

Sampling strategy for *Ciona intestinalis*

Specimens used for experiments were collected from Brest Harbour by Biological Station of Roscoff.

Locations and sampling:

1. As an example, the choice of the sampling location (Brest - Le Moulin Blanc Harbour) has been explained above presence of *Ciona* throughout the year.
2. Animals should be taken “randomly” at the same depth.
3. Sampling will be realized in diving, during which the % coverage by *Ciona* will be visually assessed according to a semi-quantitative scale set below:
0 (absence), **1** (10 %), **2** (10-30 %), **3** (30-60%), **4** (60-90 %), **5** (> 90 %)
4. T°, salinity and dissolved O₂ should be recorded at the bottom and at the surface.
5. All specimens collected from each pillar will be kept separately in a seawater tank during transport, and then put in a sea-running tank at the arrival at lab.
6. Animals will be measured and reproductive status will be assessed after careful visual examination according to a semi-quantitative scale (ASSEMBLE-JRA1-Protocol-04.00).
7. Furthermore, a standard fertilisation test will also be performed. (ASSEMBLE-JRA1-Protocol-02.00).
8. Sampling frequency: every month.

Régis Lasbielez, Laurent Leveque,
Gatean Schires
Station Biologique de Roscoff, France

Animals: Healthy adults collected from natural populations.

Apparatus: Scapels, Pasteur pipettes, petri dish, filtered seawaters.

Parameters: T°.

Edited by Francesca Paloa Cuscunà, Euan Brown