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A Study on the Factors Influencing the Growth and Survival of Juvenile Sea Cucumber, Holothuria atra, under Laboratory Conditions

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Abstract

In recent years the sea cucumber fishery has emerged as a lucrative business in Mauritius. However, few studies on these marine detritivorous animals have been carried out in the island either in the wild or for aquaculture. The aim of the present study was to investigate the influence environmental factors such as salinity and temperature and biological factors such as stocking density and feed type on the growth and survival of juvenile sea cucumbers *Holothuria atra.* The study was conducted from mid-October 2006 to end of January 2007 period. For the experiment, 150 juvenile *H. atra* of similar size and weighing (27-30 g) were collected from Le Morne and Albion Lagoons. The experiments were carried out in round polyether tanks of 2 m^3 with an area of 1.5 m^2 at Albion Fisheries

Research Centre. The juveniles *H. atra* (6 specimens per tank in duplicate) were reared at salinity 25, 30 and 35 ppt; temperature 26, 28 and 32°C; and were given two feed types (crushed algae and a formulated feed (85% crushed algae and 15% fish feed)). For studying stocking density duplicate tanks containing 6, 12 and 18 specimen (per sq m) were used. At one-week intervals, the sea cucumbers were starved for 24h and then weighed. The wet weight was used as an index for its growth performance over the experimental period. The highest specific growth rate (SGR) and survival were obtained at temperature of 28°C (1.43 ± 0.15 g and 100% respectively). At temperature 32°C, the SGR was a lower (0.78 ± 0.12 g) and a survival of 67%. Specific growth rate and survival was lowest in stocking density 18 (0.12 ± 0.23 g & 69% respectively). At salinity of 25 ppt there was decrease in SGR during the experimental period (-4.61 ± 0.29 g). Feeding *H. atra* with crushed algae produced little increase in growth (0.18 ± 0.16 g) while feeding with formulated feed the specific growth rate was notable (0.97 ± 0.14 g). Under the conditions of the experiments, growth and survival of juvenile *H.atra* were greatest at 35‰ salinity, temperature 28ºC, and a formulated feed made from algae and dried fish feed and density of 6 specimens per m^2.

Keywords: *Holothuria atra;* Salinity; Temperature; Stocking density; Feed type; Specific growth rate (SGR); Survival

**For correspondences and reprints*

1. INTRODUCTION

Recently the sea cucumbers fishery has developed into a lucrative business in the Asian countries and has just started to attract the local fisherman in the island of Mauritius (Toral-Granda, 2006).

Information on growth rates, larval ecology, habitat use, ecological role, and reproductive success, amongst others, is limited and in cases available for only a few species such as *Holothuria scabra* (Mercier *et al.*, 1999; Pitt and Duy, 2004) and *Apostichopus japonicus* (Xilin, 2004; Sun *et al.*, 2004). Few studies have been conducted on factors affecting the growth performance of post-settled juveniles of *Holothuria atra*, which may be due to the fact that they are seldom reared in captivity. Some of the studies of population growth, maturation and mortality include notes on small or young individual of sea cucumbers (Chen & Chen, 1992; Ramofafia *et al.,* 1997). Despite the commercial importance of sea cucumbers, their biology, ecology and population dynamics remain poorly understood.

The island of Rodrigues, Mauritius, has recently been established as a producer for the expanding 'bêche-de-mer' (processed-sea cucumber) industry and there is a critical requirement for information concerning its resident holothurian populations (Mrowicki, 2006). Studies of sea cucumbers in Mauritius has been done on the density distribution of some species at two lagoon only (Luchmun *et al.,* 2001) and on the asexual fission and induced spawning of *Bohadshia mormorata* and *Holothuria atra* (Laxminarayana, 2005, 2006).

The study aims to investigate the environmental factors (salinity and temperature) and biological factors (stocking density and feed type) influencing the growth and survival of sea cucumber, *Holothuria atra* under laboratory condition. The study will help in understanding the biology and would also help in a future restocking program as well as for an aquaculture possibility. The study would also help to investigate the optimum environmental and biological conditions for the culture of juvenile sea cucumbers *H. atra* under laboratory conditions.

2. MATERIALS AND METHODS

The study was carried out at Albion Fisheries Research Centre (AFRC) from 10th October 2006 to 26th January 2007. About 150 juvenile sea cucumbers *H. atra* were collected (hand-picked) from the lagoon at low tide of Albion (E57º27', S20°15') and Le Morne (E57º20', S20°28'). The juveniles of similar sizes (24-27 g) were transported to the centre in a minimum time to avoid stress in buckets and were held for two days prior to the start of experiment. A major difficulty in studying holothurians is having a valid measurement of growth. Weight measurement is difficult because of the amount of water in the respiratory trees can vary, as well as ingested sand in the gut and depend on stress condition. All the experiments were carried in duplicate so as to get an average value for each.

2.1 Experimental Setup

Experimental tanks consisted of round polyether (2 m^3 with an area of 1.5 m^2) and were filled with a layer of 15 cm of sand substratum, 1000 liters of sea water and an aerator (Figure 1). A formulated feed (FF) was prepared containing a mixture of crushed red algae (*Gracilaria species* and *Hydroclathrus clathratus*) (85%) and dry fish feed (15%). The fish feed was provided by the Albion Fisheries Research Centre. The juveniles were fed with the formulated feed twice daily (15% of the total specimen biomass per tank)

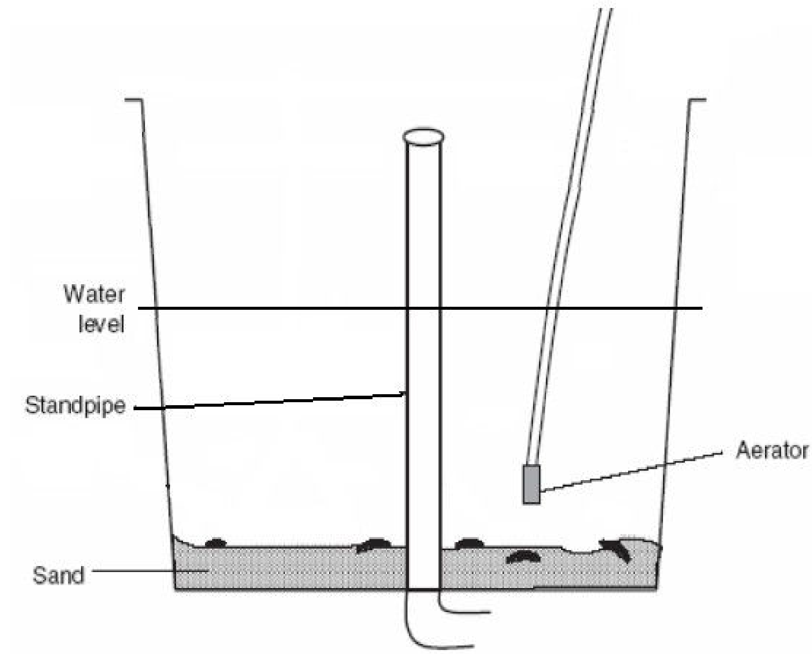


Figure 1: Tank setup, with aerator for oxygenation, standpipe for water evacuation from tank, a 15 cm layer of sand and 1000 *l* of sea water

2.2 Factors under investigation

There were 9 experimental tanks setup in duplicate including the control. The conditions in each experiment is shown in Table 1. Experiment 1 is an investigation of salinity tolerance of *H. atra.* Salinities of 25‰ and 30‰ were chosen because they induce responses such as eviceration of guts and burrowing in certain holothurians without detrimental effect (Mercier *et al.,* 1999). The required salinity was obtained by dilution and measured with a refractometer (*ATAGO S/Mill-E Japan*). The temperature in Experiment was 26 ºC and a stocking density of 6 specimens per 1.5 square metre. Experiment 2 is an investigation of the effect of temperature. Temperatures (28 and 32 ºC) were adjusted using a Rod Heater 1Kw with a thermostat (*Del Thermo, Nitto Co. Ltd, Japan*). The salinity in Experiment 2 was 35‰ (same as sea water) and stocking density of 6. Experiment 3 is an investigation of stocking density, 12 and 18 specimens per 1.5 square metre. Experiment 4 is an investigation of the effect of feeding *H.atr*a with two type of feed (formulated feed and Algal paste). In the control, a salinity of 35‰, temperature (26 ºC), a stocking density of 6 specimen were used and fed with the formulated feed

Experiment Number	Parameter under investigation		
	Factors	***Variable Conditions***	***Constant factors***
1	Salinity **(S-‰)**	25 ‰	Temperature 26º C Formulated feed Stocking density 6
		30 ‰	
2	Temperature **(T-ºC)**	28º C	Formulated feed Stocking density 6 Salinity 35‰
		32º C	
3	Stocking density (SD) (number per1.5 m^2)	12 specimens	Temperature 26 º C Formulated feed Salinity 35‰
		18 specimens	
4	Feed type (F-F)	Algal paste (F-AP)	Temperature 26º C Salinity 35‰ Stocking density 6
		Formulated feed	
	Control	Temperature 26º C, Salinity 35‰, Stocking density 6 Formulated feed	

Table 1: The factors under investigation

Every two days, water in each tank was replaced. Any excess food and excretory pellets were removed by siphoning at this time. Care was taken during sampling so as to avoid stress in these animals. To get a reliable measure of weight the specimens were placed in 30 litres plastic tanks for 24 hours in order to clear the sediment from its alimentary canal (Battaglene *et al.,* 1999; Sewell, 1991). After 24-h starvation, the external water was removed from specimens by drying them with a sponge. Specimens were weighed within 1 minute of removal from sea water (Battaglene *et al.,* 1999; Dong *et al.,* 2005). The mean weight of specimens from each experiment was used to get the growth parameters (Specific Growth Rate-SGR). Percentage survival was calculated. The values are presented as mean ± standard deviations. The growth performance was determined by:

The specific growth rate indicates the gain in biomass in the different experiment. Specific growth rate (SGR) in term of the wet weight which was calculated as the following (Dong *et al.*, 2005):

$$\text{“}SGR\ (\%\ day^{-}) = \frac{(\ln W_2 - \ln W_1)}{T} \times 100\text{”}$$

Where W2 and W1 are the final and initial wet weight of the sea cucumber, respectively; T is the duration time of the experiment in days

$$\text{“ Survival \%} = \frac{N_I - N_F}{N_I} \times 100\%\text{”}$$

N_I =Initial number of specimen & N_F =Final number of specimen

3. RESULTS

3.1 Specific Growth Rate (SGR) of juvenile sea cucumbers H. atra

The result showed different specific growth rate (SGR) of *H. atra* in the different experiments. The highest SGR (1.43 ±0.15 %) was at 28 °C and the lowest (-4.61 ±0.29 %) at 25 ‰ salinity.

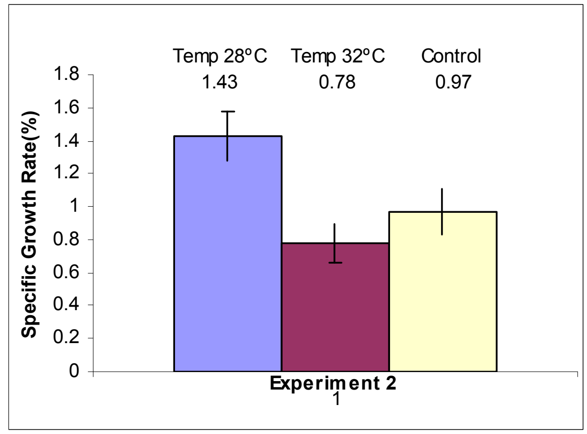
The experiment for *salinity* showed that a decrease in salinity leads to decrease in weight as well as survival (Fig. 2). The experiment on *temperature* showed that growth was greater at 28 °C than at 32 °C and temperature of 26 °C. In experiment *stocking density* 12 and 18, the SGR was very low. Growth at 12 individuals/1.5 m^2 was greater than at 18 individuals/1.5 m^2. The SGR in *SD12* and *SD18* was 0.23 (±0.20) % and 0.12 (±0.23) %. Finally in the *feed type experiment,* F-AP, the SGR was 0.18 (±0.18) % as compared to 0.97 (±0.14) % in the Control (Formulated feed-FF).

The highest SGR for the *Salinity experiment* was in Control (35‰). While there was no perceptible reaction in the tanks with salinity 30 ‰ and 35‰ (Control), there was a clear, uniform response to decreasing salinity. The low salinity of 25‰ resulted such that there decrease growth rate leading to a decrease in weight and survival. As for salinity 30‰, the growth rate was lower than the control.

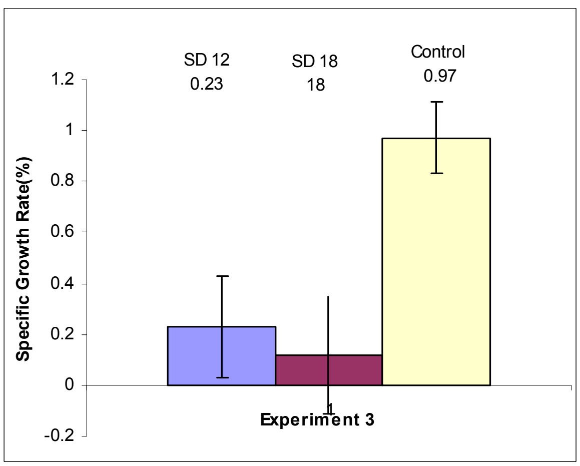
At ambient temperature 26ºC (Control), the growth rate of the juveniles *H. atra* increased as the temperature increased to 28ºC. However, a further increase in temperature led to a decrease in growth rate. In the present study higher growth and survival were observed in temperature 26ºC and 28ºC whereas in temperature 32ºC the juvenile *H.atra* had a low growth rate together with a decrease in survivorship was obtained.

The growth of juveniles was highly variable and density-dependent. The juvenile sea cucumbers held at higher densities (SD12 and SD 18), subsequently grew very slowly. Some specimen appeared to be stunted as a result of being held at higher density.

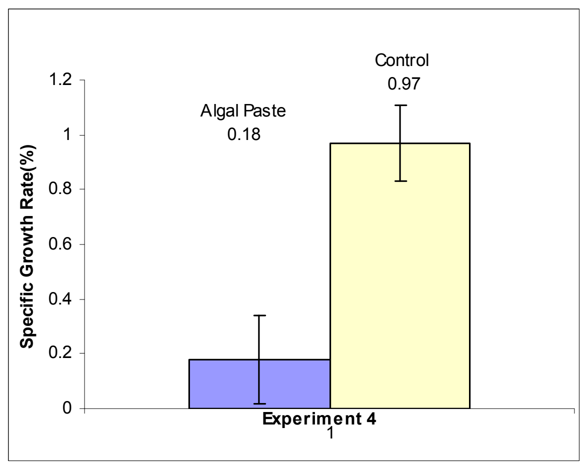
Salinity



Temperature



Stocking Density

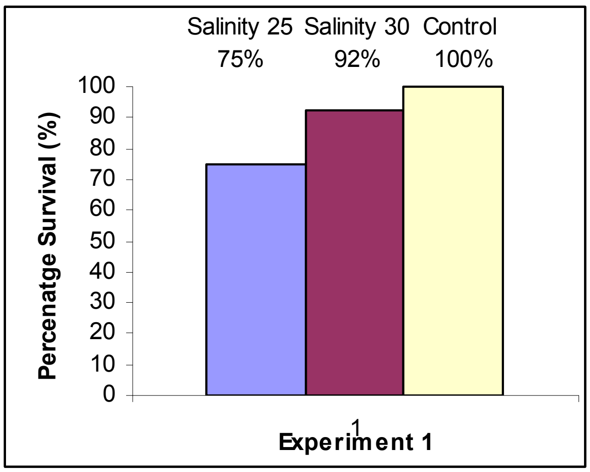


Feed type

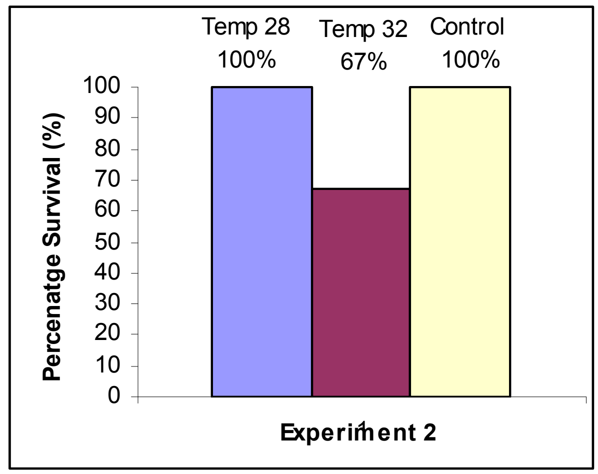
Figure 2: Specific Growth Rate (SGR) of juvenile sea cucumbers H. atra.

3.2 Percentage Survival

The mean percentage survival (Fig. 3) of juvenile sea cucumbers *H. atra* varied such that at temperature 28ºC, salinity 35‰ and stocking density 6, it was highest whereas at Temperature 32ºC, salinity 25‰ and stocking density 18, it was lowest(69%). As for the Control, the survival rate was 100%. The lowest survival rate was 67% in *Temp 32* followed by 92% in both *Salinity 30* and *Feed type F-AP*. In *SD18*, the survival rate was 69% as compare to *SD12* at 88%.

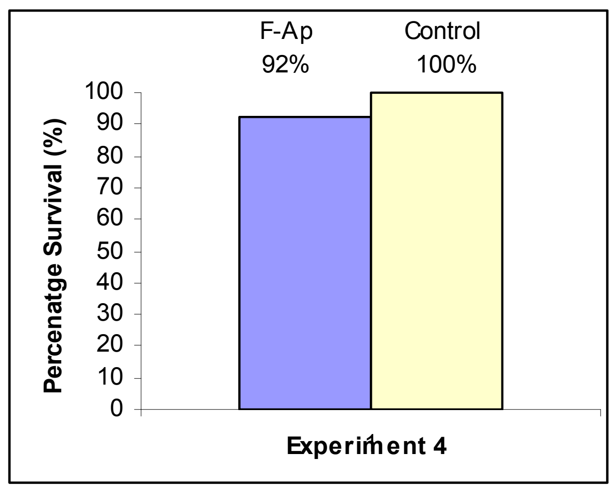


Salinity



Temperature

Stocking Density



Feed type

Figure 3: Percentage Survival of juvenile sea cucumbers H. atra in different experiments.

4. DISCUSSION

The juvenile sea cucumbers *H. atra* had different growth rates under the different conditions. The juvenile *H. atra* studied in the low salinity (25‰) and under high stocking density of 12 and 18 had a low specific growth rate (SGR). These conditions were highly unfavorable culture of sea cucumber. However in the experiment temperature 28º, salinity 30‰ and control there was regular increase in the weight throughout the experimental period. The feeding of juvenile with algal paste did not produce any net increase in weight as it had a lower SGR compared to formulated feed. However, further investigations need to be carried out to find out whether this observation is due to quality of food or in the amount of feed consumed.

Salinity

Salinity is an important factor regulating the growth, development and reproduction (Purwatee and Luong-van, 2003) of marine organisms. The sea cucumber *H. atra* is a euryhaline organism (Wang *et al.,* 2004); it can withstand significant changes in salinity. It was likely that the decreases in salinity disrupted the growth process and increase the stress in juveniles *H. atra*. During the first two weeks of experiment the mortality rate was high in salinity 25‰ because of the gradual decrease in salinity. Purcell *et al.* (1994) reported that death of sea cucumbers *H. scabra* were most apparent in the days following the drop in salinity after cyclone. Furthermore, stress due to high large fluctuation in water temperature and salinity, in turn, may lead to an increase in mortality rates in sea cucumbers (Mackey and Hentched, 2001). The influence of salinity on foraging activities of echinoderms is poorly known, although direct correlations have been found in carnivorous species. For example, the number of non-feeding starfish *leptasterias hexactis* was found to increase at low salinity while activity and feeding rate of starfish *Luidia clathrata* decrease during exposure to low salinity (Mercier *et al., 1*999).

The results showed that salinity 35‰ is the most suitable for normal growth and development of *H. atra*. Asha and Muthiah (2005) reported that the required salinity for normal growth in sea cucumbers *H. spinifera larvae* was also 35‰ while Chen and Chian (1990 cited Asha and Muthiah, 2005) reported normal growth under same salinity for sea cucumbers *Stichopus spinefera* and *Actinopyga echinites.* The result also suggests that salinity 25‰ was below the tolerance level of juveniles *H. atra* and consequently caused negative growth. Mercier *et al.* (1999) reported that decrease in salinity causes burrowing in *H. scabra* and that the burrowing effect at low salinity helps them to better equilibrate ionic concentration of coelomic fluid. As compared to *H. atra,* it has not evolved such mechanism to regulate the ionic concentration and it is directly affecting the metabolism of food.

Temperature

The temperature was within the ecological tolerance for *Holothuria atra*, as the specific growth rate increase from temperature 26ºC to 28ºC from 0.97 (±0.14) %

to 1.43 (±0.15) %. However beyond the 28ºC, growth seems to decrease gradually. Certainly the temperature 32ºC was unfavorable for growth of juvenile *H. atra*. One of reason is that dissolved oxygen (DO) level varies with temperature. As the temperature increases, the DO level decreases (James, 2004). Thus a high temperature in the tank may have lowered the DO level during the experiment, and consequently altered the habitat. The reason for the poor growth performance must be due to low oxygen content of water in treatment temperature 32ºC.

At present there are different views concerning the temperature effects on the growth and survival of aquatic organism. Both increase and decrease in temperature can alter the growth of aquatic organisms, such zooplankton (Dong *et al.,* 2005). The growth rate of sea cucumbers is closely related to the water temperature. The body weight of an adult sea cucumber, *Apostichopus japonicus* decrease roughly by half when the water temperature is above 22-24 °C during summer at Daliann, China (Chang *et al.*, 2004). In the present study, temperature 28ºC was ideal for growth and survival in juvenile sea cucumbers *H. atra*. Giraspy and Ivy (2005) reported that the optimum water temperature range from 26–29°C was the ideal for brood stock of *H. scabra*. Similar results were obtained by Therkildsen and Petersen (2006) who reported that the in tropical sea cucumbers the growth rate is correlated with an increase in sea water temperature. Temperature seems to play a positive role in growth in H. atra and helping in the faster metabolism.

Stocking density

The growth rate of *H.atra* is density-dependent. At higher density, there is accumulation of nitrogenous waste as well as low Dissolved oxygen available. There is also competition for food and space. Battaglene (1999 cited Pitt and Duy, 2004) also observed that growth of juveniles sea cucumbers of *H. scabra* in tanks slowed down or stopped when density reached about 200-225 g/m^2. While for the present study, densities were 428-430 g/m^2 (SD 12) and 706-707 g/m^2 (SD 18) whereas in the control, it was 218-230g/m^2. Obviously, the growth nearly cease in stocking 12 and 18 since very little change was observed in both. The growth of juvenile *H. atra* must have stopped at an average weight of about 250-350 g/m^2. The high mortality rates were caused by high density, a low DO, faeces on the settling substrate and competition for space amongst the specimens (Xilin, 2004). The optimum density for rearing juveniles *H. atra* is 6 speciemn/m^2 or 225g/m^2. Using proper stocking density would avoid problem such as overcrowding which induces slow growth rates and a great variability in body length, as well as causing deformities, "rotten-stomach" (Liu *et al.*, 2004). Overcrowding reduces available space and food availability, causing malnutrition, slow growth rates and size variability.

Feed type

The low SGR for the juvenile *H. atra* fed with algal paste may be due to the little cellulose activity in their and that they did not assimilated the algal paste before it was decomposed by bacteria and fungi. Other studies have shown that algae are not the major source of food for sea cucumbers (Battaglene *et al.,* 1999). Growth in juveniles *H. atra* fed with algal paste was very poor as compared to juveniles fed with formulated feed (control). Juvenile *H. atra* fed formulated feed produced a higher SGR as compared when juvenile *H. atra* fed with algal paste. However further investigations need to be carried out to find whether the differences in SGR are due to the amount of feed consumed or quality of the two feeds.

Sea cucumbers feed on organic matter and microorganisms, such as diatoms, foraminifera, radiolarian, small crustaceans and gastropods typically found in the sand (Li, 2004). The correct feeding rate is essential to ensure a high survival rate of the juvenile sea cucumbers (Xilin, 2004). As far the effect of salinity, temperature and stocking density on metabolism of food eaten by sea cucumbers, more research still need to be carried out.

Under the conditions of the experiments for the culture of juvenile sea cucumbers *H. atra,* use of salinity of 35‰, temperature 28ºC, stocking density of 6 specimens per m^2 and a formulated feed containing algae and fish feed results in high growth rate and survivorship. The results obtained can be useful in the aquaculture of juvenile sea cucumbers *H. atra*. However, future studies to establish the optimal conditions for culture of *H. atra* can be carried out with more replicates and using a combinations of factors.

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