

This document is a list of all the contributions presented considering dissemination events (local, regional, national and international), scientific events and courses or schools organised by the partners within the EAW project. A short description of the events is present in the list, followed by a more detailed information of the contributions sorted by the kind of events.

List of all EAW events

- 18th -19th May 2021: workshop organised by the EAW consortium
- two-days' online workshop (9th -10th/12/2020) organised by the EAW consortium
- 1st September 2020, the conference "Environmental DNA for fish species detection" was held at INRAE (EAW partner), in Thonon-les-Bains
- In August 2020 radio interview of Nico Salmaso and Giulia Riccioni from FEM
- ISPRA participated with a virtual stand to the “European Night of Researchers 2020”
- ISPRA organised a virtual tour during the “National Geographic Science Festival”
- During the “Science Slam” on 8.11.2019, Christian Vogelmann (LfL) present the Eco-AlpsWater project
- From 17.10. till 21.10.2019 a national event for protection and rescue in Postojna, Slovenia
- “Science Day” organized by INRAE in Thonon-les-bains
- On 9th of October 2019, the National Institute of Biology (NIB, Ljubljana, Slovenia) organised an open-day
- European event “Society next” attended by ISPRA

- “Science Week” (Scienza Insieme) organised in different ISPRA locations in Italy
- On Wednesday 24th February 2021, seminar organised by INRAE in Thonon-lesBains
- Training seminar for fishery masters organised by EAW partner LfL
- NIB presentation on 20th November 2020 to the Biotechnical Faculty students of microbiology
- On May 2020 NIB organised on-line lectures for students from the College of Environmental Protection in Velenje, Slovenia
- ARPAV (EAW partner) organised activities for students
- Within the “Science Day”, INRAE and OFB organized, at Thonon-les-bains, 2 workshops dedicated to middle school classes
- Three projects of School-Work Alternation were organised by ISPRA
- Partners LFL and LfU organised dedicated seminars for a professional school for fishermen on 13.-24.5.2019
- Demonstration of lake water sampling activities (06/05/2019) of the Italian partner ARPAV to IPSAR LUIGI CARNACINA, Istituto Professionale per i Servizi Alberghieri e della Ristorazione
- Seminar held by ARPAV partner on 26.2.2019 for a secondary school
- The IVY Foundation (The Interreg Volunteer Youth Initiative) was involved by NIB in the EAW project
- A one-day seminar “Quo vadis, la biosurveillance basée sur l'ADN?” organised by INRAE in partnership with Cost DNAquaNet also addressed to stakeholders
- NIB attended the online final event of the Interreg Europe project "From biodiversity data to decisions: enhancing natural values trough improved

regional policies" (BID-REX)

- The first of July 2020 first meeting in videoconference of the Working Group of the SNPA, coordinated by ISPRA.
- On October the 16th 2019, the Second Italian Eco-AlpsWater regional meeting in Rome was organised
- Slovenian observers (Fisheries Research Institute of Slovenia) were involved in the fish eDNA sampling with the partner responsible for sampling
- On 25.6.2019 the German partner LfL organised an event for local and external stakeholders
- Eco-AlpsWater Italian partners (ARPAV, FEM, ISPRA) and Swiss partner (SUPSI) organised a big and resonant local event in Verona
- Local information meeting on 6th February 2019 organised by LfL and LfU
- LfL attended on the 19th -20th November 2018 a workshop for inland fisheries
- On 19th-20th/11/2018 NIB attended the “DNAquaNet Micro & Meiofauna” meeting in Prague
- 12th Symposium for European Freshwater Sciences, 25th – 30th July 2021:
 - ❖ Nico Salmaso (FEM), oral presentation
 - ❖ Giulia Riccioni (FEM), oral presentation
 - ❖ Camilla Capelli (SUPSI), poster
- XXV AIOL congress held on 30th June- 2nd July:
 - ❖ Nico Salmaso (FEM): oral presentation
 - ❖ Giulia Riccioni (FEM): oral presentation
 - ❖ Leonardo Cerasino (FEM): oral presentation
- 1st DNAQUA International Conference, 9th-11th March 2021, Giulia

Riccioni (FEM), oral presentation

- On Tuesday 1st September 2020, the conference "Environmental DNA for fish species detection" was held at INRAE
- 63rd International Association for Great Lakes Research Conference on June 9th-11th 2020, Nico Salmaso (FEM), oral presentation
- International conference UGM 2019 “DNA-Methoden im Umweltmanagement” held on 4th -5th December 2019, Ute Mischke (LfU), oral presentation
- International Mountain Conference 2019, September 8th – 12th, 2019, Rainer Kurmayer (LFUI), an oral presentation and a poster
- 7th European Phycological Congress in Zagreb, Croatia, on 27th August 2019, Tina Elersek (NIB), poster presentation
- International conference “Advances in eDNA-based Approaches to Fish Ecology and Management in Hull”, UK, July 2019, Hans Rund (LFUI), oral presentation
- XXIV AIOL congress (Congresso dell’Associazione Italiana di Oceanologia e Limnologia), Bologna 5th-9th June 2019:
 - ❖ Jonas Bylemans (FEM) oral presentation
 - ❖ Adriano Boscaini (FEM) poster presentation
- 11th International conference of toxic cyanobacteria (Krakow, Poland), 6th May 2019, Nico Salmaso (FEM) oral presentation
- Congress of Health and Climate Change, Rome 3rd-5th December 2018, Serena Bernabei poster presentation
- International joint event IAGLR and ELLS in Evian in September 23rd-28th, 2018, Isabelle Domaizon (INRAE) poster presentation
- Forum Alpinum (Alpine water Conference) in Breitenwang on 5th June

2018, Rainer Kurmayer (LFUI) poster presentation

- Eco-AlpsWater SUMMER SCHOOL: *Biomonitoring of microorganisms in aquatic ecosystems: High throughput sequencing rationale and applications*. 31st August- 3rd September 2021

Dissemination events

- On 18th -19th May 2021 a workshop was organised by the EAW consortium to describe the results achieved during the project and discuss the eDNA biomonitoring implementation to all the participants represented by stakeholders, academic staff, SME representatives and simple interested citizens. The first day the event
- To promote the integration of innovative monitoring approaches in water quality assessment and management of the Alpine blue infrastructures, a two-days' online workshop (9th -10th/12/2020) was organised by the EAW consortium to show the project progress and discuss the eDNA approach biomonitoring results to all the participants represented by stakeholders, academic staff, SME representatives and simple interested citizens.
- On Tuesday 1st September 2020, the conference "Environmental DNA for fish species detection" was held at the INRAE, in Thonon-les-Bains, to illustrate the use of environmental DNA for the detection of fish species in alpine lakes. The protocols developed within the framework of the Interreg Eco-AlpsWater project were presented, as well as preliminary results obtained for Lake Bourget. 20 participants were present, with representatives from the OFB (National Office for Biodiversity), from academic institutions (INRAE, University of Savoie Mont Blanc), but also local stakeholders and end-users (lake Léman) interested by the evaluation of biodiversity in aquatic systems. The conference was also live webcast on internet and was attended by more than 200 people. This conference

was an opportunity to present the work done within the EAW project, but also to discuss with stakeholders in order to identify possible applications and the directions to follow for the future.

- In August 2020 the project coordinator Nico Salmaso and Giulia Riccioni from FEM had an interview about the use of eDNA for biomonitoring at Trento Film Festival broadcasted on Radio Dolomiti and live streaming on Radio Dolomiti facebook page (<https://it-it.facebook.com/radiodolomiti/videos/308040780452926/>, 751 visualizations).
- Italian partner ISPRA set up a virtual stand dedicated to biodiversity, habitats and ecosystems during the “European Night of Researchers 2020”. This virtual stand includes videos and materials on the management and conservation of marine and terrestrial ecosystems, information on the main threats to biodiversity, food for thought on environmental transformations, the problem of alien species and the impact of climate change and pollution on fauna. Visitors can have a tour that will deal with biology, conservation, epidemiology and genetics, highlighting the fundamental importance of a multidisciplinary approach to environmental issues.
- Activities involving also Eco-AlpsWater project have been presented in the framework of the “National Geographic Science Festival” by the Italian partner ISPRA that organised a virtual tour of the laboratories introducing genetic techniques and possible applications.
- Christian Vogelmann from EAW partner LFL presented on 8.11.2019,

“Science Slam” and he won the first price presenting the Eco-AlpsWater project at the conference “Forschungs Land Bayern – hier wächst wissen”, Biodiversität (<http://www.stmelf.bayern.de/tdf2019>). The event was held at the Bavarian State Ministry of Food, Agriculture and Forestry.

- From 17.10. till 21.10.2019 Eco-AlpsWater was introduced at a national event for protection and rescue in Postojna, Slovenia (<https://www.facebook.com/events/postojna/bogatajevi-dnevi-za%C5%A1%C4%8Dite-in-re%C5%A1evanja-2019/377699086237259/>). More than 1.500 pupils and interested public attended the event and explore the algae under microscope at our stand.
- As part of the “Science Day” at Thonon-les-bains, INRAE organized an open day that attracted more than 650 people. Among the animations proposed, one was focused on the Eco-AlpsWater project and the application of eDNA tools for ecological diagnosis of aquatic ecosystems. The objectives and strategies of the project were explained to a large public; the different steps of eDNA procedures used to obtain biodiversity inventories from aquatic samples were also illustrated. For each step of the eDNA procedure (filtration, extraction, PCR and sequencing and bioinformatics), a practical handling was organized to allow both children and adults to be involved; each step was described by a referent (from INRAE and OFB staff) who also explained either lab or computer work.
- On 9th of October 2019, the National Institute of Biology (NIB, Ljubljana, Slovenia) traditionally opened doors to wider public and schools for the whole day. Presentations and demonstrations took place at four different

locations at NIB. In the frame of this event Eco-AlpsWater was presented to almost 500 students and general public.

- ISPRA was involved to present the EAW project within the European event “Society next”, with a virtual stand dedicated to biodiversity, habitats and ecosystems curated by Claudia Greco (ISPRA, EAW partner).
- Eco-AlpsWater activities were presented during the “Science Week” (Scienza Insieme) in different ISPRA locations in Italy. Afterward, the project activities were presented also on national TV newscast (http://www.tg2.rai.it/dl/RaiTV/programmi/media/ContentItem-ad861a86-5f2d-4b36-b60f-4836134c5706-tg2.html?fbclid=IwAR0KyR6FKtT-JGvhofrsvYe0TOJBAGGIBtKojCOdOAO_bng9iQ11gUXiMVU#p=0).

Activities for younger public and education

- On Wednesday 24th February 2021, a seminar (1/2 day long) was held at the INRAE of Thonon-lesBains. This session was prepared specifically for college students, and aimed at illustrating the use of environmental DNA for the detection of aquatic biodiversity (in alpine lakes). The objectives of the EcoAlpsWater project, and the protocols developed within the framework of the Interreg Eco-AlpsWater project, were presented.
- German partners from LfL organised a training seminar for fishery masters, which was held online. In the seminar they presented and discussed the Eco-AlpsWater project.
- Activities and objectives of Eco-AlpsWater project were presented by NIB on 20th November 2020 to the Biotechnical Faculty students of microbiology (more than 40 students) in the frame of lectures about microbial diversity and identification.
- On May 2020 Slovenian partners presented EAW activities to younger public from College of Environmental Protection in Velenje, Slovenia, organized by NIB. Online lectures were divided in 5 parts, followed by fruitful discussion.
- Italian partner ARPAV presented Eco-AlpsWater activities to all students of the school Istituto Tecnico Tecnologico "GALILEO FERRARIS" - Verona (<http://www.ferrarisfermivr.gov.it/>).

- As part of the “Science Day”, INRAE and OFB organized, at Thonon-les-bains, 2 workshops dedicated to middle school classes. During these workshops (duration: 2 half days), the Eco-AlpsWater project was presented and practical demonstrations allowing the participants to perform all the steps of a fish eDNA metabarcoding analysis were organised. They started by filtering the samples and extracting DNA. The principles of PCR and sequencing were described, and finally, they carried out simplified bio-informatics analyses on a computer to assign DNA sequences to fish species. The classes were divided into four groups (each group being accompanied to treat their environmental samples); a round table allowed to discuss not only their results but also, more globally, the potential of eDNA tools for various applications among which the ecological diagnosis of aquatic ecosystems. USB keys (with the Eco-AlpsWater logo) have been produced specifically for this scientific event.
- Within the framework of three projects of School-Work Alternation, involving the secondary schools, the project has been presented through dedicated seminars by ISPRA at:
 - Mamiani High School of Rome (06/03/2019)
 - Virgilio Redi High School of Lecce (27/03/2019)
 - Meucci High School of Aprilia (13/05/2019).
- Dedicated seminars for younger public, mostly from College of Environmental Protection in Velenje, Slovenia, were organized on 16.5.2019 by NIB. Public from the north-eastern part of Slovenia was

invited to this seminar by local newspaper. All the participants attended the seminars, showing great curiosity and interest in cutting edge methods.

- In the week 13.-24.5.2019 German partners LFL and LfU organised dedicated seminars for a professional school for fishermen.
- Italian partners ARPAV presented the Eco-AlpsWater project in IPSAR LUIGI CARNACINA, Istituto Professionale per i Servizi Alberghieri e della Ristorazione - Bardolino (<http://www.carnacina.edu.it/>) and carried out a demonstration of lake water sampling activities (06/05/2019).
- Eco-AlpsWater project was introduced and described during a seminar for a secondary school in the Municipality of Asiago - Vicenza (Veneto Region) held by ARPAV partner on 26.2.2019 (<http://www.istitutosuperioreasiago.it/>).
- Youth participation in the Eco-AlpsWater project within the IVY Foundation. The NIB institute, EAW partner, collaborated with a student of molecular and functional biology through the IVY Foundation (The Interreg Volunteer Youth Initiative).

Activities for stakeholders and observers

- A one-day seminar entitled “Quo vadis, la biosurveillance basée sur l'ADN? » was dedicated to the presentation of methodological advancements in the domain of eDNA biomonitoring and to a specific workshop with stakeholders and other potential end-users of these tools. This seminar was organized in partnership with the Cost DNAquaNet on the 12th March 2021. The program was structured around 3 sessions:

- Session 1 (morning - English language):

“Overview of DNA-based method implementation”

F. Leese & A. Bouchez (Cost DNAquaNet)

- Session 2 (morning – French language):

“Diatomées et ADNe, où en sommes-nous? Développements et exemples”

F. Rimet (INRAE)

“Poissons et ADNe, où en sommes-nous? Développements et exemples”

M. Vautier & I Domaizon (INRAE -EcoALpsWater)

“Les méthodes basées sur l’ADNe: perspectives et ouvertures”

I. Domaizon (INRAE- EcoALpsWater)

- Session 3 (afternoon- French language):

Workshops organised in parallel to collect feedbacks from stakeholders and end-users regarding their needs for the future implementation of eDNA.

60 participants to the workshops and national presentations: 1/3 of stakeholders, 1/3 of private companies involved in environmental

biomonitoring and 1/3 of academics involved in the development of eDNA tools.

5 topics were discussed during the workshops:

- 1- Method development
- 2- Training / Tutoring
- 3- Transfer of information / protocols
- 4- Standardization
- 5- Advice and help for the choice of method

- On 29th September 2020 Eco-AlpsWater partner NIB attended the online final event of the Interreg Europe project "From biodiversity data to decisions: enhancing natural values through improved regional policies" (BID-REX).
- The first of July 2020 took place the first meeting in videoconference of the Working Group of the National System for Environmental Protection (SNPA) coordinated by Italian National Institute for Environmental Protection and Research (ISPRA). This working group is constituted by 12 regional agencies, some of them partners of Eco-AlpsWater project, and carried out technical meetings and field activities in order to test the e-DNA metabarcoding methods developed by EAW partners for the Alpine Space outside the Alpine area.
- On October, the 16th 2019, Italian partners organised the Second Italian

Eco-AlpsWater regional meeting in Rome with dedicated seminars describing the EAW activities:

-Microscopia classica: limiti dell'approccio tradizionale

N. Salmaso

-Tecniche di Next Generation Sequencing a supporto dei monitoraggi di nuova generazione

N. Salmaso

-Formalizzazione di protocolli per l'analisi del DNA ambientale in laghi e fiumi

N. Salmaso

-Strategia innovativa di valutazione ecologica e gestione delle risorse idriche per la protezione dei servizi ecosistemici nei laghi e nei fiumi alpini

S. Bernabei, M. Insolubile

-Metodi di campionamento per la metagenomica

C. Zampieri

-Sperimentazione italiana al di fuori dell'area Alpina


C. Greco, C. Martone, M. Insolubile, S. Macchio, P. Tomassetti, N. Mucci,
S. Bernabei

- Slovenian observers (Fisheries Research Institute of Slovenia) were involved in the fish eDNA sampling with the partner responsible for sampling (Slovenian Environment Agency, Ministry of the Environment

and Spatial Planning) on 15th October 2019.

- On 25.6.2019 the German partner LfL organised an event for local and external stakeholders. They presented Eco-AlpsWater activities through different contributions that were followed by participated discussions.
- Eco-AlpsWater Italian partners (ARPAV, FEM, ISPRA) and Swiss partner (SUPSI) organised a big and resonant local event in Verona. Contribution about this event was included also in the national TV.
- On 6th February 2019 German Partners LfL and LfU organised a local information meeting about EAW project.
- On the 19th -20th November 2018 a workshop for inland fisheries took place in Starnberg at the Institute for Fisheries at the Bavarian State Research Centre for Agriculture and Eco-AlpsWater project was introduced at the workshop by the project partner LfL.
- DNAquaNet Micro & Meiofauna meeting in Prague, 19th-20th/11/2018, Eco-AlpsWater activities were presented by Eco-AlpsWater communication manager Tina Eleršek (NIB).

Scientific contributions

	<p>12th SEFS</p>
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- 12th Symposium for European Freshwater Sciences, 25th – 30th July 2021 (online event), contributing with two oral presentations and a poster:

“Microalgae-based biodiversity and biomonitoring: Strengths and gaps of high-throughput sequencing applications”

Dr Nico Salmaso, Dr Rainer Kurmayer, Dr Ute Mischke, Dr Camilla Capelli, Dr Tina Elersek, Dr Fabio Lepori, Giulia Riccioni, Dr Frédéric Rimet, Dr Marine Vautier, Dr Isabelle Domaizon.

Abstract

The development of culture-independent high throughput sequencing (HTS) has opened new horizons in the study of aquatic biodiversity for fundamental and applied research. The limitations in the use of traditional approaches to the identification of organisms, which are based on the identification of morphological traits,

have promoted several studies that aimed to integrate new HTS technologies in regulatory biomonitoring, such as WFD (CE) and WPO (CH). In the Alpine region, one of the main objectives of the Interreg Alpine Space Eco-AlpsWater project is to develop and apply HTS methods for monitoring cyanobacteria and bacteria, microalgae, and fish, integrating and harmonizing conventional approaches. Here, we will focus on the results obtained for "algal" assemblages, i.e., cyanobacteria and microalgae in plankton and biofilms, analysed using 16S and 18S rRNA marker genes.

The survey was conducted in 37 lakes and 23 river sites. The specific points discussed in this investigation include the overall diversity recovered by HTS and light microscopy (LM); the taxonomic coverage of 16S and 18S rDNA sequences in NCBI taxonomic databases; the fraction of species identified by LM that are represented with at least one corresponding molecular marker (16S-18S) in the NCBI databases; the definition of methods to evaluate the reliability of LM classifications based on their correspondence with HTS sequences; the definition of phylogenetic approaches to fine-tune the taxonomic classifications obtained from the bioinformatic pipelines. The results are building a solid knowledge base for evaluating the range of applications and complementarities of HTS in the next generation biomonitoring systems.

“eDNA metabarcoding biodiversity of freshwater fish in the Alpine area”

Dr Giulia Riccioni, Dr Isabelle Domaizon, Dr Andrea Gandolfi, Dr Massimo Pindo, Dr Marine Vautier, Dr Rainer Kurmayer, Dr Peter Hufnagl, Dr Stefanie Dobrovolny, Dr Valentin Vasselon, Hans Rund, Dr Jonas Bylemans, Dr Nico Salmaso, Dr Josef Wanzenböck.

Abstract

Environmental DNA (eDNA) based methods are proving to be a promising tool for freshwater fish biodiversity assessment in Europe within the Water Framework Directive (WFD, 2000/60/EC) especially for large rivers and lakes where current fish monitoring techniques have known shortcomings. Many freshwater fish are experiencing critical population declines with risk of local or global extinction because of intense anthropogenic pressure and this can have serious consequences on freshwater ecosystem functioning

and diversity. Within the EU project Eco-AlpsWater, advanced high throughput sequencing (HTS) techniques are used to improve the traditional WFD monitoring approaches by using environmental DNA (eDNA) collected in Alpine waterbodies. An eDNA metabarcoding approach specifically designed to measure freshwater fish biodiversity in Alpine lakes and rivers has been extensively evaluated by using mock samples within an intercalibration test. This eDNA method was validated and used to study fish biodiversity of eight lakes and six rivers of the Alpine region including four EC countries (Austria, France, Italy, Slovenia) and Switzerland. More in detail, this metabarcoding approach, based on HTS sequencing of a section of the 12S rRNA gene, was used to assess freshwater fish biodiversity and their distribution in the different habitats. These data represent the first attempt to provide a comprehensive description of freshwater fish diversity in different ecosystems of the Alpine area confirming the applicability of eDNA metabarcoding analyses for the biomonitoring of fish inhabiting Alpine and perialpine lakes and rivers.


“Metabarcoding vs. morphological identification for aquatic ecosystem biomonitoring: a case study”

Dr Camilla Capelli, Dr Isabelle Domaizon, Dr Tina Eleršek, Dr Rainer Kurmayer, Dr Fabio Lepori, Dr Ute Mischke, Federica Rotta, Dr Marine Vautier, Dr Nico Salmaso.

Abstract

Phytoplankton and benthic diatoms are frequently used as biological quality indicators. However, conventional methods for species

identification (light microscopy) are time-consuming and require a high level of taxonomic expertise. The Interreg Alpine Space Eco-AlpsWater project (EAW) has the aim of overcoming these limits, exploring the application of high throughput sequencing (HTS)-based DNA metabarcoding for water quality assessment in the Alpine region. Here, preliminary results obtained from Lake Lugano (CH-IT) are used to evaluate the potential of DNA based approaches. For this purpose, in 2019, 10 littoral sites were sampled for the study of benthic diatoms and one pelagic site was sampled monthly for the study of eukaryotic phytoplankton and cyanobacteria. The HTS sequences of 16S rRNA (V3-V4), 18S rRNA (V4), and rbcL marker genes obtained from eDNA extracted from water and biofilm samples was compared with conventional identification by light microscopy. The genetic approach showed a good consistency with conventional methods, in particular for dominant taxa, although the correspondence between approaches was lower at the genus and, especially, the species level. Moreover, the HTS led to the identification of a higher number of indicator taxa and was found to be a sensitive tool for the detection of rare or invasive species. Therefore, the approach proposed within the EAW project, and HTS methods in general, can be effective in the study of the biodiversity and in the improvement of the ecological quality assessment of waterbodies.

	XXV AIOL
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- XXV AIOL congress held on 30th June- 2nd July contributing with 3 oral presentations (online event):

“Patterns of geographical distribution of toxigenic cyanobacterial species and oligotypes in the perialpine lake district”

Nico Salmaso, Adriano Boscaini, Giulia Riccioni, Giorgio Franzini, Giampaolo Fusato, Federica Giacomazzi, Chiara Zampieri, Silvia Costaraoss, Giovanna Pellegrini, Sabrina Pozzi, Renate Alber, Hannes Rauch, Alberta Stenico, Samuel Vorhauser, Elisa Zanut, Fabio Buzzi, Serena Bernabei, Claudia Greco, Paolo Tomassetti, Leonardo Cerasino.

Abstract

Eco-AlpsWater (EAW) is a major European project co-financed by the European Regional Development Fund (ERDF) through the Interreg Alpine Space program (www.alpine-space.eu/projects/eco-alpswater). The aim of the initiative is to integrate traditional water monitoring approaches implemented in the Alpine region and in Europe (Water Framework Directive-WFD) with high throughput sequencing technologies (HTS). In this work we will present the rationale and results obtained in the Italian hydrographic network, with a focus on large subalpine lakes and cyanobacterial communities determined on samples collected in pelagic areas and rocky-shore biofilms (Lake Garda). Overall, the pelagic and biofilm samples showed distinct communities, with only a few shared species and oligotypes (amplicon sequence variants) mostly belonging to the Chroococcales. One of the most widespread pelagic species in the Italian district and the whole Alpine region was *Planktothrix rubescens*. In contrast, *Tychonema bourrellyi* showed consistent populations only in the southern subalpine lake district. The normalized DNA sequence abundances of these two species were highly correlated with the

microcystin and anatoxin-a concentrations, demonstrating a high consistency of the results obtained by HTS and metabolomic profiling, and a high ability of HTS to predict the toxigenic potential due to the production of hepatotoxins and neurotoxins in inland waters.

“Freshwater fish biomonitoring in the Alpine area using eDNA metabarcoding”

Giulia Riccioni, Isabelle Domaizon, Andrea Gandolfi, Massimo Pindo, Adriano Boscaini, Marine Vautier, Rainer Kurmayer, Peter Hufnagl, Stefanie Dobrovlny, Valentin Vasselon, Hans Rund, Jonas Bylemans, Nico Salmaso, Josef Wanzenböck.

Abstract

Many freshwater fish are experiencing critical population declines with risk of local or global extinction because of intense anthropogenic pressure and this can have serious consequences on freshwater ecosystem functioning and diversity. Current fish monitoring techniques for large rivers and lakes, however, have known shortcomings. Within the Water Framework Directive (WFD, 2000/60/EC) environmental DNA (eDNA) based methods are proving to be a promising tool for freshwater fish biodiversity assessment. Within the EU project Eco-AlpsWater, advanced high throughput sequencing (HTS) techniques are used to improve the traditional WFD monitoring approaches by using environmental DNA (eDNA) collected in Alpine waterbodies. An eDNA metabarcoding approach has been evaluated by using mock samples within an intercalibration test, and has been used to study fish biodiversity of eight lakes and six rivers of the Alpine region including four EC countries

(Austria, France, Italy, Slovenia) and Switzerland. This approach, based on HTS sequencing of a section of the mitochondrial 12S rRNA, allowed to assess freshwater fish biodiversity and their distribution in the different habitats. These data represent the first attempt to provide a comprehensive description of freshwater fish diversity confirming the applicability of eDNA metabarcoding analyses for fish biomonitoring in Alpine and perialpine lakes and rivers.

“First report of cyanotoxins in benthic mats from Lake Garda”

Leonardo Cerasino, Adriano Boscaini, Nico Salmaso.

Abstract

Cyanobacteria represent a health hazard in aquatic environments due to their ability to produce a range of toxic metabolites, which can cause either immediate illness or long-term effects in both humans and animals. In the large subalpine lakes planktonic species *Planktothrix rubescens* and *Tychonema bourrellyi* are the main responsible of toxin production (microcystins and anatoxins, respectively) in pelagic environments. Considering Lake Garda, the largest Italian water basin, toxin measurements conducted in the last decade with a monthly frequency indicate a constant presence of both microcystins and anatoxins, yet at concentrations well below the safety thresholds indicated by the World Health Organization. Moreover, in this time span, anatoxin-a has become dominant, reaching annual maximum concentrations even two orders of magnitude higher than microcystins. However, a recent investigation, conducted in the frame of the Eco-AlpsWater project (financed by the Interreg Alpine Space programme), has revealed the presence of

cyanotoxins also in benthic samples; in particular, remarkable quantities of anatoxins have been found in biofilms collected from rocks in different sites of Lake Garda shores. Considering that benthic cyanobacteria can grow in high abundance in mats, anatoxins can reach dangerous concentrations in this material and constitute a potential threat for people and animals nearby.

- At the “1st DNAQUA International Conference”, the first results of an intercalibration exercise performed within the Eco-AlpsWater project was presented with a 3 minutes presentation currently available on the youtube channel of the event:

“Alpine freshwater fish biodiversity assessment: an intercalibration test for metabarcoding method set up”

Giulia Riccioni, Isabelle Domaizon, Andrea Gandolfi, Massimo Pindo, Marine Vautier, Rainer Kurmayer, Peter Hufnagl, Stefanie Dobrovolny, Valentin Vasselon, Hans Rund, Jonas Bylemans, Cuong Q. Tang, Josef Wanzenböck.

Abstract

Environmental DNA (eDNA) based methods (Fig.1) are proving to be a promising tool for freshwater fish biodiversity assessment in Europe within the Water Framework Directive (WFD, 2000/60/EC) especially for large rivers and lakes where current fish monitoring techniques have known shortcomings. Freshwater fish are actively involved in aquatic ecosystems functioning and diversity, contributing to the health, well-being and economy in every geographic realm. Unfortunately, many freshwater fish

are experiencing critical population decline with risk of local or global extinction because of intense anthropogenic pressure. Within the EU project Eco-AlpsWater, advanced high throughput sequencing (HTS) techniques are used to improve the traditional WFD monitoring approaches by using environmental DNA (eDNA) collected in Alpine waterbodies. To evaluate the performance of the metabarcoding approach specifically designed to measure freshwater fish biodiversity in Alpine lakes and rivers, an intercalibration test was performed. This exercise forecasted the use of mock samples containing either tissue-extracted DNA of different target species or water collected from aquaculture tanks to mimic real environmental water sampling and processing. Moreover, three water samples collected in Lake Bourget (France) were used to compare the efficiency of taxonomic assignments in natural and mock community samples. Our results highlighted a good efficiency of the molecular laboratory protocols for HTS and a good amplification success of the selected primers, providing essential information concerning the taxonomic resolution of the 12S mitochondrial marker. As further confirmation, different concentration of species DNA in the mock samples were well represented by the relative read abundance. This preliminary test confirmed the applicability of eDNA metabarcoding analyses for the biomonitoring of freshwater fish inhabiting Alpine and perialpine lakes and rivers.

- On Tuesday 1st September 2020, the conference "Environmental DNA for fish species detection" was held at INRAE, in Thonon-les-Bains, to illustrate the use of environmental DNA for the detection of fish species in alpine lakes. The protocols developed within the framework of the Interreg Eco-AlpsWater project were presented, as well as preliminary results

obtained for Lake Bourget. Despite the restrictions related to the COVID19 crisis, 20 participants were present, with representatives from the OFB (National Office for Biodiversity), from academic institutions (INRAE, University of Savoie Mont Blanc), but also local stakeholders and end-users (lake Léman) interested in the evaluation of biodiversity in aquatic systems. The conference was also live webcasted on internet and was viewed by more than 200 people. This conference was an opportunity to present the work done within the EAW project, but also to discuss with stakeholders in order to identify possible applications and the directions to follow for the future.

- 63rd International Association for Great Lakes Research Conference on June 9th-11th 2020 contributing with an oral presentation:

“The large lakes south of the Alps: Current limnological status, with a special focus on Lake Garda”

Nico Salmaso

Abstract

The large lakes south of the Alps (DSL: Garda, Maggiore, Como, Iseo and Lugano) are one of the most important lake districts in Europe. In the last decades, the DSL showed a tendency to oligotrophication, warming of the water column, decrease in the frequency of full mixing episodes followed by a lower supply of nutrients to the upper layers. In Lake Garda, the decrease of nutrients caused a decline of the mesotrophic cyanobacterium *Planktothrix rubescens* (microcystin producer), which was partially replaced by *Tychonema bourrellyi* (anatoxin-a producer), a “new” species

identified in 2014. The discovery of *Tychonema* can be considered a paradigmatic example of the unknown biodiversity in the DSL. To solve this gap, high throughput sequencing has been recently used to analyse bacteria, cyanobacteria, protists and fish. The new approach has been extended to the whole Alpine region within the EU Alpine Space project Eco-AlpsWater (www.alpinespace.eu/projects/eco-alpswater).

- International conference UGM 2019 “DNA-Methoden im Umweltmanagement” held on 4th -5th December 2019 in Innsbruck, oral presentation:

“Wer findet was: Metabarcoding vs WRRL im Eco-AlpsWater Projekt”.

Ute Mischke, Jochen Schaumburg, Nico Salmaso

Abstract

Im Zentrum des Interreg-Projektes Eco-AlpsWater steht die Ermittlung von Süßwasserarten mittels Metabarcoding im Vergleich und zur Ergänzung zu den EU-WRRL-Methoden. Es wurde ein Netzwerk von Wissenschaftlern und Überwachungsbehörden mit über 100 Personen aus 6 Ländern gebildet. Über 20 Gewässer wurden durch die 12 Projektpartner ausgewählt, um aus ihnen im Jahr 2019 Fisch-, Biofilm- und Planktonproben nach einheitlichen Protokollen zu entnehmen. Das Bayerische Landesamt für Umwelt wird die im nächsten Jahr erwarteten Analyseergebnisse besonders für den Starnberger See und den Fluss Wertach zusammenstellen und den Nutzen der eDNA-Methode für die Wasserwirtschaft bewerten.

- At the International Mountain Conference 2019, held in Innsbruck

(September 8th – 12th, 2019) Rainer Kurmayer (LFUI) introduced the Eco-AlpsWater project with a poster.

“Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers (Eco-AlpsWater)”

Rainer Kurmayer, Adriano Boscaini, Jean-Marc Baudoin, Serena Bernabei, Camilla Capelli, Isabelle Domaizon, Stefanie Dobrovolny, Tina Elersek, Claudia Greco, Giorgio Franzini, Peter Hufnagl, Aleksandra Krivograd Klemenčič, Fabio Lepori, Ute Mischke, Špela Remec-Rekar, Nico Salmaso, Jochen Schaumburg, Michael Schubert, Karmen Stanic, C. Vogelmann, Josef Wanzenböck, C. Zampieri.

Abstract

A project recently co-funded through the Alpine Space program with the aim to improve surface water quality monitoring, by (i) advanced DNA sequencing techniques enabling metabarcoding of aquatic biota relevant for the implementation of the EU Water Frame Work Directive and the Swiss Water Protection Ordinance, and (ii) novel technologies in data processing (automation in data processing, data storage, information retrieval).

Along with the identification of gaps in the monitoring approaches across the Alpine regions, the new technologies will allow to define improved experimental monitoring protocols to be applied in selected areas (including large perialpine lakes and smaller waterbodies, and key rivers). The transnational approach fills the scientific divide between academia and governance agencies, putting into practice the EUSALP agenda, i.e., capacity building of research institutions, networks and infrastructure with an alpine region dimension.

More info can be found at www.alpine-space.eu/eco-alpswater.

- 7th European Phycological Congress in Zagreb, Croatia, on 27th August 2019 contributing with a poster:

“Assessment of biodiversity and water quality in the alpine region with innovative approaches”

T. Elersek, J.M. Baudoin, S. Bernabei, A. Boscaini, A. Bouchez, J. Bylemans, C. Capelli, L. Cerasino, S. Dobrovolny, I. Domaizon, C. Donati, G. Franzini, C. Greco, P. Hufnagl, A. Krivograd Klemenčič, R. Kurmayer, F. Lepori, M. Logez, U. Mischke, M. Pindo, S. Remec-Rekar, N. Salmaso, J. Schaumburg, M. Schubert, P. Tomassetti, C. Vogelmann, J. Wanzenböck, C. Zampieri.

Abstract

Innovative approaches for water monitoring and better management of lake and river ecosystems is the object of a new project - Eco-AlpsWater, co-financed by the European Regional Development Fund through the Interreg Alpine Space programme. The project, which began in April 2018, will last three years, involving 12 partners belonging to 6 countries in the Alpine region (Austria, France, Germany, Italy, Slovenia and Switzerland). One of the main objectives is to develop and apply state of the art methods for the monitoring of microalgae, cyanobacteria, bacteria and fish based on the use of High Throughput Sequencing (HTS) techniques, complementing traditional approaches and anticipating the route in the development of new generation water monitoring systems. Traditional monitoring approaches suffer many drawbacks due to limitations in the correct microscopic

recognition of diacritical characters. The new HTS technologies are providing a comprehensive picture of taxonomic composition and biodiversity of biological elements in the Alpine region based on the analysis of samples collected in over 50 lakes and rivers.

- In July 2019 Hans Rund from LFUI (EAW partner) contributed to the international conference “Advances in eDNA-based Approaches to Fish Ecology and Management in Hull”, UK, with a poster describing aims and activities of Eco-AlpsWater.

“Development and establishment of molecular genetic (eDNA) monitoring methods for fish in waterbodies in the alpine region, and comparison with traditional ecological status assessment methods”

Rund, H., Kurmayer R. & Wanzenböck J.

Abstract

Within the Eco-AlpsWater project, the implementation of recently developed monitoring approaches aims to complement traditional methods for ecological status assessment for waterbodies in the alpine region with novel molecular methods and to provide a fish census of lake and river biodiversity. Ecosystem services provided by lakes and rivers are facing serious threats under the pressure of anthropogenic impacts, climate change, loss of biodiversity and occurrence of invasive species. The assessment of impact caused by these pressures are based on the criteria of traditional ecological status assessments, which are regulated by the Water Framework Directive (WFD). These traditional approaches are known to

be expensive, selective and time-consuming. By using next generation sequencing technologies to analyze environmental DNA samples, collected in rivers and lakes in alpine regions, a fast and cost-effective tool will be implemented to create taxonomic inventories at an unprecedented level. Furthermore, spatial distribution as well as seasonal changes in the fish community will be assessed. Various DNA extraction protocols were tested and optimized and the most promising protocol will be presented in the context of my contribution. The data acquired by the new approach will be compared to comprehensive data sets based on traditional fish ecological status assessment survey, to identify the limitations of the different approaches.

	<p>XXIV AIOL</p>
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- XXIV AIOL congress (Congresso dell'Associazione Italiana di Oceanologia e Limnologia) held in Bologna from the 5th to the 9th June 2019 contributing with a poster and an oral presentation:

“The use of environmental samples for High-Throughput Biodiversity monitoring: Current knowledge and future perspectives”

Jonas Bylemans

Abstract

Obtaining accurate inventories of species biodiversity is fundamental to gain a better understanding of ecological processes and to mitigate the impact of biotic and abiotic

stressors. A diverse array of monitoring tools is available for biodiversity monitoring but conventional tools all require the physical capture of species and are often unsuitable for rare and cryptic species. In recent years, research has shown that by applying High-Throughput Sequencing technologies to DNA extracts from environmental samples (eDNA) highly detailed biodiversity inventories can be obtained. This approach, often referred to as eDNA metabarcoding, is highly suitable for the detection of rare and cryptic species and workflows allow for rapid processing of samples thus reducing labour and cost requirement. For aquatic biodiversity in particular metabarcoding has become a highly popular monitoring tool with current studies focusing on a wide range of taxa (i.e., ranging from plants to vertebrates). During this presentation I will outline the current knowledge and applications of eDNA metabarcoding. In particular, some of the activities undertaken within the EU Interreg project ‘Eco-AlpsWater’ will be highlighted which specifically aims to promote the implementation of these technologies into traditional monitoring surveys. Finally, I will discuss some of the current challenges and future perspectives.

“Innovative approaches for the evaluation of the ecological conditions and ecosystems functionality of alpine lakes and rivers: the Interreg Alpine Space project Eco-AlpsWater”

Boscaini A., Cerasino L., Bylemans J., Gandolfi, A., Franzini G., Fusato G., Giacomazzi F., Zampieri C., Pozzi S., Pellegrini G., Alber R., Vorhauser S., Rauch H., Zanut E., Buzzi F., Bernabei S., Greco C., Salmaso N.

Abstract

Eco-AlpsWater is a project co-financed by the European Regional Development Fund (ERDF) through the Interreg “Alpine Space” programme. The aim of the project is to improve the traditional water monitoring approaches utilized in the Alpine region (Water Framework Directive-WFD in EU countries and Water Protection Ordinance-WPO in Switzerland) with innovative technologies, providing solid knowledge to support lake and river management plans. The new approach will make use of Next Generation Sequencing (NGS) techniques to analyse environmental DNA (eDNA) extracted from samples collected in lakes and rivers. These new techniques, based on the amplification and analysis of millions of DNA sequences, allow rapid and low-cost identification of aquatic organisms. The new generation monitoring will permit to carry out one of the most extensive census of lakes and rivers biodiversity of the Alpine region based on the analysis of hundreds of samples collected in over 50 water bodies. The investigations will focus on the study of bacteria and cyanobacteria, phytoplankton, periphytic communities (including diatoms) and fish. The collected data will allow to identify the areas at risk of toxic cyanobacteria, pathogenic bacteria and alien or potentially invasive organisms. The project, started in 2018 and operational until April 2021, involves 12 partners belonging to 6 countries in the Alpine region (Austria, France, Germany, Italy, Slovenia and Switzerland). The research, started in the first months of 2019, in the Italian context will in particular evaluate the ecological quality of two key environments representative of the great lakes (Garda) and rivers (Adige) south of the Alps. During the summer months, the survey will be extended to a greater number of lakes and rivers, including other great lakes of the network LTER IT08-Subalpine lakes, small lakes (for example Ledro, Caldaro, Ragogna) and rivers.

- On the 6th May 2019 Eco-AlpsWater project was presented at the 11th International conference of toxic cyanobacteria (Krakow, Poland) with an oral contribution:

“Innovative approaches for the study of biodiversity and water quality assessment in the alpine region: the interreg alpine space project eco-alpswater”

N. Salmaso, J.M. Baudoin, Bernabei S., A. Boscaini, A. Bouchez, C. Capelli, L. Cerasino, S. Dobrovolny, I. Domaizon, C. Donati, T. Elersek, G. Franzini, C. Greco, P. Hufnagl, A. Krivograd Klemenčič, R. Kurmayer, F. Lepori, M. Logez, U. Mischke, M. Pindo, S. Remec-Rekar, J. Schaumburg, M. Schubert, K. Stanic, P. Tomassetti, C. Vogelmann, J. Wanzenböck, C. Zampieri.

Abstract

The adoption of innovative approaches for monitoring and safeguarding lake and river ecosystems is the object of a new project - Eco-AlpsWater - co-financed by the European Regional Development Fund through the Interreg Alpine Space programme. The project, which began in April 2018, will last three years, involving 12 partners belonging to 6 countries in the Alpine region (Austria, France, Germany, Italy, Slovenia and Switzerland). One of the main objectives is to develop and apply state of the art methods for the monitoring of cyanobacteria and bacteria, microalgae and fish based on the use of High Throughput Sequencing (HTS) techniques, complementing traditional approaches and anticipating the route in the development of new generation water monitoring systems. Owing to the

ability to produce a wide variety of toxins, a specific attention is given to the identification of potentially toxigenic cyanobacteria. Traditionally, their monitoring in aquatic ecosystems was based on microscopic examinations. Nevertheless, traditional approaches suffer many drawbacks due to limitations in the correct microscopic recognition of diacritical characters. The new HTS technologies are providing a comprehensive picture of taxonomic composition and biodiversity of cyanobacteria and other biological elements in the Alpine region based on the analysis of samples collected in over 50 lakes and rivers. In order to evaluate general patterns in cyanobacteria composition related to cyanotoxin production, the survey is completed by the concurrent examination of sample aliquots for the determination of a wide variety of cyanotoxins in water samples and biofilms in lakes and rivers.

- Congress of Health and Climate Change (<https://healthclimate2018.iss.it/>), Rome 3rd-5th December 2018 poster presentation:

“Eco-AlpsWater, Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers”

Serena Bernabei

Abstract

The main objective of Eco-AlpsWater is to integrate the traditional monitoring approaches used in the Alpine region and at the European level with advanced and innovative methods, providing solid and qualified knowledge to further support water resources management plans. The new

approach will use Next Generation Sequencing - NGS technologies to analyze environmental DNA (eDNA) extracted from water samples collected in lakes and rivers. These new techniques, based on the amplification and analysis of millions of DNA sequences and on the use of smart technologies including automation in data processing and storage, and information retrieval, allow a rapid and low-cost identification of aquatic organisms, from bacteria to fish. The data will, in particular, identify the areas most at risk due to the presence of toxic cyanobacteria, pathogenic bacteria, and invasive or potentially invasive organisms. The project runs from April 2018 to April 2021.

- International joint event IAGLR (International Association for Great Lakes Research) and ELLS (European Large Lakes Symposium) in Evian in September 23rd-28th, 2018. The meeting was the first IAGLR meeting held outside North America, and in conjunction with the 5th European Large Lakes Symposium. EAW consortium contributed with a poster:

“Application of eDNA methods for Biomonitoring: the Eco-AlpsWater project

for innovative ecological assessment of Alpine Lakes and Rivers”

Domaizon Isabelle, Eco-AlpsWater consortium

Abstract

The aim of the project Eco-AlpsWater is to complement the traditional monitoring approaches used in the Alpine region (Water Framework Directive and, in Switzerland, the Water Protection Ordinance) with innovative technologies. The new approach will make use of high

throughput sequencing (HTS) to analyze environmental DNA in waterbodies. Along with the identification of gaps in the monitoring approaches across the Alpine regions, the new technologies will allow to define new monitoring protocols to be applied in selected areas (including large perialpine lakes and smaller waterbodies, and key rivers). The focus will be on 4 biological elements used to assess water quality, and ecological status i.e., bacteria/cyanobacteria, phytoplankton, phytobenthos and fish. The transnational approach is essential to foster interactions and fill the scientific divide between academia and governance agencies, putting into practice the EUSA, LP agenda, i.e., capacity building of research institutions, networks and infrastructure with an Alpine Region dimension; and the preservation and valorisation of natural resources at a transnational scale.

- Congress of the Bavarian State Research Centre for Agriculture (LfL) in Grub near Munich on 8th of July 2018.
- Forum Alpinum (Alpine water Conference) in Breitenwang, Tirol, on 5th of June 2018, contributing with a poster.

“Innovative ecological Assessment and Water Management Strategy for the Protection of ecosystem Services in Alpine lakes and rivers (ecoAlpsWater)”

Rainer Kurmayer

Abstract

A new project co-funded through the Alpine Space program with the aim to improve surface water quality monitoring by *i*) advanced DNA

sequencing techniques enabling metabarcoding of aquatic biota relevant for the implementation of the EU Water Framework Directive and the Swiss Water Protection Ordinance, and *ii*) novel technologies in data processing (automation in data processing, data storage, information retrieval). Along with the identification of gaps in the monitoring approaches across the Alpine regions, the new technologies will allow to define improved experimental monitoring protocols to be applied in selected areas (including large perialpine lakes and smaller waterbodies, and key rivers). The transnational approach fills the scientific divide between academia and governance agencies, putting into practice the EUSALP agenda, i.e., capacity building of research institutions, networks and infrastructure with an Alpine Region dimension.

Schools and courses

- Eco-AlpsWater SUMMER SCHOOL: *Biomonitoring of microorganisms in aquatic ecosystems: High throughput sequencing rationale and applications*. 31st August- 3rd September 2021

Lectures contribute by Nico Salmaso (FEM), Isabelle Domaizon (INRAE), Massimo Pindo (FEM), Alessandro Cestaro (FEM), Valentin Vasselon (OFB) with hands-on exercises.