Cultivation techniques for the urchin *Lytechinus variegatus* and potential use of its early developmental stages as larval food

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The marine aquaculture industry is diversifying to fill the need for food, ornamentals and byproducts created by consumer demand or by collapsing fishery stocks that are occurring globally. Properly executed, aquaculture is environmentally sound and can relieve or supplant natural fish stocks. As new species are determined to be commercially viable or needed for stock enhancement programs, new techniques for larval rearing will be needed to improve survivability. Often these new culture techniques will come from variations on standard aquaculture methods or by incorporating systems from cross-disciplines. Further research is also needed to determine optimal food for larval stages of current and future species of targeted marine aquaculture organisms. To advance these goals, new larval food sources and techniques for culture must be explored. Different systems and methods tested and modified for sea urchin culture are described here.

Paddle System

The sea urchin, *Lytechinus variegatus*, was chosen to establish protocols for future research on this and other classes of echinoderms with similar larval stages and commercial viability for the marine aquaculture industry. The paddle system (Strathmann 1987) used here is a common technique for the culture of sea urchins in research facilities, but it has potential for use as a replacement for airlift systems for many types of marine organisms that are adversely affected by high-volume air bubbles used for aeration and/or excess water motion. The water movement produced in this paddle system, as modified by Wolcott, is random and multi-directional as opposed to the persistent circular movement produced by airlift systems. The movement in the paddle system promotes thorough mixing of both larvae and food, while producing a mild surface agitation that should increase gas exchange relative to surface area. In contrast, the air bubbles in an airlift system attract proteinaceous waste and concentrate this waste at the air/water interface, reducing surface gas exchange.

The paddle aeration system (Figure 1), modeled after that of Strathmann (1987), consisted of a frame constructed of 1.27 cm PVC pipe, mounted on a 61 cm^2 sheet of 1.27 cm plywood.

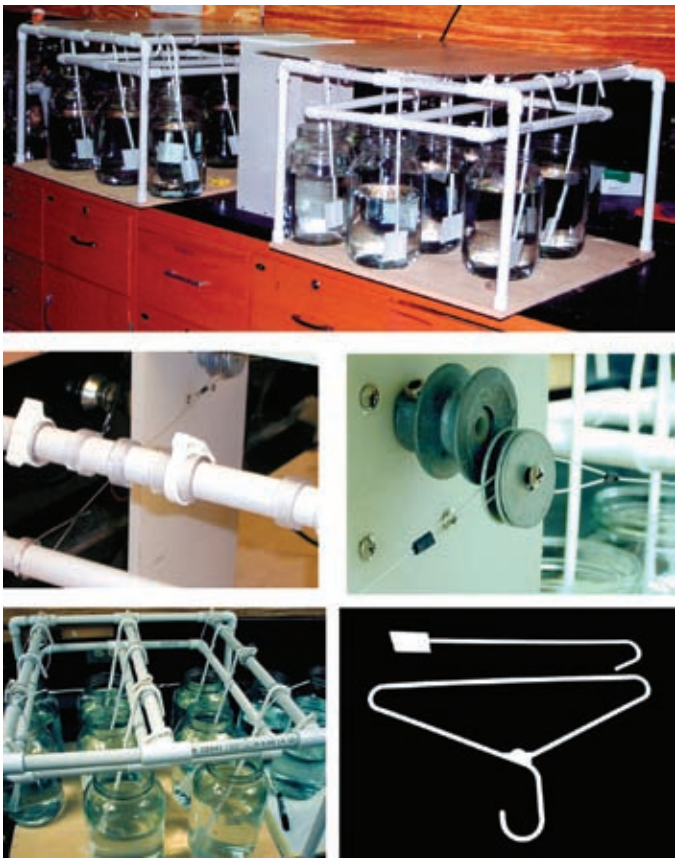

Fig. 1. Top: Paddle system. Middle left: Monofilament line attaching lower (swing) bar to offset pulley. Middle right: Close-up of offset pulley. Bottom left: Overhead view. Bottom right: Plastic hanger section used to create the paddle (upper) with the addition of sheet plastic.

Fig. 2. Lytechinus variegatus. *Top left: several color variations of adults. Top right: oral view showing KC1 injection area (arrow). Middle left: aboral view of egg release. Middle right: aboral view of sperm release. Bottom left: fertilized eggs showing induction ring. Bottom right: four-armed larval stage, blue area is a line on a sheet of writing paper.*

Table 1. Expected time post-fertilization for *Lytechinum variegatus* development at 24°C using *Rhodomonas lens* as a food source starting at the pluteus stage.

Developmental Stage	Time Post-Fertilization
2-cell	42 min.
4-cell	70 min.
8-cell	100 min.
16-cell	114 min.[a]
32-cell	147 min.[a]
64-cell	180 min.[a]
Blastula	270 min.
Gastrula	570 min.[a]
Prism	815 min.[a]
Pluteus	1025 min.[a]
2-Arm	1 day
4-Arm	2 days
6- Arm	5 days
8-Arm	7 days
Rudiment	8 days
Settled	9 days

[a] data from Amy (1983)

Paddles were fashioned from 5 cm^2 white plastic squares, plastic coat hangers and aquarium sealant. A movable rack, designed to move the paddles, was suspended from the frame by cable ties. A 5 rpm electric instrument motor[2] was fitted with a 5.1 cm pulley wheel. A small hole was drilled near the outer edge of the outer lip of the pulley wheel. A metal screw, wrapped with Teflon threading tape to smooth the surface, was attached through the hole to make a camshaft. Monofilament line was connected from the camshaft to the movable frame to produce the motion that drove the paddles. The rhythm (motor rpm) of the paddle system produces 1-2 seconds of mechanical induced movement followed by 10 seconds of diminishing motion. This calming period permits the organism to properly orient itself and feed effectively.

Urchin Culture Techniques

Sexually mature (5-9 cm) *Lytechinus variegatus* (Figure 2) were collected from near-shore seagrass beds around Virginia Key in Biscayne Bay (Miami, Florida USA) during July, 2001. Spawning was induced by injection of potassium chloride (3 ml at 0.55 M)[3] through the peristomial membrane and into the perivisceral cavity. The eggs from a single female were rinsed three times with carbon filtered and UV sterilized offshore seawater, used throughout this research, to remove debris and organisms, allowing the eggs to settle (within 30 minutes after each rinse). Sperm from a single male was collected dry and kept refrigerated until egg rinsing was complete. Four drops of sperm were placed in 200 ml of sterile seawater to activate the sperm, stirred and debris was allowed to settle (less than 15 minutes). The eggs in 500 ml of sterile seawater and 10 ml of the suspended sperm were placed in a 1 l beaker and stirred. When the eggs had settled, as much water as possible was removed and the eggs were rinsed twice to prevent polyspermy. The eggs were placed in a 20 cm diameter glass dish, to prevent egg layering. The eggs were covered with 3 cm of sterile seawater and the dish was covered with a paper towel, allowing aeration but not water motion or airborne contamination.

Free-swimming blastulas were present in the water column within 24 hours (see accompanying table) and these were poured into a 3.8 l glass jar containing sterile seawater to a total volume of 3 l. Blastulas were added to 16, 3.8 l jars (four replicates per algal species) each containing 3 l sterile seawater, at a concentration of 600 blastulas/l. The alga *Micromonas pusilla* was added at a concentration of $3x10^4$ cells/ml and at $1x10^4$ cells/ml for the other three algae (*Pavlova pinguis, Porphyridium cruentum*, and *Rhodomonas lens)*. Those cell levels were maintained throughout larval development. Every two to three days, 75 percent of the water was siphoned out (125 micrometer mesh) and the jars were re-filled with sterile seawater to a total of 3 l/jar. After settlement, the juveniles were removed from the jars by first removing all water, and then they were stimulated to release their hold on the glass by exposing them to a gentle stream of seawater, after which they were moved to the growout system (Figure 3).

Growout Methods

A custom acrylic aquarium (Figure 3) 112 x 19 x 19 cm was

set up with 3.8 cm of surface substrate from Virginia Key and planted with the seagrass, *Thalassia testudinum,* and the alga *Halimeda* sp. from the same area. Water circulated from one end to the other, with supplemental oxygen from an air bar. Light was supplied by two 112 cm cool white fluorescent bulbs with a photoperiod of 12:12 hours (on and off). After four months, the substrate and plants were removed and commercial feed from Wenger Manufacturing[4], was used exclusively to maintain growth.

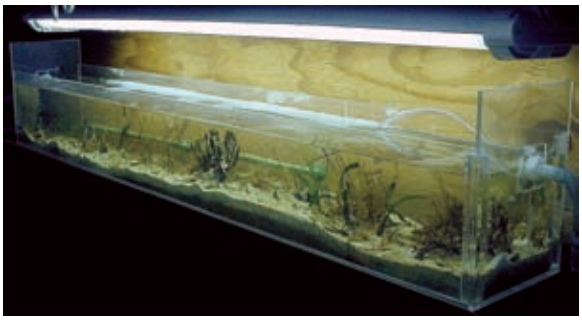

Fig. 3. Grow-out system for Lytechinus variegatus *post-larvae.*

Algae Feeding

Four algal species (Figure 4), rarely or never before used in marine aquaculture (*Micromonas pusilla*, *Pavlova pinguis, Porphyridium cruentum*, and *Rhodomonas lens*) were tested in this research. Those species were selected as genera or representatives of classes (divisions) because they possess unique and/or abundant fatty acids, polysaccharides and vitamins. Nine days after fertilization, larvae feed *Rhodomonas lens* had either developed a rudiment or had settled and begun metamorphosis. The experiment was discontinued at that time and all 16 replicates were examined to determine urchin larval development stages for each algal species. Larvae fed *Pavlova pinguis* had reached the fully developed pluteus stage. Those fed *Porphyridium cruentum* were at the eight-armed pluteus stage. Larvae fed *Micromonas pusilla* were at the six-armed or early eight-armed stages. At the algal concentrations used in this research, *Rhodomonas lens* produced superior results; however, these results may be correlated with algal cell volume, *Rhodomonas lens* largest to *Micromonas pusilla* smallest. Further research would be necessary to determine if an inadequate nutritional profile, and/or total available food biomass, may have been contributing factors in these results.

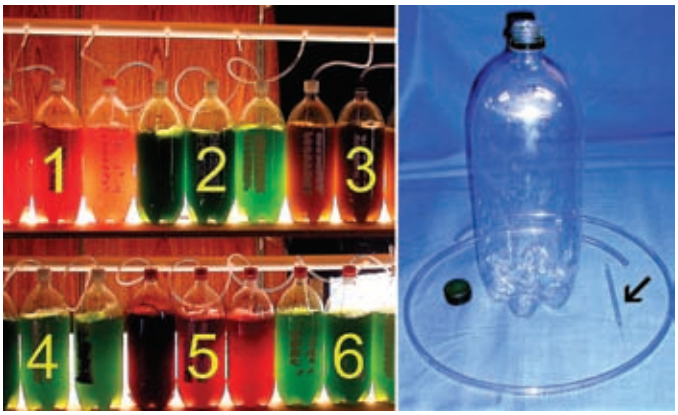

Fig. 4. Algal system. Left: Algal species shown: 1. Rhodomonas lens*, 2.* Micromonas pusilla*, 3.* Pavlova pinguis*, 4.* Nannochloris *sp., 5.* Porphyridium cruentum *and 6.* Nannochloropsis *sp. Right: Culture unit parts (note Pasteur pippette used as airstone replacement).*

Stock and Broodstock Maintenance

Natural conditions for *Lytechinus variegatus* are (Lawrence 2001):

1. Shallow water with a temperature range of 11 – 35°C
2. Low to full sunlight (covering has been noted in bright light)
3. Sexual maturity at 40 mm (1year)
4. 3-4 year life span
5. Gonochoric
6. Year-round spawning (influenced by temperature)
7. Up to 636 indiduals/m^2 (sea urchin front)

These conditions can be maintained with standard aquaculture techniques. Furthermore, with the development of commercial feeds for *L. variegatus* (Lawrence *et al*. 2001), it is now possible to maintain broodstock in captivity year round, without depending on a constant supply of natural food.

Conclusions

The present research showed that *Lytechinus variegates*, and probably other species, can be successfully cultured using a simplified paddle system and novel algae species. It was realized that the copious urchin eggs and larval stages can be valuable as food for other organisms. With the ability to maintain broodstock and induce spawning year round there is the potential to use unfertilized eggs, fertilized eggs and various urchin larval stages as food for various larval stages of other aquacultured organisms. Typical egg (through gastrula stage) size for *L. variegatus* is about 106 μm depending on female size and food profile (George *et al*. 2001), comparable to the more commonly used instar 1 phase of the calanoid copepod *Acartia* spp. (Schipp *et al*. 1999) and the rotifer *Brachionus rotundiformis* (135 μm; Fielder *et al*. 2000). Variations in egg size are found between genus and species of urchins, such as *Strongylocentrotus purpuratus* 80μm, *Dendraster excentricus* 125μm, and *Strongylocentrotus droebachiensis* 150 μm (McEdward and Herrera 1999), presenting the possibility to match urchin species to the targeted organism. Both the blastula and gastrula stages of *L. variegatus* are motile (by means of cilia) and may be a suitable replacement or supplement for copepod nauplii or rotifers if the nutritional profile is suitable for the target organism. Production of fertilized urchin eggs and subsequent stages should be less costly and more reliable than copepod nauplii. Additionally, a large female *L. variegatus* can produce nearly 3 million eggs (George *et al*. 2001).

At the two arm stage of *L. variegatus* the larvae have a body length of about 210 μm (McEdward and Herrera 1999), which is comparable to *Artemia* instar 1 about 250 μm (Tucker 1998), *Brachionus plicatilis* (260 μm; Fielder *et al*. 2000), and *Acartia* spp. nauplius instar 6 (250 μm; Schipp *et al*. 1999).

Further development of larvae of *L. variegatus* stops at the 4 arm larval stage in the absence of food. Although larvae survive without food for six days, they stop growing and developing at day two (McEdward and Herrera 1999). If the size range from egg to 4 arm larval stages is appropriate for the targeted species, then algal cultures and their resultant problems could be eliminated. Research is also needed on potential uptake by urchin larvae of Super SelcoÒ and similar enrichments that are currently used with rotifers and *Artemia* (Sorgeloos *et al*. 2001).

Notes

[1]Nova Southeastern University Oceanographic Center, Dania Beach, Florida 33004 USA Hubbard's Fish Anatomy: http://fishanatomy.net. E-mail: rlh@fishanatomy.net or larviculture@hotmail.com

[2]Hurst Manufacturing Division of Emerson Electric Company, model PA.

[3]The use of 3 ml of potassium chloride at 0.55 M resulted in a death rate of about 25 percent in injected urchins within one week, the rate of 1-2 ml of potassium chloride at 0.55 M, depending on adult size (George *et al*. 2001), should increase survivability.

[4] Wenger Manufacturing, Kansas City, Mo. U.S.A.

Acknowledgments

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