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Effects of temperature, salinity and pH on larval growth, survival and development of the sea cucumber *Holothuria spinifera* Theel

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Abstract

For large-scale seed production of sea cucumbers through a hatchery system, it is imperative to know the effects of environmental parameters on larval rearing. Auricularia larvae (48 h post-fertilization) were obtained from induced spawning of *Holothuria spinifera* and used in experiments to ascertain the effects of temperature, salinity and pH on the growth and survivorship of the larvae. The larvae were reared for 12 days at temperatures of 20, 25, 28 and 32 °C; salinities of 15, 20, 25, 30, 35 and 40 ppt; and pH of 6.5, 7.0, 7.5, 7.8, 8.0, 8.5 and 9.0. The highest survivorship and growth rate and fastest development of auricularia indicated that water temperature of 28–32 °C, salinity of 35 ppt and pH of 7.8 were the most suitable conditions for rearing larvae of *H. spinifera*.

Keywords: *Holothuria spinifera*; Sea cucumber; Larval rearing; Salinity; Temperature; pH; Growth

1. Introduction

Environmental conditions play a vital role in invertebrate larval development. Chia (1977) stated that invertebrate larvae are physiologically "competent" when they are capable of metamorphosis under the proper environmental conditions. Smiley et al. (1991) and Yanagisawa (1998) reported that settlement and metamorphosis of holothurian larvae are critical life stages. The physical conditions in which the successful rearing of larvae of *Actinopyga echinites* (Chen and Chian, 1990), *Holothuria scabra* (James et al., 1994; Battaglene, 1999), *Isostichopus japonicus* (Ito and Kitamura, 1998) and *Holothuria spinifera* (Asha and Muthiah, 2002) can be carried out under prevailing hatchery conditions have been elucidated.

As in the rest of the Indo-Pacific region, sea cucumbers form an important part of a multi-species fishery in India. As per the statistics of the Marine Products Export Development Authority (MPEDA), Government of India, the beche-de-mer product exported from India during the period 1992–2000 varied from 10.5 to 140 t and ranks first among the dried marine products sold (MPEDA, 1998, 2003). The beche-de-mer industry mainly depends on *H. scabra* followed

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by *H. spinifera* (Chellaram et al., 2003). James and Badrudeen (1997) reported estimated annual landings of 460 t of fresh *H. spinifera* along the southeast coast of India. However, the export of beche-de-mer has declined from 70 t in 1996–1997 to 3.8 t in 2001, primarily due to over-exploitation. The Ministry of Environment, Government of India, has banned both the export and fishery of sea cucumbers from June 2001 onwards. Considering its important role in the commercial fishery, seed production of *H. spinifera* through a larval hatchery is essential to carry out sea ranching and for replenishing the natural stock.

While rearing the larvae of *H. spinifera* for the first time ever, Asha and Muthiah (2002) reported that the slipper-shaped, pelagic early auricularia larva, with a mean (± S.E.) length of 498 ± 32 μm (n = 25) developed 48 h after fertilization. On day 9, late auricularia attained a mean size of 809 ± 123 μm (n = 25). On day 10, non-motile, barrel-shaped doliolaria (468 ± 57 μm) (n = 25) metamorphosed to the creeping pentacula stage. Larval rearing in *H. spinifera* was carried out at temperatures of 29–31 °C, salinities of 34.8–36.0 ppt and pH of 8.1–8.2 (Asha and Muthiah, 2002). In developing methods for rearing the larvae of holothurians, apart from the quality and quantity of food, the range of temperature, salinity and pH must be considered. In the southeast coast of India, the larvae of *H. spinifera* in the field experience a surface water temperature of 25.5–31.2 °C, salinity of 30.3–35.9 ppt and a pH of 7.8–9.0 (Marichamy and Siraimeetan, 1979). Considerable reduction has been observed in the temperature (22 °C), salinity (14 ppt) and pH (7) due to fresh water influx during the monsoon season in this area.

Hamel and Mercier (1996) reported on the effects of pH, temperature and salinity on the embryonic development of *Cucumaria frondosa*. Kashenko (2002) observed the reactions of larvae of *Apostichopus japonicus* in different salinities. Aside from these few studies, very little is known about the conditions for larval rearing of sea cucumbers. For large-scale seed production of sea cucumbers in a hatchery system, it is imperative to know the effects of temperature, salinity and pH on larval growth and survivorship. In this study, the effects of temperature, salinity and pH on larval growth and survivorship of *H. spinifera* were determined. While at present there is no commercial scale culture of *H. spinifera,* the results of this study will be useful for future mass production of juveniles for aquaculture production and/or wild stock reseeding.

2. Materials and methods

Auricularia larvae (48 h old) from the same brood, of mean (± S.E.) length 474 ± 7.8 μm (n = 25), were utilized for the experiments. For first 48 h, the larvae were reared at 28 °C, 35 ppt and pH 7.8. The larvae were transferred to the various experimental treatments by slowly changing the condition over an adjustment period of 1 h. The larvae were reared under static conditions under an ambient photoperiod regime of 12 h of light and 12 h of dark at a density of 0.5 ind ml^{-1}. They were reared in 3-l plastic, circular aquarium bowls (20 cm diameter; 16 cm height) filled with sand-filtered seawater.

The seawater in the rearing bowls was exchanged completely on alternate days and on other days only half of the water was changed. During whole water changes, the larvae were retained on a 40-μm sieve and transferred to 3-l glass beakers. After thorough mixing, a 1-ml sub-sample was taken and the number of larvae enumerated for purposes of determining survival rate. The lengths of 10 randomly chosen larvae were measured using a microscope having a precalibrated micrometer. Then, the larvae were transferred back to their respective bowls and provided with *Isochrysis galbana* at a concentration of 20,000 cells ml^{-1} as feed.

Three replicate bowls were established for each temperature, salinity and pH level. The experiments were conducted for 12 days, after which the larvae in most treatments metamorphosed into non-feeding doliolaria. The mean size and number of larvae were recorded on the 4^{th}, 8^{th} and 12^{th} day. The mean differences in the size and number of 8-day-old larvae for the temperature and 12-day-old larvae for salinity from the initial value of 2-day-old larvae were considered for each treatment in the one-way analysis of variance (ANOVA). The data normality was judged by Z-test, and the homogeneity of variances was evaluated using Cochran's test. The differences among treatment means were tested for significance by a post-hoc multiple comparisons (Fisher's LSD) test.

The larvae were reared at four different temperatures (20, 25, 28 and 32 °C). For the exposure at 20 °C, the larval rearing bowls were kept in a 7 × 10-m

room with an air conditioner and the temperature varied from 20 to 21 °C. For the 25 °C treatment, the rearing bowls were kept in a BOD incubator with a temperature range of 24–26 °C. For the 28 °C treatment, the bowls were kept in the laboratory at room temperature and the water temperature ranged from 28 to 29 °C. For the 32 °C treatment, the plastic rearing bowls were placed in an aquarium where the surrounding water was heated by a porcelain-cased heating element with Jumo thermometer and the thermostat was fixed at 32 °C.

The larvae were reared at 15, 20, 25, 30, 35 and 40 ppt salinity. The salinity was measured using a refractometer (ATAGO, Tokyo, Japan). Desired salinities <35 ppt were created by adding fresh water to ambient seawater (35 ppt). The highest salinity treatment was created by adding NaCl to ambient seawater. While NaCl would not mimic the exact composition of natural seawater because of the lack of various elements, it did give the desired salinity value.

The larvae were reared at pH of 6.5, 7.0, 7.5, 7.8, 8.0, 8.5, and 9.0. The pH>7.8 was adjusted by adding 1 N sodium hydroxide and pH<7.8 by adding diluted hydrochloric acid to ambient seawater. The pH was measured with a pH pen (Hanna Instrument, Woonsocket, Portugal).

3. Results

3.1. Temperature

On days 4 and 8, the larvae reared at 32 °C had the highest survival rate of 96.8% and 90.8%, respectively (Table 1). On day 12, the larvae reared at 32 °C retained the same survival rate of 90.8%, followed by 76.7% at 25 °C and 71.1% at 28 °C (Table 1). The survival rate of the larvae reared at 20 °C drastically decreased from 61.8% on day 8 to 29.1% on day 12 and was the lowest of all treatments on both days (Table 1). The one-way ANOVA on differences (between 2 and 8 days) in the mean survival rate of the larvae reared at different temperatures indicated a high level of significance ($p<0.001$) (Table 2). All pairwise comparisons among temperature treatments were

Table 1
Mean (±S.E., $n=30$) survival percentage of the auricularia of *H. spinifera* at different temperatures

Days	Temperature (°C)			
	20	25	28	32
4	75.2±5.9	81.7±1.2	83.3±2.4	96.8±0.6
8	61.8±5.2	79.9±1.06	74.4±3.6	90.8±3.2
12	29.1±7.2	76.7±2.4	71.1±2.0	90.8±3.2

Table 2
ANOVA table on number of *H. spinifera* survived on day 8 at different temperatures with results of LSD post-hoc multiple comparisons test

Treatments	Sum of squares	*df*	Mean Square	*F* ratio	*p* value
Between groups	1310.281	3	436.760	16.983	<0.001
Within groups	205.738	8	25.717		
Total	1516.019	11			
LSD					
	25	28	32		
20	<0.005	<0.05	<0.001		
25		>0.05	<0.05		
28			<0.005		

Table 3
Mean (±S.E., $n=30$) growth rate (μm day^{-1}) of the auricularia of *H. spinifera* at different temperatures

Days	Temperature (°C)			
	20	25	28	32
4	51.6±7.1	52.9±11.9	53.1±3.5	82.7±1.8
8	29.1±1.8	38.2±0.5	42.7±1.2	62.6±0.7
12	nil	32.6±2.1	32.8±0.72	–[a]

[a] All larvae attained doliolaria stage.

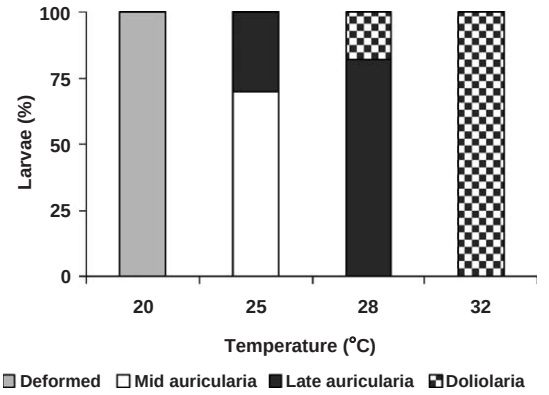

Fig. 1. Percentage of mid- and late-stage auricularia, doliolaria and deformed larvae of *H. spinifera* on day 12 at different temperatures.

Table 4
ANOVA table on growth rate of *H. spinifera* larvae on day 8 at different temperatures with results of LSD post-hoc multiple comparisons test

Treatments	Sum of squares	*df*	Mean square	*F* ratio	*P* value
Between groups	1805.555	3	601.852	228.548	<0.001
Within groups	21.067	8	2.633		
Total	1826.622	11			
LSD					
	25	28	32		
20	<0.001	<0.001	<0.001		
25		<0.01	<0.001		
28			<0.001		

significantly different except between 25 and 28 °C (Table 2).

On day 4, the larvae showed a maximum growth rate of 82.7 μm day^{-1} at 32 °C and a minimum of 51.6 μm day^{-1} at 20 °C (Table 3). On day 8, the growth rate of the larvae at 32 °C was the highest at 62.6 μm day^{-1} (with a mean length of 850 μm). The larvae reared at 32 °C developed lipid spheres on day 6, and 90% of the larvae had metamorphosed to doliolaria by day 8 and 100% by day 12 (Fig. 1). At 28 °C, 5% of the larvae attained late auricularia stage with lipid spheres by day 8 and 82% of larvae were late auricularia and 18% metamorphosed to doliolaria by day 12 with a growth rate of 32.8 μm day^{-1}(Fig. 1, Table 3). At 25 °C, with a growth rate of 32.6 μm day^{-1}, only 2% and 30% of the larvae attained the late auricularia stage by days 8 and 12, respectively (Fig. 1, Table 3). At 20 °C, larvae only reached the mid-auricularia stage by day 8 and became deformed completely by day 12 (Fig. 1). A high degree of significance ($p<0.001$) was observed in the mean growth rates of the larvae held at the different temperatures (Table 4). All pair-wise comparisons among treatment means were significant (Table 4).

3.2. Salinity

On day 4, the survival rate was maximum (88.4%) at 35 ppt and minimum (13.1%) at 15 ppt (Table 5). The larvae reared at 15, 20 and 25 ppt became deformed on days 5, 6 and 9, respectively. On day 8, maximum survival of 83.3% was observed at 35 ppt. On day 12, 72.3% survival was noticed in the larvae reared at 35 ppt, 25.3% at 30 ppt and 11.8% at 40 ppt (Table 5).

On day 4, the larvae registered the maximum growth rate of 56.3 μm day^{-1} at 35 ppt and minimum of 14.7 μm day^{-1} at 25 ppt (Table 6). The growth rate was nil for the larvae reared at 15, 20 and 25 ppt on day 8 because of larval deformities. The maximum growth rate on day 8 of 36.7 μm day^{-1} was registered among the larvae reared at 35 ppt (Table 6) and the mean larval size was 768.1 μm. On day 10, the larvae reared at 30 ppt had a growth rate of 26.6 μm day^{-1}, which declined to 3.6 μm day^{-1} on day 12 (Table 6). Whereas on day 12, the larvae reared at 35 ppt registered the maximum growth rate of 33.6 μm day^{-1} and

Table 5
Mean (± S.E., $n=30$) survival percentage of the auricularia of *H. spinifera* at different salinities

Days	Salinity (ppt)					
	15	20	25	30	35	40
4	13.1 ± 4.0	34.6 ± 7.1	42.6 ± 4.9	86 ± 1.7	88.4 ± 4.4	86.7 ± 2.4
8	nil	nil	4.0 ± 0.3	59.8 ± 11.3	83.3 ± 2.4	54.4 ± 4.5
12	nil	nil	nil	25.3 ± 2.5	72.3 ± 6.7	11.8 ± 6.5

Table 6
Mean (± S.E., $n=30$) growth rate (μm day^{-1}) of the auricularia of *H. spinifera* at various salinities

Days	Salinity (ppt)					
	15	20	25	30	35	40
4	15.1 ± 3.0	22.1 ± 3.4	14.7 ± 1.4	31.4 ± 10.5	56.3 ± 3.7	41.3 ± 1.5
8	nil	nil	nil	29.1 ± 3.6	36.7 ± 4.2	13.8 ± 1.0
12	nil	nil	nil	3.6 ± 3.9	33.6 ± 1.3	nil

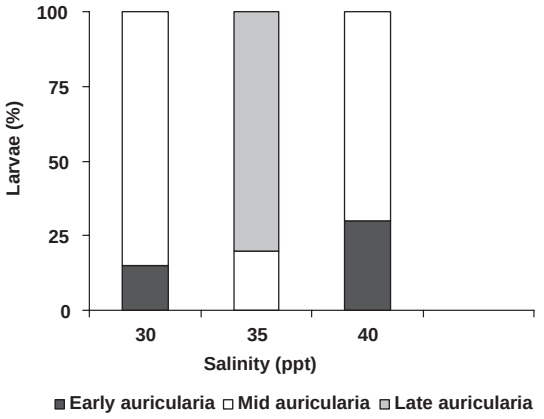

Fig. 2. Percentage of early-, mid- and late-stage auricularia larvae of *H. spinifera* on day 8 at different salinities.

nil at 40 ppt (Table 6). On day 8, 85% and 70% of larvae were in the mid-auricularia stage at 30 and 40 ppt, respectively (Fig. 2). On day 8 at 35 ppt, 80% of the larvae attained the late auricularia and 100% on day 12 (Fig. 2).

One-way ANOVAs indicated highly significant differences among the mean survival and growth rates of the larvae reared at different salinities (Tables 7 and 8). For mean survival rate, all pair-wise differences were significantly different except 15 and 20; 15 and 25; and 20 and 25 (Table 7). Similarly, except the differences in the mean growth rate of the larvae between salinities 15 and 20, 15 and 25, 20 and 25 and 30 and 40 ppt, all other pair-wise differences were significant (Table 8).

Table 7
ANOVA table on numbers of *H. spinifera* survived on day 12 at different salinities with results of LSD post-hoc multiple comparisons test

Treatments	Sum of squares	*df*	Mean square	*F* ratio	*P* value
Between groups	20184.922	5	4036.984	84.694	<0.001
Within groups	571.983	12	47.665		
Total	0756.905	17			
LSD					
	20	25	30	35	40
15	>0.05	>0.05	<0.001	<0.001	<0.001
20		>0.05	<0.001	<0.001	<0.001
25			<0.001	<0.001	<0.001
30				<0.05	<0.001
35					<0.001

Table 8
ANOVA table on growth rate of *H. spinifera* on day 12 at different salinities with results of LSD post-hoc multiple comparisons test

Treatments	Sum of squares	*df*	Mean square	*F* ratio	*P* value
Between groups	9993.5139	5	798.703	76.045	<0.001
Within groups	126.036	12	10.503		
Total	4119.54	17			
LSD					
	20	25	30	35	40
15	>0.05	>0.05	<0.001	<0.001	<0.001
20		>0.05	<0.001	<0.001	<0.001
25			<0.001	<0.001	<0.001
30				<0.001	>0.05
35					<0.001

3.3. pH

The mean percent survival of larvae on day 7 reared at pH 7.8 was highest at 83.3%, followed by 60.3%, 56.7% and 53.6% at pH 8.0, 8.5 and 7.0, respectively (Fig. 3). The larvae reared at pH 9.0 became deformed and disintegrated on day 2 onwards. At pH 6.5 and 7.5, percent survival was 51.0% and 46.8%, respectively (Fig. 3). Since deformity of the larvae except those reared in pH 7.8 occurred, no growth studies could be carried out. The larvae in 7.8 attained a mean size of 858.5 μm with a growth rate of 37 μm day^{-1} on day 12.

4. Discussion

Significantly higher mean survival and growth rates were observed in larvae reared at 32 °C than at the other temperatures and no significant difference was observed in the mean survival rate between 25 and 28 °C. Moreover, quick development of auricularia, followed by early metamorphosis on day 8, occurred at 32 °C in comparison to the slow growth and delayed metamorphosis on day 12 at 28 °C. This indicates that water temperatures >28 °C would be optimal for the normal growth and development of auricularia larvae of *H. spinifera*. James et al. (1994), Battaglene (1999), Chen and Chian (1990) and Ramofafia et al. (1995) reported water temperatures between 27 and 30 °C as the optimum for the larvae of the tropical sea cucumbers *H. scabra*, *A. echinites* and *H. atra*, respectively. Sim-

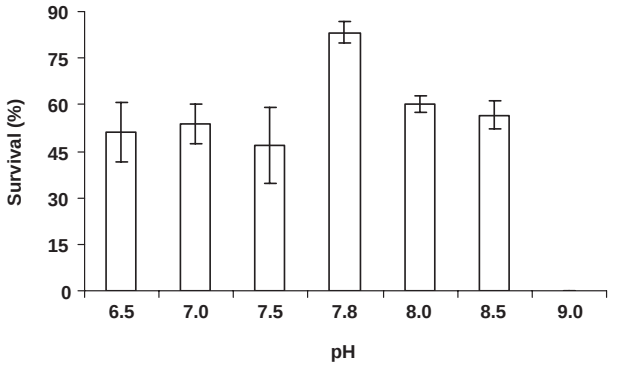

Fig. 3. Mean (± S.E.) survival percentage ($n = 30$) of the auricularia of *H. spinifera* on day 7 at various pH. There was 0% survival at pH 9.0.

ilarly, Hamel and Mercier (1996) and Ito and Kitamura (1998) also reported faster development of the larvae of *C. frondosa* and *I. japonicus*, respectively, at higher water temperatures than the prevailing natural environmental conditions.

Results indicated that a salinity of 35 ppt is optimum for the normal growth and development of *H. spinifera* larvae. Asha and Muthiah (2002) and Chen and Chian (1990) also reported on normal larval development at 35–36 ppt of *H. spinifera* and *A. echinites*, respectively. Lower survival of the auricularia in salinities 15–25 ppt on day 4 and subsequent occurrence of deformed larvae leading to disintegration on day 8 may be attributed to the inability of the mid-auricularia to rapidly react to sharp changes in the environment as Kashenko (2002) pointed out for the larvae of *A. japonicus*, which becomes vulnerable and perish at 20 ppt. The decrease in larval growth from 26.6 μm day^{-1} at 30 ppt on day 10 to 3.6 μm day^{-1} on day 12 may be due to the preferential response of late auricularia to salinity changes as envisaged by Kashenko (2002).

In the pH experiment, only larvae in the normal seawater pH (7.8) exhibited high survival and growth, indicating that auricularia of *H. spinifera* were very sensitive to pH changes. Similarly, Hamel and Mercier (1996) observed that the pentactula development of *C. frondosa* takes longer at pH 9 than at pH 8. Thus, the highest survival and growth rate and most rapid development of auricularia inferred that water temperature of 28–32 °C, salinity of 35 ppt and pH 7.8 are the optimum conditions for rearing the larvae of *H. spinifera*.

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