Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 –6131
Vol.25(3): 635–646 (2021)
www.ejabf.journals.ekb.eg

Effect of feeding regime and stocking density on the growth performance of the sea cucumber *Holothuria scabra*

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ARTICLE INFO

Article History:
Received:Feb. 24, 2021
Accepted:March 30, 2021
Online: June 25, 2021

Keywords:
Sea cucumber;
aquaculture;
echinoderms;
intensive culture;
feeding behaviour

ABSTRACT

The rapid expansion of intensive sea cucumber farming makes it necessary to optimize the culture procedures to maximize the production in a profitable economic method. In this study, the effects of feeding rhythms and two stocking densities on the growth of sea cucumber *Holothuria scabra* were examined under controlled conditions. Specific growth rate, total weight increment, weight gain day^{-1} were determined for the sea cucumber fed in day-time; night-time and on two stocking densities (10 and 20 juveniles m^{-2}). The impact of the feeding rhythm and stocking density on sediment quality was also evaluated. The feeding regimes did not affect the growth performance of sea cucumber, although the night feeding showed faster growth and highest specific growth rate and daily weight gain when compared to the day-time feeding. Stocking densities tested showed a decrease in specific growth rate with the increase of stocking density, although it was not significant. In conclusion, this study showed that stocking of sea cucumber juveniles up to 20 individuals m^{-2} is viable for farming under supplemental feed.

INTRODUCTION

Due to the high demand for sea cucumbers in Asian markets, the natural stocks in the ocean are depleting at an alarming rate (**Purcell *et al.*, 2013**). To minimize the harvest from the wild stocks, it is necessary to develop efficient aquaculture systems for sea cucumbers. The sandfish *Holothuriascabra*, Jaeger, 1833, is a popular candidate species for aquaculture in the tropical regions due to high demand and good price (up to US$ 1668 kg^{-1}) (**Robinson & Pascal, 2009; Purcell, 2014**). The farming of sea cucumber *H. scabra* is a fast-growing aquaculture sector in many regions (**Sun *et al.*, 2015**). Among the culture practices, land-based intensive farming is potentially a good method for sea cucumber with a high yield but received little attention (**Chang, 2003; Kang *et al.*, 2003**).

In intensive culture, the stocking density is critical due to its influence on growth performance, yield and health of the cultured organisms (**Rowlanda*et al.*, 2006; Qin *et***

***al.*, 2009**). In sea cucumber culture, low stocking density may lead to the wastage of available space, but if the density is high that may also affect the growth (**Li *et al.*, 2007**). The food is one of the limiting factors for the lower growth performance of sea cucumbers at high stock density, however, providing a highly nutritious diet could yield a higher production rate (**Qin *et al.*, 2009**). Further, the stocking density affects the feed conversion and protein efficiency ratio of the sea cucumbers (**Xia *et al.*, 2017**). However, the role of stocking density on the growth rate of sea cucumber such as *H. scabra* has not been extensively studied.

Some invertebrates exhibit behavioral response in relation to changing environmental conditions such as salinity, temperature, wave action, food supply, light etc (**Barkai 1991; De'ath & Moran 1998; Hudson *et al.*, 2005; Eckert, 2007**). Photoperiod (light and dark cycle) is considered as a significant factor that regulates the biological rhythm in aquatic organisms (**Sun *et al.*, 2015**). In the sea cucumber, *H. scabra*, which is a nocturnal deposit feeder, the biological rhythm is controlled by the photoperiod (**Yamanouchi, 1939; 1956; Mercier *et al.*, 1999**).

The juveniles of *H. scabra* show the behavioral response to light; during daytime (around sunrise), they begin to burrow and emerge from the burrows after sunset (**Mercier *et al.*, 1999**). The burrowing period is also affected by other environmental factors such as temperature which may lead to early or late burrowing (**Barkai, 1991; Mercier *et al.*, 1999**).

This burrowing activity of sea cucumbers in response to light is one of the determining factors for their movement and feeding. In general, after sunset, they will stay on the surface for feeding, while in burrowed condition, which is a stationary state, the sea cucumbers will not take food (**Mercier *et al.*, 1999**). Similarly, in a previous study, photoperiod is reported as one of the environmental factors for determining the feeding and digestive processes of sea cucumber, *A. japonicas* (**Dong *et al.*, 2010; Sun *et al.*, 2015**). The peak feeding activity of *A. japonicus* was observed between 2.00 to 4.00 am, and the peak enzyme activities were noticed 2-4 h before the feeding period (**Dong *et al.*, 2010; Sun *et al.*, 2015**).

The effects of feeding regime and stocking density on *H. scbra* are essential due to the increase in intensive farming practices of this sea cucumber. Previous studies investigated feeding at different times (**Yamaguchi *et al.*, 2016; Xia *et al.*, 2017**) and stocking density (**Qin *et al.*, 2009; Pei *et al.*, 2012; Asha & Diwakar, 2013)**. Such information could be useful for planning the optimal feeding schedules and stocking densities to increase production and minimize feed waste. Therefore, the objective of the present study was to investigate the effect of different feeding time and stocking density on the growth performance of sea cucumber, *H. scabra*. The results obtained from this study will be useful for formulating the feeding regime or stocking density for getting better growth performance inintensive aquaculture of sea cucumbers.

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MATERIALS AND METHODS

Animal source and acclimatization

Two experiments were conducted for a period of 60 days in the fish farm at the Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia under lab-controlled condition. Sea cucumber juveniles were obtained from pre-growout ponds of the National Aquaculture Group, Saudi Arabia. Animals were transported to the experimental site in a 300L tank with seawater (40 psu salinity) and provided with pure oxygen and kept at 26°C. Sea cucumbers were acclimatized under laboratory conditions in1.9 m- diameter FRB tanks for 8 days. The bottom of the acclimation tank was covered with 5 cm dune sand. Continuous over-flow water exchange was done during the acclimatization period, and animals were fed once per day with the compound diet at the ratio of 2% of body weight.

Experimental design

Experiment I

The study was conducted to assess the effect of feeding behavior on the growth performance of juveniles of sea cucumber, *H. scabra*. The experiment was designed in a way that the animals were fed during daytime (day feeding, control) and night (night feeding, treatment) at a stocking density of 8 animals per tank (10 individuals m^{-2}) represented as 27.7 g m^{-2}. Each control and treatment was achieved in triplicate. After taking the initial individual weight of sea cucumbers (average weight: 2.77±0.31g), the juveniles were distributed into 6 fiberglass tanks of 500 L water volume (0.8m^2 bottom area) filled with 5 cm dune sand. Both control and treatment tanks were fed with a dry diet (14.5% protein, 4.3% lipid, 38% ash and 2395 kcal kg^{-1} energy) at the ratio of 2% of body weight divided into two equal portions. Control tanks were fed during the day at 8:00 am and 2:00 pm while the treatment tanks were fed during the night at 6:00 pm and 12:00 midnight. The diet was soaked for 12 hours in water before feeding.

Experiment II

This experiment was conducted to evaluate the effect of stocking density on the growth performance of sea cucumber juveniles. The study was designated for two stocking densities: SD10 at stoking density of 10 individuals m^{-2} and SD20 at 20 individuals m^{-2}.

After assessing the initial individual weight of sea cucumbers (average weight:2.99±0.25g), the animals were distributed into 6 fiberglass tanks of 500L volume and 0.8m^2 bottom area covered with 5 cm dune sand as substratum. 8 juveniles were stocked per tank in SD10 and 16 juveniles per tank in SD20 which represented the biomass of 29.9 and 59.8 gm^{-2} in SD10 and SD20, respectively. Both treatments were in triplicate. Feeding was done with a formulated diet at a ratio of 2% of body weight, and divided into two equal portions at 6:00 pm and 12:00 am. The diet was soaked for 12 hours in water before feeding.

Growth performance analysis

The growth performance such as average body weight, monthly weight gain, specific growth rate, monthly increment rate was assessed every 29 days. An electronic digital balance (resolution=0.01g) was used for taking the individual body weight of sea cucumbers.

Parameters of the experimental tanks

The same procedures were followed for both experiments. Daily water exchange of total 70% was carried out two times at 7:00 am and 5:00 pm using 10-micron filter bag. The parameters such as dissolved oxygen, temperature and pH were monitored at 7:00 am and 3:00 pm before carrying out the water exchange using a multiparameter probe (YSI Pro 1020). The experimental tanks were continuously enriched with aeration to maintain the DO of about 5.0 mg L^{-1}, salinity 40 psu and the photoperiod was maintained as 12L:12D throughout the study. The oxic-anoxic sediment interface of experimental tanks was measured bi-weekly to evaluate the quality condition. The sediment core was collected from the base to the surface using a modified syringe (Fig. 1). Depth of sediment oxic-anoxic interface was measured in centimeter (using a standard plastic ruler) referring to the sediment layer colour change between oxic (light brown) and anoxic (grey) and measured values were changed to percentage from the total depth of sediment.

Fig. 1. Measurement for oxic-anoxic layer in the experimental tanks using soil core.

Data analysis

The growth performance of the sea cucumbers cultured with two feeding regimes and stocky densities were analyzed using two-way ANOVA (analysis of variance). The differences in total weight and monthly weight gain were subjected to two-way ANOVA with days and treatment (day vs night feeding, or SD10 vs SD20) as factors. Furthermore, one-way ANOVA was conducted to find out the variation of daily weight gain and specific growth among the treatments. The statistical tests were carried out using the STATISTICA program (ver.13) and $P<0.05$ was considered as significant.

RESULTS

Experiment I

The growth performance of sea cucumber juveniles fed with different regimes during the study is shown in Table (1). Overall body weight of juveniles showed a significant difference (Table 2) between control (day feeding) and treatment (night feeding). The highest average body weight (ABW) observed after 60 days was 5.95g for treatment and 4.18g for control. The obtained results indicated that the highest weight gain was 2.91g for treatment with a final gain increment of 97% whereas the control recorded 1.68g weight gain and 66% gain increment. The highest daily weight gain was observed in the control group with 0.05g, while in the treatment group the weight gain per day was 0.03g. One-way ANOVA results indicated that the variation in daily weight gain between control and treatment was not significant ($F_{(1,4)}=2.11, P=0.21$).

The specific growth rate (SGR) of the studied groups achieved 0.82 and1.12% d^{-1} in both control and treatment, respectively. The SGR did not show significant variation between the control or the treatment groups ($F_{(1,4)}=1.55$, $P=0.27$). The highest weight gain recorded during the first 30 days was in the treatment group of night feeding with 1.43g and 48% gain increment (Table 2). After 60 days of culture, the treatment group showed 1.48g weight gain with a 34% increment. Whereas, the weight gain in the control group after 30 days was 0.80g with 32% increment and 0.88g (28% increment) after 60 days of culture. ANOVA results indicated that the monthly weight gain did not show significant variation between the control or treatment (Table 2).

Table 1. Growth performance of sea cucumber juveniles subjected to day-time and night feeding (ABW=average body weight, SGR=specific growth rate).

Experiment I	**Control** (Day feeding)	**Treatment** (Night feeding)
Initial ABW (g)	2.50±0.11	3.04±0.13
Final ABW (g)	4.18±1.08	5.95±0.54
Final weight gain (g)	1.68±0.97	2.91±0.66
Gain increment (%)	66±36	97±26
Weigt gain/ day (g)	0.03±0.02	0.05±0.01
SGR (% d-1)	0.82±0.35	1.12±0.21
<u>30 Days</u>		
ABW (g)	3.30±0.16	4.47±0.63
Monthly weight gain (g)	0.80±0.27	1.43±0.74
Gain increment (%)	32±12	48±27
<u>60 days</u>		
ABW (g)	4.18±1.08	5.95±0.54
Monthly weight gain (g)	0.88±1.23	1.48±0.09
Gain increment (%) DOC 60	28±40	34±6

Table 2. Two-way ANOVA results for the sea cucumbers grown under two feeding regimes and stocking densities.

	Feeding regime						**Stocking density**					
	Total weight			Monthly weight gain			Total weight			Monthly weight gain		
Effects	Df	F	P	Df	F	P	Df	F	P	Df	F	P
Days	2	24.822	0.000	1	0.023	0.881	2	9.437	0.003	1	0.945	0.359
Treatment	1	18.937	0.000	1	2.103	0.185	1	3.166	0.100	1	0.771	0.405
Days*Treatment	2	1.784	0.209	1	0.002	0.960	2	0.618	0.554	1	0.810	0.394
Error	12			8			12			8		

Experiment II

The growth performance of sea cucumber juvenile cultured in two different stocking densities during the study is shown in Table (3). Overall body weight of juveniles did not show a significant difference between SD10 (8 animals tank^{-1}) or SD20 (16 animals tank^{-1}) (Table 2). The highest average body weight (ABW) observed after 60 days was 5.95 for SD10 and 4.80 for SD20. The obtained results indicated that the highest weight gain was 2.91g for SD10 with 97% gain increment; whereas, SD20 recorded 1.85g weight gain and 65% gain increment. The highest daily weight gain was in SD10 recording 0.05g while in SD20 was 0.03g. Further, ANOVA results revealed that the difference in total weight gain and daily weight gain of the sea cucumbers between SD10 and SD20 was not significant (total weight gain: $F_{(1,4)}=0.64, P=0.46;$ daily weight gain: $F_{(1,4)}=0.39, P=0.56$).

The specific growth rate (SGR) of the studied groups achieved 1.12, 0.72% d^{-1} in SD10 and SD20, respectively (Table 3). However, one-way ANOVA indicated that the SGR between SD10 and SD20 groups was not significant ($F_{(1,4)}=0.73, P=0.43$). The highest weight gain recorded during the first 30 days was in SD10 with 1.43g and 48% gain increment which further recorded 1.48g weight gain (34% increment)after 60 days of culture. The weight gain in control after 30 days was 0.36g with 14% increment and 1.49g and 42% in gain increment after 60 days. Like other growth performance parameters, the monthly weight gain also did not differ significantly between SD10 and SD20 groups (Table 2).

Table 3. Growth performance of sea cucumber juveniles cultured under two stocking densities (ABW=average body weight, SGR=specific growth rate).

Experiment II	**SD10** (8 animals/ tank)	**SD20** (16 animals/ tank)
Initial ABW (g)	3.04±0.13	2.94±0.37
Final ABW (g)	5.95±0.54	4.80±2.13
Final weight gain (g)	2.91±0.66	1.85±2.19
Gain increment (%)	97±26	65±72
Weight gain/ day (g)	0.05±0.01	0.03±0.04
SGR (% d-1)	1.12±0.21	0.72±0.78
30 days		
ABW (g)	4.47±0.63	3.30±0.36
Monthly weight gain (g)	1.43±0.74	0.36±0.65
Gain increment (%) 0	48±27	14±24
60 days		
ABW (g)	5.95±0.54	4.80±2.13
Monthly weight gain (g)	1.48±0.09	1.49±1.84
Gain increment (%)	34±6	42±51

Water and soil quality parameters of the culture tank

The daily physical water parameters are presented in Fig. (2) while the soil quality during the culture period is presented in Table (4). Although the anoxic sediment condition in experiment I varied in the first 6 weeks; showing a lower increase in treatment when compared with the control, but at the end of experiment the anoxic condition registered the same level with 25%. In experiment II, the anoxic level condition in the sediment at the end of 8 weeks was at the highest in SD20 with 22% whereas in SD10 recorded 15%.

Table 4. Anoxic soil layer interface (%) observed in the experimental tanks.

Experiment I

Week	Control	Treatment
0	0±0	0±0
2	7±2	5±0
4	7±2	8±1
6	22±1	14±1
8	25±1	25±3

Experiment II

Week	SD10	SD20
0	0±0	0±0
2	5±0	4±1
4	8±1	16±4
6	11±2	19±1
8	15±1	22±1

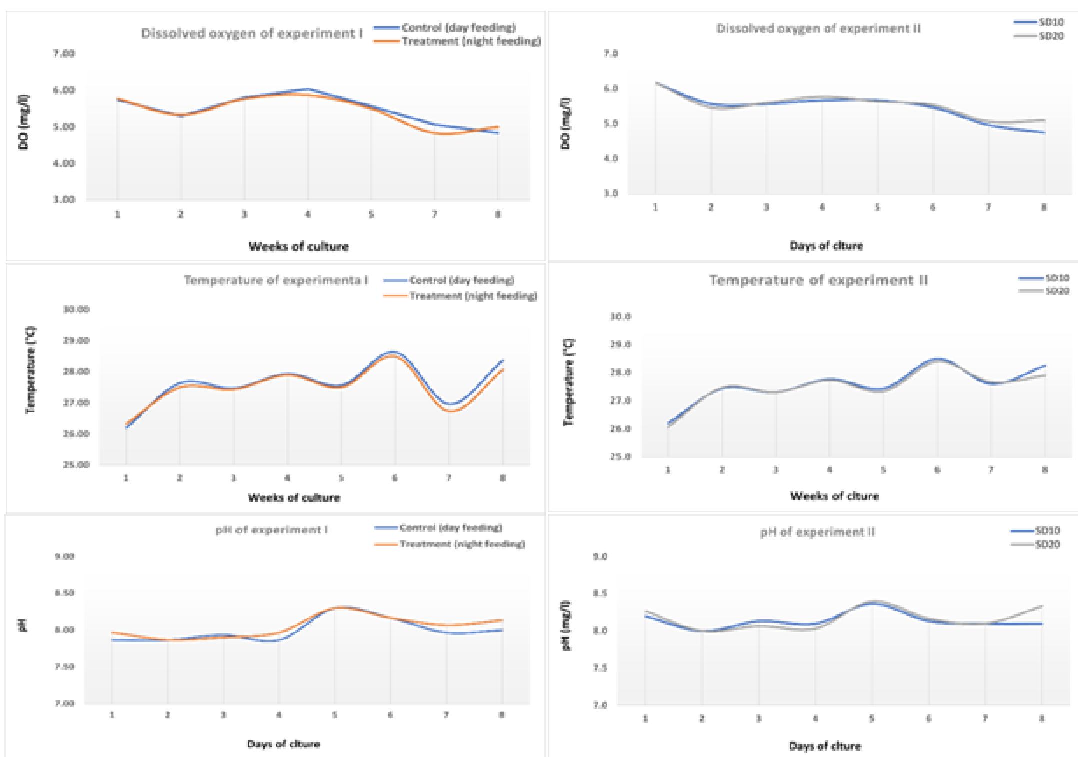

Fig. 2. Water quality parameters of the culture tanks during the experiments.

DISCUSSION

The aim of the aquaculture system is to optimize the productivity of the flow of energy to biomass production. Feeding rhythm plays an important role in the aquaculture of many aquatic animals to optimize growth performance, the efficacy of feed consumption and minimize feed waste. The results of the study indicated that the final weight gain of sea cucumber juvenile was not significantly affected by feeding rhythm. The daily activity rhythms in many species of echinoderm have been reported in previous studies (**Hammond, 1982; Mercier *et al.*, 1999; Dong *et al.*, 2010**). A report concluded that juveniles of sea cucumber, *H. scabra*, exhibits daily burrowing activity which is strongly associated with the , but no obvious evidence of movement was detected when juveniles were sheltered (**Mercier *et al.*, 1999**). This information is conflicting with the report of **Wiedemeyer (1992)** who demonstrated that primary feeding activity of *H. scabra* appeared at sunrise and sunset in southern Japan, and concluded that they feed at night and while burrowing . Moreover, **Yamaguchi *et al.* (2016)** revealed that juveniles of *A. japonicus* did not show a clear feeding rhythm but it can be modified by adjusting the feeding time while adults show this feeding rhythm.

In the current study, the obtained SGR was 0.82 and 1.12 % d^{-1} for day feeding and night feeding, respectively. **Xia*et al.* (2017)** reported on juveniles of *A. japonicas* registered SGR ranged between 1.2-1.4% d^{-1}at a stocking density of 10 animals $0.7m^{-2}$ fed for 3 times (8:00am, 4:00pm and 12:00am). It was previously reported that bacteria can account for the most majority of what sea cucumbers feed on and confirmed that heterotrophic bacteria, autotrophic microorganisms and dissolved nutrients function as feed supply for juveniles of *H. scabra* (**Yuan *et al.*, 2006; Plotieau *et al.*, 2013, 2014**). These findings could explain the non significant difference on the growth performance between feeding in different times because sea cucumber juveniles not only depend on the direct applied feed but may use the organic matter in sediment as feed source as well (**Slater & Jeffs, 2010; MacTavish*et al.*, 2012**). Further, all sizes of sea cucumber juveniles are selective feeders based on sediment characteristics (**Mercier *et al.*, 1999**).

Stocking density influence directly on survival, growth performance, health and feeding on cultured sea cucumber (**Rowlanda*et al.*, 2006**). Previous studies reported that stocking density has a different effect on many aquatic organisms and may arise due to three factors; food competition; water quality and stress from crowding (**Blackburn & Clarke, 1990; Kebus*et al.*, 1992**). The crowding stress of sea cucumber can induce the endocrine system of animals which increases the levels in the celomic fluid, in return, speed up energy intake, change the energy budget model and eventually plays a negative role in the growth and biochemical composition (**Pei *et al.*, 2012**). It was reported by **Qin *et al.* (2009)** that, availability of feed is a significant factor in assessing the growth performance among different stocking densities of sea cucumber juvenile *A. japonicus*. The result of this study indicated that, the final weight gain of sea cucumber juvenile was not significantly affected by the two stocking densities used in the work.

Previously, **Pei *et al.* (2012)** found that sea cucumber juvenile of *A. japonicas* (7g body weight) recorded a significant decrease in SGR of 1.1, 0.9, 0.6. 0.6 and 0.2 % $d^{-1}$with increase of stocking density 2, 4, 8, 16, 32 individuals m^{-2}, respectively. The current study registered 1.1 and 0.7 % d^{-1} for the stocking density of 10 and 20 individuals m^{-2}, respectively although the decrease in SGR was not significant because of the high variation of individual growth of animals. This is similar to **Pei *et al.* (2012)** who found that the stress resulted from crowding can significantly enlarge the variation of individual growth between the experimental animals. The previous authors also demonstrated that big individuals dominated the bottom of tanks and were more active for feeding, while the subdominant small animals groveled on the walls and were less active for feeding. **Qin *et al.* (2009)** assessed that the growth performance of sea cucumber was decreased with higher stocking density and SGR declined from 4.43 % d^{-1}(5 ind. m^{-2}) to 2.23 % d^{-1} (35 ind. m^{-2}) under feeding regime.

In the present study, the water quality was maintained in a good condition by carrying out the daily water exchange as described in the methodology, indicating that the variations in growth were not a result of the decline in water quality. Equivalent sufficient feed was provided, so overall lack of feed was not a factor to affect the growth performance. **Qin *et al.* (2009)** demonstrated the optimal stocking density of sea cucumber juvenile (5g initial body weight) is 22 individuals m^{-2}. Furthermore, **Asha and Diwakar (2013)** reported that 2-20 individuals m^{-2} and 10 juvenile m^{-2} is the optimally used density. Those findings are similar to the accepted stocking density of the current study of 10 and 20 individuals m^{-2}, and the possible explanation of why both stocking densities did not show a significant difference is because of the small sized animals used and that biomass might not have exceeded the carrying capacity for animals to compete for feed or living space.

In conclusion, the current study showed that sea cucumbers that were feeding at different times did not differ significantly in growth as sea cucumber can assimilate the accumulated organic matter from the matter as a food source. Stocking densities tested in the current study showed a decrease in specific growth rate although it was not significant. In addition, results indicated that the stocking sea cucumber juvenile up to 20 individuals m^{-2} is viable for farming under supplemental feed.

ACKNOWLEDGEMENTS

We thank the Managing Director of National Aquaculture Group for extending the support for this study. The authors are also thankful to the Dean, Faculty of Marine Sciences, King Abdulaziz University, for the facilities provided at KAU Fish Farm, Obhur.

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