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Effects of single and combined microalgae on larval growth, development and survival of the commercial sea cucumber *Holothuria spinifera* Theel

P S Asha & P Muthiah
Tuticorin Research Centre of Central Marine Fisheries Research Institute, Tuticorin, Tamil Nadu, India

Correspondence: P S Asha, Tuticorin Research Centre of Central Marine Fisheries Research Institute, South Beach Road Extension, Karapad, Tuticorin 628 001, Tamil Nadu, India. E-mail: ashasanil@yahoo.com

Abstract

The results of the study on the suitable algal feed for the mass rearing of holothurian larvae through hatchery system are presented. Auricularia larvae, after 48 h of fertilization, obtained from induced spawning of *Holothuria spinifera*, were fed with different algae *Isochrysis galbana*, *Nanochloropsis salina*, *Pavlova lutheri*, *Tetraselmis chuii* and *Chaetoceros calcitrans* as well as *I. galbana*+*C. calcitrans* to ascertain the effect of single and combined microalgal diet. The rate of feeding was 2 × 10^4 cells larvae^{-1}.day^{-1} for a period of 9–12 days. The growth rate of 59 μm day^{-1} with 90% and 43 μm day^{-1} with 100% occurrence of late auricularia in the larvae fed with *C. calcitrans* alone and *I. galbana*+*C. calcitrans*, respectively, indicated that *C. calcitrans* itself or in combination with *I. galbana* is the effective feed for the larvae of *H. spinifera*.

Keywords: *Holothuria spinifera*, Auricularia, *Isochrysis galbana*, *Chaetoceros calcitrans*

Introduction

As in many countries of Indo-Pacific areas, dried holothurians are an important export commodity in India also. Because of increasing demand and inadequate fishery management, the sea cucumber resources are overexploited; hence, the Ministry of Environment, Government of India, has banned both fishery and export of 'beche-de-mer' from India since 2001. The release of hatchery-produced juveniles into their natural habitat is being considered as a suitable method to enhance the wild stock for sustainable yield (Munro & Bell 1997; Yanagisawa 1998; Battaglene & Bell 1999).

In India, the 'beche-de-mer' industry mainly depends on *Holothuria scabra* and *Holothuria spinifera* (Chellaram, Samuel & Patterson Edward 2003). James and Badrudeen (1997) estimated an annual landing of 460 t of fresh *H. spinifera* along the south-east coast of India. Considering the role of *H. spinifera* in the commercial fishery and indiscriminate exploitation, hatchery production of juveniles and subsequent reseeding to natural habitat are the alternatives for natural as well as in-captive stock enhancement.

The success of large-scale seed production of sea cucumber through hatchery techniques mainly depends on suitable larval feed. Generally, nanoflagellates of Chryophyceae or Haptophyceae either singly or in combination are provided for the larvae of sea cucumbers (James, Gandhi, Palaniswamy & Rodrigo 1994). Chen and Chian (1990), James, Rajapandian, Gopinathan and Baskar (1994) and Morgan (2001) reared the larvae of *H. scabra* and *Actinopyga echinites* with a higher survival and growth rate by providing *Isochrysis galbana* as feed. Ito and Kitamura (1997) reared the larvae of *Stichopus japonicus* with *Chaetoceros calcitrans*. James, Rajapandian *et al.* (1994) and Battaglene (1999) reported that mixed algae dominated by *C. calcitrans* enhanced the larval growth of *H. scabra*.

Although the larvae of *H. spinifera* were reared successfully by providing a mixture of *I. galbana*, *C. calcitrans* and *Nanochloropsis salina* by Asha and Muthiah (2002), the suitability of other single and combined algal feed was not tested. The present work was initiated to assess the suitability of various microalgal feed either single or combined, for the mass rearing of *H. spinifera* through a hatchery system.

Materials and methods

The 48-h old auricularia larvae, obtained from the induced spawning of *H. spinifera*, were reared in triplicate at 0.5 mL^{-1} in 3 L plastic aquarium bowls containing sand-filtered sea water of 34.8–35.5 gL^{-1} salinity and a temperature of 28–30 °C for both single as well as combined feed experiment. Sea water was completely changed on alternate days and only half the quantity was changed on other days. When a complete water change was made, the larvae were retained in a 40 μm sieve and transferred to a 3 L glass beaker. After thorough mixing, a 1 mL subsample was taken in a counting chamber to estimate the survival rate. The growth rate was calculated by measuring the length of 30 larvae representing 10 in each aquarium for each treatment, using a microscope fitted with a micrometer. The maintenance and mass culture of the algae were carried out following the serial dilution technique (Gopinathan, 1982). The experiment was conducted till the auricularia larvae metamorphosed into a nonfeeding doliolaria stage.

The experiment on the single algal diet was conducted for 9 days. The initial size of the larvae was 362 ± 4.4 μm. The different algae provided were *I. galbana*, *C. calcitrans*, *N. salina*, *Pavlova lutheri* and *Tetraselmis chuii*. The concentration of feed given was 2 × 10^4 cells mL^{-1}, as suggested by Morgan (2001) and Asha (2004). The larvae were fed daily and the feed was provided as a single dose. The length of the larvae was measured on the third, fifth, seventh and ninth day.

For the combined feed experiment conducted in triplicate for 12 days, the larvae were provided with *I. galbana* and *C. calcitrans* in equal concentrations. For comparison, one set of larvae were reared with *I. galbana* alone and another with *C. calcitrans* alone at a feed concentration of 2 × 10^4 cells mL^{-1}. The initial length of the larvae was 474 ± 7.8 μm. The larval length measurements were taken on the sixth, 10th and 12th day.

From the initial values, the mean differences in the size and number of 9-day-old larvae for the single algal diet experiment and 12-day-old larvae for the combined diet experiment were considered for each treatment in the one-way analysis of variance (ANOVA). The differences between treatments were tested for significance by multiple comparisons using the SPSS 7.5 program.

Results and discussion

Single algal feed

On days 3 and 5, the larvae fed with *I. galbana* showed the maximum growth rate of 232.4 and 97.7 μm day^{-1}, followed by 210.9 and 66.4 μm day^{-1} by those fed with *C. calcitrans* and 203.9 and 60.5 μm day^{-1} by those fed with *N. salina* respectively (Table 1). The growth rate of the larvae fed with *T. chuii* gradually reduced from 142.7 to 43.3 μm day^{-1} on day 5 and to 19.9 μm day^{-1} on day 7. On day 9, the larvae reared with *C. calcitrans* registered the maximum growth rate of 59.3 μm day^{-1} (mean length: 836 μm) followed by 39.0 μm day^{-1} (673.7 μm) with *I. galbana* and 27.6 μm day^{-1} (563 μm) with *P. lutheri*. On day 9, the growth rate was nil in the larvae reared with *N. salina* and *T. chuii* (Table 1) and the mean larval lengths were 355 and 289 μm respectively. The one-way ANOVA on the differences in the mean growth rate of larvae fed with different single algal diet indicated statistical significance ($P < 0.001$) (Table 2). In the multiple comparisons, except for the differences between *I. galbana*-fed larvae and *N. salina*-fed larvae and also *N. salina*-fed and *T. chuii*-fed larvae, all other values were significant ($P < 0.001$) (Table 2).

On day 9, 90% of the larvae fed with *C. calcitrans* and 43% fed with *I. galbana* reached the late auricularia stage with a lipid sphere, whereas 90% of the larvae fed with *P. lutheri* reached only up to the mid-stage and became deformed later. Such deformities were noticed on the fifth day with *T. chuii* and on the eighth day with the *N. salina* feed (Fig. 1).

Table 1 Mean (± SE, $n = 30$) growth rate (μm day^{-1}) of the auricularia of *Holothuria spinifera* fed with single microalgae

Days	*I. galbana*	*C. calcitrans*	*P. lutheri*	*N. salina*	*T. chuii*
3	232.4 ± 4.6	210.9 ± 5.1	175.9 ± 22.9	203.9 ± 29.1	142.7 ± 49.1
5	97.7 ± 7.5	66.4 ± 6.9	57.1 ± 12.8	60.5 ± 10.4	43.3 ± 6.1
7	59.2 ± 11.1	66.1 ± 3.6	32.5 ± 1.6	31.4 ± 11.4	19.9 ± 1.9
9	39.0 ± 8.3	59.3 ± 8.8	27.6 ± 5.3	Nil	Nil

I. galbana, *Isochrysis galbana*; *C. calcitrans*, *Chaetoceros calcitrans*; *N. salina*, *Nanochloropsis salina*; *P. lutheri*, *Pavlova lutheri*; *T. chuii*, *Tetraselmis chui*.

Table 2 ANOVA table on growth rate of *Holothuria spinifera* larvae on day 9 fed with single microalgae, with results of the LSD *post hoc* multiple comparisons test

Treatments value	Sum of squares	D.f.	Mean square	*F* ratio	*P*
Between groups	7513.991	4	1878.498	15.757	<0.001
Within groups	1192.173	10	119.217		
Total LSD	8706.164	14			

	N. salina	*P. lutheri*	*T. chuii*	*C. calcitrans*
I. galbana	<0.05	>0.05	<0.05	<0.05
N. salina		<0.05	>0.05	<0.001
P. lutheri			<0.05	<0.05
T. chuii				<0.001

I. galbana, Isochrysis galbana; *C. calcitrans, Chaetoceros calcitrans*; *N. salina, Nanochloropsis salina*; *P. lutheri, Pavlova lutheri*; *T. chuii, Tetraselmis chui*, ANOVA, analysis of variance; LSD, least significant difference.

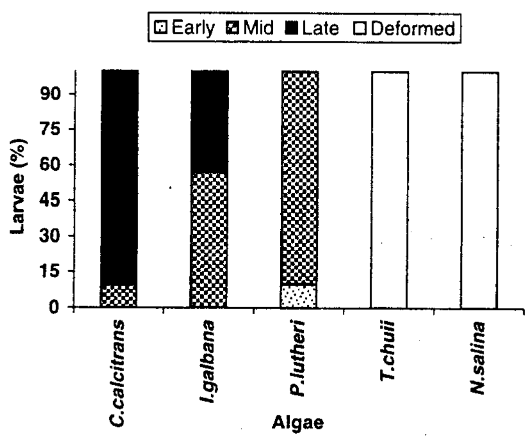

Figure 1 Percentage of early-, mid- and late-stage auricularia of *Holothuria spinifera* fed with single microalgae on day 9.

On day 9, the larvae fed with *I. galbana* showed the highest survival percentage of 67.8%, followed by 64.4% for *C. calcitrans*, 44.4% for *N. salina* and 34.3% for *P. lutheri* (Table 3). The survival of the larvae fed with *T. chuii* drastically decreased from 83.0% to 33.5% on the fifth day and further to 9.1% on day 9, which is the lowest (Table 3). The ANOVA on differences between the mean survival rate of larvae fed with different single microalgae indicated significance ($P<0.001$), and in the multiple comparison test, the differences in the survival rate of *I. galbana*-and *C. calcitrans*-fed larvae to the *T. chuii*, *P. lutheri* and *N. salina* were highly significant ($P<0.001$) (Table 4).

From the one-way ANOVA and the multiple comparisons of *I. galbana*, *C. calcitrans* alone-fed larvae, it was noticed that there was no significant difference in the survival rate, whereas the differences in the growth rate between them were highly significant ($P<0.05$). Moreover, occurrence of 90% of late auricularia in *C. calcitrans*-fed larvae indicated *C. calcitrans* as the most suitable single algal diet for the larvae of *H. spinifera*. Similarly, Ito and Kitamura (1997) and Battaglene (1999) found *C. gracilis* and *C. mulleri* to be the best larval feed for *S. japonicus* and *H. scabra* respectively.

Combined feed experiment

On days 6 and 10, the larvae fed with a combined feed registered the maximum growth rate followed by *C. calcitrans* and *I. galbana*. On day 12, the larvae fed with a combined feed registered the highest growth rate of 42.5 μm day^{-1} (mean length: 942 μm), followed by 37.0 μm day^{-1} (882 μm) with *C. calcitrans* and 32.6 μm day^{-1} (832 μm) with *I. galbana* (Table 5). And the differences in the growth rates were highly significant ($P<0.001$) (Table 6).

In the larval development, 40% of the larvae fed with *I. galbana*, 85% with *C. calcitrans* and 100% with combined feed attained the late auricularia stage on day 12 (Fig. 2).

On day 6, the larvae fed with *I. galbana* registered the maximum survival percentage of 92.6%, followed by 88.9% with combined feed and 71.1 μm with *C. calcitrans* (Table 7). On day 12, the larvae fed with a combined feed of *I. galbana*+*C. calcitrans* registered the highest survival of 72.6% followed by 67.8% with *I. galbana* and 51.3% with *C. calcitrans* (Table 7).

In the combined algal diet experiment, treatment of ANOVA showed that the differences in the mean growth and survival rates of larvae were highly significant ($P<0.05$) (Table 6 and 8). In the multiple comparisons, all differences in the mean survival and growth rates of the larvae were found highly significant ($P<0.01$) except for the differences in the mean survival rate between the larvae fed with *I. galbana* and *I. galbana*+*C. calcitrans* (Table 6 and 8).

The 100% survival of late auricularia with a 42.5 μm day^{-1} growth rate and a 72.6% survival rate in the *I. galbana*+*C. calcitrans*-fed larvae than either of the single algal diets indicated combined algal feed as being effective for the larvae of *H. spinifera*. Similarly, Shuxu and Gongchao (1981) reported that larvae of *S. japonicus* fed with a mixture of *Dicrateria*,

Table 2 ANOVA table on growth rate of *Holothuria spinifera* larvae on day 9 fed with single microalgae, with results of the LSD *post hoc* multiple comparisons test

Treatments value	Sum of squares	D.f.	Mean square	*F* ratio	*P*
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Within groups	1192.173	10	119.217		
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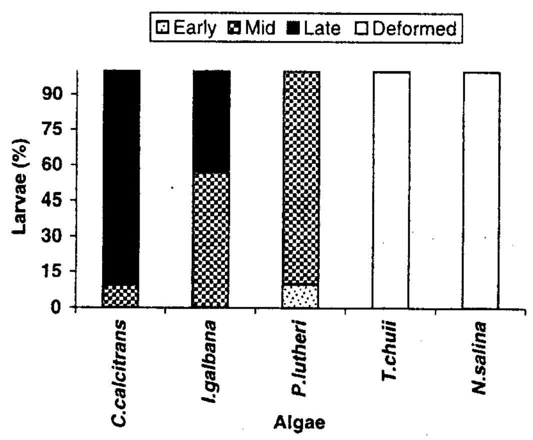
	N. salina	*P. lutheri*	*T. chuii*	*C. calcitrans*
I. galbana	<0.05	>0.05	<0.05	<0.05
N. salina		<0.05	>0.05	<0.001
P. lutheri			<0.05	<0.05
T. chuii				<0.001

I. galbana, Isochrysis galbana; C. calcitrans, Chaetoceros calcitrans; N. salina, Nanochloropsis salina; P. lutheri, Pavlova lutheri; T. chuii, Tetraselmis chui, ANOVA, analysis of variance; LSD, least significant difference.



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The 100% survival of late auricularia with a 42.5 μm day^{-1} growth rate and a 72.6% survival rate in the *I. galbana*+*C. calcitrans*-fed larvae than either of the single algal diets indicated combined algal feed as being effective for the larvae of *H. spinifera*. Similarly, Shuxu and Gongchao (1981) reported that larvae of *S. japonicus* fed with a mixture of *Dicrateria*,

Table 3 Mean (± SE, $n = 30$) survival percentage of the auricularia of *Holothuria spinifera* fed with single microalgae

Days	*I. galbana*	*C. calcitrans*	*N. salina*	*P. lutheri*	*T. chuii*
3	87.4 ± 7.3	92.7 ± 5.1	79.6 ± 1.4	85.5 ± 2.2	83.0 ± 6
5	83.3 ± 7.1	84.9 ± 7.3	74.5 ± 1.5	67.0 ± 8.1	33.5 ± 10.7
7	73.9 ± 8.6	78.9 ± 5.1	65.1 ± 2.5	50.1 ± 6.8	17.3 ± 8.1
9	67.8 ± 3.6	64.4 ± 7.2	44.4 ± 6.8	34.3 ± 7.6	9.1 ± 6.2

I. galbana, *Isochrysis galbana*; *C. calcitrans*, *Chaetoceros calcitrans*; *N. salina*, *Nanochloropsis salina*; *P. lutheri*, *Pavlova lutheri*; *T. chuii*, *Tetraselmis chui*.

Table 4 ANOVA table on numbers of *Holothuria spinifera* larvae surviving on day 9 fed with single microalgae with results of LSD *post hoc* multiple comparisons test

Treatments value	Sum of squares	D.f.	Mean square	F ratio	P
Between groups	6526.824	4	1631.706	21.276	<0.001
Within groups	766.940	10	76.694		
Total LSD	7293.764	14			

	N. salina	*P. lutheri*	*T. chuii*	*C. calcitrans*
I. galbana	<0.05	<0.001	<0.001	>0.05
N. salina		>0.05	<0.001	<0.05
P. lutheri			<0.05	<0.05
T. chuii				<0.001

I. galbana, *Isochrysis galbana*; *C. calcitrans*, *Chaetoceros calcitrans*; *N. salina*, *Nanochloropsis salina*; *P. lutheri*, *Pavlova lutheri*; *T. chuii*, *Tetraselmis chui*, ANOVA, analysis of variance; LSD, least significant difference.

Table 5 Mean (± SE, $n = 30$) growth rate ($\mu m\,day^{-1}$) of the auricularia larvae of *Holothuria spinifera* fed with combined microalgae

Days	*I. galbana*	*C. calcitrans*	*I. galbana* + *C. calcitrans*
6	53.5 ± 0.9	54.3 ± 4	63.9 ± 2.8
10	37.5 ± 3.9	41.8 ± 0.3	47.5 ± 0.2
12	32.6 ± 0.8	37.0 ± 0.8	42.5 ± 0.7

I. galbana, *Isochrysis galbana*; *C. calcitrans*, *Chaetoceros calcitrans*.

Platymonas, *Nitzschia*, *Dunaliella* and *Torulopsis* showed better survival and growth rate than those fed with *Dicrateria shanjiangensis*. James, Gandhi *et al.* (1994) and Ramofafia, Gervis and Bell (1995) also reported combined microalgae as the suitable feed for the larvae of *H. scabra* and *H. atra* respectively. Battaglene (1999) also observed that the mixture of *Rhodomonas salina* and *C. mulleri* is more effective for feeding the larvae of *H. scabra* than either of the microalgae alone.

Table 6 ANOVA table on growth rate of *Holothuria spinifera* larvae on day 12 fed with combined microalgae with results of LSD *post hoc* multiple comparisons test

Treatments value	Sum of squares	D.f.	Mean square	F ratio	P
Between groups	149.866	2	74.933	60.780	<0.001
Within groups	7.397	6	1.233		
Total LSD	157.263	8			

	C. calcitrans	*I. galbana* + *C. calcitrans*
I. galbana	<0.05	<0.001
C. calcitrans		<0.001

I. galbana, *Isochrysis galbana*; *C. calcitrans*, *Chaetoceros calcitrans*, ANOVA, analysis of variance; LSD, least significant difference.

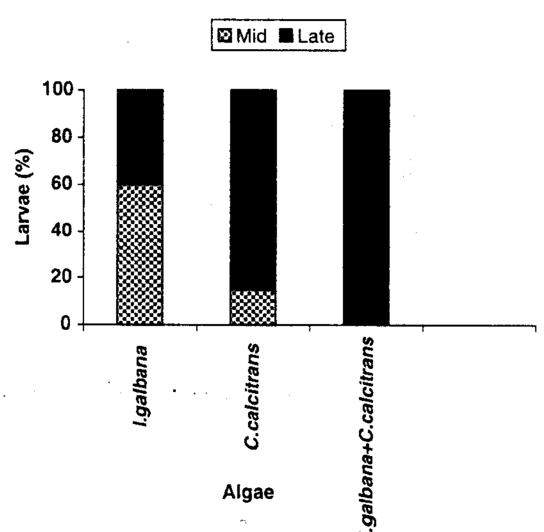

Figure 2 Percentage of mid- and late-stage auricularia of *Holothuria spinifera* fed with combined microalgae on day 12.

The development of seed production techniques through a hatchery system has generated significant interest in recent years as the alternate way for carrying out the stock enhancement program for the

Table 7 Mean (± SE, $n = 30$) survival percentage of the auricularia of *Holothuria spinifera* fed with combined algae

Days	*I. galbana*	*C. calcitrans*	*I. galbana*+ *C. calcitrans*
6	92.6 ± 3.1	71.1 ± 1.8	88.9 ± 3.6
10	70.9 ± 7.7	61.4 ± 5.5	80.6 ± 4.5
12	67.8 ± 7.6	51.3 ± 3.4	72.6 ± 2.1

I. galbana, Isochrysis galbana; C. calcitrans, Chaetoceros calcitrans.

Table 8 ANOVA table on number of *Holothuria spinifera* larvae surviving on day 12 fed with combined microalgae with results of LSD *post hoc* multiple comparisons test

Treatments value	Sum of squares	D.f.	Mean square	*F* ratio	*P*
Between groups	742.502	2	371.251	7.589	<0.001
Within groups	293.511	6	48.919		
Total LSD	1036.013	8			

	C. calcitrans	*I. galbana*+ *C. calcitrans*
I. galbana	<0.05	>0.05
C. calcitrans		<0.05

I. galbana, Isochrysis galbana; C. calcitrans, Chaetoceros calcitrans; LSD, least significant difference.

exploited sea cucumber population in most of the countries. The pelagic larva being the vulnerable stage in the culture of sea cucumbers. Ramofafia, Battaglene and Byrne (2000) suggested that the optimum feed and feeding regimes determine the success in the seed production in hatchery. In the present study, the highest growth rate (59.3 and 42.5 μm day^{-1}) and more occurrence of late auricularia (90% and 100%) in the larvae fed with *C. calcitrans* alone and *I. galbana*+*C. calcitrans* fed larvae, respectively, indicated that *C. calcitrans* alone or in combination with *I. galbana* is an effective feed for the larvae of *H. spinifera*. Thus, refining and standardization of hatchery techniques will be beneficial for the large-scale production of juvenile sea cucumbers, which could be utilized for farming or enhancing the natural stock.

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