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EFFECTS OF TEMPERATURE, SALINITY AND FEED ON THE SURVIVAL AND GROWTH OF JUVENILE SEA CUCUMBER, *Bohadschia marmorata*

M.J. Chu Yuan Kee[1], *C. Appadoo*[2*]

Department of Biosciences, Faculty of Science, University of Mauritius, Reduit, Mauritius.

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ABSTRACT

The survival and growth of tropical sea cucumber, Bohadschia marmorata (Jaeger) was investigated. Juvenile B. marmorata were collected in the wild, from La Prairie, in the south west coast of Mauritius and was reared at Albion Fisheries Research Centre (AFRC). The sea cucumbers were stocked at a density of six individuals per tank in three separate experiments: temperature (26, 28 and 32^{0}C), salinity (25, 30 and 36‰) and feed (macro algal paste and a mixture of macro algal paste supplemented with 15% powdered fish feed). During temperature and salinity treatments, B. marmorata were fed with a macro algal paste only. Temperature and salinity treatments were monitored for 63 days and feed experiment for 35 days. B. marmorata showed a negative growth in all salinity and temperature treatments. Survival of B. marmorata was 91.67 and 66.65% in temperature 28 and 32^{0}C, respectively. All B. marmorata survived at temperature 26 ^{0}C and had a specific growth rate of –0.78% day^{-1}. The specific growth rate of B. marmorata at temperature 28^{0}C and 32^{0}C was –1.03% day^{-1} and –1.23% day^{-1} respectively. B. marmorata displayed 100% survival rate at salinities 25 and 36‰, and 91.67% at salinity 30‰. Specific growth rates of B. marmorata showed a descending trend in the order of salinity 25‰ (-1.07% day^{-1}) >30‰ (-1.05 % day^{-1}) >36‰ (-0.78 % day^{-1}). Better growth was obtained when B. marmorata were fed with a mixture of algae and fish feed (0.31% day^{-1}), as compared with a feed containing pure algal paste only (–1.34%day^{-1}). This study provides some indications on the rearing conditions for B. marmorata, but more studies are needed for mariculture purposes.

Keywords: *sea cucumber, Bohadschia marmorata, temperature, salinity, feed, survival, growth*

***Correspondence** : Phone : 230-4541041, Fax : 230-4656928, email chandani@uom.ac.mu

INTRODUCTION

Sea cucumber, also known as holothurian is of important commercial value. Its product, bêche-de-mer, is considered as a delicacy product in various Asian countries and is also consumed for its medicinal and pharmaceutical properties (James, 2001). Today sea cucumbers form a large, vauable export fishery throughout most South Pacific, Asian countries, and lately New Zealand, Africa, South America and North America. The Western Central Pacific Ocean is the main producing region in the world, which exports bêche-de-mer mainly to Hong Kong, Singapore, Taiwan, China inland as

well as Europe and North America (Conand, 2004). Due to over exploitation and high bêche-de-mer market demands, sea cucumbers have become popular aquaculture animals in several countries (James, 2004).
Temperature and salinity, besides nutritional factors, are important environmental parameters influencing growth of sea cucumbers. A number of works have focused on the growth of mainly larvae and juveniles in the wild as well as in captivity (Ramofafia *et al.* 1996; Battaglene *et al.* 1999; Sun *et al.* 2004; Asha and Muthiah 2005; Yuan *et al.* 2006; Yang and Yuan 2007). However no published data is available on the growth of the tropical sea cucumber *Bohadschia marmorata*.

In Mauritius, sea cucumbers also known as "Barbara", are hand picked by fishermen for local consumption. They are also sold to hotels and to companies, which export the processed products to Asian countries. A survey done in two lagoons of Mauritius (Luchmun *et al.*, 2001) have shown that *B. marmorata* was one of the most abundant species found. It is also one of the commercially important species in the island among *Actinopyga echinites*, *Holuthuria atra*, *Holothuria nobilis*, *Holothuria scabra*, *Thelenota ananas* and *Stichopus chloronotus*. Sea cucumber fisheries in Mauritius have been growing to scale largely in the past few years and there are about 11 edible species in the island (Conand, 1998).
Bohadschia marmorata is known for its burying behaviour and for releasing cuvierian tubules when disturbed. Clouse (1997) worked on the taxonomy of *B. marmorata* from Micronesia and described its burying rhythm as circadian. Laxminarayana (2005) studied its seed production and also attempted induced asexual reproduction, but no endeavour was made on its culture. The current study aims in providing some data on the survival and growth of juveniles *B. marmorata* under different environmental conditions. Understanding the growth of this species can eventually enable culturists to manage its culture in a view to supply the bêche-de-mer market and this without the need to dip from natural stocks.

MATERIALS AND METHODS

The experimental work was carried out at Albion Fisheries Research Centre (AFRC), from October 2006 to February 2007. Collection (hand-picking) of juvenile *B. marmorata* was done at low tide by snorkeling from La Prairie Lagoon, in the South West of Mauritius (latitude 20°29'18.88"S; longitude 57°21'41.39"E). They were found at about 2 m depth. Individuals had body length 9-15 cm. The animals were transported in buckets in a minimum time to Albion Fisheries Research Centre where they were maintained in an outdoor circular tank (diameter 300 cm x height 75cm) containing seawater for 1 week prior to the beginning of experiments. The salinity of the seawater in the tank was 36‰ and temperature was 26.2^{0}C, similar to the collection site of sea cucumbers. Sea cucumbers fed on algae (*Dictyota* spp. and *Hydroclathrus clathratus*) that had colonized the pool.

General experimental design and set-up

Three experiments were conducted indoors. Salinity and temperature experiments were carried out simultaneously and different feed experiment afterwards. Circular polycarbonate tanks of 500-L and diameter 95 cm x height 75 cm were used. Tanks were washed with seawater and labeled. A layer of sand 5-6 cm deep was spread on the bottom of each tank to enable the sea cucumbers to bury themselves. Seawater in each tank was then brought to 500 L. For each treatment, six *B. marmorata* were stocked per tank.
The tanks were aerated continuously to allow water mixing and ensure adequate dissolved oxygen (DO). One aerator was provided for

each tank, the end of which was fixed with an air stone. A daily water exchange was carried out in all tanks in order to ensure good water quality. The water exchange was done with great care so that sea cucumbers were not injured or stressed. Sand in each tank was stirred, water swirled and 50% of water was exchanged at each water replacement. The sides of the tanks were regularly cleaned using a sponge, and excess food and faeces were prevented from accumulating by siphoning the bottom of tanks each morning. As for the sand substratum, it was changed every fortnight. A photoperiod regime of 12 hours light and 12 hours darkness were used as per Ponce-Palafox *et al.* (1997). Light was automatically switched on at 6.00 and switched off at 18.00.
The daily ration of feed in all experiments was equivalent to 10% of the wet biomass of the sea cucumbers per tank.

Experimental set-up
Effect of temperature on growth and survival of B.marmorata

The survival and growth of juvenile *B. marmorata* were assessed at two temperature levels; 28^{0}C and 32^{0}C (Table 1). A control treatment was also conducted (room temperature, 26^{0}C). Juveniles *B. marmorata* used in this experiment were randomly selected from the outdoor pool. 24 sea cucumbers with wet weight range 45.27 – 79.56g (64.77 ± 13.62g, mean ± S.D.) were used. The sea cucumbers were then stocked in four tanks, such that in each tank there were 6 individuals. For temperature treatment 28^{0}C (T_{28}) and 32^{0}C (T_{32}), the water temperature of the four tanks was initially at 26^{0}C. To increase water temperature, titanium rod heaters (1000W) with a thermostat (DEL THERMO, Japan) were used. The thermostat controls the on/off switch of the heater. Both rod heater and thermostat were suspended in the water. During the temperature acclimatisation period, the water temperature of all four tanks was gradually increased by a rate of 1^{0}C per day so as to avoid any thermal shock to the sea cucumbers. Within two and six days, desired temperatures of 28^{0}C and 32^{0}C were achieved respectively. After the tanks have attained final temperatures, the sea cucumbers were reared for 7 more days making the acclimatisation period 13 days (Zhang *et al*., 2006). As for the control treatments, 12 *B. marmorata* of wet weight range 38.71 – 46.56g (42.78 ± 2.66g) were used. 6 animals were stocked per tank. Temperature acclimatisation period for the control treatment was 7 days.

Effect of salinity on survival and growth of B. marmorata

Two different salinity levels were used: 25‰ and 30‰ (Table 1). Another salinity level was set at 36‰ that acted as the control. Desired salinities were obtained by mixing seawater (36‰) with fresh water (Ponce-Palafox *et al.* 1997). Prior to the experiment, 24 *B. marmorata* with wet weight range 65.27 – 101.83g (85.88 ± 11.17 g, mean ± S.D.) were taken from the outdoor pool. The sea cucumbers were divided into two groups of 12 and 6 individuals were stocked per tank. The sea cucumbers were used for salinity treatment 25‰ (S_{25}) and 30‰ (S_{30}). The salinity level in all tanks was initially 36‰. Gradually, the salinity of the water was decreased from 36‰ to the desired level, that is, 25‰ and 30‰ at a rate of 2-3‰ per day (Zhang *et al.* 2006). The final salinity level was reached after 3 and 5 days respectively. The sea cucumbers were then reared for a further 7 days in the attained salinity level. Thus the period of acclimatisation was 12 days. For the control treatment 12 sea cucumbers with wet range 38.71 – 46.56g (42.78 ± 2.66g, mean ± S.D.) were used. 6 individuals were stocked in each two tanks. Acclimatisation of *B. marmorata* totalled 7 days. Salinity of seawater in the control tanks was 36‰.

Effect of feed on survival and growth of B. marmorata

The effect of feed on survival and growth of juvenile *B. marmorata* was investigated by using two types of feed (Table 1). The first type of feed (Feed A) was composed of ground algal paste only (a mixture of *Hydroclathrus clathratus, Padina boryana* and *Dictyota* species). The other feed (Feed B) was made up of 85% Feed A which was supplemented with 15% powdered fish feed. Prior to the experiment, 24 *B. marmorata* of wet weight range 14.17 – 50.26g (29.2 ±13.51g, mean ± S.D.) were transferred from the outdoor pool into tanks. 6 sea cucumbers were stocked per tank. The animals were allowed to acclimatise for 7 days during which one group was fed with Feed A (F_A) and the other group with Feed B (F_B).

Analysis of water sample

For all experiments, salinity and temperature were recorded twice a day; at 9:00 and 15:00, whilst pH and DO were measured every two days. Salinity was measured with a portable refractometer (ATAGO S-MILL E, Japan), temperature with a standard mercury thermometer and pH with a HM-5S pH meter (TOA, Japan). Water samples were taken from each tank before the next water exchange to measure pH and DO. The amount of DO was obtained by using the Winkler-sodium azide modification method. A condition of 6.04 - 6.27 mg L^{-1} DO was maintained throughout the experiments, which is above the DO requirement for aquaculture (>5 mg L^{-1}). As for the pH, it was kept within pH range for aquaculture (7 - 8.5).

Collection of data

Changes in the wet weight of *B. marmorata* were recorded throughout the experiments. Weighing was done at one week's interval and at similar time during the day. The sea cucumbers were removed using nylon net and transferred into bare tanks (seawater only) for about 18-20 h prior to weighing. This process enabled sea cucumbers to eject their faeces while preventing them from ingesting sand. *B. marmorata* were removed from each tank and left outside for about one and a half minute. The animals were then stimulated to expel excess water remaining in the respiratory trees by gently squeezing the posterior half. They were then blotted dry with a sponge and weighed before they were placed back in their respective tank. Each sea cucumber in each tank was weighed individually. Weighing was done with an electronic balance and recorded to the nearest 0.01 g. The handling technique was standardized as much as possible.

Data processing

Survival and specific growth rate (SGR) were calculated as follows:

$$\text{Survival (\%)} = \frac{\text{Number of sea cucumbers at the end of experiment}}{\text{Number of sea cucumbers at the start of experiment}} \times 100$$

$$\text{SGR (\%day}^{-1}) = 100\,(\ln W_2 - \ln W_1)/\,T$$

Following Dong *et al.* (2006).

Where W_1 and W_2 are mean initial and final wet weight of sea cucumbers in each tank, and T is the duration of the experiment in days.

SGR was calculated separately for each treatment and its duplicate, after which both SGR was combined and a mean calculated. This mean was used to compare SGR among treatments.

Statistical analysis

Statistics were performed using software SPSS version 10.0. Possible inter-treatment differences in specific growth rate were analysed using one-way analysis of variance (ANOVA). Differences were considered significant if $p<0.05$.

Table 1: Factors under investigation. All set ups were run in duplicate and a stocking density of 6 individuals was used.

Factors under investigation	*Variable conditions*	*Constant conditions*	*Experimental Duration*
Temperature (T-°C)	28°C	Feed A Salinity: 36‰	63 days
	32°C		
Salinity (S-‰)	25‰	Feed A Temperature: 26°C	63 days
	30‰		
Feed Type (F)	Algal paste (Feed A): (*Hydroclathrus clathratus, Padina boryana* and *Dictyota* spp)	Salinity: 36‰ Temperature: 26°C	35 days
	Feed B: 85% Algal paste: (*Hydroclathrus clathratus, Padina boryana* and *Dictyota* spp) + 15% powdered fish feed		
Control		Salinity: 36‰ Temperature: 26°C Feed A	63 days

RESULTS DAN DISCUSSION

Results

Survival

Survival of juvenile *B. marmorata* reared in different temperature, salinity and feed treatments is given in **(Fig. 1)**. In experiments where mortalities were observed, dead animals were removed from tanks but not replaced. Among the three temperature treatments in this experiment, survival was highest (100%) in control (26^{0}C) followed by temperature 28^{0}C (91.67%) and temperature 32^{0}C (66.65%). Survival of juvenile *B. marmorata* ranged from 91.67% to 100% for salinity experiment. Death of 1 sea cucumber at salinity 30‰ occurred in the first week of experiment, and from then on, no mortality was further observed. On the other hand, all sea cucumbers survived at salinity 25‰ and in the control treatment. No mortality occurred during the course of the 35-day feed experiment.

Growth

Specific growth rate of *B. marmorata* varied in different temperature treatments **(Fig. 2).** At temperature 28^{0}C, SGR of *B. marmorata* was found to be –1.03% day^{-1} while in temperature treatment 32^{0}C, sea cucumbers showed a negative growth rate of –1.23% day^{-1} which was the greatest of all three treatments.

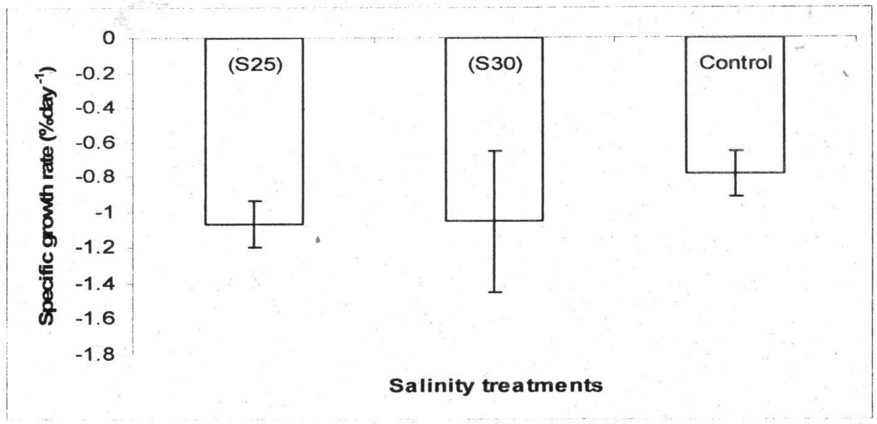

Fig.3 Specific growth rate of *B. marmorata* in different salinity treatments during 63-days experiment (Bars represent standard deviations of the means).

Results showed that growth rate of juvenile *B. mamorata* fed with Feed A were negative with a value of -1.34% day^{-1}. On the other hand, juvenile *B. marmorata* showed a daily positive growth rate of 0.46% when fed with Feed B **(Fig.4).** The difference between mean SGR of the two feed treatments was statistically significant (p = 0.007, F = 8.6 df = 2).

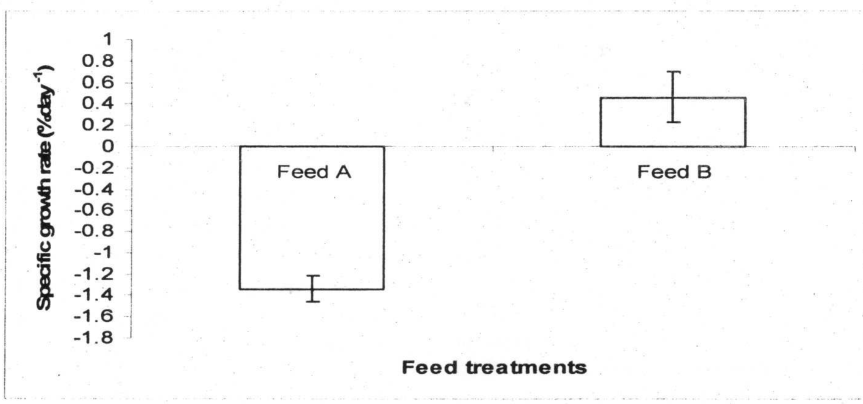

Fig. 4 Specific growth rate of *B. marmorata* reared in different feed treatments for 35 days. (Bars represent standard deviation of means).

Discussion

The study showed that temperature had a considerable effect on the survival and growth of *B. marmorata.* The animals seemed to cope well with a temperature of 26^{0}C with no mortality, but as temperature increased *B. marmorata* became more vulnerable since SGR became more negative and survival declined further. Previous studies showed that best growth in sea cucumbers is species-specific and that temperature outside tolerance range could reduce feeding activity. *Holothuria atra* showed best growth rate when reared at temperature 28^{0}C (Seeruttun *et al*., 2007). The appropriate temperature for *A. japonicus* growth was shown to be between 12 and 21^{0}C, and the optimum was 16-18 ^{0}C (Dong *et al*. 2006). Optimal temperature tolerance for *S. japonicus* was reported to range between 5-15^{0}C, while below 3 and above 17^{0}C, its feeding activity declined sharply (Chen, 1990). Temperature is known to be an important regulator of energy flow and therefore affects growth rate in marine organisms. Landau (1992) mentioned that a temperature below and above optimal could result in declined and raised metabolic rate respectively, while affecting respiratory, feeding and assimilation rate. Therefore, it might be possible that a higher temperature of 28 and 32^{0}C reduced the feeding activity in *B. marmorata* such that they lost more weight as compared in the control treatment.

Except for one sea cucumber reared at salinity 30‰, results indicate that *B. marmorata* can tolerate a salinity range of 25-36‰. However, growth of *B. marmorata* was negatively affected in all salinity treatments. Although sea cucumbers showed negative growth in all three salinity treatments, a lower salinity of 25‰ and 30‰ increased further the rate at which they lost weight. Sea cucumbers have poor osmotic regulation, and since they lack osmotic organs a salinity fluctuation can bring about alterations in their physiology. Osmotic stress faced by *B. marmorata* reared in salinity 25‰ and 30‰ might have also affected their burrowing activity as reported by Mercier *et al*. (1999) for *H. scabra*. *B. marmorata* might have adopted the same strategy as *H. scabra* in that they remained buried for longer time period in the sand.

In the control treatment, it was expected that *B. marmorata* would show positive growth because the conditions of water temperature and salinity were close to that where the sea cucumbers were collected. However, specific growth rate of *B. marmorata* was negative. This negative growth could due imposed stress on the animals under laboratory conditions. Menge and Sutherland (1987) reported that stress could affect organisms at the molecular and cellular level (cited in Sanford 2002). Changes in the sea cucumber physiology due to stress could have resulted in altered body normal functioning, which could account for the negative growth. Stress associated with frequent handling during this study caused *B. marmorata* to release their cuvierian tubules. It might be possible that *B. marmorata* spent most of their energy mainly to regenerate cuvierian tubules rather than spending it on tissue growth. Another explanation could be that *B. marmorata* might not be adapted to grow in captivity. Similarly, Conand (1990) found that *A. echinites* lost weight when they stayed more than a few months in the aquarium. In the same way, Shelley (1981) attempted the farming of juveniles *H. scabra* and reported that although the conditions closely resemble the natural environment, the individuals failed to gain weight (cited in Conand 1990). Indeed in several studies, holothurians usually decrease in weight when held in captivity (Ebert, 1978, Conand, 1983, Conand 1989a, Wiedemeyer, 1992 as cited by Ramofafia *et al*. 1996).

A significant difference in specific growth rate was observed when sea cucumbers fed on the two different feeds. *B. marmorata* fed on the formula of 85% ground algae and 15% powdered fish feed had a significantly higher growth rate than those fed on pure algal paste only. In their natural habitat, deposit-feeding holothurians like *B. marmorata* feed on other organisms other than macro algae. Sediments ingested by deposit-feeding holothurians comprise mainly inorganic compounds, organic detritus (seagrass, algae, dead and decaying animals), micro organisms (bacteria, diatoms, protozoans and cyanophyceans) and faecal pellets of other animals or their own faecal pellets (Massin, 1982 cited in Dar and Ahmad 2006). This might suggest that *B. marmorata* rely on a combination of animal

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and plant food sources for optimal growth, may be with a preferential utilisation for animal protein. Wang and Cheng (2004) reported that a mixed food diet is preferred when rearing sea cucumbers. A balanced feed not only increases survival rate, but also accelerates growth of juvenile sea cucumbers (Sui, 2004). A study done on the nutrient requirement of *A. japonicus* showed that growth rate and digestion efficiency of the cultured animals increased with the protein content contained in the formulated feeds (Sun *et al.* 2004). Likewise, *B. marmorata* fed with a supplement of protein showed a better growth. Fish feed are high sources of essential amino and fatty acids, especially formulated to meet the high protein level requirement in fish (Anderson *et al.* 1993). Highest growth rate in *A. japonicus* (Sun *et al.* 2004) and *S. japonicus* (Joo-Young *et al.* 2007) were observed when they were fed with 21.5% and 30% protein, respectively.

CONCLUSION

The outcome of the separate experiments revealed that juvenile *B. marmorata* are difficult to rear in captivity. The sea cucumbers were likely to lose weight than to increase in size. However when fed with a source of protein like fish feed, *B. marmorata* showed promising results for cultivation. The current study gives some indications on the factors to be considered for mariculture of *B. marmorata*, but further studies still need to be carried out to establish the optimal conditions for culture of *B. marmorata.*

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