SEVENTH FRAMEWORK PROGRAMME

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THEME 6: Environment (including Climate Change)

REFRESH

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

Collaborative Project (large-scale integrating project)
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Deliverable 2.1b: Protocol for standardised methodology and taxonomy for drought experiments in rivers

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Drought experiment protocol

1. Site selection 3
2. Experimental design 4

Appendix

Appendix 1 Discharge transects 9
Appendix 2 Water chemistry 11
Appendix 3 Macroinvertebrate sampling 12
Appendix 4 Macrophyte inventory 20
Appendix 5 Pool plankton development 22
Appendix 6 Measurement of stream metabolism (optional) 23

1. Site selection

1..1. Objective:

To understand the effect of climate change induced low flow and drought processes and disturbances in streams.

1..2. Questions:

a. How long can 1) low flow (stagnation) or 2) drought last before effects on the stream ecosystem become apparent?
b. What are the differences between low flow and drought effects?
c. Do wet soils or remnant pools offer refuge?

1..3. Experimental site:

In each country two experimental sites are selected- one nutrient-low site, where phosphorus is <0.15 mgP/l and the nutrient-high site, where phosphorus is >0.15 mgP/l. Overall in the whole experiment (all countries) the high and low nutrient class should statistically be different.
In Denmark and in Sweden, at both sites experiments will be conducted in 2011, while in Spain, Germany and The Netherlands experiments are conducted at one site in 2011 and at the second site in 2012. The sites were selected according to the following criteria:

- Only climate is differs along the European Atlantic climate gradient (S will position its site around Uppsala (as north as possible but within the deciduous forest area, Spain will concentrate on permanent streams).
- Permanent, lowland stream (high priority criterion).
- Width ranges between 2-5 m (depth is too variable as parameter to be included).
- The site is situated in a half-open landscape with deciduous vegetation.
- The stretches their selves are partly unshaded.
- Current velocity ranges between 15-25 cm/s.
- The slope ranges 0.5-1 m/km (altitude lower priority; plateau like situation).
- The substrate consists of sand or gravel (low priority!).
- Discharge is characterized as "less" dynamic.

2. *Experimental design*

2.1. Introduction

At both the nutrient-rich stream site and the nutrient-poor site, a control and an impact stretch are selected. Two experiments are conducted in a stream at the same time. The low flow/stagnation experiment at the upstream part of the impact stretch and the drought experiment at the downstream part of the impact stretch. For both experiments the same control stretch is used.

2.2. Site construction

The control stretch is minimally 34m long and it is situated upstream from the impact stretch (Figure 1). The impact stretch is minimally 68m long. The major habitat patterns in the both impact and control stretch are comparable.

The impact stretch consists of an original by-pass that can be an old meander or a ditch like channel that acts as a by-pass. An alternative is using a tube, or digging out a new bypass.

A weir is installed at the upstream end of the impact stretch and will be closed for the duration of the stagnation/drought phase. In this phase, water flow is redirected to the by-pass. After the stagnation/drought phase the weir is opened again. The weir can be a dam build using sand bags of wood or concrete.

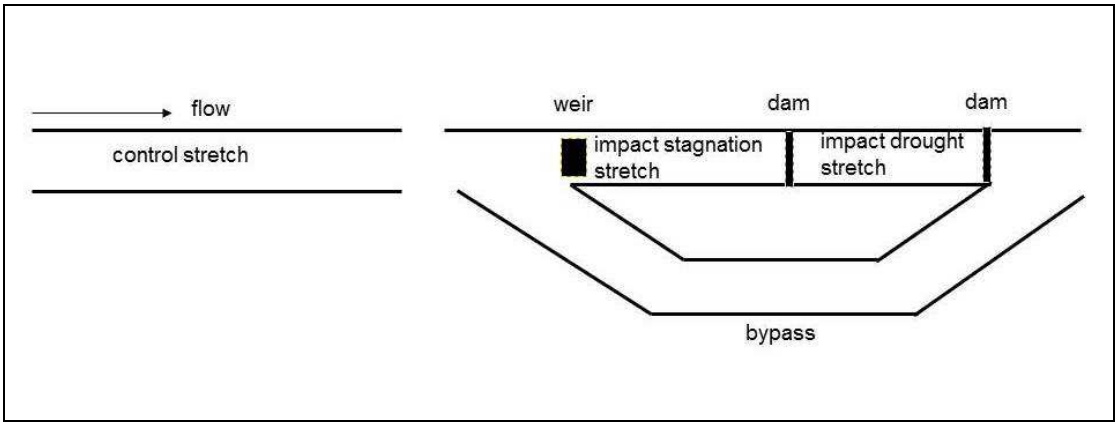

Figure 1. Schematic site situation

The impact stretch is divided in two experimental parts- a stagnation part and a drought part. The two parts are divided by a low dam (threshold). Another low dam is constructed at the downstream of the impact part. The later dam prevents water from downstream entering the impact stretch. Both dams can be constructed using sand bags.

2.3. Experiment

During the stagnation period the water in the stagnation part does not flow or flows minimally. The stagnation part is fed only with the volume of water necessary to compensate for the water loss due to infiltration and evaporation. If the stream is located in a seepage area or after heavier rain, it might be necessary to create an additional bypass or use a pump in order to remove excess of water from the part. While pumping out the water make sure not to pump out animals as well (e.g. first drain by using a perforated bucket, and only pump water out of this bucket, hereby use a gauze in front of the tube of the pomp).

The drought experiment is conducted at the downstream part of the impact stretch. During the drought period the stream bed is dry and the water is only present in the remnant pools*. It might be necessary to pump out the water from the drought part mechanically at the beginning of drought phase. Additionally, if the stream is located in a seepage area or after heavier rain it might be necessary to remove excess of water by a pump.

Preferably the control, stagnation and the drought stretches are each divided in 15 sections (10 sections to be sampled and 5 "spare" sections). There is at least 2 m space between the most upstream/downstream sections and each weir/dam.

> **Both – the dry stream bed and remnant pools are of our interest and therefore the presence of pools during the drought experiment should be secured. This can be done by digging containers that would act as pools into the streambed before start of the experiment. Such containers should have the same surface as the area sampled with the surber sampler. At sampling, the whole container is taken out or its content is pumped out.*

2.4. Timing

The combined drought and stagnation experiments last fifteen weeks: five weeks before the stagnation/drought phase and 10 weeks during the stagnation/drought phase (Table 1). The starting date of the stagnation/drought phase is variable along climate gradient and is decided by degree day and pragmatic arguments.

Table 1. Timing of the stagnation and drought experiments – Dutch example

	May	June				July					August				September
week number	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
experiment	before phase					stagnation and drought/pools phase									

3. Measurements

3.1. Continuous measurements:

3.1.1. Discharge

Discharge hydrologic data are available for at least for the whole sampling period. However, it is even better if data from previous years are available. Historical records can provide information on interannual variation in stream hydrology, and will allow examining whether experiment was undertaken at either average, wet or dry conditions.

At each experimental site discharge is measured continuously in control and both impact stretches. Use the nearest gauge station and install a continuous water level recorder in the control and both impact parts. Calibrate the water level loggers by measuring water velocity over different (preferably weekly) hydrological conditions that occur during the experiment (flood, low water, etc…).
If no gauge station is available or a continuous water level recorders cannot be installed, discharge measurements can be made manually see Appendix 1. Using this option, measurements should be taken weekly. If measurements are performed more frequently it is even better.

3.1.2. Water temperature

Water temperature is continuously measured at the control and both impact stretches by using a temperature logger (HOBO UA-002-64 logger or similar logger), with 20 min interval. At the control stretch the logger is placed in well mixed water. At the stagnation stretch the logger is placed in the middle of the stretch. At the drought stretch two loggers are placed, one in a pool and one in a drying up spot. The loggers in the stagnation stretch and pools should be located within a shaded spot to avoid heating up. In total, 4 loggers are used. During the experimental period loggers should be checked regularly to secure they are not covered by material, are still operational and measure correctly.

3.1.3. Air temperature

Use data from a nearby metrological station. If no station exist nearby, air temperature can be logged using HOBO UA-002-64 logger (or similar logger). The loggers should be placed 150 cm above the ground, in a shaded area and checked regularly.

3.1.4. Oxygen

Oxygen is measured continuously* at the control and both impact stretches, with 20 min interval. At the control stretch the logger is placed in well mixed water. At the stagnation stretch the logger is placed in the middle of the stretch. At the drought stretch the logger is placed in a pool. Thus in total 3 loggers are used. During the experimental period loggers should be checked regularly to secure they have not been covered by material, overgrown by algae, are still operational and measure correctly.

**If continuous oxygen measurements are not possible, 24 hours measurements are carried out on each sampling day.*

3.2. Interval measurements:

3.2.1. Water chemistry

Water samples are taken at each macrofauna sampling moment (thus at 10 sampling occasions). In the before phase, at each occasion 2 water samples per stream are taken– one at the control stretch and one at the impact stretch. In the stagnation/drought phase, at each occasion 3 water samples per stream are taken – one at the control stretch, one at the stagnation stretch and one at remnant pools.

Minimally pH*, EC*, O_2*, t-P, t-N, ammonium, nitrate, dissolved inorganic phosphorus (PO_4-P), calcium, chloride, iron and sodium (Appendix 2) are analyzed.
The sampling bottles are filled completely and closed under the water. Water samples are placed in the freezer (-18°C), directly after returning to the lab.

> **pH, EC, O_2 measurements can be done directly in the field, using pH, EC, O_2 meter. O_2 measurements are used to secure the continuous measurements.*

3.2.2. Substrate cover estimation:

Substrate cover is estimated at control, stagnation and drought stretch. At each stretch three 5m long sections are set for the substrate estimation and the substrate cover is estimated at same sections 5 times in the before phase (weekly) and 5 times in the stagnation/drought phase (2 weekly).

The method of estimation is chosen by the partner and 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 3).

3.2.3. Loss of water in the stream bottom – drought phase:

Core samples of the stream bottom are taken at each visit during the drought phase, thus 5 times (2 weekly). Three core samples are taken at each sampling occasion. All three samples are taken in the drought section.
The relative amount of organic material in every sample (%) is determined by dividing the weight of the dry weight of the sample by wet dry weight of the sample.Wet weight is the weight of the sample befor drying (g). In order to calculate the dry weights, samples are dried at 105°C for 6 days and weighed (g).

3.3. Macrofauna sampling:

Macrofauna sampling is carried out weekly in the before phase and two weekly in the stagnation/drought phase of the experiment (Table 2). During the before phase, macroinvertebrates are sampled from one randomly selected (beforehand) section of each: control, stagnation and drought stretch. One sample consists of 3 Surber samples (25x25 cm). The sample is taken from the 3 dominant substrates present in the randomly selected (beforehand) section (Appendix 3), one Surber from each dominant substrate. The three Surber samples are combined in one composite sample.

During the stagnation/drought phase macroinvertebrates are sampled from one randomly selected section of control and stagnation stretch. In the drought stretch, two samples are taken at a randomly selected section; one sample from the dry bottom and one sample from the remnant pools. One sample consists of 3 Surber samples (25x25 cm) or 3 core samples (of the same area as Surber samples). The sample is taken from the 3 dominant substrates present in the randomly selected (beforehand) section (Appendix 3), one Surber/core from each dominant substrate. The three Surber/core samples are combined in one composite sample.

At each visit different sections are sampled – thus none of the sections are sampled twice.

At each Surber, depth, current velocity and substrate type are measured/described. At each sampled section width is measured and cover percentage of substrates present in the section are noted down.

Table 2. Macrofauna sampling scheme

week of the experiment	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	15 weeks
number of samples	3	3	3	3	3		4		4		4		4		4	35 samples
period	before phase					stagnation and drought/pools phase										

3.4. Macrophyte inventory

Macrophyte inventory is carried out at the beginning and end of the before and stagnation/drought phase. The control, stagnation and drought stretches are described (Appendix 4).

3.5. Pool plankton development

Phytoplankton is sampled 5 times in the before phase (weekly) and 5 times in the stagnation/drought phase (2 weekly).
During the before phase, phytoplankton is sampled from one randomly selected (beforehand) section of each: control and impact stretch. Thus in the before phase stagnation and drought stretches are treated as one.
During the stagnation/drought phase in control and stagnation stretch, phytoplankton is sampled from the same section where macrofauna samples were taken. In the drought stretch, water from remnant pools where macrofauna was sampled is used for the phytoplankton sample.

To sample phytoplankton, filter 10l of stream water through a phytoplankton net (Appendix 5).

Table 3. Phytoplankton sampling scheme

week of the experiment		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	
number of samples	control	1	1	1	1	1		1		1		1		1		1	25 samples
	stagnation	1	1	1	1	1		1		1		1		1		1	
	pools							1		1		1		1		1	
	period	before phase					stagnation and drought/pools phase										

3.6. Metabolism (optional)

Measured in one location in the control, one in the stagnation and one in the drought stretch (weekly data by interval between first and second sample). (Appendix 6).

3.7. The following parameters are common for WP2 and WP4 and are included in the sampling protocol of WP4:

3.7.1 shoreline beetles
3.7.2 decomposition

Appendix 1 Discharge

1. Location

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

2. Transect measurements

The width of the stream is 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).

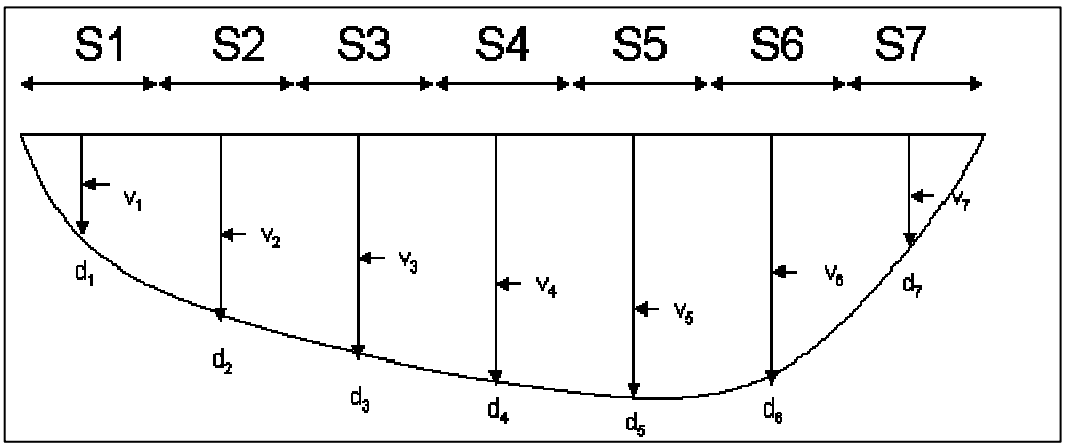

Figure 1. Transect for discharge calculation (S – section, d – water depth, v- current velocity)

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		Date	Stream
Stretch	Researchers		

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 2 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 replicates of water samples at each stretch. 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 weekly in the before phase and 2 weekly in stagnation/drought phase.

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

Appendix 3 Macroinvertebrate sampling

1. Introduction

This protocol describes the common handlings for macroinvertebrate sampling in the 'Drought experiment'.

2. Sampler

Macroinvertebrates are sampled by using either a Surber sampler with a net mesh width 500 μm, or a core with the same sample surface area. Sizes of the Surber sampler square are 25 by 25 cm (0,0625 m^2).
At the control reach and during the before phase the surber sampler is used while the core is used in the impact stretch during the period of stagnation/drought.

3. Sampling

The randomly selected sections are identified and marked on a map of the stretch before beginning of sampling. Sampling starts at the downstream end of the stretch and proceeds upstream. Three surber (or core) samples should be collected in the selected section and these are combined to one replicate (composite sample).

In order to sample the different habitat types present in each section, the three surber samples should be taken from the three most dominant substrate types present in the section. Sampled substrate types are noted on the field protocol (according to the AQEM/STAR habitat classification).

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
- sampled stretch name (control, stagnation,drought,pools)
- section code
- 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 500 μm 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.

All groups are sorted. Animals 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 section:

- width (average position)
- substrate type

Per stretch:

- pH
- EC
- oxygen
- water sample. Parameters to be analysed: nutrients t-P, t-N, ammonium, nitrate, calcium, chloride, iron and sodium (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.)

List of equipment per stream:

- Surber sampler (25x25)
- Corer (0.0625 m2)
- 4 containers/buckets per stream
- 4 covers for the containers/buckets
- field forms
- 4 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 and EC meter
- oxygen meter
- 3 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 Drought experiment		Date	Stream
Sample code:		Digital photos taken (yes/no)	Researchers
Stretch		Section	Width (m):
Measured parameters			
replicate	water depth [cm]	current velocity [m/s]	
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	**1.1.2. 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 in 3 stretches

General information			
REFRESH Drought experiment			Date
Stream			Researchers
Abiotic variables			
	control	stagnation	drought
pH			
EC			
O_2 [mg/l]			
O_2 [%]			
T [^oC]			
water sample code			

Appendix 4 Macrophyte inventory

Protocol for macrophyte inventory

- Macrophyte species abundance will be estimated in the control, stagnation and drought 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: Drought
Macrophyte inventory
Tick the box to indicate dominance category for each species

General information		
Date	Stream	Surveyors
Stretch	Section	Total plant cover

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

Appendix 5 Phytoplankton sampling and filamentous algae development

1. Introduction

This protocol describes the common handlings for phytoplankton sampling and the filamentous algae estimation in the 'Drought experiment'.

2. Sampling

At the control stretch 5 phytoplankton samples in before phase and 5 phytoplankton samples in stagnation/drought phase are taken.
At the impact stretch during before phase 5 samples are taken and during stagnation/drought phase 10 samples are taken (5 samples in stagnation impact stretch and 5 samples from pools in drought impact stretch). In the before phase samples are taken weekly and in stagnation/drought phase 2 weekly. Preferably, phytoplankton samples are taken at the same sections as the macrofauna samples.

2.1 Filamentous algae mats estimation

Estimate cover percentage of the filamentous algae mats in sampled section or pool and write it down on the field form along with the phytoplankton sampling information.

2.2 Phytoplankton sampling

Before phase (control and impact stretch) and stagnation/drought phase (stagnation impact stretch)
To sample phytoplankton, collect 10 times 1l water (thus 10 l water in total) without disturbing the bottom/bank substrate and pour it through the plankton net (45 um mesh). Water is collected from the same section where marofauna is sampled, at half of the stream depth. Filamentous algae mats are not taken into the phytoplankton sample, but cover is estimated and written down on the field form. Material from the net is washed in to a bottle.

Stagnation/drought phase (drought impact stretch)
To sample phytoplankton from the pools during the stagnation/drought phase, mix water from the 3 sampled pools together in one bucket and pour it through the plankton net (45 um mesh). Place material from the net in a bottle. Make sure that all macroinvertebrates are taken out from the water before taking the phytoplankton sample.

3. Sample processing

Each sample is fixed in the field or transported alive to the lab. To fix 1 litre sample add 2-5ml of basic Lugol solution to it. Sample fixed with Lugol solution should have a colour as cognac. If the Lugol solution is overdosed, organisms will be stained what makes the identification more difficult. Samples are stored in a dark place, at 4°C. If a sample will be analysed within one week, store it in dark place at room temperature.

Samples that will be stored for longer than one year are fixed with formaline. To fix 1 litre sample add 5-10 ml of 40% formaline to it. Formaline fixed samples are stored in a dark place, at 4°C.

4. Lugol solution

Ingredients:
potassium iodide (KI)
iodine (I2)
sodium acetate (NaAc)
distilled water

Preparation:
Dissolve 150g of potassium iodide and 50g of iodine in 1demiliter of distilled water. Add 100g of sodium acetate to the solution.

List of equipment

- 10l bucket
- 1l bucket
- phytoplankton net (45 um mesh)
- 3 bottles
- 3 labels
- field form
- pencils

Field form:

<table>
<tr><th colspan="4">General information</th></tr>
<tr><td colspan="2">REFRESH Drought experiment</td><td colspan="2">Date</td></tr>
<tr><td colspan="2">Stream</td><td colspan="2">Researchers</td></tr>
<tr><th colspan="4">Phytoplankton sampling</th></tr>
<tr><td></td><td>sample code</td><td colspan="2">filamentous algae mats coverage</td><td>volume of water</td></tr>
<tr><td>control</td><td></td><td colspan="2"></td><td></td></tr>
<tr><td>stagnation</td><td></td><td colspan="2"></td><td></td></tr>
<tr><td rowspan="3">drought (pools)</td><td rowspan="3"></td><td>1</td><td></td><td rowspan="3"></td></tr>
<tr><td>2</td><td></td></tr>
<tr><td>3</td><td></td></tr>
<tr><td>drought (pools) total</td><td></td><td colspan="2"></td><td></td></tr>
<tr><th colspan="4">Drawings/notes</th></tr>
<tr><td colspan="4"></td></tr>
</table>

Appendix 6 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. <u>Material needed</u>

- 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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