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Mass production of competent larvae of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea)

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Abstract. We evaluated the mass production of competent larvae of the sea urchin *Lytechinus variegatus* cultured at three initial densities (0.25, 0.5, and 1 larvae per ml) and fed *Chaetoceros muelleri*. Survival, length, dry weight of larvae, and larval stage index (LSI) were estimated in each treatment as a function of the density. Density decreased during the experiment due to mortality, but the percentage was similar in all three treatments (68.5, 66.7, and 76.0%). The experiment was stopped at 13 days after fertilization, when most of the larvae were competent and had settled. There were no significant differences in survival (exceeded 65% in all treatments), length and larval stage index among treatments. However, larvae weight in the two low density treatments (1.1 ± 0.11 mg and 1.2 ± 0.05 mg, respectively) was greater than the high density treatment (0.59 ± 0.376 mg). This study demonstrates that competent larvae of *Lytechinus variegatus* can be produced with less than 25% mortality in 13 days when cultures are started at densities of 0.25–1 larvae/ml. Culturing at higher densities (0.5–1 larvae/ml) had no apparent disadvantages and would reduce the cost of production.

Introduction

Sea urchins are part of the fishing industry in many countries around the world. Their gonads are a valuable seafood product in Asian and European markets, where they are often sold at more than 100/kg at wholesale markets (McBride 2001). The global catch of sea urchins has declined during recent years, though it remains high due to its increased fishing effort and the high global demand. The highest catch in 2002 was in Japan (12455 m) and the USA (6006 m) (FAO 2000). The high exploitation rate has endangered urchin resources in many countries, and has created an interest in the developing cultivation techniques (Bustos and Olave, 2001; Grosjean et al., 1998; Kelly et al., 2000).

The sea urchin, *Lytechinus variegatus* (Lamarck), is widely distributed throughout the western Atlantic and Caribbean Seas, from North Carolina, USA, to Rio Grande del Sur, Brazil (Moore et al. 1963). The low abundance of this species can only support a small fishing industry. Fishing occurs in Atlantic tropical zones, e.g., around the islands of Margarita and Coche, northeastern Venezuela, where the gonads are smoked prior to consumption (Gómez 2002).

Lytechinus variegatus grows rapidly, and attains sexual maturity and a test diameter of 40–50 mm within a year (Watts et al. 2001). Adults are easily cultivated by feeding them formulated diets (Klinger et al. 1994; George et al. 2001).

Studies of larval development for *L. variegatus* have previously been conducted using small volumes, e.g., Petri dishes (Hinegardner 1969), and mid-sized volumes, 1–4-l jars (McEdward and Herrera 1999; George et al. 2001), to elucidate morphometric changes in successive larval stages, and to examine how larval quality is affected by nutrition. The present study extends this work by exploring techniques for the massive production of competent *L. variegatus* larvae under controlled conditions.

Materials and methods

Collection of organisms, spawning, and fertilization

Adult sea urchins, *L. variegatus*, were collected by diving in the Punta de Piedras lagoon on Margarita Island, Venezuela (10 °54′ 27″ N and 64 °06′ 29″ W) during August 2002 when a high proportion of the animals are typically fully mature (Gómez 2002). The sea urchins were fed *ad libitum* with macroalgae of the genus *Ulva* over 8 days in a 4000-l tank at the Department of Aquaculture, Marine Research Station (EDIMAR) of the La Salle Foundation for Natural Sciences (FLASA) on Margarita Island, Venezuela. This tank contained seawater that had passed through a 20-μm filter.

Following this acclimatization period, 30 adults of 53.4 mm (SD = 4.81) diameter and 45.6 g (SD = 8.31) weight were induced to spawn by injecting 1–3 ml of 0.5-M KCl through their peristomal membrane and into their haemocoel. Individual sea urchins were kept within 250 ml beakers for the collection of gametes. Eggs from all urchins were pooled for fertilization in a 50 l container with 40% seawater that had passed through a 1-μm filter and had been sterilized with ultraviolet light. We mixed one volume of sperm to 30 volumes of eggs and attained a fertilization success of 98%.

Culture

After 24 h, the embryos were collected with a 60-μm sieve and placed in 10 l of seawater (1 μm filtered and UV treated). Five subsamples of 1 ml each were

counted in a Sedwick-Rafter chamber using an optical microscope. Cultures at three different densities (0.25, 0.5, and 1 larva/ml) were then established, and each density was replicated three times. All nine treatments were cultivated in 100 l cylindrical fiberglass tanks, at a temperature of 27.5°C (SD = 0.6) and gently aerated from the bottom (200–400 ml/min). Larvae culture were static with a water exchange rate of 50% perday.

The larvae were fed the microalgae *Chaetoceros muelleri* (GGB) which is routinely stocked by the phytoplankton laboratory EDIMAR/FLASA. The microalgae were cultivated within a discontinuous system using seawater that had been sterilized by filtration and enriched with Walne medium.

Cellular density was estimated using a Neubauer camera. Following the recommendations for *Loxechinus albus* culture (Bustos and Olave 2001), the tanks were provided with 20,000 cells/ml for larvae with two or four arms; 40,000 cells/ml for larvae with six arms; 60,000 cells/ml for larvae with eight arms and for competent larvae. Feeding occurred every 12 h, so as to maintain a stable density of microalgae, ensuring that an excess of food was constantly available for the larvae at any density.

Culture assessment

To evaluate the effects of initial culture density we quantified survival, the time required to reach different larval stages, and increases in length and mass. A 100 ml sample was extracted daily from each replicate and concentrated using a 60-μm sieve. The number of sieved larvae was counted and the stage of each larvae was recorded photographically.

Larval development was evaluated using the larval stage index (LSI) of Maddox and Manzi (1976), where LSI = (number of larvae in each stage)* (stage number)/(total number of larvae sampled). Moreover, on every third day a sample of ten larvae from each replicate was extracted to measure larval length, (the combined length of the body and longest arm). At the end of the experiment, after 13 days of cultivation, all larvae of each replicate were harvested using a 200-μm sieve and 30 larvae were selected at random and weighed. They were separated by filtration in order to avoid rupturing and washed in a solution of 3% ammonium formate to remove adhered seawater. The larvae were transferred to tared weighing paper and heated to 60°C for 24 h, before determining their dry weight.

Statistical analysis

Variations in maximum length (from the tips of the larval arms to apex), in dry weight, and survival were evaluated using the non-parametric analysis of Kruskal–Wallis ($p < 0.05$), followed by multiple comparisons among treatments (larval densities) as described by Siegel and Castellan (1988).

Results

Twenty females and ten males spawned. The females produced 24.2 (SE = 15.82) eggs per gram of body mass, equivalent to 1.1* 10^6 eggs per female. After 24 h, the harvested equinopluteus larvae had an initial average length of 286.6 (SE = 22.13) μm. Total length increased similarly for larvae in the densities of 0.25 and 0.5 larvae/ml (Figure 1). Larvae at a density of 1 larvae/ml exhibited the greatest variability of growth throughout the study, but by the end of the experiment, their lengths were similar to those at lesser densities ($p < 0.1$). The average length was 628.1 (SE = 38.71) μm, which compares with 632 (34.3) μm for the low density and 639 (29.6) μm for the intermediate density.

There were no significant differences in the LSI among treatments (Figure 2). The proportions of larvae in the different larval stages were similar among treatments for each day of the experiment (Table 1). However, in the last two

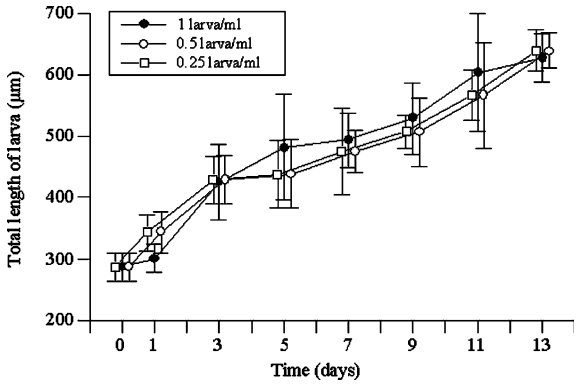

Figure 1. Total lengths of *Lytechinus variegatus* larvae in densities of 0.25, 0.5, and 1 larva/ml. Vertical lines record the statistical error with respect to the mean (n = 30 per treatment).

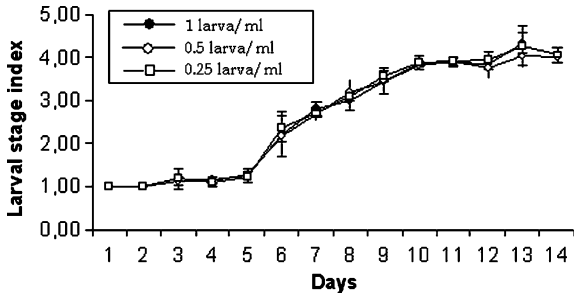

Figure 2. Larval Stage Index (LSI) of *Lytechinus variegatus*. Vertical lines record the statistical error with respect to the mean.

Table 1. Proportions of larvae in the different larval stages during the time of the experiment. Values are mean percentage and the standard deviation. Pictures of larvae in each larval stage are shown below. 2pl = two arm larvae. 4pl = four arm larvae, 6pl = six arm larvae, 8pl = eight arms larvae, 8r = competent larvae.

Level stage	Lar/ml	Days					
		1	3	5	7	9	12
2pl	1	100.0 ± 0.0	85.6 ± 7.7	28.2 ± 20.6	6.2 ± 5.1		
	0.5	100.0 ± 0.0	89.2 ± 6.4	27.2 ± 16.0	2.3 ± 5.6		
	0.25	100.0 ± 0.0	92.1 ± 7.1	17.6 ± 11.2			
4 pl	1		14.4 ± 7.7	29.4 ± 13.1	23.5 ± 21.1	6.0 ± 12.5	
	0.5		10.8 ± 6.4	26.9 ± 11.7	8.6 ± 7.8		
	0.25		7.9 ± 7.1	29.8 ± 10.6	7.5 ± 10.0	1.7 ± 2.7	
6 pl	1			42.4 ± 32.7	50.8 ± 16.9	13.8 ± 2.9	8.8 ± 8.7
	0.5			45.9 ± 25.7	57.2 ± 20.7	19.3 ± 9.7	13.6 ± 12.9
	0.25			52.6 ± 19.2	74.8 ± 13.0	9.0 ± 10.1	4.2 ± 10.2
8 pl	1				19.6 ± 13.5	80.3 ± 14.2	49.6 ± 30.3
	0.5				32.0 ± 21.6	80.7 ± 9.7	69.2 ± 26.4
	0.25				17.6 ± 5.2	89.3 ± 12.0	65.3 ± 27.4
8r	1						41.6 ± 26.1
	0.5						17.2 ± 24.6
	0.25						30.6 ± 40.0

days, there is a trend towards faster development in the treatment containing larvae at densities of 1 larvae/ml (Table 1).

In contrast to larval length, the dry weight measured at the end of the experiment did vary with initial density ($p < 0.001$). The average weight of the larvae from the low and medium density treatments (1.2 SE = 0.05 and 1.1 SE = 0.11 mg respectively) was about 50% greater than in the high density treatment (0.6 (SE = 0.38) mg) (Figure 3).

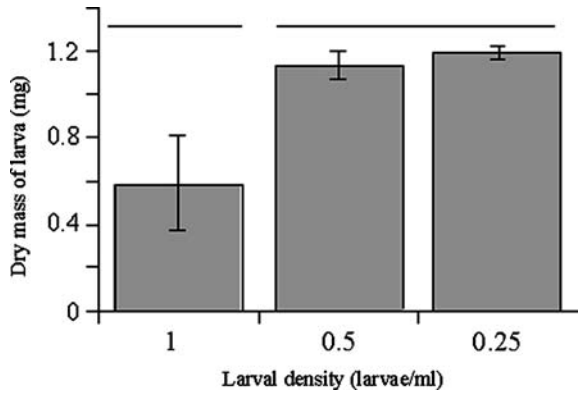

Figure 3. Dry weight of the *L. variegatus* larvae at the end of the experiment.

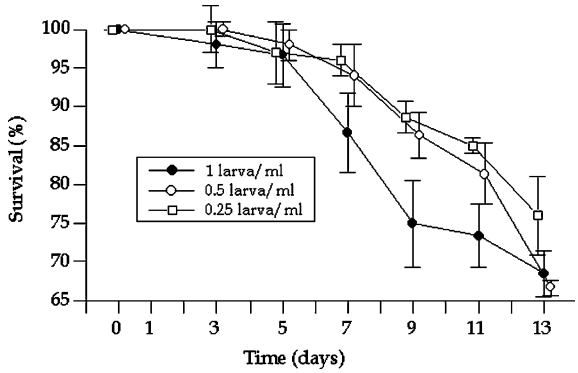

Figure 4. Survival rate of *Lytechinus variegatus* through the term of the experiment.

Survival rate showed little variation among the different densities up to day 5 (Figure 4). Thereafter, survival of larvae in the 1 larva/ml density treatment decreased markedly until day 9 but did not decrease significantly between days 9 and 1 day 13 ($p < 0.1$), when it was 68.5 (SE = 7.61). For the other two densities, the survival rate began to decrease on day 7 and continued to decrease until the end of the experiment (day 13) with a final value of 76.0 (SE = 1.13) for the low density treatment and 66.7 (SE = 3.12) for the intermediate density treatment. At the end of the experiment, there were no significant differences among the three treatments ($p < 0.05$).

Discussion

Our study demonstrates that competent larvae of *L. variegatus* can be produced in 12–13 days with a survival rate of 60–76%, when embryos are cultured at a density of 0.25–1 larvae/ml in 100 l tanks. Previous work has shown that *L. variegatus* might reach the metamorphic stage in a shorter interval (9–10 days) if cultivated at a lower density (0.19 larvae/ml) and smaller volume (1.6 l) by feeding them *Dunaliella tertiolecta* and *Rhodomonas lens* (McEdward and Herrera 1999). The larvae in our study reached a smaller length (633 μm compared to 1252 μm) than those cultivated by McEdward and Herrera (1999). Similarly, George et al. (2001) produced competent larvae at a culture density of 0.13 larvae/ml in 3.75 l jars within 9–13 days, depending upon the amount of *Dunaliella tertiolecta* being fed to them. The lower density used in these studies, 2–8 times lower than used in the present study, may have affected the final size of, and the time taken to acquire competent larvae. Alternatively, the different microalgae used as feed in our studies may have affected the larval development of *L. variegatus*.

McEdward and Herrera (1999); George et al. (2001) and our study, all produced larvae of *L. variegatus* which showed a large variation in size at the

competent stage. This plasticity should be studied further in order to see all size classes will show equal survivorship at metamorphosis.

Although these studies obtained competent larvae two days earlier than our study, the culture densities would be too low for commercial production. For example, the equivalent production in 100 l culture containers would be approximately 713 larvae, which is 24 times less than in our study. Similar low production would be obtained from extrapolations of other studies where embryos were cultivated at low densities, e.g., Cameron and Hinegardner (1974) who used larval densities of 0.066 larvae/ml to cultivate two species of sea urchin (*Lytechinus pictus* and *Arbacia punctulata*) to metamorphosis. González et al. (1987) used a density of 0.05 larvae/ml to cultivate larvae of the red Chilean sea urchin, *Loxechinus albus*, by feeding them various microalgae within 5 l containers. Standard cultivation of *L. albus* in 500 l tanks involves initial densities of 1.5–1.6 larvae/ml and results in survivorship of just 40% (Bustos and Olave 2001), well below that of the present study.

The high survival rate of *L. variegatus* (70%) in our study shows that this urchin can tolerate the high culture densities that would be needed for commercial culture. Experiments are needed to see if juveniles can also be cultured at high densities. In our trials, survival was largely independent of the environment until day 5 when most larvae had developed four arms. This represents a critical point in larval development, as at this stage the larvae begin to feed externally rather than using internal reserves. Prior to this point, there were no differences in either arm length or the larval stage, Index so external influences must have been minor compared to endogenetic factors (stored energy).

The substantial decline in the survival in the high density treatment (1 larva/ml) after day 5 (estimated density of 0.87 larvae/ml) was possibly due to limited space. It is possible the larvae in this treatment were subjected to more mechanical abrasion than were those in the low density treatments. This was suggested because more larvae in the 1 larvae/ml treatment had arms that were slightly bent compared to those in the other treatments. This could have limited their acquisition of food. Such a spatial limitation would be unlikely within the first 24 h, as the arms are not developed up to this stage.

At the end of the experiment treatments showed differences in weight only. Weight was lower in the high density treatment, suggesting that food availability was lower in this treatment because all treatments were fed at the same rate and frequency. The larvae with higher weight (from treatments of 0.25 and 0.5 larvae/ml) may have had a better physiological condition, suggesting a better chance of success at metamorphosis.

The present study demonstrates that mass production and high survival (70%) of competent larvae of *Lytechinus variegatus* is feasible within 12–13 days using initial densities of 0.25–1 larvae/ml. Future work should address the optimization of larval density densities and continuing the experiment into the juvenile phase. In addition, we recommend further experiments to test

different microalgal diets, as better diets may reduce the time needed to achieve metamorphosis, and thereby maximize the production of juveniles.

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