

SOP

Echinoderm Reproduction Lab

Purpose:

Describes the procedure for stimulating sand dollars and sea urchins to release gametes.

Policy:

This method is recommended for sand dollars and sea urchins only. High concentrations of KCl (potassium chloride) are highly stressful to animals and an overdose will result in death of the echinoderm.

Responsibility:

Public education assistants, animal care specialist, public education coordinator

Materials:

Large finger bowls or similar sized containers

0.5M KCl

Syringe with 25-gage or smaller needle (multiple needles, one for each animal)

Warm seawater bath (15-18 degrees Celsius)

2 Pasteur pipettes (labeled eggs and sperm)

3 250ml beakers (labeled eggs, sperm, fertilized)

Filtered seawater

Approx. 12 animals

Tote with ice

Procedures:

Note: Slowly warming up echinoderms is the less stressful method of stimulating them to release gametes. However, if this method does not work, injection of low doses of KCl will cause contraction of the gonads and the release of gametes.

1. Place each animal in large finger bowl filled with seawater. Place the finger bowls in large yellow tubs filled with approximately 16-18C water.
2. Leave the sea urchins or sand dollars in the water baths for approximately 4-5 hours.
3. If gametes are released, collect with Pasteur pipette and put into a glass beaker half filled with filtered seawater. Once eggs and sperm are collected into separate

beakers. (Be careful not to mix the eggs and sperm; use separate pipettes for eggs and sperm)

4. If gametes are not released after this period of time, then KCl can be administered. Note that the literature suggests using larger doses of KCl. From past experience, we have observed that administering these higher doses results in death of the animal within 1 to 14 days.

Approximately 0.1ml of KCl can be injected through the mouth into each gonad. Initially, inject only 3 gonads. The injection site of the gonads is approximate based on the animal's body symmetry. Be very careful not to damage tissues of animal by wiggling the needle around. If this amount of KCl still does not work then 0.1 ml place them back into filtered seawater and watch for gametes (male=white, female=yellow),

Note: inverted "dry-spawning" appears to result in higher mortalities.

After the above amount of KCl has been administered, there is no point in administering more, as this will surely cause undue stress and ultimate death. Gametes may be obtained but they will not be viable as they will be immature. You are better off to go on to the next animal instead of over dosing the same one. Some people advise rotating the animal in your hands after injecting the KCl.

5. A third beaker, half filled with filtered seawater, can be used to mix eggs and sperm in. To prevent polyspermy (fertilization of egg by many sperm) use a very small amount of sperm to eggs (maybe one drop of sperm to a pipette full of eggs.)
6. Once gametes have been collected, return the urchins to sea table and freshly flowing seawater. Keep those animals that have been injected with KCl separate from those that have not been spawned.
7. Lab safety is extremely important. Injections should not take place with young students present. It is best to come early and have sperm and eggs available for fertilization when students arrive.
8. Sharps should be disposed of into appropriate containers.
9. KCl should be kept in a fume hood, and clearly labeled. It should not be handled with students present. Contact BMSC staff for disposal problems.