

Effect of stocking density on the hatching rate, larval and early juvenile rearing of edible sea cucumber *Holothuria scabra* (Jaeger, 1883)

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Fertilized eggs, auricularia larvae and three month old juveniles of *H. scabra*, obtained from induced spawning were used for various experiments to assess the effect of stocking density on hatching rate, larval and early juvenile rearing of commercially important sea cucumber *H. scabra*. Experiments were conducted for two days on hatching rate of fertilized eggs, for ten days on larval survival, growth and development and for 120 days on juvenile's growth rate. An inverse relationship was observed between stocking density and hatching rate. Hatching rate varied between 15.1% at 8 eggs mL⁻¹ to a maximum of 66.4% at 0.5 eggs mL⁻¹. High survival, growth rate and fastest development of auricularia at stocking density 1 larvae mL⁻¹ indicated the optimum larval rearing conditions. Maximum growth rate in length and weight, suggested the stocking density 4.2 Nos. m⁻² is good for juvenile rearing. One way ANOVA on the differences in the hatching rate, larval growth and survival rate, juvenile growth rate both in length as well as in weight at different stocking densities indicated high level of significances (P<0.001).

[**Keywords:** Hatching rate, *Holothuria scabra*, larval rearing, juvenile rearing]

Introduction

Sea cucumbers or their processed dried form (beche-de-mer) have been a dietary delicacy and a medicine for Asians over many centuries. The industry related to it existed along Gulf of Mannar of south east coast of India. Among the seven commercial species recorded from Gulf of Mannar and Palk Bay, the industry once existed was primarily depended on *Holothuria scabra* and *H. spinifera*¹.

Aquaculture and stock enhancement have been evaluated as the possible solution for stabilizing the sea cucumber harvest. *H. scabra* commonly called sand fish is considered as a high valued tropical species with wide distribution. Since the mass production of juvenile *H. scabra* through hatchery system has been proved successful^{2,3}, it is considered as an ideal candidate for wild or captive stock enhancement programmes.

Ramofafia *et al.*⁴ indicated that hatching, metamorphosis and settlement are the crucial stages in the culture of sea cucumbers. Morgan⁵ reported that quality of eggs determines the holothurians larval life span, growth, survival and proportion of larval metamorphosis. Liu *et al.*⁶ stressed that overcrowding of holothurians larvae and juveniles reduces available space and food availability causing mal nutrition,

reduced growth rate and size variability. Juvenile sea cucumbers are highly vulnerable during early rearing stages and highest mortality rates are caused by high stocking density⁷. Battaglione and Bell⁸ also highlighted that cost effective production of holothurians juveniles through hatchery system requires large surface area to forage for detritus and bacteria. Hence the rearing density plays a key factor determining the success of holothurians hatchery system at different stages of life history. Stocking densities in which successful larval rearing of *H. scabra* can be carried out under hatchery conditions have been elucidated^{9,3}. Present study was conducted to find out the effect of varied stocking densities on hatching rate of fertilized eggs, larval and early juvenile survival and growth rate of this species, so as to redefine the existing methodology for mass production of juvenile holothurians.

Material and Methods

Twenty numbers of broodstock of *H. scabra* collected by skin divers were maintained in 1000 L FRP tanks having 150 mm thick coral sand at the bottom. Water was exchanged daily and sand every fortnight and the brooders were fed with *Sargassum* sp. powder at the rate of 5% of their body weight.

Brooders were induced to spawn by addition of feed constituted by rice bran, soya powder and *Sargassum* sp. powder (4:1:2) at a ratio of 50 g 500L⁻¹ as specified by Asha and Muthiah¹⁰. Fertilized eggs obtained were washed through a 40 µm sieve and used for the experiment on hatching rate up to the stage dipleurula. Forty eight hour old auricularia larvae of mean size 390.1 ± 9.2 µm and 90 days old juveniles of size ranging 60-113mm in length and 9.3-104g in weight from the same brood, were used for the experiment on the effect on larval and juvenile rearing.

Stocking densities selected for the experiments on hatching rate were 0.5, 1, 2, 4, 6 and 8 eggs mL⁻¹ and for larval rearing were 0.25, 0.5, 1, 1.5, 2 and 3 larvae mL⁻¹. Experiments were conducted in 3l plastic circular aquarium bowls (20 cm diameter and 16 cm height) filled with sand filtered sea water. Three replicate bowls were maintained for each treatment. Three stocking densities (4.6 Nos.m⁻², 6.2 Nos.m⁻² and 7.7 Nos.m⁻²) were selected for juvenile rearing experiments. Experiments were conducted in concrete tanks (1.3m × 1m) and three replicates were maintained inside the tank by partitioning with asbestos cement sheets at the tank bottom. Continuous flow through system of sea water was used for juvenile rearing.

The effect of hatching rate was assessed after 48 hours and the percentage of dipleurula stage was estimated in each treatment by counting the numbers in three 1 mL sub samples. In the larval rearing experiment, 100% water change was done on alternate days and 50% on other days. While during the 100% water change, the larvae retained on a 40 µm sieve were transferred to 3l glass beakers. The enumeration of larvae was carried out as suggested by Asha and Muthiah¹¹. Larvae were fed with micro algae *Chaetoceros* sp. at concentrations as specified by Asha¹² and the experiment was conducted for ten days.

For juvenile rearing experiment, 100% water was changed daily and the growth rate both in length and weight were estimated every month and the experiment was continued for 120 days. Juveniles were fed with *Sargassum* sp. powder and fine sand at a proportion of (1:2) at 5% of the body weight sieved through 120 µm sieve.

Mean difference in the number of fertilized eggs hatched on 2nd day, mean difference in the size and number of 8th day old larvae, 120th day old juveniles from the initial value for each treatment were considered for one way analysis of variance (ANOVA). The differences among treatment means

were tested for significance by a post – hoc multiple comparisons (Fisher's LSD) test.

Results and Discussion

An inverse relationship was observed between stocking density and hatching rate of fertilized eggs. Hatching rate was maximum of 66.4% at 0.5 eggs mL⁻¹ followed by 52.13%, 38.5%, 29.3% and 22.6% at stocking densities 1, 2, 4 and 6 eggs mL⁻¹ respectively (Fig.1). One way ANOVA indicated high level of significance in the hatching rate at different stocking densities and among the pair wise comparisons, except between stocking densities 2 and 4; 4 and 6; 6 and 8 all pair wise comparisons showed high level of significances.

In the present study, the highest hatching rate observed at rearing densities 0.5 - 1 eggs mL⁻¹ is in conformity with 0.8 - 1 eggs mL⁻¹ and 0.5 - 1 eggs mL⁻¹ for *H. scabra*^{2,13}; 0.5 eggs mL⁻¹ for *H. spinifera*¹, but much lower than 2.5 eggs mL⁻¹ and 1 - 9 eggs mL⁻¹ for *H. scabra*⁴, and 2.7 eggs mL⁻¹ for *H. atra*¹⁴. Yanagisawa¹⁵ indicated that hatching appears to be the most vulnerable stage in *H. scabra* and mortality during hatching can be reduced by stocking embryos at lower densities, hence experiments on hatching at still lower densities below 0.5 eggs mL⁻¹ should be trialed to arrive at the optimum level.

On day four, at the stocking density of 0.25 larvae mL⁻¹, 44.1% of the larvae survived, which decreased to 20.7% on day 6 and 9.6% on day 8 and absolute mortality observed on day 10, whereas at 3 larvae mL⁻¹, the survival rate was decreased from 70% to 53.8% on day 6 and absolute mortality observed on day 7. Larvae were survived throughout the experimental days at stocking densities 0.5, 1, 1.5 and 2 larvae mL⁻¹.

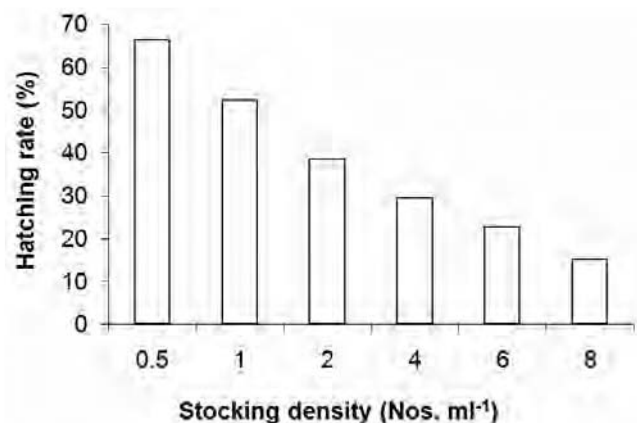


Fig. 1—Hatching rate of fertilized eggs of *Holothuria scabra* on day 2 at different stocking densities

Maximum percentage of larval survival (31.3%) was observed at concentration of 1 larvae mL⁻¹ followed by 27.2%, 17.63% and 15.3% at stocking densities 1.5, 2 and 0.5 larvae mL⁻¹ (Table 1). One way ANOVA indicated high level of significance in the survival rate of larvae at different stocking densities ($p < 0.001$) and among the pair wise comparisons, except between stocking densities 1 and 1.5; 1.5 and 2; 0.25 and 3, high significant difference was observed in the survival rate of larvae in others ($p < 0.001$).

At stocking density 0.25 larvae mL⁻¹, the growth rate of the larvae was decreased from 108.5 $\mu\text{m day}^{-1}$ on day 4 to 24.47 $\mu\text{m day}^{-1}$ on day 8 and absolute mortality was observed on day 10. At stocking density 3 larvae mL⁻¹, the growth rate was decreased from 64.9 $\mu\text{m day}^{-1}$ to 28.41 $\mu\text{m day}^{-1}$ and absolute mortality was observed on day 7. On day 10 the highest growth rate of 72.23 $\mu\text{m day}^{-1}$ was observed for larvae at 1 mL⁻¹ followed by 63.7 $\mu\text{m day}^{-1}$ at 1.5 larvae mL⁻¹, 61.3 $\mu\text{m day}^{-1}$ at 2 larvae mL⁻¹ and 60.6 $\mu\text{m day}^{-1}$ at 0.5 larvae mL⁻¹ respectively (Table 2). The one way ANOVA indicated high level of significance in the growth rate at different stocking density treatment ($p < 0.001$) and except between stocking densities 0.5 and 1.5; 0.5 and 2.0; 1.5 and 2.0 and 0.25 and 3.0 high level of significance ($p < 0.001$) was observed among all pair wise comparison of growth rate differences.

On day 10, absolute deformity were observed for the larvae reared at stocking densities 0.25 larvae mL⁻¹ and 3 larvae mL⁻¹. 70% of the larvae attained the late auricularia stage at stocking density 1 larvae

mL⁻¹ followed by 60%, 55% and 50% at 1.5, 2 and 0.5 larvae mL⁻¹. 10% of the late auricularia stage metamorphosed to doliolaria stage at 1 larvae mL⁻¹ followed by 5% each at 1.5 and 2 larvae mL⁻¹ and only 2% at 0.5 larvae mL⁻¹ (Fig. 2).

Highest survival, growth rate and fastest development of auricularia stage was observed at stocking density 1 larvae mL⁻¹ followed by 1.5 larvae mL⁻¹, which is in conformity with the larval stocking density of 0.75 larvae mL⁻¹ for *H. scabra* versicolor⁹ and 1 larvae mL⁻¹ for *H. scabra*⁸, but higher than 0.3-0.4 larvae mL⁻¹; 0.1-0.2 larvae mL⁻¹ and 0.5 larvae mL⁻¹ for *Stichopus japonicus*^{16,17,18}. The difference might be due to the species wise variations in the size of auricularia larvae. Ramofavia *et al*⁴ also indicated that larval density of *H. scabra* will be lowered as the rearing days were advanced.

The growth rate both in length and weight for the juveniles were maximum of $0.323 \pm 0.047 \text{ mm.day}^{-1}$ and $0.417 \pm 0.011 \text{ gm.day}^{-1}$ at stocking density 4.6 Nos. m⁻² followed by $0.203 \pm 0.85 \text{ mm.day}^{-1}$ and $0.34 \pm 0.025 \text{ gm.day}^{-1}$ at stocking density 6.2 Nos. m⁻² and $0.173 \pm 0.0108 \text{ mm.day}^{-1}$ and $0.263 \pm 0.011 \text{ gm.day}^{-1}$ at 7.7 Nos. m⁻² respectively (Fig.3). The one way ANOVA indicated high level of significance in the growth rate both in length and weight at different stocking densities ($p < 0.01$) and among the pair wise comparisons, except in the growth rate in length between stocking densities 6.2 and 7.7, all pair wise comparisons showed high level of significances.

In the present study, the highest growth rate ($0.323 \text{ mm.day}^{-1}$ in length and $0.417 \text{ gm.day}^{-1}$ in

Table 1—Mean (\pm S.E., n = 30) survival percentage (%) of the auricularia larvae of *Holothuria scabra* at various stocking densities (Nos.ml⁻¹).

Days	Stocking densities					
	0.25	0.5	1	1.5	2	3
4	44.1 \pm 7.01	59.7 \pm 14.01	83.6 \pm 5.9	79.8 \pm 7.32	80.73 \pm 11.8	70 \pm 4.9
6	20.77 \pm 2.5	43.23 \pm 8.25	54.07 \pm 1.2	56.8 \pm 11.01	45.5 \pm 1.7	53.8 \pm 1.44
8	9.6 \pm 2.41	19.9 \pm 5.83	43.5 \pm 4.3	43.1 \pm 6.7	29.7 \pm 8.4	—
10	—	15.3 \pm 3.6	31.3 \pm 5.7	27.17 \pm 3.98	17.63 \pm 5.32	—

Table 2—Mean (\pm S.E., n = 30) growth rate ($\mu\text{m day}^{-1}$) of the auricularia larvae of *Holothuria scabra* at various stocking densities (Nos.ml⁻¹)

Days	Stocking densities					
	0.25	0.5	1	1.5	2	3
4	108.5 \pm 10.09	93.4 \pm 12.009	84.7 \pm 4.41	84.4 \pm 6.115	71.73 \pm 1.98	64.9 \pm 4.995
6	39.78 \pm 0.441	61.87 \pm 2.52	72.9 \pm 2.205	76.1 \pm 2.78	50.83 \pm 4.4	28.41 \pm 1.7
8	24.47 \pm 4.5	43.3 \pm 2.4	58.3 \pm 2.88	56.93 \pm 0.29	50.73 \pm 1.14	—
10	—	60.6 \pm 4.69	72.23 \pm 2.21	63.7 \pm 0.78	61.27 \pm 1.31	—

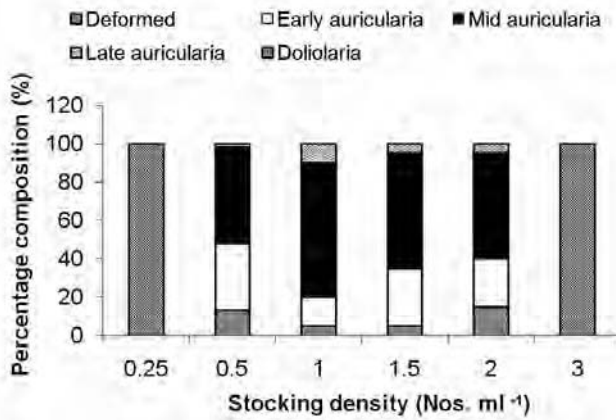


Fig. 2—Proportion of auricularia larvae of *Holothuria scabra* on day 10 at different stocking densities

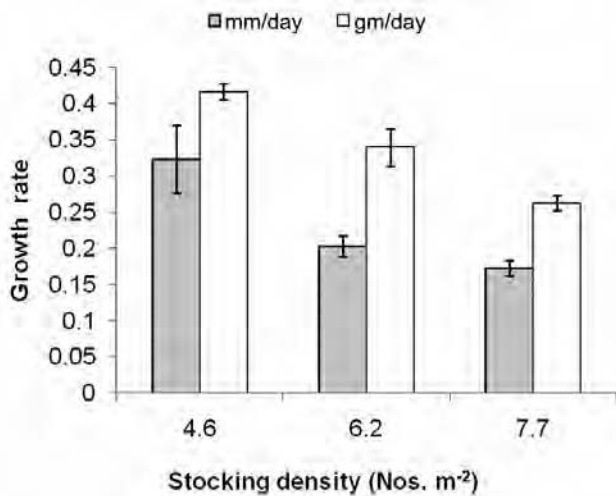


Fig. 3—Growth rate of *Holothuria scabra* juveniles on day 120 at different stocking densities

weight) obtained for juvenile is more or less similar to the reported growth rates of 0.4 gm. day^{-1} ¹⁹, $0.2\text{-}0.8 \text{ mm day}^{-1}$ ⁸, but higher than $0.062 \text{ gm. day}^{-1}$ ²⁰, $0.05 \text{ gm. day}^{-1}$ ²¹ and 0.2 gm. day^{-1} ²² for *H. scabra* juveniles. An inverse relationship was also observed between stocking density and growth rate of juveniles both in length and weight. Pitt and Duy¹³; Pitt *et al.*²²; Battaglione and Bell⁸, and Ramofafia *et al.*²³ also reported such negative relationships between stocking density and growth rates in their previous studies.

The recommended stocking density for obtaining maximum growth rate of holothurians juveniles varied substantially. Sui *et al.*⁷ recommended 10 Nos. m^{-2} for *S. japonicus*. For *H. scabra* juveniles, stocking densities recommended differently as 5 Nos. m^{-2} ⁸; 20

Nos. m^{-2} ⁵; 2 Nos. m^{-2} ²⁴ and 10 Nos. m^{-2} ²⁵. Chen²⁶ indicated that stocking density depends on the size of the seeds, habitat condition including the natural feed availability and sea water exchange etc., hence the present work warrants the need for standardizing the optimum rearing densities for juvenile holothurians of varied size and age groups along with its suitable feed and feeding regimes.

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