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SPAWNING, LARVAL REARING AND EARLY GROWTH OF HIPPOPUS HIPPOPUS (LINN.) (BIVALVIA: TRIDACNIDAE)

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The results of rearing laboratory-spawned eggs and larvae of the giant clam Hippopus hippopus (Linn.) in the laboratory are described. Veliger larvae reared at stocking densities of 1.2 to 5/ml and fed with the unicellular algae Isochrysis galbana and Tetraselmis sp. developed into 3-4-month-old juveniles. The survival rates of 3-4-month-old juveniles from veligers ranged from 0.03 to 2.13%. For successful mariculture, larval mortality rates must be reduced.

Tridacnid clams are presently the subject of mariculture efforts in a number of laboratories in the Indo-Pacific region, primarily for the purpose of preventing their extinction and augmenting existing stocks for food (see Munro and Gwyther, 1981; Fitt et al., 1984; Heslinga et al., 1984). Hippopus hippopus, one of the seven extant species of tridacnid clams in the Philippines (Rosewater, 1965, 1982), is being studied for possible culture for its adductor muscles and mantle as food and for its shell as a decorative item.

Hippopus hippopus is hermaphroditic and has been induced to spawn in the laboratory most consistently by the introduction of freshly macerated or freeze-dried gonad (Wada, 1954; Jameson, 1976; Gwyther and Munro, 1981; Fitt et al., 1984) and by the intragonadal injection of serotonin (Braley, 1985; Crawford et al., in press). Fitt et al. (1984) studied the early development from fertilized eggs to the veliger stage and Jameson (1976), from fertilized eggs to the 58-day juvenile stage.

The present paper deals with the spawning, larval rearing and early growth of Hippopus hippopus. Our study is part of the research program on the culture of giant clams for restocking of coral reefs participated in by James Cook University, Australia, the Fisheries Division, Fiji Ministry of Primary Industries, the University of Papua New Guinea, Port Moresby, the University of the Philippines Marine Science Institute and the Silliman University Marine Laboratory, Dumaguete City, Philippines.

MATERIALS AND METHODS

Broodstock.

Mature-sized Hippopus hippopus (18-23.3 cm long) wer collected from Sumilon Island, near Cebu Island, in October 198 and August 1985, the Cagayan Islands, Sulu Sea in April 1985 ar Campuyo, Manjuyod, Negros Oriental in August and November 1985 The clams were held in a laboratory tank 2m long x 1m wide x 0.5 deep, and provided with unfiltered sea water for a few days t six months. Water temperatures in the holding tank fluctuate between 27 and 34° C, and salinity between 31 and 33 ppt.

Spawning.

Only broodstock clams with more than 50% mature eggs wer used in the spawning experiments. Mature eggs (spherical in shap under the microscope) were removed from the gonads with a huma biopsy needle, following the method of Crawford et al. (i press). The clams were induced to spawn by either pouring 10-20 ml of macerated gonad material into the water or injecting int the gonad 1-4 ml of 2mm serotonin (crystalline serotonin [5 hydroxytryptamine, creatine sulfate complex]) dissolved i filtered sea water. The serotonin solution was used immediatel after preparation or stored at -4°C before use. In some instance serotonin injection was followed by the addition of gona material. The experimental clams (usually two per experiment were induced to spawn in 60-l glass aquaria containing filtere sea water, mostly in the afternoon between 1400 and 1700 h.

The induced clams were allowed to release sperm until th water was dense with it. They were then repeatedly transferre to new aquaria, following the method of Jameson (1976), unti they spawned eggs. This procedure was intended to separate th sperm from the eggs. The egg-water mixture was gently aerated t disperse the eggs evenly in the aquarium.

To fertilize the eggs, 100 ml of sperm suspension from th first aquarium of the other clam was added to the egg-wate mixture. This procedure ensured cross fertilization and mini mized polyspermy.

For spontaneous spawnings, the eggs or fertilized eggs wer scooped with a bucket or filter bag (70 µm mesh size) from th holding tank and transferred to 60-l aquaria filled with filtere seawater.

To estimate the density and number of eggs and the number o fertilized eggs, the volumetric technique of Castagna an Kraeuter (1984), widely used in bivalve mariculture, wa followed. The eggs and larvae were measured under th microscope using an ocular micrometer.

arval rearing.

The fertilized eggs were allowed to develop in aerated aquaria to the veliger stage. Samples of the larvae were taken daily for stage determination, size measurement and density determination. Size measurements were expressed in means ± standard deviation and range. Density was estimated from ten 1-ml samples, following the method of Castagna and Kraeuter (1984). Two liters of unicellular algal culture (mixture of Isochrysis galbana and Tetraselmis sp. at a density of 10^5 cells/ml) were added once to the aquaria as food for the veligers. Water temperature remained between 25 and 28.5 °C, and salinity, 31 to 34 ppt.

When most of the larvae (>50%) reached the pediveliger stage, they were transferred to 1m x 2m x 0.5m tanks with 450 liters of filtered seawater. Pediveligers from the three spawnings were stocked at different densities: 4/ml, 3/ml and 1/ml. The rearing tanks were supplied with fresh filtered seawater at a rate of 156.5 - 194.6 l/min for at least 8 hours per day. Five liters of mixed Isochrysis galbana and Tetraselmis culture (10^5 cells/ml) were added to each tank every other day. Transparent plastic roofing excluded rain from the tanks.

The substrate for larval settlement in the three tanks differed. One had a few pieces of coral rubble and stones; the second had coral fragments, pebbles and stones occupying about 50% of the bottom surface; the third had plastic matting. The bottom of the second tank was divided into five equal areas of 1 m^2, four of which contained different substrates: coral fragments, smaller coral rubble, pebbles and stones; the fifth area was bare. The intent was to assess differential use of substrate by juveniles.

RESULTS

Spawning.

A total of 18 spawnings occurred in the laboratory from December 1984 through April 1985 (numbers in parentheses): February (2), March (1), April (2), May (1), July (1), August (1), September (3), October (3), November (2) and December (1). Five (27.8%) spawnings occurred spontaneously and 13 (72.2%) were induced with serotonin only, macerated gonad only, or both (Table 1). All spontaneous spawnings occurred in 1985, involving an unknown number of clams. In four of these spawnings, the water temperature in the holding tanks had risen to 30-34 °C from about 1200 h to late afternoon (1730 h) and spawning must have occurred between 1545 and 1650 h. In the fifth instance there was no appreciable rise in water temperature, and spawning probably occurred between 1800 and 1900 h.

Table 1. Summary of data on Hippopus hippopus spawning induction experiments. All clams used beginning July 25, 1985 showed mature eggs by biopsy. Broodstock were held in laboratory tanks from few days to several months.

Date of Experiment	Number of Clams Induced	Date Collected (Number)	Spawning Stimulus	Result
10 Dec 84	6	20 Oct 84	Macerated gonad	Two released sperm
05 Feb 85	8	20 Oct 84	Macerated gonad	All released sperm
12 Apr 85	6	20 Oct 84	Macerated gonad	Three released sperm
09 May 85	3	20 Oct 84	Macerated gonad	Two released sperm
25 Jul 85	3	20 Oct84(2) 24 Jul85(1)	Macerated gonad	All released sperm
14 Aug 85	2	13 Aug 85	Macerated gonad	One released sperm, the oth released sperm & then eggs normal development to juveni stage
16 Sep 85	3	Apr 85	Macerated gonad	All released sperm
21 Sep 85	3	20 Oct 84	Macerated gonad	All released sperm
26 Sep 85	1	Apr 85	Macerated gonad	Released sperm
20 Nov 85	3	16 Nov 85	Serotonin	All three released sperm, on one released few eggs.
28 Nov 85	2	26 Nov 85	Macerated gonad	Only one released sperm an few ripe eggs, which develop to one-day old trochophore larvae
28 Feb 86	2	Apr 85	Serotonin followed by macerated gonad after 2 hr	Only one released sperm aft addition of macerated gona
27 Mar 86-01 Apr 86	3	20 Mar 86	Serotonin followed by macerated gonad after 5 days	Only one released sperm after addition of macerate gonad

Table 2. Data on survival of Hippopus hippopus larvae reared in the laboratory .

	DATE SPAWNED		
	14 Aug 1985	30 Aug 1985	15 Oct 1985
Number of eggs spawned	59,940,000 (933/ml)	2,000,000 (33/ml)	-
Number of fertilized eggs	1,200,000 (20/ml)	300,000 (5/ml)	4,500,000 (75/ml)
Number of trochophore larvae	480,000 (8/ml)	250,000 (4/ml)	300,000 (5/ml)
Number of veliger larvae	300,000 (5/ml)	70,000 (1.2/ml)	240,000 (4/ml)
Density of pediveliger larvae	4/ml	1/ml	3/ml
Number of juveniles	90 (3.5 mo. old)	1,493 (3 mo. old)	4,357 (4 mo. old)
Percent survival rate of fertilized eggs to veliger	25.0	23.3	5.3
Percent survival rate of fertilized eggs to 3-4 mo. old juveniles	0.0075	0.497	0.0968
Percent survival rate of veligers to 3-4 mo. old juveniles	0.0375	2.13	1.82

Note: Numbers of eggs and larvae are estimates determined by the volumetric count method of Castagna and Kraeuter (1984); numbers of juveniles were determined by actual count.

Only sperm were released in ten induced spawnings, althou biopsy showed that at least 50% of the eggs were mature, f experimental clams used beginning July 1985. Spawning of sper and eggs occurred during or near the full moon (4 out of 8), t new moon (3) and the first quarter (1). Sperm were released a all phases of the moon.

Larval development.

Mature eggs of Hippopus hippopus are spherical in shape measuring 143.16 ± 7.26 µm in diameter (n=10; range, 127.5-150. µm, excluding the membrane). One clam 183 mm long released abo 60 million eggs upon induction with macerated gonad. The numbe released by clams which spawned spontaneously was not kno because of the undetermined number of spawning individuals.

Larval development was observed for two batches of eg released on 14 and 30 August 1985. The fertilized eggs underwe cleavage after one hour, developed into trochophore larvae with 20 hours, straight-hinge veligers in 22-26 hours and pediveliger in about five days (range 4 to 7 days). Pediveligers swam a crawled on and near the bottom. They settled on about day (ranging from day 6 to day 10) and generally metamorphosed day 9 (ranging from day 8 to day 12). Zooxanthellae were clear visible in 17-day old juveniles.

Substrate and larval settlement.

The numbers of three-month old juveniles which were fou attached to the four types of substrate in one of the larva tanks were as follows: 633 on coral fragments (length 4.2-10. cm), 211 on the coral rubble (length 1.8-5.0 cm), 215 on ston (dia. 2.5-7.4 cm), 320 on pebbles (dia. 0.5-1.5 cm) and 114 the bare area. (Most clams in the area were found attached objects which were accidentally introduced [a piece of wood, broken piece of PVC pipe and a leaf] and only 24 to the concre floor.) The clams were unevenly distributed; the largest numbe attaching to the coral fragments (chi-square from a contingen table = 539, df=4, p <.001).

Larval survival.

Larval survival rates are shown in Table 2. For the thr batches of trochophore larvae stocked at densities 8/ml, 4/ml a 5/ml, the survival rates to veligers were 62.5%, 28% and 80 respectively. Veliger survival rates to 3-4-month-old juvenile were 0.03%, 2.13% and 1.82%, respectively. The second and thi batches, with about 2% survival rates, were provided wi substantial settlement substrate, but the first batch was no This batch had the lowest survival rate. The influence of su strate on settlement is not yet known.

arval and early juvenile growth.

Growth of the larvae and juveniles up to the day-56 juvenile was observed for two batches. Two-day-old straight-hinge veligers had a mean length of 189.3 µm ± 24.0 (range 151-220 µm; n=10). Seven-day-old pediveligers measured 223.5 µm ± 12.7 (range 209-[illegible] n=7). Juveniles on day 17 had a mean length of 281 µm (range [illegible]-301 µm; n=3) and on day 56, 382-452 µm (n=2).

Subsequent data on juvenile growth included individuals from the three batches of spawn. Those from the first batch had a length of 464-475 µm (n=2) on day 62 and a mean length of [illegible] mm ± 2 (range 1-9 mm; n=22) on day 120. Individuals from the second batch were much longer, with a mean of 12 mm ± 3.42 (range [illegible] mm; n=100) on day 110. Individuals from the third batch had a length of 0.5 -1 mm (n=2) on day 98 and a mean length of 7.13 mm ± 3.0 (range 1-15 mm; n=100) at day 134. A wide variation in growth in length is evident among individuals in a batch and between individuals belonging to different batches.

DISCUSSION

Spawning.

Of the 22 clams induced to spawn with macerated gonad and/or serotonin, four (18.2%) did not respond and 18 (81.8%) released either sperm only or both sperm and eggs. Only three of the 18 (16.7%) spawned eggs. In comparison, Fitt et al. (1984) recorded two out of ten (20%) success for gonad induction; Braley (1985) reported 12 out of 23 (52.2%) for serotonin induction and Alcazar (unpubl. data) gave 12 out of 14 (85.7%), also for serotonin induction. Despite biopsy data showing the presence of mature eggs in our experimental animals, only a small proportion of our clams produced eggs, comparable to the results of Fitt et al. (1984) but much smaller than those of Braley (1985) and Alcazar (unpubl. data); these differences are significant (chi-square = 18.226, df= 3, $p < .001$). The reason for the low proportion of our clams producing eggs is not known. It should be noted, however, that the samples of Fitt et al.(1984) came from the Caroline Islands at about the same latitude as the south-central Philippines, while those of Braley (1985) and Alcazar (unpubl. data) were from the Great Barrier Reef.

Larval behavior and growth.

In general, the behavior of the larvae followed that described by Jameson (1976) and by Fitt et al. (1984). Trochophore and veliger larvae were free-swimming, and pediveligers crawled and swam on and near the bottom. Settlement occurred 6-10 days after fertilization, mostly on day 7.

Metamorphosis to juveniles occurred between days 8 and 12, most on day 9, when the larvae were about 231-234 µm in length. Fi et al. (1984) gave 185-195 µm as the size at metamorphosis. T juveniles were first seen to harbor zooxanthellae on day 17. veligers and pediveligers were slightly larger than those Jameson (1976) and Fitt et al. (1984), but our juveniles on da 56 and 62 were much smaller than those of Jameson (1976) on d 58.

Larval survival.

Larval (veliger) survival rates to juveniles for our thr batches of spawn varied from a low 0.03% to a relatively high 2 The low rate is tentatively ascribed to inadequate settlement substrates in the rearing tanks. Survival and growth rates m have been enhanced if the veligers were fed with a nutritio supplement (vitamins, yeast extract) in addition to t unicellular algae, following Fitt et al. (1984).

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