BiodiversIRES: Global biodiversity of intermittent rivers and ephemeral streams

The second coordinated effort of the 1000 Intermittent Rivers Project

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1. Key questions:

Are IRES unique from a biodiversity perspective?
What are the global and local drivers of IRES biodiversity?
How will climate change alter riverine biodiversity through increased drying of river networks?

2. Context:

Rivers and streams are among the most endangered hotspots of biodiversity on Earth (Reid et al., 2019). Rivers and streams that do not flow permanently, hereafter intermittent rivers and ephemeral streams (IRES), are abundant on Earth, representing ~half of our river network length (Datry et al. 2017). For decades, IRES have been considered as a subclass of rivers with low values for society (Steward et al. 2012). This is still the case in many countries (e.g., USA, Marshall et al. 2018), where governments and lobbies are trying to remove IRES from jurisdictional protections. This is mostly based on the assumption that IRES have low biodiversity values (Larned et al. 2010, Steward et al., 2012, Acuna et al. 2014). However, recent evidence from local studies has shown that IRES can be rich in both aquatic and terrestrial organisms (e.g. Steward et al. 2017, Stubbington et al. 2017). To better recognize the biodiversity of IRES and its underlying drivers, a global, standardized and coordinated effort is urgently needed.

Here, we aim to quantify aquatic and terrestrial biodiversity in IRES at the global scale. To do so, we will sample >200 IRES across the globe using sampling techniques and protocols, including i. Surber sampling and dry sediment rewetting for aquatic invertebrates, ii. pitfall traps for terrestrial invertebrates, iii. soils and sediment eDNA metabarcoding for micro-organisms and meiofauna, iv. water eDNA metabarcoding for fish, and v. phototraps and track analyses for

mammals and megafauna. At each sampling reach, relevant environmental factors will be measured/estimated including flow regime, climate condition, geomorphic setting, land-use, etc. Global maps of IRES will also be generated through concurrent projects, under current conditions and future climate change scenarios. This will allow us to predict future riverine biodiversity of IRES. If you think you will contribute, please fill this short document.

3. Methods

3.1 Reach selection

Along one river, select a pair of reaches, one perennial, the other experiencing drying (flow cessation or complete disappearance of surface water). A reach is defined as 10×the average width of the active channel. Ideally, the perennial reach should be upstream, but a downstream reach is also acceptable. If the whole river network is intermittent, select the closest perennial reach in another river.

The paired reaches should be morphologically as similar as possible in terms of substrate, channel width, topography, as well as exposure to human impacts. Avoid sites where there are bars in the channel or other obstacles.

For participants who were involved in the previous coordinated 10000IRP experiment, try to focus on the same reaches so you already have many environmental variables estimated/measured.

3.2 Sampling protocol:

The general sampling design is shown in **Figure 1**. Each reach should be visited and sampled twice: during the flowing and non-flowing phases. The non-flowing phase refer to dry riverbeds **with or without** disconnected pools in the intermittent reach. **Details for each sampling protocol are described in section 5**.

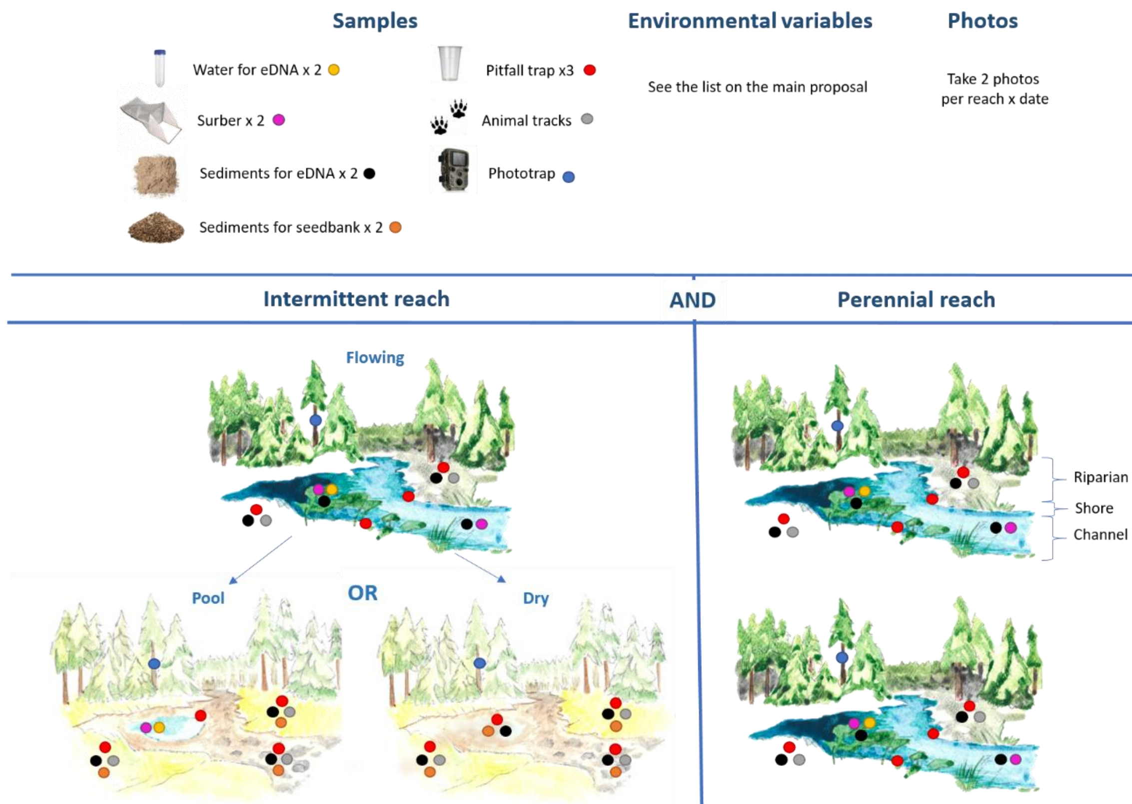

Figure 1. Sampling design for the BiodiversIRES project

3.3 Aquatic biodiversity:

For each phase with water (i.e. the flowing phase and non-flowing phases with disconnected pools), collect 4 Surber samples (see details in section 5): 2 Surber samples from 2 riffles and 2 Surber samples from 2 pools from both the intermittent reach and the perennial reach. If the available pools are too small (i.e. < 1 x 1 m), do not consider them.

For each phase with water (see above), collect water for eDNA analyses in 2 pools (for each reach) by passing water through a 0.22 µm filter using Sterivex cartridges (section 5).

During the non-flowing phase, collect sediments from 4 locations (2 from pools and 2 from dry riffles) in the dry riverbed and soils from 4 locations in the adjacent riparian zone (two on each side of the river channel and < 20 m from the riverbed) for seedbank analyses (section 5).

3.4 Terrestrial biodiversity:

During both the flowing and non-flowing phases, collect 5 pitfall traps (section 5) in the riverbed at each reach and 5 in the adjacent riparian zone. During the flowing phase, pitfall traps should be placed along the shore.

For each phase, collect 4 sediment samples (dry for non-flowing phases, wet for flowing phases): 2 from pools and 2 from riffles from the riverbed for terrestrial eDNA analysis (section 5). Collect also 4 soil samples from the adjacent riparian zone (two on each side of the river channel and < 20 m from the riverbed).

For the larger fauna, install a photo-trap (section 5) at the site and record the presence of animals over 3 month (or more) during each phase.

During the non-flowing phase, in addition to the photo-trap, record animal tracks (section 5).

3.5 Environmental variables:

To be part of the project, you must also complete the Excel file (provided with protocol, and available on our website) to describe the following variables:

At the catchment scale (upstream of the sampling reach):

Strahler stream order, stream length, distance to source, distance to downstream main confluence, distance and direction to the closest flow recorder, distance to the closest perennial site, number and location of active flow recorders in the catchment, catchment area, climate zone (Köppen system, http://en.wikipedia.org/wiki/K%C3%B6ppen_climate_classification), land uses (forest, agricultural land….), % of the network that is intermittent (estimations), average annual precipitation, average annual temperature.

At the reach scale (10×the wetted width):

Active channel width, width of the floodplain when relevant, estimated % cover of silt/sand/gravel/cobble/boulder/bedrock substrates across the reach, riparian cover (estimated visually as a %), riparian vegetation (absent/herbs/shrubs/trees), the 3 dominant riparian species if possible, the estimated dry phase duration prior to the time of sampling (for pitfall, terrestrial eDNA and seedbank sampling), estimates of the flowing period duration prior the time of sampling (for Surber and aquatic eDNA samples), distance between the intermittent and perennial reaches, the type of non-flowing phase (complete drying or presence of pools), GPS coordinates in WSG84, and altitude (m asl).

Take enough **photos** to represent the study sites.

Soil and sediment moisture and organic matter content:

For each reach (perennial, intermittent) and each phase (flowing, non-flowing), take small (ca. 100 g) sediment subsamples (from stream channel) and soil subsamples (from riparian zone) close to each spot used for pit fall trap deployment. Collect only the first 10 cm of sediment or soil, then pool for each reach and each phase all sediment subsamples from channel and all soil samples from the riparian zone into one composite sample (a. 600 g). Store composite

samples in closed zip bags or plastic containers and in a cooler to minimize evaporation.

Along with the samples and data being sent, we require a statement that you have all the requested permissions to 1) collect samples at the selected reaches, and 2) ship material and/or data overseas (including through the Nagoya protocol). Without this, we will not be able to use your data!

3.6 Sample processing:

Surber and pitfall samples
Process Surber and pitfall samples to the lowest taxonomic resolution possible. The knowledge for terrestrial invertebrates can be poor, but the family level is a minimum to be reached. Use morphospecies in case you can.

Soil and sediments for eDNA
Wearing gloves, clean the sampling equipment (e.g. shovel, sieves) with 10% bleach between samples. In the laboratory, sieve soil or sediment samples at 2 mm using material cleaned between samples (using 10% bleach). Take a subsample of about 15 g from the sieved and homogenized 2-L sample and store it at -20°C in a sterile 50-mL Falcon tube correctly labelled (see the 1000IRP recommendation for labelling, below). Avoid cross contamination of samples during the sieving procedure.

Labelling and shipment of samples
Make sure the samples shipped contain labels with a code, which you will also report on the Excel spreadsheet. For the code, use the following system:
COUNTRY–NAME–RIVER–REACH–HABITAT–REP–TYPE–DATE–PHASE
COUNTRY: Country in which the sample was collected
NAME: Surname of the responsible participant
RIVER: Name of the river where the sample was collected
REACH: Reach where sample was collected (Perennial = “P”, Intermittent = “I”)
HABITAT: Habitat where sample was collected (Channel = “Ch”, Riparian = “Ri”)
REP: Number of replicate sample (“1” or “2”)
DATE: Date sample was collected, in format DD-MM-YY
PHASE: Hydrological phase in which sample was collected (Flowing: “FLOW”, Non-flowing = “DRY”)
TYPE: Type of material (water eDNA, sediment/soil eDNA)

Shipment of eDNA samples:
Sterivex cartridges and soil/sediment samples should be stored at -20°C before shipment. Samples should be sent to on dry ice (ensure that you use enough dry ice so that the samples stay frozen during transport). The address is
DATRY Thibault

INRAE, 5 rue de la Doua
CS 20244, 69625 Villeurbanne Cedex
France

Samples should be sent on Monday and arrive in less than 36 hours. All necessary permits and authorizations should be included with the shipment (sample collection, import/export). **Contact your national focal point to obtain the authorization to collect and exchange samples in agreement with the Nagoya protocol (https://absch.cbd.int/search?documentSchema=CNA) before the sampling period.**

4. Rules to be included in BiodiversIRES:

To be included in the further steps of the project (i.e. analyzing data and contributing to the publication/s), the minimum **per participant*** is to

1) collect and analyse **Surber** and **pitfall** samples,
2) conduct the **seedbank experiment**
3) collect **eDNA samples from soil and sediments**
4) document the **mandatory environmental variables**
5) provide the **permissions of sampling and sharing genetic resources**

from at least 1 river with paired perennial/intermittent reaches and covering 2 hydrologic phases (flowing and dry),

Tracks, photo-traps and water eDNA samples **are strongly encouraged** though and would provide higher recognition in the main manuscript and allow contributing to the additional manuscripts.

*even if multiple people from your lab are involved in the different phases of the process, **only one person will be recognized as a participant per river sampled**. If you want to include more colleagues from your lab (e.g., PhD, postdocs, etc), please replicate the effort following the rules stated above.

5. Details for each sampling method

5.1 %moisture and % ash free dry mass (AFDM)

In the laboratory, sieve samples (2mm). Samples are then weighed, dried at 60°C for 24 h, and re-weighed, to calculate water content (%), and combusted at 550°C for 4 h to estimate AFDM.

In total, these samples comprise:

Flowing phase: 1 sediment sample (6 pooled subsamples) from channel and 1 soil sample (6 pooled subsamples) from riparian habitat

Non-flowing phase: 1 sediment sample (6 pooled subsamples) from channel and 1 soil sample (6 pooled subsamples) from riparian habitat
Total number of samples: 8 = 2 reaches (1 perennial + 1 intermittent) * 2 hydrological phases (flowing + non-flowing) * 2 habitats

Determine % water content and %AFDM for each for the 8 samples.

5.2 Surber samples

During the flowing phase, collect 2 Surber samples (sample area 0.1 m^2, mesh size: 250 μm) from 2 riffles and 1 Surber samples from each of 2 pools in the intermittent reach, and the same (another 4 samples) in the perennial reach. During the non-flowing phase, if any disconnected pools are present, sample 2 pools if you can choose, or from 1 if only 1 is left. If the pools are too small, i.e., < 1x1 m, disregard them. Surber samples are taken by vigorous manual disturbance of the substrate within the Surber quadrat for one minute. To generate flow, some agitation by hand is needed to transfer disturbed invertebrates into the net and have an effective sampling in lentic conditions. Use a comparable method in the riffle and pool habitats in the perennial reach For each sample, empty the contents of the net into a container and preserve in a 70% ethanol. Nets should be thoroughly checked and any invertebrates remaining on the net should be removed and put in the sample container.

Invertebrates will later be identified and counted by each participant team (in your own lab) to the lowest possible identification level. Invertebrate data, including taxon list and counts, along with information on the hydrological phase, should then be computerized and sent to the core team of the 1000IRP project.

5.3 Pitfall traps

For each reach (perennial, intermittent) and each phase (flowing, non-flowing), establish 6 spots, located a certain distance apart from each other (i.e. 2 ×mean channel width) and perpendicular to the thalweg channel. At each spot, sample two habitat types (channel and riparian). The channel habitat is defined as (i) the center of the dry stream channel in the intermittent reach during the dry phase, and as (ii) the shoreline of the stream channel (i.e. the exposed stream sediments located a few 10-20 cm centimeters from the water's edge) in the intermittent reach during the dry phase and in the perennial reach during both phases. The riparian habitat in both types of reaches and for both hydrological phases is defined as the area located from the edge of the high-water channel and bankfull to the edge of upland, characterized by the presence of a distinctive riparian vegetation and substrate type. Within each habitat type at each spot, deploy 1 pitfall trap. Distribute shoreline and riparian spots evenly on both sides of the channel (i.e. 3 on right side and 3 on left side). Pitfall traps are plastic containers

(height: approx. 80 mm, diameter: approx. 80mm) inserted into the sediments and filled to 3/4 with glycol (cooling liquid for cars, very toxic). Record the exact height and diameter of the containers used. Position a plastic cover over each trap to prevent rain, falling leaf litter and other debris from blocking the trap. Set the traps for one week (min. 5 days, max 15 days). Pool the contents of all collected pitfall traps from each habitat at one site, transfer the collected specimens into plastic containers filled with 70% ethanol, and store until further analysis. Record the number of pitfall traps pooled for each habitat, in case some were lost during sampling (e.g. destroyed by animals).

In total, these samples comprise:
Flowing phase: 1 sample (6 pooled pitfall traps) from channel and 1 sample (6 pooled pitfall traps) from riparian habitat
Non-flowing phase: 1 sample (6 pooled pitfall traps) from channel and 1 sample (6 pooled pitfall traps) from riparian habitat
Total number of samples: 8 = 2 reaches (1 perennial + 1 intermittent) * 2 hydrological phases (flowing + non-flowing) * 2 habitats

In the lab, identify the sampled terrestrial invertebrates to the lowest taxonomic level possible. See Corti et al. 2013 for details. Invertebrate data, including a taxa list and counts, along with information on the hydrological phase, should then be computerized and sent to the core team of the 1000IRP project.

5.4 eDNA sampling from the water column

During the flowing and non-flowing phases, collect eDNA in 2 pools (for each reach) by filtering water through a 0.22 µm filter using Sterivex cartridges (http://www.merckmillipore.com/FR/fr/product/Sterivex-GP-Pressure-Filter-Unit,MM_NF-SVGPL10RC). Filtration should be done in the field using a peristaltic pump or a 50-mL sterile syringe until the filter clogs, and the duration of the filtration procedure (in seconds) and volume filtered (in mL) recorded. At the end of the filtration step, remove excess water from the Sterivex cartridge by pumping air through the filter. **The volume of water filtered and the duration of the filtration procedure are mandatory information**. Label (using the 1000IRP recommendations explained in section 3.6) your Sterivex cartridge and place the cartridge in a sealed zip-lock bag with the same label. Store filtered water samples at -20°C until shipping (see below).

In total, the required eDNA samples comprise:
Flowing phase: 2 samples from pools
Non-flowing phase: 2 samples from pools
Total number of samples: 8 = 2 reaches (1 perennial + 1 intermittent) * 2 hydrological phases (flowing + non-flowing) * 2 pools

5.5 Soil and sediment samples for eDNA analyses

Collect soil and sediment samples (2 L in volume) in 3 locations within a circular sampling area with 1 m radius and at depths of 0-10 cm using a shovel. For the flowing and non-flowing phases, 4 sediment samples are collected from the riverbed (2 from pools and 2 from riffles) and 4 soil samples are collected in the adjacent riparian zone (two on each side of the river channel and < 20 m from the riverbed) at each site for eDNA analyses. Avoid cross contamination of samples during the sieving procedure. Wear gloves and clean the material used for soil/sediment sampling with ethanol (shovel, sieves) between each sample. In the laboratory, sieve soil or sediment samples at 2 mm. Take 2 subsamples of about 15 g from the sieved and homogenized 2 L sample and store them at -20°C in 2 sterile 50 mL labelled Falcon tubes. For each sample, send 1 Falcon tube to Irstea and retain the other (replicate subsample) at -20°C as a backup.

In total, the required eDNA samples comprise:
Flowing phase:
- Riverbed: 2 sediment samples from pools, 2 sediment samples from riffles
- Riparian zone: 4 soil samples (two on each side of the river channel and < 20 m from the riverbed)

Non-flowing phase:
- Riverbed: 2 dry sediment samples from pools, 2 dry sediment samples from riffles
- Riparian zone: 4 soil samples (two on each side of the river channel and < 20m from the riverbed)

Total number of samples: 32 = 2 reaches (1 perennial + 1 intermittent) * 2 hydrological phases (flowing + non-flowing) * 8 (2 pools, 2 riffles, 4 riparian)

5.6 Invertebrate seedbank:

The riparian habitat is defined as the area located from the edge of the high-water channel and bankfull to the edge of upland, characterized by the presence of a distinctive riparian vegetation and substrate type. During the non-flowing phase, each sediment sample (2.5 L in volume, sieved to retain the fraction < 5 mm) is collected at depths of 0-10 cm using a shovel. Estimate the time since the last flowing period (even if with some uncertainty, i.e. ± 1 week). Samples are placed in individual plastic buckets of 10 L, returned to the laboratory, and if possible, placed in an environmental chamber at room temperature (20°C) with a day-night cycle corresponding to the conditions at the sampling reach. Then, 5 L of tap water, previously dechlorinated by standing it for 24 h in the lab, is added to each bucket. After water is added, each bucket is gently shaken, and the surface water poured through a 250-μm metal sieve. This initial sample is used to collect any living aquatic or terrestrial invertebrates and predators that may prey on viable eggs and is preserved with 70% isopropyl alcohol. Each bucket is then refilled

with tap water previously dechlorinated to an equivalent 5-L level, an air-stone is placed on the sediment surface to keep the water oxygenated, and each bucket is covered with a plastic screen (1-mm mesh) to keep any emerging insects from escaping. The total incubation/inundation period is 21 d, as a compromise between time for invertebrates to emerge and preventing anoxia to develop. After 21 d, sediments in each bucket are gently elutriated 5 times with each elutriate decanted through a 250 µm mesh sieve. The material retained on the sieve is preserved with 70% isopropyl alcohol for later analysis. All invertebrates are handpicked and identified to the lowest practical level and counted. See more details in Larned et al. 2007, Datry et al. 2012. Send the list of invertebrates and eggs counted and identified to the core team of the 1000IRP project, along with information on the hydrological phases.

5.7 Photo Traps:

During the whole study period, each sampling reach is monitored using a photo-trap. This device records animal movements when they cross the 'field' of the camera, allowing their identification from the recordings later on. To do so, a device should be placed in a nearby tree, so it can cover a maximum of the riverbed, including when dry. The frequency of downloading the images recorded is device-dependent, so this is up to each participant. For more information, see Findlay et al. 2017. After the study period send to the core team of the 1000IRP project the list of animals recorded along with information on number of individuals and in which phase and time the animals appeared.

5.8 Tracks:

For each phase, identify 2 habitat types: dry riverbed and riparian zone habitats, separated from each other by approximately 100 m. In each habitat, select 3 stations each comprising 1 circle of 0.7 m in diameter, which will be covered with a 2-5 mm layer of smoothed white marble or fine sand dust. Try to place one station in the range of the phototrap installed (see 5.7). In case of rain, the layer may need to be reset. Stations are checked every 2 days to observe the tracks of terrestrial vertebrates (optimum would be 5 visits per station). After documenting the number and the direction of tracks, the dust surface is resmoothed.

To document tracks:

-take photos

-note the direction of the tracks: upstream, downstream, perpendicular to the river on the right, perpendicular to the river on the left, no clear direction

-classify the animal according to its taxonomic group: micromammals (Orders Rodentia and Insectivora), lagomorphs (Order Lagomorpha), marsupials (Diprotodontia), carnivores (Order Carnivora), ungulates (Order Artiodactyla), reptiles (Class Reptilia), and birds (Class Aves)

- use the latin name for the species/genus, whenever possible.

For more information, see Sánchez-Montoya et al. 2016. After the study period, send the list of animal tracks identified to the core team of the 1000IRP project, along with information on number of individuals and in which phase tracks were recorded.

6. Literature:

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