Maintenance of Amphioxus and induction of spawning

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Additional information:
Fuentes et al. *2007 J Exp Zool B Mol Dev Evol. 308:484-93. and Fuentes et al. 2004 J Exp Zool B Mol Dev Evol. 302:384-91.*

1- Amphioxus adults (B. lanceolatum) showing developed gonads can be sampled from February to July in coastal waters close to Argele`s-sur-Mer (France) at a depth of 5-10m from sandy substratum approximately 50-100m offshore. The latitude and longitude of the collecting place are, respectively, 421 32' 53'' N and 31 03' 27'' E. Collection is performed with a research boat from the "Laboratoire Arago, Observatoire Océanologique, Université Pierre et Marie Curie/C.N.R.S," Banyuls-sur-Mer, France, using a "Charcot-Picard" (60cm *x* 20cm, mesh size 50μm) sediment dredge.

2- Amphioxus adults are collected from the sand using a sieve (1.25mm mesh) and distributed into 3l tanks (around 30 animals per tank). Tanks are filled with clean sand as a substratum.

3- The tanks are kept in a water bath at a constant 18°C temperature, under a 10/14hrs night/day cycle (light from 7:00 to 21:00hrs)

4- Water in the tanks is completely renewed automatically four times per day by pumping new sea water at 18°C into each tank for 20 min.

5- Animals are fed with crushed Tetramicrofood (0.5 g/feeding) (www.tetra-online.-com) supplemented with 15.000–40.000 cells/mL/feeding of mixed algae (equal parts of *Dunaliella tertiolecta, Isochrysis galbana* and *Tetraselmis suecica*). Algal cultures are grown in f/2 medium as described by Guillard *et al*. (Guillard and Ryther, 1962. Can J Microbiol 8:229–239). The f/2 medium was eliminated by centrifugation before feeding the algae to the amphioxus.

6- Spawning can be induced by placing amphioxus at 23°C at 18:00hrs of day -1. During the afternoon of day 0, the animals are separated individually in plastic cups and spawning occurs naturally around 45 min after the light has been turned of (spawning at 21:45hrs of day 0).

7- Once the animals spawn, use separate pipettes to collect eggs and sperm. Transfer spawned eggs into a Petri dish containing FSW. Add 10-20 µL of concentrated sperm to one batch of eggs (several thousand) in the Petri dish. Mix the sperm with the eggs by gently swirling the dish. Monitor the eggs under a dissecting microscope to check for signs of fertilization. Developmental staging at 19°C can be obtained from Bertrand *et al.* 2011 Development 138:4819-30.

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