1. PROTOCOL FOR YELLOW AND SILVER EEL SAMPLING IN RIVERS

The following protocol describes the guidelines to conduct eel surveys to estimate density of yellow and silver eels at each pilot basin using electric fishing. In addition to presenting the criteria for establishing the location of sampling sites and procedures for conducting the fish surveys, this protocol also defines the methods to collect biometric information on eels, data on other fish species, and environmental variables.

Yellow and silver eels should be taken to the laboratory to implement other protocols. The *Protocol for otolith preparation and age reading* should be implemented to meet one of SUDOANG's objectives (**mandatory**), while the *Protocol to assess the infection by Anguillicola crassus and the Swimbladder Degenerative Index (SDI)* and the *Protocol to sample gonads for sex ratio assessment,* are **facultative**.

1.1. Timing of surveys

Sampling to estimate the density of yellow and silver eels should be conducted in **late summer/early autumn** to ensure the capture of silver eels. Thus, it is expected that, depending on the location (latitude) of the river basin, sampling occurs when eels are already in a silver stage **BUT** before their escapement, which takes place in autumn/winter.

1.2. Site selection

Electric fishing will be conducted in each pilot basin, only in freshwater, both in the main river and in its tributaries (up to 3rd order tributaries). The **reach to be sampled should be representative of the river segment** covering existing physical diversity and containing at least one riffle if there is one in the segment. For a better understanding, a schematic representation and the definitions of the terms are presented.

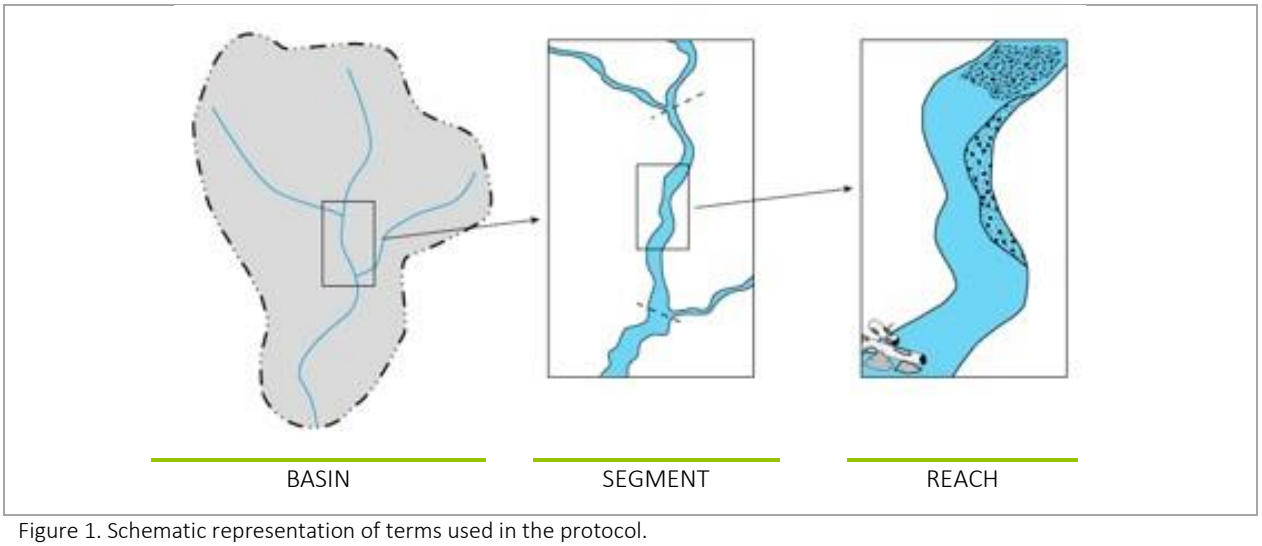


Figure 1. Schematic representation of terms used in the protocol.

BASIN: The entire watershed.

SEGMENT: section of a stream within the basin with similar biotic and physical properties (e.g. similar gradient and discharge).

REACH: A section of a stream within a segment from where biological data will be collected.

Sampling reaches should be **photographed** and **georeferenced** with a GPS, so that they can be recognized.

One possible criteria to **define** the **number and location** of sampling sites that are representative of the river segment, is presented. Three steps should be followed:

1. **Divide the river basin in hydrological segments**
   - Changes in gradient, discharge (tributary confluences) and/or disturbance (reservoirs) will be used to establish segments;
   - If gradient type is repeated, then the longest segment or the one that best represents the downstream stream gradient will be used;
   - If stream gradient is very low, then changes in discharge (tributary confluences) will be used to designate segments;
   - If gradient is low and there are no tributaries, segments will be placed at equal distances from each other;
     Segments should not be placed in reservoirs.

2. **Measure every hydrological segment**
   - Segments >10 km in length can be divided according to the slope into minimum, medium and maximum
     - Slope indicates the stream gradient, which in turn influences sediment transport and discharge characteristics.
     - Slope is defined as the difference in elevation at the upstream (Elv_{Upst})and elevation at the downstream (Elv_{Dowstr}) ends of a stream segment, divided by the length of that segment ($Length_{seg}$):

$$\frac{(Elv_{Upstr} - Elv_{Dowstr})}{Length_{seg}}$$

3. **Place sampling reaches within a segment**
   - A reach will have to be placed in the centre of a gradient or discharge segment and should be representative of that segment.

– If a **segment is less than 60 km in length**, the number of reaches should be as follows:

Length	Number of reaches
30-60 km	3
11-29 km (< 30)	2
1-9 km (< 10)	0 - 1

– If the **segment length is equal to or greater than 60 km** place one reach per each 20 km as follows:

Length	Number of reaches
60 km	3
80 km	4
100 km	5

1.3. Length of the sampling reach

Spatial scale is a critical aspect in any sampling protocol. Regardless of where sampling takes place, the vast majority of species likely to be present in the range of the electric field should be captured within this length of the stream. To standardize the fishing protocol the sampled reach length should be defined. The following lengths should be adopted:

Stream Type	Length of reach	Minimum	Maximum
Wadeable Stream	20 times the wetted stream width	100 m	300 m
Non-wadeable Stream	10 times the wetted stream width	300 m	500 m

1.4. Sampling procedures

Fish sampling will be conducted by electrofishing. The conductivity will determine the initial voltage setting selected. It is recommended to select the following voltages as maximum values, depending on the water conductivity: **400 V** for **high conductivity** (> 300 µS/cm); **800 V** for **medium conductivity** (100 - 300 µS/cm); **1000 V** for **low conductivity** (< 100 µS/cm).

- A team of at least four (4) people is desirable. One (1) operator carrying the anode and three (3) carrying auxiliary dip nets to capture any stunned or fleeing fish, and place them in a bucket. Fish should be removed as soon as possible from the electrical field.
- It is recommended to use **Direct current** (DC) because it is less harmful to fish and mortality and injury of fish needs to be kept to a minimum (voltage and intensity must be recorded).
- When **sweeping**, you should do the sampling by leaving the **anode 30 s in the water** and then release the button, **OR**, in areas of high density, leave the anode until fish are attracted; The anode should move in a circular way (~1m diameter). The mesh size of the anode should be small enough (1-2 mm x 1-2 mm) to retain eels of all ranges.

- Per sampling reach, **at least 2 passes** should be done with **an interval of 30 min between them**. **If** the **2nd pass collects more individuals** than the 1st, **a 3rd pass should be conducted**, again with an interval of 30 min. The quantity of eels caught in each pass should be recorded separately.
- **After each pass**, all eels sampled should be **measured** and **weighted** as soon as possible. Bycatch should be identified, and the number of individuals recorded. All specimens captured in successive passes should be kept in a container placed in the river (equipped with a cover and small openings allowing the water renewal but preventing small fish escape) until the end of sampling, and should only be returned to the water at the end of all biometric measurements.
- Regardless of the depth of the reach (see below) fishing should **ALWAYS be conducted** in an **upstream direction**. Fishing should be carried out differently depending on the river depth:

In shallow rivers (< 0.8 m depth)

- In **narrow rivers** (width < 15 m), fishing should be carried out across the entire river, aiming to include both margins and the centre of the river. However, in **wide rivers** (width ≥ 15 m), fishers should walk slowly along the upstream course, describing a **zig-zag** between the two margins, while covering all existing habitats and taking out the fish that are sheltered.

In deep rivers (≥ 0.8 m depth)

- The electrofishing will be carried out ONLY in the margins because efficiency is extremely reduced in deeper areas, especially if the target species is benthic, as it is the case with the eel.
- Electric fishing by wading is limited to the depth at which wading can be safely carried out. It is not advisable to place the anode head deeper than you can see.

1.5. Environmental data to collect in the field

During sampling, the following parameters should be measured and recorded **whenever the habitat changes**:

- Depth (m);
- Current speed (m/s);
- Water temperature (°C);
- Conductivity (µS/cm);
- Dissolved oxygen (mg/L **OR** %);
- The type of **substrate** and of **vegetation in the margins** and **instream cover** should also be recorded (format and classes in Data template);
- Also, the **area** sampled (m^2), and the **sampling time** (min) should be recorded.

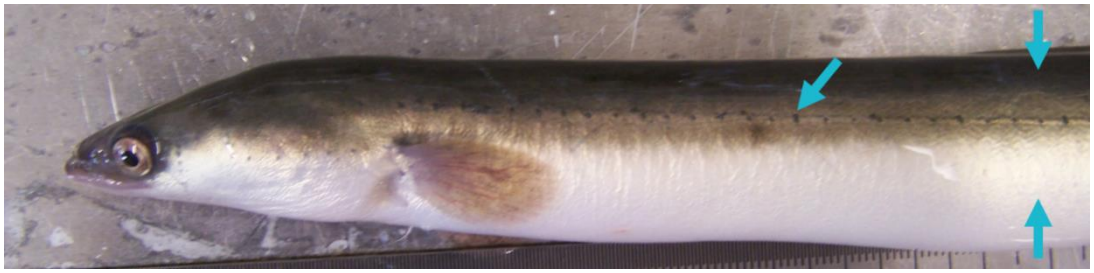
1.6. Biological data to collect in the field at each fish pass

The following data should be recorded in the field for each eel:

- Total length (mm);
- Total weight (0.01 g);

– Identification of the phase of the eels (yellow or silver) by:

1. **Visual inspection:**
   - presence of a conspicuous lateral line;
   - body color and contrast between dorsal and ventral parts.



2. **For silver eels and all eels with length larger than 300/350 mm, you should measure:**
   - Length of pectoral fin;
   - Vertical and horizontal diameters of the eye (always **left eye**, unless malformed or defective. In such case, the right eye should be used, but this information should be noted in the observations field);

The limit 300/350 mm should be assessed according to the latitude. It corresponds to the lower silvering size limit for eels in the southern distribution range.

- To confirm the silver eel stage, you should use the classification by Durif et al., (2009). The R **script** to calculate/identify the silver eel stage is in the end of the protocol.

– Bycatch should also be identified and counted (Fish species and *Procambarus clarkii*) at each pass.

1.7. Samples for laboratory analysis

Mandatory protocol

To meet the objectives proposed in the SUDOANG project, **20 silver eels and 60 yellow eels/pilot basin/year** should be collected and analyzed in the laboratory for age determination (*Protocol for otolith preparation and age reading*). The **sample of yellow eels** should be **stratified** to cover all sizes and therefore, increase the variety of ages. These yellow eels should be taken from the lower, middle and upper reaches of the catchment (20 eels from each area=60 eels) and be representative of the sizes at each area.

Facultative protocols

If other protocols are to be implemented (*Protocol to assess the infection by Anguillicola crassus and the Swimbladder Degenerative Index* and the *Protocol to sample gonads for sex ratio assessment*), eels should be analyzed as soon as possible (preferably still fresh).

To reduce animal sacrifice, eels collected for age determination should also be used for the analysis of *Anguillicola crassus* infection, whereas only eels that do not have a typical male or female appearance, should be used to assess sex ratio (molecular and histological analysis).

The identification of each eel taken to the laboratory and location of sampling should be recorded and kept constant for all analyses.

1.8. Field equipment

- Electrofishing apparatus (up to 1000 V);
- Anode and cathode;
- Rubber gloves;
- Waders;
- Auxiliary dip nets (3x);
- Containers (*e.g.* buckets) to place the fish collected;
- Ruler and scale;
- Precision balance;
- Multiparametric probe;
- Current meter;
- GPS and camera;
- Digital calliper.

1.9. R script to identify the silver eel stage from Durif *et al.* (2009)

The stacomi project is an open access bundle (Postgres database, JAVA, R) to treat migration monitoring information. One of the class method developed in this package allows to calculate Durif's stages.

This class contains a dataset with Durif coefficient, and you can use some internal function from the package to calculate stage. To use the function fun_stage_durif you need to create a dataset with columns

*Body Length **BL** (mm)*

*Weight **W** (g)*

*Vertical eye diameter **Dv** (mm)*

*Horizonal eye diameter **Dh** (mm)*

*Pectoral fin length **FL** (mm)*

```
require(stacomiR)

# Load the coefficients from Durif
data("coef_durif")

########################################
# To use the function fun_stage_durif manually
# create a matrix with columns BL","W","Dv","Dh","FL"
##############################################
# here it is extracted from the data at hand
silver_eel<-as.matrix(r_silver@calcdata[[1]][,c("BL","W","Dv","Dh","FL")])
head(silver_eel) # to see the first lines
#>        BL    W    Dv    Dh    FL
#> 25710 830 1074  8.14  8.70 39.79
#> 25711 714  740  8.24  8.52 38.04
#> 25712 720  755  6.92  6.87 34.01
#> 25713 860 1101 10.53 10.43 44.47
#> 25714 716  752  7.42  8.76 33.78
#> 25715 690  622  7.83  9.25 29.58
stage <- fun_stage_durif(silver_eel) # apply the function to the matrix
stage[1:10] # look at the first 10 elements in vector silver
#>  25710  25711  25712  25713  25714  25715  25716  25717  25718  25719
#> "FIII" "FIII" "FIII"  "FIV" "FIII" "FIII"   "FV"   "FV" "FIII" "FIII"
```

References

Durif C., Guibert, A., & Pierre, E. (2009). Morphological discrimination of the silvering stages of the European eel. In J. M. Casselman & D. K. Cairns (Eds.), Eels at the Edge. Science, Status, and Conservation Concerns (pp. 103–111). Bethesda, MA: American Fisheries Society Symposium 58.

1. Yellow and Silver Eel Sampling

Site name:				Site code:
GPS Coordinates	Lat:	Long:	Coordinate system:	Date:
Photos (ref):			Team:	

Atmospheric conditions

Air temperature (°C):				
Nebulosity:	☐ *clear*	☐ *slightly cloudy*	☐ *averagely cloudy*	☐ *fully cloudy*
Wind:	☐ *null*	☐ *light*	☐ *moderate*	☐ *strong*
Rain:	☐ *yes*	☐ *no*	Before sampling:	

Sampling reach

River Gallery	Right river bank	0 % ☐ *absent*	*0%-25%* ☐ *sparse*	*25%-50%* ☐ *intermediate*	50%-75% ☐ *semi-continuous*	75%-100% ☐ *continuous*
	Left river bank	☐ *absent*	☐ *sparse*	☐ *intermediate*	☐ *semi-continuous*	☐ *continuous*
Vegetation in river bed	Macrophytes / hydrophytes	(0%) ☐ *absent*	*(0%-25%)* ☐ *sparse*	(25%-75%) ☐ *intermediate*	(75%-100%) ☐ *abundant*	
	dominant type:	☐ higher plants		☐ moss	☐ filamentous algae	
Large woody debris in river bed:		(0%) ☐ *absent*	*(0%-25%)* ☐ *sparse*	(25%-75%) ☐ *intermediate*	(75%-100%) ☐ *abundant*	

Type of river bed cover (choose the ones that are present):					Dominant type:	
☐ mud	☐ sand	☐ gravel (coffee grain - egg)	☐ small stones (A5 - A4)	☐ bigger stones (A4 - A3)	☐ blocks/ rocks (> A3)	☐ flagstone

Total instream cover: _____%				River width (average - m):
Habitat:	Pool _____%	Run _____%	Riffle _____%	River depth (average - m):

Sampling equipment

			Model:	Manufacturer:
Type:	☐ battery		☐ dorsal generator	☐ non-dorsal generator
Electric current:	☐ pulse (frequency: _____Hz)		☐ DC	☐ PDC ☐ AC
Cathode:	☐ ring (diameter: _____cm)		☐ cable	☐ other (which?):
Fishing net area (m^2):		***or*** length (cm):		width (cm):

(start) (end)	*(start) (end)*	*(start) (end)*
1st pass: ___H___ - ___H___	***2nd pass:*** ___H___ - ___H___ *half hour after 1st pass*	***3rd pass:*** ___H___ - ___H___ *only if 2nd pass collects more indiv. than 1st*

Environmental parameters	**Environmental parameters**	**Environmental parameters**
Water temperature (°C):	Water temperature (°C):	Water temperature (°C):
Dissolved oxygen (mg/L **or** %):	Dissolved oxygen (mg/L **or** %):	Dissolved oxygen (mg/L **or** %):
TDS (mg/L):	TDS (mg/L):	TDS (mg/L):
Conductivity (µS/cm):	Conductivity (µS/cm):	Conductivity (µS/cm):
Current speed (m/s):	Current speed (m/s):	Current speed (m/s):

Sampling parameters	**Sampling parameters**	**Sampling parameters**
Sampling area (m^2):		
Fishing time (min):	Fishing time (min):	Fishing time (min):
Voltage (V):	Voltage (V):	Voltage (V):
Amperage (A):	Amperage (A):	Amperage (A):

Remarks

Site code: Date:

Species	Nr. indiv. in each pass 1st	2nd	3rd	Species	Nr. indiv. in each pass 1st	2nd	3rd	Species	Nr. indiv. in each pass 1st	2nd	3rd
Acipenser baerii				*Dicentrarchus labrax*				*Petromyzon marinus*			
Abramis brama				*Esox lucius*				*Phoxinus bigerri*			
Abramis brama				*Fundulus heteroclitus*				*Phoxinus phoxinus*			
Achondrostoma arcasii				*Gambusia affinis*				*Phoxinus septimaniae*			
Achondrostoma occidentale				*Gambusia holbrooki*				*Pimephales promelas*			
Achondrostoma oligolepis				*Gasterosteus aculeatus*				*Platichthys flesus*			
Achondrostoma salmantinum				*Gobio alverniae*				*Poecilia reticulata*			
Acipenser sturio				*Gobio gobio*				*Pseudochondrostoma duriense*			
Alburnoides bipunctatus				*Gobio lozanoi*				*Pseudochondrostoma polylepis*			
Alburnus alburnus				*Gobio occitaniae*				*Pseudochondrostoma willkommii*			
Alburnus alburnus				*Gobius paganellus*				*Pseudorasbora parva*			
Alosa alosa				*Gymnocephalus cernua*				*Pungitius laevis*			
Alosa fallax				*Hucho hucho*				*Pungitius pungitius*			
Ambloplites rupestris				*Hypophthalmichthys molitrix*				*Rhodeus amarus*			
Ameiurus melas				*Iberochondrostoma lemmingii*				*Rutilus rutilus*			
Ameiurus nebulosus				*Iberochondrostoma lusitanicum*				*Salaria fluviatilis*			
Anaecypris hispanica				*Iberochondrostoma olisiponensis*				*Salmo cettii*			
Aphanius baeticus				*Iberochondrostoma oretanum*				*Salmo rhodanensis*			
Aphanius fasciatus				*Iberocypris palaciosi*				*Salmo salar*			
Aphanius iberus				*Ictalurus punctatus*				*Salmo trutta*			
Atherina boyeri				*Lampetra alavariensis*				*Salvelinus alpinus*			
Australoheros facetus				*Lampetra auremensis*				*Salvelinus fontinalis*			
Barbatula barbatula				*Lampetra fluviatilis*				*Salvelinus umbla*			
Barbatula quignardi				*Lampetra lusitanica*				*Sander lucioperca*			
Barbus barbus				*Lampetra planeri*				*Scardinius erythrophthalmus*			
Barbus haasi				*Lepomis gibbosus*				*Silurus glanis*			
Barbus meridionalis				*Leucaspius delineatus*				*Squalius alburnoides*			
Blicca bjoerkna				*Leuciscus aspius*				*Squalius aradensis*			
Carassius auratus				*Leuciscus bearnensis*				*Squalius carolitertii*			
Carassius carassius				*Leuciscus burdigalensis*				*Squalius castellanus*			
Carassius gibelio				*Leuciscus leuciscus*				*Squalius cephalus*			
Chelon auratus				*Leuciscus oxyrrhis*				*Squalius laietanus*			
Chelon labrosus				*Lota lota*				*Squalius malacitanus*			
Chelon ramada				*Luciobarbus bocagei*				*Squalius pyrenaicus*			
Chelon saliens				*Luciobarbus comizo*				*Squalius torgalensis*			
Chondrostoma nasus				*Luciobarbus graellsii*				*Squalius valentinus*			
Cobitis bilineata				*Luciobarbus guiraonis*				*Syngnathus abaster*			
Cobitis calderoni				*Luciobarbus microcephalus*				*Telestes souffia*			
Cobitis paludica				*Luciobarbus sclateri*				*Thymallus thymallus*			
Cobitis taenia				*Luciobarbus steindachneri*				*Tinca tinca*			
Cobitis vettonica				*Micropterus salmoides*				*Triplophysa coniptera*			
Coregonus lavaretus				*Misgurnus fossilis*				*Umbra pygmaea*			
Cottus aturi				*Mugil cephalus*				*Valencia hispanica*			
Cottus duranii				*Oncorhynchus kisutch*				*Vimba vimba*			
Cottus gobio				*Oncorhynchus mykiss*				*Zingel asper*			
Cottus hispaniolensis				*Osmerus eperlanus*							
Cottus perifretum				*Pachychilon pictum*							
Cottus petiti				*Parachondrostoma arrigonis*							
Cottus rondeleti				*Parachondrostoma miegii*							
Cottus sabaudicus				*Parachondrostoma toxostoma*							
Ctenopharyngodon idella				*Parachondrostoma turiensis*							
Cyprinus carpio				*Perca fluviatilis*							

Interreg Sudoe SUDOANG

1. Yellow and Silver Eel Sampling

Page ___/___

Site code: Date:

Biometric data

*(**T**otal **L**ength - mm; **T**otal **W**eight - g (0.01g);* ***Visual inspection:*** *presence of a **C**onspicuous **L**ateral **L**ine – yes/no; **B**ody **C**olor and **C**ontrast between dorsal and ventral parts- yes/no;* ***If Silver eel AND if eels > 300/350 mm****: **E**ye **D**iameter-**V**ertical, **E**ye **D**iameter-**H**orizontal, **P**ectoral **F**in **L**ength – mm, **S**ilver **S**tage –* Durif *et al.*, 2009; ***Remarks****: **R**eleased - **R**; Retained for further **A**nalyses - **A**)*

ID of eel	Nr of pass	TL	TW	*If Silver eel OR if eels > 300/350 mm*						Remarks
				CLL	BCC	ED-V	ED-H	PFL	SS	