



Project BIOLAWEB

Deliverable D3.1

Reports from joint events

Acronym:	BIOLAWEB
Full title:	Boosting Institute of Chemistry, Technology and Metallurgy in Water Biomonitoring
Grant No:	101079234
Call:	HORIZON-WIDERA-2021-ACCESS-03
Topic:	HORIZON-WIDERA-2021-ACCESS-03-01
Type of action:	HORIZON Coordination and Support Actions (HORIZON - CSA)
Granting authority:	European Research Executive Agency
Starting Date:	01/10/2022
Project Duration:	36 months



This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101079234.





Deliverable D3.1

Reports from joint events

Deliverable data sheet

Deliverable number:	D3.1
Deliverable title:	Reports from joint events
Work package:	Training and networking (WP3)
Lead Beneficiary:	NIVA
Type:	R — Document, report
First submission on:	30/01/2024
Due month:	16
Dissemination level:	PU - Public

Version history

Version	Date	Main author(s)	Summary of changes
V01	04/01/2024	Dr Susanne Schneider, Dr Miloš Ćirić	First version created
V01	30/01/2024	Dr Miloš Ćirić, Dr Susanne Schneider	Final version compiled

Quality control

Activity	Name	Date
Created	Dr Susanne Schneider, Dr Miloš Ćirić	04/01/2024
Reviewed	Dr Miloš Ćirić, Dr Susanne Schneider, Dr Andreas Ballot	22/01/2024
Revised	Dr Aleksandra Marković, Dr Miloš Ćirić, Dr Susanne Claudia Schneider, Dr Andreas Ballot, MSc Dragana Zlatović, Dr Danijela Vidaković, Dr Clarisse Lemonnier, Dr Frédéric Rimet	26/01/2024





Deliverable summary

In this deliverable, we report joint events which were organized within WP3 “Training and networking” of the BIOLAWEB project. Specifically, we report the activities within Task 3.1: Methods for developing ecological status indices, Task 3.2: Metabarcoding of diatoms and phytoplankton, and Task 3.3: eDNA of macrophytes.

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1. Introduction

The objectives of WP3 – networking and training of the BIOLAWEB project are:

- To train UB-ICTM in the development of status indicators that are in accordance with the demands of the EU Water Framework Directive;
- To train UB-ICTM in the application of metabarcoding and the sampling and analyses of eDNA;
- To train UB-ICTM in developing new methods for ecological status assessment.

The BIOLAWEB project aims to reach these objectives through the following three tasks:

- Task 3.1: Methods for developing ecological status indices
- Task 3.2: Metabarcoding of diatoms and phytoplankton
- Task 3.3: eDNA of macrophytes

In this deliverable, we report the activities which have been performed within these three tasks during the first 16 months of the BIOLAWEB project. The links to publicly available teaching material (PPT presentation or YouTube video) for each activity within WP3 are provided in this deliverable.

2. Report of activities within WP3

Task 3.1: Methods for developing ecological status indices

Subtask 3.1.1 Workshop on index development

The workshop on index development was divided into: 1) online introduction, performed on April 25, 2023, followed by 2) a course during May 2-5, 2023, which was held physically at UB-ICTM. The workshop was led by Dr Susanne Schneider, NIVA.

Table 1. Information on the target audience for the workshop on index development

Target groups	No of participants (foreseen)	No of participants (achieved)
<i>Students, scientists and interested stakeholders</i>	15	Online introduction: 24 Online and onsite: 17

The online course covered a general introduction into the principles of bioindication, followed by an assignment of seminar papers to all course participants. In the two weeks between the online introduction and the physical course in Belgrade, each course participant, working in teams and with a background in published manuscripts, prepared a presentation on a selected example of a biotic index, which was developed in different countries in Europe.

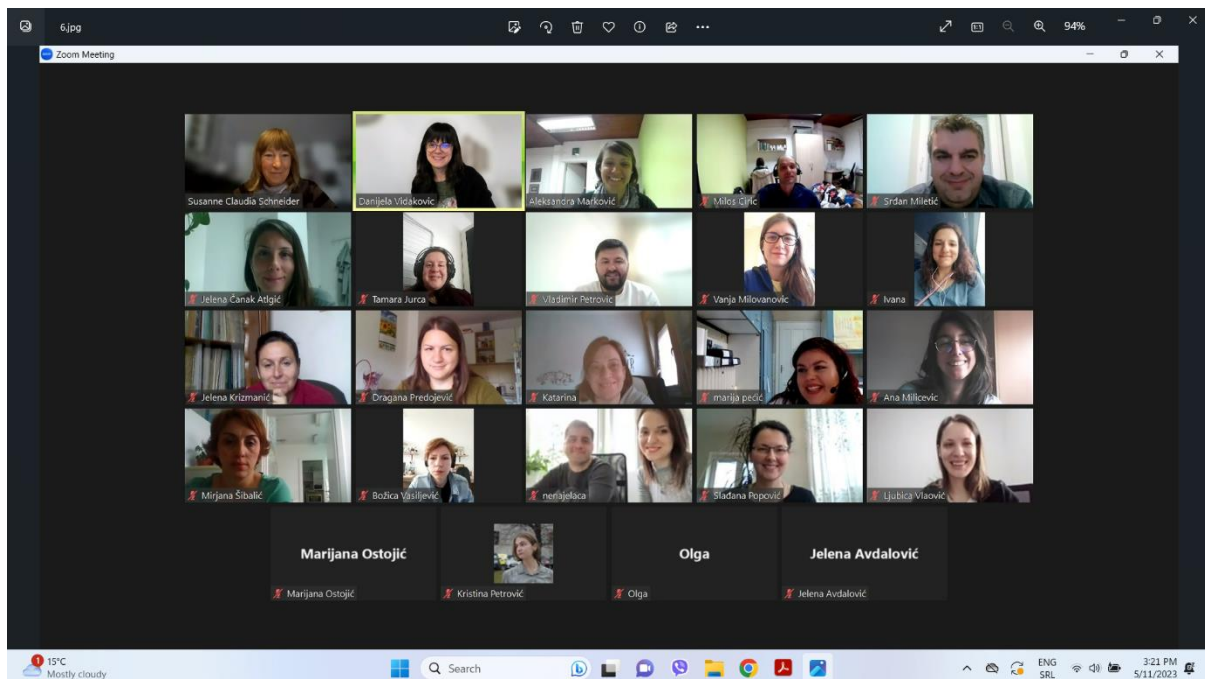


Figure 1. Workshop on index development (on-line, 25th April 2023)

The second course part, which was held physically in Belgrade, started with an in-depth explanation of important principles for bioindication. In the next step, each course participant presented a biotic index, based on the seminar papers which were assigned to the participants. The advantages and disadvantages of each index were then discussed with all course participants. The course ended with a summary of the most important lessons learned.

The first, online part of the course, was followed by **24 participants** (Figure 1). The second, onsite course, was attended by **17 participants** (which followed online part as well): 11 researchers (UB-ICTM, Faculty of Biology and Institute for Biological Research "Siniša Stanković"), 4 students (Faculty of Biology), 1 professor (Faculty of Biology), and 1 biologist (Serbian Environmental Protection Agency).

The course material is publicly available from the following website:

<https://biolaweb.com/work-package/wp3-training-and-networking>

Subtask 3.1.2 Summer school and field exercise

UB-ICTM hosted **The Blue Green Future (BgF) Summer school 2023** between 2nd - 13th October 2023 in Belgrade, Serbia. During this 10-day event we had **71 participants** who had the pleasure and opportunity to listen to **18 lecturers** from **5 countries** (France, Norway, the UK, the Republic of North Macedonia, and Serbia).

The programme of the BgF summer school was divided in two parts: the 1st part (WP3: **Subtask 3.1.2** – Summer school and field exercises including **plenary lectures**, **Subtask 3.2.2** – The second workshop and **Subtask 3.3.1** – The first eDNA workshop), the 2nd part (WP5: **Subtask 5.4.2** – Virtual training and **Subtask 5.5.2** – Seminars). The 1st part was organized in form of plenary lectures, workshops, and field exercises (Appendix 1). The 2nd part included workshops in project management. The report on the 2nd part of the BgF summer school will be included in the deliverable D5.2 – Training materials.

Table 2. Information on the target audience for the BgF summer school with field exercises

Target groups	No of participants (foreseen)	No of participants (achieved)
<i>Students, scientists and interested stakeholders</i>	The 2 nd workshop: >10	The Plenary session: 23
	The 1 st eDNA workshop: 12	The 2 nd workshop: 15
	Field exercises: 12	The 1 st eDNA workshop: 15
	The BgF summer school: 15-20	Field exercises: 12
		In total for the BgF 1 st part: 24

The 1st part of BgF summer school was held between 2nd - 6th October 2023 and it was attended by **24 participants** and **6 lecturers**. It started with three plenary lectures at the Center for the Promotion of Science (CPN) in Belgrade (Figure 2):

Dr Zlatko Levkov, Ss. Cyril and Methodius University, Faculty of Biology, North Macedonia:

World of diatoms - how to make a problem out of something wonderful

Dr Frédéric Rimet, INRAE:

Diatom metabarcoding for biomonitoring and for basic ecology

Dr Martyn Kelly, Bowburn Consultancy:

Use of metabarcoding for ecological assessment of benthic algae in the UK



Figure 2. The BgF summer school – The Plenary session (photo taken on 2nd October 2023)

During the BgF summer school different workshops on eDNA (described in the following sections) were combined with hands-on outdoor training. Three field exercises were conducted on 5th October 2023 (Savsko lake, Belgrade), 12th October 2023 (Plava banja, Kikinda) and 13th October 2023 (Pečena Slatina, Baranda). In total, **12 participants** took part in the field exercises (researchers, students of biology and one representative of the Serbian Environmental Protection Agency). On the shore, attendees learned how to filtrate water samples collected from the lake using pressure assisted filtration (PAF, Niras, Allerød, Denmark) and syringes with Sterivex filters, for eDNA of macrophytes and metabarcoding of phytoplankton, respectively (Figures 3 and 5). Also, the participants had the opportunity to learn how to sample diatoms from the shore, and phytoplankton from the boat for subsequent analyses following the protocols developed by INRAE (Figure 4).



Figure 3. Field exercise on Savsko lake in Belgrade (5th October 2023)



Figure 4. Field exercise on Plava banja near Kikinda (12th October 2023)



Figure 5. Field exercise on Pečena slatina near Baranda (13th October 2023)

Task 3.2: Metabarcoding of diatoms and phytoplankton

Subtask 3.2.1 The first workshop

The workshop *Metabarcoding of diatoms and phytoplankton for biomonitoring* was the first knowledge transfer event within WP3 and it attracted 10 times more participants than was initially planned. After the first announcement on 20th of January 2023 and the high number of received registrations, the BIOLAWEB team decided to enable online participation and organized the workshop as a hybrid event. With 99 participants from 21 countries and 3 continents, the first workshop on metabarcoding was a very successful event.

Table 3. Information on the target audience for the 1st Workshop – Metabarcoding of diatoms and phytoplankton for biomonitoring

Target groups	No of participants (foreseen)	No of participants (achieved)
<i>Scientists, graduate students and interested stakeholders</i>	>10	Onsite: 41 Online: 58 In total: 99

The first workshop was held from 29th to 31st March 2023 in Belgrade, Serbia and was open to researchers, students (undergraduate, MSc and PhD), university professors, and other interested stakeholders (e.g., experts from the water sector etc.). The workshop programme

was divided into three parts: Part I – Introduction to metabarcoding, Part II – Metabarcoding of diatoms and Part III – Metabarcoding of phytoplankton (Appendix 2).



Figure 6. The 1st Workshop – Metabarcoding of diatoms and phytoplankton for biomonitoring (photo taken on 31st March 2023)

Twelve different sessions consisted of interactive talks given by four experts from the French partner institution:

Dr Agnès Bouchez, INRAE:

General introduction (DNA – reminders; DNA Barcoding – main principles and examples; DNA Metabarcoding – main principles and examples)

Diatom metabarcoding for biomonitoring – 2nd part (Barcode choice, sample preservation, DNA extraction, sequencing) (with Dr Frédéric Rimet)

Diatom metabarcoding for biomonitoring – 4th part (Impact of biovolumes, application to monitoring networks, intercalibration exercise) (with Dr Frédéric Rimet)

Metabarcoding for biomonitoring (technology transfer) (with Dr Frédéric Rimet)

Dr Frédéric Rimet, INRAE:

Diatom metabarcoding for biomonitoring – 1st part (Reminders - biology, taxonomy, biomonitoring)

Diatom metabarcoding for biomonitoring – 3rd part (Reference library – Diat.barcode, proof of concept)

Phytoplankton for biomonitoring reminders – 1st part (Biology, diversity of phytoplankton; Classical methodology for phytoplankton biomonitoring)

Phytoplankton metabarcoding for biomonitoring – 2nd part (Barcode selection – in silico, test with mock communities) (with Dr Clarisse Lemonnier)

Dr Clarisse Lemonnier, INRAE:

Metabarcoding, main steps – 1st part (Presentation of the main steps; Barcode: definition and selection; Reference databases)



Metabarcoding, main steps – 2nd part (Lab work - DNA extraction and PCR; Bioinformatic analysis - OTUs, ASVs, taxonomy affiliation)

Phytoplankton metabarcoding for biomonitoring – 3rd part (Protocols: field, extractions, PCR)

Dr Benjamin Alric, INRAE:

Phytoplankton metabarcoding for biomonitoring – 4th part (Phytool reference library; Tests on environmental samples)

All experts participated in a course and skill evaluation at the end of the workshop.

The course video material is publicly available on the BIOLAWEB YouTube channel:

<https://youtu.be/GCXumUKsd00?si=Yob-Ec--b7m2hFdC>

Subtask 3.2.2 The second workshop

The second workshop *Introduction to bioinformatics (R programme with examples and R codes)* was organized during the BgF summer school from 2nd to 4th October 2023 in Belgrade. This interactive course was given by **3 lecturers** from INRAE. Before the workshop, participants were asked to register and provide information on their educational level. The plan was to adjust the course to suit the needs of potential attendees. Prior to the onsite training, they received a detail instruction how to install R program, specific R packages, MEGA software, and all necessary links.

Table 4. Information on the target audience for the workshop – Introduction to bioinformatics

Target groups	No of participants (foreseen)	No of participants (achieved)
Scientists and graduate students	>10	Onsite: 15

INRAE lecturers combined theoretical knowledge of diatom and phytoplankton taxonomy, ecology, and biogeography with practical exercises in the R program. The workshop was divided into three sections: 1) data processing, 2) taxonomic composition and diversity, and 3) eco-phylogenetic analyses and started with a minor introduction to R language. The end of the program was dedicated to the course evaluation. The event was followed by **15 participants** onsite: 7 researchers and 8 students, including two PhD students (Figure 7).

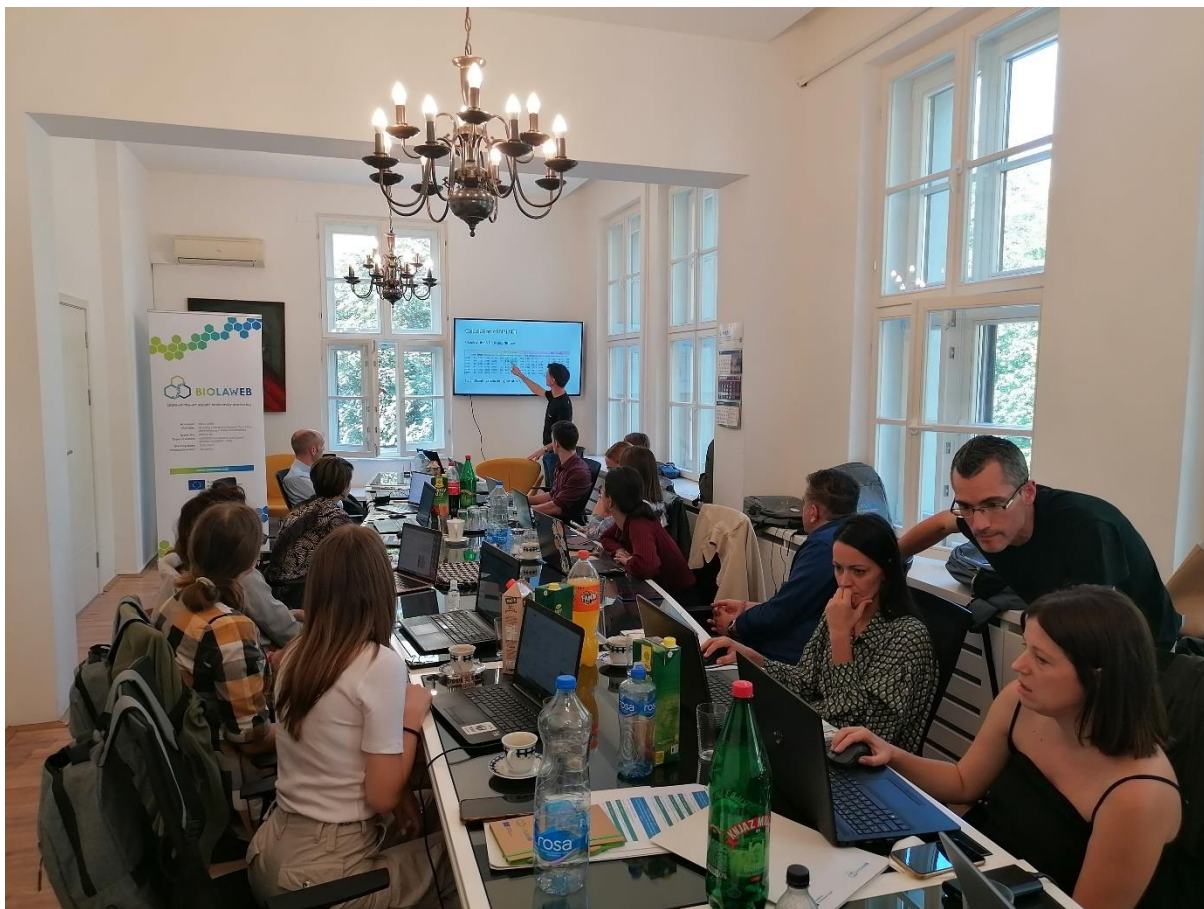


Figure 7. The 2nd Workshop – Introduction to bioinformatics, the BgF summer school (photo taken on 4th October 2023)



During these three days, all major parts of ecological analysis of metabarcoding data were covered. At the end participants acquired both theoretical knowledge and practical examples to answer specific ecological questions regarding diatom or phytoplankton biomonitoring.

The list of lecturers:

Dr Frédéric Rimet, INRAE:

Eco-phylogenetic analyses (with examples and R codes) 1st part

Eco-phylogenetic analyses (with examples and R codes) 2nd part

Eco-phylogenetic analyses (with examples and R codes) 3rd part

Course evaluation

Dr Clarisse Lemonnier, INRAE:

Data processing, normalization of data (with examples and R codes) 1st part

Data processing, normalization of data (with examples and R codes) 2nd part

Taxonomic composition (with examples and R codes)

Alpha diversity (with examples and R codes)

Dr Benjamin Alric, INRAE:

Beta diversity (with examples and R codes)

Differential abundance analysis (with examples and R codes)

Course presentations are available on the BIOLAWEB website:

<https://biolaweb.com/work-package/wp3-training-and-networking>

Task 3.3: eDNA of macrophytes

Subtask 3.3.1 The first eDNA workshop

The first workshop on eDNA of macrophytes was held from 4th to 6th October 2023 in Belgrade, Serbia, during the BgF summer school and was open to researchers, students (undergraduate, MSc and PhD), university professors and other interested stakeholders (e.g., experts from the water sector etc.) (Table 5).

Table 5. Information on the target audience for the workshop – eDNA with the focus on macrophytes

Target groups	No of participants (foreseen)	No of participants (achieved)
Scientists, graduate students and interested stakeholders	12	Onsite: 15

The purpose of this workshop was to impart knowledge to interested students and scientists how new methods based on eDNA can support or substitute traditional methods to investigate waterbodies for the presence of selected macrophyte species. The workshop programme was divided into 5 parts: Part I – Metabarcoding macrophytes - General introduction DNA, Part II – Aquatic macrophytes as indicators for the ecological status of lakes, Part III – Sampling of eDNA of aquatic macrophytes (Charophytes), Part IV – DNA extraction, Part V - Barcoding/Metabarcoding. The five sessions consisted of interactive talks given by Dr Andreas Ballot from the Norwegian partner institution – NIVA (Appendix 1). The course taught the basics of DNA and how eDNA samples are taken and analyzed to determine the presence of macrophytes (*Chara*) in a simple and quick way.

The workshop was attended by **15 participants** onsite: 8 students, 6 researchers and 1 biologist (Serbian Environmental Protection Agency) (Figure 8).

Workshop presentations are available on the BIOLAWEB website:

<https://biolaweb.com/work-package/wp3-training-and-networking>



Figure 8. Workshop – eDNA with the focus on macrophytes, the BgF summer school (photo taken 6th October 2023)



3. Appendix 1 – Agenda for the BgF summer school, 1st part



BgF summer school

First part 2nd – 6th October 2023, Belgrade

Agenda

DAY 1 (2 nd October) 09:00 – 14:35	
The Center for the Promotion of Science (CPN) Kralja Petra 46, Belgrade, Serbia	
PART I	
09:00 – 10:00	Registration of participants
10:00 – 10:05	Welcome speech Dr Dejan Opsenica, President of the Scientific Board, UB-ICTM
10:05 – 10:15	General overview of the BIOLAWEB project Dr Miloš Ćirić, coordinator of the BIOLAWEB project, UB-ICTM
10:15 – 11:15	Plenary lecture 1 – World of diatoms - how to make a problem out of something wonderful Dr Zlatko Levkov, Ss. Cyril and Methodius University, Faculty of Biology, North Macedonia
11:15 – 11:45	Coffee break
11:45 – 12:05	Plenary lecture 2 – Diatom metabarcoding for biomonitoring and for basic ecology Dr Frédéric Rimet, INRAE
12:05 – 12:15	Q & A
12:15 – 13:15	Plenary lecture 3 – Use of metabarcoding for ecological assessment of benthic algae in the UK Dr Martyn Kelly, Bowburn Consultancy
13:15 – 13:30	Discussion
13:30 – 13:35	Closing plenary session Dr Miloš Ćirić, coordinator of the BIOLAWEB project, UB-ICTM
13:35 – 14:35	Lunch break



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DAY 1 (2nd October) 15:30 – 17:45

The Institute of Chemistry, Technology and Metallurgy - National Institute of the Republic of Serbia (UB-ICTM, Serbia), Conference room, 1st floor, Njegoševa 12, Belgrade, Serbia

PART II

15:30 – 16:30	<i>Data processing, normalization of data (with examples and R codes) 1st p</i> Dr Clarisse Lemonnier, INRAE
16:30 – 16:45	Coffee break
16:45 – 17:45	<i>Data processing, normalization of data (with examples and R codes) 2nd p</i> Dr Clarisse Lemonnier, INRAE

DAY 2 (3rd October) 08:30 – 18:30

The Institute of Chemistry, Technology and Metallurgy - National Institute of the Republic of Serbia (UB-ICTM, Serbia), Conference room, 1st floor, Njegoševa 12, Belgrade, Serbia

PART II

08:30 – 09:00	<i>Registration of participants</i>
09:00 – 10:00	<i>Taxonomic composition (with examples and R codes)</i> Dr Clarisse Lemonnier, INRAE
10:00 – 10:30	Coffee break
10:30 – 11:30	<i>Alpha diversity (with examples and R codes)</i> Dr Clarisse Lemonnier, INRAE
11:30 – 12:30	Lunch break
13:00 – 15:00	<i>Beta diversity (with examples and R codes)</i> Dr Benjamin Alric, INRAE
15:00 – 15:30	Coffee break
15:30 – 17:30	<i>Differential abundance analysis (with examples and R codes)</i> Dr Benjamin Alric, INRAE
17:30 – 18:30	<i>Eco-phylogenetic analyses (with examples and R codes) 1st part</i> Dr Frédéric Rimet, INRAE



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DAY 3 (4th October) 08:30 – 18:30

The Institute of Chemistry, Technology and Metallurgy - National Institute of the Republic of Serbia (UB-ICTM, Serbia), Conference room, 1st floor, Njegoševa 12, Belgrade, Serbia

PART II

08:30 – 09:00	<i>Registration of participants</i>
09:00 – 10:30	<i>Eco-phylogenetic analyses (with examples and R codes) 2nd part</i> Dr Frédéric Rimet, INRAE
10:30 – 11:00	<i>Coffee break</i>
11:00 – 12:30	<i>Eco-phylogenetic analyses (with examples and R codes) 3rd part</i> Dr Frédéric Rimet, INRAE
12:30 – 13:30	<i>Lunch break</i>
13:30 – 14:00	<i>Course evaluation</i> Dr Frédéric Rimet, Dr Clarisse Lemonnier, Dr Benjamin Alric
14:00 – 16:00	<i>Consortium meeting</i>
16:00 – 16:30	<i>Coffee break</i>
16:30 – 18:30	<i>Introduction to eDNA focus on macrophytes</i> Dr Andreas Ballot, NIVA

DAY 4 (5th October) 08:30 – 17:00

The Institute of Chemistry, Technology and Metallurgy - National Institute of the Republic of Serbia (UB-ICTM, Serbia), Conference room, 1st floor, Njegoševa 12, Belgrade, Serbia

PART III

08:30 – 09:00	<i>Registration of participants</i>
09:00 – 10:00	<i>Introduction to aquatic macrophytes</i> Dr Andreas Ballot, NIVA
10:00 – 10:30	<i>Coffee break</i>
10:30 – 11:30	<i>Introduction to field sampling of eDNA aquatic macrophytes</i> Dr Andreas Ballot, NIVA
11:30 – 12:30	<i>Lunch break</i>
14:00 – 17:00	<i>Field exercise on Savsko lake</i> Dr Andreas Ballot, NIVA



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DAY 5 (6th October) 08:30 – 17:00

The Institute of Chemistry, Technology and Metallurgy - National Institute of the Republic of Serbia (UB-ICTM, Serbia), Conference room, 1st floor, Njegoševa 12, Belgrade, Serbia

PART III

08:30 – 09:00	<i>Registration of participants</i>
09:00 – 10:00	<i>Introduction to DNA isolation, PCR preparation, (meta)barcoding 1st part</i> Dr Andreas Ballot, NIVA
10:00 – 10:30	Coffee break
10:30 – 11:30	<i>Introduction to DNA isolation, PCR preparation, (meta)barcoding 2nd part</i> Dr Andreas Ballot, NIVA
11:30 – 12:00	<i>Discussion and course evaluation</i> Dr Andreas Ballot, NIVA
12:00 – 12:15	<i>Closing</i> Dr Danijela Vidaković, UB-ICTM Dr Miloš Čirić, UB-ICTM



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4. Appendix 2 – Agenda for the 1st workshop



1st Workshop: Metabarcoding of diatoms and phytoplankton for biomonitoring

29th- 31st March 2023

The Institute of Chemistry, Technology and Metallurgy - National Institute of the Republic of Serbia (UB-ICTM, Serbia), Njegoševa 12, Belgrade, Serbia

Agenda

DAY 1 (29 th March) 09:00– 18:00	
PART I (Introduction)	
09:00-10:00	Registration of participants
10:00 – 10:15	<i>Introduction, presentation of the project and the speakers</i> Miloš Ćirić, coordinator of the BIOLAWEB project, UB-ICTM Danijela Vidaković, UB-ICTM
10:15 – 11:15	<i>General introduction (DNA – reminders; DNA Barcoding – main principles and examples; DNA Metabarcoding – main principles and examples)</i> Agnès Bouchez, INRAE
11:15 – 11:45	Coffee break
11:45 – 12:45	<i>Metabarcoding, main steps – 1st part (Presentation of the main steps; Barcode: definition and selection; Reference databases)</i> Clarisse Lemonnier, INRAE
12:45 – 13:45	Light lunch break (finger foods included)
13:45 – 14:45	<i>Metabarcoding, main steps – 2nd part (Lab work - DNA extraction and PCR; Bioinformatic analysis - OTUs, ASVs, taxonomy affiliation)</i> Clarisse Lemonnier, INRAE
14:45 – 15:00	Questions
DAY 1 - PART II (DIATOMS)	
15:00 – 15:30	<i>Diatom metabarcoding for biomonitoring – 1st part (Reminders - biology, taxonomy, biomonitoring)</i> Frédéric Rimet, INRAE
15:30 – 16:00	Coffee break
16:00 – 17:30	<i>Diatom metabarcoding for biomonitoring – 2nd part (Barcode choice, sample preservation, DNA extraction, sequencing)</i> Frédéric Rimet, INRAE Agnès Bouchez, INRAE
17:30 – 18:00	Questions



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DAY 2 (30 th March) 09:00– 16:45	
PART II (DIATOMS)	
09:00-09:30	Registration of participants
09:30 – 10:30	<i>Diatom metabarcoding for biomonitoring – 3rd part (Reference library – Diat.barcode, proof of concept)</i> Frédéric Rimet, INRAE
10:30 – 11:00	Coffee break
11:00 – 12:00	<i>Diatom metabarcoding for biomonitoring – 4th part (Impact of biovolumes, application to monitoring networks, intercalibration exercise)</i> Frédéric Rimet, INRAE Agnès Bouchez, INRAE
12:00 – 12:15	Questions
12:15 – 13:15	Light lunch break (finger foods included)
DAY 2 - PART III (PHYTOPLANKTON)	
13:15 – 14:00	<i>Phytoplankton for biomonitoring reminders – 1st part (Biology, diversity of phytoplankton; Classical methodology for phytoplankton biomonitoring)</i> Frédéric Rimet, INRAE
14:00 – 14:30	<i>Phytoplankton metabarcoding for biomonitoring – 2nd part (Barcode selection – in silico, test with mock communities)</i> Clarisse Lemonnier, INRAE Frédéric Rimet, INRAE
14:30 – 15:00	Coffee break
15:00 – 15:35	<i>Phytoplankton metabarcoding for biomonitoring – 3rd part (Protocols: field, extractions, PCR)</i> Clarisse Lemonnier, INRAE
15:35 – 16:15	<i>Phytoplankton metabarcoding for biomonitoring – 4th part (Phytool reference library; Tests on environmental samples)</i> Benjamin Alric, INRAE
16:15 – 16:45	Questions



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DAY 3 (31 st March) 09:00– 12:15	
09:00 – 09:30	Registration of participants
09:30 – 10:15	<i>Metabarcoding for biomonitoring (technology transfer)</i> Agnès Bouchez, INRAE Frédéric Rimet, INRAE
10:15 – 11:00	<i>Skill evaluation and Open discussion</i> Agnès Bouchez, INRAE Clarisse Lemonnier, INRAE Frédéric Rimet, INRAE Benjamin Alric, INRAE
11:00 – 11:30	Coffee break
11:30 – 12:00	<i>Course evaluation</i> Agnès Bouchez, INRAE Clarisse Lemonnier, INRAE Frédéric Rimet, INRAE Benjamin Alric, INRAE
12:00 – 12:15	<i>Closing</i> Danijela Vidaković, UB-ICTM Miloš Čirić, coordinator of the BIOLAWEB project, UB-ICTM



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