

Consortium Agreement

for the project

“DNAqualMG: Innovating transnational aquatic biodiversity monitoring using high-throughput DNA tools and automated image recognition”

(Project number: Biodiversa2022-738)



BETWEEN:

(1) University of Duisburg-Essen (UDE)

- the Coordinator –

Executing bodies:

- (a) Professor Florian Leese
- (b) Professor Daniel Hering
- (c) Professor Bank Beszteri

- (2) Finnish Environment Institute (SYKE),
- (3) French National Research Institute for Agriculture, Food and the Environment (INRAE),
- (4) University of Lodz (UniLodz),
- (5) University College Dublin (UCD),
- (6) University of Jyväskylä (JYU),
- (7) Associacao Biopolis (BIOPOLIS),
- (8) Massaryk University (MU),
- (9) FH Kaernten – Gemeinnützige GmbH (CUAS),
- (10) Aarhus University (AU),
- (11) University of Natural Resources and Life Sciences, Vienna (BOKU),
- (12) Botanic Garden and Botanical Museum (BGBM),
- (13) Swedish University of Agricultural Sciences (SLU),

and

University of Nis (UNFSM)

- as a subcontractor of party (8) MU -

- hereinafter, jointly or individually, referred to as "Parties" or "Party" -

relating to the Project entitled

» **DNAqualMG: Innovating transnational aquatic biodiversity monitoring using high-throughput DNA tools and automated image recognition** «

in short

»**DNAqualMG**«

hereinafter referred to as "Project".

Preamble:

The Parties, having considerable experience in the field of biodiversity monitoring, have submitted a proposal for the Project to the Granting Authority as part of the European Biodiversity Partnership (Biodiversa+) – call BiodivMon (2022-2023). With the project, the Parties aim to advance biodiversity monitoring in stream ecosystems with genetic and image-based methods. The Parties wish to specify or supplement binding commitments among themselves in addition to the provisions of the rules and those of their national funding agencies.

NOW, THEREFORE, IT IS HEREBY AGREED AS FOLLOWS:

Section 1: Definitions

"Background" means information which is held by the Parties prior to their accession to this agreement, as well as copyrights or other intellectual property rights pertaining to such information, reports, inventions, software, ideas, methods, solutions, devices and materials, the application for which has been filed before their accession to this agreement, and which is needed for carrying out the *Project* or for using *Foreground*;

"Defaulting Party"

Defaulting Party means a Party which has been identified to be in breach of this Consortium Agreement and the Rules as specified in Article 4.2 of this Consortium Agreement.

"Foreground" means the results, including information, whether or not they can be protected, which are generated under the *Project*. Such results include rights related to copyright; design rights; patent rights; plant variety rights; or similar forms of protection.

"National Funding Body"

means the national agency that provides the funding to a Party to participate in the Project.

"Needed" means:

For the implementation of the Project:

Access Rights are Needed if, without the grant of such Access Rights, carrying out the tasks assigned to the recipient Party would be impossible, significantly delayed, or require significant additional financial or human resources.

For Use of own Foreground:

Access Rights are Needed if, without the grant of such Access Rights, the Use of own Foreground would be technically or legally impossible.

"Software"

Software means sequences of instructions to carry out a process in, or convertible into, a form executable by a computer and fixed in any tangible medium of expression.

"State Aid Rules"

State Aid Rules means article 107-109 of the Treaty of the Functioning of the European Union (as may be amended) and all applicable laws, legislation, directives, regulations, guidelines, procedures that are derived from article 107, including all rulings of the courts of both the EU and Ireland, and including also, for the avoidance of any doubt, the Communication from the EU Commission entitled "Framework for state aid for research and development and innovation", reference 2022/C 414/01 (the "RDI Framework").

Section 2: Purpose

The purpose of this Consortium Agreement is to specify with respect to the Project the relationship among the Parties, in particular concerning the organisation of the work between the Parties, the management of the Project and the rights and obligations of the Parties concerning inter alia liability, Access Rights and dispute resolution.

Section 3: Entry into force, duration and termination

3.1 Entry into force

An entity becomes a Party to this Consortium Agreement upon signature of this Consortium Agreement by a duly authorised representative.

This Consortium Agreement shall have effect from (01.03.2024).

A new Party may enter the Consortium upon signature of an accession document signed by the new Party and the Coordinator. Such accession shall have effect from the date identified in the accession document.

3.2 Duration and termination

This Consortium Agreement shall continue in full force and effect until complete fulfilment of all obligations undertaken by the Parties under the Rules of the respective National Funding Agencies and under this Consortium Agreement.

However, this Consortium Agreement or the participation of one or more Parties to it may be terminated in accordance with the terms of this Consortium Agreement or the Rules or the Terms of the respective National Funding Agencies.

If the National Funding Agency does not award the Funding or terminates it, this Consortium Agreement shall automatically terminate in respect of the affected Party/ies, subject to the provisions surviving the expiration or termination under Art. 3.3 of this Consortium Agreement.

3.3 Survival of rights and obligations

The provisions relating to Access Rights and Confidentiality, for the time period mentioned therein, as well as for Liability, Applicable law and Settlement of disputes shall survive the expiration or termination of this Consortium Agreement.

Termination shall not affect any rights or obligations of a Party leaving the Consortium incurred prior to the date of termination, unless otherwise agreed between the consortium and the leaving Party. This includes the obligation to provide all input, deliverables and documents for the period of its participation.

Section 4: Responsibilities of Parties

4.1 General principles

Each Party undertakes to take part in the efficient implementation of the Project, and to cooperate, perform and fulfil, promptly and on time, all of its obligations under the National Funding Agreement entered into by such Party and this Consortium Agreement as may be reasonably required from it and in a manner of good faith as prescribed by Belgian law.

Each Party undertakes to notify promptly, in accordance with the governance structure of the Project, any significant information, fact, problem or delay likely to affect the Project.

Each Party shall promptly provide all information reasonably required by a Party or by the Coordinator to carry out its tasks.

Each Party shall take reasonable measures to ensure the accuracy of any information or materials it supplies to the other Parties.

4.2 Breach

In the event a responsible Consortium Body identifies a breach by a Party of its obligations under this Consortium Agreement (e.g.: a Party not delivering required work [Milestones and Deliverables in Work Packages as defined in the Proposal] or producing poor quality work), the Coordinator or the Party appointed by the Consortium by simple majority if the Coordinator is in breach of its obligations under this Consortium Agreement will give written notice to such Party requiring that such breach be remedied within 30 calendar days.

If such breach is substantial and is not remedied within that period or is not capable of remedy, the General Assembly may decide to declare the Party to be a Defaulting Party and to decide on the consequences thereof which may include termination of its participation.

4.3 Involvement of third parties

A Party that enters into a subcontract or otherwise involves third parties in the Project remains solely responsible for carrying out its relevant part of the Project and for such third party's compliance with the provisions of this Consortium Agreement. It has to ensure that the involvement of third parties does not affect the rights and obligations of the other Parties under this Consortium Agreement.

4.4 Specific responsibilities regarding data protection

Where necessary, the Parties shall cooperate in order to enable one another to fulfil legal obligations arising under applicable data protection laws (the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data and relevant national data protection law applicable to said Party) within the scope of the performance and administration of the Project and of this Consortium Agreement.

In particular, the Parties shall, where necessary, conclude a separate data processing, data sharing and/or joint controller agreement before any data processing or data sharing takes place.

Section 5: Liability towards each other

5.1 No warranties

In respect of any information or materials (incl. Foreground and Background) supplied by one Party to another under the Project, no warranty or representation of any kind is made, given or implied as to the sufficiency or fitness for purpose nor as to the absence of any infringement of any proprietary rights of third parties.

Therefore,

- the recipient Party shall in all cases be entirely and solely liable for the use to which it puts such information and materials, and
- no Party granting Access Rights shall be liable in case of infringement of proprietary rights of a third party resulting from any other Party exercising its Access Rights.

5.2 Limitations of contractual liability

No Party shall be responsible to any other Party for any indirect or consequential loss or similar damage such as, but not limited to, loss of profit, loss of revenue or loss of contracts, provided such damage was not caused by a wilful act or gross negligence.

A Party's aggregate liability towards the other Parties collectively shall be limited to once the Party's share of the total costs of the Project as identified in the financial plan of the accepted proposal provided such damage was not caused by a wilful act or gross negligence.

The terms of this Consortium Agreement shall not be construed to amend or limit any Party's statutory liability.

5.3 Damage caused to third parties

Each Party shall be solely liable for any loss, damage or injury to third parties resulting from the performance of the said Party's obligations by it or on its behalf under this Consortium Agreement or from its use of Foreground or Background.

5.4 Force Majeure

No Party shall be considered to be in breach of this Consortium Agreement if such breach is caused by Force Majeure. Each Party will notify the competent Consortium Bodies of any Force Majeure without undue delay. If the consequences of Force Majeure for the Project are not overcome within 6 weeks after such notification, the transfer of tasks - if any - shall be decided by the competent Consortium Bodies.

6 Coordinator

6.1 The Coordinator shall be the intermediary between the Parties and Biodiversa+ and shall perform all tasks assigned to it as described in this Consortium Agreement and the accepted proposal.

6.2 In particular, the Coordinator shall be responsible for:

- Monitoring compliance by the Parties with their obligations
- Keeping the address list of Members and other contact persons updated and available
- Collecting, reviewing and submitting information on the progress of the Project and reports and other deliverables to the Project,
- Preparing the meetings, proposing decisions and preparing the agenda of meetings, chairing the meetings, preparing the minutes of the meetings and monitoring the implementation of decisions taken at meetings, transmitting promptly documents and information connected with the Project,
- Providing, upon request, the Parties with official copies or originals of documents which are in the sole possession of the Coordinator when such copies or originals are necessary for the Parties to present claims.

6.3 The Coordinator shall not be entitled to act or to make legally binding declarations on behalf of any other Party.

6.4 The Coordinator shall not enlarge its role beyond the tasks specified in this Consortium Agreement and the accepted proposal.

Section 7: Financial provisions

The detailed total budget for the project is outlined in the accepted proposal and the National Funding Guidelines. Each party is responsible for complying with their respective budgets and reports any significant deviations to the Coordinator in the consortium. If the deviations are estimated to have a significant impact on project execution, the parties shall at a joint meeting discuss and agree on means to resolve the problems. This meeting will be arranged by the Coordinator.

A Defaulting Party leaving the Consortium shall, within the limits specified in Article 5.2 of this Consortium Agreement, bear any reasonable and justifiable additional costs occurring to the other Parties in order to perform its and their tasks. Any additional costs which are not covered by the Defaulting Party shall in principle be apportioned to the remaining Parties pro rata to their share in the total costs of the Project as identified in the Consortium Budget.

Each Party will be responsible to its National Funding Body and will indemnify the other Parties in relation to any claims, liabilities, losses, expenses and damages (including legal costs) which might arise as a result of or in connection with that Party's obligations to its National Funding Body.

Section 8: Foreground

8.1. *Foreground* shall be the property of the *Party* carrying out the work generating that *foreground*.

8.2. Where several *Parties* have jointly carried out work generating *foreground* and where their respective share of the work cannot be ascertained, they shall have joint ownership of such *foreground*. They shall establish an agreement regarding the allocation and terms of exercising that joint ownership.

However, where no joint ownership agreement has yet been concluded, each of the joint owners shall be entitled to use the joint foreground and to grant non-exclusive licences to third parties, without any right to sub-licence, subject to the following conditions:

- a) at least 45 days prior notice must be given to the other joint owner(s); and
- b) in case of a commercial use of the joint foreground market prices must be provided to the other joint owner(s). Any contribution of the Party to the creation of the joint foreground will be deducted from the market price.

8.3 Dissemination

8.3.1 Publication

8.3.1.1 Dissemination activities including but not restricted to publications and presentations shall be governed by the following procedure:

Prior notice of any planned publication shall be given to the other Parties concerned at least 45 days before the publication. Any objection to the planned publication shall be in writing to the Coordinator and to any Party concerned within 30 days after receipt of the notice. If no objection is made within the time limit stated above, the publication is permitted.

8.3.1.2 An objection is justified if

- (a) the objecting Party's legitimate academic or commercial interests are compromised by the publication; or

(b) the protection of the objecting Party's Confidential Information, Foreground or Background is adversely affected.

The objection has to include a precise request for necessary modifications.

8.3.1.3 If an objection has been raised the involved Parties shall discuss how to overcome the justified grounds for the objection on a timely basis (for example by amendment to the planned publication and/or by protecting information before publication) and the objecting Party shall not unreasonably continue the opposition if appropriate actions are performed following the discussion.

8.3.2 Publication of another Party's Foreground or Background

For the avoidance of doubt, a Party shall not publish Confidential Information, Foreground or Background of another Party, even if such Confidential Information, Foreground or Background is amalgamated with the Party's Foreground, without the other Party's prior written approval. For the avoidance of doubt, the mere absence of an objection according to 8.3.1 is not considered as an approval.

8.3.3 Cooperation obligations

The Parties undertake to cooperate to allow the timely submission, examination, publication and defence of any dissertation or thesis for a degree which includes their Foreground or Background subject to the confidentiality and publication provisions agreed in this Consortium Agreement.

8.3.4 Use of names, logos or trademarks

Nothing in this Consortium Agreement shall be construed as conferring rights to use in advertising, publicity or otherwise the name of the Parties or any of their logos or trademarks without their prior written approval.

Section 9: Access Rights

9.1 General Principles

9.1.1 Each Party shall implement its tasks in accordance with the Consortium Plan and shall bear sole responsibility for ensuring that its acts within the Project do not knowingly infringe third party property rights.

9.1.2 Parties shall inform the Consortium as soon as possible of any limitation to the granting of Access Rights to Background or of any other restriction which might substantially affect the granting of Access Rights (e.g. the use of open source code software in the Project).

9.1.3 If the Consortium considers that the restrictions have such impact, which is not foreseen in the Consortium Plan, it may decide to update the Working Plan accordingly.

9.1.4 Any Access Rights granted expressly exclude any rights to sublicense unless expressly stated otherwise.

Access Rights shall be free of any administrative transfer costs.

Access Rights are granted on a non-exclusive basis, if not otherwise agreed in writing.

9.1.5 Foreground and Background shall be used only for the purposes for which Access Rights to it have been granted.

9.1.6 All requests for Access Rights shall be made in writing.

The granting of Access Rights may be made conditional on the acceptance of specific conditions aimed at ensuring that these rights will be used only for the intended purpose and that appropriate confidentiality obligations are in place.

9.1.7 The requesting Party must show that the Access Rights are Needed.

9.2 Access Rights for implementation and for Use

Access Rights for project implementation:

Access Rights to Foreground and Background Needed for the performance of the own work of a Party under the Project and for the exclusive use in research and teaching shall be granted on a royalty-free basis, unless otherwise agreed.

Access Rights for Use

Access Rights to Foreground or Background if Needed for Use of a Party's own Foreground including for third-party research shall be granted on market conditions.

Access rights for internal research activities shall be granted to the research performing institution Parties on a royalty-free basis

In respect of Access Rights to Background if Needed for Use of a Party's own Foreground the Parties shall conclude a separate bilateral agreement.

A request for Access Rights may be made up to ~~twelve~~ twelve months after the end of the Project or, in the case of Art. 9.4.2.1.2, after the termination of the requesting Party's participation in the Project.

9.3 Additional Access Rights

For the avoidance of doubt any grant of Access Rights not covered by this Consortium Agreement shall be at the absolute discretion of the owning Party and subject to such terms and conditions as may be agreed between the owning and receiving Parties.

9.4 Access Rights for Parties entering or leaving the Consortium

9.4.1 New Parties entering the Consortium

All Foreground developed before the accession of the new Party shall be considered to be Background with regard to said new Party.

9.4.2 Parties leaving the Consortium

9.4.2.1 Access Rights granted to a leaving Party

9.4.2.1.1 Defaulting Party

Access Rights granted to a Defaulting Party and such Party's right to request Access Rights shall cease immediately upon receipt by the Defaulting Party of the formal notice of the decision of a simple majority of the consortium to terminate its participation in the Consortium.

9.4.2.1.2 Non-defaulting Party

A non-defaulting Party leaving voluntarily and with the other Parties' consent shall have Access Rights to the Foreground developed until the date of the termination of its participation. It may request Access Rights within the period of time specified in Art. 9.2.

9.4.2.2 Access Rights to be granted by any leaving Party

Any Party leaving the Project shall continue to grant Access Rights pursuant to this Consortium Agreement as if it had remained a Party for the whole duration of the Project.

9.5 Compliance with State Aid Rules

The grant of any assignment of, or licence to, Foreground pursuant to Section 8 and/or Section 9 is subject to compliance with EU State Aid Rules and the Parties shall use all reasonable endeavours to ensure that the terms of any such assignment or licence do not give rise to unlawful state aid. If it is necessary to amend any of the provisions of this Agreement to ensure compliance with State Aid law Rules then the Parties (or the relevant Parties, as the case may be) shall amend the arrangements accordingly.

9.6 Specific Provisions for Access Rights to Software

For the avoidance of doubt, the general provisions for Access Rights provided for in this Section 9 are applicable also to Software.

Parties' Access Rights to Software do not include any right to receive source code or object code ported to a certain hardware platform or any right to receive respective Software documentation in any particular form or detail, but only as available from the Party granting the Access Rights.

Section 10: Non-disclosure of information

10.1 All information in whatever form or mode of transmission, which is disclosed by a Party (the "Disclosing Party") to any other Party (the "Recipient") in connection with the Project during its implementation and which has been explicitly marked as "confidential", or when disclosed orally, has been identified as confidential at the time of disclosure and has been confirmed and designated in writing within 15 days from oral disclosure at the latest as confidential information by the Disclosing Party, is "Confidential Information".

10.2 The Recipients hereby undertake in addition for a period of 5 years after the end of the Project:

- not to use Confidential Information otherwise than for the purpose for which it was disclosed;
- not to disclose Confidential Information to any third party without the prior written consent by the Disclosing Party;
- to ensure that internal distribution of Confidential Information by a Recipient shall take place on a strict need-to-know basis; and
- to return to the Disclosing Party on demand all Confidential Information which has been supplied to or acquired by the Recipients including all copies thereof and to delete all information stored in a machine readable form. If needed for the recording of ongoing obligations, the Recipients may however request to keep a copy for archival purposes only.

10.3 The Recipients shall be responsible for the fulfilment of the above obligations on the part of their employees and shall ensure that their employees remain so obliged, as far as legally possible, during and after the end of the Project and/or after the termination of employment.

10.4 The above shall not apply for disclosure or use of Confidential Information, if and in so far as the Recipient can show that:

- the Confidential Information becomes publicly available by means other than a breach of the Recipient's confidentiality obligations;
- the Disclosing Party subsequently informs the Recipient that the Confidential Information is no longer confidential;
- the Confidential Information is communicated to the Recipient without any obligation of confidence by a third party who is in lawful possession thereof and under no obligation of confidence to the Disclosing Party;
- the Confidential Information, at any time, was developed by the Recipient completely independently of any such disclosure by the Disclosing Party; or
- the Confidential Information was already known to the Recipient prior to disclosure or
- the Recipient is required to disclose the Confidential Information in order to comply with applicable laws or regulations or with a court or administrative order, subject to the provision Art. 10.7 hereunder.

10.5 The Recipient shall apply the same degree of care with regard to the Confidential Information disclosed within the scope of the Project as with its own confidential and/or proprietary information, but in no case less than reasonable care.

10.6 Each Party shall promptly advise the other Party in writing of any unauthorised disclosure, misappropriation or misuse of Confidential Information after it becomes aware of such unauthorised disclosure, misappropriation or misuse.

10.7 If any Party becomes aware that it will be required, or is likely to be required, to disclose Confidential Information in order to comply with applicable laws or regulations or with a court or administrative order, it shall, to the extent it is lawfully able to do so, prior to any such disclosure

- notify the Disclosing Party, and
- comply with the Disclosing Party's reasonable instructions to protect the confidentiality of the information.

- 10.8** The confidentiality obligations under this Consortium Agreement shall not prevent the communication of Confidential Information to the National Funding Agencies.
- 10.9.** The Parties undertake to cooperate to allow the timely submission, examination, publication and defense of any dissertation or thesis for a degree that includes their Results or Background subject to the confidentiality and publication provisions agreed in this Consortium Agreement.

Section 11: Miscellaneous

11.1 Attachments, inconsistencies and severability

This Consortium Agreement consists of this core text and

Attachment 1: Grant proposal

In case the terms of this Consortium Agreement are in conflict with the terms of the Rules or the National Funding Guidelines, the terms of the latter shall prevail. In case of conflicts between the attachments and the core text of this Consortium Agreement, the latter shall prevail.

Should any provision of this Consortium Agreement become invalid, illegal or unenforceable, it shall not affect the validity of the remaining provisions of this Consortium Agreement. In such a case, the Parties concerned shall be entitled to request that a valid and practicable provision be negotiated which fulfils the purpose of the original provision.

11.2 No representation, partnership or agency

The Parties shall not be entitled to act or to make legally binding declarations on behalf of any other Party. Nothing in this Consortium Agreement shall be deemed to constitute a joint venture, agency, partnership, interest grouping or any other kind of formal business grouping or entity between the Parties.

11.3 Notices and other communication

Any notice to be given under this Consortium Agreement shall be in writing to the addresses and recipients as listed in the most current address list kept by the Coordinator.

Formal notices:

If it is required in this Consortium Agreement that a formal notice, consent or approval shall be given, such notice shall be signed by an authorised representative of a Party and shall either be served personally or sent by mail with recorded delivery or email with receipt acknowledgement.

Other communication:

Other communication between the Parties may also be effected by other means such as e-mail with acknowledgement of receipt, which fulfils the conditions of written form.

Any change of persons or contact details shall be notified immediately by the respective Party to the Coordinator. The address list shall be accessible to all concerned.

11.4 Assignment and amendments

No rights or obligations of the Parties arising from this Consortium Agreement may be assigned or transferred, in whole or in part, to any third party without the other Parties' prior formal approval.

Amendments and modifications to the text of this Consortium Agreement require a separate agreement between all Parties.

11.5 Mandatory national law

Nothing in this Consortium Agreement shall be deemed to require a Party to breach any mandatory statutory law under which the Party is operating.

11.6 Language

This Consortium Agreement is drawn up in English, which language shall govern all documents, notices, meetings, arbitral proceedings and processes relative thereto.

11.7 Applicable law

This Consortium Agreement shall be construed in accordance with and governed by the laws of Belgium excluding its conflict of law provisions.

11.8 Settlement of disputes

The Parties shall endeavour to settle their disputes amicably.

Any dispute, controversy or claim arising under, out of or relating to this contract and any subsequent amendments of this contract, including, without limitation, its formation, validity, binding effect, interpretation, performance, breach or termination, as well as non-contractual claims, shall be submitted to mediation in accordance with the WIPO Mediation Rules. The place of mediation shall be Brussels unless otherwise agreed upon. The language to be used in the mediation shall be English unless otherwise agreed upon.

If, and to the extent that, any such dispute, controversy or claim has not been settled pursuant to the mediation within 60 calendar days of the commencement of the mediation, it shall, upon the filing of a Request for Arbitration by either Party, be referred to and finally determined by arbitration in accordance with the WIPO Expedited Arbitration Rules. Alternatively, if, before the expiration of the said period of 60 calendar days, either Party fails to participate or to continue to participate in the mediation, the dispute, controversy or claim shall, upon the filing of a Request for Arbitration by the other Party, be referred to and finally determined by arbitration in accordance with the WIPO Expedited Arbitration Rules. The place of arbitration shall be Brussels unless otherwise agreed upon. The language to be used in the arbitral proceedings shall be English unless otherwise agreed upon.

The award of the arbitration will be final and binding upon the Parties.

Nothing in this Consortium Agreement shall limit the Parties' right to seek injunctive relief in any applicable competent court.

Section 12: Signatures

AS WITNESS:

The Parties have caused this Consortium Agreement to be duly signed by the undersigned authorised representatives in separate signature pages the day and year first above written.

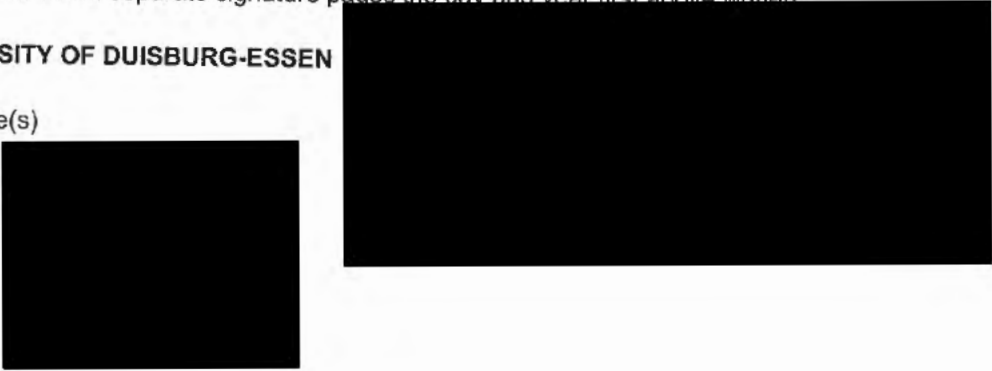
UNIVERSITY OF DUISBURG-ESSEN

Signature(s)

Name(s)

Title(s)

Date



FINNISH ENVIRONMENT INSTITUTE

Signature(s)



Title(s) Development manager

Director of Administration

Date 21.02.2024

23.02.2024

INSTITUT NATIONAL DE LA RECHERCHE POUR L'AGRICULTURE L'ALIMENTATION ET
L'ENVIRONNEMENT

Signature(s)

Name(s)

Title(s)

Date

UNIVERSITY OF LODZ

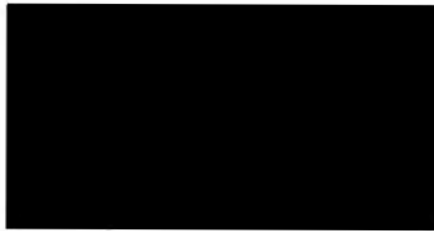
Signature(s)

Name(s)

Title(s)

Date

1/03/2024 ✓



UNIVERSITY COLLEGE DUBLIN

Signature(s)

Name(s)

Title(s)

Date



UNIVERSITY OF JYVÄSKYLÄ

Signature(s)

Name(s)

Title(s)

Date



ASSOCIACAO BIOPOLIS

Signature(s)

Name(s)

Title(s)

Date



MASARYK UNIVERSITY

Signature(s)

Name(s)

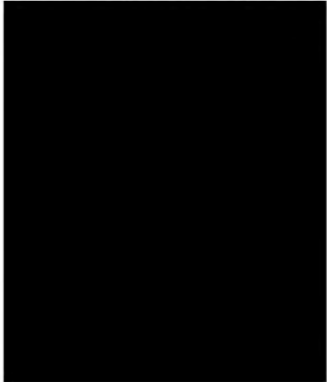
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FH KAERNTEN - GEMEINNUETZIGE GmbH

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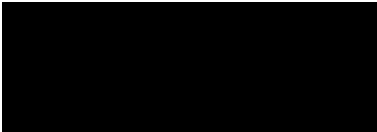
Name(s)

Title(s)

Date

AARHUS UNIVERSITY

Signatur



Name(s)

Title(s) Professor, Head of Department

Date:



UNIVERSITY OF NATURAL RE

Vienna

Signature

Name:

Title(s):

Date: 16/02/2024

**BOTANIC GARDEN AND BOTANICAL
MUSEUM, Freie Universität Berlin**

Signature

Name(s)

Title(s) Dr.

Date 28.02.2024

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

Signature(s)

Name(s)

Title(s)

Date



Attachment 1: Proposal

DNAqualMG: Innovating transnational aquatic biodiversity monitoring using high-throughput DNA tools and automated image recognition

(Proposal submitted to the 'BiodivMon' call: Biodiversa2022-738)

A. Detailed description of the research area and research plan and approach to stakeholder engagement and expected societal and/or policy impact

1. Main research questions and explanation of the planned research novelty

Comprehensive and reliable data are essential to understand biodiversity status and drivers, predict trends, and guide management and restoration in the context of European and international regulations, such as the European Green Deal (EGD), the Nature Restoration Law (NRL), Biodiversity Strategy and the Post-2020 Global Biodiversity Framework¹. The framework aims at achieving the UN Convention on Biological Diversity (CBD) vision of “living in harmony with nature by 2050”. These action plans are central in the UN Decade on Ecosystem Restoration and aim to put Europe’s biodiversity on a path of recovery by 2030. Maintaining species diversity and ecosystem functioning while considering also the intraspecific genetic diversity of organisms is recognized as crucial in biodiversity conservation² and emphasized in the main goals of the Post-2020 Global Biodiversity Framework. All these international directives or strategies depend on reliable monitoring data. However, many monitoring approaches implemented so far are insufficient in terms of spatial and temporal resolution to accurately infer biodiversity trends³. Therefore, harmonized monitoring schemes and networks are needed to produce the data required for effective transnational biodiversity monitoring. The by far most extensive biodiversity assessment programme worldwide is implemented under the European Water Framework Directive (2000/60/EG, WFD); here, freshwater data for ecological quality monitoring are generated through a network of institutions (environmental agencies, research institutes, consultants) in all 27 member states⁴. Benthic macroinvertebrates (BMI) and benthic diatoms (DIA) are among the organism groups (Biological Quality Elements; BQEs) to be monitored for assessing the ecological quality. Although these biomonitoring data are intercalibrated at the level of status classes and thus comparable across countries⁵, they are not interoperable in terms of taxonomy as they differ in spatial, temporal and, taxonomic ‘school’ used for identification, and most importantly, taxonomic resolution. Status classes and associated ecological quality ratios (EQRs) – as reported to the EU – aggregate original taxonomic information and are thus unsuitable for systematic biodiversity monitoring. In other words, **the WFD monitoring data that are compiled with tremendous effort do not live up to their full potential**. Currently WFD biomonitoring data are only used to assess surface water status, but they could be used for many other purposes, e.g. biodiversity monitoring and be integrated to serve multiple EU legislatives and global biodiversity strategies. Currently, new biodiversity assessment tools are emerging that boost information content – in particular taxonomic resolution – and are able to support upscaling of spatial-temporal coverage by automating individual steps in biodiversity sampling and assessment workflows^{6,7}. **For aquatic bioassessment and monitoring, two approaches hold particular strengths: molecular^{8,9} and automated image-based methods^{10,11}.**

Molecular biodiversity assessment: Molecular methods have some obvious advantages for biodiversity assessments over traditional methods of taxa identification. In particular, the identification relies on DNA molecules. Thus, the process of identification is successful even if only parts of organisms are available or if the collected life stage has characters that do not allow for morphological identification. The sequence of a characteristic fragment (“DNA barcode” marker) is compared against sequences obtained from taxonomically determined specimens as references. Through this reference database comparison, the taxonomic name of the species, or at least of the genus, is retrieved. Moreover, even undescribed or morphologically nondistinctive species can be detected and formally assigned based on their unique genetic sequences¹². This is important as many organismal groups consist of so-called “dark taxa”, i.e. species-rich groups of great ecological importance, like many oligochaete worms, small insects like chironomids and several microalgae, for which many species are not described yet or identification with a morphological traits is extremely challenging^{13,14}. Another key aspect is that sample processing can also be faster, cheaper and automated using environmental specimen samples or environmental DNA (eDNA) samples collected from environments¹⁵. Furthermore, samples, DNA extracts and sequences can be made available through biobanks or data repositories, thereby contributing to FAIR principles, open science and open data. The key aspect of DNA barcoding, however, is assigning sequences to species names. This identification lives up to its full potential if reference databases are complete. For Europe, the completeness of reference databases such as the Barcode of Life Datasystems¹⁶ differs among taxonomic groups, but for freshwater invertebrates and fish species data availability is high (~60% and >95%)¹⁷. EU-funded initiatives are working towards the completion of reference databases, in particular through the Bioscan-Europe initiative. With complete

reference sequence (“barcode”) databases, identification to species level using DNA-sequences is more precise and objective than with traditional identification methods.

Different molecular biodiversity assessment methods exist. DNA metabarcoding is the most popular approach to determine species composition based on specimen or biofilm samples, or environmental DNA (eDNA) sampled from water or sediment. The approach has reached a level of scientific and technical maturity that already provides a backbone for breakthrough advances towards a more holistic understanding of biodiversity changes^{18,19}. Successful automation of the molecular analysis workflow has already been demonstrated in a number of case studies^{15,20}. The increasing availability of platforms for storing, accessing and analysing data opens new opportunities for biodiversity monitoring^{16,21,22}.

Like other methods, DNA-based assessments also come with restrictions, in particular the lack of abundance/biomass quantification²³. Thus, where quantitative data are needed, DNA metabarcoding must be complemented efficiently to unlock the full potential for biodiversity monitoring. While several challenges remain^{9,24,25}, technology readiness levels of DNA metabarcoding are quite advanced and implementation has already started in national and international monitoring standards (e.g. CEN/TC230/WG28). The most important gap hindering implementation into regulatory monitoring frameworks is the lack of transnationally standardized approaches, quality assurance and quality control (QA/QC). Through an exponential increase in studies world-wide there is a continuous diversification of the approaches used for metabarcoding²⁶. To compile the existing evidence and derive best-practice recommendations as part of formal standards thus is of critical importance for biodiversity monitoring⁹.

Image-based biodiversity bioassessment: Although image-based taxa recognition methods are conceptually close to “traditional” manual taxa recognition, their development lag behind that of molecular approaches. Recent advances in deep learning are now making image-based biomonitoring realistic. Nevertheless, the technological and algorithmic components for high throughput, partially automated image based taxonomic assessment are still only available for certain organism groups, such as selected invertebrate taxa and mounted diatoms. Progress has been made both for aquatic macroinvertebrates and for diatoms, reaching the technology readiness level allowing for broader application. A key benefit of image-based approaches are their low running costs and rapid data acquisition. Accumulating evidence demonstrates that imaging-based methods can complement molecular approaches, particularly by providing reliable estimates of taxon-specific sizes, size structure and biomass and for diatoms biovolumes^{27,28}. Also, image data hold a particular added value for transnational biodiversity monitoring: image data do not underlie restrictions imposed by the Nagoya protocol or CITES regulations, and can be internationally transferred. As some monitoring agencies store and archive samples taken for monitoring purposes, these are potentially reusable for follow-up analyses. Thus, samples already routinely collected for WFD purposes could not only be used/re-used in fundamental biodiversity and ecological research, but also for biodiversity monitoring on a wide geographic scale. The key challenges to such implementation are mostly technical, like automation of the processes of imaging, counting, and sorting to speed up the analyses of macroinvertebrate samples²⁹, but also conceptual, like dealing with high species richness, e.g. for diatoms, and infrastructure-related, like issues with data management.

Through the integration of two innovative monitoring technologies, DNAqualMG will explore novel options and propose concepts for transnational biodiversity monitoring. The focus will be on 150 freshwater samples of benthic macroinvertebrates and diatoms routinely collected as part of the European transnational water quality monitoring as part of the WFD. Through the objectives proposed, **DNAqualMG** will advance the current state of biodiversity monitoring by proposing automation, integration of workflow and transnational harmonisation of approaches. Scientifically, **DNAqualMG** will link biomonitoring (ecological status classes) and biodiversity monitoring data using high-resolution taxonomic information and quantitative (image-based) data to assess how status classes reflect the biodiversity state holistically.

2. Scientific objectives, main research questions in relation to the theme call

The scientific objective of **DNAqualMG** is to explore the individual and combined potential of molecular and image-based approaches to advance biodiversity monitoring. A focus will be on European rivers under different stressor impacts (agricultural/urban land-use vs. natural land-cover) as well as restoration after stressor release (reservoir/dam removal). The three research questions (**RQ1-3**) addressed are:

- **RQ1:** What changes in macroinvertebrate and diatom biodiversity, i.e. losses and gains other than the WFD indicator taxa, occur with change in WFD ecological status?
- **RQ2:** Do restorations that raise the WFD status class to good or high also represent an equivalent recovery of lost biodiversity?
- **RQ3:** Can molecular and image-based methods be used to support biodiversity monitoring and help identify novel biodiversity indicators?

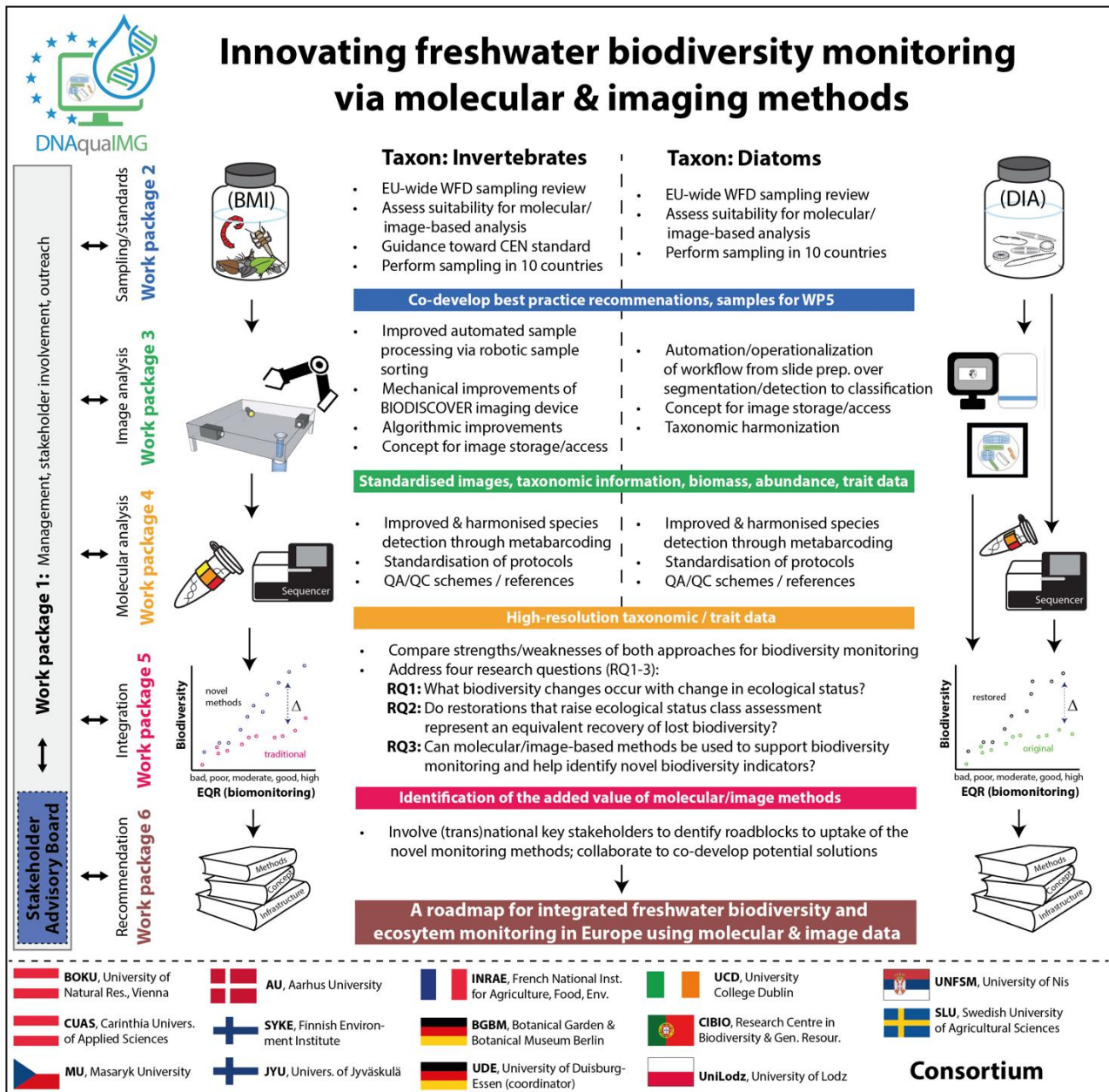


Fig. 1: Overview of DNAqualMG's tasks and deliverables related to the six work packages (WPs). Stakeholder engagement is key to all WPs and will be coordinated primarily through WP1.

DNAqualMG aligns well with the Biodiversa+ BiodivMon call as it integrates novel methods and concepts, i.e. molecular and image-based taxa recognition, into existing European freshwater biomonitoring (WFD). This generates high-resolution, quantitative and comparable biodiversity data. The project addresses primarily **Theme 1** of the Biodiversa call (*Innovation & harmonisation of methods/tools for collection & management of biodiversity monitoring data, 50%*), on aspects of methodological advancements, harmonisation and international standardisation. Through ten transnational use cases, DNAqualMG explicitly addresses **Theme 2** (*Addressing knowledge gaps on biodiversity status, dynamics, and trends to reverse biodiversity loss, 30%*) by correlating biomonitoring (i.e. ecological status class change) with high-resolution biodiversity data for two aquatic key groups. Finally, **Theme 3** (*Making use of available biodiversity monitoring data, 20%*) is addressed through the link to existing transnational infrastructures, i.e. through a compilation of storing processes of WFD samples and suggestions for routines to enable their broad usage, and re-use of collected samples or data for WFD assessment. Here, DNAqualMG will consult, involve and collaborate with regional, national, European and global stakeholders to make sure the developments find entry into legislative bio(diversity) monitoring. Beyond that, solutions proposed go beyond the European WFD biomonitoring context (Biodiversity Strategy, NRL, Marine Strategy Framework Directive, MSFD) and can be adapted to invertebrate and algal samples from other ecosystems. Eventually, DNAqualMG will pave the way for a much more extensive use of monitoring data that are collected for the WFD and will be made suitable for biodiversity monitoring.

3. Detailed description of the approach and methodology

To address the method innovation objective of the call and the three research questions (RQ1-3), **DNAqualMG** will organize its work in six interconnected work packages (**WPs**, Fig. 1). **WP1** coordinates the project, oversees the progress, communication, data management as well as the identification and involvement of stakeholders. **WP2** deals with the practical questions regarding sample collection in the context of WFD to co-develop standard procedures for sampling and storing with stakeholders. It is also in charge of delivering 150 quality samples from the 10 countries for image-based (**WP3**) and molecular (**WP4**) analysis. **WP3** will develop key technological breakthrough developments from the engineering perspective (sample sorting through computer vision and robotic arm) and artificial intelligence (classification of taxa) to upscale biodiversity monitoring from benthic macroinvertebrate samples (BMI) as well as diatoms (DIA), for which technological readiness levels are still at or just below demonstrator level. **WP4** will bring the existing advanced metabarcoding methods into formal standardisation using established QA/QC frameworks of CEN and ISO. **WP5** will address the key research questions on how WFD samples extend our understanding of biodiversity responses to stressor increase (ecological gradients) and release (restoration). It will identify the key added value of the DNA and image-based methods for biodiversity monitoring individually and in combination. Finally, in **WP6** the **DNAqualMG** consortium together with international stakeholders will co-develop a roadmap for the novel methods' uptake as part of WFD and other key directives for improved and harmonized biodiversity monitoring in Europe to support better decision-making and more effective measures.

Work package 1 Management, stakeholder involvement, outreach (Lead: UDE Leese, AU)

Objectives: WP1 is led by UDE Partner 1 (Leese) and AU (deputy lead) who will coordinate the project, overlook tasks, milestones and deliverable achievements together with all WP leads. UDE is in charge of organising the kick-off, mid-term and final project meetings as well as coordinating the project outreach and dissemination. Stakeholders at all levels will be identified and involved as a common exercise. Stakeholders will also actively engage with **DNAqualMG** through their involvement in the Stakeholder Advisory Board. Detailed description of **WP1 tasks**, see Section C.

Work package 2 Sampling, standardisation (Lead: BOKU, SLU)

Objectives: Review the present fit of existing WFD biomonitoring protocols for the application of molecular/image-based taxa identification methods and propose standardized advancements to fill gaps with respect to integrated biodiversity monitoring requirements of key legislation and international agreements (WFD, NRL and GBF/CBD). WP2 will determine relevant implementation steps to fill the identified gaps through the co-creative process developed in WP1 with key European, and national stakeholders. Focus will be on identifying and prioritising key procedural steps that require unified and internationally standardized methodological approaches for BMI and DIA and that can be advanced through their introduction as work items into the dedicated standardisation working groups of CEN/TC230 or ISO/TC 147. The identified optimal procedures will feed into WPs 3 and 4 (image and molecular processing) as well as into WP6, which will consider their implementation by countries in accordance with national monitoring procedures and capabilities.

Key stakeholders of WP2: National and regional environmental agencies in charge of implementing the WFD. Interaction: Consult & interact to allow for DNA/image analysis-conform sampling on the ground.

Tasks incl. milestones and deliverables

Task 2.1 (BOKU, BGBM, SLU, SYKE): Assess suitability of currently applied sample preservation and potential for re-use in transnational biodiversity monitoring and broader scale scientific investigations by imaging and DNA-methods. To do so, T2.1 will develop a stakeholder questionnaire to compile national procedures to sample, preserve and store BMI and DIA samples in European countries.

- **Milestones:** M2.1a. Questionnaire on sampling and storage procedures produced, translated and sent to stakeholders (M2). M2.1b. Overview table of methods and rating of DNA/image-based analysis produced (M6). M2.3c. National stakeholders consulted on feasibility of harmonized protocols (M9).

Task 2.2 (BOKU, MU, INRAE, all partners): To test the DNA/image-based approaches in an explicitly transnational framework, we will set up a sampling campaign obtaining 15 samples per partner country. Sampling strategy will focus on dominant stream types per country and provide samples across a broad ecological status class gradient (3-4 samples per ecological status class, key focus on status classes 2 and 3). Sampling will be coordinated by BOKU using an initial standardized protocol and performed by partners in collaboration with the relevant national stakeholders in charge of the WFD assessment. In some cases, we will use existing material (PT, SWE). Data will be used to address RQ1 and RQ3.

- **Milestones:** M2.2a. Samples from all countries taken (M5). M2.2b. BMI/DIA identified to the taxonomic level requested by country/WFD (M14).
- **Deliverable:** D2.2. Preserved and identified BMI from 10 partner countries for DNA and image-based analysis. DIA preserved for molecular and image-based analysis (M14).

Task 2.3 (UniLodz, BOKU, INRAE SYKE, BGBM, SLU): Based on the questionnaire (T2.1) and sampling experiences (T2.2) we will identify and propose methodological process steps necessary to implement DNA-based analysis into current national WFD macroinvertebrate sampling pipelines, and prepare precursors of European standards by introducing new working item proposals into the workflow of European standardisation group for water quality CEN/TC230/WG28 or its international standardisation technical committee ISO/TC147.

- **Milestones:** M2.3a. Feedback collected from stakeholders through online-workshop (M18) to: M2.3b. identify additional challenges and roadblocks from the sampling conducted in year 2 (outside of DNAqualMG) (M20).
- **Deliverable:** D2.3a. Report/paper on specific roadmap steps needed to sample and store WFD samples (BMI, DIA) to allow for downstream image/DNA analysis in accordance with stakeholders (M22). D2.3b. Adapted precursor for BMI/DIA preservation and storing for CEN TC230 (diatom and macroinvertebrate working groups as well as WG28 on DNA) or ISO/TC147 (M22).

Work package 3 Image-based biodiversity assessments (Lead: AU, UDE Beszteri)

Objectives: Advance image-based biodiversity assessments to improve routine applicability for *i.* complete benthic macroinvertebrate bulk samples (BMI) based on the BIODISCOVER imaging device²⁷, as well as using mounting-methods for species-level identification of the ecologically highly relevant and diverse yet often neglected group of non-biting midges (Chironomidae)³⁰, and *ii.* diatom light microscopic slides (DIA)¹¹; test compatibility with samples from WFD monitoring. WP3 will substantially extend availability of taxonomically annotated, high quality image data for future applications of image-based biodiversity monitoring of benthic macroinvertebrates and diatoms.

Key stakeholders of WP3: DIA: collaborating with local-regional water authorities (practical interlinking to routine monitoring workflow); EuropaBON and follow-up EU organisations under the Knowledge Centre for Biodiversity for high level coordination with other biodiversity monitoring activities.

Tasks incl. milestones and deliverables

Task 3.1 (BMI; AU): Automatize invertebrate specimen sorting. To automatize and scale-up BMI bulk sample processing T3.1 consists of three subtasks: *i)* Spreading of specimens: different types of mechanical motion, spreading as well as bin picking methods will be tested to derive best practice recommendations. *ii)* Distinguishing specimens from debris: to effectively sample only specimens and not debris, computer vision will be used to identify specimens for picking. Specifically, we will use a hyperspectral camera or a simpler 2D classification algorithm on the bin picking tray to only transfer the specimens. *iii)* Develop a robotic arm for automated sorting: We will develop robotic end effectors for successful pickup of different sizes and shapes of specimens using automated tweezers and suction systems. This system is an add-on removing the need for manual feeding of BIODISCOVER.

- **Milestone:** M3.1a. Prototypes of robot arm and specimen sorting device developed and tested in the full system test on BMI samples from 10 partner countries (5 samples with 50 BMI as demonstrator, these will be provided directly through T2.2 via subsampling) (M12).
- **Deliverable:** D3.1. Publication of design and technical specifications for a BMI specimen sorting device (M32).

Task 3.2 (BMI; AU, JYU, UDE Leese): Enhance the prototype of the BIODISCOVER scanning system to accommodate larger specimens and enable fast, high resolution, multi-angle, automatic scanning of specimens over a broad size range; develop a calibration method to ensure interoperability among copies of the system (available at JYU and UDE). The latter is key for upscaling the approach at transnational level.

- **Milestones:** M3.2a. Broad size range specimen scanner developed and tested in the full system test on BMI bulk samples from each partner country (M18), also mentioned in Task 3.1. M3.2b. Calibration method developed (M24).
- **Deliverable:** D3.2. Publication of design and technical specifications for a BMI specimen scanner and calibration method (M26).

Task 3.3 (BMI; AU, JYU): Develop and implement a mechanism for collection and labelling of the classified specimen in the form of a rack movable in X and Y plane with multiple 50+ containers for sorting specimens into a large number of species groups. A solution for removing excess ethanol from moving specimens into containers from BIODISCOVER will be developed. A laser edging system for the tubes in the rack will be

applied to create physical barcodes for each sample. This system is an add-on for BIODISCOVER for classification-dependent collection instead of bulk storage.

- **Milestone:** M3.3. Automated specimen collection and labelling device developed and tested in the full system test on BMI bulk samples from each partner country (M28), also mentioned in Task 3.1.
- **Deliverable:** D3.3. Publication of design and technical specifications for a BMI specimen collection and labelling device (M32).

Task 3.4 (BMI; JYU, UNFSM, MU, UDE Leese, AU): Optimize deep-learning models for species identification and biomass estimation from high-resolution images taken by the BIODISCOVER device. Incremental and transfer learning techniques will be applied. This work will focus on labelled image datasets collected from four countries (DE, AT, FI, CZ) and on a smaller set of dried and weighed samples. The transferability of the trained models from one country to another will be evaluated and further advanced. For biomass estimation, simple regression models will be used as baseline and compared to more advanced deep learning models.

- **Milestones:** M3.4a. Classification software prepared (M6), M3.4b. Biomass estimation software prepared (M12).
- **Deliverables:** D3.4a. Image analysis software including trained classification and biomass estimation model published (M18), D3.4b. Publication covering the applied machine learning techniques (M30).

Task 3.5 (BMI; MU, UNFSM). Estimation of macroinvertebrate biomass/size categories based on the known relationship with morphological parameters. Compilation of macroinvertebrate images recognized as morphotaxa. Image area of specimen body will be linked to size category of defined morphotaxa (e.g. Baetidae). Biomass variability within size category of morphotaxa will be evaluated based on information

- **Milestones:** M3.5a. Phenotypic trait data compiled for BMI for selected countries (CZ, DE, AT, FIN) (M30). M3.5b. Review of relationships applicable for biomass/size categorisation of specimens (M18).
- **Deliverable:** D3.5. Morphotaxa catalogue (M30).

Task 3.6 (DIA; UDE Beszteri, INRAE, SLU, UniLodz): Perform transnational web-based taxonomic intercalibration^{31,32} on diatom virtual slides to assess inter-observer variability in taxon identification at the level of individual microscopic specimen and for generating an agreed upon taxonomic “gold standard” for automatic identification (ca. 3-500 taxa). Test and address any obstacles related to sample preservation or density relevant for routine monitoring in discussion with local-regional monitoring stakeholders.

- **Milestone:** M3.6. Intercalibration with experts from 5 different countries performed and analysed (M12).
- **Deliverable:** D3.6. Annotated and curated training data set for Task 3.7. Report on suitability of WFD samples/slides for slide scanning microscopy (M12).

Task 3.7 (DIA; UDE Beszteri): Apply pre-trained segmentation model³³ and train classification model¹¹ on “gold standard” image set, extended by already available training data from slide scanning microscopy images of diatoms collected from two German stream systems.

- **Milestones:** M3.7a. Segmentation and classification models with data from Task 3.6 trained (M24). M3.7b. Application of models on samples obtained from WFD stakeholders / project partners (M30).
- **Deliverable:** D3.7. Best practice recommendations for a digital microscopy-based workflow for diatom biodiversity monitoring (M30).

Task 3.8 (DIA, BMI; AU, UDE Beszteri, JYU): Archive and publish obtained collections of taxonomically annotated high-resolution specimen images for future re-use, contribute to the development of metadata requirements for BIODISCOVER datasets as well as diatom slide-scans along with guidelines achieving the minimum image quality and metadata required for being accepted into the main database (WP6).

- **Deliverable:** D3.8. Final collection of images (intercalibration “gold standard” from T3.6 and evaluation set from T3.6) publicly available (M36).

Work package 4 Molecular biodiversity assessments (Lead: CUAS, INRAE, UniLodz)

Objectives: Innovate and harmonize transnational biodiversity monitoring of freshwater invertebrates and diatoms using DNA metabarcoding workflows. Identify suitable infrastructures at national and transnational level, test workflows and suggest a validation scheme. Link efforts with international initiatives (e.g. BGE/BIOSCAN-Europe, BiCIKL, EcoAlpsWaters, DNAqua-Net, eDNAquaPlan).

Key stakeholders of WP4: Joint Research Centre(s), Knowledge Center for Biodiversity, ECOSTAT, SMEs/Service providers of DNA-based analyses.

Tasks incl. milestones and deliverables

Task 4.1 (BIOPOLIS, INRAE, UniLodz, CUAS, UDE): Evaluate marker and primer sets used for well-resolved species determination. Identify markers and primer pairs for metabarcoding standardisation of each target group based on bibliographical data, ongoing studies by consortium members, and *in silico* testing. Supplement barcode database with barcodes of missing indicator taxa (i.e. chironomids) for the given

markers. Propose a standard approach for implementation of these primers in Europe-wide metabarcoding efforts for diatoms and macroinvertebrates.

- **Milestone:** M4.1. Ranking of existing markers and primer pairs for DNA metabarcoding of European DIA and BMI in a comprehensive table (M9).
- **Deliverable:** D4.1. Open-access publication of recommended markers and primer pairs ('white list') for diatom and macroinvertebrate metabarcoding (M12).

Task 4.2 (CUAS, INRAE, SYKE, UDE, SLU): Prepare a guideline for (trans)national and regional infrastructure for effective DNA metabarcoding with established semi-automated sample processing and analyses using sufficient quality controls that can be suggested as a new work item proposal to CEN TC230 or ISO TC147. Include protocols with minimum requirements for key analysis steps, comprising DNA extraction, PCR, sequencing, bioinformatics. The guideline will be based on a literature review and inputs from experts and outputs of ongoing European and national projects contributing to DNA metabarcoding standardisation. Consult stakeholders relevant for DNA-based data acquisition and analysis. Publish guidelines and make it available to stakeholders (mostly small and medium enterprises [SMEs] involved) and the scientific community.

- **Milestones:** M4.2a. Draft guideline for DIA and BMI metabarcoding using WFD samples that can be distributed to key stakeholders for their feedback (M15). M4.2b. Collect responses of key stakeholders (M18).
- **Deliverable:** D4.2. Guideline for DIA and BMI metabarcoding using WFD samples (M24).

Task 4.3 (INRAE, UniLodz, SYKE, CUAS, UDE, BGBM): Develop and perform a lab ring-test with the transnational consortium to identify key aspects of infrastructure and analysis components that impact accuracy and species detection sensitivity^{34,35,21,22}. Two separate tests (1 for DIA, 1 for BMI) will be carried out by consortium labs and external labs. The test will benefit from experiences gathered by the consortium partners on earlier intercomparison exercises for diatoms and macroinvertebrates between European laboratories (DNAqua-Net initiative). Test will identify procedural gaps and proposed feed into guidelines developed in Tasks 4.1 and 4.2.

- **Milestone:** M4.3. Launch of 2 proficiency tests (1 for diatoms, 1 for macroinvertebrates) (M15).
- **Deliverable:** D4.3. Completed ring test and identified components for differences in species detection (M30).

Task 4.4 (UDE, INRAE, SYKE, SLU): Propose Quality Control (QC) criteria including reference materials, validation schemes and laboratory accreditations. QC criteria (e.g. equipment performances, material cleanliness, DNA quantity, sequencing technology, sequencing depth, replicates and controls, staff training) will be proposed based on the agreed **protocols** in T4.2 and the first results of T4.3. In addition, QC criteria typology will incorporate existing international standards giving a framework for the competence of testing laboratories (e.g. ISO 17025).

- **Milestones:** M4.4. Outlined QC criteria for metabarcoding analyses of BMI and DIA (M32).
- **Deliverable:** D4.4. Publication of the QC criteria in open-access (M36).

Work package 5 Integration of data streams from biodiversity assessment (Lead: MU, JYU)

Objectives: WP5 assesses the expected added value of the molecular and image-based methods alone and in combination for transnational biodiversity monitoring through use-cases along gradients of ecological impact and restoration for which the transnational consortium already has extensive expertise and access to sites. By this, WP5 addresses the three research questions (RQ1-3) based on the best practices developed and implemented in WP2-4.

Tasks incl. milestones and deliverables

Task 5.1 (MU, BOKU, INRAE, UCD, UDE, UniLodz): Test how comprehensive species diversity data obtained via automated image-recognition and molecular data correlate with the ecological status using the 150 samples provided by the consortium. Quantify loss of species (and gain of tolerant species) per taxonomic group when the ecological status deteriorates. For RQ1 sampling sites will be selected together with stakeholders, sites should span a gradient of ecological quality classes (see T2.2).

- **Milestone:** M5.1. Collected reports from use cases on added value of image-based/molecular data (M30).
- **Deliverable:** D5.1. Report on the added value of the novel monitoring methods (M36).

Task 5.2 (MU, UNFSM, INRAE, UDE Leese & Hering): Testing of novel macroinvertebrate indicators (enhanced by molecular and image-based analyses) for sensitivity to environmental parameters altered by stream degradation (RQ1) and restoration (RQ2). Chironomid life cycles (automated image recognition of instars) and taxa richness (DNA analysis) will be investigated in impounded rivers before and after restoration (alteration of hydropeaking regime CZ, removal of hydromorphological/urban stressors DE). DNA-based taxa

richness of chironomids and automatically identified BMI body size will be tested in indicators of taxonomic and functional diversity and their response to anthropogenically altered patterns of nutrients and organic matter.

- **Milestones:** M5.1a. Manuscript submitted on added value of image-based/molecular data for chironomids (M30). M5.1b. Manuscript submitted on application of novel indicators for assessment of complex effects of agriculture and urbanisation (M34).
- **Deliverable:** D5.2. Report on the practical advantages of the novel monitoring methods (M36).

Task 5.3 (JYU, UDE Leese): Develop and compare image-based machine learning techniques that can enhance the recognition accuracy using the corresponding molecular data (e.g., the list of species recognized via molecular techniques). The work will be done using the already available dataset from Finland and later tested on samples from Germany and Austria that have both modes of data available.

- **Milestones:** M5.3a. Implementation of techniques for using the molecular data for enhancing the image-based classification accuracy (M18). M5.3b. Manuscript submitted on technical image-recognition enhancement using molecular data (M24).
- **Deliverable:** D5.3. Codes & paper on technical image-recognition using molecular data published (M30).

Task 5.4 (UNFSM, SYKE, JYU, UDE, BIOPOLIS): Assess, list and rate the individual benefits of biodiversity data generated from molecular and image-based methods compared to the nationally applied classical WFD assessments. Test reliability of quantitative and functional parameters gained through image-based methods and how the combined analysis workflow alters biodiversity monitoring also in terms of speed and costs (RQ3).

- **Milestone:** M5.4. Manuscript submitted on added value of image/molecular data (M30).
- **Deliverable:** D5.4. Paper on added value of image/molecular data published (M36).

Work package 6 Roadmap of implementation (Lead: UCD, SYKE)

Objectives: WP6 compiles and outlines a stepwise process to implement best-practice recommendations of WPs 2-5 and input from key stakeholders to improve the integrated use of biological samples collected for ecological status assessment under the WFD. The recommendations will delineate pathways to support the uptake of new molecular and image-based taxa recognition tools for integrated biodiversity monitoring of key legislation, like the MSFD, the WFD and NRL. The finalized roadmap will synthesize country-specific suitability analysis, identification of prioritized standardisation needs (WP2), innovative and harmonized assessment approaches (WP3-4), and performed use cases (WP5).

Key stakeholders: national, European and international stakeholders, in particular WFD, ECOSTAT, EEA, EU Knowledge Center for Biodiversity, EuropaBON, IPBES, UNEP, other relevant BioDivMon projects and the Biodiversa+ partnership.

Tasks incl. milestones and deliverables

Task 6.1 (UCD, SYKE, BOKU, all partners). Identify roadblocks and possible solutions to novel method implementation. We will co-develop a questionnaire with key stakeholders (Tab. 1) to assess the procedural roadblocks that the environmental biomonitoring sector needs to overcome to integrate the novel methods into assessment/monitoring programs for biodiversity monitoring. Co-development is chosen to assure that the questions are pertinent to the goals of key stakeholders. This query will be sent out to national stakeholders and responses will be compiled into a report. Following the report, a workshop will be held in each of the consortium's partner countries with key national stakeholders (including key businesses/private consultants) to co-develop solutions to overcome the identified implementation obstacles. All national workshops will follow the same design and reporting format and involve agencies responsible for WFD monitoring, preparation of river basin management plans, conservation of protected species and habitats. The project team will compile the co-developed solutions on the support and mechanisms of integration into a report that will feed into the roadmap to implementation in WP6.2. This will involve consideration of Essential Biodiversity Variables (EBVs) to effectively measure change in response to environmental change or restoration and other interventions across space and time. Important aspects that will be included are standardisation needs of data collection process and storage and publishing approaches as well as an analysis on how to improve and better support reporting and inform policy.

- **Milestones:** M6.1a. Stakeholder questionnaire co-developed (M26). M6.1b-d. National stakeholder Workshop (M27, M28, M29).
- **Deliverables:** D6.1. Summary report on solutions to roadblocks at national level based on national stakeholder workshop reports (M30).

Task 6.2 (SYKE, BOKU, UDE, UCD, AU). Prepare and perform a hybrid international stakeholder workshop to further develop the national solutions to the main gaps identified in T6.1. This workshop will specifically focus on the steps needed to attain integrated implementation with respect to goals of key international

agreements (CBD/GBF) and EU legislation (WFD, NRL). Results will be summarized alongside findings of WPs 2-4 to provide action points, a time schedule and cost estimates in a roadmap on where and how the novel methods can be implemented to address the goals of key international stakeholders/policies. These include ECOSTAT (to initiate formal compliance check), the EU Knowledge Centre for Biodiversity (facilitates implementation of the EU Biodiversity Strategy), members of EuropaBON (identifies user and policy needs for biodiversity monitoring), DG Environment (environmental policy development and implementation), GBIF (international biodiversity network and data infrastructure) the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES, interface between science and policy on biodiversity), UNEP's World Conservation Monitoring Centre, Biodiversity Indicators Partnership (BIP, interface of biodiversity science, policy, and practice) as well as transnational bodies such as ICPER (International Commission for the Protection of the Elbe River), ICPDR (International Commission for the Protection of the Danube River), and ICPO (International Commission for the Protection of the Odra River against Pollution). In terms of monitoring the EBVs will be included in the discussions on the roadmap. Feedback from Task 6.1 will help inform where the roadmap may need to be tailored to the needs of the stakeholders in the various partner countries.

- **Milestones:** M6.2a. International stakeholder workshop (M30). M6.2b. Dissemination of the roadmap at an JRC / ECOSTAT meeting (M36).
- **Deliverables:** D6.2. Publication of the roadmap to implementation for the new biodiversity tools and their integration in existing WFD monitoring including guidance on best practices (M36).

4. Added value of the project (describe how the project will build on previous work)

This project builds to a large extent on conceptual and practical advances that the European COST Action DNAqua-Net (CA15219) has initiated. The aim of DNAqua-Net was to nucleate researchers and practitioners developing DNA-based methods for bioassessment of aquatic ecosystems in Europe and beyond.² It was successful in bringing experts together and even launching a Working Group within CEN on the topic (CEN TC 230/WG 28 “eDNA and DNA methods”). However, to advance routine implementation of molecular methods at the European scale still requires coordinated action at many ends. Three central Horizon Europe projects deal with general method advancements (BGE – Bioscan Europe), metadata standards (BiCIKL) and setup of reference libraries for DNA/eDNA data (eDNAqua-Plan). **DNAqualMG** embraces advances made by these transnational EU projects but focuses on the habitat and the legislative context, for which the application of molecular methods in routine monitoring is most advanced, namely freshwater habitats and the Water Framework Directive). **DNAqualMG** builds strongly on the progress of DNAqua-Net and several national and transnational pilots initiated by DNAqua-Net and consortium members (e.g. Freshbar, GeDNA, dbDNA, EnvMetaGen, HYDROGen, SCANDNAnet, SYnAQUA). It aims to overcome well documented limitations of DNA-based methods with respect to quantification and to provide robust solutions for DNA-metabarcoding to become truly quantitative. The key innovation of **DNAqualMG** is to leverage the strengths (quantitative, speed, cost-effectiveness, retention of virtual copies) that image-based methods have shown to offer by members of the team^{27,29,30} to complement the strengths of DNA metabarcoding (taxonomic resolution, speed, costs). Consortium partner's experiences with the organisation of international proficiency testing, development of national QA/QC and the definition of precursors of minimum criteria for method standardisation as well as formulation of co-produced national strategy documents will be important experiences to build on^{18,35} and to extend standardisation beyond onto the ISO level to maximize project work impact and alignment with international activities.

5. Transnational added value of the project and of collaboration

Transnational biodiversity monitoring schemes and comparable methodology are essential to the successful implementation and impact evaluation of the Global Biodiversity Framework, the European Green Deal and the EU 2030 Biodiversity Strategy. The EC has launched several initiatives, such as the Biodiversa+ partnership and the flagship project EuropaBON, to design a European-wide biodiversity monitoring program to single out effective holistic solutions for drivers such as urbanisation, agriculture and infrastructure that improve large scale resilience to a broad range of perturbations on biodiversity. These initiatives have been mapped and strongly rely on innovative monitoring methods which need to be co-developed, harmonized, tested and implemented across European countries. With this call, Biodiversa+ explicitly addresses this need, and through the transnational **DNAqualMG** consortium we will explicitly address this for the combination of novel DNA and image-based biodiversity monitoring methods. **DNAqualMG** is rooted in and builds on previous and on-going transnational efforts to develop scientific and technical innovations that can be – by inclusion of selected stakeholders – standardized but still respect the needs of countries with different socio-economic and environmental contexts. Our project leverages already ongoing ecological monitoring under the WFD to minimize costs and to maximize transnationally comparable biodiversity data.

Complementarity of the expertise in the consortium: **DNAqualIMG** requires expertise in five fields to reach the impact needed.

1. *Traditional freshwater bioassessment and biodiversity monitoring:* Partners from BOKU, BIOPOLIS, INRAE, MU, SYKE, SLU, UCD, UDE Hering, UniLodz and AU have decade-long experience with freshwater biodiversity assessment as well as proficiency testing, standardisation and intercalibration of methods. They were also responsible for developing approaches for the national bioassessment under the WFD and are involved in related transnational networks.
2. *DNA-based method development:* Partners from BGBM, BIOPOLIS, CUAS, INRAE, SLU, SYKE, UDE, UniLodz have leading expertise in the development of DNA-based methods from BMI and DIA biodiversity assessments using DNA and eDNA metabarcoding. They are members of international networks such as international Barcode of Life, Bioscan Europe, etc.
3. *Algorithms for image-based biodiversity assessments:* Partners from AU, JYU, UDE and UNFSM are leading experts in the development of high-throughput algorithmic aspects (especially deep-learning approaches) required to reliably classify organisms into the respective taxonomic groups and to quantify phenotypic and functional traits such as body-size, volume and shape.
4. *Engineering:* Partners from AU; JYU, SYKE, and UDE Beszteri are developing technical solutions for image-based bioassessment. From AU the current BIODISCOVER machine is produced. UDE developed the slide scanner approach for diatom assessment.
5. *Science-Policy dialogue:* Partners from BOKU, BIOPOLIS, MU, UCD, UDE Leese & Hering, SYKE have long and successful experience in stakeholder engagement and approaches to bridge the science application gap, i.e. guide implementation of novel methods into implementation. With such a knowledgeable and experienced consortium, **DNAqualIMG** will ensure maximal scientific, policy and societal impact and put the EU into a pioneering position in biodiversity monitoring.

6. Approach to stakeholder engagement and expected societal and/or policy impact

Early key transnational stakeholder engagement is vital to agree on common approaches to modify current, or develop new, minimum requirements for DNA and image-based biodiversity monitoring of freshwaters using samples collected within the context of the WFD framework. If no such early agreements are jointly reached, member states will separately develop a range of national approaches that will require painstaking intercalibration such as was the case with results from method approaches for water quality status assessment under the WFD. Due to the sensitivity of both genetic and image-based methods, separately developed national requirements will result in transnationally incompatible data and fail to single out effective holistic solutions that improve large scale resilience to a broad range of perturbations on biodiversity and genetic diversity. Thus, the expected policy impact of **DNAqualIMG** is to provide technical and conceptual pathways to the implementation of two novel biodiversity monitoring methods within the existing WFD policy context. Through wide stakeholder involvement project innovations can be adopted in practice also to service other legislative contexts, irrespective of the current idiosyncrasies of national WFD monitoring programs.

To do this, **DNAqualIMG** makes stakeholder engagement a key task in all WPs. The stakeholder identification (WP1 & 2) will be done together with the proposed members of the Stakeholder Advisory Board in M3 in an online-meeting. Specific tasks of stakeholder engagement are then subject to the different WPs and WP leads will follow specific pathways of engagement. E.g. WP2 will consult relevant actors about their experiences with traditional and novel monitoring approaches and will involve national stakeholders to co-develop technical innovations and solutions for sampling and storing of samples. WP2 will collaborate with them to actually perform sampling (M5) and collect feedback on the practicability of the approaches. WP4 will consult stakeholders that are relevant for DNA-based data acquisition and analysis. Thus, the proposed pre-cursors will be sent out for review and to collect feedback from SMEs/service providers (M18). Especially WP6, where pathways of integration of the novel monitoring methods into existing bioassessment routines are developed, the involvement of transnational key-stakeholders from national environmental agencies, European and global environmental institutions (DG environment, EEA, JRC ECOSTAT, GEOBON, UNEP) is key. WP6 will co-develop a questionnaire to identify the conceptual and practical roadblocks together with them (T6.1). To develop solutions, a workshop will be held to make sure the roadmap of implementation, which is the practical final result of **DNAqualIMG**, is based on co-identified pathways. A list of identified stakeholders who already agreed to engage and collaborate with **DNAqualIMG** is presented in Table 1 (non-exhaustive list due to space restrictions).

Societal impact: The impacts of **DNAqualIMG** for society are manifold. Two key aspects are:

1. *Knowledge on biodiversity loss and change:* **DNAqualIMG** will generate a mechanism to generate biodiversity data in unprecedented depths through high taxonomic resolution and holistic assessment (DNA) and quantitative features (Imaging). This is essential to better understand where and how biodiversity changes and to devise effective management actions for sustainable development and the

UN goal of living in harmony with nature.

2. **Effective and efficient use of resources:** DNAqualMG proposes an efficient and effective approach to monitor biodiversity. Specifically, it uses synergies with a broad-scale and fully-implemented environmental assessment directive (WFD) in order to substantially extend this into a fully-operational freshwater biodiversity monitoring framework using novel monitoring technologies. Furthermore, it will connect existing national, European and international infrastructures with respect to data generation, storage, analysis and decision making to be cost-effective.

Tab. 1: Selection of key stakeholders who will collaborate with DNAqualMG including their contact details. Further key stakeholders will be identified (WP1) and involved (WP2-6) in the project.

| Stakeholder group / level | Contacts (selection) | Directive / Engagement | Outcome / benefits for stakeholders |
|---|----------------------|---|---|
| <u>Regional:</u> Environmental protection agency monitoring expert consultants in charge of conducting WFD biomonitoring | | WFD sample collection (sampling/preservation): consult, involve, collaborate | Feasible field protocols for adequate sampling |
| <u>National:</u> Environmental agencies and Ministries responsible for reporting EU legislation | | WFD, Biodiversity Strategy (national and EU): involve, collaborate | Synergies on national strategies to collect and produce biodiversity data from WFD samples; better compliance with legislation goals; integrated, clear and harmonized data reporting |
| <u>European:</u> Central EC infrastructure as well as EU networks and projects developing biodiversity monitoring | | EU Biodiversity Strategy, Green Deal, Post2020 GBF: inform, collaborate | Better compliance with legislation goals |
| <u>International:</u> Global biodiversity networks, DNA barcoding initiatives overseas EPAs | | GBF, Post2020: involve, collaborate | Reliable, harmonised biodiversity data (according to the FAIR principles) |

7. References

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B. Communication and outreach plan

Target audience of DNAqualMG: i) National and international environmental agencies, national ministries, environmental policy and decision makers, government departments, and national and international environmental agencies and organisations (e.g. DG Environment, KCBD, EEA, ECOSTAT, JCR, HELCOM, IPBES, international Env. Agencies, Conservation Agencies); ii) Environmental and planning professionals in industry, local governments and administrations, water utilities, agriculture, biodiversity conservation, recreation and tourism bodies; iii) Academic researchers.

Outputs of DNAqualMG and relevance: DNAqualMG will deliver novel monitoring methods to be implemented as part of regulatory WFD assessment to be scaled up to harmonised European biodiversity monitoring with novel methods. It will provide practical and scalable solutions for sampling, storing and analysing through DNA and automated image-recognition methods. These methods are key to derive biodiversity trends for different groups and make informed environmental decisions possible. Outputs will in particular be i) stakeholder reports, ii) scientific publications, iii) software or analysis scripts, iv) guidance documents (precursors to international standards) as well as a variety of easily understandable information pieces (e.g. blog features, podcasts, flyers, social media contributions) to involve the general public.

How and when will communication take place: The dissemination, exploitation and impact management of the project will be coordinated in WP1 and start at the project start (M1). Principles and practises established in the Biodiversa+ Stakeholder Engagement Handbook will be applied throughout.

On-site communication: DNAqualMG will use more targeted forms of communication to engage differentially with its specific target groups. Workshops and other face-to-face interactions with substantive stakeholders in WP4 will be a key to engaging local stakeholders and communities. There will also be workshops with instrumental environmental decision-makers and planners. A Stakeholder Advisory Board will be established (M3) to fine-tune the work programme and ensure that the produced results will meet the needs of end-users. Members of the project team have close links with the agencies described above and selected representatives have already provisionally agreed to join the Stakeholder Advisory Board. The Stakeholder Advisory Board will meet the project team annually in conjunction with Plenary Assemblies. Its members will receive regular updates and invitations to input throughout the project and will be asked to facilitate dissemination to their wider networks.

Online formats: From the outset, DNAqualMG will also use all appropriate means of online communication to inform, consult, involve project partners and relevant stakeholders as well as to collaborate on the subjects. For example, to co-develop updated sampling and preservation methods, DNAqualMG WP2 will perform two workshops with the national monitoring experts. One prior to sampling, and one after one independent monitoring cycle using the proposed draft methodology. To keep stakeholders informed, besides email-based communication and updates, DNAqualMG will build and maintain a website (M3), with key information about the project, participatory activities, feature articles, and network information (responsible: UDE Leese). The website will host current and archived infographics, e-newsletters, and podcasts, as well as online handbooks, toolkits, and project updates and deliverables. The consortium will maintain social media feeds, including – as appropriate for different target audiences – LinkedIn, Twitter/Mastodon, Instagram and for training videos also YouTube. Visibility of data and outputs will also be maximised through engaging with platforms such as the Freshwater Information Platform (FIP), where we can use all its dissemination outlets, e.g. the widely read Freshwater Blog or the (meta)data publishing unit (see Section E, which also includes approaches to data protection and timelines for open access).

Communication of relevant project outputs will take place in a variety of appropriate forms throughout the project lifetime: dedicated e-newsletters (all) and policy postcards (policymakers), through open access

platforms, databases, maps and repositories (researchers and environmental professionals) and through conference presentations and publications in peer reviewed journals (researchers).

C. Description of project coordination and management

The project will be coordinated by UDE (Leese). Together with WP co-leads, project progress, milestones, stakeholder engagement will be monitored. Potential risks to project success are identified (Tab. 2).

Tab. 2: Potential project risks and the management of risks in DNAquaIMG.

| Description of risk (likelihood/impact) | WPs | Proposed risk-mitigation measures |
|---|-----|--|
| 1. Disciplinary and geographical scope too broad to enable effective integration (low/low) | All | <ul style="list-style-type: none"> ▪ DNAquaIMG already involves 10 different partner countries. ▪ Furthermore, all partners have extensive experience of interdisciplinary and international collaboration as part of other projects (e.g. MERLIN, DNAqua-Net, eDNAqua-Plan, BioScan-Europe etc.) ▪ Quarterly WP leader meetings will monitor progress towards integration, and instigate peer-to-peer mentoring where necessary. |
| 2. Delays in project deliverables (low/medium) | All | <ul style="list-style-type: none"> ▪ The schedule for project deliverables will be agreed in the Consortium Agreement, and monitored by WP1 and the project coordinator on a continuous basis. ▪ At critical points in the project, contingency plans to remediate any parts of the WPs behind schedule may be implemented, including redirection of partner resources, and the coordinator's direct involvement, as impacts could be medium. |
| 3. High dependency of work packages on others (low/medium) | All | <ul style="list-style-type: none"> ▪ This is low risk, as consortium partners will work across work packages and case study regions in complementary ways. For example, research teams for WP2 will conduct fieldwork independently and can process samples before the availability of others, so one work package is not dependent upon completion of another. ▪ Where a work package is dependent upon others (e.g. WP5 relying on WP2-4), the teams for those work packages will work directly with each other to advise and assist with data collection. The project coordinator will monitor progress of all interdependent tasks to ensure that they remain on schedule. ▪ Several partners have additional material from previous work that is intended to be used, so in case of delay or dropout of a partner's samples, there is sufficient material available. |
| 4. Little or no uptake from stakeholders (low/medium) | All | <ul style="list-style-type: none"> ▪ Consortium partners know from existing relations with stakeholders in case study regions that there is strong interest in the complementary use of DNA-based methods for biomonitoring. Therefore, there is little risk of no-uptake. For image-based assessment technology readiness level is behind that of DNA-based methods. Therefore, the uptake of these methods, in the absence of service providers, is definitely higher (medium). ▪ The consortium will set out demonstrable measures of stakeholder engagement for each task, and monitor engagement performance to ensure take-up is substantial, balanced, and inclusive. The Committee, as well as all partners, will also seek advice from stakeholders and the Advisory Board about measures to remedy any low levels of engagement, and indeed to maximise engagement and impact. |
| 5. Many other networks are working on similar solutions leading to entropy of biodiversity monitoring approaches (low/medium) | | <ul style="list-style-type: none"> ▪ Consortium partners are actively involved in national and international projects that develop the tools proposed. Close collaboration in particular with eDNAquaPlan, BGE Bioscan-Europe, International Barcode of Life, MAMBO will guarantee that the solutions we develop build on these networks but are unique. ▪ Through the engagement with EuropaBON and their specific feedback, the developed methods will be useful for their novel biodiversity monitoring concept. |

The steering group will meet every month for a 1-2h online meeting to update the consortium about progress, risks (delays etc.) and identify and implement remediation measures. UDE Leese is also in charge of finalising the Data Management Plan (DMP) and the Consortium Agreement (CA) with input from all partners. Through WP1 also support for the organisation of national workshops in the countries of partners without funding is provided (UDE Leese).

Task 1.1 (UDE, AU): Organisation of project meetings. Kick-off meeting (M3), mid-term meeting (M18) and final meeting (M36) will be organized by WP1.

- **Milestones:** M1.1a-c. Three consortium meetings held (M3, M18, M36).

Task 1.2 (UDE): Develop a data management plan (DMP) that lists all aspects along the data generation and publication life cycle.

- **Milestones:** M1.2a. Data Management Plan established and operational (M6). M1.2b-d. updated versions (M12, M24, M36).

Task 1.3 (UDE, UCD, SYKE, BOKU): Identify and involve stakeholders engaged in traditional and DNA-based monitoring of freshwaters identified and involvement/collaboration within **DNAqualMG**. This will be done in collaboration with stakeholder members from the Advisory Board (**Fig. 1**). In the project initiation phase, WP1 will perform a stakeholder mapping approach in collaboration with collaborative EU projects (eDNAqua-Plan, BIOSCAN-Europe, MERLIN) to maximize synergies among projects and identify relevant actors for EU biodiversity monitoring.

- **Milestone:** M1.3. Table with relevant stakeholders complemented and contacts established (M6).

Task 1.4 (UDE): Communicate project progress to academic and applied target audiences. Especially communicating the project findings in a non-technical fashion through a website, reports and social media.

- **Milestone:** M1.4. Website, LinkedIn account, Twitter/Mastodon/Instagram social media accounts setup (M3) and updated after each of the Milestones and Deliverables of WP2-6 is delivered.

D. Interconnection to national and transnational research projects and programmes

PIs of **DNAqualMG** collaborate with stakeholders in many national, European and truly international projects dealing with biodiversity monitoring using novel methods. We will list the most important projects here and explain planned connections and collaborations with these projects and research programs.

DNA and image-based project interconnection: In Germany, the Federal Environment Agency funds currently two projects for the advancement of DNA methods as part of Water Quality assessment: GeDNA and dbDNA. PIs Leese/Hering/Zimmermann are partners of GeDNA, Leese/Hering/Schmidt-Kloiber of dbDNA. Through GeDNA, experiences with good-practice protocols (and protocols failures) have been collected, which puts us at a stage where already from the start of **DNAqualMG** we have a proposition of advanced methodology for T2.1-T2.3. dbDNA (2022-2025) will extend the reference databases for taxonomic assignment and develop a pilot for DNA-based ecological status class assessment on the German analysis platform "Gewaesser-bewertung-berechnung"). In Austria, Austrian Research Promotion Agency (FFG) is funding the project BioMONITec (Švara/Jungmeier), focusing on the implementation of novel technologies and approaches in regular monitoring of biodiversity. Approaches include metabarcoding of freshwater bulk samples and eDNA, as well as provision of support with these approaches to stakeholders. Further imagin projects that we will link to are TIMED (Finland, Raitoharju), FinBIF FIRI (Finland, Meissner), AIAQUAMI (Serbia, Milošević). At international level, members of **DNAqualMG** are involved and plan collaborations with several key projects or networks, e.g. HEU Project MAMBO on novel image-based methods, BioScan-Europe (Biodiversity Genomics Europe, UniLodz and BIOPOLIS PIs in the project), international Barcode of Life project, the iTrackDNA project (Canada, UDE Leese on advisory board), as well as the eDNAquaPlan project that was recently granted that will develop eDNA library infrastructures for aquatic biodiversity monitoring (SYKE, BIOPOLIS, UDE, INRAE PIs).

International projects on biodiversity monitoring general: In addition to the discipline-focused networks on image and DNA-based methods, DNAqualMG will collaborate with key projects and networks EuropaBON is a key network for biodiversity monitoring in Europe; the last year of EuropaBON falls into the first funding year of DNAqualMG (BIOPOLIS is PI). EuropaBON is also partner of Biodiversa+. Contacts to GEOBON also exist and will be intensified; especial with FWBON (freshwater). Partner Schmidt-Kloiber (BOKU) is regional coordinator of FWBON, which will guarantee the uptake, also regarding the IUCN GLOSAM (standardisation sampling protocols internationally). Similar direct ways of uptake can be made with the Alliance for Freshwater Life where partner Schmidt-Kloiber (BOKU) is steering group member. Outcomes of **DNAqualMG** will be disseminated through cooperation with the Freshwater Information Platform (FIP), of which Schmidt-Kloiber/Hering are founding members.

F. Proposed Data Management Approach

Data production: **DNAqualMG** will produce four major types of data, of which three originate from a biological sample, i.e., tissue and DNA (1-3) and one type from computational processing (4): 1) digital sequence data through DNA metabarcoding, 2) digital image data, 3) descriptive metadata (sampling event, location, primers, indexed, sequencing platform (and engine)) and 4) code and scripts. The raw digital sequence data will be computationally processed and produce two consecutive sub-data-types a) sequence clustering to i) operational taxonomic units (OTUs) or ii) amplicon sequencing variants (ASVs) and b) taxonomic information tables for the clustered sub-data types (a). In addition, this computational process generates code and scripts. The analysis of digital image data (2) will produce a) taxonomic information tables and b) codes. Metadata will be acquired during sample acquisition and used for ecological data analysis. DNAqualMG will acquire biological data compliant with the national and international (Nagoya, CITES) legislation and transfer the biological samples according to Nagoya protocol requirements (national PIC procedures). After project completion, the biological samples will be provided for long-term storage within the GGBN (Global Genomics Biodiversity Network) repositories.

Data infrastructure and security: **DNAqualMG** will draw on UDE's central Research Data management and server facilities, such as the established object storage system (central backup) and in-house infrastructure (in-house backup solution). Additional physical data storage can be purchased for 100 Euros per TB per year.

FAIR principles: **Findability** of the above-mentioned datasets will be achieved by publishing all occurrence data on international platforms like the FIP and GBIF using persistent identifiers (DOI for ecological data, Project numbers from ENA) as well as publishing scientific articles (with accompanying datasets). A statement will indicate how to access the datasets in each article, either through a link in the data availability section, a DOI link to open access archives, or via supplementary materials. **Accessibility** of the datasets and metadata will be achieved by ensuring the public availability of both scientific articles and datasets. Scientific articles will be uploaded to publicly open pre-publication platforms such as bioRxiv prior to submission to peer-reviewed journals (with an update of versions along the review process), and the final version of the article will be deposited in national and open archives. WP1 will ensure that all research outputs are linked to the project's website. Digital sequence data will be uploaded to ENA using metadata formats suggested by international consortia (BioScan ERGA, GSC). Protocols used will be shared openly via protocols.io (including DOI). For **interoperability**, we will use the BIOME format proposed by GCS, and supply requested metadata. No biodiversity-specific broadly accepted standard repository exists for large-scale imaging data. Among generic repositories, Zenodo is, to our knowledge, the most suitable candidate for the archival and publication of imaging data to be acquired in **DNAqualMG**. Zenodo accepts data set sizes that apply to image datasets and has been successfully used by WP3 participants. Developments in related projects will be followed to identify possible emerging new alternatives. Imaging data sets will be published with accompanying metadata and in standard formats (specimen image collections and/or large-scale virtual slides in the COCO format). In parallel, the diatom image data will also be made available through the BIIGLE 2.0 image annotation platform. All units will be explicitly detailed in an accompanying description of the datasets. Finally, **reusability** will be secured via a) detailed documentation of data generation including code/scripts e.g., scripts (electronical labjournal eLabFTW) for data processing, b) metadata and c) by depositing datasets in standard and open formats, mostly .txt or .csv files, and uploading outputs to GBIF/FIP. Additionally, the documentation will be concertedly published with the datasets in public archives.

Data responsibility and data management: Leese, as the PI and Schmidt-Kloiber, will ensure the development, implementation, and update of the data management plan (DMP) with the support of UDE's data stewards. As the main PI, UDE will define terms of use in the Consortium Agreement and – if needed – an IP agreement. UDE will ensure that all published papers indicate a link toward this common archive for accessing datasets and scripts. These datasets will be accessible only to all members of the project before publication, and they will be publicly available after publication over the long term through international platforms (see Findability).