AMBER Field Manual

Best Guidance on River Habitat Assessment

AMBER

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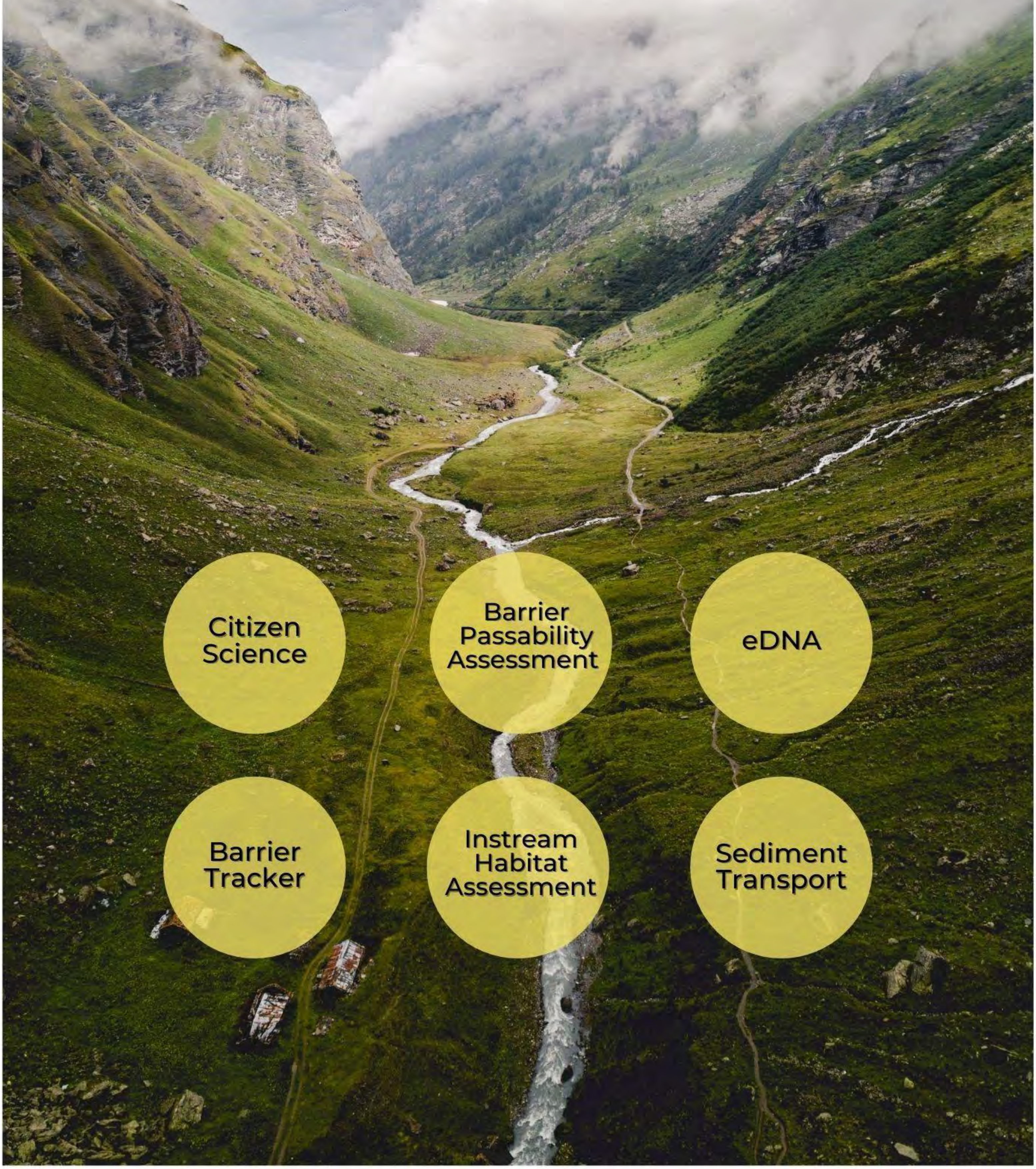

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1. Introduction

This document reviews best guidance on field data acquisition for adaptive barrier management utilising some of the tools developed during the AMBER project.

The AMBER Tools have been developed to help resource managers quantify stream fragmentation, assess barrier impacts and benefits, and make better, informed decisions on existing and future barriers based on what if scenarios.

Three types of tools were developed, depending on the problems they help to solve:

1. **Data Acquisition Tools (DATs)**
   To collect and harmonize data, to fill gaps, and to make sense of disparate information

2. **Mapping and Assessment Tools (MATs)**
   To assess barrier effects, to predict changes, and to turn information into knowledge

3. **Decision Support Tools (DSTs)**
   To consider trade-offs, to inform decisions, and to turn knowledge into application based on what if scenarios

A summary of some of the tools described in the AMBER Field Manual can be found in AMBER Policy Brief 2 (https://amber.international/policy-briefs/).

These acronyms are used throughout the text in reference to the practical application of the AMBER Tools described:

3D	Three dimension
ADCP	Acoustic Doppler Current Profiler
ADIZ	Air Defense Identification Zone
AMBER	Adaptive Management of Barriers in European Rivers – project acronym
API	Application programming interface
ATZ	Aerodrome Traffic Zone
BAC	benzalkonium chloride
BVLOS	Beyond Visual Line of Sight
CRS	Common Reporting Standard
CSV	Comma Separated Value
CTR	transport and business aviation group
EASA	European Aviation Safety Agency
EC	European Community
eDNA	Environmental DNA – genetic analysis of DNA found in environment (e.g. water)
EEA	European Economic Area
EFC	Expected Fish Communities
EPSG	European Petroleum Survey Group
ESRI	Environmental Systems Research Institute
EUROCONTROL	pan-European, civil-military organisation dedicated to supporting European aviation
FB	Face book
FCMacHT	fish macrohabitat community
GA	Grant Agreement
GIS	Geographic information system

GPS	Global Positioning System
HMU	Hydro-morphological units
ICAO	International Civil Aviation Organization
ICE	French national barrier pass ability assessment protocol
ICF	Spanish national barrier pass ability assessment protocol
ID	Identification
IOS	iPhone Operating system
IT	Information Technology
JARUS	Joint Authorities for Rulemaking on Unmanned Systems
JRC	Joint Research Centre
MATLAB	Matrix Laboratory
MATZ	Military Aerodrome Traffic Zone
MCR	compiler runtime package for MATLAB
MesoHABSIM	Mesohabitat Simulation Model
MRT	Military Route
OS	Operating system
PAE	Pre-Exposed Area Electrofishing Grids
PANSA	Polish Air Navigation Services Agency
PC	Personal computer
PEA	Pre-exposed area
PHABSIM	Physical habitat Simulation Model
PVC	Polymerizing vinyl chloride
QGIS	Quantum Geographic information system
RC	Remote control
RMZ	Radio Mandatory Zone
ROL48	the Government Protection Bureau issues flight requirements
SfM	Structure from motion
SNIFFER	Scotland and Northern Ireland Forum for Environmental Research
SSIFI	The Stanisław Skaowicz Inland Fisheries Institute
TMA	Terminal Manoeuvring Area
TRA	Temporary Reserved Area
TS	Transversal Sections
TSA	Temporary Segregated Area
UAV	Unmanned aerial vehicles
UAVS	Unmanned Aircraft Vehicles Systems
USGS	United States Geological Survey
UTC	Universal Time Coordinated
UV	Ultra Violet
VLOS	Visibility Line of Sight
WARP	Reprojection QGIS tool
WGS	World Geodetic System
WMS	Web Map Service
WP1	Work package no 1 of the AMBER project

2. Citizen Science Programme and Barrier Tracker (DAT1)

2.1. The purpose and background of the tool

Traditionally, science was brought closer to citizens through environmental education, however, in recent decades, a new methodological approach has emerged. Citizen science is a means to establish new links between scientists, citizens and the environment.

This new concept defines public participation in the production of scientific knowledge and at the same time provides society with a better understanding of the environment, ecosystem service or environmental risk, often promoting greater involvement in the conservation of the environment. It therefore benefits both the scientific community and citizens.

Citizen science includes projects designed by scientists where citizens have the opportunity and tools to participate in data collection. This way, citizens' messages are transferred "bottom up" in different ways: towards centralized systems, using communication technologies such as mobile applications, or putting citizens in contact with environmental managers and researchers. This has implications that go beyond purely technological ones: they constitute a de facto increase in the power of society, which can lead to changes in governance models, especially in the field of environmental policies.

Statement of Purpose of the 'Barrier Tracker' app

The AMBER Citizen Science Program (Barrier Tracker app and website portal) has been developed to collect citizen science data on longitudinal physical instream barriers throughout the 31 EEA member countries as well as Switzerland and Andorra.

Data collected via the Barrier Tracker can be used to:

1. Assist in the estimation of barrier numbers in different countries;
2. Raise public awareness and understanding of the importance of river connectivity
3. Provide data on barrier characteristics and incidence of obsolete barriers.

The citizen science strategy has added data to the AMBER Barrier Atlas of stream barriers (AMBER Policy Brief 1, https://amber.international/policy-briefs/) in a way that would not be possible without a much greater investment in staff and resources. Combining the AMBER Barrier Tracker smartphone application with the Citizen Science Programme allows for the collection of more data and greater spatial coverage of records than would be possible using conventional surveys. At the same time, it makes citizens more aware of current rates of stream fragmentation and engages the public in reconnecting rivers.

Data collected by volunteers must add value and be relevant, and this is the leitmotiv of citizen science that differentiates it from environmental education. To do this end, citizen scientists must be provided with appropriate tools for collecting data. The key for successful citizen science programmes is the adoption of simple methodologies that do not require prior knowledge and that generate useful data. The Barrier Tracker app was designed to be accessible throughout the EEA, and to meet the following criteria:

1. **No need for registration**
   To facilitate use, no registration details are required to use the app. Registration is only required to interact on the AMBER web-interface such as leader-boards, viewing user data by username or other users by username (not actual name). Rigorous data protection procedures have been employed in the development of the app and within the app itself).

2. **Minimum data input requirements**
   The app is intended to be used by non-specialists. Users can use the app by simply taking photos of barriers (which are geo-located and dated). Having only a geolocated photo still fulfils the prime purpose of the app, which is to obtain a more realistic estimate of the number of barriers in different EEA Member States. Users are also prompted for information on barrier use and size (Tier 1) and experienced users are able to supply additional information on barrier features (Tier 2).

3. **A simple interface**
   An intuitive app with clearly understandable symbols and little or no text typing was considered necessary for ease of use, to simplify translations, and for speed of input. Short descriptions of barrier types with photo examples were integrated to ensure that non-specialist users could use the app without any previous barrier knowledge. Additional information on barrier types and the importance of monitoring barriers is provided on the online Citizen Science web-interface (https://portal.amber.international/).

4. **Useable without good internet or GPS connection**
   Many barriers are located in remote areas, so it was important that the app could work without signal reception. The app allows users to upload barrier photos at a later time (if there is no internet connection) and to locate or improve the accuracy of the location of the barrier on a map manually (if GPS signal is weak or absent).

5. **Useable on different platforms and different countries**
   The app is available for both Apple iOS (version 8 and above) and Android (version 4.4 and above), It uses a hybrid code, i.e. one code-base deployed and optimised for both platforms. Users can login via standard username/password, Open Authentication (Twitter, Google, FB) or submit without prior authentication or registration. Users can also select one of 12 European languages.

2.2. Instructions

Installation

The AMBER Barrier Tracker smartphone app is available from the Google Play Store & App Store for IOS & Android (search for "**Barrier tracker**" in store), or it can be or directly downloaded from:

- iOS: https://itunes.apple.com/al/app/barrier-tracker/id1246829944?mt=8
- Android: https://play.google.com/store/apps/details?id=com.natural_apptitude.amber&hl=en

The website portal is hosted on the AMBER website (http://amber.international/) or can be directly accessed from https://portal.amber.international/.

Operation
Barrier Tracker app
The AMBER Barrier Tracker records and submits data and stores it in a backend system. Submitted records can be verified by experts from the AMBER consortium in this backend system. Data can be exported or imported into other systems via an API key. This Citizen science data is merged with data from the AMBER Barrier Atlas and forms a separate special layer). The app developer fulfils the data protection regulations for storing any personal data.

- **Tier 1 Interface**

The app features:

1. "Home": record a New Obstacle (**Figure 1**) or View Map

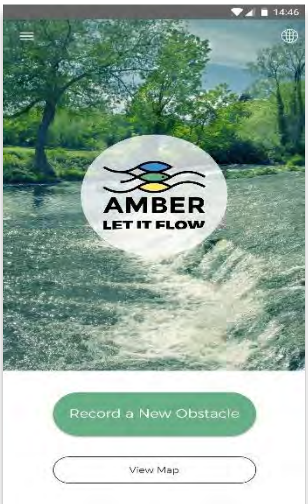

Figure 1. Home page of Barrier Tracker smartphone application. Photo: P. Parasiewicz, SSIFI.

2. A drop down **(Figure 2)** menu featuring:

- "Maps"
- "My Records" page(s)
  - Pending records
  - Submitted records: view how many barriers have been submitted in total by user
  - Record status: user status based on the number of their submitted records that have verified, i.e. beginner (<5), explorer (5-20) or expert (>20)
- "My Account"
  - Rank
  - Submitted records
  - Verified records
  - Total AMBER records
- "Information"
  - About AMBER: project information
  - Connect with us: project social media
  - Data privacy: data policy page, including personal data protection
  - Health and Safety advice page
  - App information
  - App Guide: guide on how to use the app most effectively

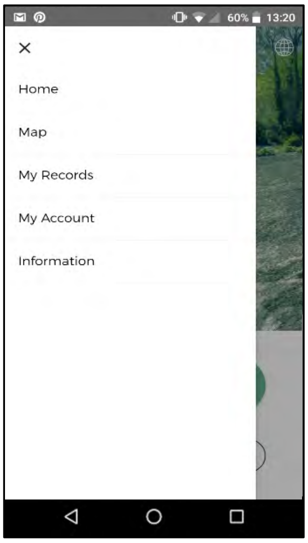

Figure 2. Drop down menu on home page. Photo: P. Parasiewicz, SSIFI.

When selecting “Record a New Obstacle”, the user is asked to complete the information described in **Table 1**. Examples of the pages to be completed when recording a new obstacle are provided in **Figure 3**.

Table 1. Attributes of the tier one "Record a New Obstacle" page(s).

Feature	Record entry	Automatic or manual	Answer types
Barrier Photo	Obligatory	Automatic opening, manual shutter control and option to retake image: camera opens upon opening "record obstacle" page	User defined (photo)
Date of record	Obligatory	Automatically, upon taking photo	Date in format Hours/minutes/seconds & Day/month/year
Barrier Type	Optional	Manual	Weir, dam, culvert, ford, sluice, ramp (with images to aid in making the choice)
Barrier Height	Optional	Manual	Height categories: <0.5 meter, 0.5 - 1.0 meter, 1.0 – 2.0 meter, 2.0 – 5.0 meter, 5.0 – 10.0 meter, >10.0 meter*
Does the barrier extend across the entire watercourse?	Optional	Manual	Yes/no
Is the barrier in working condition?	Optional	Manual	yes/no/don't know
Please add any additional notes	Optional	Manual	
Barrier Location (Geo-location of obstacle)	Obligatory	Automatic, upon taking photo. Prompt for GPS (locate) to be used if not switched on. App records whether location was taken based on GPS, phone signal or both.	Lat./long. coordinates via GPS chipset on phone and where there is a suitable signal, the phone signal.
Please add any additional notes	Optional	Manual	Text

(a)

(b)

(c)

(d)

Figure 3. (a) barrier Types (with user photo of barrier behind); (b) in-app information on barrier type; (c) example of how data is input; barrier height; (d) map atlas data under street layer. Photo: P. Parasiewicz, SSIFI.

Tier 2 Interface

This is for river managers, practitioners and more expert users who have contributed more than 20 verified records. This allows more detailed information, specific to each barrier, to be entered **(Table 2).**

Table 2. Attributes of the Tier two 'record obstacle' page(s).

Category selected following 'obstacle type' question	Additional information collected (pt1)	Additional information collected (pt2)
Weir	Vertical, sloped, stepped	-
Dam	overflow dam; wing dam; check dam; arch dam; barrage; embankment dam; don't know	-
Culvert	(1) width of culvert; don't know	-
Ford	(1) depth category: dry; shallow (<15cm); deep (>15cm); don't know	-
Sluice	(1) width of sluice gate; don't know	(2) depth of sluice gate; don't know
Ramp	-	-

Another page for additional information beyond the barrier type is also available (optional) (**Table 3**).

Table 3. Additional attributes in the Tier two 'record obstacle' page(s).

Question	Additional information collected (pt1)
Fish pass present?	yes/no/don't know
River width	Estimate in meters; don't know
River name	Name of rive
Flow conditions	Flow condition at time of recording: Low/regular/ high

User Types

- **Unregistered users**
  Unregistered users can record and submit barrier data from the Tier 1 interface anonymously, however they cannot see their scores in the "my records" page and are prompted to register upon submitting records.
- **Registered users**
  Registered users can record and submit barrier data from the Tier 1 interface, see their scores in the "my records" page.
- **Beginner users (<5 records)**
  registered users automatically become beginner users.
- **Explorer users (5-20)**
  registered users automatically become explorer users when the number of records submitted exceeds 5.
- **Expert users (>20)**
  registered users automatically become expert users when the number of submitted records by a user exceeds 20. Once these records are validated, users have access to the Tier 2 interface.

Website portal
This portal (**Figure 4**) relates to the AMBER Barrier Tracker app and allows users to:

- View all app recorded barriers on a map interface and see information about each barrier
- Use a satellite map to look for barriers and enter their locations manually
- Classify barriers already submitted via the app
- View an "ID Guide" on the barriers, as featured in the app
- View information on the app and download it
- Share some information about the project via social media
- Learn more about barriers and the importance of maintaining river connectivity

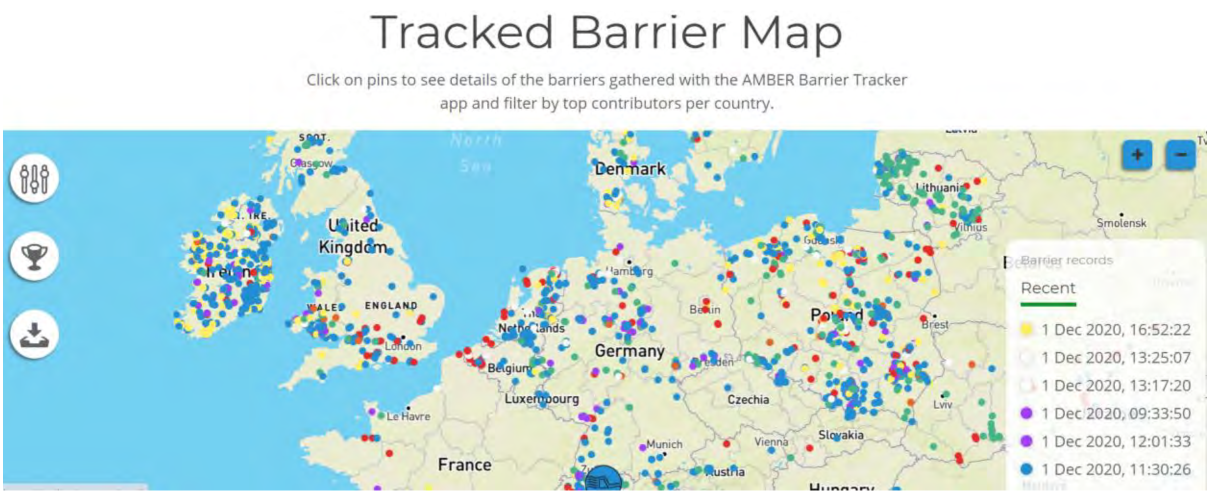

Figure 4. Map of tracked barriers on the AMBER Citizen Science Portal. Source: AMBER website (https://amber.international/european-barrier-atlas/, accessed 06/12/20).

2.3. Equipment needed

A smartphone and an internet connection (to upload barrier data).

2.4. Preparation for fieldwork

No preparation is needed.

2.5 Data collection process

Sending data from app
Data is uploaded to the Coreo backend system. The app allows the data to be uploaded after the data has been collected, i.e. when the phone has an internet connection (many field surveys will take place in locations where there is no phone reception or internet connection).
Data Storage
The app developer will maintain the data within Coreo both during the lifecycle of the project and after the project end date. The data are also hosted at the AMBER website and at Joint Research Council at the European Commission.

Verification of data
Data are verified by a group of validators within the AMBER project who have the ability to log on to the back-end system.

2.6. Data security

Users can choose to register or use the app anonymously. The information collected by the Barrier Tracker consists of recorder name, email address, username, identification skill level, GPS location, date and time of record, photograph of barrier, location name, barrier type and sub-types and a number of other optional data relating to the barrier and water course. Organisations with full access to the data set are members of the AMBER project and Natural Apptitude Ltd. Users can submit as many records as desired and withdraw the personal data at any time by contacting info@fishmigration.org.

3. Barrier passability assessment (MAT1)

3.1. The purpose and background of the tool

Due to limited resources, precise enumeration of the impact of individual or multiple barriers on river connectivity using empirical telemetry techniques is generally only undertaken at large hydropower dams (Chanseau & Larinier 1998; Gowans *et al*. 2003; Rivinoja *et al*. 2006). Although these empirical studies provide valuable information on barrier passability at a small scale, they are generally very resource-intensive, and with some notable exceptions (Winter & Van Densen 2001; Ovidio *et al*. 2007; Lucas *et al*. 2009), they generally focus on salmonids and/or larger barriers. There is a need for a rapid coarse-scale passability assessment tool that can be implemented quickly and cheaply at catchment, national or international scales to facilitate prioritisation of restoration actions.

Around the world, numerous passability assessment protocols have been formulated to meet these requirements, but these have generally been developed in an ad hoc fashion, resulting in major inefficiencies, and duplication of effort and frequent repetition of mistakes (Kemp and O'Hanley, 2010). Within Europe, three key protocols exist that are well developed, widely accessible and are fully or partially available in English (the SNIFFER [UK], ICE [French) and ICF [Spanish] protocols). These protocols were tested and critically reviewed by Kerr *et al.* (2016). The French ICE protocol is the least subjective and produces passability scores for the most species (n=47), whilst requiring less physical measurements to be recorded than other fine-scale protocols (for example. SNIFFER) (Kerr *et al.,* 2016). Recent work has also highlighted that the ICE protocol produces comparable passability scores to the finer scale SNIFFER protocol and is much quicker and easier to undertake (Barry *et al.,* 2018). The ICE protocol has been chosen as the protocol of choice for use by the AMBER consortium and for wider promotion as a European standard for barrier assessment as it produces scores for a wide range of species, it is the least subjective, and it is quick to undertake whilst producing comparable score to the higher-resolution protocols that require more measurements to be recorded.

One limitation of the ICE protocol is that passability scores are produced through a decision tree process, which can be time consuming to complete, especially as this has to be repeated for each species assessed. The Barrier Passability and Hydropower Potential Assessment Software Tool presented in this chapter automates the process, rapidly calculating the passability scores and reasons for limited connectivity for all species at the click of a button based on a few simple input parameters. Scores produced range from 0 – 1: 0 (total barrier), 0.33 (high-impact partial barrier), 0.66 (medium impact partial barrier), 1 (low-impact passable barrier). In addition to generating ICE passability scores, the tool estimates the hydropower potential (Watts) at the site through a simple assessment of discharge and head drop. As such, the tool produces data that can be used to populate barrier mitigation prioritisation tools (see Kerr *et al.,* 2016) and efficiently help with catchment level management decisions.

3.2. Instructions

Installation

The software was developed in MATLAB but functions as a standalone tool regardless of whether the user has a MATLAB licence. To run the software a MATLAB compiler runtime (MCR) package is required. This MCR is contained within the software installation file and will be automatically installed with the main software:

1) Download the software from the AMBER website (https://amber.international/software).

2) Run the Barrier assessment tool.exe. This will guide you through the process of installation and by default install the software within 'C:\\program files\University of Southampton\Barrier Assessment Tool'.

Operation
See **Figures 5** and **6**.

1) Create a new Microsoft Excel (.xlsx) file for the data to be loaded into by pressing the "Create new file" button within the "Select file" panel. Alternatively select an existing file to export the data into using the "Select existing file" button.

2) Enter in all available barrier information within the "Barrier details" panel within the "Settings tab". A unique "Barrier I.D." is required for each barrier.

3) Identify if the barrier is made up of multiple Transversal Sections (TSs) or not and fill in the total number of TSs and TS I.D. as appropriate. For more information select the relevant "?" button in the "Barrier details" panel.

4) For each TS select the appropriate barrier type tab and fill in the required details.

5) Press the calculate button to calculate the passability score for that TS. Scores produced range from 0 – 1: 0 (total barrier), 0.33 (high-impact partial barrier), 0.66 (medium impact partial barrier), 1 (low-impact passable barrier).

6) Export the data to your selected excel file by pressing the export button within the relevant tab.

7) Repeat the process until passability scores are logged for each TS.

8) If the barrier consists of more than one TS make sure the correct barrier I.D. is entered within the settings tab and then press the "Compute score for multiple TSs" button. This will generate the passability score for each species for the whole barrier and output it into the selected Excel spreadsheet.

9) Clear all previously logged details and repeat for next barrier.

10) Periodically backup the Excel file to ensure overwriting errors cause no loss of data.

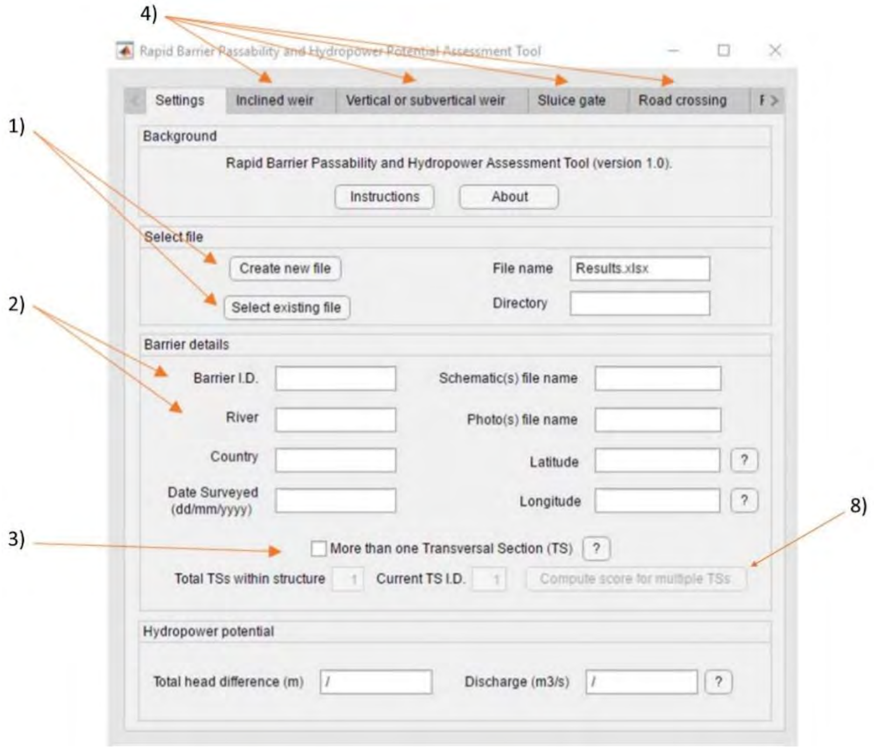

Figure 5. Settings tab of the Rapid Barrier Passability and Hydropower Assessment Tool. Source AMBER D2.3.

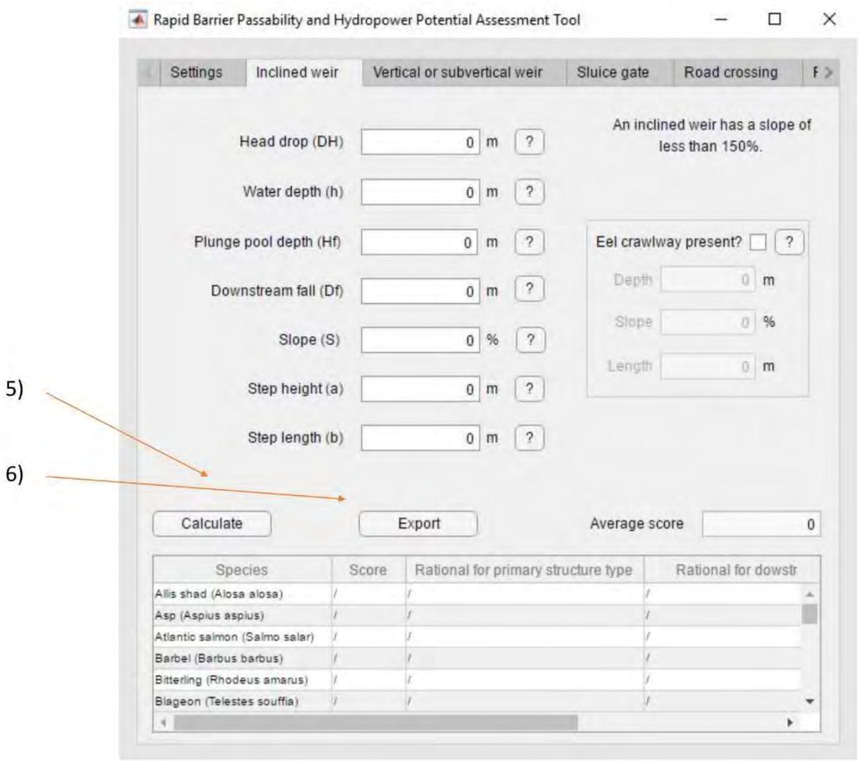

Figure 6. Inclined weir tab of the Rapid Barrier Passability and Hydropower Assessment Tool. Source AMBER D2.3.

3.3. Equipment needed

An example of the equipment that may be needed to collect the relevant barrier measurements is provided in **Figure 7**. Note that velocity measurements are only required under certain flow conditions for road/rail crossings (for example, culverts) so this device is not always needed.

Figure 7. Suggested equipment for collecting barrier measurements. Source AMBER D2.3.

3.4. Preparation for fieldwork

Permits and certificates

The Barrier Passability and Hydropower Potential Assessment Software Tool does not require equipment or procedures subject to legal qualifications or permits for their use.

Collecting the data required by the tool may involve access to private lands, depending on the purpose of the study and the site selection for sample collection. The right of the public to roam across private land exists in only a few jurisdictions (for example, Scotland), while in most countries is a contentious issue that has often led to court action. To prevent inconvenient encounters with landowners or legal issues, check the local regulations and fill in the relevant forms to obtain permission to access private lands.

Safety

Obtaining barrier data may require access into the river and onto the barrier and the work can be dangerous in some circumstances. In particular, staff may be working in remote locations and need to access flowing water. Health and safety issues relating to collecting barrier data are responsibility of each individual person intended to use the Barrier Assessment Tool. Acknowledging and undertaking a formal risk assessment of the hazards involved in working in/near flowing water and means by which then can be minimized is important in preventing accidents and ensuring the safety of the users collecting the data. This encompasses following the recommended precautions with regards weather, heat, dehydration, wading, working at height etc., paying special attention to the hazards and risks that may occur in the vicinity of the barrier. Personnel, with their line managers, should assess their suitability for the task, for example, inability to swim increases the risk in the event of suddenly plunging into the water.

Channel dimensions tends to be restricted at barriers causing increases in flow speed. In addition, the purpose of many structures is to maintain upstream water levels, which inherently results in a head-drop between up and downstream water levels. As such working a height is often a key consideration when assessing the risks involved in collected data at river barriers. Individuals undertaking fieldwork should also be aware of the temperature, precipitation and the risk of high flow and flash flooding during fieldwork.

When working in polluted waters, follow the corresponding guidelines to protect yourself from contact with the water to avoid exposure to dangerous chemicals or microbes. Always, when possible, work with someone to reduce accident risk, particularly in remote locations or those associated with a higher risk of accidents and ensure that others are aware of your schedule of fieldwork and study locations.

An example of equipment that may be required to safely collect data for impact assessment with the Barrier Assessment Tool is provided in **Figure 8**.

Figure 8. Suggested equipment required for impact assessment using the Barrier Assessment Tool. Source AMBER D2.3.

3.5. Additional data collection requirements

The assessment tool is designed to be used as standalone software installed on a field laptop or tablet, that enables users to record all relevant barrier data without the need for additional paper forms. However, it is advised that user produce a detailed field sketch of the barrier and site containing the following information:

- Date
- Time
- Field operative names
- North arrow
- River name
- Scale
- Flow direction
- Delineation of Traversal Sections
- Flow conditions (low, medium, high)

This field sketch can then be photographed onsite or scanned once offsite and the name and location of the image file recorded within the software.

Several photos of the barrier should also be taken during the visit. At minimum, an upstream and downstream photo of the barrier should be taken if possible. Preferably, multiple shots focussing on key features of the barrier (for example, crawlways) and individual TSs should be taken as well and appropriately stored. Photo file names can be logged within the software.

3.6. Background information needed

It is recommended that the ICE protocol be undertaken under “hydrological conditions most common during the migratory period of the given species”. However, high flow conditions can make barriers inaccessible, and even if the barrier is accessible, key features are often submerged making assessment difficult. These factors should be considered when planning a trip to assess passability for a species that typically migrates under high-flow conditions. If a broader multi-species assessment of a barrier is required, then it is recommended that low flow summer conditions are targeted. As such, monitoring discharge at the site and predicted rainfall is essential when planning a trip to undertake a barrier assessment. Depending on the country, discharge values can often be obtained remotely through government webpages designed to provide flood warnings. For example, in the UK, frequently updated river level data is available from https://riverlevels.uk. Annual flow data may also be available from archive sites such as http://nrfa.ceh.ac.uk/.

Predicted rainfall can be obtained from international weather services such as https://www.yr.no/ or https://www.metoffice.gov.uk/.

It is recommended that users access and check whether such data is available for the region of interest to plan their site visit and maximise the chances of being able to safely collect data.

3.7. Logistics

Before going to the field:

1. Ensure that the software is loaded onto an appropriate field computer and is functioning correctly.
2. Ensure that you have all the data collection and safety equipment required.

3. Check the forecast. If there is a high probability of excessive precipitation coinciding with or immediately prior to the planned sampling, it should be re-scheduled since this may result in rapidly changing flow conditions that increase the risk of working at the site.
4. Confirm that batteries are fully charged for electronic devices such as GPS. Carry spare ones in case they run out.
5. Carry any permits needed.
6. Schedule your time in advance and always add some extra time for any unexpected eventuality.

Once you are in the field:

1. From the bank, locate the barrier and undertake a preliminary assessment of the site; noting access points, escape routes and locations where equipment can be safely deployed (for example, levelling equipment).
2. Be aware of safety risks and take appropriate preventive actions to avoid any issue.
3. Undertake field sketch of the site, delineate any lateral heterogeneity into separate labelled Transversal Sections, and classify the sections according to barrier type.
4. Carry out data collection according to the systematic collection protocol above.
5. Remember to take photographs and log all required metadata before leaving the site.
6. Before leaving site check that, all equipment and rubbish has been collected. Make sure all gates access points are left as they were found.

3.8. Data collection process

Number of people

It is recommended that at least two people undertake data collection at a barrier.

Troubleshooting and unusual situations

The Barrier Assessment Tool covers five commonly occurring barrier types, and the majority of barrier types encountered should fall within one of these categories. It may not be possible to assess the anomalous barriers that do not fit into one of the five categories. Users must use their discretion to judge whether a barrier can be categorised into one of the five categories. Further guidance with this can be gained by reviewing the relevant section within the ICE protocol guidance document (Baudoin *et al.,* 2014).

Lateral heterogeneity along the width of the barrier is accounted for in the tool by delineating different Transversal sections (TSs) and calculating passability scores for each individual section. The final passability score for the entire barrier is considered equivalent to the TS with the highest passability score. To compute the barrier score for multiple TSs, calculate and export the score for each TS separately (numbering them appropriately), make sure the Barrier I.D. data entry field is correct and press the ‘Compute score for multiple TSs’ button. This creates a new line of data in the output data file, with the TS I.D. listed as ‘ALL’ and the barrier type as ‘Combined’.

The impact of complex structures that consist of multiple different barrier types longitudinally (one after the other) cannot be cumulatively assessed using the Barrier Assessment Tool, although passability of each individually barrier type should be calculated and exported. As long as the structure consists of less than five distinct longitudinal barrier types the overall passability score can be estimated manually F(see p142, ICE protocol, Baudoin *et al.,* 2014).

Software bugs and faults may occur and should be reported to Dr Jim Kerr (j.kerr@soton.ac.uk).

3.9. Data security

It is recommended that the output from the spreadsheet is frequently backed up. This is to ensure that the risk of data loss through overwriting errors is kept to a minimum.
It is recommended that 10% of data undergo a quality control process whereby the results are crossed checked back against the original ICE decision tree protocols. This can either be for 10% of the species for a single barrier or 10% of assess if multiple assessments are being undertaken. Any output errors that are identified should be reported to Dr Jim Kerr (j.kerr@soton.ac.uk).

4. eDNA (MAT2)

4.1. Purpose and background

Environmental DNA (eDNA) is DNA that has been actively or passively released by any organism into its environment. Sources of eDNA include, but are not limited to, cellular and subcellular debris from skin, scales, mucus, gametes, carcasses, faeces and urine. eDNA analysis has been shown to be an effective method of determining the presence of a wide variety of aquatic macro organisms including fish, amphibians and macro-invertebrates among others (Carew *et al.,* 2013; Hänfling *et al.*, 2016, Lim *et al.,* 2016; Thomsen *et al.,* 2012; Valentini *et al.,* 2016). It provides a sensitive, fast and relatively inexpensive sampling approach for detecting target organisms.

Sampling eDNA is straightforward. It simply involves collecting water in a container while accounting for critical issues such as contamination and DNA preservation to ensure sample integrity and thus data reliability. A range of tools and protocols have been successfully used. However, as eDNA becomes more widely used through its recognition as a cost-effective tool for biomonitoring, the need for standardization also grows. Implementing practical, agreed guidelines promotes such standardization.

The aim of this chapter is to provide practical, standardized and proven field guidance for researchers and managers in the collection, concentration (i.e. filtration), and preservation of eDNA samples from lotic environments (i.e. flowing water). It includes a planning and sampling workflow diagram, general considerations for sampling design, a step-by-step sampling protocol for flowing-water step-by-step and a list of the material and equipment needed to perform the described eDNA sampling protocol.

The proposed protocol can be adapted as required, depending on factors such as target taxa and their ecology, the environmental conditions of the system being sampled, etc. Over time, we have refined the proposed protocol to minimize contamination while maximizing species detection and sampling efficiency. It is intended to be convenient for a broad range of end users and its design has been guided by the need for field equipment and sampling kit to be portable and to allow for rapid sampling and immediate preservation (to minimize eDNA degradation), enabling the collection of many samples in a single day.

4.2. Before you start

The flow diagrams in **Figures 9, 10** and **11** and associated text in this section provide a step-by-step decision-making guide. This will help to establish whether eDNA surveys may be beneficial in replacing or supplementing current traditional survey methods, the type of sample to be collected, the downstream sampling technique to be applied, and other aspects associated to sampling design.

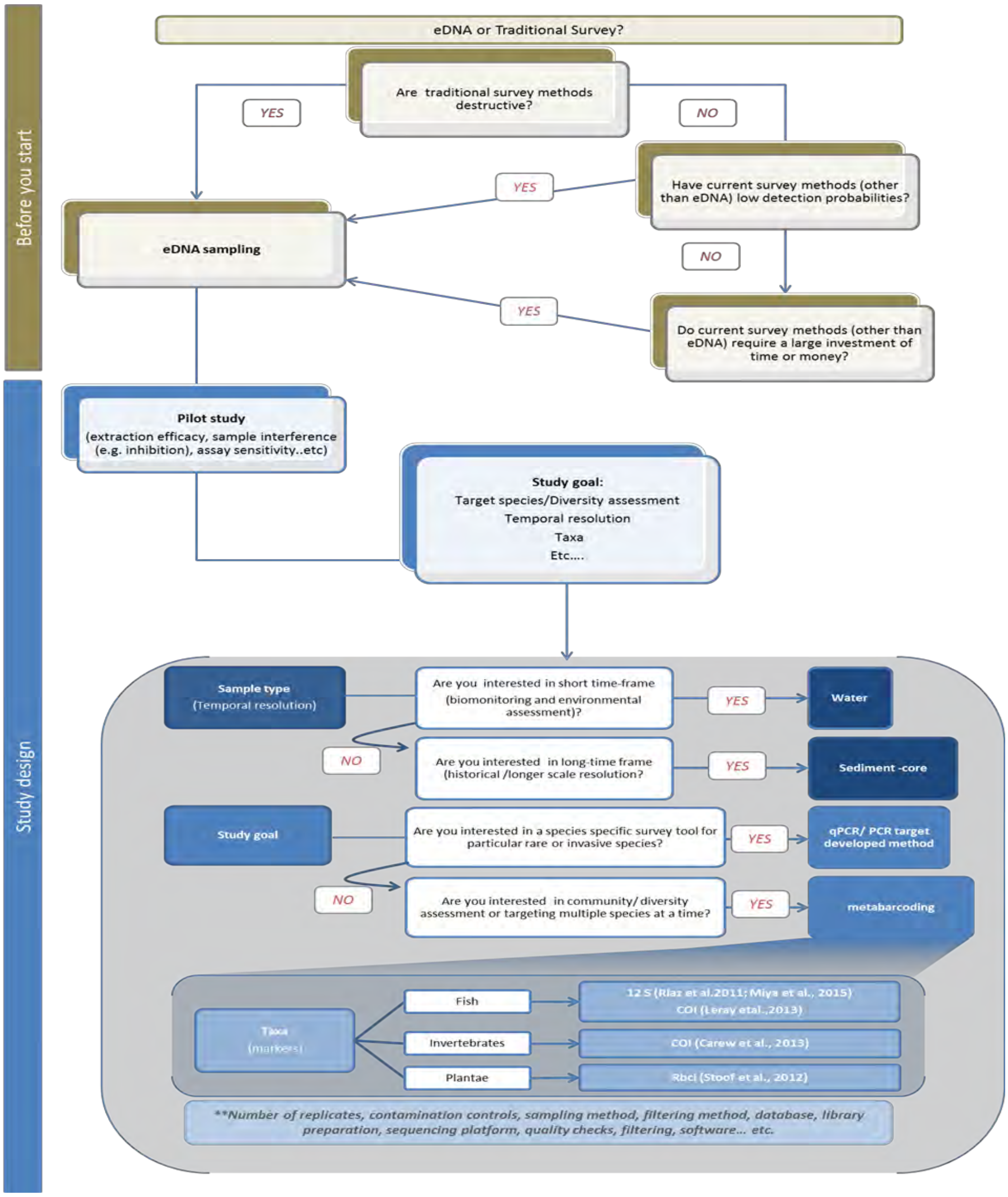

Figure 9. Decision flow chart and guidance for eDNA surveys (from Goldberg *et al.,* 2015a).

4.3. eDNA sampling or traditional surveys?

In most assessment scenarios traditional surveys:

- provide lower detection probabilities than eDNA based methods
- can be detrimental / destructive to the habitat
- require extensive training and taxonomic knowledge of the personnel conducting the surveys
- are costly and time consuming compared to eDNA sampling.

Consequently, eDNA can be more sensitive, faster and more cost-effective than traditional survey methods. However, eDNA may not always be the method of choice, for example:

- when surveying for a particular species for which the current method (other than eDNA) provides high detection rates with a relatively low cost and investment of time (Goldberg *et al.,* 2015a).
- when targeting a species whose presence is limited to a very specific timeframe during which environmental conditions hamper the feasibility of carrying out eDNA sampling, as eDNA needs to be sampled following a rigorous procedure to avoid sample contamination.
- when demographic information or developmental stage of the target species is needed.

4.4. Study design

The application of eDNA in the detection of species and biodiversity assessment may vary considerably depending on study goals, characteristics of the taxa of interest, and the features of the system to be sampled. It is always advisable to conduct a pilot study to determine the optimal sampling design. Critical considerations for study design include how to prevent contamination in the field and the laboratory, and the specific needs related to sample-type, study goals, timing, location, etc. (Goldberg *et al.,* 2016).

Sample type –water sample or sediment core?

Several studies have shown that DNA particles in natural environments can be detected for a relatively short timeframe (Seymour *et al.,* 2018; Shogren *et al.,* 2017; Wilcox *et al.,* 2016). This makes water samples ideal for biomonitoring and environmental assessment, since we can be highly confident that we are getting a snapshot of the community localized in a very short timeframe (Deiner *et al.,* 2017; Shaw *et al.,* 2016). Compared to aqueous eDNA, sedimentary eDNA could provide a more abundant and longer-lasting source of genetic material, reflecting a longer timescale biodiversity representation, being useful for inferring past site occupancy by aquatic macrofauna, as it has been commonly used as a source of historical and ancient DNA (Deiner *et al.,* 2017; Goldberg *et al.,* 2016; Turner *et al.,* 2015).

Study goal- species-specific markers or eDNA metabarcoding?

Species-specific and taxon-specific eDNA assays are the most appropriate approach when targeting a particular species (commonly referred to as “barcoding”). This will be especially true if the target species occurs at very low densities or is difficult to distinguish from similar species. This is because a targeted approach should in most cases give higher sensitivity and discrimination power over a multi-species approach. eDNA metabarcoding, a rapid multi-species biodiversity survey tool, holds huge potential for holistic biodiversity assessment and routine freshwater monitoring (Creer et al., 2016; Deiner *et al*., 2017; Lim *et al*., 2016; Thomsen *et al*., 2012; Valentini *et al*., 2016). This multi-taxa approach is a more appropriate freshwater monitoring tool than species-specific surveys when dealing with wider habitat questions, for example, assessment of the effects of loss of stream connectivity across a wide range of species. eDNA metabarcoding allows whole communities to be surveyed, enabling to gauge fluctuations in species richness while still obtaining information on the presence/absence and “eDNA relative abundance” of species of particular interest.

eDNA-based metabarcoding protocols have been tested and validated within AMBER and are ready to be used. The eDNA toolkit has been used to examine barrier effects in relation to Aquatic Invasive Species (AIS) in the Iberian Peninsula (Clusa *et al*., 2017a, b, Fernandez *et al*.,2018) and in Great Britain (Robinson *et al*, 2019a,b) and also in relation to fish community composition in the rivers Nalón and Guadalhorce (Spain), rivers Garry, Afan and Tawe (UK), and river Allier in France (Deliverable D2.5).

Sampling design- When, where and how?

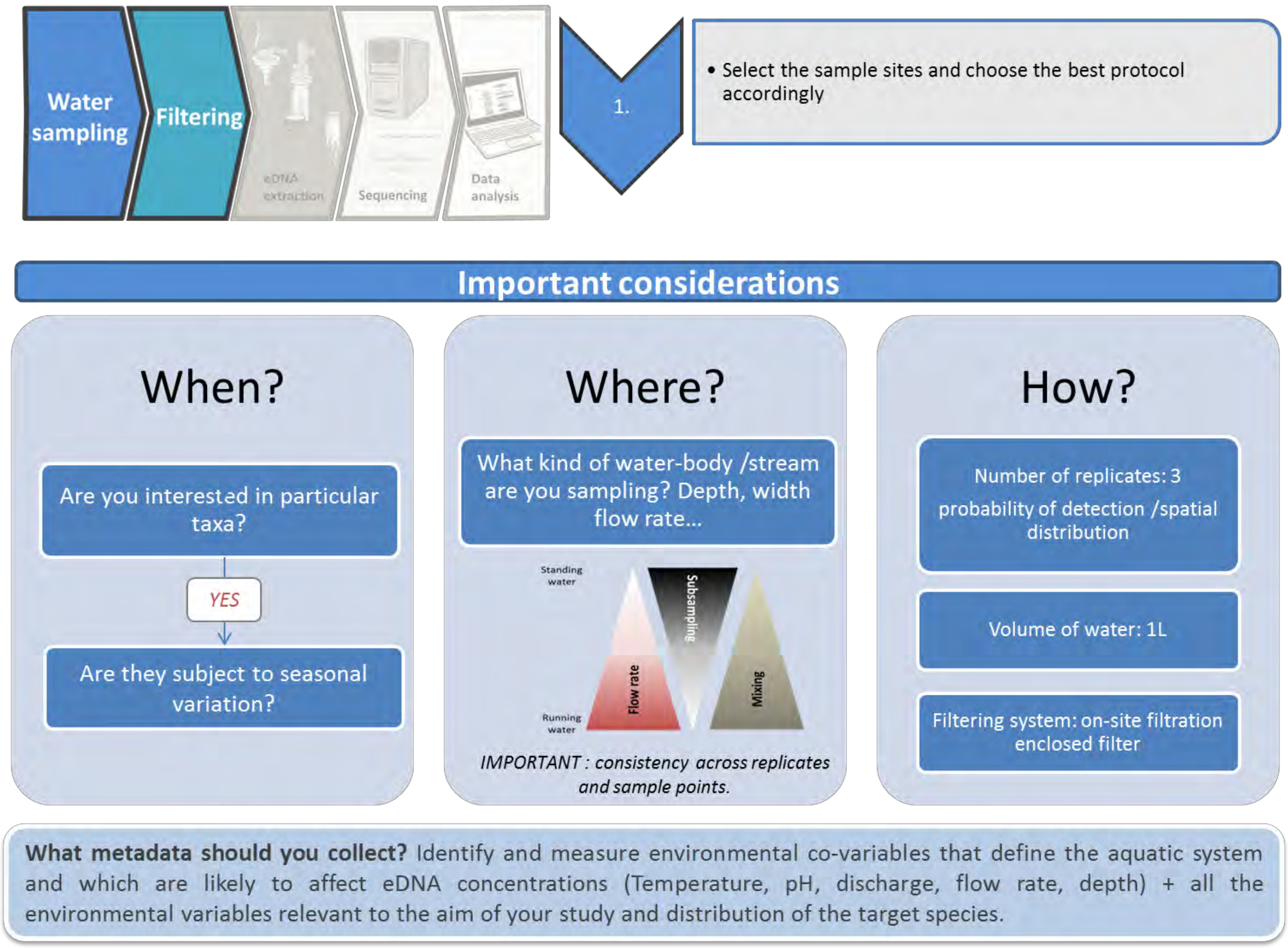

Figure 10. Important aspects to be considered associated to sampling design. Source: AMBER D2.5.

When

The timing of sampling can be critical, as some species are subject to seasonal variation in their presence or relative abundance, which leads to changes in eDNA levels in the water. Therefore, seasonal variation should be considered when scheduling the sampling. This is imperative when undertaking species-specific detection on migratory taxa that are not present all year round. When scheduling sampling time, environmental covariates should also be considered, since detection probability can experience seasonal fluctuations associated to dilution, transportation, and degradation (Bista *et al.,* 2017; Buxton *et al.,* 2018). In this regard, timing will generally differ in relation to latitude and local water-body dynamics.

Note: Were high discharge and extreme climatological events to coincide with the planned sampling, it should be re-scheduled, as the input of allochthonous eDNA from outside the study area might increase significantly, with potential to inflate diversity estimates.

Where

The spatial arrangement of the sampling will be system-dependant. Much is still unknown about eDNA dynamics in lotic systems. The best location will be dependent on the species distribution in the water column and the degree and pattern of mixing of the water. If organisms tend to congregate in slower waters, higher detection power might be realized in the stream margins though mixing will be higher in the thalweg (centre of the main current). Depth, width and flow rate are the key parameters to consider when deciding where to sample. Subsampling, i.e., sampling water from different locations in close proximity within the same sample site, followed by pooling, is the recommended approach. The lower the flow rate, the lower the mixing, therefore the higher the recommended number of subsamples to be taken (**Figure 12**).

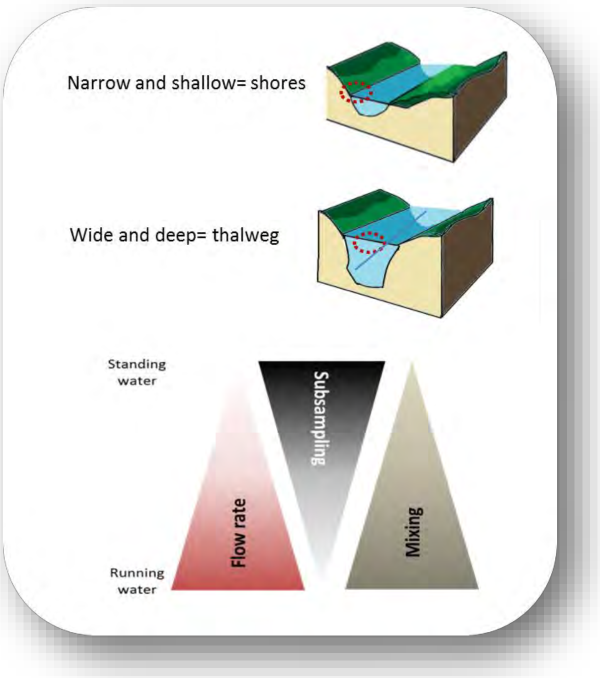

Figure 11. Site selection for collection of water for eDNA analysis. Source: AMBER D2.5.

Note: The most important consideration in sampling is consistency across replicates and sample points (i.e., for a given study all samples must be carried out following the same criteria).

How

Number of replicates: A minimum number of 3 replicates (**ideally 6**) at each sampling site is recommended. This allows assessment of detection probabilities and control for sampling variability.

Volume: 1L is a reasonable and commonly used volume (for example, Inui *et al.,* 2017; Shaw *et al.,* 2016; Spens *et al.,* 2017). Although smaller volumes are easier to process and can give good results, they may reduce detection probability. On the other hand, higher volumes may increase detection probability but are more time consuming. The volume chosen will be a compromise between detection probability and time efficiency. Water turbidity will affect how much water can be filtered before the filter clogs and affects either the volume filtered or the choice of filter pore diameter. Samples collected from turbid streams with abundant impurities (for example, tannins and organic materials) may obstruct the filter before the total suggested volume (1L) can be filtered using the recommended filter (pore diameter 0.22 µm). Filters with a greater pore diameter (0.45 to 1 µm) could decrease filter congestion in such circumstances. Filter pore size selection is a trade-off between the volume of water that can be filtered and the proportion of DNA retained on the filter, since small water volumes can achieve equivalent eDNA recovery relative to filtration of higher volume through a larger pore sized filter (Turner *et al*. 2014). Pre-filtering is also an option in very turbid waters.

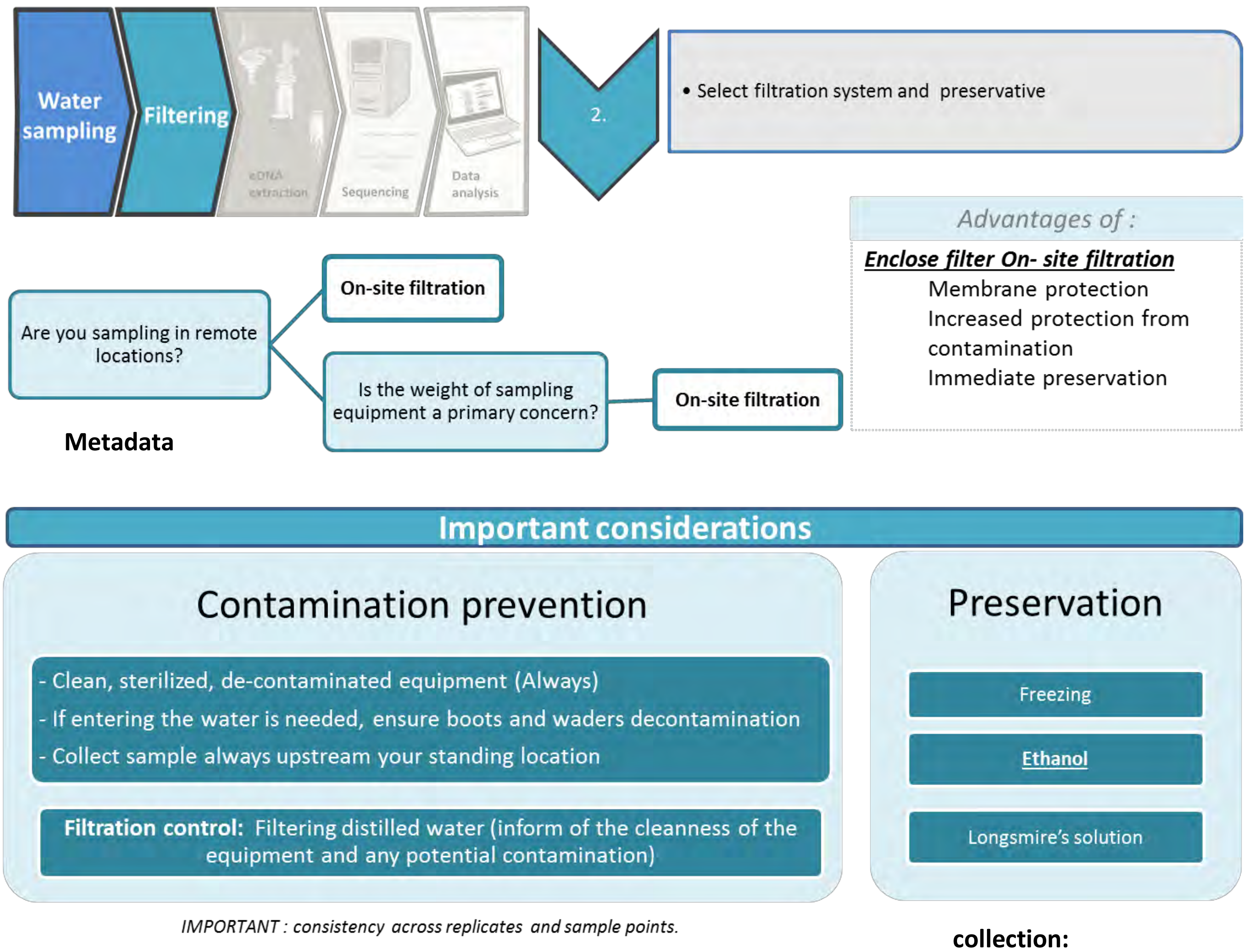

Figure 12. Important aspects to be considered associated to sampling process. Source: AMBER D2.5.

It is important to identify, measure and report the abiotic environmental co-variables likely to affect eDNA concentrations. These include temperature, pH, discharge, and flow rate (Goldberg *et al.,* 2016; Strickler *et al*., 2015; Seymour *et al*., 2017), as well as any environmental variables relevant to species distribution and the aim of the study.

Filtering system: We recommend on–site filtration, either with pump or manually (syringe) using encapsulated filters. The latter reduce the risk of contamination. Encapsulation of the filter limits its handling, protects the membrane from other external insults and contamination, and allows immediate preservation. This makes it the preferred option for standardized routine monitoring (Spens *et al.*, 2017).

Sample Preservation: Filters can be preserved by freezing or cold storage (Jerde *et al.,* 2011; Santas *et al.,* 2013), by immersion in ethanol (Goldberg *et al.,* 2011; Spens *et al.,* 2017), Longmire's solution (Renshaw *et al.,* 2015; Spens *et al.,* 2017; Williams *et al.,* 2016) or lysis buffer (Majaneva *et al*., 2018) or by drying on silica gel (Majaneva *et al.*, 2018). Ethanol is widely available, inexpensive and it can be readily used, making it a preferred alternative. Ethanol must be absolute (≥99.8%) molecular biology grade (DNase and contaminant free) to guarantee eDNA preservation.

Contamination prevention: Contamination can arise from many sources at different steps during the sampling process. It is critical for the success of the eDNA analysis and reliability of the results to prevent contamination and follow strict procedures. A series of guidelines are provided in section 0. to ensure contamination is eliminated or minimized.

4.5. Equipment needed – Sampling kit

Figure 13. Sampling kit. The displayed kit contains the material needed to perform the proposed eDNA sampling in flowing water. (See Appendix 8.3 for a detailed list of suggested supplies, manufacturers, description and alternative suppliers. Source: AMBER D2.5.

1. 1L Sterile bags or bottle (WhirlPak® stand -up Sample Bag)
2. Filter holder with filter
3. 50 ml disposable syringes (Thermo Scientific National Sci.)
4. 10 ml disposable syringes
5. Filter caps
6. Preservative (**Absolute Ethanol (Molecular grade)** or *Longmire's solution)
7. Resealable plastic back
8. Nitrile gloves
9. Indelible ink markers
10. Distilled ultrapure water (Filtration blank)
11. Bleach (10% solution)
12. Duffel bag Plastic coloured bag (-for clean equipment/material)
13. Plastic black bag (-for contaminated equipment/material)
14. Outflow bucket
15. Telescopic pole
16. GPS/cell phone with GPS app
17. Multi-parameter probe/ Flow meter (recommended)*
18. Rubber boots
19. Waders

4.6. Preparation of fieldwork – before going to field

Permits, certificates and forms

eDNA sampling is a non-invasive sampling method that does not require equipment or procedures subject to legal qualifications or permits for their use.

Traditional surveys such as electrofishing or trapping can be detrimental to populations and of particular concern to endangered species. The use of eDNA avoids this concern as it eliminates the need for removing individuals from their habitat, and the necessity of permits and licences.

eDNA sampling may involve access to private lands, depending on the purpose of the study and the site selection for sample collection. The right of the public to roam across private land exists in only a few jurisdictions (e.g. Scotland), while in most countries is a contentious issue that has often led to court action. To prevent inconvenient encounters with landowners or legal issues, check the local regulations and fill in the corresponding forms to obtain permission to access private lands.

Safety

Water samples may be collected under a wide range of conditions and the work can be dangerous in some circumstances. In particular, staff may be working in remote locations, and need to access flowing water. Health and safety issues relating to water sampling are responsibility of each individual person intended to perform eDNA sampling. However, acknowledging and undertaking a formal risk assessment of the hazards involved in water sample collection and means by which they can be minimized is important in preventing accidents and ensuring the safety of the users collecting the samples. This encompasses following the recommended precautions with regards weather, heat, dehydration, wading, etc., paying special attention to the hazards and risks that may occur in the vicinity of the sampling site (Yobbi *et al.*, 1996: A Guide to Safe Field Operations-U.S. Geological

Survey). Personnel, with their line managers, should assess their suitability for the task, for example, an inability to swim increases the risk in the event of suddenly plunging into the water. At bridge sites, ensure that you are not blocking the traffic, wear high visibility vests with reflective strips, post appropriate signs, and be vigilant of wide loads and large trucks which may extend over the walkway. Individuals undertaking fieldwork should also be aware of the temperature, precipitation and icing risk, as it can increase accident probability. In general, ensure the selected location is flat and stable enough to support your body and equipment. When working in polluted waters, follow the corresponding guidelines to protect yourself from contact with the water to avoid exposure to dangerous chemicals or microbes. Always, when possible, work with someone to reduce accident risk, particularly in remote locations or those associated with a higher risk of accidents and ensure that others are aware of your schedule of field work and study locations.

Contamination prevention

One of the most critical aspects of eDNA sampling is preventing contamination to ensure accuracy of eDNA results. Contamination can occur as a result of any sort of contact with eDNA beyond the water sample being filtered. This can include foreign DNA left over on hands, clothes, waders, boots, or any other sampling material and equipment used at previous sampling sites. This makes it essential to follow the contamination prevention guidelines summarized below.

CONTAMINATION PREVENTION GUIDELINES

- Use clean, sterilized, de-contaminated equipment.
- Keep clean and contaminated items in separate bags.
- Employ strict de-contamination protocols for all equipment and clothing if it needs to be re-used. [To decontaminate submerge the material in 10% bleach solution and rinse thoroughly with distilled water and let dry.]
- Whenever possible samples should be collected in such a way as to avoid entering the water body to reduce the probability of contaminating the site, boots and clothing, and (or) sampling equipment. If it is necessary to enter the water at the sampling site to access the right location, ensure you have decontaminated your rubber boots and waders before entering the stream, and always collect the sample upstream from your standing location to minimize potential contamination.
- Choose a flat location to lay out the sampling equipment to minimize the risk of contamination.
- Use Filtration controls. They indicate the general cleanness of the equipment and any potential contamination during the sampling.

WARNING! If decontamination has to take place in the field, be extremely cautious to minimize any potential hazard to the environment and/ or wildlife in case of spill. Bleach should not be disposed of into the river or nearby environments. If it is inevitable, it must be further diluted before disposal.

Logistics
Before going to the field:

1. Ensure that you have all the equipment, materials and reagents needed. The sampling kit described in section contains all the materials needed to collect water samples for eDNA assays. Prepare single points sampling kit bags. Always carry spare individual eDNA sampling kits accounting for any potential contamination issues.

   Note: Filtration system (filter holders plus selected membrane filter) need to be assembled and sterilized beforehand. It must be assembled in the lab under laminar flow hood. Place the membrane filters in the filter holders, autoclave the filters once assembled wrapping them individually in aluminium foil following manufacturer's recommendations and take the appropriate precautions to avoid contamination, then expose them to UV for twenty minutes and place them in individual bags for sampling.

2. Follow the contamination prevention guidelines described in the previous section of this guide (0)
3. Check the forecast. If there is a high probability of precipitation coinciding with the planned sampling, it should be re-scheduled since it may considerably increase the input of allochthonous eDNA from outside the study area, which can inflate diversity estimates.
4. Confirm that batteries are fully charged for electronic devices such as GPS, thermometers, Multi-parameter probes, etc. Carry spare ones in case they run out.
5. Carry any permits needed.
6. Schedule your time in advance and always add some extra time for any unexpected eventuality. When designing your sampling, if carrying out on-site filtration as suggested, take into consideration that filtering on-site using syringes is time consuming and physically demanding specially if water is turbid.

Once you are in the field:

1. Identify the exact sampling location. The flatter the terrain, the better, to facilitate laying out the equipment, minimizing the risk of contamination, reducing the risk of falling into the stream or contaminating it with any DNA on gear or personnel.
2. Be aware of safety risks (0) and take appropriate preventive actions to avoid any issue.
3. Maximize contamination prevention during sampling by following the guidelines in section 0.
4. Carry out sampling following the step-by-step collection protocol (Muha *et al*., 2019)
5. Collect and record all metadata (temperature, pH, flow, turbidity, etc.) after the water sample has been collected and saved in a closed re-sealable bag.

4.7. Data collection process

Number of people:
Sampling is recommended to be carried out in pairs to ensure safety, guarantee sampling procedure is rigorously followed, and minimize contamination issues.

Task Allocation
One person should physically collect the water, entering the stream (only when strictly needed). The other should carry out the on-site filtering following the protocol described in the section below.

4.8. Sample collection Protocol

As indicated, a minimum of 3 replicates per sampling point is recommended. Proposed volume: **1L** for each replicate consisting of the pooling of five 200ml subsamples collected within 100m around the sampling point (= 3x 1L per sampling point) (**Figures 14, 15** and **16**).

Water sample collection
1. Fill up the Whirlpak bag (or sterile bottle) with water by holding it into the stream. Place it in a well-mixed portion of the flow. **If subsampling and pooling, fill up the Whirlpak bag (or sterile bottle) of smaller capacity (i.e., 200ml) by holding it into the stream in the different spots the subsamples are to be taken till 1L had been collected and pool them and mix them within the 1 L Whirlpak bag (or sterile bottle).*

Filter

2. Take up 50ml of water with the 50ml syringe
3. Attach the syringe to the filter inlet.
4. Press the plunger and push the water through the filter.
5. Remove the filter from the syringe and repeat steps 2 to 4 until 1 litre of water has been filtered. **If the filter is clogged (for example, if the water is turbid and contains high levels of suspended sediments, tannins and organic materials), change the filter and use a new one. Make a note and specify which volume has passed through each filter.*

Dry the filter

6. Remove the filter from the syringe, fill the syringe with air. Re-attach the syringe to the filter system and push the air through to dry out the filter. Repeat this step until the filter seems dry (i.e., no water drops are expelled from the filter holder).

Add preservative

7. Take a sterile syringe filled with ethanol beforehand and pass it through the filter. Stop when it starts to emerge in the outlet of the filter. Other suitable preservatives are Longmire's solution or other lysis buffer solutions.

Cap

8. Seal the outlet and inlet with the caps.
9. Place the capped filters into the re-sealable bag and note down geo-location (GPS) and date (Ideally, also temperature and estimated flow rate)

Note. At each sampling site, after filtering the samples, filter a sample of distilled water as a field filtering control and label it as such.

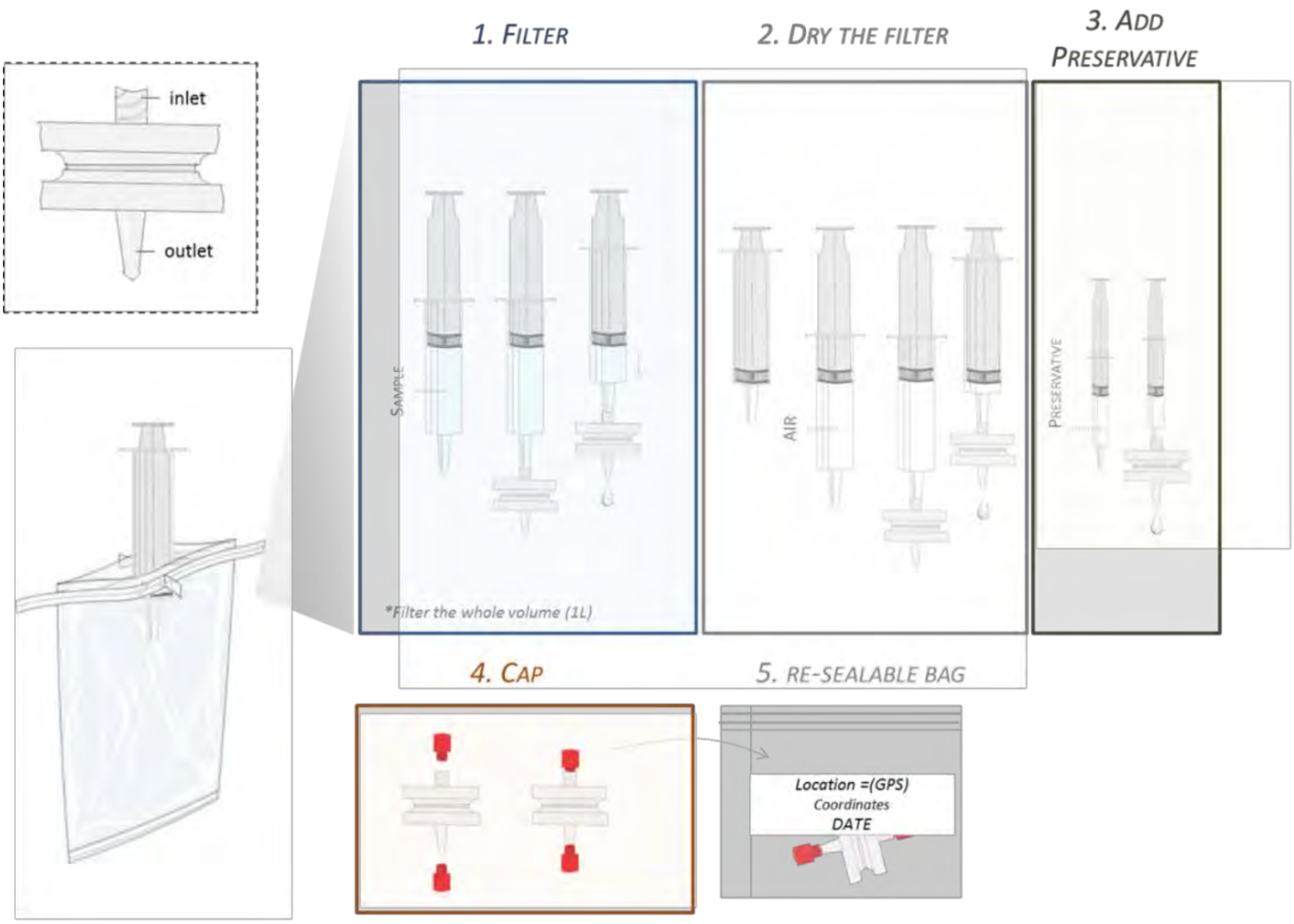

Figure 14. Graphical summary of on-site filtration protocol. Source: AMBER D2.5.

Figure 15. Photographic summary of on-site filtration protocol; water sample collection and filtration. Source: AMBER D2.5.

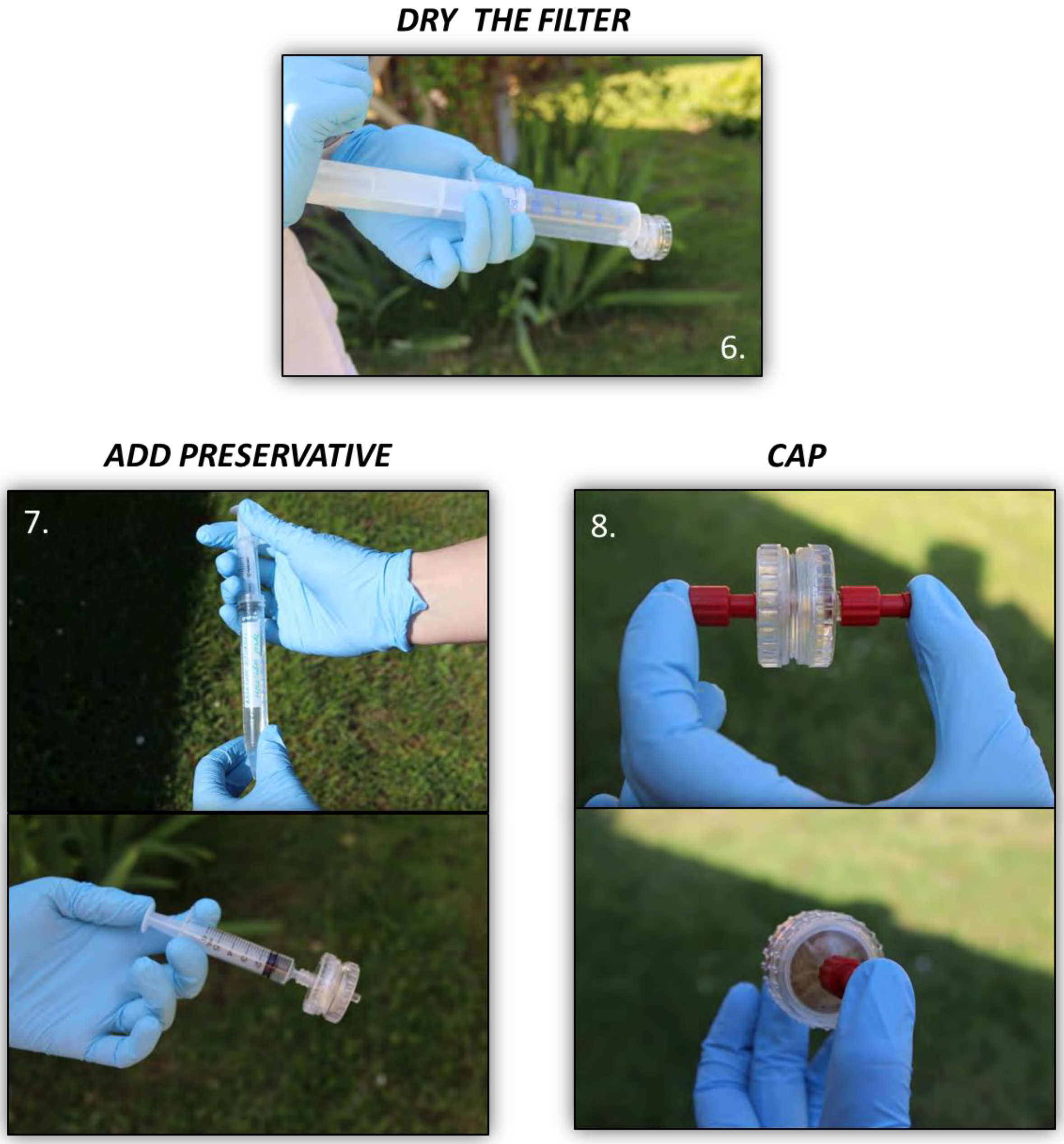

Figure 16. Photographic summary of on-site filtration protocol; dry filter, add preservative and cap. Source: AMBER D2.5.

4.9. Troubleshooting

If the sample collection kit (filtering system) gets contaminated or contamination is suspected, discard (place in a contaminated material black bag), take a new clean kit and repeat.

If equipment and waders need to be re-used (for example, if we are sampling different sites on the same day) de-contaminate by submerging the material in 10% bleach solution, rinse thoroughly with distilled water and let dry before using it again (between sites). A decontamination tub can be used for this purpose. Alternatively, sprayer containing 10% bleach solution can be used to atomize it over the surface to be decontaminated, which must be thoroughly rinse after. Bleach should not be disposed of into the river or nearby environments. If it is inevitable, it must be further diluted before disposal. River water can be used to that purpose. An outflow bucket will be handy in these circumstances.

As specified in point 5 of the sampling protocol, if filters get clogged during filtration before the total volume (1-L) can pass through the filter, use a new filter system to resume sampling until the total volume has been filtered. Label the plastic sample bags of each filter to indicate both the order of use and the approximate volume filtered (for example, 2 filters used for location 1 for replicate 1. Filter 1 used for that sample would be labelled “Loc1-sample 1-rep1A- 700 mL” and filter 2: “Loc1-sample 1-rep1B- 300 mL”)

After sample collection

- Keep the collected sample (encapsulated filters with added preservative) in a dry place, away from water, heat, and sunlight.
- Environmental DNA samples are stable for a variable period of time. Samples should be either processed (DNA extraction) or archived at -20 °C -80 °C soon after collection.
- If samples are going to be processed by an external laboratory, send them as soon as possible so DNA can be extracted, and down-stream processing performed.

5. Instream Habitat Assessment (MAT3)

5.1. The purpose and background of the tool

The Mesohabitat Simulation Model (MesoHABSIM) is an approach for modelling instream habitats. It consists of a data collection strategy and analytical techniques that allow the user to compute how much habitat is available for target fauna (typically fish) under specific environmental circumstances. It builds upon pre-existing physical habitat simulation models (for example, PHABSIM) to predict an aquatic community's response to habitat modification. MesoHABSIM modifies the data acquisition technique and analytical approach of similar models by changing the scale of resolution from micro- to meso-scales. Due to this increase in scale, the model takes variations in stream morphology along the river into account. Mesohabitat types are defined by their hydromorphological units (HMUs), such as pools and rapids, geomorphology, land cover and other hydrological characteristics. Mesohabitats are mapped under multiple flow conditions at extensive sites along the river. This allows modelling of available fish habitat at a range of flows. For model calibration, fish data is collected in randomly distributed mesohabitats where habitat surveys are also conducted.

The results of MesoHABSIM create the framework for integrative analyses of many aspects of the ecosystem. It also allows managers to recreate reference conditions and evaluate possible instream and watershed restoration measures or alterations, such as dam removals or changes in water withdrawals. From the perspective of resource managers, it not only allows for quantitative measures of ecological integrity, but also creates a basis for making decisions where trade-offs between resource use and river restoration need to be considered.

Research has shown that the combination of streamflow variability and river morphology (along with other factors such as water quality, energy inputs and biotic interactions) determine the composition and dynamics of stream ecosystems (Karr *et al*. 1986; Statzner *et al*. 1988; Minshall 1988; Townsend & Hildrew 1994; Poff & Ward 1989). Following the development of the ecological niche theory (Hutchinson 1957), it is clear that living organisms are affected not only by habitat quality but also by its variability. The development of physical habitat models builds on the observation that aquatic biota respond to physical changes in a stream at population level over long time periods but over a shorter timeframe. Physical variables are not the only factors affecting the abundance and health of riverine organisms (Karr *et al*. 1986), but their response to anthropogenic impacts are well known and are the easiest to measure (Stalnaker 1995).

In general, modelling of riverine physical habitat consists of two major procedures that together lead to an assessment of the impacts of various management options. Biological sampling is applied to determine habitat use by selected fish and/or invertebrate species. Spatial measurement provides a description of the morphologic/hydraulic habitat conditions in the study water body at a range of flows. Changes in these conditions with changing discharge can then be determined and variation in habitat suitability thus evaluated.

The selection of physical variables in habitat models stems from empirical studies which demonstrated the association of fish (and invertebrates) with particular physical and chemical aspects of their available habitat (Binns & Eisermann 1979; Jungwirth 1988; Milner *et al*. 1985; Rabeni & Jacobson 1993: Wright *et al*. 1993). The step was taken to link simple physical habitat use/description models to hydraulic models capable of predicting the variation of the key habitat variables in an incremental fashion (Bovee 1982; Gore & Nestler 1988). This allowed simulation of the relationship between stream discharge and an aggregate physical habitat quantity for life stages of a target aquatic species (for example, fish). Thus, researchers simplified the analysis of anthropogenic changes (Orth 1987) by modelling only the key habitat variables likely to change (which can also be measured relatively easily) and linking these with the preferences of aquatic species.

Data types

There are three major types of physical attributes that are incorporated in the habitat modelling process: hydraulics, geometry and habitat dressing. Hydraulics includes the basic data on hydrology of the investigated river section, including characteristic multiannual average flows (average low flow, low flow threshold, medium flow threshold). The first step in preparation of a MesoHABSIM survey is to select the range of flows that will be closely analysed in the model. Field surveys are conducted usually at low flows, up to a low/medium flow threshold. These may vary with project objectives as well as with the time of year of the investigation. The MesoHABSIM surveys consist of multiple observations of at least three flows within this range. This is the lowest number of observations necessary to develop habitat flow rating curves. At each flow condition, a set of measurements of water velocity and depths is taken in every HMU to assess the hydraulic parameter distribution.

The geometry of the river channel determines the number and distribution of HMUs on an investigated river section. This is assessed by the HMUs identification and measurement of water depth and substrate composition. In deep and narrow riverbeds runs, fast-runs, rapids and pools prevail, while in rivers with broad channels and flat banks, glides, backwaters, riffles, or side arms are more frequent. River slope is also a key factor for river channel geometry and habitat variability.

Habitat dressing describes the complexity and diversity of the HMU, related to suitability for various groups of aquatic organisms. Features such as canopy shading, woody debris, water vegetation, boulder rocks, shallow margins, undercut banks, etc. are noted for each HMU in a simple scale (absent, present or abundant). Land use on both shores is also registered.

Model types

The European Macrohabitat River Types (FCMacHT) was based on a number of physio-geographic factors associated with Expected Fish Communities (EFC). The nine river types distinguished at a continental scale are however broad, and various combinations of HMU types may be recorded in each type. The habitat survey with help of MesoHABSIM modelling is a next step to more precise determination of the actual conditions for aquatic organisms at a given site under various hydrological conditions. The next level of model complexity concerns bio-periods corresponding to various seasonal changes of aquatic ecosystems. This includes, for example, fish requirements for various life stages: spawning, juvenile growth, foraging and overwintering habitats. Fish assemblages are considered good indicators of river environmental state as well as riverine habitat suitability and availability (Schmutz *et al*. 2007a,b, Harrison and Whitfield 2004). Hence, fish fauna could be regarded as an "umbrella group" for other aquatic organisms and model evaluation is usually based on fish sampling. On occasion, in parallel with the fish survey, complementary assessment of macroinvertebrate distribution in various habitat types is conducted for more precise model adjustment. The HMU suitability for particular fish species or functional guilds can be assessed from the literature data on their habitat preferences. This could be done for various fish life stages or different bio-periods.

The flow diagram in **Figure 17** provides a step-by-step procedural guide.

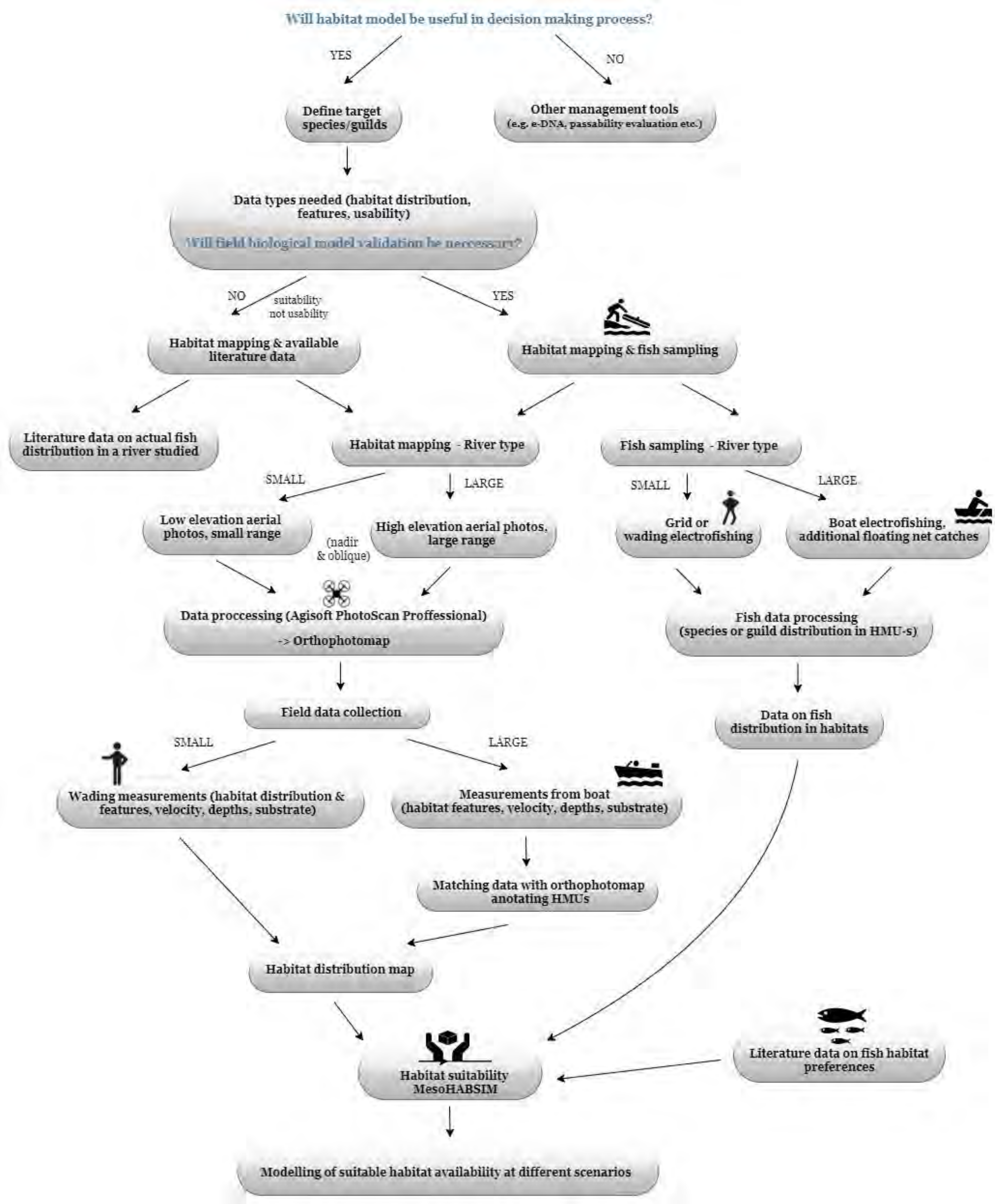

Figure 17. Decision tool and guidance for habitat model surveys (Study design). Author: K. Suska, SSIFI.

5.2. Equipment needed

Reconnaissance

Unmanned aerial vehicles are the tool used for creating current orthophotomaps of monitored river sections. These maps are used as a detailed background for the on-the-ground data collection. Multirotors such as "Phantom" (**Figure 18**), can be used to make orthophotomaps of small lowland rivers. To work on large rivers drone with increased range and stability for example UAV" Birdie" plane (**Figure 19**) may be necessary.

Figure 18. DJI "Phantom 4 Professional". Photo: J. Ligięza, SSIFI.

Figure 19. Plane UAV "Birdie", https://www.flytechuav.com/, accessed 03/11/2020.

Habitat mapping

The habitat mapping is conducted with commercially available mapping software and GPS equipped field computer, tablet or smartphone. For Windows computers, ARC Pad software (ESRI) can be used and on the android systems, T-Map application (TaxusIT). For depth and velocity measurements on small, wadable rivers a Dipping Bar can be used (Jens 1968, **Figure 20**) or electromagnetic flowmeter (for example, Marsh Mcbirney flow mate, **Figure 21**).

Figure 20. Jens Dipping Bar. Photo: K Celejewska.

Figure 21. Measurement of velocities with electromagnetic flowmeter Marsh McBirney. Photo: J. Ligięza, SSIFI.

Figure 22. Echosounder Hummingbird SI 998c mounted on a boat. Photo: P. Parasiewicz, SSIFI.

To collect the hydraulic data on large rivers, a commercially available Acoustic Doppler Current Profiler (ADCP) probe and Side Scan sonar (**Figure 22**) can be used for recording of riverbed structure. Both instruments are mounted on the boat, typically an inflatable type with an outboard motor. To avoid damage to the impellers, it is advisable to use the propeller's guard cage or jet engine.

For ADCP, River Surveyor or an equivalent unit with GPS and Bluetooth data connection can be used. As the Android application for operating the River Surveyor is no longer available, a Windows Mobile Tablet or ruggedized field computer is required. The River Surveyor Life software is free and available on the Sontek website (www.sontek.com). The software is designed specifically for the measurement of cross sections for discharge measurement, and therefore edge parameters (starting and ending shore distance) must be specified for each measured transect. Any one-digit number can be entered in the application.

For surveying riverbed structure, the Humminbird 998C SI Combo unit with external GPS can be used, however, there is a wide variety of similar equipment available. The Hummingbird has the ability to record the picture of the river bottom every second with GPS location. The data can be viewed and exported with third party software such as SonarTRX (Leraand Engineering Inc.).

For each electronic device, an appropriate power supply and backup power supply (for example, a power-bank) should be provided.

Fish sampling

For electrofishing with pre-exposed area electrofishing grids (PAE), a set of 2-5 grids is used (Bain *et al*. 1985). For small rivers, a grid has an area of 9m^2 with 6m length and 1.5m width. For larger rivers, it can be scaled up to 9x2m or larger. The grid consists of two cables – electrodes that are parallel to each other. These cables are attached to two PVC pipes at ends of each cable to maintain the same distance of 1.5m between electrodes. The PAE are plugged to the generator (for example, 1kW) and transformer (i.e., 15A), and this device may generate alternative (400V) or direct current (according to country-specific regulations).

Electrofishing in the case of the wading method (**Figure 23**) is conducted using portable aggregate which supplies 1.5kW. Fishing from the boat (**Figure 24**) is done with help of stationary 5kW aggregate. The anode is a round net with knotless mesh (5mm) netting, while the cathode is a plaited copper wire with a length of 1-2m (varying in line with water conductivity).

Figure 23. Wading electrofishing in a small river. Photo: J Szlakowski SSIFI.

Figure 24. Boat electrofishing on a large river. Photo: K Suska SSIFI

A floating net of 100m length, 2m height and mesh size of 40-80mm should be used, equipped with a start net buoy that will be well visible on water surface.

Additional fishing equipment consists of:

- devices for fish transport and storage (opt. 10-20l buckets and 70-100l containers)
- aerating system
- landing nets (5mm mesh), measure for fish length, protocols (digital or paper)
- portable seats and table

- additional fuel tank for aggregates and engine
- for large rivers fishing a boat with engine and paddles
- a set of 10 buoys with rope attached anchors for transect marking
- waterproof marks with numbers for samples identification

5.3. Preparation for fieldwork

Certificates

UAVS

In many countries a license is required to authorize the use of unmanned aerial vehicles. For example, in Poland, where aviation law is very strict, to obtain a UAV pilot licence (VLOS – Visibility Line of Sight/BVLOS), you must have: a current aeronautical-medical certificate, current liability insurance, theoretical and practical training and pass the theoretical and practical exam. In other countries it is necessary to contact the main aviation office regarding licensing requirements and requirements for obtaining the license.

Electrofishing

Due to risk of electrocution, the electro-fishing crew leader should have appropriate qualifications. In Poland, to lead an electro-fishing group, a qualification certificate for operating of electro-fishing tools is required. Also, the equipment used to electro-fishing must have a valid certificate issued by an authorized electrician.

Permits and Safety

UAVS

In many countries, aviation law sets the requirements for the flight of an unmanned aerial vehicle. The airspace is divided into zones where it is possible to fly without a permit, with a permit under certain conditions or where there are no flights, regardless of the weight of the flying model or unmanned aircraft.

In Poland, for example, major zones are distinguished (**Table 4**).

Table 4. Flight requirements in Poland.

ROL48 – the Government Protection Bureau issues flight requirements	
CTR – flights are coordinated with the Polish Air Navigation Services Agency (depending on the distance from the airport border, different requirements must be met and various flight conditions are issued)	
Other structures of the airspace where operations are carried out in the range of visual visibility:	
Solid structures (non-elastic) – most active H24 • TMA (Terminal Manoeuvring Area) • EP P (Prohibited Area) • EP R (Restricted Area) • ADIZ (Air Defense Identification Zone) • RMZ (Radio Mandatory Zone)	**Flexible structures (time of activity (UTC) given in the Airspace Use Plan on website (amc.pansa.pl)** • TSA (Temporary Segregated Area) • TRA (Temporary Reserved Area) • EP D (Danger Area) • ATZ (Aerodrome Traffic Zone) • MATZ (Military Aerodrome Traffic Zone) • MRT (Military Route) • EA (Exercise Area) • Regions of the Air Sport and Recreation Activity • Temporary areas of Increased Aviation Activity

The operator is obliged to:

- maintain direct eye contact with the unaided eye with an unmanned aerial vehicle
- secure and adapt take-off and landing area
- ensure that the unmanned aircraft gives priority to manned aircraft
- bear responsibility for the decision to perform the flight and its regulatory compliance
- check the technical condition of the unmanned aerial vehicle before the flight
- check the weather conditions and information about limitations in air traffic before the flight. (permanent and temporary).

Operating requirements for unmanned aerial vehicles (**Figure 25**) when used for purposes other than sport and recreation, include:

- marking of aircrafts by placing on them a rating plate containing information of the aircraft owner
- the unmanned aircraft must be equipped with warning lights to provide an all-round light emission, visible from above and below when flying earlier than 30 minutes before sunrise and later than 30 minutes after sunset
- when performing flights operator must have an operating manual together with preventive recommendations of the President of the Civil Aviation Office
- unmanned aircraft must be equipped with a Failsafe system programmed in a manner consistent with the preventive recommendations
- operator is required wearing a warning vest

During flights UAV operator must have on site:

- original and valid aeronautical-medical certificate
- original and valid UAV license
- valid document with a photo of the UAV operator
- the number of the valid insurance policy

Figure 25. Main rules for unmanned aerial vehicles, http://www.ulc.gov.pl, accessed 21/06/2018.

To explore a large area in zones separated from airspace open to aviation it is possible in Poland for example, to perform UAV flights out of the operator's eyesight (BVLOS – Beyond Visual Line of Sight). It is required that PANSA (Polish Air Navigation Services Agency) are contacted a minimum of 120 business days prior to scheduled flights.

Further information on the use of unmanned aerial vehicles and flying models in Poland and other countries can be found on the website of the Civil Aviation Authority (http://www.ulc.gov.pl, accessed 21/06/2018). There are also contacts with organizations and international agencies related to UAV, for example, the European Commission (http://ec.europa.eu/enterprise/sectors/aerospace/uas/index_en.htm), ICAO (https://www.icao.int/Search/pages/results.aspx?k=rpas&s=All%20Site), EASA (https://www.easa.europa.eu/easa-and-you/civil-drones-rpas/drones-regulatory-framework-background) and EUROCONTROL (https://www.eurocontrol.int/remotely-piloted-aircraft-system-rpas).

Habitat mapping

No specific permits except permission to access the premises are necessary. This is most often the case when working in National Parks and protected areas.

Electrofishing

To conduct electrofishing, it is necessary to have permission from the local government authorities responsible for environmental protection. In Poland for example, fishing permission is issued by the Voivodeship Marshal's Office, Regional Directorate for Environmental Protection and district fisheries. In the case of conducting research in a nature preserve, permission from the Minister of the Environment is required.

Electrofishing safety:

- the fishing group should be led by person with an appropriate qualification certificate
- the leader of the fishing group is obliged to train the employees of the group on operating the electro-fishing device and safety
- a member of the fishing group should know how to provide the assistance to an electrically injured person
- electro-fishing has to be conducted with a visibility of at least 25m, a temperature from minus 5°C to plus 35°C and a wind speed up to 4° on the Beaufort scale
- electrofishing is restricted in heavy wind, stormy weather and rainfall
- the fishing group should wear gloves and footwear not conductive of electricity (rubber boots, waders), polarized sunglasses are recommended
- during boat catches, the fishing crew have to be dressed in life jackets, it also recommended when wading in rivers with deeper sections (> 1m)
- it is forbidden to immerse any part of the body into water at a distance of less than 20m from working electrodes
- the electro-fishing equipment should be switched off when any bystander is less than 15m from working electrodes

Forms

Habitat mapping is most effectively accomplished using digital data collection tools, which allow drawing and annotating the observation on aerial photography. For this purpose, data collection software such as on Windows OS customized ARCMap, QGIS plugin Stream Map or on Android customized T-Map Application is required. The three options are presented below according to development chronology.

ARCMap

The customized mapping forms can be downloaded from www.rushingrivers.com. These should be opened with ArcMap software. Below, tabs on the forms that pop-up on the screen after the HMU polygon has been drawn on the aerial photograph in the ArcPAD software are described (**Figures 26** to **30**).

Site Details

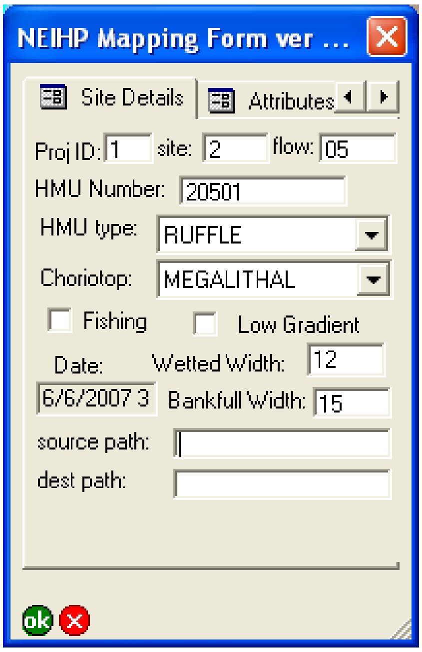

Figure 26. The site details tab is the first tab seen after completing a HMU polygon and includes some of the most important information for that polygon. Photo: P. Parasiewicz, SSIFI.

The information to be completed on the site details tab is:

- **Date:** Date of mapping
- **HMU Number:** Unique sequential numbering for that day
- **HMU Type:** See HMU Definitions section
- **Choriotop:** See Choriotop Definitions section
- **Fishing:** Check box if mapping during fishing survey
- **Low Gradient:** Check if surveying a low-gradient rivers.
- **Wetted Width:** Enter the current wetted width (in meters) obtained using the range finder.
- **Bankfull Width:** Enter the bank-full width obtained using the range finder.

Attributes

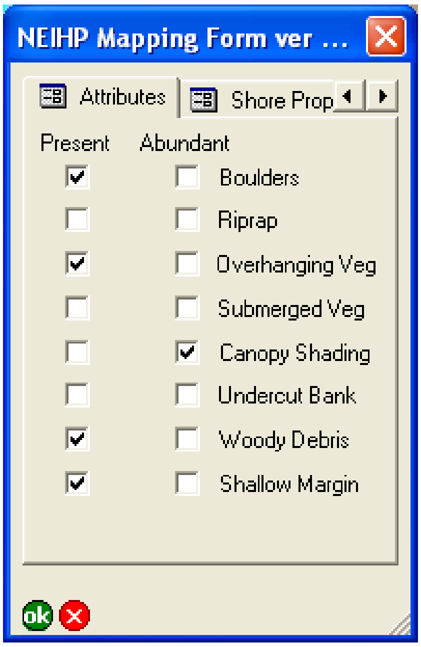

Figure 27. The attributes table. Photo: P. Parasiewicz, SSIFI.

Check the boxes to indicate whether the following are present or abundant: Boulders, Riprap (manufactured concrete erosion control), Overhanging Vegetation, Submerged Vegetation, Canopy Shading, Undercut Bank, Woody Debris, and Shallow Margin. There is no need to check both abundant and present; if abundant, presence is implied.

Shore Properties

Figure 28. The shore properties table. Photo: P. Parasiewicz, SSIFI.

Record Left Shore Use looking downstream; then repeat process for the Right Shore Use. Shore Use should be the adjacent land-use for that section, not necessarily the characteristic of embankment. These are: Agriculture, Field, Forested, Pasture, Residential, Road, Shrub brush, or Urbanized. Take into account the potential effects of local land-use in the watershed. Check boxes for: Eroded, Stabilized (non-rip-rap erosion control), Irregular Shoreline, Clay.

Comments

Figure 29. A text box is available for writing any comments associated with the mapping file. Photo: P. Parasiewicz, SSIFI.

This is helpful for locating specific features like temperature probes or unexpected stream alterations.

Help

Figure 30. The help table gives definitions of the dominant choriotop types entered in the Site Details table. Photo: P. Parasiewicz, SSIFI.

MapStream application
tMap application for android
The tMap application can be downloaded from http://www.taxusit.com.pl/en/Tmap. It is a GIS type Android app, where raster and vector data are presented in the form of layers. The raster data may be online maps from WMS (Web Map Service) or any orthophotomap.

In the upper right corner, there is a scroll-out menu to open project manager. The template project MesoHABSIM mapping template should be selected. This will upload two basic layers: mapping and hydro (**Figure 31**). Layer Mapping is a polygon layer, with an HMU attribute table attached (**Figure 32**). The hydro layer is a point layer with a table consisting of fields for entering measured hydraulic attributes (depth and velocity), substrate class and its embeddedness. Heading meaning are provided in **Table 5**.

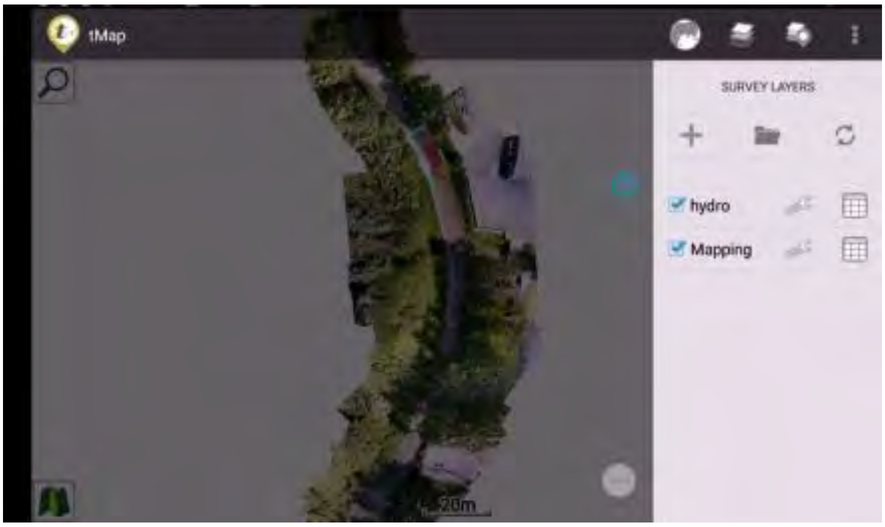

Figure 31. View of tMap display with survey layers menu scrolled down. Photo: P. Parasiewicz, SSIFI.

Figure 32. HMU attributes table associated with Mapping layer. Photo: P. Parasiewicz, SSIFI.

Table 5. Meanings of each heading

Name	Meaning
HMUNum	HMU Number (it is recommended that this number consists of 5-6 digits of which the last two indicate unit number, the middle two the estimated flow value during the survey and the first one or two the site number)
HMUType	Type of the unit
Choriotop	Dominant unit's choriotop (substrate)
FISHING	If the unit was fished (T/F)
BANKWIDTH	Average bankfull width of the unit (m)
WTTDWIDTH	Average wetted width of the unit (m)
LOWGRADIEN	Is the site on low gradient river type
PBOULDERS	Presence of boulders (T/F)
PRIPRAP	Presence of riprap at the banks (T/F)
POVERHGVEG	Presence of oerhangin vegetation (T/F)
PSUBMDVEG	Presence of submerged vegetation (T/F)
PCANOPY	Presence of canopy cover shading (T/F)
PUNDERCUT	Presence of undercut banks (T/F)
PWOODYD	Presence of woody debris (T/F)
PSHALLOW	Presence of shallow margins (T/F)
ABOULDERS	Abundance of boulders (T/F)
ARIPRAP	Abundance of riprap at the banks (T/F)
AOVERHGVEG	Abundance of overhanging vegetation (T/F)
ASUBMDVEG	Abundance of submerged vegetation (T/F)
ACANOPY	Abundance of canopy cover shading (T/F)
AUNDERCUT	Abundance of undercut banks (T/F)
AWOODYD	Abundance of woody debris (T/F)
ASHALLOW	Abundance of shallow margins (T/F)
LSHOREUSE	Left shore use
LCLAY	Clay on left bank
LERODED	Left bank eroded
LSTABLE	Left bank stabilized
LIRRGRSHOR	Irregular shore at left bank
RSHOREUSE	Right shore use
RCLAY	Clay on right bank
RERODED	Right bank eroded
RSTABLE	Right bank stabilized
RIRRGRSHOR	Irregular shore at Right bank
POSITION NUM	Number of the vertical measured in HMU
CHORIOTOP	Substrate type at vertical location (1m^2 radius)
TSE	Tauchstabeinheiten – torque units of Jens Dipping bar
DEPTH	Column depth in vertical (cm)
EMBEDDED	Embededness (loose, embeded,s olid)
VELOCITY	Mean column velocity in vertical (cm/s)
Flow	Estimated survey flow (in flow/watershed area)
COMMENT	Comment

These tables can be easily modified by adding additional fields. This function is provided in the layer menu of the programme (**Figure 33**).

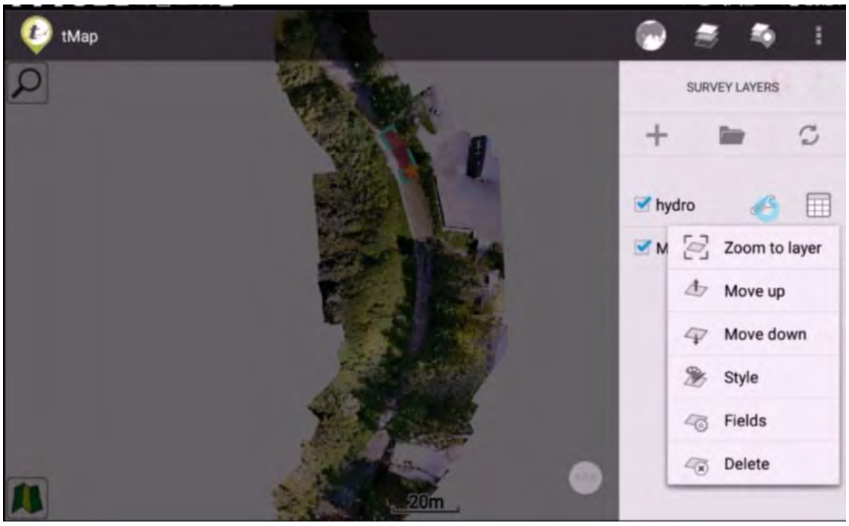

Figure 33. tMap display with layer setup menu scrolled down. Photo: P. Parasiewicz, SSIFI.

The tables are the background for the data entry forms that appear when you draw the polygon or a point (see below). The final option for data collection is sketching the HMU on printed aerial imagery and entering the data into a paper form presented below (**Figure 34**).

Mapping sheet

Date:________ Hour:________ Name:___________________________

River/Site/Place:____

HMU No:_____

Type of hydromorphological unit:

rapid cascade riffle ruffle fast run run glide plunge pool sidearm pool backwater complex low complex high

Gradient: ☐ *low* ☐ *medium* ☐ high

Substrate (choose/select occurring in significant quantities):

☐ Megalithal (>40cm, big boulders)
☐ Macrolithal (20-40cm, hand to head)
☐ Mesolithal (6-20cm, fist to hand)
☐ Microlithal (2-6cm, egg to fist)
☐ Akal (fine gravel)
☐ Psammal (sand)
☐ Detritus (organic debris)
☐ Xylal (branches and roots)
☐ Sapropel (organic sediment)
☐ Phytal (plant remains, floating mate/plants)
☐ Debris (eg. shells)
☐ Pelal (silt, clay)

Covers (present = check, abundant = fill in the square):

☐ underwater vegetation ☐ shallow margin left shore ☐ undercut bank ☐ canopy shading
☐ boulders ☐ shallow margin right shore ☐ woody debris ☐ rip rap
☐ overhanging vegetation

Depth [cm]	TSE	V [cm/s]	Substrate	Embedment rate (solid; embedded; loose)

Figure 34. Form used for mapping containing substrate classification. P. Parasiewicz, SSIFI.

Background information needed (temperature, flow etc.)

Before starting the field work, the flow and depth of the water on the investigated section of the river should be checked.

Habitat mapping is carried out most often at low flow conditions (below low flow pulse threshold obtained with Indices of Hydrologic Alteration software available from The Nature Conservancy, www.conservationgateway.org). The equipment used should be adequate for the size of the river and the current depth of water. Prior to taking pictures with the UAV, the availability of airspace areas, detailed weather forecast (wind, temperature, rainfall, humid air) and current solar activity needs to be verified. Prepare the background aerial imagery obtained by drones and upload the background map layer as a raster file to the mapping application. WMS map or import aerial photography collected with drones may be used. **Figure 35** demonstrates the tMap view with uploaded orthophotomap.

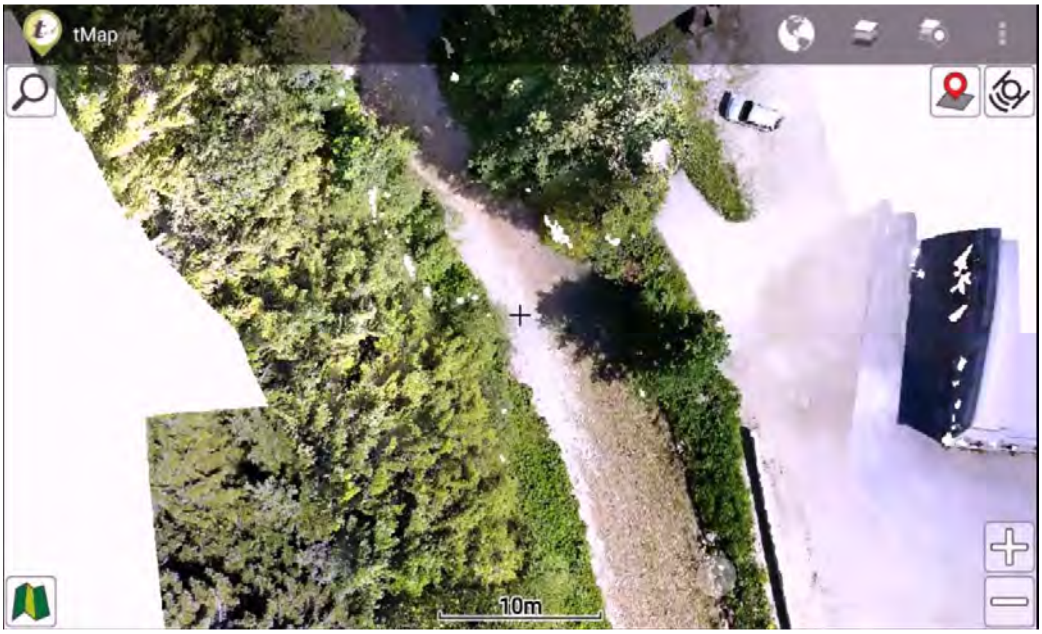

Figure 35. View of the aerial image from drone imported to tMap. In the top right corner are project settings menus. In the lower left edit menus. Photo: P. Parasiewicz, SSIFI.

Caution: if the raster files have high resolution and are therefore very large it will take very long time for application to upload it and it will be very difficult to navigate through the map. The resolution in the GIS environment can be lowered. In QGIS you can use the WARP tool for this purpose.

Procedure:

a) Open the obtained raster file in QGIS, highlight and choose "**WARP**" procedure from **"Raster/Projections"** menu. The menus presented in **Figure 36** and **37** will pop up.

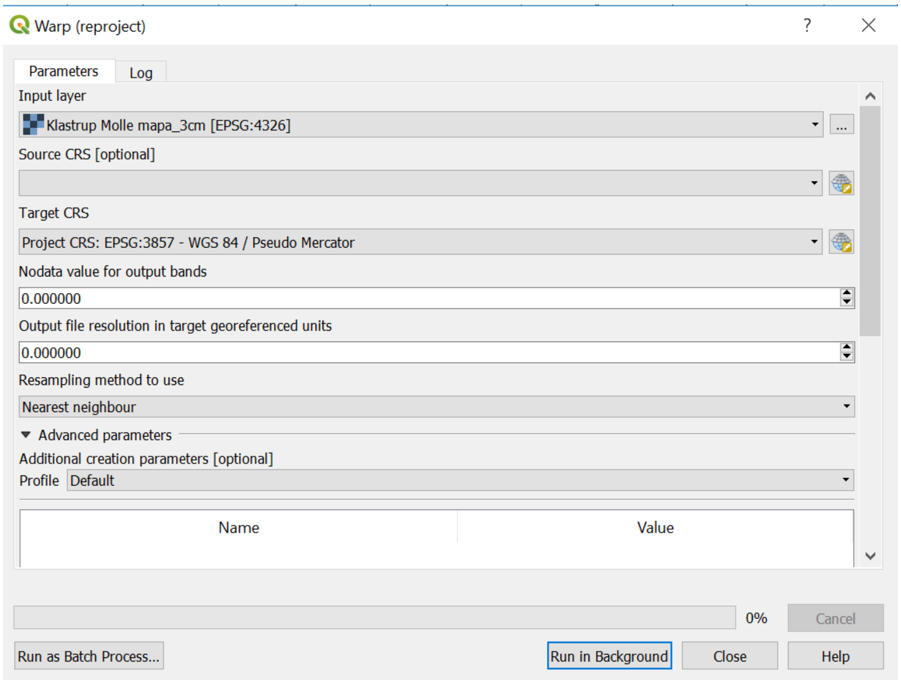

Figure 36. Upper section of the WARP tool dialog window. Photo: P. Parasiewicz, SSIFI.

b) Choose target CRS that is supported by Tmap (for example, EPSG:3857 – WGS 84/ pseudo Mercator
c) Choose output file resolution (for example, 0.05 for 5 m)
d) Scroll down to advanced parameters

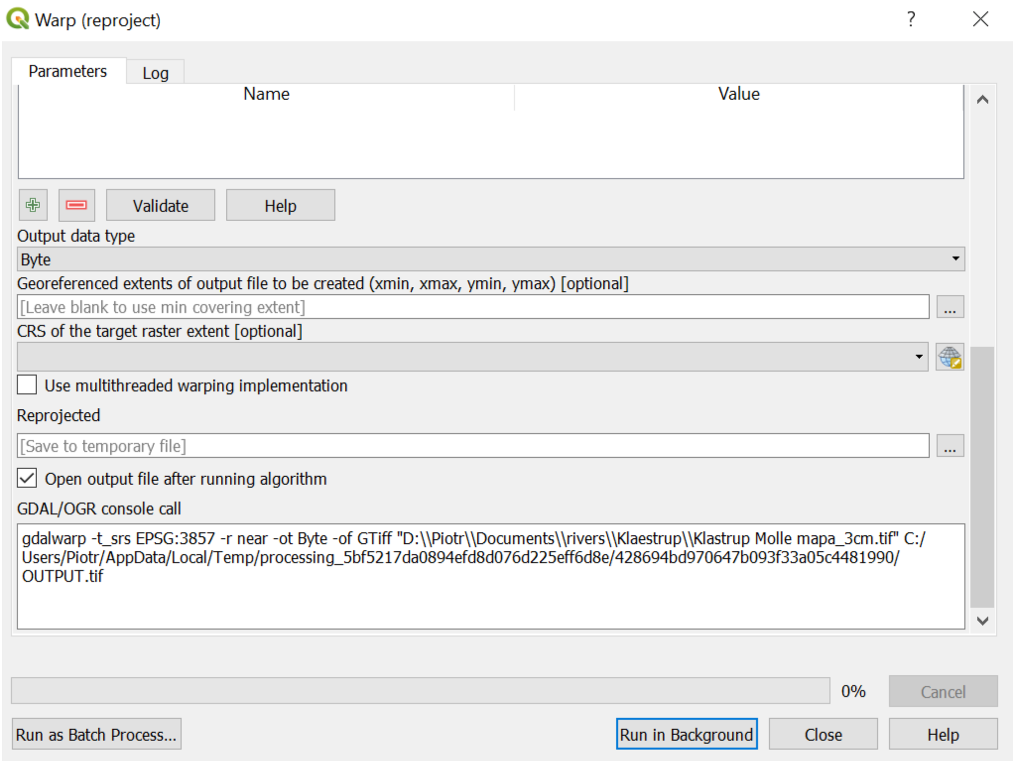

Figure 37. Lower section of the WARP tool dialog window. Photo: P. Parasiewicz, SSIFI.

e) Setup "**Output data type**" to **Byte**.
f) If the layer size is over 150,000KB, the photo will need to be broken down into smaller sections. To do this, select "**…**" next to "**Georeferenced extents of output file to be created**" and pick "**Select extent on canvas**" from the scroll out menu. Select the appropriate portion of the map (**Figure 38**).

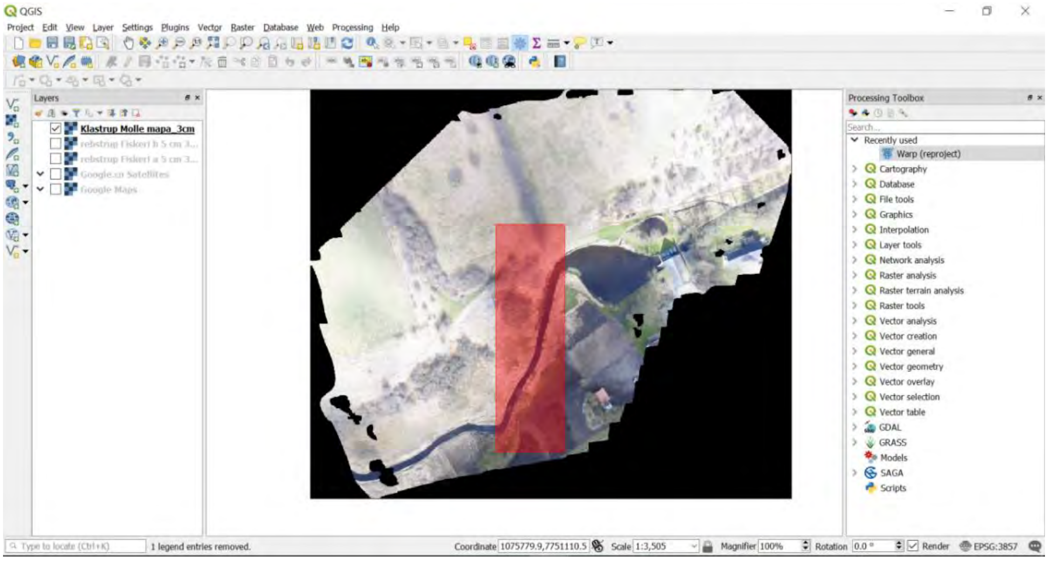

Figure 38. View of drone obtained orthophotomap in QGIS with the selection box. Photo: P. Parasiewicz, SSIFI.

g) Select “**CRS of the target raster extent”** (same as before)
h) Hit **“…”** next to “**Reprojected”** and chose save to file, then enter the output file name.
i) Hit “**Run in Background”** (**Figure 39**)
j) Copy created raster file to the to the Tablet and import to Tmap.

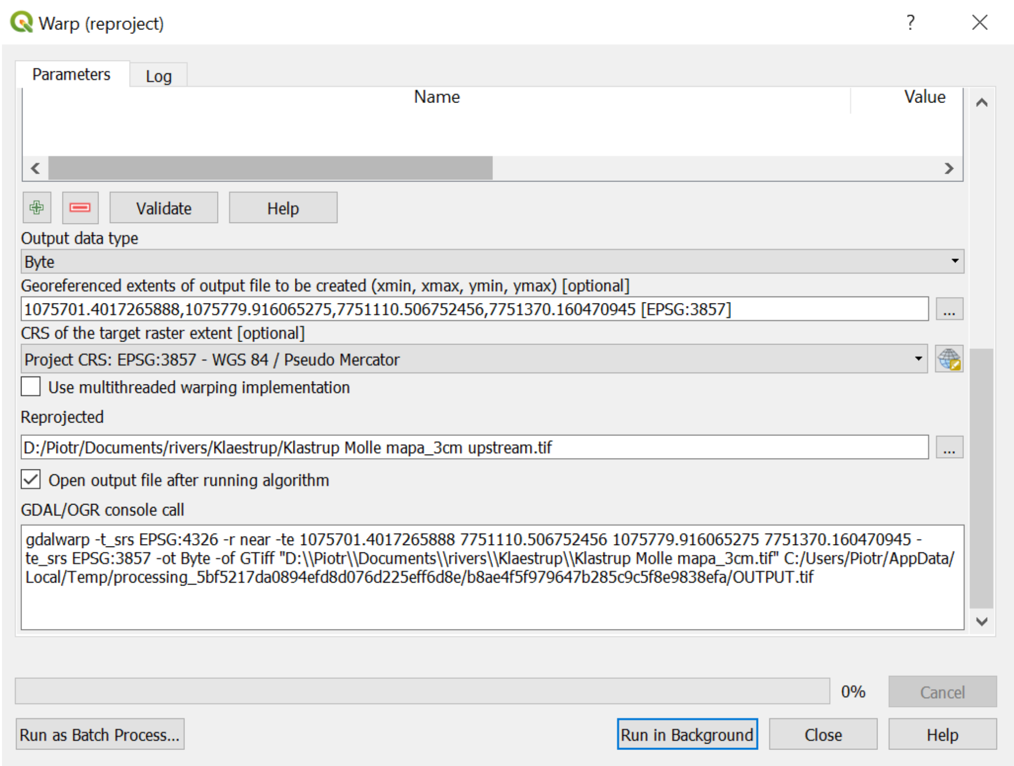

Figure 39. View of completed lower part of WARP dialog box. Photo: P. Parasiewicz, SSIFI.

Caution: ensure that the right projection is used, such that the orientation and size of the map matches GPS locations. Prior to leaving for the fieldwork, you should test the proper location in GIS environment or on in tmap by importing one of the Online WMS maps (for example, Google maps).

Location selection
When planning the collection of data from a given area, it is necessary to plan the order of the examined sections of the rivers, establish the targeted flow conditions and consequently the number of necessary surveys. It is recommended that a table is prepared with target flow, site and survey date to keep track of project progress and to estimate the possible time frame and schedule for the fieldwork. In the case of taking aerial photos, the number of missions (dependant on the size of river and UAV) needs to be planned and appropriate battery supply secured.

Depending on the size of the river and its surroundings, the locations of survey starting points should be identified: a place for launching a boat or a safe descent into the water. Logistics for the number of team members in the field, need to be prepared, for example, lodging close to the survey site. During mapping surveys, it is important to use daylight as much as possible. Therefore, an early start time is recommended, with it being preferable to not leave the site until the survey is completed.

5.4. Data collection process

Small rivers

Habitat mapping
The habitat survey's goal is to determine the spatial proportions of mesohabitat units in selected river sections. Mesohabitat units or Hydro-morphological Units (HMUs) are river sections with similar morphologic, hydraulic, and cover attributes (i.e. pools, riffles, runs). For each HMU, the location is determined with GPS in conjunction with high-resolution aerial photographs, creating a detailed map of selected sites on the river. The outlines of each HMU are drawn as geo-referenced polygons on a surveying unit such as an Android tablet.

Mapping teams are made up of one HMU mapper and at least one hydraulic sampling technician. The HMU mapper is the team leader and responsible for delineating the unit, relaying information to the hydraulic technician, and recording all of the data for the unit.

For the mapper: at the beginning of the survey, open the Layers menu in tMap and select layer "Mapping" (**Figure 40**).

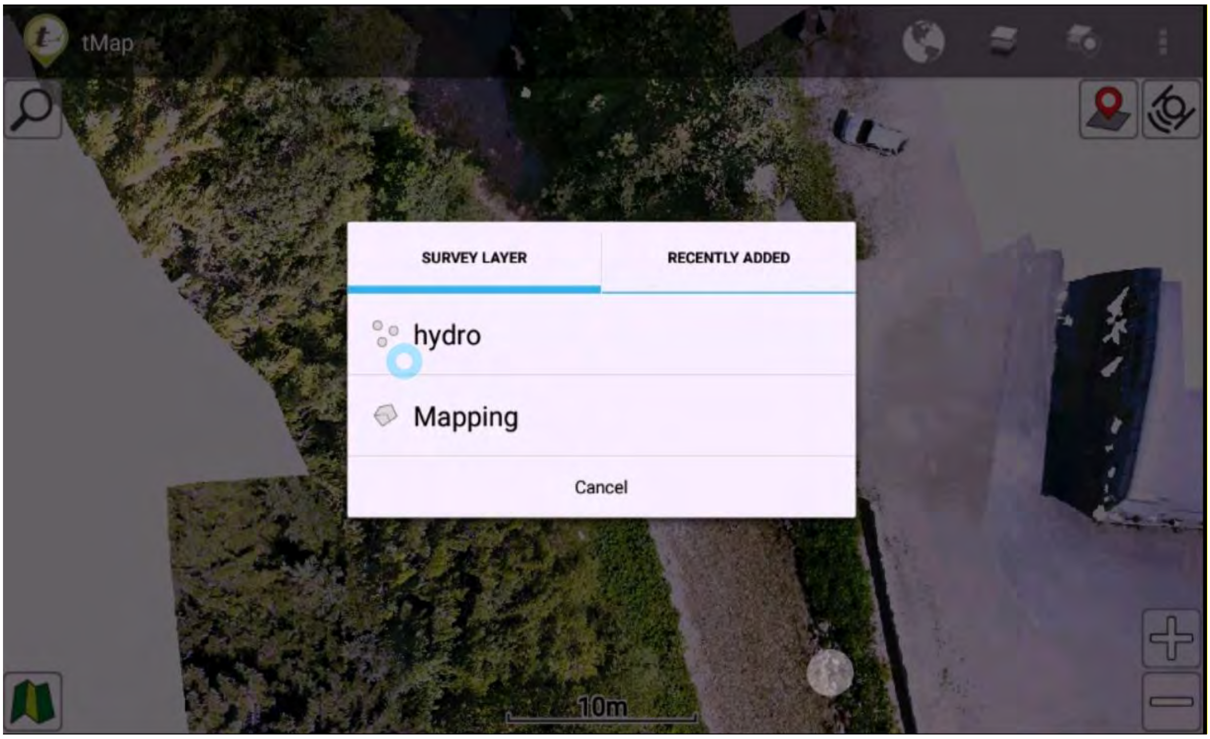

Figure 40. Layers menu of tmap. Photo: P. Parasiewicz, SSIFI.

Begin mapping an HMU by walking through the HMU to identify boundaries. Locate the beginning and end of the HMU. Hit the red location mark button (upper right) to start the survey. Draw the polygon on the aerial image as accurately as possible, clicking points along the boundary of the HMU (**Figure 41**). Click the green check sign and the HMU data collection form will appear (**Figure 42**).

Figure 41. Screenshot of the T-map settings during polygon annotation. In the left lower corner, input devices can be selected: GPS, laser range finder or finger. The second button allows the drawn polygon to be edited. The middle button is activated to allow drawing and the one to the right of this allows removal of the most recently drawn point. The green check mark closes the polygon and opens the attribute table. Photo: P. Parasiewicz, SSIFI.

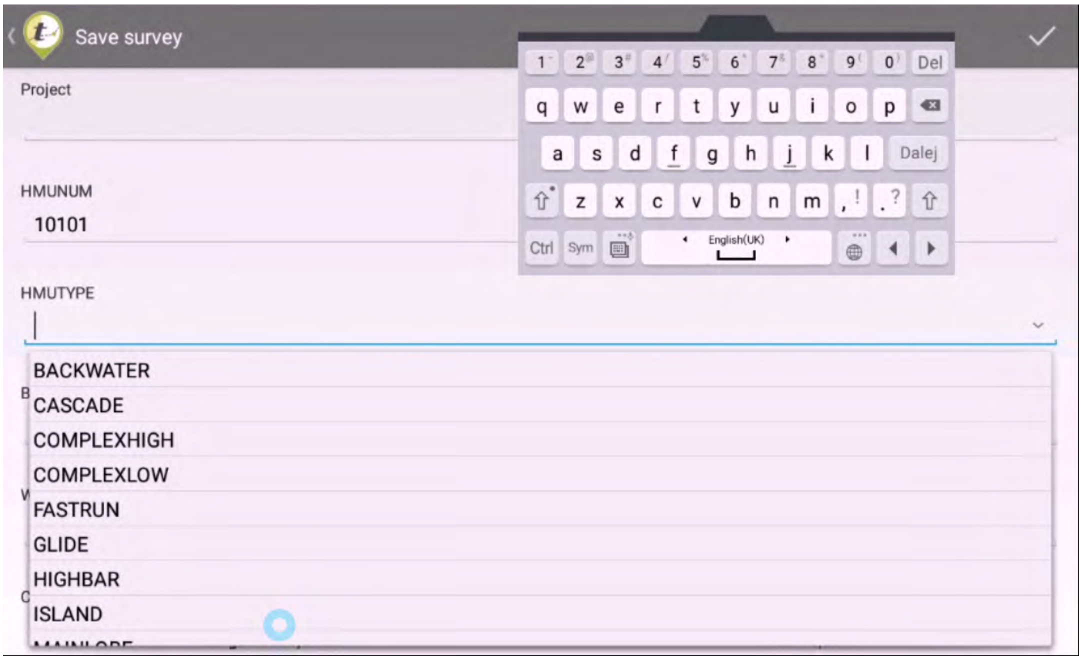

Figure 42. Form for entering HMU attributes. The items can be entered or selected from scroll out menus. Photo: P. Parasiewicz, SSIFI.

Enter the project name, enter the HMU number and select HMU type from scroll out menu (**Figure 42** and **Table 6**), as well as the dominant choriotop. If it is low gradient, indicate that here.

Table 6. Characteristics of HMU – hydro-morphological units (Dolloff *et al*. 1993; Bisson and Montgomery 1996; Perkins 2002; Parasiewicz 2007a, Parasiewicz *et al*. 2013).

HMU	Hydromorphological unit characteristics
Riffle	Shallow areas, steep gradient, convex streambed shape, moderate water velocity, surface turbulence.
Rapid	High gradient reaches, convex streambed shape, fast water velocity, coarser substrate, strong surface turbulence.
Cascade	Stepped rapids with small waterfalls and pools below boulders
Ruffle	Similar to rapids, but at lower water flow, habitats in transition to either run or riffle
Plungepool	Main flow falls from a step and scour the streambed making a deep pool with high turbulence and back current
Fast run	Fast-flowing stream channels with thalweg shape visible on the water surface,
Run	Uniform stream channels with well-determined thalweg. Streambed is laterally concave (U-shape or V-shape)
Pool	Deep water areas with concave streambed shape, slow current and no turbulence
Glide	Stream stretches of uniform, flat streambed, with laminar flow and lack of turbulence
Backwater	Stagnant water areas, sometimes with slow back current, placed along channel margins, behind obstructions
Sidearm	Channel branches (smaller than half river width), usually located at other elevation than main riverbed.
Complex-low	Low lying sand or gravel bars interspersed with shallow channels with low velocities and generally shallow waters. The “Low” refers to the ratio of water to wet sand (< 50% of unit is water)
Complex-high	Low lying sand or gravel bars interspersed with shallow channels with low velocities and generally shallow waters that contains a “High” ratio of water to sand (> 50% of unit is water). Typically, there are wider channels separated by small, partially submerged bars and the depths and velocities of the unit are higher.

For each HMU, information on habitat features relevant to aquatic organisms is gathered during the field survey. This includes absence, presence or abundance of the following cover elements: submerged vegetation, woody debris, boulder-rocks, shallow margins, riparian vegetation cover, etc. (see **Figure 43**). In the forms provided, absence is assumed as a default value, it is only necessary to make a selection of the attribute value if it is present or abundant. Abundance is assumed if this attribute occurs at more than 30% of unit area or on both shores.

Figure 43. Form for entering HMU attributes. The items can be entered or selected from scroll out menus. Photo: P. Parasiewicz, SSIFI.

Carefully note the bank attributes, always mapping facing downstream. If additional comments such as point source pollution, drainage pipe, or any other important feature about the HMU are present, proceed to the **Comment** page (**Figure 44**).

Figure 44. Form for entering HMU attributes related to shore attributes. The presence of an attribute can be indicated by choosing "yes" from be indicated by choosing "yes" from the scroll out menu. Photo: P. Parasiewicz, SSIFI.

Tap **check** when all the information is filled in. Clicking **Cancel** will delete all information, including the polygon that has just been drawn.

For the sampling technician; measure hydraulics at least 7 stratified random location in each HMU. For this, where the HMU ends must be confirmed. First, visually estimate hydraulic units or strata (areas with similar hydraulics, for example, shallow, slow) and the proportional area in the HMU. According to these proportions, allocate the number of measurements for each strata and random locations within a series of hydraulic measurements are taken. These measurements comprise of substrate type, substrate embedment, water depth and mean water column velocity. Communicate the measurements to the mapper. To enter the data on the tablet, the mapper is to open the hydro layer and draw a point at the estimated location of the measurement (see **Figure 45**).

Figure 45. Screen shot of the stream map with annotated hydraulic measurement point. Photo: P. Parasiewicz, SSIFI.

The appropriate data entry form then appears, and the mapper enters the information into the form (**Figures 46** and **47**).

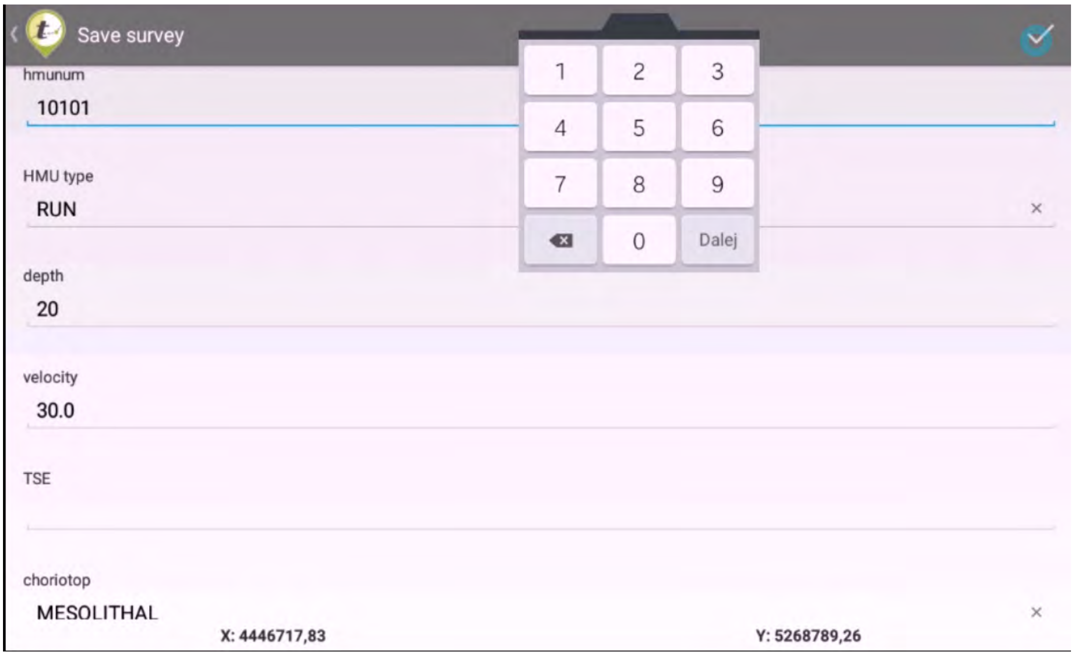

Figure 46. Top of hydraulic data entry form for hydraulic data. Depth and velocity are entered in cm and cm/s respectively. Photo: P. Parasiewicz, SSIFI.

Figure 47. Bottom of hydraulic data entry form for hydraulic data. Position number indicates the number of the measurement in HMU. Photo: P. Parasiewicz, SSIFI.

The check mark in the upper right corner saves the data and indicates that the next point may be proceeded to. The tMap settings menu allows the setup so called "**Persistent attributes"**, which means that the data just entered will be used as a default for the next measurement. In this case, it should be noted that counting position numbers should be restarted as soon as the team move to the next HMU.

If there is no available data regarding flow conditions at a given site, this should be measured. For this purpose, a uniform river profile should be selected, and a series of depth and velocity measurements taken every 50cm of river width. Substrate classification uses the Austrian Standard ÖNORM 6232 (1995). It is worth noting that same HMU type may look different in various river typesm for example, in a mountain river, riffle is usually formed by stones and gravel, while in a lowland river it may occur on woody debris accumulated between the shores.

Habitat survey for fish

The electrofishing surveys are conducted parallel with hydromorphological unit mapping on the river. For this purpose, pre-exposed area electrofishing grids (PAE) are usually used. Once the grids are deployed in HMUs, it is necessary to wait at least 10 to 15 minutes before electrofishing is started. This time is the minimum period for fish to return to their habitat (Bain *et al*. 1985). In each of the selected HMUs, from 2 to 5 grids are located depending on the area of investigated habitat. HMU complexity (for example, amount of cover, water velocity and depth or substrate variability) should be represented within the sampled area. The species of all captured fish is determined, and total length is measured with accuracy to within 1mm. After the measurements are taken, fish are released to the river downstream from the analysed reach to avoid catching them in the next samples. For each grid location, specific environmental variables on HMU type, river location, habitat characteristics: stream depth and velocity, substrate, gradient and covers are recorded as described above.

The other available method that may be applied is electrofishing in accordance with fish based ecological status monitoring standards (CEN EN 14011 2003). When the wading method is used, samples are taken using portable aggregate. The operator moves against the river current in an upstream direction, and in the case of narrow watercourses, by moving diagonally from one bank to the other. The minimum length of selected sampling sites is 100m. To assess fish distribution in particular HMUs, this method should be modified. Prior to electrofishing, HMUs are delineated and all measurements are carried out as described above. In each HMU, a transect of known lengths (five to 20m depending on habitat patch size) covering habitat diversity is marked with small buoys. Every transect is sampled separately by wading against the current (the minimum time for fish return to their habitats before sampling starts should be maintained). All other procedures are same as for PAE sampling.

Both electrofishing methods are applicable and effective in rivers of a depth up to 2m.

Support Team Size

River habitat mapping could be performed by at least a two-person team, while electrofishing with PEA and the wading method require a three to four-person team. Five person teams are optimal, taking into account that one site should be mapped and fished the same day (at uniform water flow conditions).

Task Allocation
Habitat mapping involves at least two persons who are wading in the river: one is operating the field computer with an application allowing recording of HMU features descriptions and marking of GPS points. The second person is taking measurements of water depth and velocity, as well as checking the bottom substrate composition and embedment rate (visually or by foot kicking) and reporting results to the first person.

During PAE electrofishing, teams of two persons are placing the grids in delineated HMUs. Then, after the time for fish accommodation, one person is operating the generator (and safety pedal switching it off), while two persons are catching fish with landing nets and the forth person is collecting the catch with a bucket and transporting it to grid-specific containers on the shore. After one grid is sampled, the generator is plugged to the next grid and the fishing is repeated. When one HMU is fished, the team starts taking fish measurements: one person takes fish length measurements, the second person notes the results (separately for each grid) and optimally the third one handles the fish and releases them. The process is repeated in the next habitats.

When wading or boat electrofishing is performed, one or two persons are placing the buoys marking the transect in delineated HMUs. Then, after the time for fish accommodation, one person is carrying the portable aggregate, a second person is operating the anode (and safety switch), the third catches fish with a landing net and the forth collects the catch with a bucket and transports it to a transect-specific container on the shore. The fish measuring scheme is as described above.

Step by step

1. Taking photos by UAV and background map preparation

2. Mapping:
    - upload the digital map to the field computer
    - delineate HMUs as polygons
    - describe HMU dressing
    - take depth, velocity, substrate measurements and mark point locations on the map (minimum,7 replicates)

3. Electrofishing PAE:
    - Locate the grids in delineated HMUs
    - prepare the electrofishing device, while waiting for fish accommodation
    - plug the generator to a grid
    - set the fishing team near a grid
    - fish for and collect the catch
    - move to the next grid – procedure repeated
    - take fish measurements after one HMU is sampled
    - take depth, velocity and substrate measurements in grid corners
    - move grids to the next HMU (optimally parallel to fish measurements)

4. Electrofishing wading:
   - mark and locating the transects in delineated HMUs
   - prepare the electrofishing device, while waiting for fish accommodation
   - fish on transects in one HMU and collect the catch
   - take fish measurements after one HMU is sampled
   - procedure repeated in next HMU (optimally transects marking this prepared parallel to fish measurements)
   - take depth, velocity, substrate measurements at transect edge points

Large rivers

Habitat mapping on a large river needs to be conducted in post-processing. The mapping procedure utilizes data collected during the survey consisting of aerial imagery and depth and velocity measurements using GPS positioned ADCP on the boat.

Taking UAV photos

The UAV is equipped with a GPS system and after calibration by the operator, it receives a signal from navigation satellites. After successfully connecting to at least six satellites, the vehicle can switch to ready-to-fly mode. The operator then can cover the chosen area with sufficient accuracy, enabling orthophotomap creation. The operator must control the camera and ensure that it is facing downward during taking pictures *(***Figure 48***).* The operator should continuously check the information transfer between the remote-control device and UAV, and also oversee the proper battery charging for the drone, remote control and tablet. During the flight, the operator is constantly supported by the information given by the observer. Finally, after each mission, the operator must safely bring the UAV to the ground.

Figure 48. DJI 'Phantom 4 Professional' taking photos above Polish large lowland river near Wloclawek dam, Vistula River. Photo: J. Ligięza SSIFI.

Preparing the orthophotomap

From the collected photos, an ortho-photomap should be created. Commercially available software such as Agisoft PhotoScan Professional can be used. It consists of eight main commands for map creation (in the 'Workflow' window):

- "Add Photos/Add Folder"
- "Align Photos"
- "Build Dense Cloud"
- "Build Mesh"
- "Build Texture"
- "Build Tiled Model"
- "Build Dem"
- "Build Orthomosaic"

Each of these commands have different parameters that can be changed and adjusted to current photos. Finally, "**Export Orthomosaic"** command in a '**File**' window is used to create a georeferenced ortho-photomap. The entire process of operation is explained in detail in the User Manual on the Agisoft website (http://www.agisoft.com/downloads/user-manuals/, available 28.06.2018).

Collecting hydraulic and bed structure data

Prior to beginning a hydraulic survey, ensure that all batteries are charged, and the equipment is functional. Mount the instruments on the boat and make sure all are securely attached. Both probes are setup for the same time and synchronized.

The task is to collect sufficient data samples to support habitat classification and habitat modelling. Since, at the time of the survey, the distribution of HMUs may not yet be determined, as much ground as possible should be covered to obtain sufficient data to calculate depth and velocity distributions. The most effective method is collecting data moving the boat in diagonal “zigzags” up and down stream, such that a series of cross section is measured in a distance from each other shorter than half of the river width (**Figure 49**). Backwaters and deep shore pools should all be captured. In shallow areas, fall back on measuring 10 or more verticals with a current profiler using procedures such as the one for smaller rivers.

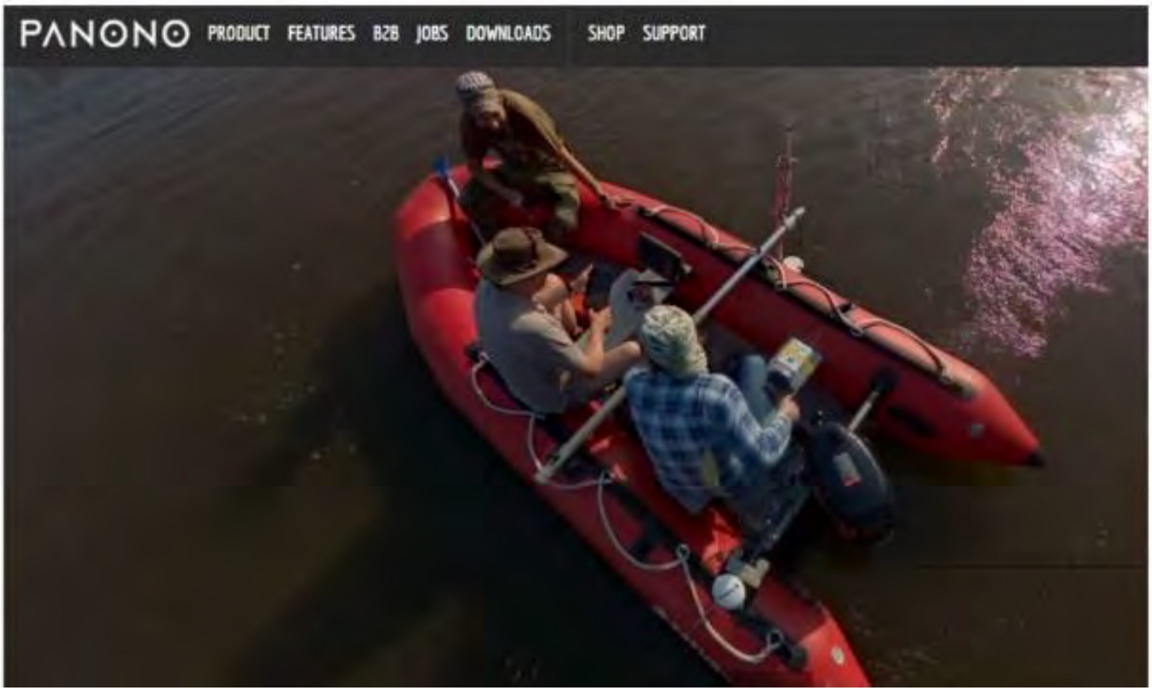

Figure 49. Hydraulic survey using Acoustic Doppler Profiler (mounted to the side) and side scan sonar and spherical camera. Photo: P. Parasiewicz, SSIFI.

If there is any doubt about the flow in the river (i.e., no recording gauge station in proximity to the site), use the equipment to measure current discharge at at least one cross section at the beginning and at the end of the survey.

When collecting data with River Surveyor units, use the River Surveyor Live software. Follow the manufacturer instructions to gather the velocity and depth data (for example, SONTEK/YSI 2010).

Habitat sampling for fish

Habitat-specific fish sampling at large rivers needs prior HMU delineation based on aerial mapping and measurements of depth, velocity and substrate composition as described above. The fish sampling should be done at almost the same flow conditions as mapping, so optimally this should be conducted the next day. On large rivers, electrofishing should be conducted with certified 5KW aggregate from a boat moving slowly along earlier-marked sampling lines. In each HMU, transects of known lengths (10-50m depending on habitat patch size) set out pairs of buoys connected with a 12m long rope to demarcate fishing plots. The first buoy is anchored and the second floats on the rope (see schematic, **Figure 50**). Starting downstream, sample one, 2m wide pass along the rope. In large HMUs, multiple plots can be sampled. For each fished plot, the catch is collected in separate containers. Fish measurements are conducted at the shore after one HMU is sampled.

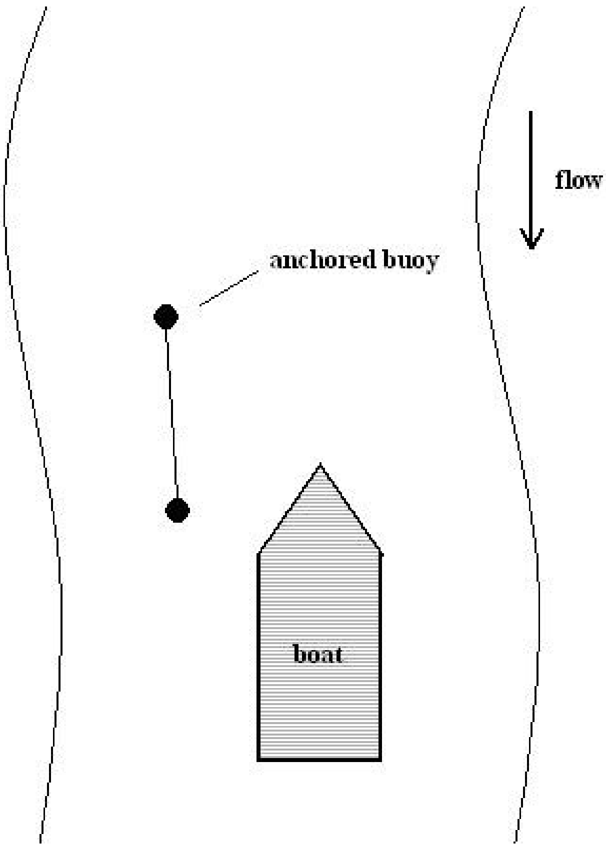

Figure 50. Schematic design of electrofishing in large rivers. Author: P. Parasiewicz, SSIFI.

It needs to be emphasized, that in large rivers electrofishing methods have some limitations. In deep and fast flowing river sections, the effectiveness of this technique is low, especially for large fish specimens of bream, barbel, asp or catfish. For such habitats, only near-shore zones with juvenile fish assemblages can be sampled to assess the general species composition, but not the exact abundance and size structure. Some additional information could be gathered by observations of fish behaviour, for example, predator activity. For a detailed model verification, a combination of electrofishing and floating net catches is recommended. The chosen location for conducting the floating net catch should be characterized by a uniform bottom profile (lack of submerged trees, coarse boulders etc.) to avoid damaging and tangling the net.

Support Team Size

- For data collection with a UAV, two persons are needed: operator and observer.
- For hydraulic data collection, two persons are needed: boat operator and surveyor.
- For boat electrofishing a team of at least five persons is needed. All of them should be experienced in boat operation.
- Additional fishing with floating nets requires at least two persons.
- Optimally, two to three additional persons should proceed fish measurements, while the boat team continues electrofishing.

Task Allocation

While taking photos by UAV, the operator controls the flight using a remote control (RC) connected to a tablet, and the observer keeps eye contact with the UAV. The observer is extremely useful for collecting data, especially from large rivers. While the UAV is above the ground, the observer controls the airspace and informs the operator about approaching manned and unmanned aircrafts, as well as birds that may be dangerous to the drone and. The observer confirms that the UAV does not go beyond eyesight range.

While fish sampling is performed, one person handles the boat, two persons operate anodes and two collect fish with landing nets and place them into transect specific containers. Fish measurements are organized as described above. When fishing with floating nets is conducted, one person operates the boat and field computer while the second is handling the net.

Step by step

1. Taking photos by UAV:
    - GPS system calibration
    - switch UAV to the ready to fly mode
    - planning the mission
    - process of taking pictures
        - control of flight correctness and proper information transfer
        - control of the battery level
    - collecting the data from UAV flight
    - software data processing (Agisoft PhotoScan Professional)
    - export georeferenced orthomosaic
        - control of the camera’s work - downward position
        - bring safely the UAV to the ground

2. Othomosaic preparation

3. Hydraulic measurement:
    - mount the ADCP on the boat and prepare the connection with field computer

- calibrate the compass
- setup the local time in tablet settings and on the probe
- fire up the probe and set up instrument depth, salinity and water temperature
- mark the GPS location of starting point (in tMap for example)
- setup edge parameters and start moving
- move the boat slowly in zigzags up or downstream such that you will measure a cross section in a distance from each other approximately equivalent to HMU width
- mark the GPS location of transect end points
- stop the system
- download the data to the computer and check the file content. If you use external GPS, make sure that time is properly recorded

4. Classification of riverbed texture:
   - mount side scan sonar on the boat
   - setup time
   - turn the recording on
   - move the boat slowly in zigzags up or downstream such that you will measure a cross section in a distance from each other approximately equivalent to HMU width

5. Boat electrofishing:
   - marking and locating edge points of the plots in delineated HMUs
   - preparing the electrofishing device, while waiting for fish accommodation
   - fishing on plots in one HMU and collecting the catch
   - fish measurements after one HMU is sampled
   - procedure repeated in next HMUs (optimally transects marking parallel to fish measurements)

6. Additional floating net catch:
   - marking start points location of net in delineated HMUs
   - releasing the start net buoy and moving downstream in line with it to reel of the whole net
   - slow float downstream with restriction to preserve the same velocity and distance between boat and a start net buoy
   - marking end points location and collecting the catch from the net
   - fish measurements after one HMU is sampled

Downloading hydraulic data

Depending on the equipment used, follow manufacturer’s instructions for data download. Usually, the data is in a text file in comma separated values format (CSV), which can be easily uploaded to GIS as a point cloud. Control the traces after upload to ensure these fall in the right places on the aerial image taken with drones.

Determining the distribution of habitats on the river

Having measured velocities, depths and substrate assigned to an ortho-photomap (for example using QGis), habitat distribution can be determined in accordance with the rules given in the MesoHABSIM method (described above). The analysis of oblique pictures of the shores allows the determination of the cover attributes and depth, while velocity distribution helps to better delineate observed habitat units. It is most convenient to use tMap software for mapping while observing the orthophoto map with plotted depths and velocities for identification of HMUs and oblique photos for determination of habitat dressing (**Figures 51** and **52**).

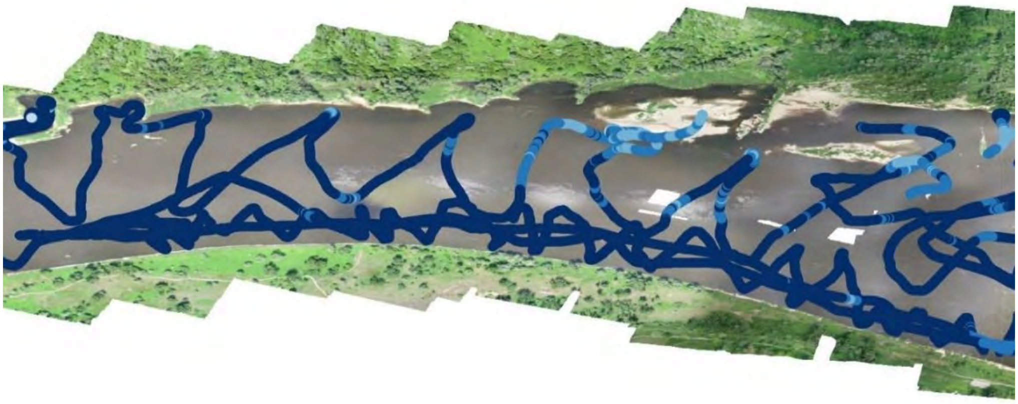

Figure 51. Traces of depth measurements taken with ADCP plotted on nadir aerial photograph captured the under Włocławek Dam on the river Vistula. The darker the blue, the deeper the river. Author K. Suska, SSIFI.

Figure 52. Oblique photos of backwater area taken with Phantom 4 professional, documenting habitat dressing consisting of irregular shore and some woody debris. Photo: K. Suska SSIFI.

Troubleshooting and unusual situations
During the UAV flight, there may be many unpredictable meteorological situations: wind blows, clouds (shadows on a surface), rainfall, increased air humidity (white frost or icing of UAV propellers), too much sunlight (reflections of the sun's rays from the water surface, too much heat for the multirotor's engines and battery), inadequate angle of sun rays (a uniform colour of the water surface because flight is too early or too late in the day) and too high or too low temperature (difficult operator focus, shortened UAV flight time by faster battery discharged).

Other sudden situations may be associated with the appearance of another unmanned or manned aircraft in the airspace to close to our UAV, or the UAV being attacked by birds. The operator must also consider, for example, passers-by, dogs, bicycles, cars, etc. in the near vicinity.

There can be many issues associated with the connection between the registering units and measuring instruments, and these should be referred to the user's manuals of each instrument. It is a good practice to check the status of device communication prior to leaving for the field.

5.5. Data security

Data Handling

Immediate actions
Photos should be saved on the hard drive after each flight and survey, from the UAV memory card. If this cannot be done in the field, all materials must be saved on an external disk after the end of the working day. It is good practice to record all collected data for each day in new folder named for the survey date.

When using tMap software, open the layer menu and execute export features menu (**Figure 53**). When asked about the location choose folder on the internal memory card. Then copy it to external card with file utilities or transfer to PC.

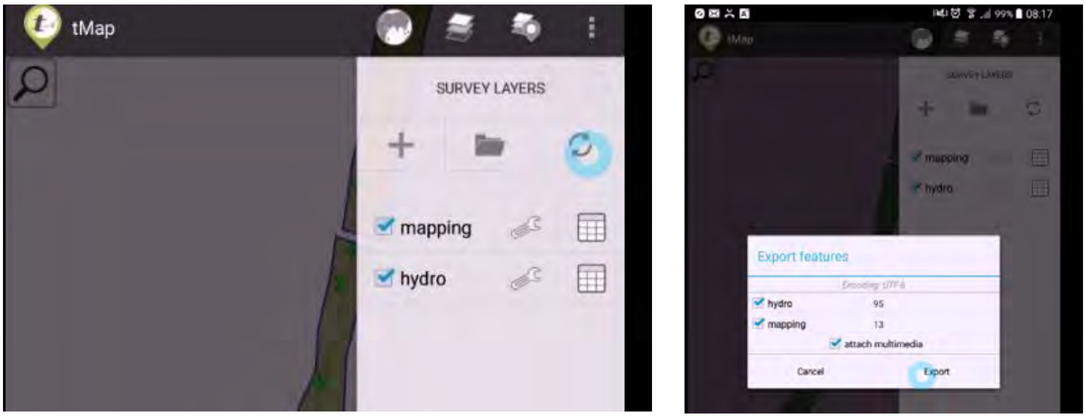

Figure 53. Exporting data from tMap. Photo: P. Parasiewicz, SSIFI.

Transfer the collected mapping data to a desktop or laptop computer at the end of each day to perform the first level of quality assurance/quality control. This involves redrawing or editing the polygons in the GIS system to ensure that they fit together and confirming that no polygon HMU data were accidentally lost.

Preparation for post processing

A data review should take place after each working day. Data from the UAV are divided into folders: various missions of given heights, riverbank images, films, low-height photos and perspective photos.

Quality Control

All data will be saved on the separate drive, and if possible, submitted at the end of the day to the ftp server. For quality analysis, ensure that the data are complete, reasonable, legible, and verified by the investigator(s).

Data Storage

Data should be maintained at the office in digital form in an organized directory specific to that project.

The following structure is proposed
:

1. Raw data - mapping and fishing files stored by date
2. Edited data - field data corrected in the lab
3. Field book hydrology data - in both hard copy and database form
4. All data will be backed up onto another medium or hard drive

Make a copy of the data

Before any corrections are made to the data, two copies should be made. Under a project folder, a folder containing the project river name and the word “raw’ will contain one copy of the data. Perform the first level of editing using the second copy of the data. Field book data will be entered into a Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheet or established database.

6. Sediment transport

6.1. Grain size sampling in alluvial rivers

Grain size is a fundamental descriptor for many fluvial and ecological processes. Consequently, a range of methods have emerged over the past decade allowing for the measurement of clast dimensions. These clast dimensions are organised in what is known as the 'Wentworth' scale (**Figure 54**). This scale classifies particles of loose sediment in a base-2 logarithmic fashion going from small particles of clay to the largest boulders. The measurement of particle sizes can be performed with either manual or image-based methods. Manual-based methods involve the physical measurement of sediment grains. At a basic level, grains are conceptualised as triaxial ellipsoids (**Figure 55**). In this model, it is imagined that a sediment clast, of any size, can be measured with three axis, a, b and c, that bound an ellipsoidal body in 3D space. By convention, the a-axis denotes the longest axis, b, the intermediate and c, the shortest. However, in most environmental applications, the measurement of the intermediate axis b is the standard procedure to quantify the dimensions of a clast. Actual methods for the measurement of sediment are very diverse and depend on the size and location of the bed material. The focus of the study can also have an impact. For the details of classic grain size measurement methods, refer to Bunte and Abt (2001). This is a comprehensive report on all aspects traditional grain size measurement, advantages and disadvantages of methods, potential methods involving imagery are also foreseen.

6.2. Grain size measurement

The field of image-based, remotely sensed, grain size measurement has undergone considerable progress in the last two decades. There are now methods to measure particles with photos taken from the ground with a hand-held camera (Detert and Weitbrecht, 2012; Graham *et al*., 2005a, 2005b; Rubin, 2004) from airborne imagery using piloted aircraft (Buscombe *et al*., 2010; Carbonneau *et al*., 2005, 2004; Dugdale *et al*., 2010; Rubin, 2004) and also using drones (Carbonneau et al., 2018; Woodget *et al*., 2018; Woodget and Austrums, 2017). For example, Carbonneau *et al*. (Carbonneau *et al*., 2018), demonstrated that low-cost drones could replace labour-intensive field surveys by using drones operated at very low altitudes in combination with existing grain-size measurement software by Detert and Weitbrecht (2012b) as illustrated in **Figure 56**. In this approach to particle sizing, a low-cost drone is flown near the surface of a river bar. At very low altitudes, below 10m, the images taken by the drone then become useable in particle identification software (**Figure 57**).

Readers therefore have a very wide choice of methods. If only sparse data is required, manual pebble counts, see Bunte and Abt (2001), are often the most straightforward approach. However, in cases where highly detailed, small scale measurements are required, a ground-based approach using an image-based approach is recommended, for example, Detert and Weitbrecht, 2012 and Graham *et al*., 2005b. For medium scale approaches requiring of few hundred meters of river length of continuous data per day, a drone-based approach is recommended such as robotic photo-sieving (Carbonneau *et al*., 2018). For slighter larger scales, going from 1 to 10km of river length per day, higher altitude flights will be required and users should move away from robotic photo-sieving and consider the methods of Woodget *et al*., 2018 and Woodget and Austrums, 2017. Finally, for large scale measurements of grain size, full-size aircraft remain the only viable option (Carbonneau *et al*., 2005, 2004). In fact, Carbonneau *et al*. (Carbonneau *et al*., 2005) demonstrated that even submerged grain measurements can be reliably made with image-based methods.

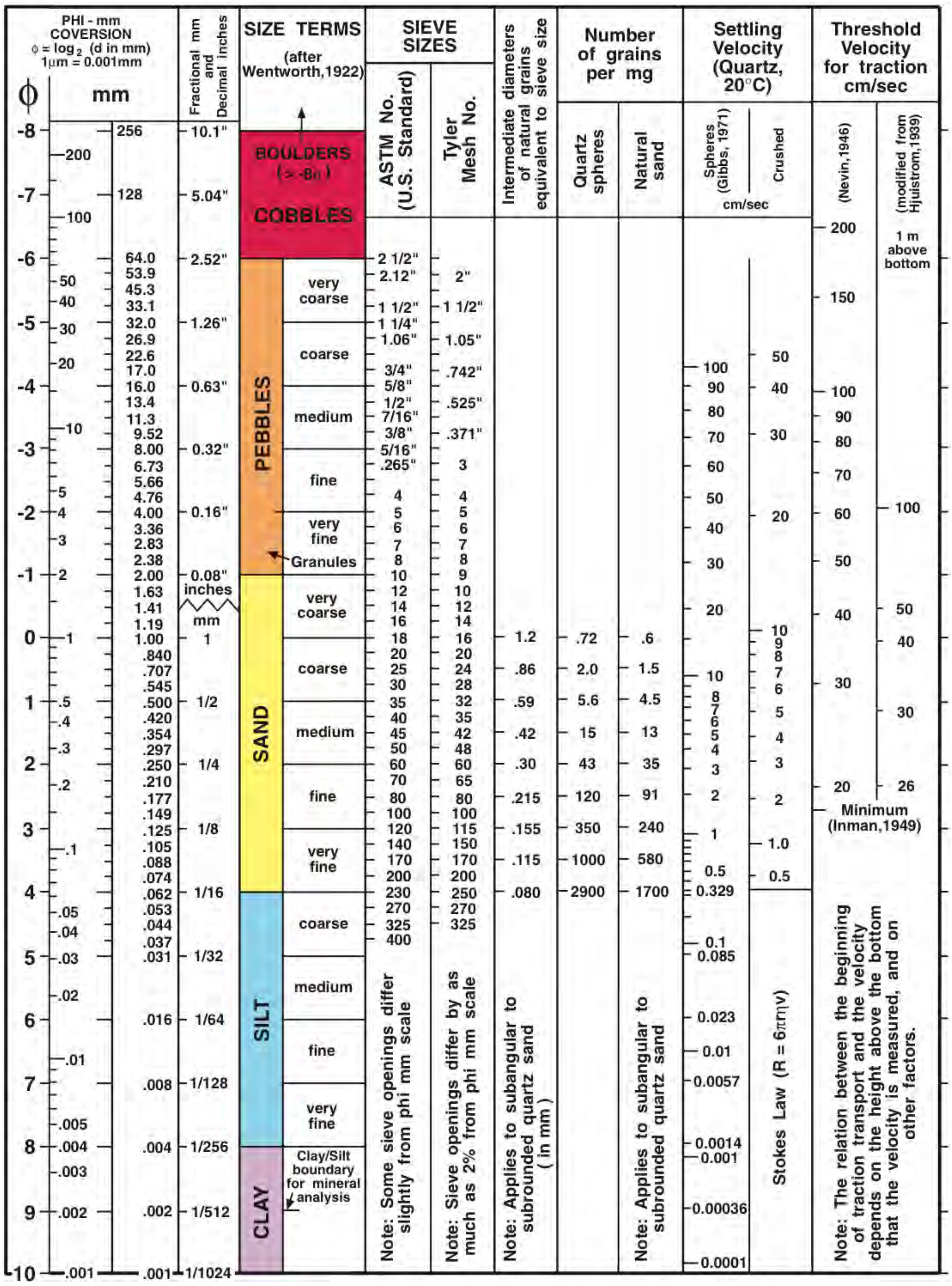

Figure 54. The Wentworth scale of grain size classification. Source: USGS.

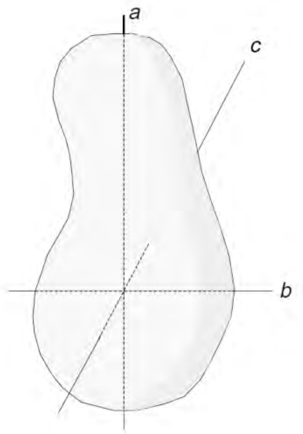

Figure 55. The triaxial ellipsoid model of particle shape with axis a, b and c. Source: Bunte and Abt (2001).

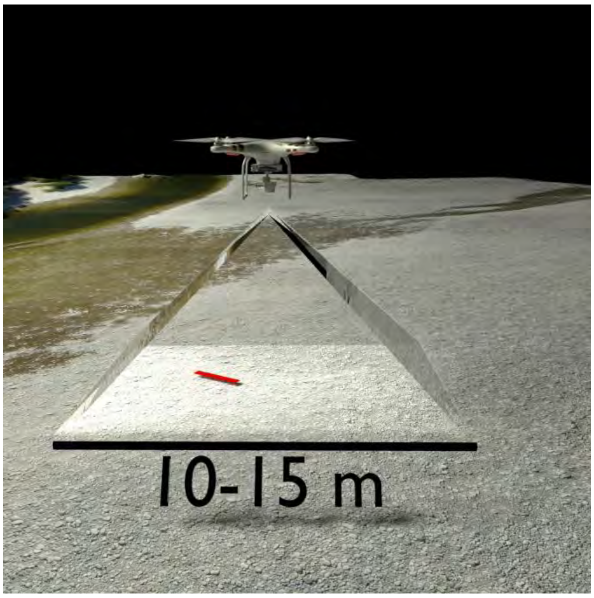

Figure 56. Robotic Photosieving procedure. A drone is flown very close to a gravel bar in order to collect mm-resolution imagery capable of distinguishing individual clasts. Source: AMBER D2.4.

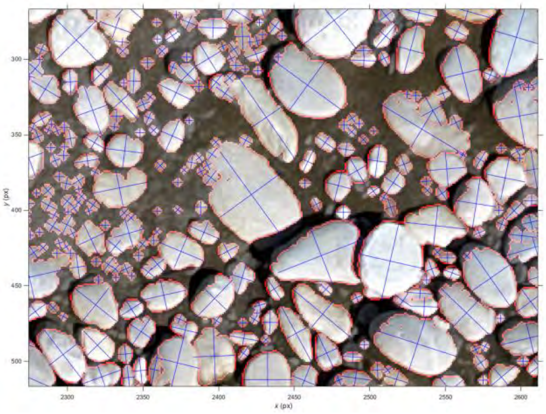

Figure 57. Sample output from BASEGRAIN by Detert and Weitbrecht (2012*).*

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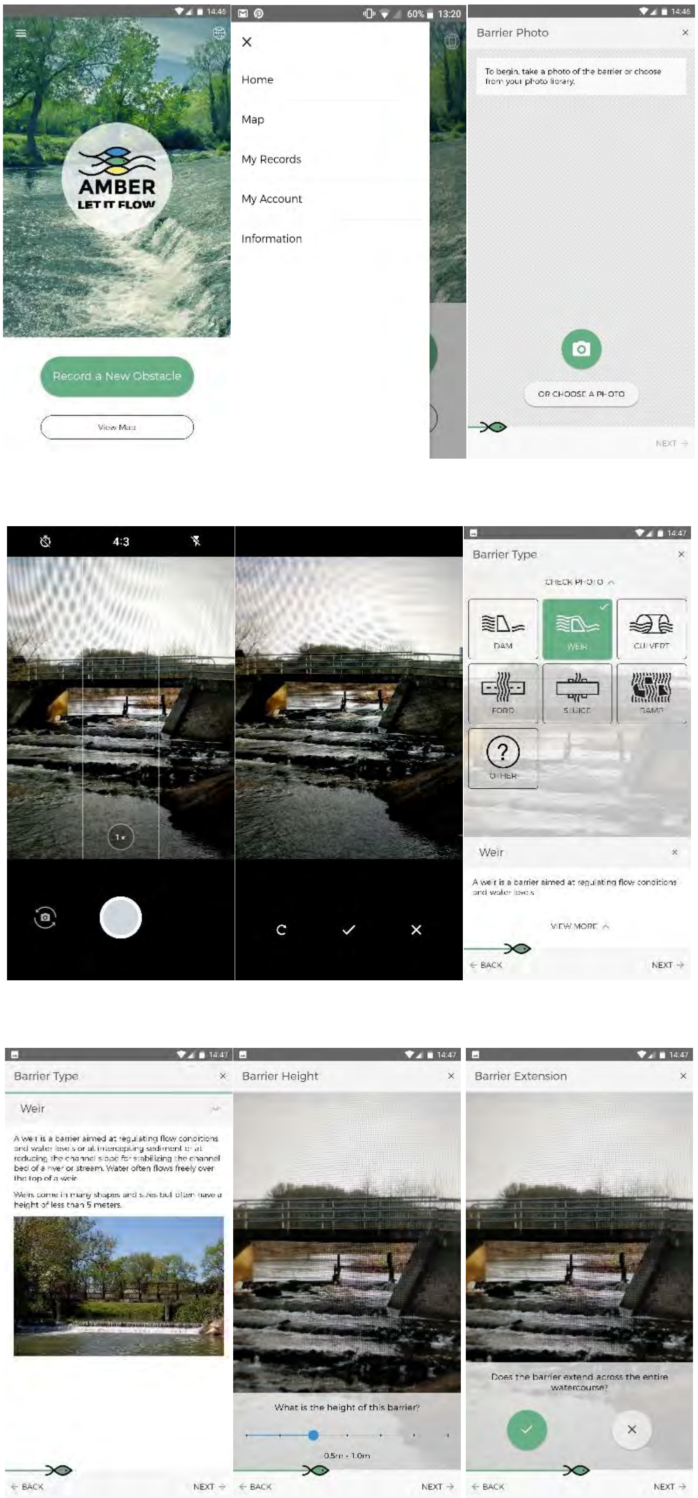
8. Appendices

8.1. Barrier Tracker App



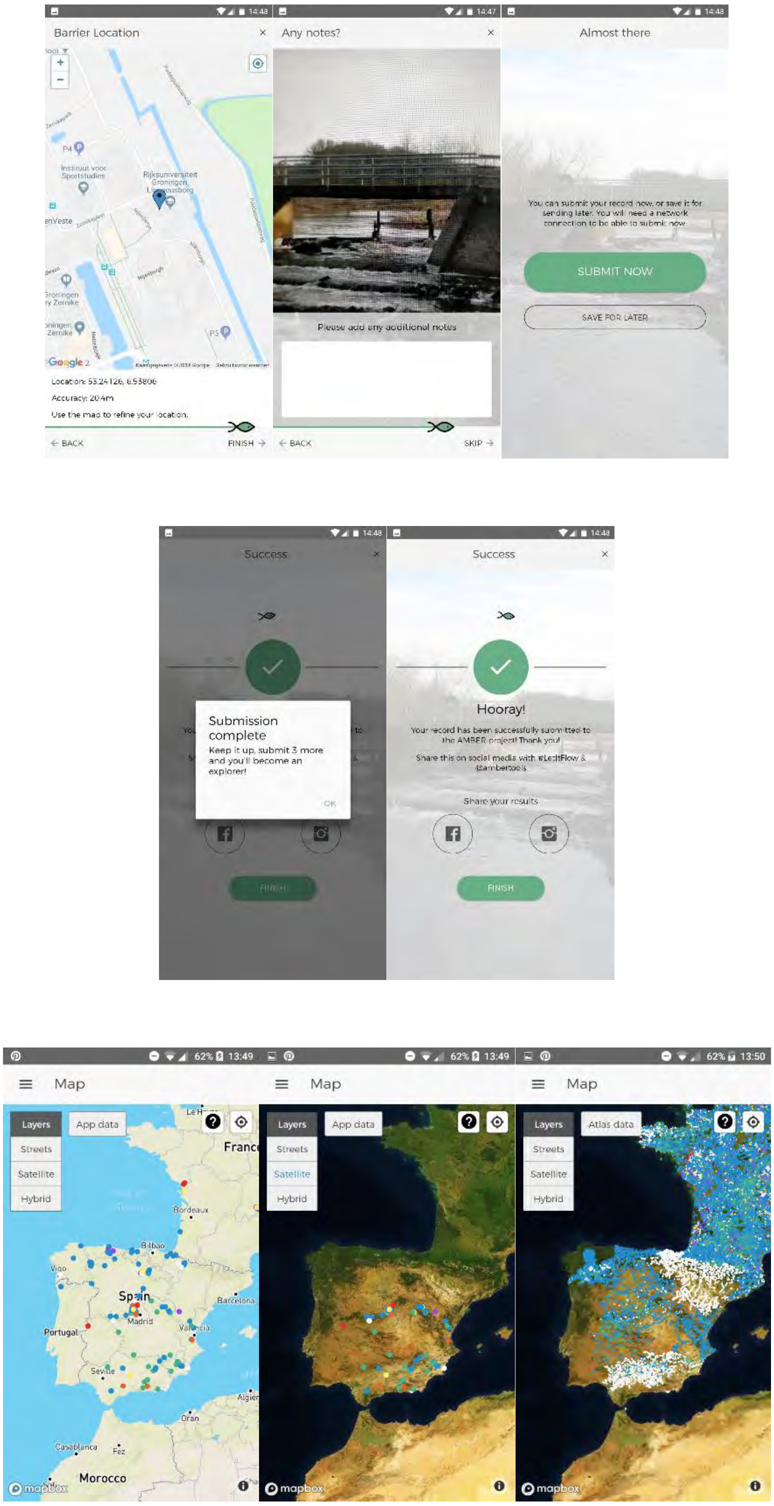
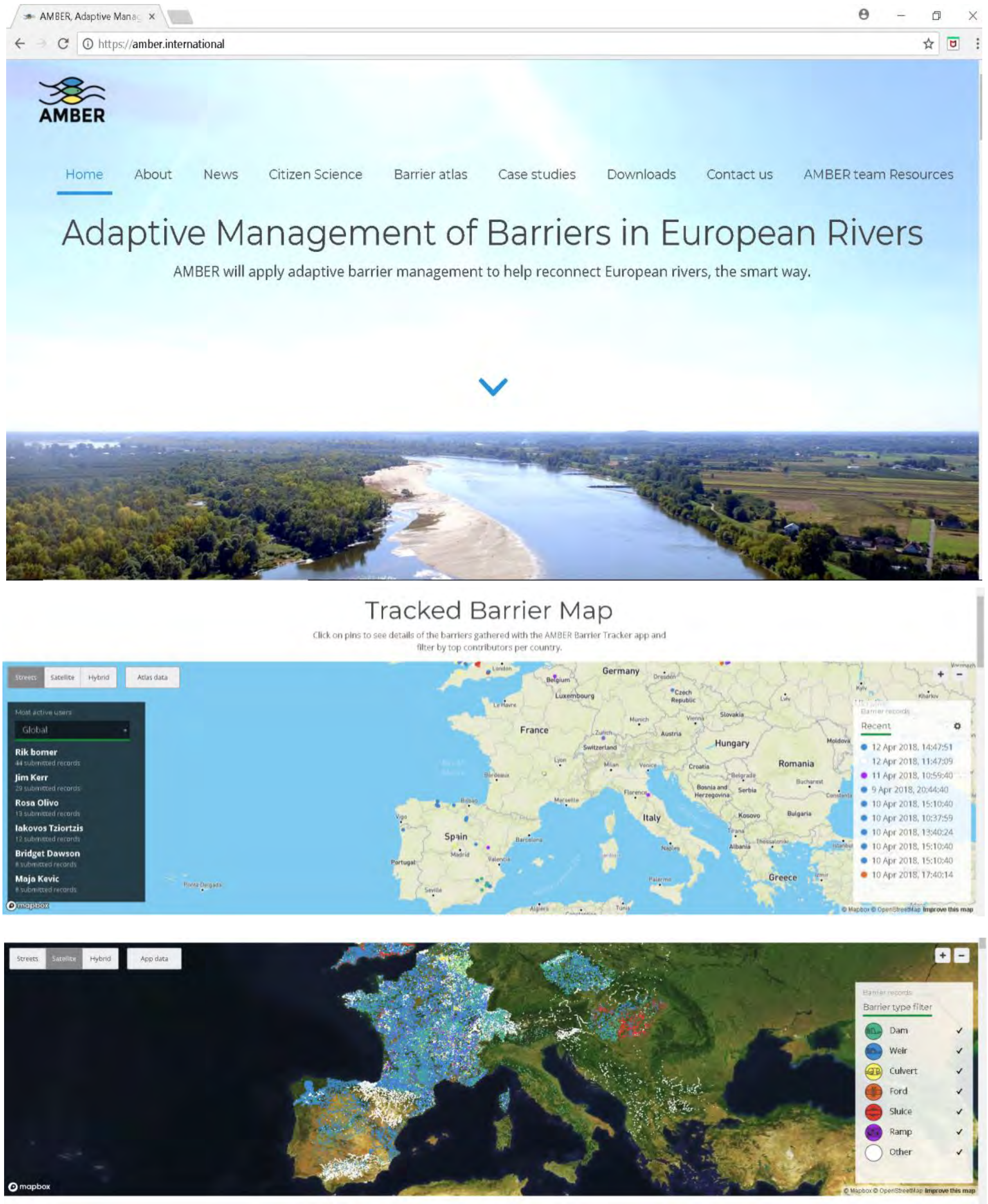
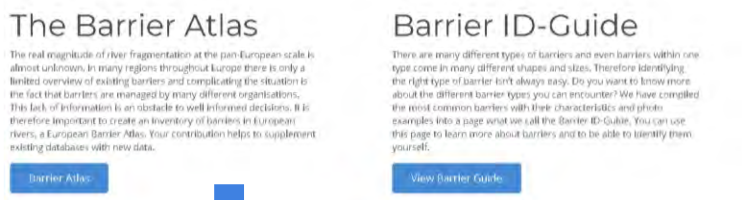


Figure 58. Example images from the Barrier Tracker app in use, including data input, geolocation and record submission. Photo: P. Parasiewicz, SSIFI.

8.2. Website Portal





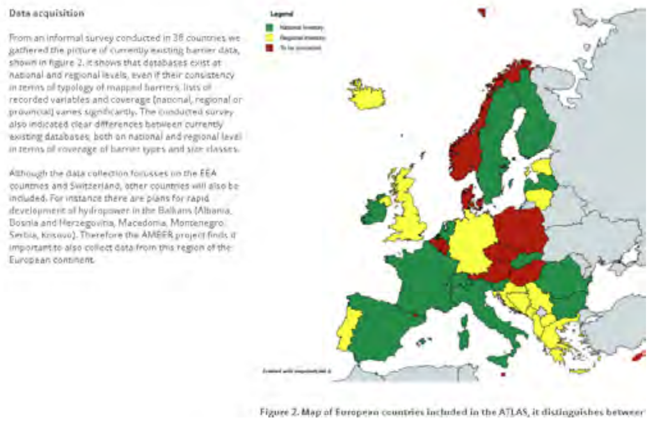


Table 1. Key parameters that we propose to be compiled for the ATLAS.

Key parameters	Definition
ATLAS_ID	New ID defined within AMBER
Source_ID	ID of the source (national, regional) database
URL	Link to data source. It can be, e.g.: the web address of the owner institution, the available web address of the national/regional DB
Country	EU country or EU area, e.g. Balkans, Danube...
X_coord	Latitude
Y_coord	Longitude
River	Name of the river
Basin	Name of river basin
Height	Barrier height (m), i.e. the vertical distance between the lowest point on the crest of the barrier and the lowest point in the original streambed
Type	Dam, weir, spillway, etc.
Year	Date of building (end)

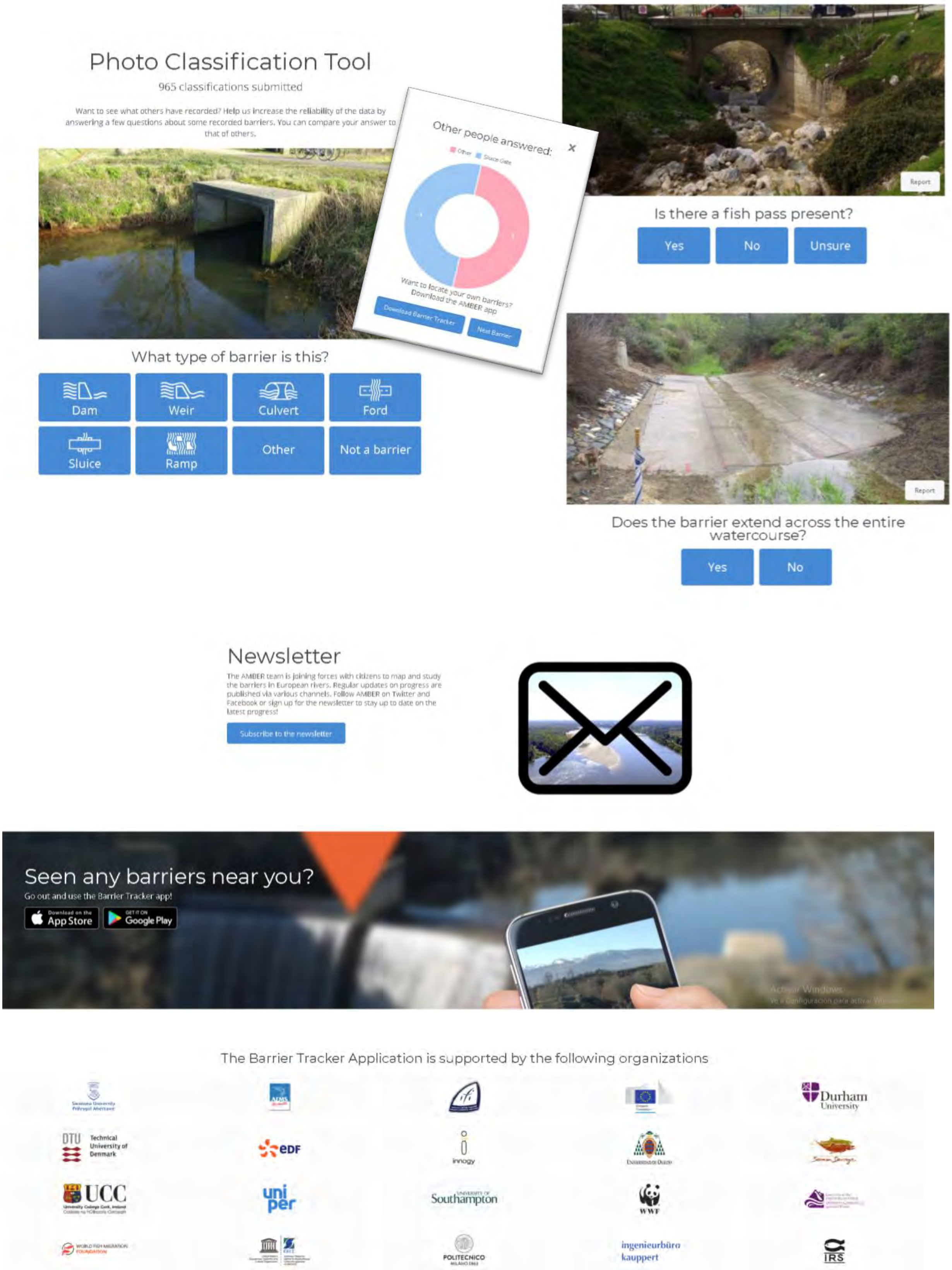


Figure 59. Images from the AMBER website and Citizen Science portal (https://portal.amber.international/ accessed 21/06/2018).

8.3. Suppliers of material for environmental DNA sampling

Table 7. List of items, vendor, item description, catalogue reference and indicative pricing for eDNA sampling material (Source Deliverable D2.5).

**Item	Supplier /Vendor	Item description	Catalogue reference (UK)	Orientative pricing	link
Whirl-Pak® Stand-Up Bag	SIGMA-Aldrich	Whirl-Pak® Stand-Up Bag 1065 mL capacity(36 oz)-pkg of 250	WPB01449WA-250EA [Mfr. No. B01449WA (Nasco)]	86.30£ 99.03€	https://www.sigmaaldrich.com/catalog/product/sigma/wpb01449wa?lang=en&region=GB&cm_sp=Insite-_-recent_fixed-_-recent5-5 https://www.enasco.com/p/B01449WA
50 ml Syringe	SIGMA-Aldrich	NORM-JECT-Syringe PP/PE without needle luer slip ip, eccentric, capacity 50 mL, graduated- 2 mL, sterile	Z118400-30EA	79£ 90.65€	https://www.sigmaaldrich.com/catalog/product/aldrich/z118400?lang=en&region=GB&cm_sp=Insite-_-prodRecCold_xviews-_-prodRecCold10-9
	Fisher scientific	Terumo™ 3-Part 50mL Luer Lock Syringes, ,graduated- 1 ml ,sterile 25/pk	15349067	14.56 £ 19.75€	https://www.fishersci.ie/shop/products/3-part-50ml-luer-lock-syringes/15349067
Filer Holder	Cole-Parmer	Polycarbonate Filter Holder, 25 mm, 12/pk	UY-29550-42	102£ 117.05€	https://www.coleparmer.co.uk/i/polycarbonate-filter-holder-25-mm-12-pk/2955042
	Cole-Parmer	Polypropylene Filter Holder for 25-mm membranes, 6/pk	UY-06623-32 (Advantec – Mfr # 43303010)	94.80£ 108.48€	https://www.coleparmer.co.uk/i/advantec-43303010-polypropylene-filter-holder-for-25-mm-membranes/0662332?PubID=UY&persist=true&ip=no&keyword=&gclid=Cj0KCQjwuMrXBRC_ARIsALWZrIioYDadDsEEyrqRvQkUPTxFuO_ONuMdKh6B7IIg-

**Item	Supplier /Vendor	Item description	Catalogue reference (UK)	Orientative pricing	link
					GuoYEEQMqqOu3QaAqxTEALw_wcB
Filter	Merk-milipore	Millipore Express PLUS Membrane Filter, polyethersulfone, Hydrophilic, 0.22 µm, 25mm (100 u)	GPWP02500	123.00£ 141.15€	https://www.merckmillipore.com/GB/en/product/Millipore-Express-PLUS-Membrane-Filter-polyethersulfone-Hydrophilic-0.45nbspm-25nbspmm,MM_NF-HPWP02500
Holder Caps	AMAZON	Universal UN940 Obturator Male/Female Luer Lock Caps Red (Pack of 100)-sterile	--	4£ 4.6€	https://www.amazon.co.uk/Universal-UN940-Obturator-Male-Female/dp/B018P8V1KQ/ref=sr_1_fkmr0_1?ie=UTF8&qid=1524223121&sr=8-1-fkmr0&keywords=Universal%2BUN940%2BObturator%2BMale%2FFemale%2BLuer%2BLock%2BCaps%2BRed%2B(Pack%2Bof%2B100)-sterile&th=1

**Any Alternative manufacturer's products may be substituted. Some alternative supplies are provided.

8.4. Alternatives to syringe on-site filtration protocol

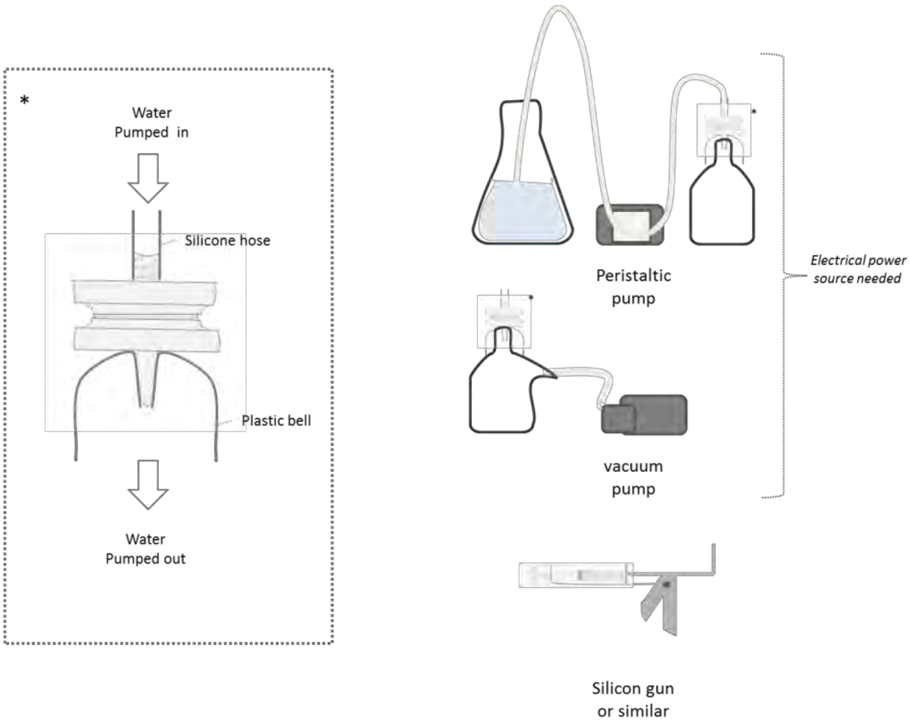


Figure 62. Alternatives to syringe use for on-site filtration of water for eDNA sampling. Source: AMBER D2.5.

Note: Using a peristaltic pump or a vacuum pump instead of the syringes is an alternative. In that case, it must be taken into account that an electrical power source is needed, and that equipment is heavy (this is not feasible when sampling remote locations and equipment weight is a concern). Manual hand pump, silicon gun or similar will be another possibility that would assist filtration in the field without the need of accessing to an electrical power source. Always decontaminate thoroughly the equipment between sample sites.

On-site filtration may not be a suitable option timewise when many sample sites need to be sampled on a short period of time. If that the case, the addition of quaternary ammonium compound benzalkonium chloride (BAC) at a final concentration of 0.01% (Yamanaka *et al.*, 2017) after sample collection followed by filtration upon arrival to the lab might be a preferred option.

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