

# Effect of seawater temperature on sea urchin (*Lytechinus variegatus*) gastrulation

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## Objective:

To test the effects of four seawater temperatures (13, 24, 27, and 30°C) on gastrulation in sea urchin (*Lytechinus variegates*) embryos.

## Background:

Sea urchins are a common indicator organism used in research, quickly showing signs of stress due to slight changes within their habitat (“*Sea Urchin Embryology*”). We will use this property to assess the affect of altering the seawater temperature post-fertilization.

Temperature plays an important role in sea urchin development. Sea urchins of the species *Lytechinus variegatus* are fertile from March to October. Grown in culture, the best temperature for normal growth is around 23°C (Mazur, 1971). This temperature would mimic the temperature embryos would be exposed to in the ocean. *L. variegatus* urchins can be found in the warmer areas of the Western Atlantic Ocean, ranging from North Carolina, southward toward Brazil. The species is rarely found in areas deeper than 50 meters and prefers calm, clear water (Norris, 2002).

Temperature is being studied, as global warming may have dire affects on the sea urchin population. The ocean temperature is not only warming, but the seawater is becoming increasingly acidic as well. In *Heliocidaris erythrogamma*, an increase of an additional 4°C has been shown to decrease the occurrence of cleavage in embryos by 40 percent. Gastrulation is also affected. Normal gastrulation only occurs in four percent of the embryos with a temperature increase of 6°C (Byrne, 2009). The ability to develop in elevated temperatures may be related to the normal environment. Species that live in more consistent environments may be less tolerant of temperature change.

## Procedure:

1. Gametes from healthy adult *Lytechinus variegates* were collected and fertilized following Gamete collection and Fertilization protocol.
  - a. Gamete Collection
    - i. 3 mL of 0.5 M KCl was injected to induce spawning.
    - ii. Using Artificial Sea Water (ASW), eggs were collected in a beaker, washed and stored at room temperature.

- iii. Sperm were stored at 4 °C in a small test tube after collection in a dry petri dish.
- b. Fertilization Protocol
  - i. 5 mL of eggs were allowed to settle in a test tube after being transferred from the beaker. All but 2 mL of ASW were pipetted out.
  - ii. Three drops of sperm were diluted in 5 mL of ASW (diluted sperm) five minutes before use because viability in water is short-term.
  - iii. Diluted sperm were observed with a compound microscope to ensure motility.
  - iv. Eggs were fertilized with 2 drops of diluted sperm.
  - v. ASW was added until the tube was 90% full 5 minutes post-fertilization.
  - vi. A depression slide was used to view 2-3 drops of eggs 10 minutes post-fertilization.
  - vii. Fertilization envelopes were observed.
- 2. Fertilized eggs were divided into four Petri dishes, labeled 13°C, 24°C, 27°C, and 30°C.
- 3. Eggs were placed in incubators according to the temperature they were labeled. The 24°C trial was moved to a secure location within the lab, as it developed at room temperature.
- 4. As embryos developed, photos were taken throughout a 24-hour period.

### Literature Cited:

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