EEL SAMPLING PROTOCOLS


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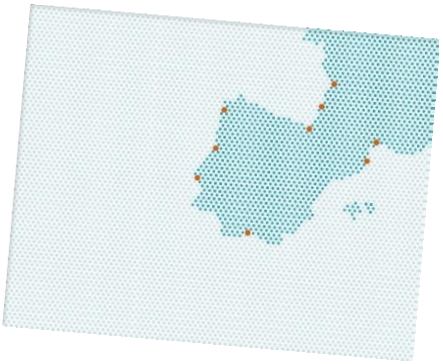

Interreg Sudoe

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SUDOANG

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PREAMBLE

The assessment of the European eel population requires the use of standardized methods to improve data quality and the efficient use of human and financial resources. Thus, the following protocols, developed within the framework of SUDOANG, establish such standardized methods to sample and analyse the eel population in the Sudoe area. Some of these protocols will be applied during the three years of the project to achieve their objectives, while others remain as a starting point to standardize eel surveys in all countries and create a baseline for the assessment of the population in the Sudoe area.

This guide contains 5 protocols:

1. Protocol for yellow and silver eel sampling in rivers;
2. Protocol to estimate glass eel recruitment;
3. Protocol for otolith preparation and age reading;
4. Protocol to assess the infection by *Anguillicola crassus* and the Swimbladder Degenerative Index (SDI);
5. Protocol to sample gonads for sex ratio assessment.

Despite created within the framework of SUDOANG, these protocols can also be implemented outside the SUDOE area and are thus useful for other agencies in charge of monitoring the eel population and any eel researcher.

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.

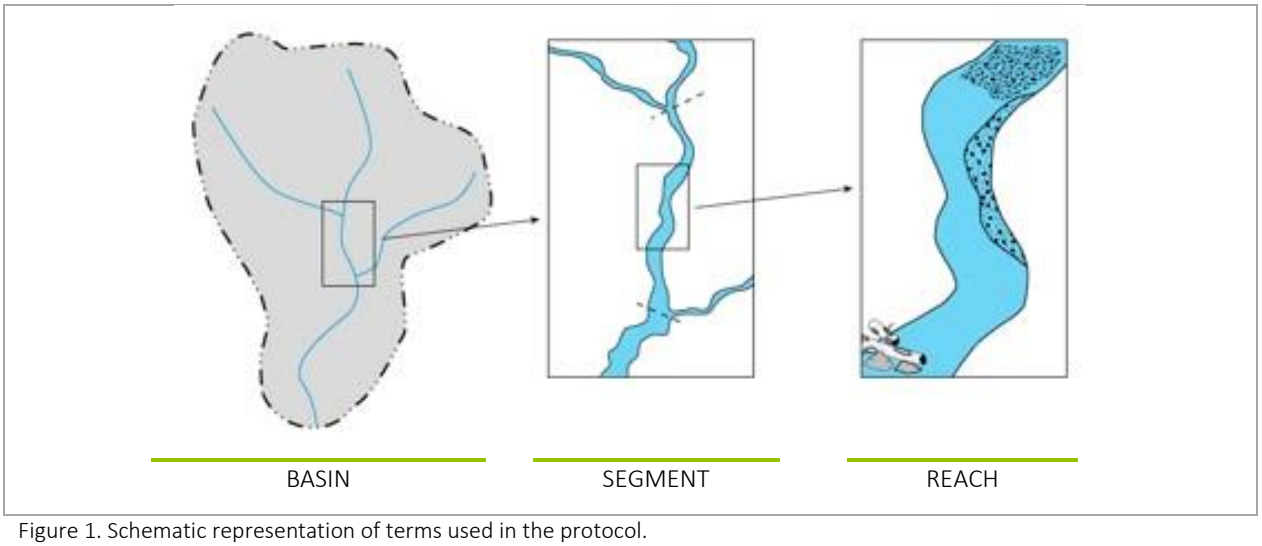


Figure 1. Schematic representation of terms used in the protocol.

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**:

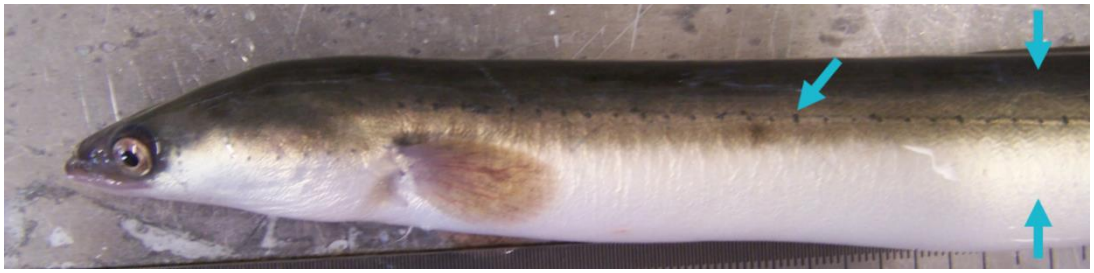
- Depth (m);
- Current speed (m/s);
- Water temperature (°C);
- Conductivity (µS/cm);
- Dissolved oxygen (mg/L **OR** %);
- The type of **substrate** and of **vegetation in the margins** and **instream cover** should also be recorded (format and classes in Data template);
- Also, the **area** sampled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

2. PROTOCOL TO ESTIMATE GLASS EEL RECRUITMENT

The aim of this protocol is to develop a standard method to calculate a glass eel recruitment index using a fishing gear (a net or a trap), preferably at the river mouth, i.e., not influenced by any anthropogenic pressure. The method also aims to provide a quantitative estimate of the glass eel recruitment for each catchment so that the levels of recruitment can be compared among basins. It is hoped that the use of this protocol could be extended to other catchments throughout the SUDOE area, to provide a nationwide recruitment index.

The conditions in the 10 pilot basins covered by the SUDOANG project are different. The basins contacting the Atlantic Ocean are strongly influenced by the tide, unlike the Mediterranean Sea where there is little tide and glass eels enter the river migrating against the current. The methods to sample glass eels and estimate recruitment have therefore, to be adjusted to the local conditions. However, regardless of the method chosen to sample glass eels, the objective is to record/ obtain a number of glass eels per volume of water (in the case of river basins influenced by the tide) or cross-section (in the case of river basins draining into the Mediterranean).

Glass eels use selective tidal transport to save energy. Hence, in the Atlantic coast, where there is the influence of the tide, glass eels should be caught during the flood tide. In the Mediterranean, where the tide influences the activity of glass eels to a lesser extent, the wind plays an important role in the recruitment process.

2.1. Timing of surveys

The fishery should be conducted **monthly** during the more **intense migration period** (ideally 6 months). Sampling should be performed in **New Moon Day.** If the weather conditions are not favourable to fishing, the fishery can be done up to a maximum of 2 days following the New Moon Day. In pilot basins that flow into the Atlantic, sampling should always be conducted during the **night flood tide**. In the catchments flowing into the Mediterranean, sampling should be conducted at similar hours of the night or around the highest water level if those conditions are considered to influence catches. In any case, the sampling protocol should be set after the initial experiments carried out during the first year and maintained in the long term to ensure the standardized collection of data for a recruitment series.

2.2. Site selection

The **fishing location** should be as close to the sea as possible, to avoid the influence of other fishermen, in case there is a fishery.

2.3. Sampling procedures

- The fishing gear should be preferably the one used by fishermen or other that has already been used;
- A sieve should be used to separate glass eels from other organisms;

- When 50 or fewer eels are caught they should be taken to the laboratory. When more than 50 glass eels are caught, a subsample of 50 glass eels should be kept for analysis;
- Water parameters should be measured at the beginning and end of each fishing period. If fishing is continuous, two records will be enough (beginning and end of fishing). If sampling is conducted repeatedly, measures should be recorded at regular intervals, i.e., at each sampling occasion.

- In the Atlantic area:
  - Sampling should start at the beginning of the flood tide and last until the end;
  - It is necessary to record the value of the flowmeter at the beginning and at the end of sampling;
  - The number of glass eels per volume of water should be recorded;
  - If fishing is continuous during the entire flood tide, it is necessary to record the value of the flowmeter at the beginning and at the end of sampling, and also the number of glass eels per volume of water;
  - If sampling can be conducted repeatedly at regular intervals during the entire flood tide, then the value of the flowmeter at the beginning and at the end of each occasion should be recorded separately; Glass eels should also be collected and stored separately on each occasion;
  - At the end of fishing, the 50 glass eels to retain for laboratory analysis should be equally distributed throughout the time intervals of sampling;
  - The cross-sectional area of the river and average water velocity should be provided to estimate the overall volume of water flowing in the river.

- In the Mediterranean area:
  - The sampling should last long enough to cover the night peak migration.
  - If a fyke-net (or similar sampling gear) is used, the cross-sectional surface sampled by this trap should be recorded, and the overall cross-sectional river at the location of the trap should be measured. If possible, tag-recapture experiments can be carried out to estimate the efficiency of the trap;
  - If a ladder trap is used, the efficiency of the ladder should be assessed, either by carrying mark-recapture experiments or by carrying out bongo tows to provide a measure of glass eel densities in water (protocol similar to the Atlantic area protocol);

2.4. Environmental data to collect in the field

The following environmental variables should also be recorded:

- Date and time of fishing;
- Tide hour and height;
- Depth;
- Water temperature and salinity or conductivity (brackish water or freshwater) – via data loggers or Van Dorn Bottles/Niskin Bottles;
- Date of New Moon day;
- Flow rates across the stream width and at trap sites;
- Length of fishing period.

2.5. Laboratory procedures

Samples taken to the laboratory should be kept in water in the refrigerator, to be analysed as quickly as possible.

In the lab, the glass eels should be placed in paper to remove extra water, and the following data should be recorded:

- Individual length (mm);
- Individual weight (0.01g);
- Pigmentation stage (according to the classification from Briand, 2009).

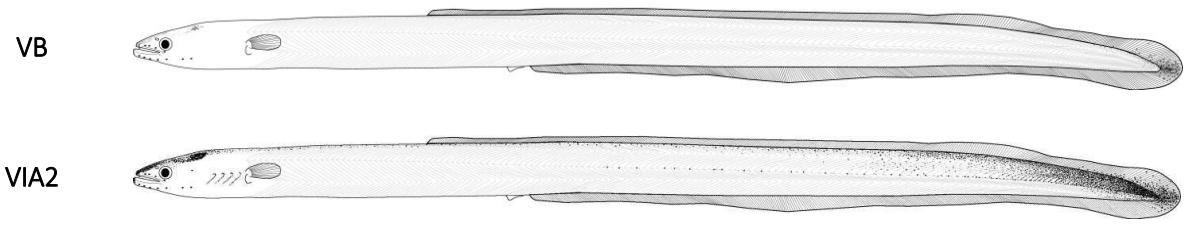
2.6. Field Equipment

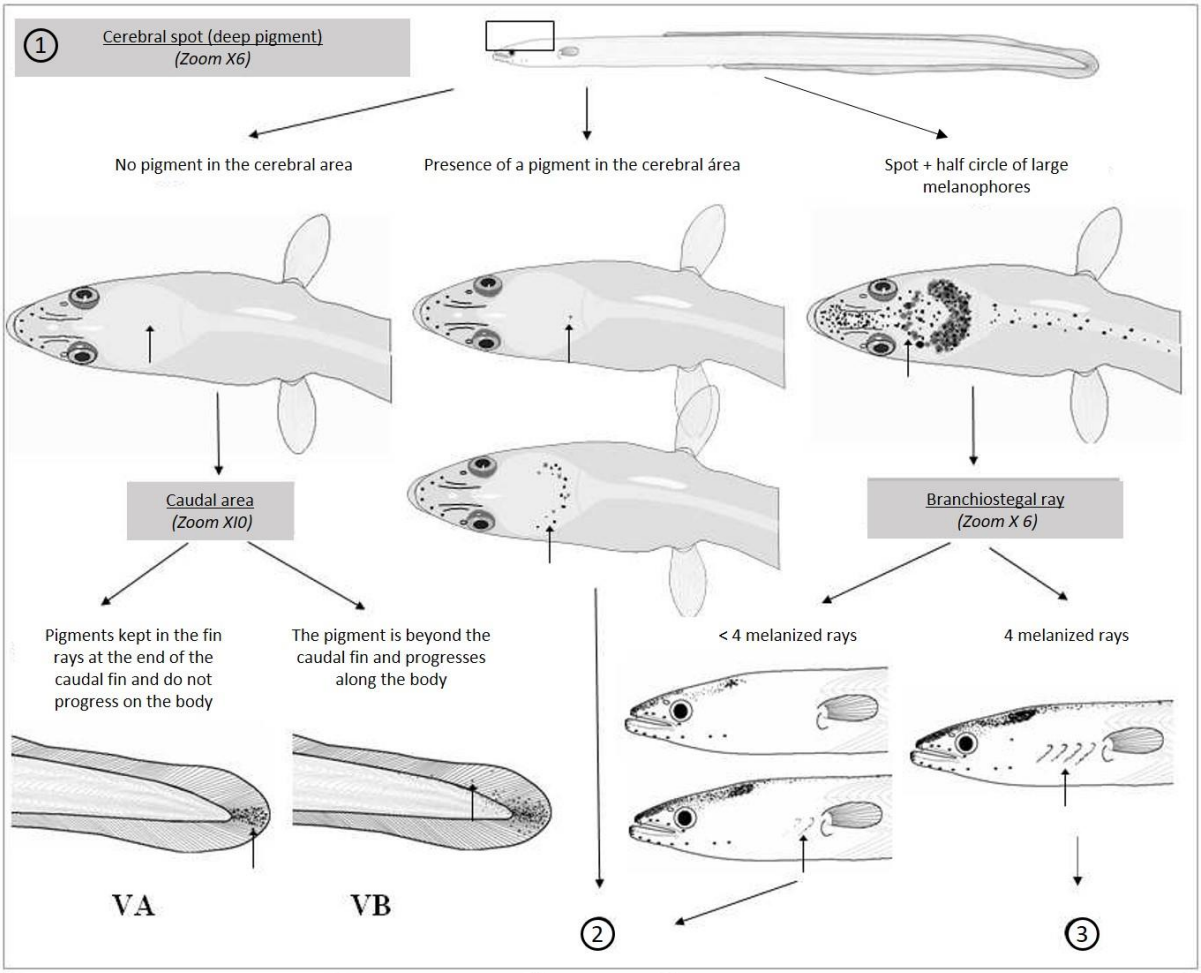
- Net/trap;
- GPS;
- Flowmeter;
- Sieve;
- Van Dorn or Niskin Bottles or Data logger.

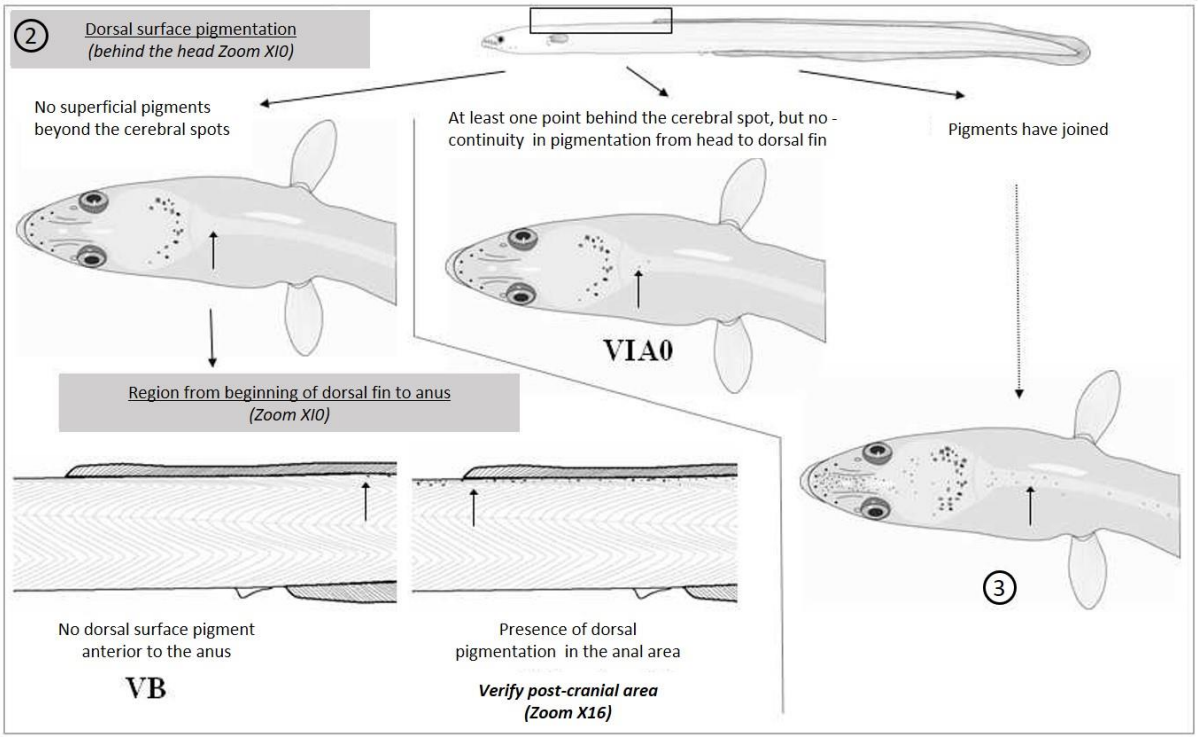
2.7. Pigmentation stages

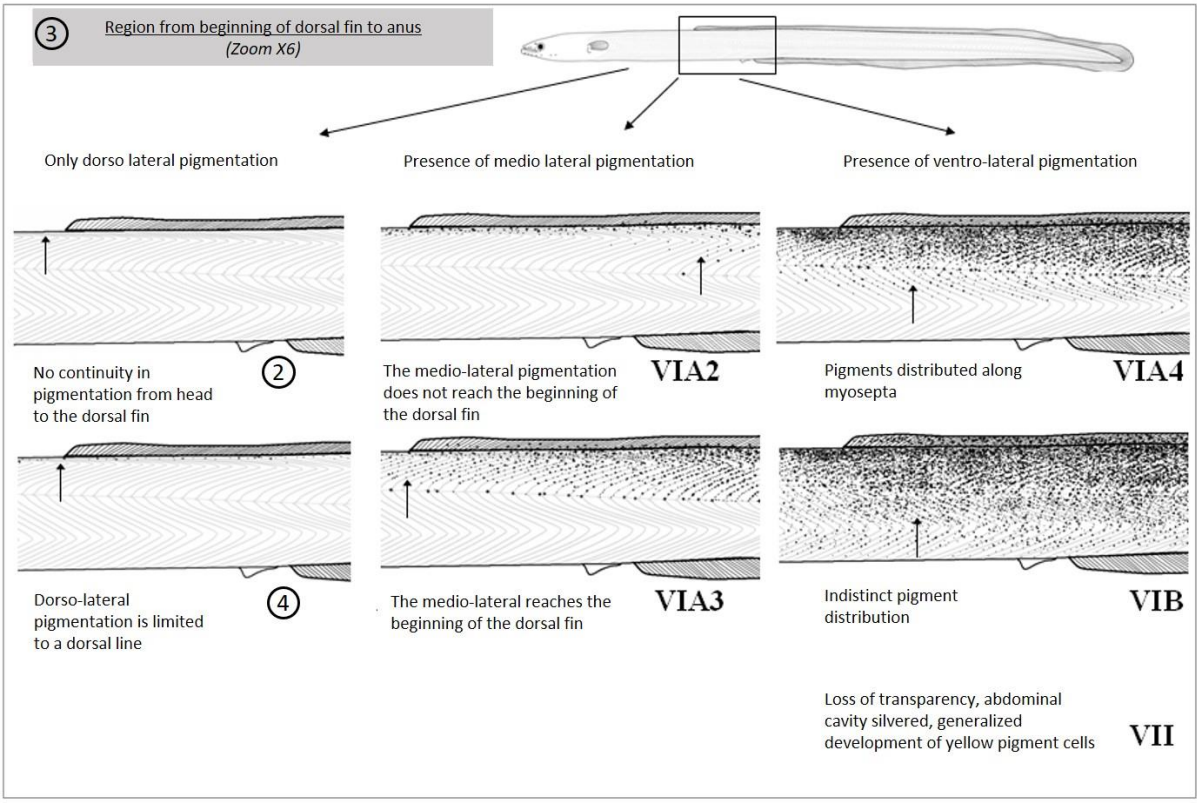
The identification of pigmentation stages should follow the classification by Briand (2009).

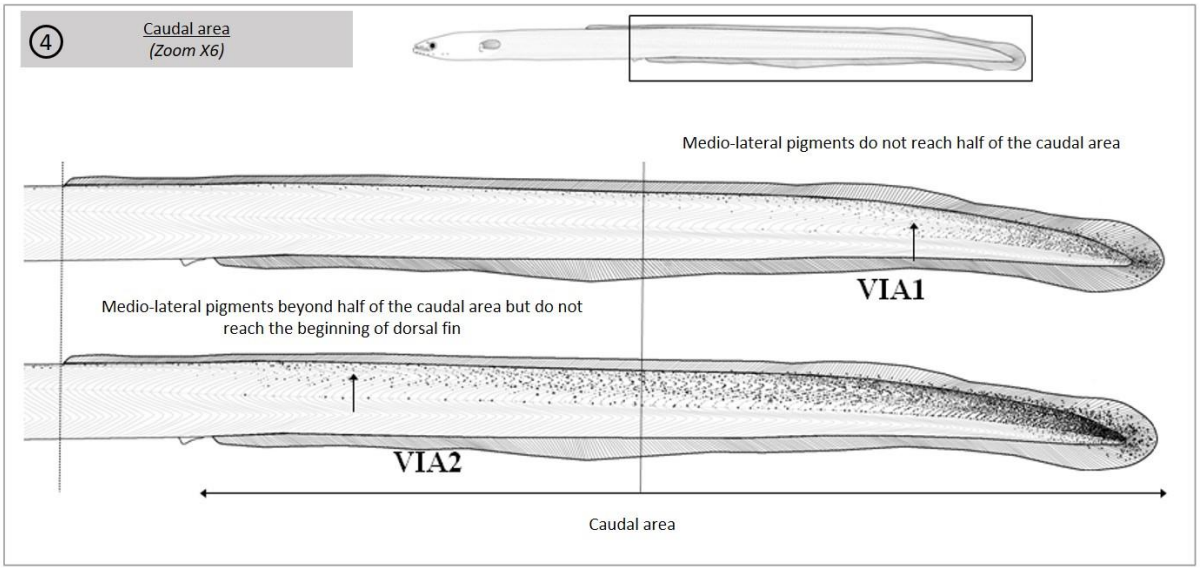
From (Strubberg, 1913; Elie et al., 1982; Lecomte-Finiger, 1983).




1
Cerebral spot (deep pigment)
(Zoom X6)
No pigment in the cerebral area
Presence of a pigment in the cerebral área
Spot + half circle of large melanophores
Caudal area
(Zoom XI0)
Branchiostegal ray
(Zoom X 6)
Pigments kept in the fin rays at the end of the caudal fin and do not progress on the body
The pigment is beyond the caudal fin and progresses along the body
< 4 melanized rays
4 melanized rays
VA
VB
2
3


2
Dorsal surface pigmentation
(behind the head Zoom XI0)
No superficial pigments beyond the cerebral spots
At least one point behind the cerebral spot, but no - continuity in pigmentation from head to dorsal fin
Pigments have joined
VIA0
Region from beginning of dorsal fin to anus
(Zoom XI0)
3
No dorsal surface pigment anterior to the anus
VB
Presence of dorsal pigmentation in the anal area
Verify post-cranial area
(Zoom X16)





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Briand C. 2009. Dynamique de population et de migration des civelles en estuaire de Vilaine. PhD thesis, Agrocampus Ouest. Rennes, France. 207p.

Elie P., R. Lecomte-Finiger, I. Cantrelle and N. Charlon. 1982. Définition des limites des différents stades pigmentaires durant la phase civelle d'Anguilla anguilla L. Vie et Milieu 32: 149–157.

Lecomte-Finiger R. 1983. Contribution a` la connaissance de l'écobiologie de l'anguille, Anguilla anguilla, L. 1758, des milieux lagunaires méditerranéens du golfe du Lion: Narbonnais et Roussillon. PhD Thesis, Université de Perpignan, France.

Strubberg A.C. 1913. The metamorphosis of elvers as influenced by outward conditions. Meddelester fra Kommissionen for Havundersøgesler, serie Fiskeri Copenhagen 4: 1–11.

3. PROTOCOL FOR OTOLITH PREPARATION AND AGE READING

This protocol was developed to harmonize methodologies for otolith preparation and age reading within the framework of SUDOANG. It is based on Manual on Age reading (ICES 2009). The sex of individuals should be identified by macroscopic analysis (See *Protocol to sample gonads for sex ratio assessment*).

3.1. Extraction and storage

- Extract both sagittal otoliths from each eel;
- Clean them with water and dry them;
- Store the otoliths dry in small containers (e.g. *eppendorfs)*, but make sure they are fully dry to avoid deterioration.

3.2. Grinding and polishing

- Choose the right otolith for consistency. If not possible choose the left, but include this information in the observations;
- Otoliths can be observed whole (without preparation) with strong transmitted light or on a dark surface with strong incident light. If there are less than 4 to 5 annual marks, the age can be read without any other preparation, except being immersed into 96% ethanol to improve the visualization of the growth marks.
- If age is more than 5 years, otoliths must be embedded in resin;
- Sagittal sections are obtained by embedding directly the otolith in resin while transverse section requires embedding in two layers of resin, so that the otolith is in the middle of the resin block;
- The grinding process must be carefully checked until the midplane of the otolith has been reached. For that, the otolith is examined under a stereo dissecting microscope, with the largest magnification possible, using a variety of light types including transmitted, reflected or polarized light;
- The otolith is ground along the sagittal (if eel is up to 12 years, and otolith is not curved) or transverse plane (requires cutting a slice of otolith along the transverse axis with a diamond saw) (depending on the curvature of the otolith so depending on the size of the otolith) until the centre of the nucleus is reached;
- Grinding can be performed manually, or by using a grinding wheel with silicon carbide sandpaper, lubricated with distilled water;
- Polish the ground surface of the otolith using a decreasing range in coarseness (1200-4000 grit) of silicon carbide wet dry sandpapers, jewellery cloths or pastes made from aluminium or diamond powder, lubricated with distilled water.

For more details on the procedures, consult the Manual for Age Reading of Atlantic eel (link below in the references).

3.3. Age reading

- After preparation, you should acquire images of the otoliths to facilitate exchange among all readers.
- Sagittal sections require etching and dying to enhance winter marks. Transverse sections do not require that process;
- Read the age, i.e., count the number of winter marks. Translucent zones (winter) are bright, and opaque zones (summer) are dark, when the otolith is viewed with **transmitted light.** If viewed with **reflected light,** opaque zones (summer) are bright, and translucent zones (winter) are dark.

3.4. Material

- Stereo dissecting microscope (with digital camera and image capture and analysis software Image J);
- Glass microscope slides;
- Fine point forceps;
- Mounted needles;
- Slide container box.
- Reagents needed for the preparation:
  - Wax, epoxy resin, to embed the otolith;
  - Alumina and diamond powder pastes for grinding the otolith.

References

ICES. 2009. Workshop on Age Reading of European and American Eel (WKAREA). Bordeaux, France: ICES CM 2009\ACOM: 48, 66 pp.

ICES 2009. Manual for the Ageing of Atlantic Eel. In Workshop on Age Reading of European and American Eel, (WKAREA) Annex 4, 57 pp.

4. PROTOCOL TO ASSESS THE INFECTION BY *ANGUILICOLA CRASSUS* AND THE SWIMBLADDER DEGENERATIVE INDEX (SDI)

1. Try not to open the swimbladder when taking it out of the eel;
2. The swimbladder is made of 2 layers (kind of socks), and we have to analyse both together;
3. Put the swimbladder in a **Petri dish** with salty water (8g/L);
4. Have a first look at the swimbladder without opening it to have an idea of the transparency-opacity (for further SDI determination);
5. Open the swimbladder on the longitudinal axis with small **scissors** (as shallowly as possible so as not to damage the parasites), from one end to the other (be careful some small parasites can hide in the bottom of one end). Also open the canal between the 2 gas glands (larvae can hide there);
6. Have a look when opening the swimbladder for possible leaking of exudate (pieces of dead worms, erythrocytes, decaying swimbladder tissue, eggs and L2 stage of *A. crassus* must be considered as exudate);
7. To determine the Swimbladder Degenerative Index (SDI) you need a **stereomicroscope** and a **calliper**.

4.1. Determine the individual level of *Anguillicola crassus* infection

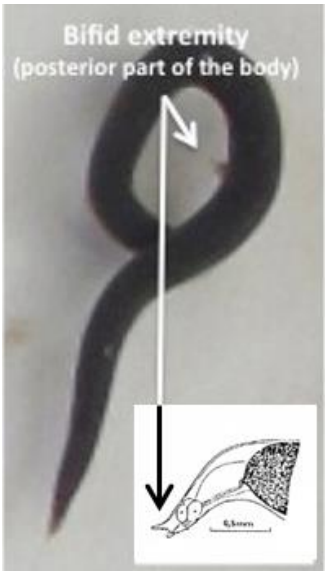
At this point you have 2 options:

1) the first one is the easiest and less time consuming: just remove the parasites and count them (but without stereomicroscope you will miss all the tiny ones (larvae for example).
2) the second one is a little bit more difficult and time consuming but better to assess the possible impact of *A. crassus*: identify the developmental stages and sex the adults if possible (necessity to stretch out the swimbladder wall and of a stereomicroscope with strong light not to miss them).

Infection by *Anguillicola crassus*

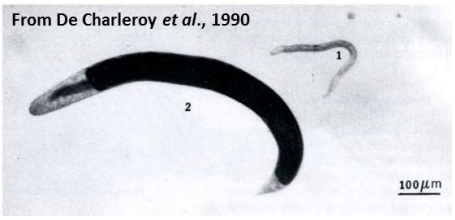
1) Removal and counting of the parasites without a steromicroscope (underestimates the infection).

2) Removal and counting of the parasites with a steromicroscope (considers all stages of the parasite)



Male

Female



L3 larvae (1) and L4 larvae (2)

L3 larvae

L4 larvae

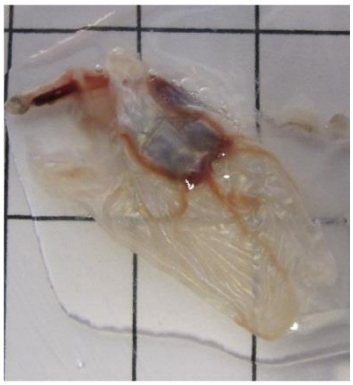
4.2. Determine the Swimbladder Degenerative Index (SDI) (modified from Lefebvre *et al.*, 2002)

Based on 3 criteria each one being coded 0, 1 or 2 (increasing degradation)

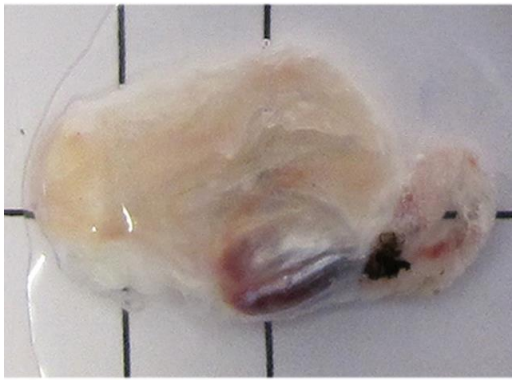
1) Transparency-Opacity of the swimbladder wall

You need a paper with bold lines printed on it. Flat the swimbladder on it and if:

- You can see the lines through the swimbladder without any magnification (natural light): score 0
- If you can't see anything with the transmitted light of the stereomicroscope: score 2
- If you cannot see with natural light and no magnification but you can see the lines with the transmitted light: score 1.



Lines clearly visible
Natural light
SCORE 0



Lines hardly visible
Transmitted light
SCORE 1

Lines not visible
Transmitted light
SCORE 2

2) Presence of pigmentation and/or exudate

Pieces of dead worms, erythrocytes, decaying swimbladder tissue, eggs and L2 stage of *A. crassus* should be considered as exudate.

Pigmentation that is following the blood vessels and/or pneumatic canal is not considered as pigmentation (like a road - - - - - - - -).

- When the swimbladder is frozen, it happens that small pieces of skin are detached (desquamation) in the lumen, but they are not exudate.
- If no pigmentation and no exudate: score 0
- If only pigmentation or only exudate: score 1
- If both pigmentation and exudate: score 2

Pigmentation (external or internal)
SCORE 1

From De Charleroy et al., 1990

Exudate alone (L2 in eggs on the right picture)
SCORE 1

SCORE 0	No pigmentation AND no exudate
SCORE 1	Pigmentation OR exudate
SCORE 2	Pigmentation AND exudate

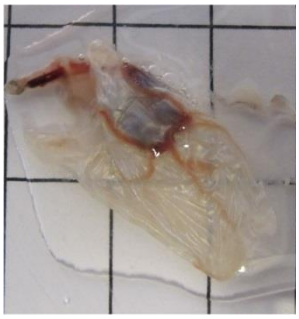
3) Thickness of the swimbladder Wall

We use a dial calliper (electronic is better).

We should not squash the wall of the swimbladder too much. It should just be squeezed enough so that the swimbladder does not fall when it is in a vertical position.

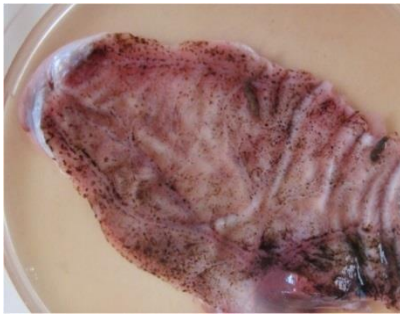
If the swimbladder has different thickness at different locations, we record the average.

- Score 0: <1mm
- Score 1: ≥ 1mm and ≤ 3mm
- Score 2: > 3mm



Less than 1 mm

SCORE 0



Between 1 and 3 mm

SCORE 1

More than 3 mm

SCORE 2

In case the analyses are not conducted in fresh material, the swimbladders should be removed and preserved at -20 °C. For that, each swimbladder should be kept in a small container (size of each container should be adapted to the size of the swimbladder) with an appropriate codification for further identification of the eel.

References

Lefebvre F., P. Contournet and A.J. Crivelli. 2002. The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology*, 124: 457-463.

5. PROTOCOL TO SAMPLE GONADS FOR SEX RATIO ASSESSMENT

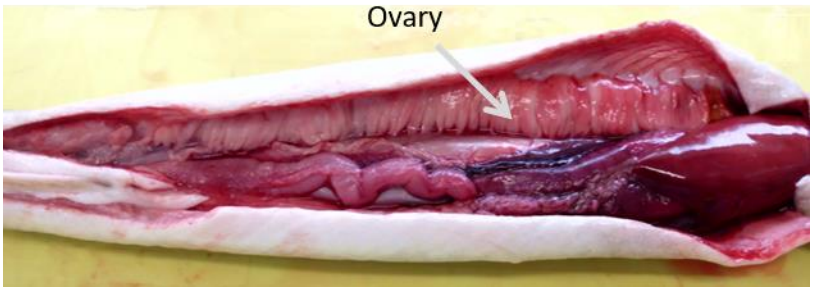
This protocol is designed to sample gonads of yellow eels for molecular and histological analyses when it is not possible to identify the sex of eels through macroscopic analysis. It should concern fish with a size encompassing 20 to 30 cm (but some fish under 30 may have gonads sufficently developped for a macroscopic assessment of the sex and some above 30 cm may have gonads not sufficently developped). Just after death, dissect the eels and inspect their gonads.

5.1. Macroscopic observation

In largest yellow eels and, in particular, in silver eels, it is easy to identify the sex (Fig.1):

- Ovaries can be identified by the presence of transverse folds, which when the gonad is more developed, divide the gonads in many small elongated compartments;
- Testis can be recognized by the presence of individual lobes, which are attached to the dorsal part of the gonad.

FEMALE



MALE

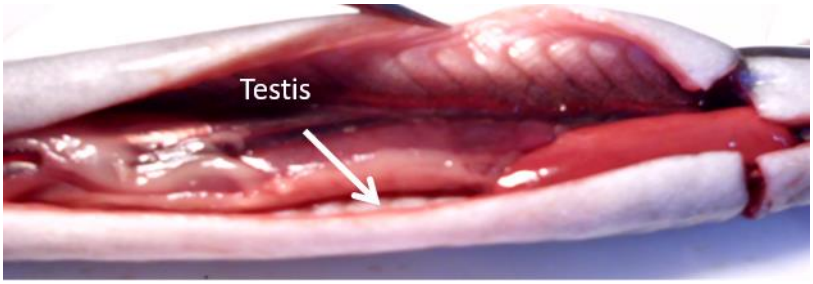


Figure 1. Location and macroscopic aspect of an ovary and a testicle in the abdominal cavity of a female and an eel male.

The morphological distinction between testis and ovaries is however difficult for small yellow eels because it is common to find undiferentiated or intersex gonads (Beullens *et al*. 1997). The different categories (undifferentiated/intersex, male and female) can be recognized by looking at Fig.2. Take care that the separation between lobes (3) should not be used as a criterion for males.

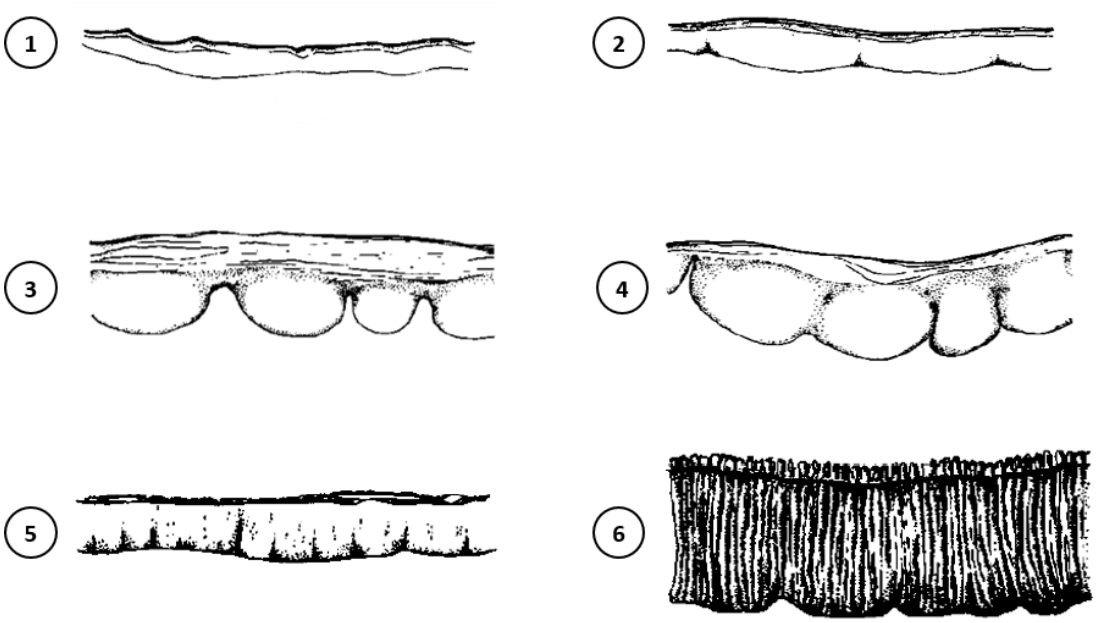


Figure 2. Morphological aspect of eel gonads: Undifferentiated (1 and 2) or intersex (3); Male (4); Female (5 and 6) (from Beullens *et al*., 1997).

If the macroscopic analysis fails to identify the sex of the yellow eels the gonads should be removed for further analyses (see Fig. 3 and section 5.2).

Figure 3. Sampling gonads in a juvenile eel.

5.2. Conditioning for molecular and histological analyses

The following procedures should be adopted:

A- Molecular analysis

- You can use small drops of RNA later to limit RNA degradation and make the gonad more visible *in situ* during dissection;
- Put promptly **one of the gonads** (the first that you extract, usually the left one) in a 1.5 ml RNA free eppendorf filled with RNAlater liquid;
- Keep the eppendorfs at low temperature (but not freeze) and incubate them at least one hour (but it can be overnight) at 4°C;
- Remove RNA later and pierce the cap (small hole) before storage at -80 °C.

B- Histological analysis

- Put the **other gonad** in a micromesh histosette;
- Immerse it in Bouin for 1 to 3 h;
- Rince 1h with clear water;
- Transfer it to formol 10%.

References

Beullens K., E.H. Eding, P. Gilson, F. Ollevier, J. Komen and C.J.J. Richter. 1997. Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguilla* L.) maintained in captivity. *Aquaculture*, 153:135-150.

TEMPLATES
to register field and laboratory data

TEMPLATES for Protocol 1
YELLOW AND SILVER EEL SAMPLING IN RIVERS

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		0 %	*0%-25%*	*25%-50%*	50%-75%	75%-100%
	Right river bank	☐ *absent*	☐ *sparse*	☐ *intermediate*	☐ *semi-continuous*	☐ *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*							

1. Yellow and Silver Eel Sampling

Page ___/___

Site code: Date:

Biometric data

(Total Length - mm; Total Weight - g (0.01g); ***Visual inspection:*** *presence of a Conspicuous Lateral Line – yes/no; Body Color and Contrast between dorsal and ventral parts- yes/no;* ***If Silver eel AND/OR if eels > 300/350 mm****: Eye Diameter-Vertical, Eye Diameter-Horizontal, Pectoral Fin Length – mm, Silver Stage –* Durif *et al.*, 2009; ***Remarks****: Released - R; Retained for further Analyses - A)*

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

TEMPLATES for Protocol 2
ESTIMATION OF GLASS EEL RECRUITMENT

2. Estimation of Glass Eel Recruitment

Site name:			**Site code:**
GPS Coordinates	Lat:	Long:	Coordinate system:
Date:	Date of New Moon:		Hours: (start - end) ___H___ - ___H___
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 section

Total fishing time (minutes):	Tide hour: ___H___	Tide height (m):
Fishing net opening area (m^2):		River width (average - m):

Environmental conditions *IF CONTINUOUS FISHING (if not, fill in next page)*

	Start	*End*		*Start*	*End*
Hour:	___H___	___H___	Conductivity (µS/cm):		
Water temperature (°C):			TDS (mg/L):		
Salinity:			Depth (m):		
Flowmeter:					

Remarks (please describe the fishing method, including the type of fishing net/ trap)

2. Estimation of Glass Eel Recruitment

Page ___/___

Site code: Date:

Sampling data *IF NOT CONTINUOUS FISHING*

*(**Repl**icates - 20 minutes each*; **W**ater **T**emperature - °C; Salinity; **Cond**uctivity - µS/cm; **TDS** - mg/L; Depth – m; *Total **W**eight fished – 0,01g; **Sub-sample** – 0,01g)*

Repl. Nr.	Hours		Flowmeter	Environmental parameters					TW (g)	Sub-sample (g)	Remarks
				WT (°C)	Salinity	Cond (µS/cm)	TDS (mg/L)	Depth (m)			
	Start										
	End										
	Start										
	End										
	Start										
	End										
	Start										
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	End										
	Start										
	End										
	Start										
	End										

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2. Estimation of Glass Eel Recruitment

Page ___/___

Biometric data

(Total Length - mm; Total Weight - 0.01g; Pigmentation stage - see protocol)

Glass Eel ID	Site code	Repl.	Date	TL	TW	Pigmentation stage	Remarks

TEMPLATE for Protocol 3
OTOLITH PREPARATION AND AGE READING

3. Age Reading

Biometric data

(*Total Length - mm; Total Weight - g; Eviscerated Weight - g;* ***Sex*** *- Male/Female/Undifferentiated*)

Eel ID	Site code	Date	TL	TW	EW	Sex	Age	Remarks

TEMPLATE for Protocol 4
INFECTION BY *ANGUILICOLA CRASSUS* AND THE SWIMBLADDER DEGENERATIVE INDEX (SDI)

4. Swimbladder Evaluation

Eel ID	Site Code	Date	Parasite *Anguillicola crassus*							Swimbladder Degenerative Index (SDI)				Remarks
			Female	Male	Unsexed	L4	L3	L2/eggs	Necrotic	Opacity	Pigmentation/ Exudate	Thickness	1 mm	

TEMPLATE for Protocol 5
SAMPLING GONADS FOR SEX RATIO ASSESSMENT

5. Sex Ratio Assessment

Biometric data

(*Total Length - mm; Total Weight - g; Eviscerated Weight - g*)

Eel ID	Site code	Date	TL	TW	EW	Nr. Molecular Analysis	Nr. Histological Analysis	Remarks (morphological aspect or other)


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