

Spawning of gametes in *Paracentrotus lividus*

1. Randomly select 4-5 adult animals (sex determination in *P. lividus* is not possible by visual inspection; only after the spawning female and male will be easily distinguished by gametes: eggs are yellow-orange, sperm are white)
2. Apply one of the following stimulus in order to induce the spawning (the 3 methods (a – c) are progressively more invasive and can be applied successively or alternatively, according to the quantity of gametes needed).
 - a) **Shaking**: shake vigorously animals wrapping them in common paper. Place the animals with the oral side down on a Petri dish. Wait for a few minutes and check the spawning of gametes from the aboral surface.
 - b) **Electrical shock**: place the urchin with the oral side down on a Petri dish with a thin layer of sea water just lapping the animal. Apply the paddle to the opposite sides of animal near the gonopore and give an electrical discharge lasting a few seconds. Repeat this electrical shock for 3 – 4 times. Wait for a few minutes and check the spawning of gametes from the aboral surface.
 - c) **Intracoelomic injection** : place the urchin with the oral side up. Inject about 1 ml of KCl 0,5M directly into coelomic cavity through the soft membrane surrounding the mouth (Aristotele lantern), in several points. Check the spawning of gametes from the aboral surface (in a short time).



3. Proceed to the gametes collection :
 - Sperm**: 1. Collect sperm “dry” by pipetting it directly from the gonopores. 2. Transfer the sperm into an eppendorf tube and keep it cold. Sperm stored cooled (at 4 °C) will be good for 24 h. 3. Dilute sperm just before use; the sperm activated (by dilution) have a short life span.
 - Eggs**: 1. To collect the eggs, invert the female sea urchin over a clean beaker filled with filtered seawater. Make sure that the gonopores are fully submerged in order to let the eggs shed directly into the seawater. 2. Let eggs settle in the beaker by gravity. 3. Change the seawater twice removing the covering water replacing it with fresh seawater. 4. Do not allow the eggs to sit for a very long time (no longer than 2-3 h) before the use.

For fertilization procedure see ASSEMBLE-JRA1-Protocol-18.00.

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Animals: Adult sea urchins collected from natural population.

Apparatus: filtered seawater 0,22μ, various size glass beakers, Pasteur pipets and pipet bulbs, Petri dishes, eppendorf tubes, syringes (2 – 5 ml) and needles.

An electrical device (9 – 12 V): two metallic paddles with insulated handles (electrodes) connected to a battery.

Chemicals: potassium chloride (KCl) 0,5M in distilled water.

Parameters: T 20 °C

Additional information:

1. The gonopores (five) are situated on the top of animal (aboral surface)
2. Generally, the ripeness of gonads is directly proportional the easiness of spawning and than inversely proportional to the inducing stimulus.
3. If only small amount of gametes are desired, it is enough to induce the spawning by shaking or electrical shock; to obtain maximal quantities of gametes, it is often necessary to inject the animal which will spawn the entire store of gametes.
4. The spawning induced by shaking and electrical shock enable the sea urchins to recover; injected animals come to death in a short time.

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