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A Comparison of Diets and Water Agitation Methods for Larval Culture of the Edible Sea Urchin, Tripneustes ventricosus (Echinodermata: Echinoidea)

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A COMPARISON OF DIETS AND WATER AGITATION METHODS FOR LARVAL CULTURE OF THE EDIBLE SEA URCHIN, *TRIPNEUSTES VENTRICOSUS* (ECHINODERMATA: ECHINOIDEA)

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ABSTRACT

Tripneustes ventricosus (Lamarck, 1816) has been harvested for human consumption in the Caribbean for centuries, where harvest rates occasionally exceed sustainability. Historically a backreef and grass-bed urchin, the species has recently been observed on the forereef where it appears to control macroalgal growth in the absence of *Diadema antillarum* (Philippi, 1845) (Woodley and Gayle, 1999). Large-scale culturing has the potential to produce *T. ventricosus* in sufficient numbers for remediation of degraded coral reefs, restocking of nearshore habitats, and development of an aquaculture industry for one or more Caribbean islands. We report the first successful culturing of *T. ventricosus* from fertilization to exotrophic juvenile and the results of experiments to measure the effectiveness of agitation methods and diets applicable to large-scale larval culture. Airlift agitation was not effective in the 3.78-L (1-gal) jars used here. Cultures were successfully reared without mechanical agitation, but paddle agitation, used successfully in many small-scale experimental designs, produced the highest survival rates. Of the five algal diets tested, *Rhodomonas* sp., and a mixture of *Rhodomonas* Karsten and *Isochrysis* Parke, produced the most rapid development (23 d to metamorphosis). *Isochrysis* aff. *galbana* (Tahitian strain) supported slower development (36 d to metamorphosis) but produced the highest (48%) survival rate.

Tripneustes Agassiz (1841), a genus of sea urchin found throughout the tropics and sub-tropics, includes two extant species: *Tripneustes gratilla* (Linnaeus, 1758) from the Pacific and *Tripneustes ventricosus* (Lamarck, 1816) in the Atlantic and Caribbean Sea (Lawrence, 2001). *Tripneustes ventricosus* occurs in a variety of habitats, including *Thalassia testudinum* Banks & Soland. ex Koenig beds (Moore et al., 1963), coral rubble (Scheibling and Mladenov, 1987), rocks and rock ledges (McPherson, 1965), and inner platform reefs (Cameron, 1986), where it opportunistically feeds on macroalgae (McPherson, 1965; Cameron, 1986; Scheibling and Mladenov, 1987). However, following the mid-1980s epizootic that killed 95% of long-spined sea urchins, *Diadema antillarum* (Philippi, 1845), a major grazer of reef macroalgae throughout the tropical western Atlantic (Liddell and Ohlhorst, 1986; Foster, 1987; Hughes et al., 1987; Levitan, 1988; Carpenter, 1990), *T. ventricosus* invaded the forereef at Discovery Bay, Jamaica, and greatly reduced abundances of fleshy macroalgae above 10 m (Woodley and Gayle, 1999). Haley and Solandt (2001) reported that at Discovery Bay, the density of *T. ventricosus* increased dramatically from 1995 to 1998 and then declined as *Diadema* recovered significantly, leading them to suggest that grazing by *T. ventricosus* may serve a vital role in reef recovery and that biological succession occurs as recovering *Diadema* stocks replace *T. ventricosus* on the forereef.

Tripneustes ventricosus is harvested at several eastern Caribbean islands, including Barbados, St. Lucia, and Martinique (Smith and Berkes, 1991). The species is subject to periodic population declines resulting from, or at least exacerbated by, over-harvesting (Scheibling and Mladenov, 1987).

No documentation exists on the successful culturing of *T. ventricosus* from fertilization through metamorphosis to exotrophic (feeding) juvenile. Pearse and Cameron (1991) reported that both Mortensen (1921) and Lewis (1958) cultured larvae through metamorphosis. However, Mortensen (1921: 32) stated: "We have a fully formed larva, which is about to begin its metamorphosis…The metamorphosis of this larva could not be followed, as the sojourn at Tobago ended by this time." Lewis (1958) only estimated time to metamorphosis from a series of larvae collected from the plankton and reared for short periods. Cameron (1986) stated that he reared *T. ventricosus* through metamorphosis but only cited Lewis' (1958) estimate. Scheibling and Mladenov (1987) stated that techniques were being developed and suggested that culturing could solve the over-harvesting problem, but they did not mention any culturing success.

Although *T. ventricosus* is not commercially reared, Lawrence and Bazhin (1998) concluded that it would be appropriate for aquaculture because it grows faster to a larger size and reaches sexual maturity sooner than other urchins. Large-scale culturing of *T. ventricosus* is thus worth investigating toward at least three uses: (1) remediation of coral reefs overgrown by macroalgae following the *Diadema* mass mortality (Stimson, 2002), (2) rehabilitation of over-harvested populations (Scheibling and Mladenov 1987), and (3) as a food source and associated industry (Lawrence and Bazhin, 1998) for several Caribbean nations. Hagen (1996) also indicated that it might prove profitable.

Given the potential for commercial aquaculture of this species, we compare two methods of water agitation and four diets for culturing *T. ventricosus* from fertilization through metamorphosis to an exotrophic juvenile stage.

Methods and Materials

Raw seawater was filtered to remove particulate matter and organisms larger than 0.5 μm across, sterilized with ultraviolet light, and stored in Nalgene® carboys for no more than 1 wk. All experiments were conducted in an air-conditioned laboratory, and temperature was regulated by the room's thermostat. The initial experiment was conducted at 23 °C after Eckert (1998), the second at 23.5 °C, and the final experiment at 24.0–24.5 °C.

Tripneustes ventricosus is capable of reproducing year-round in southeast Florida, although breeding, determined from gonad volume, seems to peak in May and December (McPherson, 1965). Adults were collected at various times from November 2000 through November 2001 offshore of Broward County, Florida.

Prior to spawning, adults were rinsed in sterile seawater (SSW) to remove as much debris, algae, and other organisms as possible. Spawning was induced by intracoelomic injection of 3 ml of 0.55 M KCl. Sperm were collected dry (without added SSW), covered, and kept refrigerated prior to activation. Eggs were collected in a 500-ml beaker containing 200 ml SSW, and then washed twice in SSW by decanting and replacing SSW (Strathmann, 1987).

Five to six drops of sperm were diluted in 200 ml SSW and allowed to stand for 5–10 min for activation immediately prior to fertilization. Fertilization was initiated by introducing several drops of diluted sperm to a 500-ml beaker containing the eggs of one female suspended in approximately 200 ml of SSW (modified from Strathmann, 1987). Fertilization was confirmed under ocular microscopy by the presence of a fertilization membrane. The fertilized eggs were then placed in a culture dish (13-cm diameter) in a density sufficient to produce a monolayer of embryos covered by approximately 3 cm SSW (Amy, 1983).

Each of the three experiments described here consisted of 3–5 treatments —each treatment a different combination of algal diet and water agitation method— with four replicates

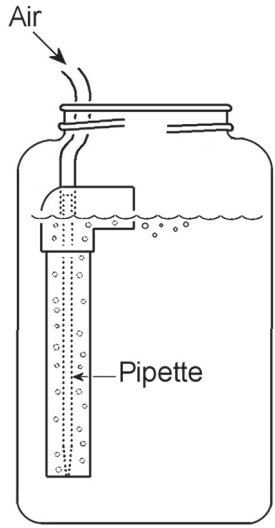

Figure 1. Airlift system. A clamp (not shown) holds the pipe against the rim of the culture jar.

of each treatment. Each replicate consisted of a 1-gal (3.8-L) culture jar filled with 3 L SSW plus approximately 1800 larvae (0.6 larvae ml^{-1}) that had reached the blastula stage (6–7 hrs post-fertilization). The jars were placed under one of two water agitation systems except for those not agitated during the final experiment. The fluorescent ceiling lights remained on 24 hrs d^{-1}, and all jars were covered with a translucent plastic sheet suspended approximately 10 cm above the tops of the jars to reduce accumulation of dust and other debris. SSW in the culture jars was changed every 2–3 d by reverse siphon. Culture jars were replaced every other time SSW was changed.

Two methods of mechanically agitating the culture water were used: airlift and paddles. English and metric equivalents are given below because materials were purchased using the former system of measurement. The airlift method was recommended (B. Baca, CSA South, Inc., Dania Beach, Florida, pers. comm.) as an effective means of maintaining larvae and algae in suspension. Flow tubes (Fig. 1) were constructed using 1-in (2.54-cm) diameter PVC pipe capped with a 90° elbow. An air tube was inserted through a 3⁄16-in (0.48-cm) diameter hole drilled in the elbow and was fitted with a glass pipette with the pipette tip flush with the bottom of the PVC pipe. The flow tubes were fastened to the inside of the culture jars.

The paddle agitation system (Fig. 2), modeled after that of Strathmann (1987), consisted of a 0.5-in (1.27-cm) PVC pipe frame, mounted on a 2-ft^2 (61-cm^2) sheet of 0.5-in (1.27-cm) plywood. Paddles were fashioned from 5-cm^2 white plastic squares, plastic coat hangers, and aquarium sealant. A movable carriage was suspended from the frame by cable ties. A 5-rpm electric motor was fitted with a 2-in (5.08-cm) pulley wheel modified to make a camshaft. Monofilament line run from the camshaft to the movable carriage produced the back and forth motion that drove the paddles. During the final experiment, four culture jars were not agitated except for that resulting from the thrice-weekly water changes and the daily addition of algal food.

Algal starter cultures were obtained from UTEX, The Culture Collection of Algae at the University of Texas at Austin, and grown in the laboratory. We used *Rhodomonas* sp. (UTEX no. 2163), *Cryptomonas* sp. (no. 2458) (both Cryptophyta); *Isochrysis* aff. *galbana* Parke (no. 2307) (Haptophyta), and *Dunaliella tertiolecta* Butcher (no. 999) (Chlorophyta). These are abbreviated below as *Rho, Cry, Iso*, and *Dun*, respectively. SSW for culturing algae was heat Pasteurized before use. Stock f/2 solutions of culture medium were prepared to industry specifications (Guillard and Ryther, 1962; Guillard, 1975). However, a higher concentration

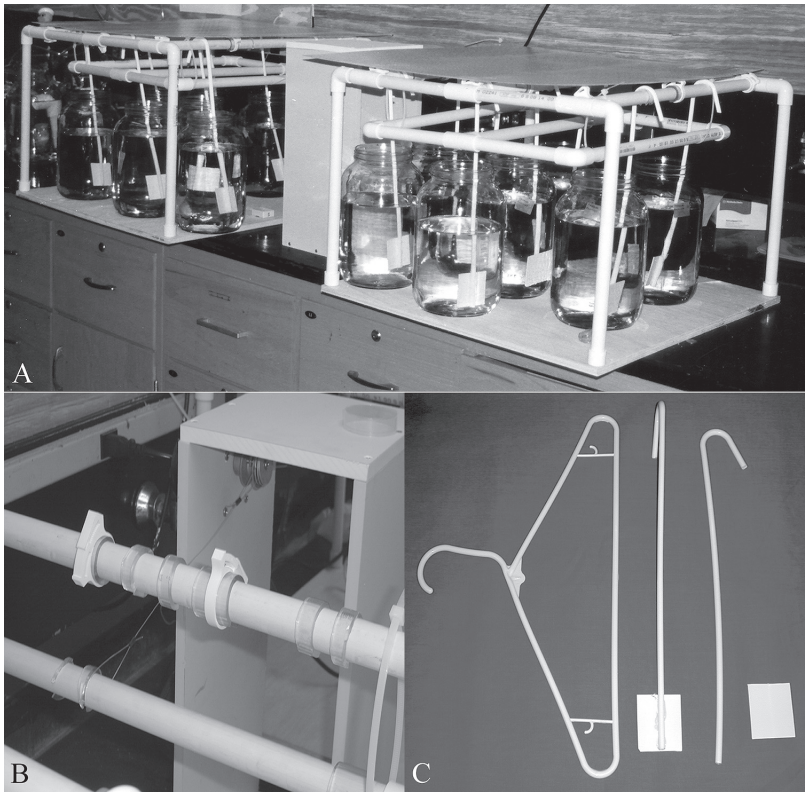

Figure 2. (A) Paddle-agitation system, (B) close up of motor stand with cam and monofilament attached to moveable carriage that drives paddle motion, and (C) paddles —constructed by gluing white plastic squares to portions of plastic coat hangers with aquarium sealant.

(f/1) of the major nutrients and vitamins was required to develop the normal red color in the cryptomonads.

Prior to each feeding, algae were poured into 50-ml plastic centrifuge tubes and centrifuged at 2000 rpm to separate cells from the culture medium. Cell density was determined by ocular microscopy using a hemacytometer. Cultures were fed daily. Density was 10 cells μl^{-1} for the initial feeding and was reduced to 7.5 cells ml^{-1} to avoid overfeeding.

Culture experiment 1 consisted of five treatments. One was airlift-agitated and fed 50% *Iso* and 50% *Rho* (*Iso/Rho*). The remaining four were paddle-agitated and fed as follows: *Rho*, *Iso/Rho*, *Iso/Dun*, and *Cry*. Culture experiment 2 consisted of three diet treatments, all paddle-agitated: *Rho*, *Iso/Rho*, and *Cry*. *Iso/Dun* was eliminated because, although this diet sustained larvae for 11 d in experiment 1 and 22 d during a failed culture preceding experiment 2, growth and development was slow in comparison with the three diets reported here. Culture experiment 3 consisted of four treatments. Three were paddle-agitated: one 100% *Iso*, one *Iso/Rho*, and one 100% *Rho*. The fourth was fed *Iso*, but received no mechanical agitation.

In experiment 3, one-way analyses of variance (ANOVA) (Sokal and Rohlf, 1998) were used to test for differences in developmental stages at day 20 and numbers of larvae surviving to settlement time among the three feeding regimens and between the two agitation methods. Experiments 1 and 2 produced no results requiring statistical analysis.

In all cases, larvae with rudiments transferred to dissecting dishes in anticipation of metamorphosis were accompanied by microscope slides that had been permitted to accumulate natural biofilms (e.g., diatoms and other microorganisms) by immersing them in a 1200-L tank with seawater circulation open to the adjacent Intracoastal Waterway.

Results

Culture Experiment 1.—Results are shown in Table 1. All larvae in the airlift culture jars died by day 8. Microscopic examination revealed that dead larvae were physically damaged (although the damage may have been post-mortem). Larval survival among the paddle-agitated treatments varied by diet. No larvae fed either *Iso/Dun* or *Cry* survived to day 11. All larvae in three of the four replicate jars fed *Iso/Rho* died by day 11; all larvae in one of the four jars fed *Rho* died by day 21, and all in another jar died by day 31. However, larvae in the two remaining *Rho* jars and one of the remaining *Iso/Rho* jars survived to day 39, but all died by day 42. Photographs (Fig. 3A) of larvae at day 18 confirm that a rudiment had developed on at least one animal fed *Rho*.

By day 12, flocculent material appeared in the water column and on the bottom of culture jars receiving *Rho* and *Iso/Rho*. The density of flocculent material increased over time and was accompanied by unidentified ciliates, which attacked dead and dying larvae.

Culture Experiment 2.—Results are shown in Table 2. Flocculent material and ciliates appeared again in cultures fed *Rho* and *Cry* and, to a lesser extent, *Iso/Rho*. Water changes initially suspended the flocculent material, but most of it settled to the bottom of the jars and remained there despite the paddle-generated water movement. On day 12, examination of culture water under a compound microscope revealed live *Rho*, an unidentified ciliate, live and dead larvae, and flocculent material, which blanketed both the larvae and the bottom of the petri dish. On day 13, three of the four cultures fed *Rho*, one *Iso/Rho* culture, and two of the four *Cry* cultures contained no living larvae. All larvae in the remaining *Cry* cultures were dead by day 25 and all had substantial amounts of flocculent material in the culture water.

On day 18, specimens taken from the remaining cultures (three *Iso/Rho* and one *Rho*) had reached the 8-arm stage and appeared to have a developing rudiment. Twelve from the *Iso/Rho* culture with more advanced rudiments were placed in a dish containing a biofilm-covered microscope slide. These larvae were continued on the *Iso/Rho* diet and placed under a constant slow drip of SSW to maintain water quality.

The first live product of metamorphosis, identified on day 23, did not resemble a typical juvenile urchin. Under magnification, it appeared as a ball of tissue with five tube feet and a few small aboral protrusions (Fig 3B). Due to its fanciful resemblance to a small pig, this stage will be referred to as an echinoporculus. Four or 5 d later, spines appeared and the animal took on the appearance of a juvenile urchin (Fig.

Table 1. Culture experiment 1. Age-at-death of *Tripneustes ventricosus* larvae by agitation method and diet. Numbers represent days following fertilization when no animals remained alive (observations made every 2–3 d). Diets were as follows: 50% *Isochrysis* and 50% *Rhodomonas* (*Iso/Rho*), *Rhodomonas (Rho),* 50% *Isochrysis* and 50% *Dunaliella* (*Iso/Dun*), and *Cryptomonas (Cry)*.

Agitation method	Algal diet	Rep 1	Rep 2	Rep 3	Rep 4
Airlift	*Iso/Rho*	8	8	8	8
Paddle	*Rho*	42	42	32	21
Paddle	*Iso/Rho*	42	11	11	11
Paddle	*Iso/Dun*	11	11	11	11
Paddle	*Cry*	11	11	11	11

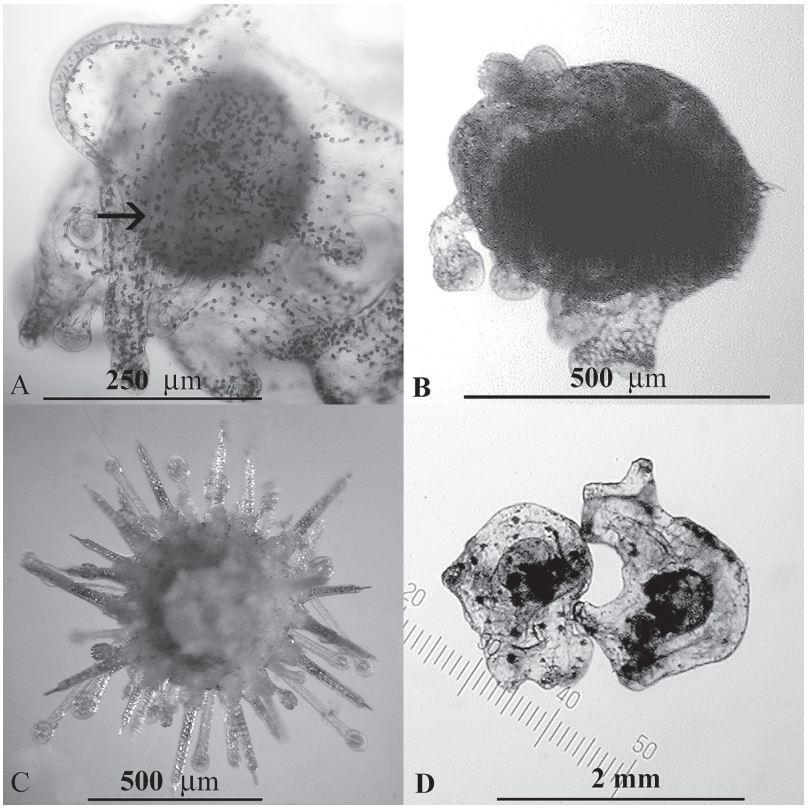

Figure 3. Larval and juvenile stages of *Tripneustes ventricosus*. (A) Mature larva with rudiment (arrow), (B) echinoporculus, (C) juvenile form following echinoporculus, and (D) misshapen larvae.

3C). On day 25, water in the remaining four culture jars (three *Iso/Rho* and one *Rho)* was reduced to ~100 ml and siphoned into petri dishes 10 ml at a time. The larvae remaining alive were counted and placed in dissecting dishes under the drip system.

By day 27, six echinoporculi and two normal juveniles were identified. By day 34, no more postlarvae had appeared, but four were juveniles and two remained in the echinoporculus stage (two had died). On day 35, test diameters of the juveniles and echinoporculi (measured using an ocular micrometer) were 0.46–0.58 and 0.39–0.47 mm, respectively. The two remaining echinoporculi became juveniles by day 40. One juvenile died on day 47. On day 54, test diameters of the remaining five juveniles ranged from 0.46 to 0.53 mm.

Culture Experiment 3.—Results are shown in Table 3. The flocculent material appeared again in both *Iso/Rho*-fed and *Rho*-fed cultures, but its density was greatest

Table 2. Culture Experiment 2. Age-at-death of *Tripneustes ventricosus* larvae by diet. All cultures used paddles as agitation method. Numbers represent days following fertilization when no animals remained alive. Figures in () are numbers of live larvae removed at day 25. Abbreviations as in Table 2.

Algal diet	Rep 1	Rep 2	Rep 3	Rep 4
Iso/Rho	25 (~325)	25 (60)	25 (~325)	13
Rho	13	13	25 (~75)	13
Cry	13	25	13	25

Table 3. Culture experiment 3. Larval development of *Tripneustes ventricosus* at day 20 following fertilization, by agitation method and diet. Numbers are percentages of larvae identified at each stage. Figures in () are total numbers of larvae at each stage from all four replicates combined. Larval pedicellaria appear prior to settlement. Abbreviations as in Table 2.

	Agitation method and algal diet			
Developmental stage	None *Iso*	Paddle *Iso*	Paddle *Iso/Rho*	Paddle *Rho*
2-arm	4.2 (1)		2.6 (1)	
4-arm	70.8 (17)	20.5 (15)		
6-arm	25.0 (6)	31.5 (23)	2.6 (1)	
8-arm		34.2 (25)	35.9 (14)	
Rudiment		1.4 (1)	41.0 (16)	100 (2)
Rudiment and pedicellaria		8.2 (6)	10.3 (4)	
Misshapen		4.1 (3)	7.7 (3)	
Total	(24)	(73)	(39)	(2)

in the 100% *Rho* cultures. Although ciliates were present in the *Iso*-fed cultures, their density appeared to be much lower than in other cultures (although none were quantified), and they were generally not as dense as in experiments 1 and 2. Misshapen larvae, typically without developed arms and/or similar in shape to degenerating larvae (Fig. 3D) and noted in all experiments, were present in samples taken on day 20 from paddle-aerated cultures fed *Iso* and *Iso/Rho* (Table 3).

Larval survival and development differed by diet and by agitation method (Tables 3 and 4). At day 20, culture water in each jar was reduced to ~750 ml, and a 10-ml sample was taken from each, totaling 40 ml for each treatment. The live larvae in each sample were counted and their developmental stages noted (Table 3). Twenty-four live larvae were found in the four samples (40 ml total) taken from the non-agitated *Iso* replicates; none had reached the 8-armed stage. The paddle-agitated samples fed *Iso* contained approximately three times as many live larvae (73); 43.8% of these had reached the 8-armed stage, including those that had also developed pedicellariae or rudiments. A paired comparison t-test of survival rates between these two agitation methods showed a significant difference ($P < 0.001$). Thirty-nine live larvae were found in the samples taken from the cultures fed *Iso/Rho*; 87.2% had reached the 8-arm stage, including 41.0% with a rudiment and 10.3% with a rudiment and pedicellariae. Only two live larvae were found in the four *Rho*-fed samples; both had reached the 8-armed stage and had rudiments.

Metamorphosis began on day 23 in experiment 2. On day 20, in anticipation of similar timing for experiment 3, larvae in the paddle-agitated *Rho*-fed jars were removed, counted, and placed in settlement dishes containing biofilm-covered microscope slides (Table 4). Slower development was anticipated for the paddle-agitated *Iso/Rho*- and *Iso*-fed cultures: they were removed from the culture jars, counted, and placed in biofilm-containing settlement dishes on day 23 and 28, respectively (Table 4). Larvae in non-agitated jars were counted and placed in dishes with biofilm-covered slides on day 33 to encourage metamorphosis prior to the experiment's termination on day 37 (Table 4).

A one-way ANOVA found significant differences in percent survival between treatments ($P < 0.001$). A Student-Newman-Keuls multiple range post-hoc test with a significance level of 0.05 indicated that significantly greater numbers of larvae survived to settlement in paddle-agitated cultures fed *Iso* relative to the other three treatments.

Table 4. Culture experiment 3. Number of *Tripneustes ventricosus* (larvae, echinoporculi, and juveniles) surviving at approximate settlement day (days following fertilization). All replicates initially contained 1,800 larvae. [1]Includes 61 echinoporculi. [2]Includes eight echinoporculi and four juveniles. Abbreviations as in Table 2.

		Agitation method and algal diet			
Settlement day	Rep	None *Isochrysis*	Paddle *Isochrysis*	Paddle *Iso/Rho*	Paddle *Rho*
Day 33	1	312			
	2	246			
	3	274			
	4	257			
Day 28	1		1,008		
	2		886		
	3		821		
	4		758		
Day 23	1			377 [1]	
	2			176 [2]	
	3			9	
	4			1	
Day 20	1				96
	2				13
	3				4
	4				1

The date metamorphosis began differed by treatment method: day 23 for *Rho*- and *Iso/Rho*-fed cultures and day 36 for paddle-agitated *Iso*-fed cultures. Metamorphosis was not observed in the non-agitated cultures because all remaining larvae, juveniles, and echinoporculi from all cultures were transferred to aquaria (in which individuals could not be observed) on day 37.

On days 24–26, 30 echinoporculi were placed in a dissecting dish to monitor their subsequent development. Over an 8-d period, 12 of the 30 eventually produced visible spines and developed the typical juvenile form. In the *Rho* and *Iso/Rho* cultures, the frequency of new echinoporculi decreased as metamorphosis progressed among the larvae. No echinoporculi were noted among the *Iso*-fed metamorphosed larvae.

Post-Experimental Observations.—At the conclusion of experiment 3, all settled animals and larvae with competent rudiments were transferred to three 122 × 15 × 20 cm aquaria containing substrate harvested from a nearshore environment and inoculated with *Iso* and *Rho*. After 60 d, 31 juveniles with test diameters of 2–4 mm were transferred to an another 122 × 15 × 20 cm aquarium without substrate, weaned using benthic diatoms and fed prepared feed manufactured by Wenger Manufacturing, Inc. Nineteen surviving juveniles with test diameters of 14–17 mm were released into the nearshore environment 6 mo post-fertilization.

Discussion

These experiments produced the first exotrophic juveniles from cultures that started with the fertilization of *T. ventricosus* eggs. Amy (1983) thoroughly documented the early, endotrophic development of *T. ventricosus* larvae. Lewis (1958) documented the exotrophic larval stages, though he never cultured the larvae from

fertilization to metamorphosis and did not recognize the echinoporculus as a viable stage in the perimetamorphic process.

Culture Temperature.—*Tripneustes ventricosus* larval development appears to be temperature sensitive. Mortality during the early larval period increases as the temperature deviates from 25 °C and reaches 100% at 20 and 30 °C (Cameron et al., 1985). Our initial experiment was conducted at 23 °C, the ambient temperature in the laboratory. As warmer weather arrived, the temperature near the culture jars increased to 23.5 ± 0.5 °C for the second experiment. The final experiment was run at 24–24.5 °C. As a result, the increasing temperature in the laboratory may have been a contributing factor to improved survival rates and development times recorded over the course of the three experiments. However, such variations do not account for the significantly greater numbers of larvae that survived to settlement in paddle-agitated cultures fed *Iso* relative to the other three treatments run simultaneously in the final experiment.

A failed fertilization attempt and an experiment preceding experiment 2 using adults collected from water > 30 °C suggest that temperature may also affect gamete viability. McPherson (1965) observed *T. ventricosus* sperm and eggs in Florida during each month of the year and concluded that spawning occurs throughout the year. The spawning results obtained during the 13 mo of this project support McPherson's (1965) conclusions. However, viability of the spawn product may be temperature-limited.

Agitation Methods.—Water movement is generated in echinoderm larval cultures by mechanical agitation via paddles or by a flow of air bubbles. Both function in maintaining larvae in suspension. The latter method, generated either by an exposed airstone or bubbler, or with the bubble stream constrained within a pipe as an airlift, may also aerate the culture. Echinoderm larvae have also been cultured successfully without mechanical agitation. Onoda (1936) successfully cultured several species in 200-ml containers; Chen and Run (1988) cultured *T. gratilla* at 25 °C in 500-ml beakers without agitation and obtained metamorphosis on day 30 with a biofilm stimulus and on day 47 spontaneously. Juinio-Menez et al. (1998) also cultured *T. gratilla* at 25–26 °C in 3-L glass jars without agitation and obtained settlement in 42 d.

Echinoderm larvae are usually cultured for scientific studies in 100–4000-ml containers with agitation via paddles or similar mechanical devices (Cameron and Hinegardner, 1974; Strathmann, 1987; Eckert, 1998). Aeration, however, is a standard aquaculture (that is, larger commercial-scale) agitation method. In Japan, the largest producer of cultured sea urchin "seed" (J. Lawrence, University of South Florida, pers. comm.), larvae are typically cultured in 1000-L tanks with one airstone at the bottom of the tank and one near the surface providing a gentle circulation (Hagen, 1996). Chilean urchin culture uses the same methods (Bustos and Olave, 2001). Grosjean et al. (1998) described a land-based, closed-cycle system used in France, in which echinoderm larvae are cultured in 200-L tanks with a central bubbler. The Dalian Fisheries University, Peoples Republic of China, routinely cultures urchin larvae in 60-L containers with a central bubbler (pers. obs., 2002).

Aeration is thus a viable agitation method in larger containers. The failure of airlift during this project may be related to the apparently stronger stream of bubbles constrained by the pipe relative to the smaller size of the culture jars. The airlift method should be reexamined for *T. ventricosus* larval culture in larger container sizes.

Experiment 3 indicates that *T. ventricosus* will develop at least to the 6-armed pluteus stage without agitation. However, when larvae are paddle-agitated, the survival rate is significantly higher and development is more rapid. Microscopic examination of larval development showed that none of the larvae cultured without mechanical agitation and fed *Iso* reached the 8-armed stage by day 20, while 43.8% of those subjected to paddle agitation and fed *Iso* had reached that stage.

Algal Diet.—Tissue was resorbed from the arms of larvae fed *Iso/Dun* in the first experiment, suggesting that the larvae were not receiving enough nourishment. As a result, the frequency of feeding was increased from thrice weekly to every day. To keep algal density below a level at which larval cilia might clog (G. L. Eckert, Univ. Alaska Southeast, pers. comm.), concentrations were reduced from 10 to 7.5 cells μl^{-1} following the initial feeding. Tissue resorption was not noticed in subsequent experiments and the larvae continued to feed until metamorphosis.

Survival and development rates during the experiments varied among feeding regimens, suggesting that the nutritional content differed among algal species. However, no consensus appears to exist regarding the relative importance to echinoderm larvae of the various macromolecules, as well as monomers, minerals, and vitamins, identified in these algae (Boidron-Metairon, 1988; J. Lawrence, pers. comm.). George et al. (2000) reported that lipids affect the nutritional value of microalgae and that lipid content of the diet correlates positively with faster development and better survival of echinoderm larvae.

The fatty acid composition of lipids is also important to growth and development of marine invertebrate larvae (Jeffrey et al., 1994). Enright et al. (1986) reported that diets deficient in fatty acids 20:5(n3) and 22:6(n3) result in poor growth of larval oysters. Brown (1991) stated that green algae, such as *Dunaliella*, are deficient in both, perhaps explaining the relative poor performance of *T. ventricosus* larvae fed this alga. Cryptomonads, including *Rhodomonas* and *Cryptomonas*, contain a relatively high total lipid level and have a very high proportion of 22:6(n3) (Enright et al., 1986). *Isochrysis* aff. g*albana* has a lipid content similar to that of the cryptophytes and has a high concentration of 22:6(n3), although the percentage appears to differ by species strain and culture technique (Jeffrey et al., 1994). These authors concluded that: "Species of *Isochrysis* … are favored [as a feed for larvae in aquaculture] because of their small size, fast growth rates, wide temperature ranges, absence of tough cell wall, and absence of toxins that could affect either the animal or the human consumer" (p. 301).

Cell wall structure may also play a role in survival of larvae fed *Rhodomonas* and *Cryptomonas*. While *Isochrysis* lacks a tough cell wall, the crytomonads not only have a double-layered periplast —a layer of proteinaceous scales beneath the cell surface (Leadbeater and Green, 1993)— but also contain ejectosomes (Bold and Wynne, 1978). To the extent that all or a portion of the periplast is not digestible by the *T. ventricosus* larvae, the flocculent material that accumulated in the culture jars may have consisted of an aggregation of millions of these scales (perhaps accompanied by bacteria and microfungi) that blanketed the larvae and prevented them from swimming freely. The ejectosomes may also have irritated the external and digestive surfaces of the larvae, perhaps contributing to their death. However, this second hypothesis is not consistent with the findings of Eckert (1998) on *D. antillarum* (R. A. Cameron, California Inst. Technol., pers. comm.) on echinoderms in general, or Hubbard et al. (2003) on *Lytechinus variegatus* (Lamarck, 1816). In all of these cases,

Rhodomonas performed well as a larval food. In the results reported here, the *Rho* and *Iso/Rho* diets resulted in more rapid growth and development compared with *Iso*. However, the *Iso* diet produced significantly higher survival rates compared with the other two diets. *Isochrysis* is thus a suitable feed for culturing *T. ventricosus*.

Ciliates.—Ciliates were noted in all replicates of each experiment and were probably introduced into the cultures during sperm and egg collection. All culture seawater was filtered to 0.5 mm and sterilized with UV light. Algal culture seawater was further heat pasteurized. Ciliate concentrations tended to be greater in *Rho* or *Cry* cultures. The lowest concentrations were observed among the 100% *Iso*-fed cultures. We do not believe ciliate concentrations had a negative impact on larval nutrition or rate of development because, had that been the case, 100% *Rho*-fed and *Iso/Rho*-fed cultures in experiments 2 and 3 should have developed at a slower rate than 100% *Iso*-fed cultures.

Perimetamorphic Period.—Although the term metamorphosis generally refers to the change in structure from larval to juvenile form, identification of the commencement and conclusion of the period has varied in the literature. Mortensen (1921) terminated his experiment after a larval rudiment appeared, but later stated that the larva had been reared to metamorphosis (Mortensen, 1943). Lewis (1958) considered larvae with a rudiment to be metamorphosing though still swimming actively in the water column. R.A. Cameron (pers. comm.) explained that in the 1980s he had limited the term metamorphosis to the rapid change in form from larva to juvenile after settlement as opposed to the lengthier period implied by Mortensen and Lewis. However, Cameron (1986) quoted Lewis (1958) when stating that *T. ventricosus* metamorphose in about 28 d, yet Lewis clearly referred to larvae swimming in the water column. In Experiments 2 and 3, paddle-agitated larvae fed *Iso/Rho* and *Rho* (Cameron's preferred diet), became juveniles and echinoporculi in 23 d.

Gosselin and Jangoux (1998) used Cameron's definition in discussing the onset of metamorphosis in *Paracentrotus lividus* (Lamarck). However, to accommodate the many changes that occur at this stage, they coined the term perimetamorphic period, which begins when the rudiment appears and ends when the juvenile becomes exotrophic. The term may be more appropriate for describing the transition to exotrophic juvenile in *T. ventricosus*.

During experiments 2 and 3, the form referred to as the echinoporculus frequently preceded the juvenile. Echinoporculi bear short spines visible under light microscopy, indicating that at least part of the juvenile structure is present. Gosselin and Jangoux (1998) described a post-metamorphic form in *P. lividus* similar to the echinoporculus, and Hubbard et al. (2003) observed the same form in *L. variegatus.* The possibility exists that immature eggs released by the 0.55 M KCl used to induce spawning may have developed into this as an aberrant form. However, Lewis (1958: 617) observed the same form, which he called a "degenerate metamorphosing larva," among larvae culled from plankton samples not derived from KCl-induced laboratory spawning. No other references to this life history stage have been found. Further research will be needed, including examination under SEM, to gain a better understanding of the developmental processes that occur in the echinoporculus stage.

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