with a multiparameter probe housed on a floating platform (Fig. 1). Challenges in the potabilization process have emerged since the reservoir's early years of use, particularly during the summer and autumn seasons, due to hypolimnetic deoxygenation and an excessive presence of algae in the epilimnion. To address these issues, various restoration measures have been implemented in Bidighinzu Lake. Notably, an aeration system was installed in 1966 around the water intake tower, and in 1987, nutrient loads from the watershed were reduced by diverting civil and industrial wastewater downstream of the dam. In 2010, an experimental activity involving a micro-bubble aeration system was conducted in Lake Bidighinzu. The objective was to maintain sufficient oxygen levels in the hypolimnetic waters, especially during the summer months, to enhance the purification process, prevent phosphorus release from sediments, and mitigate eutrophication.



*Fig. 1- Real-time remote monitoring platform in Lake Bidighinzu equipped with a multiparameter probe.*

## 1.2 DiSSCo

The “Distributed System of Scientific Collections” (DiSSCo) is an European infrastructure, which includes naturalistic collections distributed in 23 countries (Italy included), more than 170 scientific institutions and 5,000 scientists, and several thousands of stored animal and plant specimens, both dried and wet. It is now entering the Transitional Phase (DiSSCo-RI) towards the final stage of becoming an ERIC within 2026. CNR-IRSA takes part in DiSSCo with six collections, shown below.

### 1.2.1 *Tardigrada from historical Ramazzotti collection*

The Ramazzotti collection, started in 1940s, is composed of numerous slides and microscopic preparations of Tardigrada, including animals and their eggs (which are useful for taxonomic recognition), collected and prepared by Giuseppe Ramazzotti (1898-1986). Samples taken from 1938 to 1964 in Europe, Africa, North and South America by this Italian taxonomist are part of a historical collection. The majority of the samples were collected in Europe, with Italy being the primary location, followed by Germany, France, Finland, Norway, Poland, Austria, Switzerland, Sweden, the Netherlands, and the United Kingdom. Samples from Africa (Uganda and Congo), North America (United States and Mexico), and South America (Chile) are also included. 3319 records are included in the Ramazzotti collection dataset, which was created within the framework of the Italian Integrated Environmental Research Infrastructures System project (ITINERIS), aimed to build the Italian Hub of Research Infrastructures in the environmental scientific domain. This dataset is made to protect information by digitizing samples and make sharing the information collected by Ramazzotti and stored at CNR-IRSA, Verbania easier. This is why every record in the database is associated with a photo of the microscopic slide. The data have been arranged into a standardized Darwin Core Archive format, with the aim of harmonizing it and making it accessible worldwide via the Global Biodiversity Information Facility infrastructure (GBIF). Photos and metadata about the specimens are also present.

The collection is composed of ten boxes, numbered in progressive order with Roman numerals, containing a variable number of slides. There are a total of 859 slides divided as follows:

*Table 1 - Subdivision of slides in the boxes of the Ramazzotti collection.*

<i>Box</i>	<i>n° of slides</i>
I	100
II	98
III	98
IV	100
V	99
VI	94
VII	87
IX	83
X	83
XI	17

### 1.2.2 *Inhabit Oligochaeta collection*

The digitization of a new collection of Oligochaetes, collected during the INHABIT project, has begun. The INHABIT Oligochaeta collection, preserved at the Water Research Institute, is composed of numerous slides and microscopic preparations of Oligochaetes. The animals were collected during the sampling campaigns of the project Life + Inhabit (Local hydro-morphology, habitat and RBMPs: New Measures to Improve ecological quality in South European rivers and lakes), which provided the study and the detailed analysis of the four Biological Quality Elements (BQE) (phytoplankton, macrophytes, macroinvertebrates and fishes) required by the Water Framework Directive 2000/60/CE. The samples were collected in 10 Italian lakes, located in two different Italian regions

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

(Piedmont and Sardinia) and in the two Ecoregions (Alpine and Mediterranean). They are natural and artificial lakes, divided into 6 for Piedmont and 4 for Sardinia. As regard Piedmont, among the natural water bodies, lakes Viverone, Candia, Mergozzo, Sirio and L. Avigliana piccolo were sampled, while among the reservoirs L. Morasco. The Sardinian lakes were the reservoirs of Bidighinzu, Sos Canales, Posada and Liscia. The digitization provides for each organism a photo, and for mature animals multiple photos to highlight the reproductive structures.

### *1.2.3 River invertebrates collection*

The collection contains several hundreds of different freshwater benthic macroinvertebrate specimens collected, as part of research project activity, from diverse Italian regions and a wide range of river types, covering a wide altitudinal and anthropic alteration/natural condition gradient. This collection is stored at the CNR-IRSA Brugherio headquarters.

### *1.2.4 Andrea Buffagni personal collection*

The collection is mainly dedicated to Ephemeroptera faunistic and taxonomy. The collection includes larvae and adults. Samples were intermittently collected since 1989 with the aim of maximizing information on Ephemeroptera diversity mainly on river ecosystems. The collection includes a set of microscope slides for Ephemeroptera species, where disassembled specimens are mounted for high magnification detail observation. This collection is stored at the CNR-IRSA Brugherio headquarters.

### *1.2.5 Herbarium TAR*

The collection of specimens for the phycological herbarium TAR began in 1984. It is a research herbarium, with dried seaweed specimens stored on cardboard sheets. A part of Pierpaoli's herbarium is also included within the herbarium TAR. This collection is stored at the CNR-IRSA Taranto headquarters.

The phycological herbarium TAR consists of little more than 500 sheets to date, with specimens of Rhodophyta, both Bangiophyceae and Florideophyceae, Fucophyceae, and Chlorophyta. Most of the samples are from the Ionian Sea, mainly from the Mar Piccolo basin and the Mar Grande basin in Taranto. Moreover, there are some samples from the Tyrrhenian and the Adriatic Sea, the Atlantic and the Pacific Ocean, and the Arctic, which were collected on study tours. As a part of Pierpaoli's herbarium, 103 samples collected by the researcher Irma Pierpaoli in the Mar Piccolo and the Mar Grande in the Twenties are present. The collection is continuously enriched by the acquisition of new seaweed specimens during project sampling activities.

### *1.2.6 Herbarium "Irma Pierpaoli" - MBMP*

The "Irma Pierpapoli" herbarium dates back to 1909. It consists of about 660 dried specimens of seaweeds, collected by the Italian phycologist Irma Pierpaoli along the Ionian coast of the Mar Piccolo and Mar Grande of Taranto, as well as the Adriatic coast of the Ancona province until 1965. From 1962 to 1972, some additional specimens were collected along the Apulian both Adriatic and Ionian coasts by Prof. Pietro Parenzan. This collection is owned by the Marine Biological Station of Porto Cesareo (University of Salento, Lecce, Italy), but is located at the CNR-IRSA Taranto headquarter for research purposes.

## 2 ACTIVITIES

### 2.1 eLTER

The main objective to be reached through the activities performed within the eLTER infrastructure was the implementation of a cross-RI database to store data and make them open. This requires to *Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

enhance the monitoring capacity at eLTER sites, especially in the southern part of Italy. For this purpose, ad-hoc observation and measurement campaigns are seasonally conducted. The acquisition of new common equipment and sensors at different sites allowed to collect harmonized data, useful to monitor ecosystem status and changes and integrate new indicators. At the site “Lake of Bidighinzu”, lake functioning and long-term variations are being monitored. The monitoring activities are monthly carried out. The activities carried out at the eLTER sites “Collelongo-Selva Piana”, “Foce Trigno-Marina di Petacciato”, “Piano Limina”, “Central Italy coastal dunes” will be reported within the Deliverables D6.6 and D6.24 (Task 6.08). Since the measurement of EBVs and ECVs requires the in situ positioning of the equipment, and their continuous movement is neither advisable for their proper functioning nor economically viable, a temporary relocation of some of those new acquired at those sites was ordered to carry out all the planned activities.

### 2.1.1 Lake of Bidighinzu

Lake water samples were collected monthly at a station situated in the deepest part of the reservoir. Samples were taken at various depths (0 m, 1 m, 2.5 m, 5 m, 7.5 m, 10 m, 15 m, and 20 m) using a Niskin bottle (Fig. 2). Monitoring activities for the tributary began on a monthly basis in January 2023, with the exception of August due to no recorded discharge.

Water temperature, conductivity, dissolved oxygen and pH were measured in situ with a multi-parameter probe (Eureka Manta 35+) while transparency was measured with a Secchi disk (Fig. 3). The samples were stored in a cold, dark environment for laboratory analyses of alkalinity, ammonium, nitrate, nitrite, total nitrogen, reactive silica, orthophosphate and total phosphorus following Strickland and Parsons (1972), and for chlorophyll a, following Golterman et al. (1978). Dissolved inorganic nitrogen (DIN) was obtained as the sum of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ .

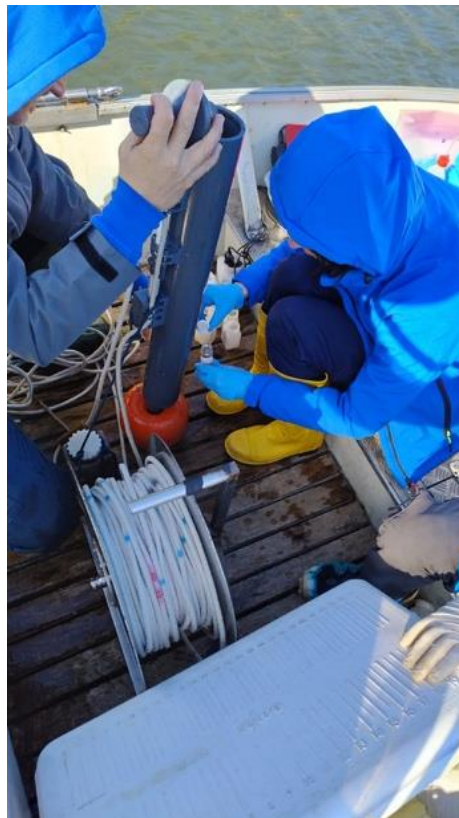


Fig. 2- Monitoring activities in Lake Bidighinzu: sample collection using a Niskin bottle.



*Fig. 3- Monitoring activities in Lake Bidighinzu: in situ measurements using a multiparameter probe.*

Samples for phytoplankton analysis were collected at 0 m, 1 m, 2.5 m, 5 m, 7.5 m, 10 m (Fig. 4). Phytoplankton samples were fixed with Lugol's solution and analysed after the sedimentation of variable volumes of water (5-10 ml) depending on phytoplankton cell density, according to the Utermöhl technique (1958) using an inverted microscope (Zeiss, Axiovert 5 with Axiocam 208) (Fig. 5). Cell counts were made at 200× magnification of the entire bottom of the sedimentation chamber for the larger and more easily identifiable species, and replicated at 400× on a selected number of fields for the smaller cells. Species were determined using several taxonomic guides. Unidentified cells were grouped as Others.

Correlative experiments to gain new insights into the effects of global warming on the planktonic food web of the lake have been also carrying out. For this activity, starting in the first fortnight of July 2024, controlled thermo-light mesocosms were set up to study the changes in the biological community at different water temperatures and different planktonic top predators (mesozooplankton or ciliates), focusing on the cell size structure of the phytoplankton. Analyses of picoplankton, phytoplankton, nanoflagellates and mesozooplankton are ongoing.

The instruments acquired through the ITINERIS project, specifically the multiparameter probe and autoclave with connected PCs, have been used for laboratory and in situ activities, as well as for data storage and calibration.



*Fig. 4 – Monitoring activities in Lake Bidighinzu, collection of phyto- and zooplankton samples.*

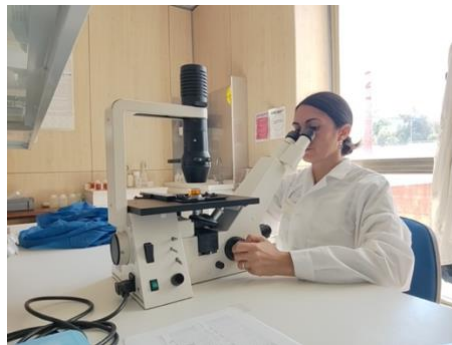


Fig. 5 - Monitoring activities in Lake Bidighinzu: determination of phytoplankton using inverted light microscopy.

## 2.2 DiSSCo

The main objective to be reached through the activities performed within the DiSSCo infrastructure was the provision of biodiversity data, also metadata, concerning specimens of animal and plant species stored in the Natural Scientific Collections at different CNR-IRSA headquarters. In particular, through the acquisition of new specific equipment, physical specimens have been digitalized and identified by Minimal Information Digital Specimen (MIDS). When available, additional information has been included (e.g. images and videos, DNA). However, due to their sensitive nature, it was best not to move those historical collections to the CNR-IRSA Taranto headquarter. In the same way, the continuous movement of instruments was neither advisable for their proper functioning nor economically viable. Therefore, a temporary relocation of some new acquired equipment at Brugherio and Verbania headquarters was ordered to carry out all the planned activities.

### 2.2.1 *Tardigrada* from historical Ramazzotti collection

Microscope slides, as well as microscopic preparations of Tardigrades, including animals and their eggs, have been photographed and scanned, and metadata (species name, locality, etc.) saved in a spreadsheet. In Google Earth, it was possible to find the coordinates by reporting the locations written on the label. Due to the unavailability of specific sampling point coordinates, the Locality centroid was utilized. WGS 84 data was used to georeference data. All data and metadata have been uploading to GBIF for open access and permanent storage.

Boxes XI, X, and IX have been photographed using a microscope (Zeiss Axioplan, with TiEsseLab TrueChrome HD IIS camera), while for box VII e VI and later a more modern instrument (Leica DM6 B, with Leica K3C camera and Leica DM4 B with Leica K3C camera) has been used. For each slide there are from a minimum of one animal (or egg) to a maximum of 177 organisms. Photos are available in BMP and TIFF formats with a size range between 5 MB and 18 MB. Each image has its own unique code, which has been renamed using the CollectionCode, the Roman numeral of the box, the slide number, and the specimen number. When there is only one animal in a slide, the last number has been omitted. ES: RamVB-I-54 = box I-slide (Fig. 6). In addition to the animals, individual slides have been also photographed, so as to keep track of the tags on them. In this case the photo has been renamed with the Roman numeral of the box, the number of the slide, and the letter S, to indicate that it is the image containing the slide (Fig. 7). The dataset was compiled using the name of the image as part of the unique code.

At the same time, the revision of the nomenclature of the species was performed using WORMS as a reference site (<https://www.marinespecies.org/>).

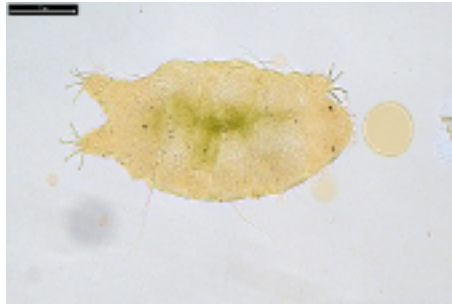


Fig. 6 – Ramazzotti Collection: *Echiniscus spinuloides* (RamVB-I-54).



Fig. 7 – Ramazzotti Collection: slide 54 Box I (RamVB-I-54\_S).

### 2.2.2 Inhabit Oligochaeta collection

The digitalization of the InO collection (Oligochaeta from lakes, collected during the Inhabit project) is in progress. This collection includes in total about 9500 animals and 297 slides divided as follows for the different lakes: Viverone 18, Mergozzo 64, Sirio 13, Candia 27, Avigliana piccolo 13, Morasco 20, Sos Canales 103, Bidighinzu 10, Posada 18 and Liscia 11. Each slide contains multiple individuals. The images were acquired using Leica DM4B microscope with Leica K3C Camera (Fig. 8). For each mature animal, more photos were taken from different parts of the body, to highlight the reproductive structures (Fig. 9). The photos are made in Tiff format and the dimensions are approximately 18 MB. Each image has a unique code, formed by the collection code (inO), the first letters of the lake name, the sampling period (C=circulation, S=stratification), the sampled area (L=littoral, S=sublittoral and P=deep), the transect number and the slide letter. In addition to the animals, individual slides have been also photographed, so as to keep track of the tags on them. The taxonomy of oligochaetes is based on the presence of reproductive structures present in mature organisms. Many specimens present in the collection are immature, for this reason it was not possible to assign the species to all the organisms, but they are often identified at the genus or family level.

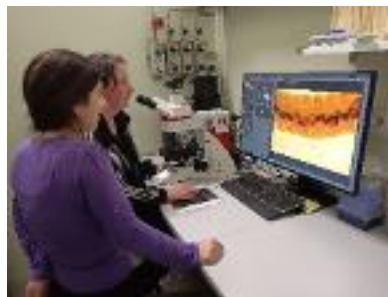


Fig. 8 – Digitalization of Inhabit Oligochaeta collection.

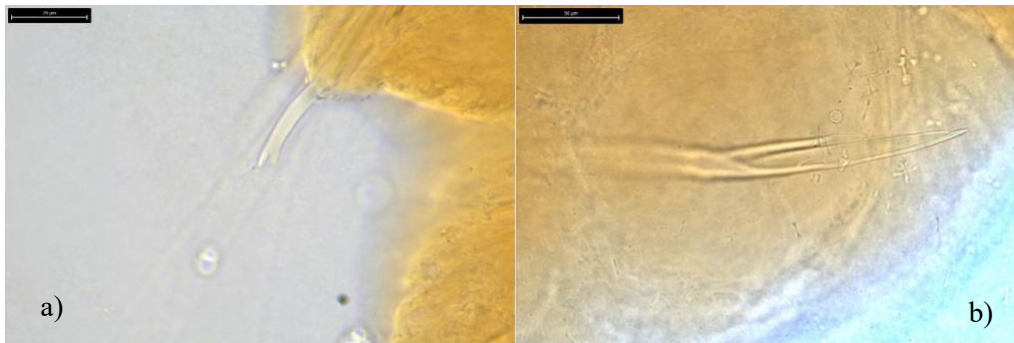


Fig. 9 – Inhabit *Oligochaeta* collection: *Potamothenix hammoniensis*. a) Pectinate chaetae, b) spermathecal chaetae.

### 2.2.3 River invertebrates collection

The digitization and the metadating of Ephemeroptera specimens slides and of multi taxa samples for a selection of rivers has been completed and data published in GBIF. Samples have been selected to cover a wide gradient of geographic distribution, site environmental quality (e.g., including near-pristine sites), altitude and local hydromorphology features. Selected areas include Sardinia Island, South Italy and the Alps.

The criteria to select samples and the data flow for digitization have been defined, and the selected format for data storing outlined. This format has been discussed and agreed among the different teams involved in ITINERIS dealing with digitization of collections. Such format takes into account GBIF system criteria, and fulfills Darwin Core system information, Minimum Information about a Digital Specimen (MIDS) and DiSSCo standards.

Selected samples have been verified. The verification process consists in confirming the correspondence between the physical sample, stored in boxes labelled by projects and/or geographical area and the information archived in CNR-IRSA databases. In doing so, preservation status of the different vials was checked and the best preserved samples selected for photos. Each record represents a taxon (mainly identified at family or genus level) collected at a site and belonging to a specific sampling unit. For each record information on MIDS level I on taxon name, collection data, sampling location, taxon abundance, determinavit are provided.

### 2.2.4 Andrea Buffagni personal collection

Data storage has been completed for a selection of samples of best preserved and representative specimens, able to provide information on Ephemeroptera distribution and in some cases endemisms. Pictures of microscope slides and single specimens have been taken.

The instruments acquired within the ITINERIS project have been used for both digitization and data storage activities, specifically the digital microscope, the optical microscope, the stereomicroscope and the connected workstations (Figs 10, 11).



Fig. 10 - Image acquisition from CAMPNP river invertebrate collection using a stereomicroscope.



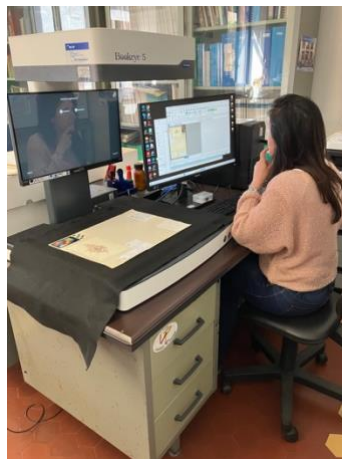
*Fig. 11 – Image acquisition of Ephemeroptera specimens using a digital microscope.*

### 2.2.5 Herbarium TAR

Dried specimens were photographed and scanned thanks to the new acquired equipment, such as the planetarium scanner and a digital camera (Fig. 12). Moreover, a digital handheld microscope was used in order to capture some details as morphological and reproductive elements. Before obtaining a digital image, 2 labels were prepared and applied on each exiccatum. The former contains information about the Institution name and the “Specimen ID”, an alphanumeric code made up of the collection code and a progressive number (e.g., TAR-000101). The latter contains information about the samples such as the species name, the place of collection, the name of the person who sampled the specimen and the name of the person who identified it. If this information was coincident in more than a sheet, a unique Specimen ID was assigned.

Data from the labels and other information (e.g. collection date and location, collector, reproductive phase) of herbarium sheets were documented in an Excel spreadsheet in order to obtain a relative database. When necessary, also the nomenclature update has been performed using Algaebase website (<https://www.algaebase.org/>).

To store temporarily the digitalized images, a Microsoft-Teams® folder was created.



*Fig. 12 – Digitization of a herbarium sheet through a planetarium scanner.*

### 2.2.6 Herbarium “Irma Pierpaoli” - MBMP

Regarding “Irma Pierpaoli” herbarium, the registration process on Index Herbariorum (<https://sweetgum.nybg.org/science/ih/>) was completed and the relative assigned code was MBMP. The codification process proceeded with the same modalities than the Herbarium TAR and to date, the digitization and the acquisition of metadata have been finished (Fig. 13). The nomenclature update was performed according to the Algaebase website (<https://www.algaebase.org/>). As for the herbarium TAR, also for MBMP collection all images were stored in a dedicated folder on Microsoft-Teams®.



Fig. 13 – Labelling of a herbarium sheet.

For both the digitalized collections the publication was carried out, using the Integrated Publishing Toolkit (IPT) that shares biodiversity data, on GBIF portal for open access and permanent storage.

## 3 DATA AND RESULTS

### 3.1 eLTER

#### 3.1.1 Lake of Bidighinzu

Previously available data sets include information on phytoplankton and limnological variables spanning the last 70 years. The data series pertains to the main limnological parameters, including Secchi depth, temperature, pH, conductivity, dissolved oxygen and saturation, alkalinity, NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, total nitrogen, soluble reactive phosphorus, total phosphorus, dissolved silica, and dissolved concentrations of Fe and Mn. Additionally, the data covers phytoplankton abundances, such as chlorophyll-a, cell densities, biomass, as well as class and species composition. Currently, data are being collected in spreadsheets based on a database setup structured into records. In the future, there is an expectation to organize the data within a shared system and distribute it through a common platform.

Results from the ITINERIS activities show compositional changes in both autotrophic and heterotrophic planktonic groups throughout the experiments with differences between treatments.

##### 3.1.1.1 Abiotic parameters

The thermal typology of Lake Bidighinzu is classified as warm monomictic. As it is characteristic of such lakes, particularly in conditions of eutrophication, its functioning is marked by a dual pattern of winter mixing and summer stratification. This pattern substantially affects the dynamics of all investigated physical, chemical, and biological variables.

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

In Lake Bidighinzu, during summer stratification, the warmer and less dense surface water (epilimnion or mixing zone) lies above the colder and denser bottom water (hypolimnion). Between depths of 8 and 15 meters, these two water masses are separated by the metalimnion, a layer characterized by a sharp temperature decline. Consequently, the hypolimnion remains isolated and, throughout the entire period of thermal stratification (typically from May to October), it cannot exchange with the atmosphere. On the other hand, the epilimnion loses contact with the sediments and the associated nutrient recycling processes. Additionally, the difference in water density slows down or even prevents phytoplankton from sinking.

Generally, the transition from periods of water circulation to stratification marks a shift from a phase with fewer management challenges and better water quality to one characterized by a significant decline in water quality and the emergence of complex usage issues.

The investigation identified a high concentration of nutrients as the primary cause of the observed issues. This excess of nutrients initiates and sustains the eutrophication process, leading to unfavorable conditions for the phytoplankton community, which is dominated by Cyanobacteria. The high cell density and species composition of this assemblage clearly indicate eutrophic to highly eutrophic conditions. More specifically, the detailed patterns of parameter variations are described as follows.

Surface temperature increased gradually from winter to summer, reaching a surface peak of 27.48 °C in August. This summer warming leads to thermal stratification, with a clear distinction between epilimnion and hypolimnion. Dissolved oxygen showed an inverse correlation with temperature: high in winter (up to 113% in February), it reached supersaturation in the summer epilimnion (up to 145% in July) due to photosynthetic activity, but dropped below 10% in the hypolimnion, indicating hypoxia. This strong reduction at the hypolimnion level is a consequence of the transfer of organic matter from the surface production areas to deeper waters, where the oxygen demand necessary for the mineralization of the organic matter produced is greater. In fact, during the summer months, the lake reached critical levels of under-saturation (>30%) starting from a depth of 5 m.

The significant correlation between the epilimnion's pH and the biotic component is not coincidental. In fact, during the months with the highest production, the pH values showed the most intense peaks (reaching up to 9.41 in June) and averaged 8 units, which is typical of a eutrophic system. Conversely, the winter months, which had the lowest productivity, also recorded the minimum pH values (as low as 7.12 in January). Conductivity showed moderate variations. It slightly increased in winter, reaching a peak of 507  $\mu\text{S}/\text{cm}$  in February, likely due to continental leaching. Conversely, it decreased in summer, reaching a minimum of 388  $\mu\text{S}/\text{cm}$  in August, probably because the biota increased its selective absorption of dissolved ions starting in spring.

The nutrients nitrogen and phosphorus exhibited a pattern consistent with the hydrophysical dynamics typical of a highly eutrophic system. For all components, high concentrations were observed in winter (often peaking in March) due to runoff. These concentrations declined in spring as a result of algal uptake, then increased again in the summer hypolimnion due to sediment release. Total nitrogen reached a peak of 3622  $\text{mg N m}^{-3}$  in March and a low of 912  $\text{mg N m}^{-3}$  in June.

Specifically, the values detected for dissolved inorganic nitrogen components, nitric nitrogen and ammonia nitrogen, were significant in terms of both the water column average and the maximum peak. For ammonia nitrogen, the average concentration during the study period was approximately 259  $\text{mg N m}^{-3}$ . Extremely high maximum values were observed in the summer due to the mineralization of biomass in the deeper layers, coinciding with hypolimnic anoxia (lack of oxygen), reaching a peak of 2201  $\text{mg N m}^{-3}$  in September. Nitric nitrogen showed a temporal pattern typically associated with eutrophy. Specifically, the values were higher in the winter and spring months (maximum column mean of 1096  $\text{mg N m}^{-3}$  in January), corresponding to increased leaching from the catchment basin. Conversely, the values were significantly lower in the summer and autumn months (minimum of 19  $\text{mg N m}^{-3}$  in November) due to intense consumption by phytoplankton, leading to algal blooms.

Throughout the study period, the detected concentrations of total phosphorus were consistently high, which led to the significant algal growth observed. The average phosphorus concentrations in the water column ranged from 166 mg P m<sup>-3</sup> in February to 289 mg P m<sup>-3</sup> in October.

The high levels of reactive phosphorus recorded in the hypolimnion, particularly during the summer months (exceeding 650 mg P m<sup>-3</sup> between August and September, with a peak of 824 mg P m<sup>-3</sup> in October), are also linked to the intense mineralization of organic matter produced in the upper water layers. Specifically, these high values, combined with anoxic conditions, likely caused substantial phosphorus release from the sediments, contributing to a significant internal nutrient load.

The fraction of phosphorus bound to certain metals, especially iron in the form of ferric phosphate, is highly sensitive to redox potentials. Under reducing conditions, trivalent iron tends to be reduced to divalent iron, releasing the bound phosphate ions (HPO<sub>4</sub><sup>2-</sup>), making them available for further algal growth. The iron concentration data indicates that this mechanism was particularly pronounced during the summer months: iron levels in the deeper waters (below 15 m) increased from approximately 200-400 mg Fe m<sup>-3</sup> in winter to a peak of 2922 mg Fe m<sup>-3</sup> in August.

Reactive silica ranged from a peak of 8.59 mg Si L<sup>-1</sup> in March to a minimum of 2.59 mg Si L<sup>-1</sup> in May.

#### 3.1.1.2 Biotic parameters

Chlorophyll a, an indicator of phytoplankton dynamics and production, exhibited a complex trend. Average values in the photic zone during the study period were high (14 mg m<sup>-3</sup>), exceeding Vollenweider's (1982) eutrophication threshold of 8 mg m<sup>-3</sup>. A peak of 107 mg m<sup>-3</sup> was observed in October. While concentrations generally decreased with depth (reaching a spring minimum of 0.54 mg m<sup>-3</sup> in April at 7.5 m), exceptions like high December values (over 12 mg m<sup>-3</sup> at 10 m) suggest a complex interplay of environmental factors.

Regarding the phytoplankton component, analytical determinations are ongoing; therefore, only data from the first half of 2024 (January-June) are currently available. Data showed an average cell density of 71.2 x 10<sup>6</sup> cells L<sup>-1</sup> in this temporal period, with a peak of 149.9 x 10<sup>6</sup> cells L<sup>-1</sup> recorded in February. Cyanophyceae were dominant, with an average density of 61 x 10<sup>6</sup> cells L<sup>-1</sup>. The peaks of density of the most represented species, *Snowella* sp. and *Aphanothece* sp., exceeded 100 x 10<sup>6</sup> cells L<sup>-1</sup>. Following this, the average cell density of Bacillariophyceae was of 4.4 x 10<sup>6</sup> cells L<sup>-1</sup>, with *Cyclotella* sp. being the most abundant species and reaching a maximum of approximately 30 x 10<sup>6</sup> cells L<sup>-1</sup>.

#### 3.1.1.3 Trophic state assessment

Models that synthesize trophic state evaluation, including the Vollenweider (OECD, 1982) model and Carlson's TSI, corroborate the significant eutrophication identified through individual chemical-physical and biotic analyses. In particular, the levels of the main descriptors indicate a condition of hyper-eutrophy. Total phosphorus places the lake in a eutrophic state according to the OECD fixed interval classification, but OECD Probability Distribution Diagrams suggests a higher probability of hypertrophy (56%), with a TSI of 79. Chlorophyll a, with a TSI of 55 and a probability of 68%, confirms the eutrophic state. The maximum peak of chlorophyll a further strengthens this assessment, highlighting a combined probability of eutrophy/hypertrophy of 99%. Although the transparency suggests hypertrophy with an 88% probability, its TSI of 63 classifies the lake as eutrophic. A discrepancy exists between the TSI calculated using total phosphorus (79) and those based on chlorophyll a (55) and transparency (63). According to Carlson (1977), this difference may result from intense zooplankton grazing, which could reduce chlorophyll a and transparency levels relative to total phosphorus.

#### 3.1.1.4 Long-Term Data Comparison

The analysis of data from the latest ITINERIS project year revealed a significant uniformity when compared to the long-term LTER series (established in 1988), falling within the typical range of variation for that series. Although a general downward trend was noted in the average, peak, and low values, the lake continues to exhibit high nutrient levels, suggesting a eutrophic to hyper-eutrophic

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

condition (Fig. 14). This underscores the necessity of implementing measures in the catchment area to decrease eutrophic inputs.

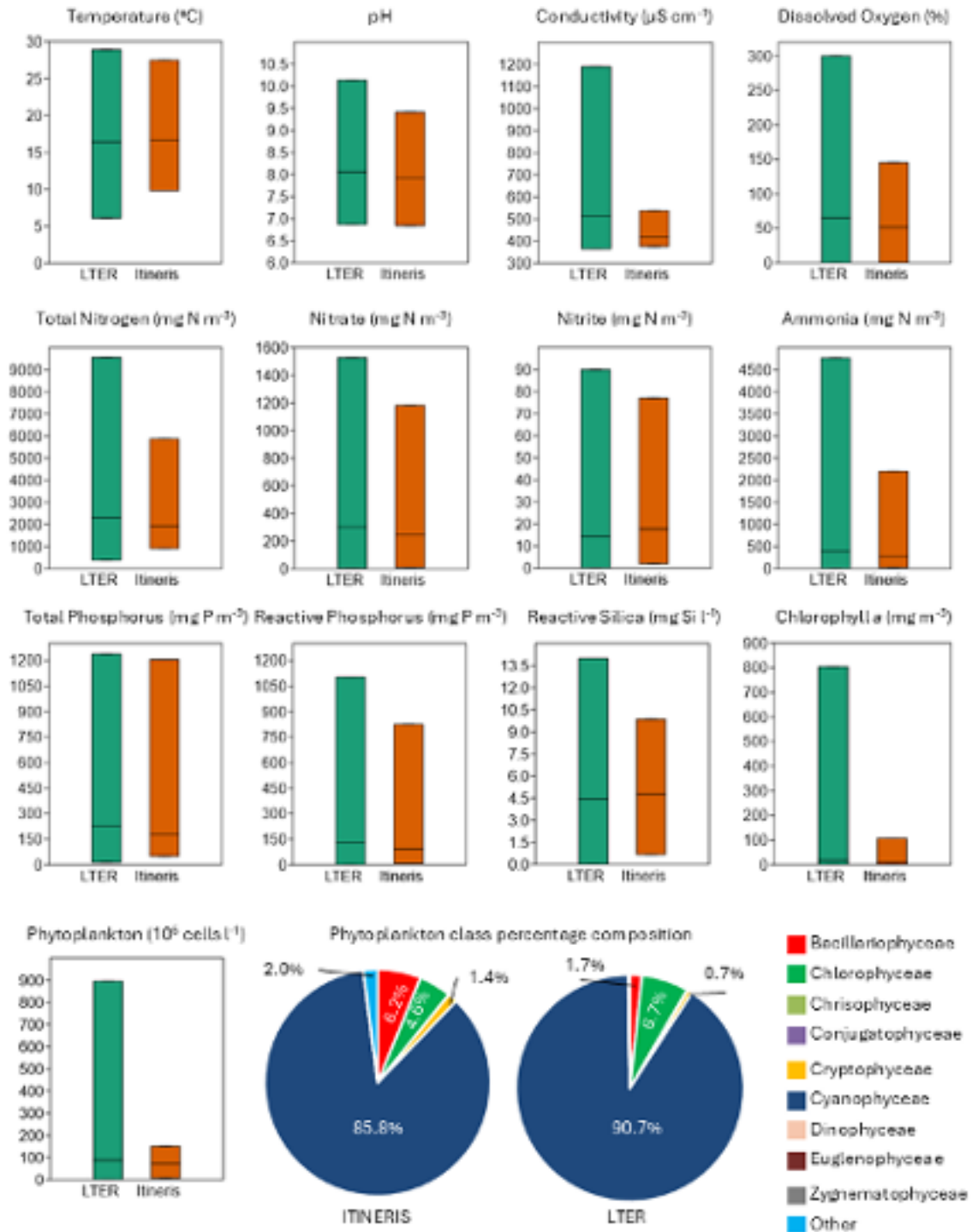


Fig. 14 - Comparative summary of the investigated parameters during the activities of the ITINERIS project and the LTER series.

- Temperature: The ITINERIS series has a narrower temperature range (9.81 °C to 27.48 °C) compared to the LTER series (6.10 °C to 28.90 °C), despite a slightly higher average temperature of 16.63 °C versus 16.39 °C for the LTER series.
- pH: The average values are similar for both series (ITINERIS: 7.93; LTER: 8.06), as are the minimum values (ITINERIS: 6.84; LTER: 6.87). However, the maximum value is lower in the ITINERIS series (9.41) compared to the LTER series (10.14).
- Conductivity: Although the ITINERIS data are within the fluctuation range of the LTER series, the maximum (ITINERIS 517 mS cm<sup>-1</sup>; LTER 1190 mS cm<sup>-1</sup>) and average (ITINERIS 417 mS cm<sup>-1</sup>; LTER 512 mS cm<sup>-1</sup>) values are significantly lower for the ITINERIS series. The minimum values are comparable (ITINERIS 375 mS cm<sup>-1</sup>; LTER 365 mS cm<sup>-1</sup>).
- Oxygen: The mean (ITINERIS 52%; LTER 65%) and minimum (0% for both series) values are similar. The maximum values, however, differ significantly (ITINERIS 145%; LTER 300%).
- Nutrients:
  - Nitrogen: Total nitrogen decreases in the ITINERIS series, with lower mean (ITINERIS: 1933 mg N m<sup>-3</sup>; LTER: 2317 mg N m<sup>-3</sup>) and maximum (ITINERIS: 5865 mg N m<sup>-3</sup>; LTER: 9571 mg N m<sup>-3</sup>) values. Minimum values are higher in the ITINERIS series (906 mg N m<sup>-3</sup>) compared to the LTER series (377 mg N m<sup>-3</sup>). The ammonia component (14% in ITINERIS; 17% in LTER) prevails over the nitrate component (13% in both series), while nitrite values are modest and comparable between the two.
  - Phosphorus: Total phosphorus values are lower in the mean (ITINERIS 178 mg P m<sup>-3</sup>; LTER 229 mg P m<sup>-3</sup>) and in the maximum (ITINERIS 1205 mg P m<sup>-3</sup>; LTER 1236 mg P m<sup>-3</sup>), while the minimum value are higher in the ITINERIS series (50 mg P m<sup>-3</sup>) than in the LTER (15 mg P m<sup>-3</sup>). The same trend is observed for the reactive form.
  - Reactive silica: The average and minimum values are comparable, while the maximum value is much lower in the ITINERIS series (9.9 mg Si L<sup>-1</sup>) than in the LTER (14 mg Si L<sup>-1</sup>).
- Chlorophyll a: A reduction in system productivity is evident, as shown by the lower average values (ITINERIS: 10 mg m<sup>-3</sup>; LTER: 18 mg m<sup>-3</sup>) and maximum values (ITINERIS: 107 mg m<sup>-3</sup>; LTER: 803 mg m<sup>-3</sup>). The minimum values remain comparable.
- Phytoplankton: The limited representativeness of the data does not allow a reliable comparison between the ITINERIS and LTER series. The total cell densities of the ITINERIS series (71.3 x 10<sup>6</sup> cells L<sup>-1</sup>) are similar to the multi-year average of the LTER series (87.9 x 10<sup>6</sup> cells L<sup>-1</sup>). However, the peaks observed in the ITINERIS series (149.9 x 10<sup>6</sup> cells L<sup>-1</sup>) are significantly lower than those observed in the LTER series (895.7 x 10<sup>6</sup> cells L<sup>-1</sup>). As regards the percentage contribution to total density of each phytoplankton class detected, the Cyanophyceae represent the dominant class in both series, with 85.8% for ITINERIS and 90.5% for LTER. Considering the other phytoplankton classes, ITINERIS presents a succession of Bacillariophyceae (6.2%) and Chlorophyceae (4.6%), while in the LTER series the percentages are reversed in favour of Chlorophyceae (6.7%), followed by Bacillariophyceae (1.7%).

### 3.1.1.5 Estimation of nutritional load and modeling evaluation of expected concentrations.

A preliminary assessment of the lake's hydrological balance outlined the current water usage and its impact on water quality. The data indicates a lake average volume of approximately 4.019 x 10<sup>6</sup> m<sup>3</sup> during the hydrological year, with a total contribution of 6.736 x 10<sup>6</sup> m<sup>3</sup> from the catchment basin. Based on experimental measurements conducted in the tributary, the nutrient loads draining from the catchment area were determined to be 4.95 t P a<sup>-1</sup> of phosphorus and 74.3 t N a<sup>-1</sup> of nitrogen. To the water discharge from the catchment area, an additional volume of 8.788 x 10<sup>6</sup> m<sup>3</sup> must be added, resulting from the transfer via the interconnection of the Alto Temo reservoir into the pipeline. The additional loads due to the transferred volumes from the Alto Temo reservoir were calculated exclusively for phosphorus, amounting to 1.04 t P a<sup>-1</sup>. The trophic response of the lake system, i.e., the expected concentrations of phosphorus and chlorophyll a induced by the territorial nutrient loads described above, was evaluated using the Vollenweider predictive model developed by the OECD, in the version adapted for shallow and flooded lakes (Shallow Lakes and Reservoirs, OECD, 1982).

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

The model focused on phosphorus, which is the most important element for primary production and for which this model, already tested in other Sardinian contexts, can reasonably be applied. This approach provides a useful tool for planning territorial interventions aimed at reducing eutrophication loads. Through subsequent territorial analysis, it is possible to identify the contributions from different sources and to hypothesize their potential selective reduction, along with the corresponding probable trophic levels that the water body would assume.

Starting from the estimated input load of  $5.99 \text{ t P a}^{-1}$  and the turnover time of 0.292 years, calculated for the period January–December 2024, the model estimates an expected annual mean phosphorus concentration in the lake of approximately  $150 \text{ mg P m}^{-3}$ . This value is lower than the concentration measured experimentally during the same monitoring period ( $178 \text{ mg P m}^{-3}$ ). This discrepancy requires further investigation and verification, but it is likely that a significant contribution originates from additional internal loads released by the sediments. Therefore, to restore the system to a more tolerable mesotrophic condition, the modeling will be useful for identifying strategies to be proposed in a load contingency plan.

## 3.2 DiSSCo

### 3.2.1 Tardigrada from historical Ramazzotti collection

The dataset contains 4178 records of occurrences from 1938-1964, 859 of which are slides (Fig. 15) and 3319 are actual samples of Tardigrada (Fig. 16). The information on the slide contains a species name for 1751 of them. Taxonomic revision is necessary for the others, because the slide contains numerous species, and a specialist is required to assign each sample to the correct one. So, a higher taxonomic level, like genus, order, or family, has been assigned to these individuals. Out of the 204 locations in the dataset, only 88 could be traced with coordinates. The dataset and all images are available and can be consulted on Global Biodiversity Information Facility infrastructure (GBIF <https://www.gbif.org/dataset/e9aeed17-b33f-4af2-8471-9be87633d7e1>) and the Collection is also registered in the Global Registry of Scientific Collections (GRSciColl <https://scientific-collections.gbif.org/collection/91ebb3a8-89cb-4403-8324-3ea2d6d445d1>).



Fig. 15 – Ramazzotti Collection: slide 8 Box II.

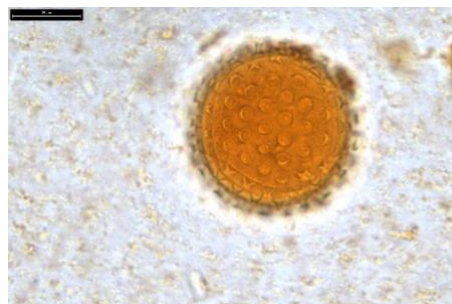


Fig. 16 – Ramazzotti Collection: egg of *Macrobiotus hufelandii*.

Additional information can be found in Deliverable 6.14 and Activity 6.06.

### 3.2.2 *Inhabit Oligochaeta collection*

At the moment, 671 images have been taken and 123 records inserted into the dedicated dataset. All the specimens digitized so far come from two Piedmont lakes: Avigliana and Candia. The specimens digitized so far come from two Piedmont lakes: Avigliana and Candia. In particular, for the first lake all the animals were photographed and all the information was inserted into the dataset, with a total of 316 photos (303 specimens and 13 slides) (Fig.17) and 123 records. For Lake Candia instead the acquisition of photos has begun, with the production of 355 images, but no information has yet been inserted into the dataset.



Fig. 17 – *Inhabit Oligochaeta* collection: a) Slide InO-AvSL1A\_S, b) specimen number 10 of the slide (InO-AvSL1A\_10).

### 3.2.3 *River invertebrates collection and Andrea Buffagni personal collection*

A total of six collections were published in GrSciCol and GBIF, from CNR-IRSA Brugherio Unit, following ITINERIS project purposes (Table 2). Two collections have been submitted from Sardinia. These include samples from slightly altered (277 specimens digitized) and pristine (reference) sites (642 specimens digitized). The dataset comprises the occurrences of 36 invertebrate families from slightly altered river sites, divided in four phyla, four classes and 12 orders. The dataset from nearly pristine (Reference) sites comprises the occurrences of 53 invertebrate families, divided in four phyla, four classes and 14 orders. For both Sardinian collections the most represented taxonomic group is the Arthropoda phylum and particularly the Insecta class. For three insect orders, namely Plecoptera, Odonata and Ephemeroptera, occurrence data are available at the genus level. More than 300 images of best preserved Sardinian specimens were captured (Figs 18, 19).



Fig. 18 - *Limnephilidae* image from Corongiu river (Sardinian slightly altered site). Image acquired with Stereomicroscope (8X).



Fig. 19 - Hydropsychidae image from Sardinian nearly pristine river site (catalogue number 00318\_). Image acquired with Stereomicroscope (32.5X).

Table 2 - List of collections provided for ITINERIS project from CNR-IRSA Brugherio Unit (River invertebrate collection and Andrea Buffagni personal collection).

Collection type	Collection name	Collection code	Institution	Institution code	Main taxa	Nr. of physical specimens digitized	Environment	DOI
Slides	Ephemeroptera BUFFAGNI-	EpheAB	Istituto di Ricerca Sulle Acque	CNR-IRSA-MI	Ephemeroptera	58	freshwater	<a href="https://doi.org/10.15468/ctauz3">https://doi.org/10.15468/ctauz3</a>
Preserved	Sardinia river invertebrates – slightly altered sites	SARDNP	Istituto di Ricerca Sulle Acque	CNR-IRSA-MI	Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Trichoptera, Diptera.	277	freshwater	<a href="https://doi.org/10.15468/uta29e">https://doi.org/10.15468/uta29e</a>
Preserved	Campania River invertebrates - slightly altered sites	CAMPNP	Istituto di Ricerca Sulle Acque	CNR-IRSA-MI	Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Trichoptera, Diptera.	236	freshwater	<a href="https://doi.org/10.15468/nnrbrqm">https://doi.org/10.15468/nnrbrqm</a>
Preserved	Piedmont River Invertebrates - slightly altered sites	PIEMNR	Istituto di Ricerca Sulle Acque	CNR-IRSA-MI	Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Trichoptera, Diptera.	611	freshwater	<a href="https://doi.org/10.15468/dtu2jv">https://doi.org/10.15468/dtu2jv</a>
Preserved	Sardinia river invertebrates – nearly pristine sites	SARDRP	Istituto di Ricerca Sulle Acque	CNR-IRSA-MI	Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Trichoptera, Diptera.	642	freshwater	<a href="https://doi.org/10.15468/32ra6q">https://doi.org/10.15468/32ra6q</a>
Preserved	Campania river invertebrates - nearly pristine sites	CAMPRP	Istituto di Ricerca Sulle Acque	CNR-IRSA-MI	Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Trichoptera, Diptera.	210	freshwater	<a href="https://doi.org/10.15468/v5c55v">https://doi.org/10.15468/v5c55v</a>

Two collections have been submitted from Southern Apennines (Campania region), these two also include samples from slightly altered (236 specimens digitized) and pristine (reference) sites (210 specimens digitized). The dataset comprises the occurrences of 28 insect families for slightly altered rivers, divided in 5 orders. The dataset from nearly pristine (reference) sites comprises the occurrences of 30 insect families, divided in 6 orders. Most records refer to family level identification, information on Ephemeroptera taxa and a few other groups is provided at genus level. About 100 images of best preserved Campania specimens were captured (Figs 20, 21).



*Fig. 20 - Baetis image from Campania slightly altered river site (catalogue number 00060\_). Image acquired with Stereomicroscope (8X).*



*Fig. 21 - Ephemera image from Campania nearly pristine river site (catalogue number 00130\_). Image acquired with Stereomicroscope (7X).*

One collection from Alpine river invertebrates was submitted, from slightly altered river sites in Piedmont region. 611 specimens were digitized and a total of more than 100 images acquired. The dataset comprises the occurrences of 43 invertebrate families, divided in three phyla, two classes and 13 orders. The most represented taxonomic group is the Arthropoda phylum and particularly the Insecta class. For three insect orders, namely Plecoptera, Odonata and Ephemeroptera, occurrence data are available at the genus level (13 genera). About 100 images of best preserved Piedmont specimens were acquired (Fig. 22).



*Fig. 22 - Rhyacophylidae image from Piedmont (catalogue number 00022A). Image acquired with Stereomicroscope. a) 6.3X; b) 20X.*

A part of Ephemeroptera slides held at CNR-IRSA in Brugherio, for a total of 58 digital specimens was submitted (Fig. 23). The list includes specimens primarily collected in Italy starting from 1989 using qualitative methods, including some Italian endemisms and covering a wide geographical

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

range. As part of the ITINERIS project and the National Biodiversity Future Centre (NBFC), Ephemeroptera samples have been curated, catalogued, and digitised to make natural history collections virtually available to the public at any level, simplifying access and sharing of information.

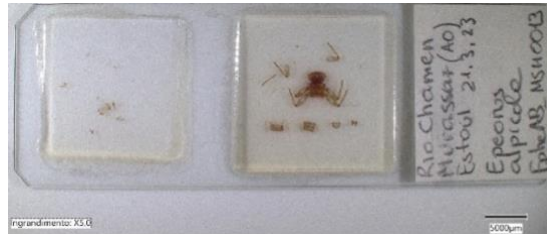


Fig. 23 - Image (slide) of *Epeorus alpicola* (catalog number *EpheAB\_MSH001*).

Further details are provided within Deliverable 6.14, Activity 6.06.

### 3.2.4 Herbarium TAR

The digitization of the herbarium TAR was completed. From a total of 507 sheets on paper, 394 have been selected and 353 digital sheets were encoded since several of them shared the same MIDS. From a taxonomic point of view, 3 Spermatophyta, 29 Chlorophyta, 47 Heterokontophyta (Phaeophyceae), 76 Rhodophyta have been digitalized.

To date a first dataset named “The Solieriaceae of the Herbarium TAR”, a small part of TAR collection, was uploaded to GBIF portal. The dataset can be visualized to the link <https://scientific-collections.gbif.org/collection/e93e42d2-6f14-424a-befd-3c4e921167f1>.

Since the TAR collection is continuously being updated with new samples, the digitization of data and images will continue over time.

Each digitalized image showed specific elements: 2 labels, a color-check and a paper ruler (Fig. 24).



Fig. 24 - Two digitalized specimens of the herbarium TAR: a) *Ulva laetevirens* (Ulvales, Chlorophyta), b) *Cystoseira compressa* (Heterokontophyta, Fucales).

Deliverable 6.14 and Activity 6.06 provide additional information.

### 3.2.5 Herbarium “Irma Pierpaoli” - MBMP

The digitization of Herbarium MBMP was also performed. From a total of 400 sheets on paper, 553 digital specimens were codified. From a taxonomic point of view, 75 Rhodophyta, 36 Chlorophyta and 29 Heterokontophyta (Phaeophyceae) species, were digitalized. To date, a first dataset named “The Rhodophyta of Taranto Seas” was uploaded on GBIF portal to share data about species

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

collected by Irma Pierpaoli during the first years of the last century. The dataset can be consulted at the following link: <https://scientific-collections.gbif.org/collection/750698d3-458b-4de1-b929-355da92cbc56>.

The digitalized images belonging to the herbarium MBMP consisted of 2 labels, one paper ruler and a color checker. In addition, other original labels were present at the bottom of the sheet. They refer to revisions done in the past (Fig. 25).



Fig. 25 - Two digitalized specimens of the herbarium MBMP: a) *Alsidium corallinum* (Rhodophyta, Ceramiales), b) *Ericaria crinita* (Heterokontophyta, Fucales).

Deliverable 6.14 in Activity 6.06 provides additional details.

## 4 INTERACTIONS WITH OTHER WPS OF ITINERIS OR OTHER PNRR PROJECTS

### 4.1 eLTER

#### 4.1.1 Lake of Bidighinzu

In the context of the ITINERIS project, researchers who are engaged in WP6 are also actively participating in WP5, Activity 5.13 - Expand biological and ecosystem observations in the eulerian platforms of the Ionian and Tyrrhenian Seas. There is also a strong interaction with PNRR NBFC, spoke 1.

### 4.2 DiSSCo

#### 4.2.1 *Tardigrada* from historical Ramazzotti collection and Inhabit *Oligochaeta* collection

There is a strong interaction with ITINERIS activity 6.6 – National network of the aquatic science collections and PNRR NBFC, spoke 3.

#### 4.2.2 *River invertebrates collection and Andrea Buffagni personal collection*

There is a strong interaction with ITINERIS activity 6.6 – National network of the aquatic science collections and PNRR NBFC, spoke 3.

#### 4.2.3 *Herbarium TAR*

There is a strong interaction with ITINERIS activity 5.13 - Expand biological and ecosystem observations in the eulerian platforms of the Ionian and Tyrrhenian Seas (eLTER) and activity 6.6 – National network of the aquatic science collections (DiSSCo), and PNRR NBFC, spoke 3.

## 5 NEW EQUIPMENT

### 5.1 eLTER

#### 5.1.1 *Lake of Bidighinzu*

New equipment was acquired to improve data acquisition and make them FAIR:

- n. 1 multiparameter probe, to measure EOVs in lake freshwater.
- n. 1 autoclave, functional to standard laboratory procedures for different analytical determinations on lake freshwater samples.
- n. 1 personal computer for the calculation, treatment and collection of data deriving from analytical determinations in the lake freshwater.
- n.1 personal computer for the management of the calibration and maintenance procedures of the multi-parameter probe.

#### 5.1.2 *Other eLTER sites*

For the activities to be carried out at “Collelongo-Selva Piana”, “Foce Trigno-Marina di Petacciato”, “Piano Limina”, “Central Italy coastal dunes” the new equipment acquired is as follows:

- n. 2 Tree Talker Cyber systems to be installed in terrestrial sites of the eLTER infrastructure, for the measurement of tree ecophysiology
- n. 20 camera traps complete with accessories for monitoring terrestrial fauna
- n. 2 field acoustic recorders for bird fauna and bats, complete with accessories, for the measurement of animal biodiversity
- n. 1 infrared CO<sub>2</sub> and H<sub>2</sub>O analyzer for flow measurement systems, with related accessories, for the measurement of eddy covariance in terrestrial ecosystems
- n. 1 energy balance sensor - four-component radiometer, for the measurement of eddy covariance in terrestrial ecosystems
- n. 1 sonic anemometer, with related accessories.

### 5.2 DiSSCo

#### 5.2.1 *Tardigrada from historical Ramazzotti collection and Inhabit Oligochaeta collection*

New equipment was acquired to improve data acquisition and make them FAIR:

- n. 1 fluorescent inverted microscope semi-motorized, DIC
- n. 1 stereomicroscope

both equipped with digicam, computer, monitor and software functional to the digitization of freshwater organism collection (i.e. freshwater invertebrates and microscope slides)

### 5.2.2 *River invertebrates collection and Andrea Buffagni personal collection*

New equipment was acquired to improve data acquisition and make them FAIR:

- n. 1 digital microscope functional to the digitization of river invertebrates.
- n. 1 optical microscope and n. 1 stereomicroscope, both equipped with digicam, functional to the taxonomic identification and cataloging of freshwater insects.
- n. 3 workstation, functional to the storage and management of the invertebrate collections.

### 5.2.3 *Herbarium TAR and Herbarium "Irma Pierpaoli" - MBMP*

New equipment was acquired to improve data acquisition and make them FAIR:

- n. 1 handled digital microscope for the magnification of morphological features of herbarium specimens.
- n. 1 digicam with accessories, lens, and tripod for the processing of herbarium sheets images.
- n. 1 planetary scanner functional to the digitization of herbarium sheets.
- n. 1 workstation with accessories for the storage of data.

## 6 COMMUNICATION AND DISSEMINATION

Project activities and results were published on scientific journals and presented at project meetings, workshops and congresses both as oral communications (OC) and posters (P).

### 6.1 eLTER

Padedda B.M., Buscarinu P., Pulina S., Satta C.T., Casiddu P., Manca B., Cherchi M., Pittalis C., Lugliè A., Petrocelli A. 2024. Long-Term Ecological Research in Lake Bidighinzu: studies on trophic state, phytoplankton, future perspectives. 2nd ITINERIS General Meeting, Rome, July 9-10 2024. (P)

Pulina S. 2025. Heatwave effects on phytoplankton morpho-functional traits. LifeWatch Italy Conference, Rome, January 29<sup>th</sup>-30<sup>th</sup>. (OC)

### 6.2 DiSSCo

Buffagni A., Cazzola M., Cislighi S., Cardoni S., Erba S., Belfiore C. Where we come from and where we would like to go: approaching the Pandora's box of Italian mayflies (Ephemeroptera). Aquatic Insects (submitted).

Cislighi S., Cardoni S., Cazzola M., Erba S., Buffagni A. 2024. Digitization, barcoding and microscope slides: coupling old-fashioned and innovative habits for biodiversity studies. XXVIII AIOL congress, Lecco 24-28 June 2024. (OC)

Cislighi S., Cardoni S., Cazzola M., Erba S., Fabrizio S., Buffagni A. 2024. New meets old: an integrated approach for Ephemeroptera biodiversity studies combining digital imaging, DNA barcoding and microscope slides. XVII International Conference on Ephemeroptera and XXI International Symposium on Plecoptera, Torino, 21-26 July 2024. (P)

Cislighi S., Cazzola M., Cardoni S., Stefani F., Erba S., Belfiore C., Buffagni A. 2024. Tassonomia e Biodiversità degli Efemeroteri delle acque dolci italiane (EpheBIO). Notiziario dei Metodi Analitici & IRSA news, Volume 1, Luglio 2024. ISSN 2465-017X

Papa L., Petrocelli A., Spada L., Cecere E. 2024. The herbaria of the CNR Istituto Talassografico di Taranto "A. Cerruti": digitization and sharing activities within the DiSSCo project. 2nd ITINERIS General Meeting, Rome, July 9-10 2024. (P)

*Activity 6.07 - Biological diversity and ecosystem function indicators (EBV, ECV) data collection and provision with a focus on freshwater ecosystems (lakes). Final report at 30/04/2025*

Papa L., Spada L., Petrocelli A., Cecere E. 2024. Digitization of algal collections at CNR-IRSA Taranto: an example of long-term enhancement. 43rd CIESM Congress, Palermo 14-18 October 2024. (P)

Papa L., Spada L., Petrocelli A., Cecere E. 2024. The importance of herbaria in long-term research: contribution of the DiSSCo infrastructure to their valorization. The Annual Scientific Meeting of the Algology Group of the Italian Botanical Society (Sbi) Ferrara, 15-16 November 2024 (OC).

Russo E., Armeli Minicante S., Cislighi S., D'Onofrio R., Buffagni A., Camatti E., Capotondi L., Cazzola M., Cecere E., Conese I., Erba S., Ferraro L., Fontaneto D., Giordano L., Grande V., Guarneri I., Maggiore F., Papa L., Petrocelli A., Sigovini M., Spada L., Zaupa S. 2024. The activities for the national network of Aquatic Science Collections (ASCs). 2nd ITINERIS General Meeting, Rome, July 9-10 2024. (P)

Zaupa S., Fontaneto D., Piscia R. & Kamburska L., 2025. Digitalization of the tardigrade collection of Giuseppe Ramazzotti. *Journal of Limnology* 84: 2217, in press.

## 7 REFERENCES

Carlson R.E. 1977. The limnetic zooplankton community of Boulder Basin, Lake Mead in relation to the metalimnetic oxygen minimum: Las Vegas, University of Nevada (Doctoral dissertation, Masters Thesis, 95 p).

Golterman H.L., Clymo R.S., Best E.P.H., Lauga J. 1988. Methods of exploration and analysis of the environment of aquatic vegetation. In: Symoens, J.J. (eds) *Vegetation of inland waters. Handbook of vegetation science*, vol 15-1. Springer, Dordrecht. [https://doi.org/10.1007/978-94-009-3087-2\\_2](https://doi.org/10.1007/978-94-009-3087-2_2)

Strickland J.D.H., Parsons T.R. 1972. *A Practical Handbook of Seawater Analysis*. 2nd edition. Ottawa, Canada, Fisheries Research Board of Canada, 310 pp. (Bulletin Fisheries Research Board of Canada, Nr. 167 (2nd ed)). DOI: <http://dx.doi.org/10.25607/OBP-1791>

Utermöhl H. 1958. Guidance on the quantitative analysis of phytoplankton—Methods. *Mitt. Int. Ver. Theor. Angew. Limnol*, 9, 1-38.

Vollenweider R.A., Kerekes J. 1982. *Eutrophication of waters. Monitoring, assessment and control*. Organization for Economic Co-Operation and Development (OECD), Paris, 156.