

## Green Sea Urchin Hatchery Protocol

Based on: Eddy, Brown & Harris, 2015. Aquaculture of the Green Sea Urchin *Strongylocentrotus droebachiensis* in North America. Chapter 8 In: Eddy, S., Brown, N. (2015). Echinoderm Aquaculture. Germany: Wiley.

Produced by Sarah Redmond, Springtide Seaweed, LLC. This material is based upon work supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, through the Northeast Sustainable Agriculture Research and Education program under subaward number FNE21-992.

### Spawning:

- **Injection:** Mature urchins are injected into the perivisceral cavity with 2-3mL of 0.5M KCl solution via 3-5 injections around the peristomial membrane. KCl stimulates the gonad wall to contract and eject ripe gametes from the top of the urchin. A syringe and 18-22 gauge needle is inserted angled radially away from the mouth, into the edge of the soft tissue surrounding the mouth on the bottom of the urchin, about 1cm in.
- **Collection:** Injected urchins are inverted over 500mL bowls and allowed to release gametes for 1hr.
- **Fertilization:** Ripe eggs (orange, free flowing, most > 105µm) are mixed from smaller females, or collected from larger females, to collect 30-120mL eggs, added to 4L filtered 10°C seawater. Sperm is diluted by adding 1mL sperm each from 2-3 males into 1L filtered seawater, then 1-2mL of sperm solution is added to the 4L egg solution for 5-10 minutes with occasional stirring.
- **Washing:** Eggs are checked for fertilization under a microscope for developing halo indicating fertilization. Fertilized eggs are washed with seawater through a 105µm screen for 5-10min until water is clear. Only batches with over 75% fertilization are incubated.

### Incubation:

- **Stocking:** Washed fertilized eggs are stocked into flat sided clear acrylic 18 liter hatching cones with 12-14 liters of filtered seawater at densities of 80-120 eggs per mL. Tanks are aerated from the bottom with filtered air to keep eggs in suspension.
- **Incubation:** Eggs are hatched at 9-10°C in 48 hours in incubation tanks. Hatched larvae are rinsed with filtered seawater for 5-10 minutes on a 105µm screen before moving to larval rearing tanks.

### Larval Rearing:

- **Initiation:** Washed hatched eggs are stocked into fiberglass clear conical cultivation tanks (230-350L) at 10-12°C, under a 16L:8D photoperiod. Aeration is supplied from the bottom to keep larvae in suspension. Stocking densities are 4-7 larvae per mL.
- **Water exchange:** Larval tanks can be flow through or static. For flow through systems, a flow of 0.2-0.4 l/min will allow for 1-2 tank exchanges per day. Static systems can be partially exchanged every 2-3 days. All water exchanges must use a 105µm screen for draining with aeration around the screen to prevent larvae from escaping or becoming trapped on the screen.
- **Feeding:** Determine survival to feeding based on the average of three 1mL sample counts. After 2-3 days, with the development of 4 arms, micro algae cultures are added to tanks to imitate feeding. Tanks are fed once per day with micro algae (ex. 5-6 liters of *Dunaliella tertiolecta* and *Rhodomonas salina*), to provide algal cell densities of 20,000-60,000 cells/mL.

### Settlement:

- Urchin settlement: When rudiment is well formed around the center, and the same length of the stomach, urchins are ready to settle. Move larvae to settlement tanks prepared with algae and biofilm for feed. Some micro algae can be added with larvae to settlement tanks. Settlement tanks should have aeration, feed, and clean seawater at 10°C. Flow through or water changes should utilize a screen to prevent escapes. Settlement tanks can include wavy polycarbonate plates, shell hash, or any other three dimensional solid structure that will allow juveniles to settle and begin grazing. Settlement tanks can be prepared before introduction of juveniles with micro stages of macroalgae, and/or larger plants.
- Macroalgal feed: Settling tanks can be prepared with macroalgae several weeks to months before settling urchins. If using kelp, spores can be obtained from reproductive sporophytes through collection of the dark band of sorus tissue. Sorus tissue is cleaned with gentle scraping, wiping, and rinsing, and wrapped in a damp paper towel to gently desiccate 12-24 hours at 10°C. Sorus tissue is submerged into filtered seawater to release spores, which can be introduced to the settlement tank for cultivation. Spores will settle, germinate, and develop into the microscopic gametophyte stage. After two weeks, with enough light, reproduction will take place and juvenile sporophytes will be produced. Both the gametophyte and juvenile sporophyte stages can be utilized as feed for settled urchins. To develop free living tumble cultures of macroalgal feed for urchins, spores can be settled on a surface, then scraped off and placed in aerated culture tanks for further cultivation. Clean filtered seawater, aeration, nutrients, photoperiodic control, lights, and regular water changes are required to cultivation and maintain macroalgal cultures for urchin feed.



Figure 1. Left, orange eggs exuded by female urchin. Right, sperm produced by male urchin.

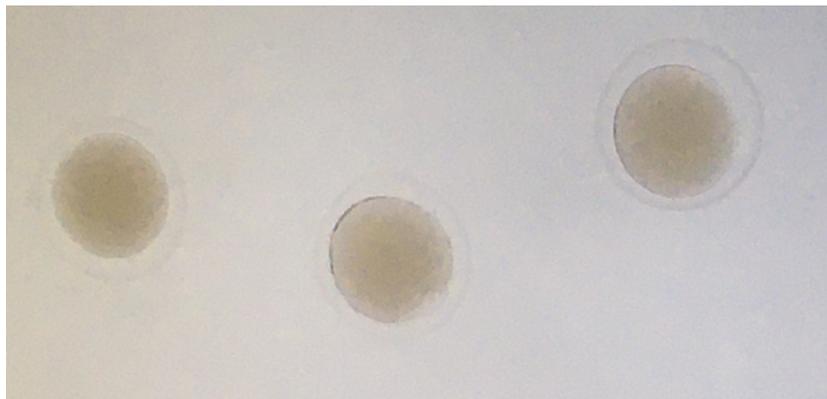


Figure 2. Fertilization envelope halo developing around fertilized eggs.

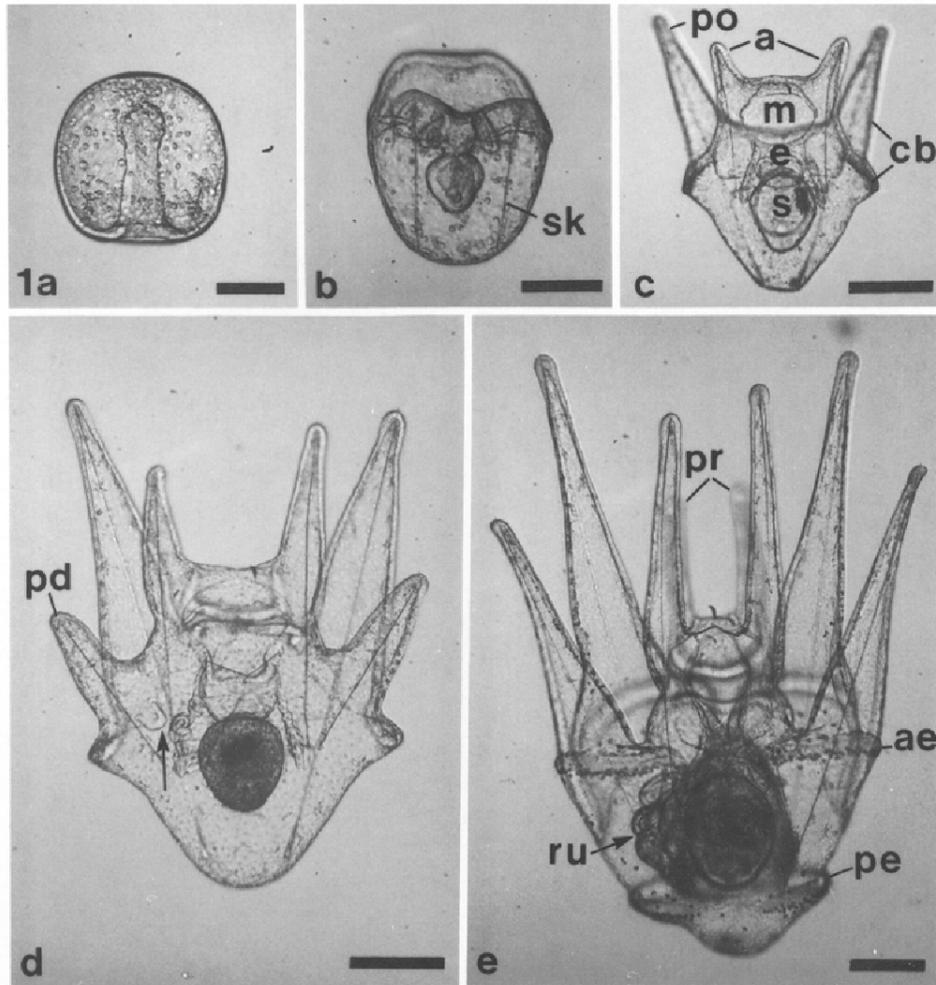


Figure 3. a-e. Developmental stages of *S. droebachiensis*. a 50-h full gastrula. b Ventral view of a 70-h prism ; sk skeleton. c Dorsal view of a 6-day four-arm pluteus; a anterolateral arms; cb ciliary band; e esophagus; m mouth; po post-oral arms; s stomach. d Dorsal view of an 11-day sixarm pluteus; arrow invaginating vestibule; pd posterodorsal arms. e Dorsal view of a 27-day eightarm pluteus; ae anterior epaulette; pe posterior epaulette; pr pre-oral arms; ru adult rudiment, a, b Bars= 100 gm. c-e Bars = 200

From: Bisgrove, B.W., & Burke, R.D. (2004). Development of the nervous system of the pluteus larva of *Strongylocentrotus droebachiensis*. *Cell and Tissue Research*, 248, 335-343.



Figure 4. Sea urchin larvae almost ready to move to settling tanks.



Figure 5. Left, hatching tank. Right, Larval rearing tank with screen for water changes.



Figure 6. Left, a small shell hash and dulse settling tank for urchins. Right, urchins produced after 7 months in settling tanks.

