SEVENTH FRAMEWORK PROGRAMME

THEME 6: Environment (including Climate Change)

Adaptive strategies to Mitigate the Impacts of Climate Change on European Freshwater Ecosystems

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Dissemination Level (add X to PU, PP. RE or CO)

PU	**Public**
PP	Restricted to other programme participants (including the Commission Services)
RE	Restricted to a group specified by the consortium (including the Commission Services)
CO	Confidential, only for members of the consortium (including the Commission Services)

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Site selection

1. Objectives:

Understand temperature processes in streams.
Identify temperature disturbance regimes in streams.

2. Questions:

What shaded stream length needed to sort effect?
Shading is more than temperature: what about habitat heterogeneity?

3. Experimental site

a. Gradient types:

Shading gradients are selected. A shading gradient consist either of:

- an open upstream and a shaded stretch downstream or
- a shaded stretch upstream followed by an open stretch downstream

b. Number of sampling sites:

In total 12 sampling sites are chosen:

shading gradient	In a nutrient gradient
open-forest	6
forest-open	6

c. Selection criteria for shading gradients:

- fragmented landscape with deciduous forest
- scale: transects of large length stream stretches (preferably 2 km shaded and 2 km open)
- stream type: 1.5-4 m width
- near-natural stream stretches
- need for both nutrient-poor and nutrient-rich stretches
- avoid strong groundwater influx (look for dry wooded banks, and avoid Alnus forests: seepage could also be interpreted by using the vegetation types present) a groundwater routing with chloride input and EC monitoring can also indicate influx
- avoid presence of tributaries
- average canopy cover in a shaded stretch must be higher then 50%
- select one gradient per stream

d. The length of the sampling site:

Each sampling site is preferably four km long –two kilometers upstream from the shaded/unshaded border and two kilometers downstream from the shaded/unshaded border.

Experimental design

1. **Water temperature measurements:**
   Within each shading gradient, 10 measurement spots are chosen and on each spot a temperature logger is installed (Appendix 1). Temperature is measured constantly year round (at least till the 1st of October 2010), with 20 minutes interval.

2. **Air temperature and light intensity measurement:**
   Air temperature and the light intensity are measured constantly for more than one year (at least till the 1st of October 2010), with 20 min interval. Position of the light logger should represent the stretch conditions – see the light and temperature loggers installation protocol (Appendix 2).

3. **Loggers readout**
   The loggers are read out every 3 months. While reading out the loggers, water depth, width and current velocity at each of the loggers are measured and discharge transect is measured at one location per stream (Appendix 3).

4. **Canopy cover**
   The canopy cover is estimated in all streams, along the whole shading gradient, in the summer – see the canopy cover estimation protocol (Appendix 4).

5. **Macrofauna sampling:**
   Macrofauna sampling is carried out in autumn*. Macroinvertebrates are sampled from all 12 streams. Later on we decide on the number of samples to process (suggestion of 8 streams). The samples are taken at each of the water temperature logger sites. This means 10 sites per stream, for each of the streams.
   At each site two habitat types will be sampled; one soft substrate sample and one hard substrate sample. These two habitat type specific samples are kept separate (Appendix 5).
   While macrofauna sampling, depth, current velocity and substrate type are measured for each surber. Water width and oxygen is measured at each logger. Ph and EC are measured per stream and two water samples in each stream are taken (one at the end of each stretch) (Appendix 5). There is no need to take water samples during macrofauna sampling, if the macrofauna sampling and diatom sampling are split in time (but are still done in the same season) and the water samples were taken during the diatom sampling.

   *Because of drought situations, the streams in Spain will be sampled in spring 2011

6. **Substrate cover estimation:**
   Substrate cover estimation is done at one location in the open and one location in the shaded stretch, preferably in the middle of the stretch. Substrate cover is estimated along 20m long stretch. The estimation method will be chosen by partner and it depends on the size of the stream and turbidity of the water. The STAR substrate classification will be used (see macrofauna sampling field form in Appendix 5).

7. **Macrophyte inventory**
   Macrophyte inventory is carried out before the leave fall. One, 20-50m long transect in the open stretch and one 20-50m long transect in the shaded stretch is inventoried (Appendix 6). The transects should be representative of the stretches.

8. **Benthic diatoms sampling:**

Benthic diatoms are sampled before the leave fall. One sample is taken at the end of open and one at the end of shaded stretch (Appendix 7). Optionally, 2 diatom samples per stretch can be taken.

The first water samples should be taken at the same time and location as the diatom samples.

The substrate from which diatoms are sampled should be the same in all stretches/streams. The best substrate is stone. If stone is not available, then macrophytes, otherwise branches or other hard substrates.

Caution: The diatom sample in the shaded stretch should be taken from a substrate located in a well shaded location. The diatom sample in the open stretch should be taken from a substrate located in a not shaded location.

9. **Water samples:**

Water samples are taken seasonally (4 per year). At each occasion we take 2 water samples per stream – at the end of shaded and open stretch, as the nutrient content can depend on the land use (runoff from the fields), so perhaps we see the differences between open and shaded stretches. Minimally nutrients t-P, t-N, ammonium, nitrate, dissolved inorganic phosphorus (PO_4-P), calcium and chloride (Appendix 8).

The bottles should be filled completely and closed under the water. Water samples should be placed in the freezer (-18°C) directly after returning to the lab.

If macrofauna samples and diatom samples are taken on different days, the water samples should be taken at the same time and location as the diatom samples. Further water sampling could be combined with the readout of the loggers.

10. **Geographical stream direction**

Elaine reports back on a possibility to define average geographical position of each stream based on the *digital elevation model* with ArcGIS. This measure gives a proxy to estimate the amount of sun radiation that directly reaches the stream.

11. **Metabolism (optional)**

Measured in one location in the open and one location in the shaded stretch, 24 hour measurement (Appendix 9).

12. **Decomposition (optional)**

Appendix needed (Spain; Eugenia)

13. **The following parameters will not be considered with the shading survey:**

    a. Shoreline beetles

Appendix 1 Installation of water temperature loggers

1. The experimental design:

At each sampling site, 10 measurement spots are selected. On each spot a HOBO UA-002-64 temperature/light logger is installed. Temperature is recorded continuously over 14 months, with 20 minutes intervals.

The loggers are preferably installed before the 15th of July. Measurement spots are set according to the following schema:

Shading gradient in km's:

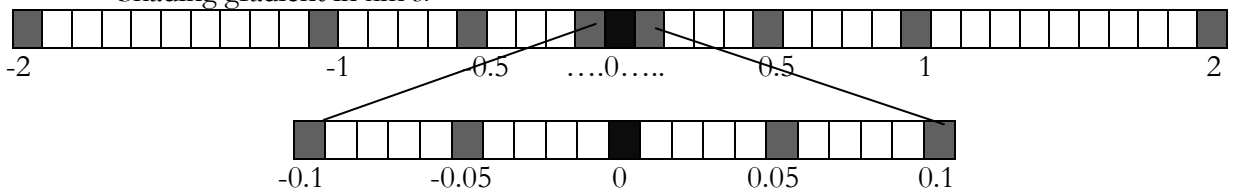

Figure 1. Distribution of the measurement spots along the shading gradient (distribution of the loggers will be modified if the length of the stretch is shorter than 4km)

Adjustment of water temperature data points to stretches shorter than 4km:

- for transects (shaded or unshaded) < 2km, but >1 km -> the last data point of 2 km is moved to the end point of the transect;
- for transects of = 1 km -> the last data point of 2 km moves to the 750 m spot;
- for transects of < 1 km -> the data point of 1 km moves to the end point of the transect, the data point of 2 km moves to 750 m.

The loggers are checked (for sand cover or damage) and readout every 3 months.

2. Programming the loggers

Before installing the loggers, the start date and hour of the measurements and the recording interval are programmed.
The same date and hour can be set for all the loggers. Preferably, 16th of July when all loggers are installed in all streams.

3. Position in the stream

A number of parameters influence the temperature measurements: habitat, current velocity, water depth, light conditions and position of the logger in the cross-section of the stream.

Preferably, the loggers are installed at the end of a 10-20 m straight stretch of the stream, where the current velocity pattern in the cross-section is less variable, the water is well mixed and due to that – water temperature is representative for the whole stream wet area.

Position the logger in the same habitat along a stream, habitats can differ between streams and countries. The logger is positioned near the bottom of the stream.

In the open stretch the logger is installed in the average light conditions of the respective stretch and in the shaded stretch in the average shaded conditions.

4. Installation method

Place a 20 – 30 cm metal pole in the sediment. Attach the logger using a plastic strip to the metal pole and place the logger close to, but above the sediment.

5. Equipment

- ✓ logger installed on a metal pole
- ✓ hammer
- ✓ GPS
- ✓ map
- ✓ field form
- ✓ pencil

Appendix 2 Installation of air temperature and light intensity loggers

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1. Introduction

As the relationship between insolation and air temperature in open and shaded stretches differ (buffering capacity of the shaded stretch on the air temperature above the stream), this would affect the relationship between air temperature and water temperature. So, we need average air temperature measurements from both unshaded and shaded stretches. In the unshaded stretch the average conditions are easily met as air temperature will be homogenously distributed along the stretch. In a shaded stretch the distribution of air temperatures will be heterogeneous (?). We do not want to research this heterogeneity but only want to know if there is a difference. The difference would be the most pronounced in a heavily shaded spot of the stretch. Therefore we should like to place one air temperature logger in this heavily shaded spot.

3. The experimental design:

Because air temperature and light loggers are combined, we need (in total) 2 of these loggers (HOBO UA-002-64 loggers) per stream. Light intensity and temperature are measured continuously for about 14 months with 20 min interval. In the open stretch, the logger is placed at an unshaded spot. In the shaded stretch, the most heavily shaded spot is chosen. The loggers are installed near the bank of the stream.

4. Installation of the loggers:

Depends on the local conditions.

5. Equipment

- ✓ logger installed on a metal pole
- ✓ hammer
- ✓ GPS
- ✓ map
- ✓ pencil
- ✓ field form

Appendix 3 Discharge

1. Location

Discharge is measured at one site per stream, preferably at every visit. Preferably, the discharge site has a straight homogenous profile (a concrete structure).

2. Transect measurements

The width of the stream should be divided in 10 equal sections. Current velocity (as m/s at 0.4 times the depth above the stream bed) and water depth are measured in the middle of each section (Figure 1).

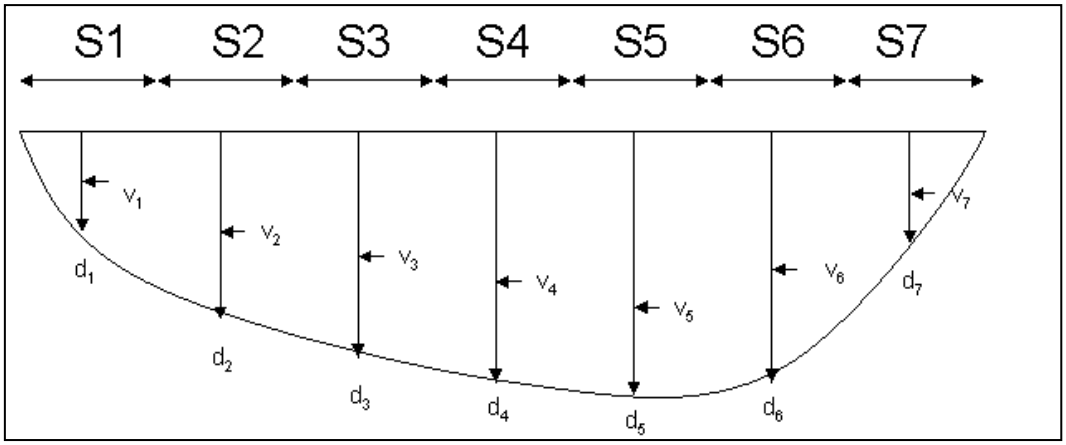


Figure 1. Transect for discharge calculation (S – section, d – water depth, v- current velocity)

3. Calculations

In order to know discharge of the stream at a given location, the following formula is used:

$$Q = \sum(v_{1\text{-}x} * d_{1\text{-}x} * w_{1\text{-}x})$$

,where:
Q- discharge (m3/s)
v- current velocity (m/s)
d- water depth (m)
w- section width (m)

4. Equipment

- ✓ measuring tape
- ✓ depth stick
- ✓ current velocity meter
- ✓ pins to install measuring tape
- ✓ field form
- ✓ pencil
- ✓ spare batteries

General information			
REFRESH Shading experiment		Date	Stream
Location	Researchers	X coordinate	Y coordinate

Discharge transects				
location/ section	section width (cm)	water depth (cm)	current velocity	
			replicate	value
1			1	
			2	
			3	
2			1	
			2	
			3	
3			1	
			2	
			3	
4			1	
			2	
			3	
5			1	
			2	
			3	
6			1	
			2	
			3	
7			1	
			2	
			3	
8			1	
			2	
			3	
9			1	
			2	
			3	
10			1	
			2	
			3	

Appendix 4 Protocol for estimating canopy cover

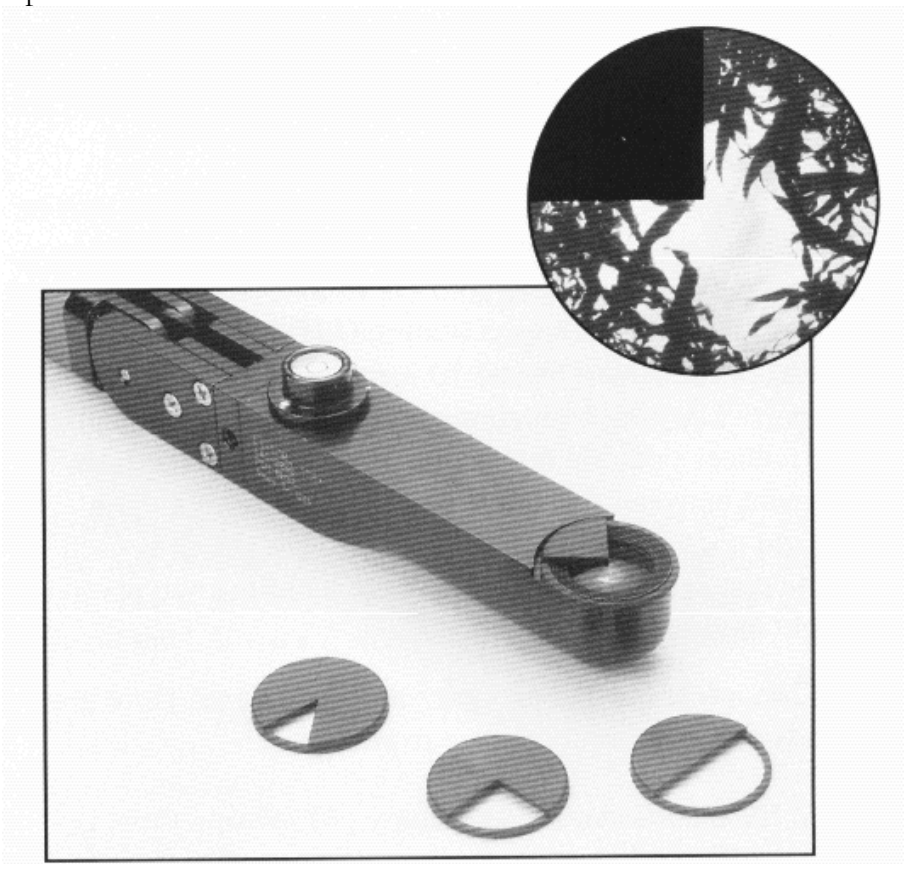
Canopy cover is using a LAI-2000 plant canopy analyzer (LI-COR).

1. General:
The protocol can be downloaded:
http://www.licor.com/env/Products/AreaMeters/lai2000/2000_intro.jsp
Read Chapter 3 and Chapter 4.

2. Background:
The LAI-2000 calculates Leaf Area Index (LAI) and other canopy structure attributes from radiation measurements made with a "fish-eye" optical sensor. Measurements made above the canopy (A reading) and below the canopy (B reading) are compared and used to determine canopy light interception at five angles, from which LAI is computed using a model of radiative transfer in vegetative canopies. LAI is dimensionless, but can be thought of as m2 foliage area/m2 ground area (see Chapter 5 in the manual).

3. Procedure:

a. Before measurements
Before measurements, the sensor is fitted with the 270 ° view cap to mask the operator , as shown on the picture.



Measurements should be preferably made when there is a full cloud cover. A readings are used as reference and are made in the open field. B readings represent the shading conditions. Check A reading before beginning B readings by doing several subsequent A reading. If A readings are inconsistent, poor measurement technique or far-from-ideal sky conditions are indicated. As

reference above-canopy readings in areas without any canopy (e.g. in the middle of a grass field) must be made.
See below how to make subsequent A readings.

Make sure the LAI-2000 is calibrated and Resolution is set on HIGH. For calibration follow the instructions in the manual. Calibration is not important when using the same sensor for A and B reading, only when using separate sensors for A and B readings.

Along each of the shaded or unshaded stretch, ten points for B readings are identified. These points are evenly spread along the entire length of each stretch. For example, if a stretch is 500 m long, conduct one B reading every 50 m.

b. Measurements

At each measurement spot the B reading is preformed at the middle of the stream. The sensor is held by the operator close to the water surface with the sensor in upward direction. This position ensures that shading by vegetation and steep banks is included in the LAI calculation.

When sky conditions are stable, one A reading will suffice for all ten B readings. If sky conditions changes, a new A reading must be made immediately before the next B reading. See below how to make a reading under either stable or changing sky conditions.

c. Operations:

How to make subsequent A readings:

Use this procedure to check for stable sky conditions and for correct operation technique.

- Turn the LAI-2000 on by pressing the "ON" key
- Press the "OPER" key.
- Press the "↑" key until "11 SET Op Mode" is on the top line of the display.
- Press "ENTER".
- Press the "↑" key until "1 sensor X" is displayed.
- Press "ENTER".

You are now prompted for the sequence of A and B readings. The display shows the following:

Seq=↑↑↓↓ (or another combination of ↑↓) NEW=

"↑" means an A reading (above canopy) and "↓" means a B reading (below canopy)

- Press "↑" once.
- Press "ENTER".
- The next prompt REPS= is for the number of times you wish to repeat the pattern before calculations. We will do 1.
- Press "ENTER" and you will return to the OPER menu.

- Press the "BREAK" key.
- Press the "LOG" key.
- The following two prompts are for identification of the data. Enter whatever you care for each of these.
- The display will now show:

```
0*T      13:49:52 (real time)
0        0.00 ± 0.00
```

- Place the sensor where you want to make an A reading. make sure the sensor is levelled (indicated by the bubble level on the sensor) and press either the "ENTER" key or the button on the optical sensor.
- Following two beeps the LAI computes the result and VIEW MODE is entered.
- Press "5" to view the results.
- The display will now show:

```
13:52:01 (real time)   1.128 (A reading)
1/    1, CHAN   A1
```

- In this case, 1.128 is the A reading.
- Repeat the above several time to check if A readings are constant.

How to make one A readings followed by ten B readings:

Use this procedure when sky conditions are stable.

- Turn the LAI-2000 on by pressing the "ON" key
- Press the "OPER" key.
- Press the "↑" key until "11 SET Op Mode" is on the top line of the display.
- Press "ENTER".
- Press the "↑" key until "1 sensor X" is displayed.
- Press "ENTER".

You are now prompted for the sequence of A and B readings. The display shows the following:

```
Seq=↑↑↓↓ (or another combination of
↑↓)
NEW=
```

"↑" means an A reading (above canopy) and "↓" means a B reading (below canopy)

- Enter "↑↓↓↓↓↓↓↓↓↓↓".
- Press "ENTER".
- The next prompt REPS= is for the number of times you wish to repeat the pattern before calculations. We will do 1.
- Press "ENTER" and you will return to the OPER menu.
- Press the "BREAK" key.
- Press the "LOG" key.
- The following two prompts are for identification of the data. Enter whatever you care for each of these.
- The display will now show:

0*T 13:49:52 (real time) 0 0.00 ± 0.00

- Place the sensor where you want to make an A reading. Make sure the sensor is levelled (indicated by the bubble level on the sensor) and press either the "ENTER" key or the button on the optical sensor.
- Do the ten B readings along the reach.
- Following the last B reading the LAI computes the result and VIEW MODE is entered.
- Use the arrow keys to scroll through the data.
- Write down LAI and SEL (std error of LAI).

How to make one A reading followed by one B reading (x10):

Use this procedure when sky conditions are unstable and change rapidly. Time lag between each A and B reading must be very short.

- Turn the LAI-2000 on by pressing the "ON" key
- Press the "OPER" key.
- Press the "↑" key until "11 SET Op Mode" is on the top line of the display.
- Press "ENTER".
- Press the "↑" key until "1 sensor X" is displayed.
- Press "ENTER".

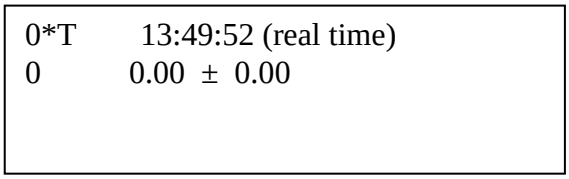
You are now prompted for the sequence of A and B readings. The display shows the following:

Seq=↑↑↓↓ (or another combination of ↑↓) NEW=

"↑" means an A reading (above canopy) and "↓" means a B reading (below canopy)

- Enter "↑↓".
- Press "ENTER".

- The next prompt REPS= is for the number of times you wish to repeat the pattern before calculations. We will do 1.
- Press "ENTER" and you will return to the OPER menu.
- Press the "BREAK" key.
- Press the "LOG" key.
- The following two prompts are for identification of the data. Enter whatever you care for each of these.
- The display will now show:



- Place the sensor where you want to make an A reading. Make sure the sensor is levelled (indicated by the bubble level on the sensor) and press either the "ENTER" key or the button on the optical sensor.
- Do the corresponding B reading.
- Following the B reading the LAI computes the result and VIEW MODE is entered.
- Use the arrow keys to scroll through the data.
- Write down LAI. No SEL (std error of LAI) will be computed as only one B reading.

Appendix 5 Macroinvertebrate sampling

1. Introduction

This protocol describes the common handlings for macroinvertebrate sampling in the 'Shading survey'.

Two basic questions need to be answered by means of the shading experiment:

Q1: How does shading affect the stream water temperature?

We use all data collected in 12 streams to answer Q1.

Q2: Do differences in temperature have effects on (selected groups of) macroinvertebrates, diatoms and macrophytes?

We use a minimum of 8 streams (4 shaded-unshaded and 4 unshaded-shaded) with temperature differences to answer Q2. We expect that not all of the 12 streams will show temperature differences between shaded and unshaded sites or will encounter other circumstances that make them unsuited for analysis.

2. Sampler

Macroinvertebrates are sampled by using either a Surber sampler with a net mesh width 500 um or 1mm, or kick sampler (deep water) with the same sample surface and net mesh size. Sizes of the Surber sampler square are 25 by 25 cm (0,0625 m^2).

3. Sampling

Macroinvertebrate samples are taken from all 12 streams. The samples are taken from each of the watertemperature logger sites. Thus 10 sites per stream, for all twelve streams are sampled.

At each watertemperature logger site a stretch of 25-50 m (depending on suited habitat availability) is marked.

At each site two habitat types will be sampled: one from soft substrate and one from hard substrate (according to the AQEM/STAR habitat classification). These two habitat type specific samples are kept separate. Sampled habitat types should be comparable among all sites within one stream.

One habitat type sample consists of three Surber samples. Before sampling a habitat type the three Surber sampling spots are randomly chosen within the marked stretch. Sampling starts at the downstream end of the reach and proceeds upstream. The whole sample (three subsamples per habitat type per logger site) thus are lumped together and treated as one sample.

In order to sample the different habitat types present in each stream, the three surber samples per section should be taken from the most dominant soft and the most dominant hard substrate types present for the whole stream. If the section has only one substrate type the three surber samples are distributed to cover the section. Otherwise the three are distributed over the area of each dominant type. Sampled substrate types should be noted on the field protocol.

To sample with a Surber sampler place it on the substrate and disturb the substrate within the 25 x 25 cm frame to a depth of about 3 cm. The current will take invertebrates and organic material into the net. Try to avoid the excess amount of detritus entering the net. In slow-flowing areas create an extra current by hand to move the invertebrates towards the net.

Rinse each Surber sample with clean stream water through the outside of the net to concentrate the sample at the bottom of the net (if you have little material you can take the second or even third Surber sample before rinsing). After taking all three samples turn the net inside out above the sample container (e.g. a bucket) and rinse the collected material into the container. Take care to only rinse water from the outside of the net as you do not want to introduce new specimens that come with the water you rinse the net with.

Large wood and stones can be removed from the sample immediately and inspected for clinging or sessile organisms. Any organisms found have to be placed into the sample container or sample vial. Generally, it is recommended not to spend time inspecting small debris in the field; however, large and fragile organisms or species that cannot be preserved can be picked out of the sample in the field. These organisms should be stored in a small separate container.
Large and rare organisms, which can easily be identified in the field (such as crayfish, large mussels), should be removed from the sample and returned to the stream.
If you sort preserved samples then you can preserve the sample with formalin (4% final concentration) or ethanol (70% final concentration) immediately after collection. Water in the sample should be decanted before adding the ethanol. Fixation is important to prevent carnivores, particularly stoneflies (Setipalpia), beetles (Adephaga), caddis larvae (e.g. Rhyacophilidae), Sialidae and certain Gammaridae, from eating other organisms.
Forceps may be needed to remove organisms from the net. The sample container should close tight.

The containers are transported to the laboratory. Life samples are stored in a fridge at about 4°C and oxygenated. All live samples should be sorted within 4 days after sampling.
Also preserved samples should be stored cool. Place a label (written in pencil, printed on a laser printer or photocopied) with the following information inside the sample container and vials:

- project name (optional)
- stream name
- site name (temperature logger code)
- site code (optional)
- habitat type
- date of sampling
- investigators name (optional)

The outside of the container should include the same information and, when preserved, the words "preservative: formalin or ethanol, respectively". If more than one container is needed for a sample, each container should be labelled with all the information of the sample and should be numbered (e.g., 1 of 2, 2 of 2, etc.).
If taxa (e.g. crayfish, large mussels) were identified in the field and returned to the stream, note their presence and abundance on the label placed in the sample container as well as on the field form. If possible, label and place the container with the rare and fragile organisms into the main sample container and note its existence on the field form.

4. Sample processing

A sample is washed from the container trough a 1 mm mesh width sieve in the laboratory or in the field. Do not wash too strong as the water will pore animals through the sieve. The sieve residue is rinsed into a transparent tray (when you use light from beneath the tray) or a white tray.

Next the (selected groups) of macroinvertebrates, are picked out the tray with a biforceps and placed in vials. 100% of the sample is sorted.

It is advised to put major taxonomic groups in separate vials, this makes the identification process more easy. The vials are labeled according to the labeling of the sample container.

Only Ephemeroptera, Plecoptera, Trichoptera, Crustacea, Odonata, Coleoptera and Mollusca (Bivalvia, Gastropoda) are sorted. These selected groups are identified to species level, except for the Odonata, the family Coenagrionidae to family level, the Bivalvia the genera Pisidium and Sphaerium to genus level.

5. Substrate type and cover within each Surber area

Substrate cover (%) is estimated at each sampled Surber area, before stirring the substrate and noted on the field form. Classification of substrates follow table 1 (the AQEM/STAR substrate classification).

6. Additional abiotic variables

The following variables are noted in the field form:
Per Surber sample:

- depth
- current velocity (m/s) at 0.4 times depth above the stream bed
- substrate type

Per 6 Surber's together:

- width (average position)
- oxygen

Per strection (shaded/unshaded):

- pH
- electric conductivity
- 2 water samples at the end of both stretches. Parameters to be analysed: nutrients t-P, t-N, ammonium, nitrate, calcium and chloride (The bottles should be filled completely and closed under the water. Water samples should be placed in the freezer (-18°C) directly after returning to the lab or analysed immediately.)
  Note: Preferably, the water samples are taken during the diatom sampling.

List of equipment per stream:

- Surber sampler (25x25)
- 20 containers/buckets per stream
- 20 covers for the containers/buckets
- 20 field forms
- 20 labels for macrofauna containers/buckets
- pencils
- waders
- gloves (optional)
- note pad
- rubber boots (optional)
- GPS
- camera (optional)
- map
- current velocity meter
- depth stick
- measuring tape
- oxygen and temperature meter
- pH meter
- EC meter
- 2 labeled bottles for water samples
- spare batteries

7. Quality control in the field

- Sample labels must be completed properly, including the sample identification code, date, stream name, sampling location, and collector's name, and placed inside the sample container. The outside of the container should be labelled with the same information.
- After sampling of a habitat type has been completed at a site, the nets should be rinsed thoroughly, examined carefully, and picked free of organisms or debris. Any additional organisms found should be placed into the sample containers. The equipment should be examined again prior to its use at the next habitat type.

Field form

General information				
REFRESH Shading experiment		Date		Stream
Location		X coordinate		Y coordinate
		Digital photos taken (yes/no)		Researchers
Sample code:		HARD/SOFT substrate		Width (m):
Measured parameters				
replicate	water depth [cm]	current velocity [m/s]	O_2 [mg/l]	O_2 [%]
1				
2				
3				
Notes/drawings				

Habitat estimations							
name	size	description	substrata type	habitat in the section (%)	sampled substrate (%) per surber sample:		
					1	2	3
Megalithal	>40 cm	(cobbles and boulders)	boulders				
Macrolithal	20 -40 cm	(head size cobbles)	blocks				
Mesolithal	6 -20 cm	(fist to hand size cobbles)	cobbles				
Microlhital	2 -6 cm	(pigeon egg to child's fist)	coarse gravel, pebbles				
Akal	2 -0.2 cm	(fine to medium size gravel)	gravel				
Psammal/ psammo-pelal	6 μm -0.2 cm	(sand and mud)	sand, mud				
Argyllal	< 0.006 mm	(silt and clay)	silt, loam, clay				
Algae		filamentous algae, algal tufts	algae				
Sub-merged macrophytes		(cover and composition)	submerged macrophytes				
Emergent macrophytes		(cover and composition)	emergent macrophytes				
Living parts of terrestrial plants		(cover and composition)	bank macrophytes				
Xylal (wood)		(dead wood, roots twigs and branches)	woody debris				
CPOM		(deposits of coarse particulate organic matter)	CPOM				
FPOM		(deposits of fine particulate organic matter)	FPOM				
Organic mud		mud and sludge (organic) = pelal	organic mud				
Leaves		(leaves)	organic				

The AQEM/STAR substrate categories.

Mineral substrates	**1.1.1. Definition**
Hygropetric sites	water layer on mineral substrata
Megalithal (> 40 cm)	upper sides of large cobbles, boulders and blocks, bedrock
Macrolithal (> 20 cm to 40 cm)	coarse blocks, head-sized cobbles, with a variable percentages of cobble, gravel and sand
Mesolithal (> 6 cm to 20 cm)	fist to hand-sized cobbles with a variable percentage of gravel and sand
Microlithal (> 2 cm to 6 cm)	coarse gravel, (size of a pigeon egg to child's fist) with variable percentages of medium to fine gravel
Akal (> 0.2 cm to 2 cm)	fine to medium-sized gravel
Psammal/psammopelal (> 6 μm to 2 mm)	sand and mud
Argyllal (< 6 μm)	silt, loam, clay (inorganic)
Biotic microhabitats	**Definition**
Phytal	floating stands or mats, lawns of bacteria or fungi, and tufts, often with aggregations of detritus, moss or algal mats (interphytal: habitat within a vegetation stand, plant mats or clumps)
Algae	filamentous algae, algal tufts
Submerged macrophytes	macrophytes, including moss and Characeae
Emergent macrophytes	e.g. *Typha, Carex, Phragmites*
Living parts of terrestrial plants	fine roots, floating riparian vegetation
Xylal (wood)	tree trunks, dead wood, branches, roots
CPOM	deposits of coarse particulate organic matter, e.g. fallen leaves
FPOM	deposits of fine particulate organic matter
Sewage bacteria and –fungi and saprobel	sewage bacteria and –fungi, (*Sphaerotilus, Leptomitus*), sulfur bacteria (e. g. *Beggiatoa, Thiothrix*), sludge
Organic mud	mud and sludge (organic) = pelal
Debris	organic and inorganic matter deposited within the splash zone area by wave motion and changing water levels, e.g. mussel shells, snail shells

Environmental parameters measured twice per stream

General information					
REFRESH Shading experiment				Date	
Stream				Researchers	
Abiotic variables					
Water sample code	shaded/open	X coordinate	Ycoordinate	EGV [µS/cm]	pH N/A

Appendix 7 Macrophyte inventory

Protocol for macrophyte inventory

- Macrophyte species abundance will be estimated in the middle of each shaded/open stretch along a 20-50 m long transect were the macrophyte composition is representative for the whole stretch.
- At each transect the macrophytes are identified to species level (except for mosses, they are just "moss"). The inventory only includes the aquatic species, so those that grow in the border between terrestrial and aquatic habitat are excluded.
- Walk the length of the transect and write down the relative abundance of every species from the scale below. Relative abundance means that the abundance of each species is set relative to the other species occurring along the transect. So even if the total cover of macrophytes is very low the most common species along the transect is noted as dominant.

Scale:

Dominant -	Highest coverage
Abundant -	Large coverage (> 20% of veg. cover).
Frequent -	Frequently present but no high coverage.
Occasional -	Spread over the transect, coverage low.
Rare -	Only one ore some specimens.

Field form: Shading experiment
Macrophyte inventory
Tick the box to indicate dominance category for each species

General information		
Date	Stream	Surveyors
Shaded/ Open	X coordinate	Y coordinate

Species name	**Dominant**	**Abundant**	**Frequent**	**Occasional**	**Rare**

Appendix 7 Diatom sampling

Protocol for the sampling of benthic diatoms from rivers

Principle

The benthic diatom flora from submerged substrates in rivers or streams are sampled in order to produce representative collection of the diatom assemblage in both shaded and open stretch. The data are suited for the calculating of relevant diatom based water quality indices and the analysis of biodiversity (relative abundance of taxa, no density data is available).
The method described below, coming within the framework of the REFRESH project, is derived from standard procedures (AFNOR, 2000; CEN, 2002) used for the bio-assessment and bio-monitoring of streams.

Field sampling equipment

· Appropriate water safety equipment and waders.
· A toothbrush (or other similar instrument) to remove diatoms from substratum.
· A large plastic container to collect the sample (to remove diatoms from stones, to shake-off diatom from Macrophytes).
· A plastic or glass tube to store the sample.
. Permanent marking pens (waterproof writing material) or other means of labeling the samples.

Preservative

It is required to stop cell division of diatoms and decomposition of organic matter. No preservative is necessary if the sample is treated within a few hours of collection. In that case, after sampling, storage should be done in dark place (and cool, if possible).
Formaldehyde 34% (ratio 10% formaldehyde / 90% sample, adjustable according to the organic matter load – a final solution of 1% to 4%) or 70% ethanol can be used. For health security, we recommend to use ethanol.

Sampling methods and choice of substratum

Hard surface samples are expected to be the most consistent, are faster to analyse and less likely to be contaminated with dead cells. **The preferable substratum is a rock** that is big enough to have remained stable under most flows, yet small enough to be picked out of the water (e.g. approx 10-30 cm diameter). The sample should be a composite of brushing from rocks at the site. At least five cobbles should be sampled. However, if cobbles are unavailable, then either 5 small boulders or 10 pebbles should be sampled. An area of approximately 10 cm² or more should be scrapped.

Sampling consists of brushing the upper part of the substratum (water exposed part) with a toothbrush into a plastic container in order to collect the biofilm (Figure 1). It is preferable to brush an area that does not have other growths (such as moss, lichen, or filamentous algae). If there is some detritus on the rock (or other substratum), the sample may actually be part "soft surface" community as well as "hard surface" community. To avoid this contamination of the hard surface sample, shake any hard surfaces (under the water) prior to sampling.

If there are no rocks at all, then the preferred substrata are macrophytes, in any case woody debris, mud and detritus must be avoided. The type of substratum used should be recorded. When several species of macrophytes are present, incorporate these species in a composite sample that is representative of the macrophytes present in the sampled reach. Submerged macrophytes are preferred over emergent ones. When emergent macrophytes are sampled, use only the submerged portions at sufficient depths to

allow for fluctuating water levels. Filamentous algae (macro-algae) can be dislodged by hand. Place a portion of the plant in a sample container with some water (clear water of the river). Shake it vigorously and rub it gently to remove algae. Remove plant from sample container. Decant the suspension. In this way one combined sample will be collected, containing the diatom films of several macrophytes or macro-algae at the site.

Finally, transfer the sample collected in the plastic container into a plastic or glass tube (figure 2). Let the sample precipitate for a while and then take of the supernatant. Add preservative to stop cell division and for permanent storage.

Figure 1: *Sampling consists of brushing the upper part of the substratum.*

Figure 2: *transfer of the sample collected in the plastic container into a plastic or glass tube.*

Sampling location

Wherever possible, **the samples should be from riffle or run sections** of the stream. It is preferable to move away from the bank in order to sample the main current flow. It is preferable (if possible) to sample from approximately 15 cm depth. This measure is also selected for maintaining consistency.

If the stream has no riffles or runs then slow-flowing sections can be sampled.

Examine all brushing tools and plastic container for residues. Rub them clean and rinse them in water after sampling and before sampling to avoid contamination between samples.

Site selection

Diatoms would be sampled in two spots before the leaves fall in each stream: 1 sample downstream the open stretch and one downstream the shaded stretch (Figure 3). Thus, at least 24 samples will be available for each partner.

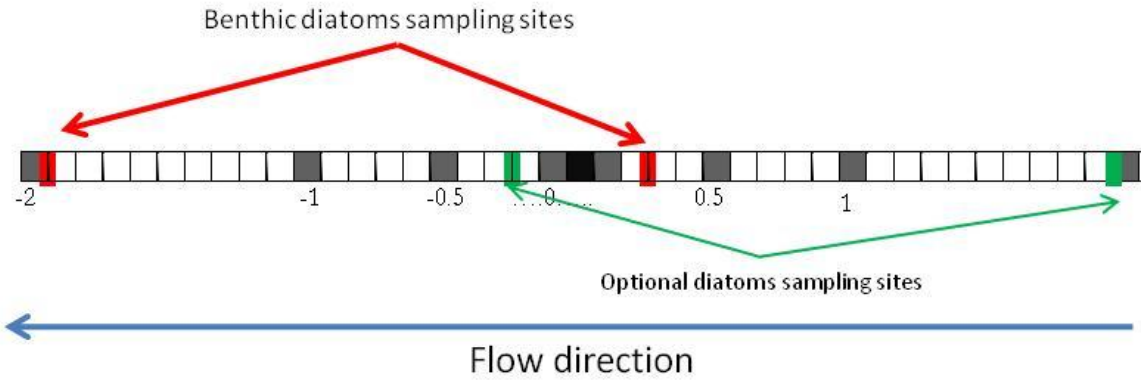


Figure 3. Location of the benthic diatoms sampling spots along the gradient.

Caution: for the consistency of the REFRESH objectives,

i) the diatom sample in the shaded stretch should be taken from a substrate located in a well shaded location; the diatom sample in the open stretch should be taken from a substrate located in a not shaded location ;

ii) it is very important that the combination substrate/current flow would be the same in the two stretches;

iii) It is advised to sample the same substrate in all 12 streams, in order to compare diatom flora between streams.

Suggestions for optional sampling:

- optional samples could be taken, one in the upper part of the stream and one in the upper part of the second stretch (figure 3)
- Some rivers could be sampled in 6 or more spots (spots 2000, 1000, 100, -100, -1000 and -2000).

Labelling

Place a permanent label on the outside of the sample tube with the following information: waterbody name, location, station number, date, substratum and habitat (running or low-flowing section). Name of collector and type of preservative can be optional.

References

AFNOR, 2000. NF T 90-354. Qualité de l'eau. Détermination de l'Indice Biologique Diatomées (IBD), 63p.

CEN, 2002. Water quality - Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers.

Appendix 8 Water chemistry

Nutrient background concentrations

Analysis to be done:

- Dissolved inorganic nitrogen (NH_4-N, NO_3-N) and phosphorus (PO_4-P).
- Total nitrogen (TN) and total phosphorus (TP).

Field protocol

a. Material needed

- Sampling vials to collect stream water (45ml)
- Glass-fiber filters (0.7 μm pore size, e.g., Whatman GFF). Filters should be combusted before the sampling (4-5 hours @ 450ºC)
- Filter holders
- 100 ml syringe

b. Procedure

1. Collect 3 water samples at the end of each experimental reach; 3 replicates for the open reach and 3 replicates for the shaded reach (a total of 6 per stream) in a mid-channel area with running water. Flush the syringe 3 times before taking the samples.
2. Samples for dissolved inorganic nitrogen (NH_4-N and NO_3-N) and phosphorous (PO_4-P) should be filtered through ashed Whatman glass fiber filters (0.7μm pore size) into 45ml tubs.
3. Samples for total nitrogen (TN) and total phosphoros (TP) should not be filtered.
4. Keep samples on ice for transport to the laboratory. Once in the lab, place them into the refrigerator if analysis will be done within the next 24 hours or freeze them for further analysis.

Lab protocol

Use standard colorimetric-related methods (APHA, 1995) or ion-exchange chromatography for the analysis of NO_3 and PO_4 except for ammonium concentration (only standard colorimetric-related methods are suitable). For TN and TP a pre-digestion is needed in order to oxidize the organic matter to nitrate and phosphate, respectively.

Sampling frequency

Take samples at least every 3 months, coinciding with the download of the HOBOs.

Related literature

APHA. 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington DC, USA.

Appendix 9 Measurement of stream metabolism

1. Measurement of stream metabolism

Task applicable to: control and treatment stream reaches under flow through conditions. Under drying conditions in the treatment stream reaches we could estimate respiration rates as described for the riparian zones. Alternatively, discrete measurements of dissolved oxygen across the reaches could be done to estimate spatial variation in this parameter in the two reaches and under different hydrologic conditions.

1.2. Introduction

To measure the stream metabolism we will use the diurnal upstream-downstream dissolved oxygen change technique. Because this technique is based on measuring *in situ* changes in dissolved oxygen concentration over a 24 h period at two points along a stream reach, it allows to estimate the daily rates of respiration and gross primary production at the stream ecosystem level.

Measurements will be done on each sampling date at the two experimental reaches. These data will provide information on the biological activity and dominance of heterotrophic and autotrophic metabolism under the different hydrologic conditions. Comparison of results between the two reaches will allow to evaluate how hydrological modifications affect the stream metabolism.

Notice that changes in dissolved oxygen (DO) concentration between the top and the bottom of the reach are due to two types of mechanisms: a) a physical mechanism due to the equilibration of DO between the water column and the atmosphere, and b) a biological mechanism due to the metabolism of photoautotrophic and heterotrophic organisms. Therefore, to estimate the rate of change in DO due only to the biological activity (that is the stream metabolism) it is necessary to have good and reliable estimates of the net changes in DO between the two sampling stations and also of the DO fluxes between the water and the atmosphere (i.e., reaeration flux). This section includes the procedures to do these two estimates.

It is recommended to conduct these measurements under stable discharge conditions; that is, avoid to sample shortly after a flood. Additionally, if discharge in your study site exhibits a pronounced daily variation, make sure to have a record of discharge at least several times over the 24 h period to correct DO fluxes for this variation.

1.3. Field protocol

1.3.1. Measurement of upstream-downstream net DO changes

a. Material needed

- Light meter
- Thermometer (included in the oxygen meters)
- 2 oxygen meters. Alternatively, Winkler bottles at least 12 for each sampling site (sampling sites are the top and bottom of each experimental reach)
- 1 or 2 data loggers (if you use the oxygen meters to record daily temporal variation of this parameter). Alternatively, data can be recorded manually.

b. Procedure

1. If you use oxygen probes, cross-calibrate both probes at a given site. Additionally, to ensure correct readings, probes can be also calibrated with the Winkler assay in the laboratory.
2. Deploy oxygen probes in the middle of the cross-section at the top and bottom part of the experimental reach. The depth of the probe tip should be approximately half way water surface/sediment surface. Make sure that probes will remain steady at a single spot during the recording period (i.e., 24 h.). If necessary, use clamps and stands to fix the position.
3. Start recording DO and temperature at both sites at sunset on first day and keep recording at least for 24 h. It is recommended to expand the recording period to capture a full day-time period and a full night-time period.
4. Data can be automatically recorded using data loggers or manually recorded.
    - If data are automatically recorded, a sampling frequency of 5 minutes is enough (shorter intervals can incorporate too much noise).
    - If data are manually recorded, ideal sampling frequency should be around the time that water takes to travel from the top to the bottom of the reach (i.e., the nominal travel time). In practice, during daytime sampling frequency should be at least every hour (better if it is more often) and at night sampling can de done every 2 or 3 h (again, it is better if it can be done more often).
5. During daytime, measure light (PAR) at a single spot representative of the reach. We recommend placing the logger at half way between the top and the bottom of the reach. IT IS IMPORTANT TO RECORD THE TIME WHEN SUN SETS AND RISES!

1.3.2. Measurement of the coefficient of reaeration

a. Material needed

- Propane gas tank with a regulator
- Tygon tubing securily connected to the propane tank at one end and to a diffuser at the other end
- conductivity meter
- sodium chloride (cooking salt)
- large plastic container
- 2 liter plastic jar to collect stream water to prepare the tracer solution
- peristaltic pump
- graduated cylinder and stop watch to check flow rate of peristaltic pump
- plastic vials to collect water for chloride concentration analyses

- gas-tight vials (5 mL is fine) to collect water for dissolved gas (propane) concentration analyses
- 5 ml syringes with needle
- GC with flame ionization detector for propane detection
- ion chromatograph for chloride analysis

b. Procedure

1. Record the total length of your experimental reach
2. Define 6 sampling points along the reach and record the distance between them.
3. Place peristaltic pump and gas tank at the top of the reach. Insert the gas diffuser in the stream and cover the stream surface of this addition site with some plastic sheet to enhance the mixing of the gas with stream water. Make sure that at this point the stream is a single channel. Ensure that tracer solution will quickly mix with stream water at this point.
4. Prepare the tracer (NaCl) solution in the carboy. Mix well the stream water with the NaCl. Take a sample of this solution to analyze chloride concentration and record the conductivity of it.
5. Collect water samples at each sampling site for background chloride concentration and gas concentration. To collect samples for gas concentration use the 5 ml syringe (without the needle). Avoid getting bubbles in the syringe. Once the syringe is filled up with the sample, plug the needle and transfer 4 ml of water into the gas-tight vials. Label vials adequately (sampling site, background). Shake the vials vigorously and keep them in a cooler with ice.
6. Prepare to simultaneously pump the chloride solution into the stream using the peristaltic pump with the injection point being a few cm upstream of the propane diffuser, thus allowing the propane bubbling to mix the sources. Set pumping rate of peristaltic pump by measuring delivery of chloride solution into a graduated cylinder at given pumping settings. Be careful not to get any solution into stream and return delivered solution to original stock.
7. Place conductivity meter at the bottom of the reach. Position probe in the middle of the stream channel. Record background conductivity.
8. Have field crew synchronize stop watches. Record date and starting time.
9. Begin experiment by releasing propane and turning on peristaltic pump.
10. Record variation of conductivity over addition time at even intervals (e.g., every 5 s or less frequent if stream flow is low) at the bottom of the reach. Alternatively, connect the conductivity meter to the data logger for continuous records of conductivity.

11. Once conductivity at the bottom of the reach is stabilized (i.e., plateau conditions) wait for about 10 minutes and collect water samples (for chloride and for gas) at each sampling point (3 replicates per site). Label the vials (site and plateau). Store the vials in the cooler.
12. Once water samples are collected, turn off the tracer addition and the gas tank. Record the time of addition shut off.
13. Keep recording conductivity over time at the bottom of the reach until it drops back to ambient levels (this step is optional and not needed for reaeration calculation).
14. Record the duration of the entire experiment.
15. In the laboratory, run chemistry analyses for Cl and propane.

Note:
Gas may diffuse to the atmosphere relatively fast. To ensure we get good data to measure the reaeration coefficient, sampling sites can be concentrated close to the addition site. Make sure you record the distance between them and the addition point.

c. Complementary measurements

For the metabolism estimates, it is important to measure light in the reach at even intervals over the 24 h period.
Measure stream channel widths and depths at each sampling site.

1.4. Lab protocol

- To analyze chloride concentration, use an ion chromatograph or the standard technique in your laboratory. Conductivity values could be used as surrogates of chloride concentration.

- To analyze propane concentration, use a gas chromatograph with the adequate column to detect propane. NOTICE THAT WE DO NOT NEED TO KNOW THE EXACT CONCENTRATION BUT ONLY THE PEAK AREA OR HEIGHT.

  1 hour prior to the gas analysis, leave the gas vials with the water at room temperature. Shake the samples several times during this hour to allow equilibration of the dissolved gas with the vial head space.
  Once the GC is ready, take a gas sample from the head space of the vial using a precise needle (ca 15 to 20 µl). Analyze the gas content for each sample and record either the peak height or the peak area.

1.5. Calculations

1.5.1. Estimate of the reaeration coefficient (Koxy)

Average background Cl concentrations (or conductivity) from all sampling sites unless you observe a consistent spatial trend among values along the reach. Average also background values (peak areas or heights) of gas (propane). For each site, calculate the average Cl concentration and gas peak values at plateau from the 3 replicates. Correct values of either Cl and gas measured at

plateau for the background values (plateau – background). For each site, calculate the natural logarithm of the gas/chloride ratio using the background corrected values. Plot these natural logarithm ratios against downstream distance and fit a curve. The slope of the line represents the proportion of gas lost per linear m reach.

The reaeration coefficient of the oxygen (Koxy, min-1) for the experimental reach is calculated using the following equation:

$$Koxy = slope * v * 60 * F$$

Where, the slope (m^{-1}) is estimated from the regression defined above, v (m/s) is the average water velocity in the reach, 60 is a conversion factor from seconds to minutes, and F is a conversion factor from propane reaeration coefficient to oxygen coefficient that corrects for the molecular size. For propane, F is 1.39. Average water velocity can be calculated from the conductivity time-curve recorded during the solute addition.

The Koxy varies as a function of water temperature. Therefore, if daily variation in water temperature is very pronounced in your study site it is recommended to correct Koxy for this parameter to get more accurate reaeration fluxes. To account for this factor, Koxy at a given temperature (T') is estimated as follows:

$$Koxy_{T'} = Koxy_{T} * (1 + ((T' – T) * 0.0241))$$

Where $Koxy_T$ is the coefficient directly estimated from the calculations, and T is the water temperature measured at the time when the gas addition was performed. Additionally, to be able to compare Koxy among our study sites we will also estimate Koxy at 20 ºC.

Notice that Koxy can be also estimate without the need of conducting gas additions using standard formulas. However, some studies have demonstrate that Koxy form indirect formulas are lower than Koxy obtained with the direct gas method, and thus, the use of those values somehow underestimate the reaeration fluxes. An alternative method quite widely used is the night-time oxygen deficit method (Marzolf et al. 1994).

1.5.2. Calculation of gross primary production and ecosystem respiration

These calculations can be done using the "metabolism calculation" excel file. However, in this section we have included a description of the different steps that are needed to estimate these parameters to help understanding the file.

First of all, to be able to measure the changes in DO concentration of a mass of water as it travels along the experimental reach we need to compare DO concentration measured at the top of the reach at a given time T with the DO concentration measured at the bottom of the reach at T plus the time that water takes to travel from the top of the reach to the bottom of the reach (i.e., nominal travel time between top and bottom). This nominal travel time is estimated based on the average water velocity and the distance between the two sampling sites (i.e., distance between sites / average water velocity).

DO concentrations at the bottom of the reach at T+travel time (being T the time of the DO reading at the top of the reach) can be directly obtained from the data logger records just by matching values measured at the top of the reach with those measured at the bottom of the reach that coincide with the T+travel time lag. If data has been recorded manually and sampling frequency does not match with this elapsed time, bottom-reach DO concentrations at T+travel time can be inferred from measurements done at two consecutive times using a simple regression between measurements and time between measurements.

Once DO concentrations at the bottom of the reach are corrected for the travel time, we can estimate the DO change rate (mg O_2/s) at a given time by multiplying the DO concentration difference between the top and the bottom of the reach (DO bottom – DO top) by the stream discharge (L/s). Notice that this DO change rate is the net result of two processes: the reaeration flux and the stream metabolism. Therefore, to estimate gross primary production and respiration (i.e., DO changes due to biological activity) the net change measured at a given time have to be corrected by the reaeration flux occurring at this particular time (i.e., (DO bottom – DO top) – reaeration flux)).

The reaeration flux (R.F., mg O_2/s) at a given time is a function of the DO deficit (mg O_2/L), the coefficient of reaeration (Koxy, min^{-1}) corrected for the water temperature at that time, the water travel time (min) between the two sampling sites, and the stream discharge (Q, L/s).

$$R.F. = DOdeficit * Koxy * Travel\ time * Q$$

The DO deficit is defined as the DO concentration at saturation minus the DO concentration measured in the stream. At a given time, measured DO concentration is the average between DO measured at the top and at the bottom (corrected by the travel time). DO at saturation depends on the water temperature (in our case, we will use the average temperature between the two sampling sites measured at a given time) and the atmospheric pressure. This value can be estimated from formulas or directly obtained from tables. Table 1 shows the percentage of DO saturation at different water temperatures, if your site is located <1000 m a.s.l. you can use these values. If the site is located at a higher altitude you should correct the DO at saturation also for the atmospheric pressure. Note that values from this table as well as the formula to correct for altitude are already incorporated in the excel file.

Table 1. Solubility of oxygen, from a wet atmosphere at a pessure of 760 mm Hg in mg O2/L, at temperatures from 0 to 35 ºC (data from Truesdale, Downing and Lowden 1955)

Temp.	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	14.16	14.12	14.08	14.04	14.00	13.97	13.93	13.89	13.85	13.81
1	13.77	13.74	13.70	13.66	13.63	13.59	13.55	13.51	13.48	13.44
2	13.40	13.37	13.33	13.30	13.26	13.22	13.19	13.15	13.12	13.08
3	13.05	13.01	12.98	12.94	12.91	12.87	12.84	12.81	12.77	12.74
4	12.70	12.67	12.64	12.60	12.57	12.54	12.51	12.47	12.44	12.41
5	12.37	12.34	12.31	12.28	12.25	12.22	12.18	12.15	12.12	12.09
6	12.06	12.03	12.00	11.97	11.94	11.91	11.88	11.85	11.82	11.79
7	11.76	11.73	11.70	11.67	11.64	11.61	11.58	11.55	11.52	11.50
8	11.47	11.44	11.41	11.38	11.36	11.33	11.30	11.27	11.25	11.22
9	11.19	11.16	11.14	11.11	11.08	11.06	11.03	11.00	10.98	10.95
10	10.92	10.90	10.87	10.85	10.82	10.80	10.77	10.75	10.72	10.70
11	10.67	10.65	10.62	10.60	10.57	10.55	10.53	10.50	10.48	10.45
12	10.43	10.40	10.38	10.36	10.34	10.31	10.29	10.27	10.24	10.22
13	10.20	10.17	10.15	10.13	10.11	10.09	10.06	10.04	10.02	10.00
14	9.98	9.95	9.93	9.91	9.89	9.87	9.85	9.83	9.81	9.78
15	9.76	9.74	9.72	9.70	9.68	9.66	9.64	9.62	9.60	9.58
16	9.56	9.54	9.52	9.50	9.48	9.46	9.45	9.43	9.41	9.39
17	9.37	9.35	9.33	9.31	9.30	9.28	9.26	9.24	9.22	9.20
18	9.18	9.17	9.15	9.13	9.12	9.10	9.08	9.06	9.04	9.03
19	9.01	8.99	8.98	8.96	8.94	8.93	8.91	8.89	8.88	8.86
20	8.84	8.83	8.81	8.79	8.78	8.76	8.75	8.73	8.71	8.70
21	8.68	8.67	8.65	8.64	8.62	8.61	8.59	8.58	8.56	8.55
22	8.53	8.52	8.50	8.49	8.47	8.46	8.44	8.43	8.41	8.40
23	8.38	8.37	8.36	8.34	8.33	8.32	8.30	8.29	8.27	8.26
24	8.25	8.23	8.22	8.21	8.19	8.18	8.17	8.15	8.14	8.13

Table 1. Solubility of oxygen, from a wet atmosphere at a pessure of 760 mm Hg in mg O2/L, at temperatures from 0 to 35 ºC (data from Truesdale, Downing and Lowden 1955)

25	8.11	8.10	8.09	8.07	8.06	8.05	8.04	8.02	8.01	8.00
26	7.99	7.97	7.96	7.95	7.94	7.92	7.91	7.90	7.89	7.88
27	7.86	7.85	7.84	7.83	7.82	7.81	7.79	7.78	7.77	7.76
28	7.75	7.74	7.72	7.71	7.70	7.69	7.68	7.67	7.66	7.65
29	7.64	7.62	7.61	7.60	7.59	7.58	7.57	7.56	7.55	7.54
30	7.53	7.52	7.51	7.50	7.48	7.47	7.46	7.45	7.44	7.43
31	7.42	7.41	7.40	7.39	7.38	7.37	7.36	7.35	7.34	7.33
32	7.32	7.31	7.30	7.29	7.28	7.27	7.26	7.25	7.24	7.23
33	7.22	7.21	7.20	7.20	7.19	7.18	7.17	7.16	7.15	7.14
34	7.13	7.12	7.11	7.10	7.09	7.08	7.07	7.06	7.05	7.05
35	7.04	7.03	7.02	7.01	7.00	6.99	6.98	6.97	6.96	6.95

Notice that positive reaeration fluxes indicate that water is under-saturated and thus, oxygen tends to move from atmosphere into the water. Negative fluxes indicate that water is over-saturated and thus, oxygen tends to move from water to the atmosphere.

Instantaneous net DO change rates corrected by the reaeration flux can be either positive (i.e., net increase in DO along the reach) or negative (i.e., net decrease in DO along the reach. Corrected DO change rates measured during night are due to the respiration of the whole stream community (heterotrophs and photoautotrphs) and should be negative. Whereas rates measured during day time are influenced by either the respiration of the whole stream community and the primary production of the photoautotrophic organisms and thus, rates can be negative (R>GPP) or positive (R<GPP).

Daily rates of respiration (R) and gross primary production (GPP) are estimated based on the instantaneous DO change rates measured over a 24 h period.

- Daily rates of ecosystem respiration (g O_2/m^2 d) are estimated by calculating the average of the instantaneous net DO change rates measured at night and extrapolating this value over 24 h (i.e., avg DO change rate at night times 24) and dividing it by the total surface stream bed area (average wet channel width times reach length). This method assumes that respiration rates are constant on a daily basis. A more refined estimate of this parameter can be done by extrapolating a line between the instantaneous rates obtained at night hours and integrating the area below rate equal zero.

- Daily rate of gross primary production (g O_2/m^2 d) is estimated considering only the instantaneous DO change rates measured during day time hours (from 1 hour before sun rises and 1 hour after the sun sets). To estimate this parameter subtract the average instantaneous DO change rate measured at night from all the instantaneous DO change rates measured during day time hours and integrate the resulting curve. Divide this value by the total surface stream bed area.

- Daily rate of net ecosystem production (g O_2/m^2 d) is defined as daily rate of GPP minus daily rate of R. Negative values indicate that R>GPP (i.e., the ecosystem

metabolism is heterotrphic-dominated), positive values indicate that R<GPP (i.e., the ecosystem metabolism is autotrophic dominated). Another way of characterizing the ecosystem metabolism is by calculating the GPP:R ratio.

Instantaneous and daily DO rates can be converted to carbon rates using the quotients suggested by Bott (2006). For photosynthesis we will use a photosynthetic quotient (PQ) of 1.2 and apply the following equation:

$$GPP\ (g\ C/m^2\ d) = GPP\ (g\ O_2/m^2\ d) * (1/PQ) * (12/32)$$

Where 12 is the atomic weight of C and 32 is the molecular weight of O2.

For respiration we will use a respiratory quotient (RQ) of 0.85 and apply the following equation:

$$R\ (g\ C/m^2\ d) = R\ (g\ O_2/m^2\ d) * RQ * (12/32)$$

1.6. Related literature

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