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

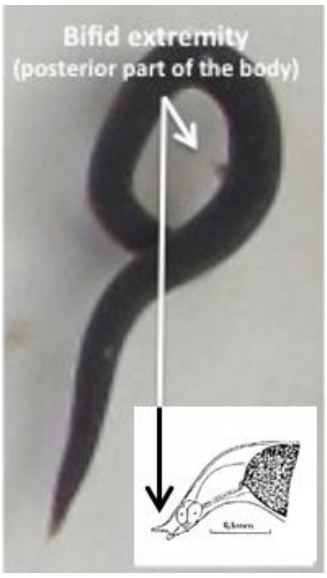
At this point you have 2 options:

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*

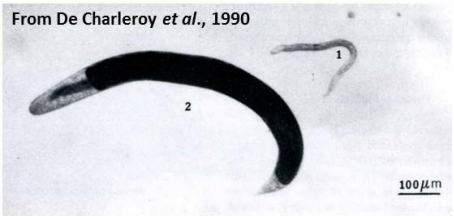
1) Removal and counting of the parasites without a steromicroscope (underestimates the infection).

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

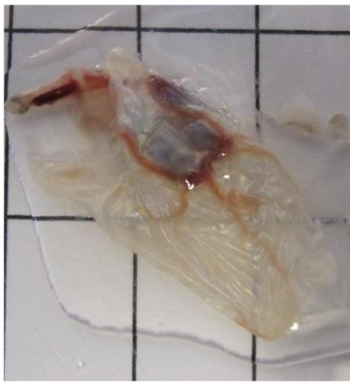
4.2. Determine the Swimbladder Degenerative Index (SDI) (modified from Lefebvre *et al.*, 2002)

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

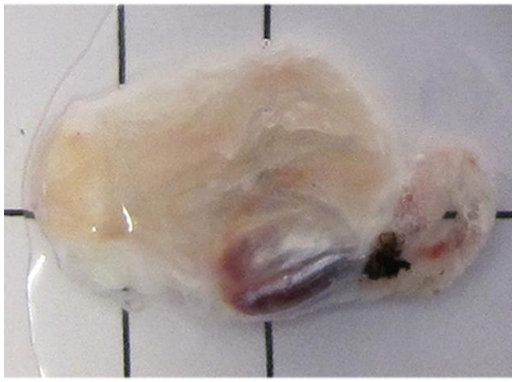
1) Transparency-Opacity of the swimbladder wall

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

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

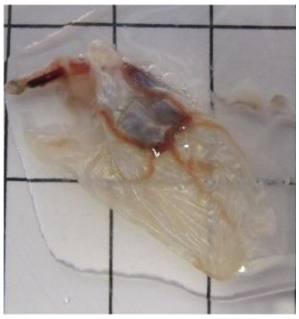
3) Thickness of the swimbladder Wall

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.

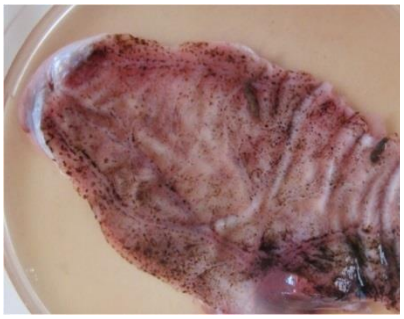
If the swimbladder has different thickness at different locations, we record the average.

- 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.

4. Swimbladder Evaluation

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