Preparation of sea urchin gametes and development of sea urchin embryos

PREAMBULE. While the following protocol has been developed to prepare gametes and obtain healthy larvae from the purple sea urchin Strongylocentrotus purpuratus, it has been successfully used for other echinoderms such as sea urchins, sea stars and brittle stars.

1. Spawning of sexually mature males and females was induced by intracoelomic injection of 0.5 mM KCl in filtered seawater (FSW).
2. Eggs and sperm were collected from injected animals placed on the top of a glass beaker filled with FSW with their aboral side facing down.
3. Eggs were placed in a 1-L glass beaker filled with FSW under slow stirring, and fertilized with 20 µl of sperm freshly collected at the surface of a spawning male.
4. Zygotes were allowed to divide once before collected and pooled into a 25-ml Falcon tube.
5. Two-cells stage embryos were placed at 14°C into E-flasks (5L) filled with FSW to achieve a density of 5-10 embryos per mL. FSW was continuously aerated to maintain oxygen concentrations close to air saturation and mixed by the slow convective current of a stream of single bubbles (approx. 100 bubbles per min).
6. When reaching the pluteus stage, larvae were fed daily with the cryptophyte algae *Rhodomonas spp.* at 20°C under a 12:12 hour light:dark cycle. Algae concentration and size were checked daily in the experimental bottles with urchin larvae using a coulter counter and adjusted to the maximum concentration of 150 µg carbon L^{-1} (~3000 to 6000 cells mL^{-1} for diameters ranging between 6 and 9 µm). Cultures were maintained at a salinity of 32‰ and a total alkalinity of 2200±30 µmol kg^{-1}.

Attention: Some variation between egg batches from individual females is commonly observed.

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Animals:
Adult sea urchins collected on the Californian coast (Kerckoff Marine Laboratory, California Institute of Technology, USA) were maintained in natural flowing deep sea water at 14°C and fed ad libitum with Ulva lactuca.

Apparatus: Electronic particle analyzer (Elzone 5380, Micrometrics).

Chemicals: KCl analytical grade (Sigma-Aldrich).

Labware: 1-L glass beakers; disposable sterile petri dishes, serologic pipettes, 25-ml tubes, 1-ml syringes equipped with 28-G needles (Falcon, Becton Dickenson)

Algae culture procedures: Rhodomonas spp. (Marine Algal Culture Centre, Gothenburg University) were grown according to Guillard and Ryther (1962). Ulva lactuca collected from the Gullmars fjord were maintained in circulating sea water under constant light.

Additional information:

***Stumpp M, Wren J, Melzner F, Thorndyke MC & Dupont S** (2011) CO_2 induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. Comp Biochem Physiol A: Mol Integr Physiol 160: 320-330.*

***Guillard RRL, Ryther JH** (1962) Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea (Cleve) Gran. Can J Microbiol 8:229-239.*

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