5. PROTOCOL TO SAMPLE GONADS FOR SEX RATIO ASSESSMENT

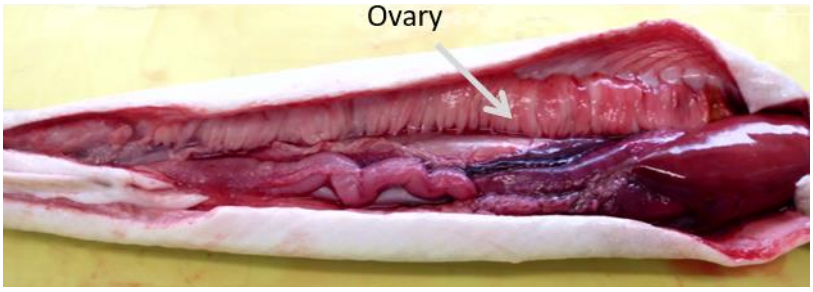
This protocol is designed to sample gonads of yellow eels for molecular and histological analyses when it is not possible to identify the sex of eels through macroscopic analysis. It should concern fish with a size encompassing 20 to 30 cm (but some fish under 30 may have gonads sufficently developped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

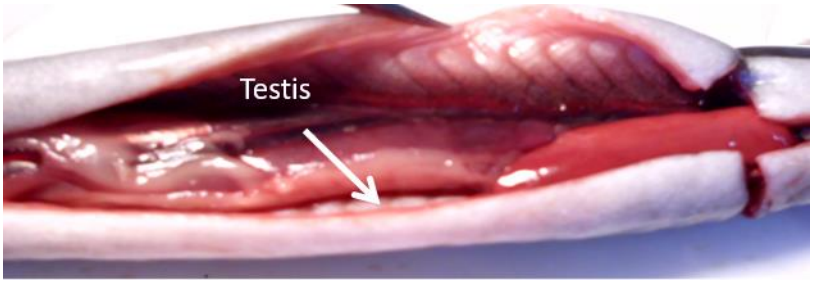


Figure 1. Location and macroscopic aspect of an ovary and a testicle in the abdominal cavity of a female and an eel male.

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.

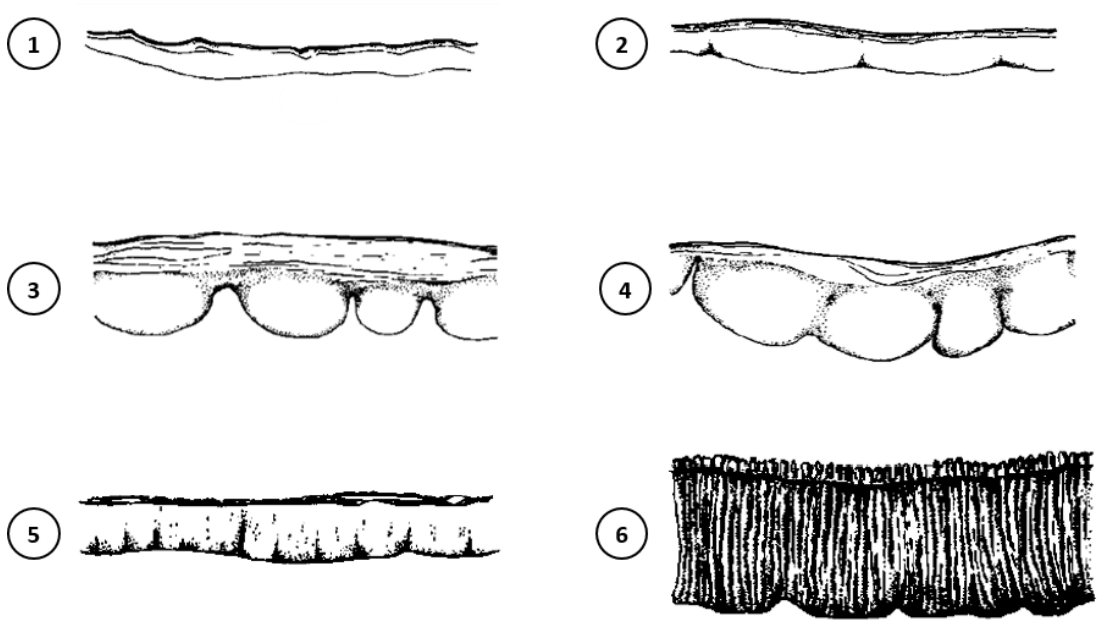


Figure 2. Morphological aspect of eel gonads: Undifferentiated (1 and 2) or intersex (3); Male (4); Female (5 and 6) (from Beullens *et al*., 1997).

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).

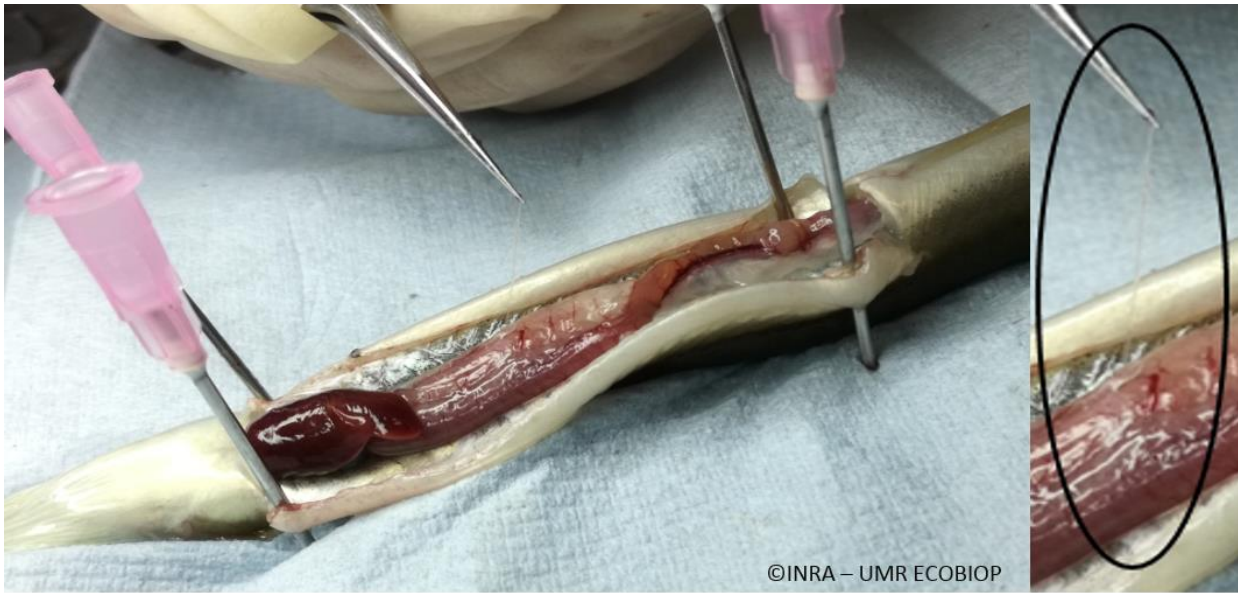


Figure 3. Sampling gonads in a juvenile eel.

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

Interreg Sudoe SUDOANG

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)