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# Research Article
# Effects of Different Macroalgaediets on Growth Performance on Impatient Sea Cucumber *Holothuria impatiens* Forskaal, 1775 (Echinodermata: Holothuroidea)

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## Abstract
**Objective:** This experimental work was aimed to study the effect of different ration of some different local macroalgae species diets on the significant growth rate and the survival rate of *H. impatient* juveniles to provide a preview for the large-scale sea cucumber aquaculture. **Materials and Methods:** A three natural seaweeds diets *Caulerpa racemosa* (green algae), *Cystoseira indica* (brown algae) and *Jania rubens* (red algae) were used as a powder (1, 2 and 3% of their initial body weight)to feed sea cucumber juveniles *Holothuria impatiens*. Growth performance of *H. impatiens* was calculated for 12 weeks to identify the optimal diet for this species. There was no significant difference between sea cucumber juvenile's weights at the beginning of the experiments. Data (Mean±SE) were analyzed with a one-way ANOVA. The SPSS version 18.0 for Windows was used. **Results:** Sea cucumbers fed on *C. indica* diets, showed SGRs were significantly higher than those fed *C. racemosa* and *J. rubens* diets ($F = 25.5$, $p<0.05$). Sea cucumbers fed 2 and 3% powdered seaweeds *C. indica* (0.5% $day^{-1}$) obtained the best growth than the other treatments ($p<0.05$). The ingestion rate was higher when fed the *C. racemosa* and *C. indica* diet than when fed the *J. rubens* diet ($p<0.01$). Ammonia-nitrogen production was much lower in *H. impatiens* fed the *C. indica* and *C. racemosa* diet than when fed in *J. rubens* diets. No mortalities were found in sea cucumber juvenile for 12 weeks. **Conclusion:** This study concluded that *C. indica* was optimum seaweeds for use in the culture of the seacucumber *H. impatiens* then *C. racemosa* seaweeds but the *J. rubens* seaweeds were the worst.

Key words: Growth performance, *Holothuria impatiens*, *Caulerpa racemosa*, *Cystoseira indica*, *Jania rubens*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Holothuria impatiens*, the impatient sea cucumber, is commonly found in shallow tropical waters, associated with coral reefs at depths up to 40 m. They distinguished by their soft flexible bodies and tube feet that have obvious soles[1,2].

At least 60 species from 1400 species of sea cucumbers all over the world are harvested by artisanal fishers and by trawl fishing[3,4]. There has been enormous commercial interest in culturing tropical sea cucumbers in countries where sea cucumber populations have been overexploited[5,6]. The production of sea cucumber juveniles in the hatchery requires suitable feeds to maximize survival rates and promote somatic growth[7,8].

Sea cucumbers processed into beche-de-mer are highly marketable as a food product an important source of income for coastal communities due to their nutritional value and for perceived medicinal benefits[9-11]. Although, *H. impatiens* has not a fishery purposes uptell now (low value), it can be expected that this species may become more popular after the depletion of other species of higher commercial importance[12]. It has been suggested that sea cucumbers *Holothuria impatiens* possess a wide range of bioactive compounds, some components isolated have antiviral and anti cancer activities[13-15].

Successful rearing of juvenile sea cucumbers requires knowledge of feed intake behavior and dietary requirements. Advances in culture methods in China affect the success of sea cucumber aqua culture especially for larval production and settlement and the critical early juvenile growth stage[16]. However, little is known about which artificial diets are capable of inducing rapid growth and healthy conditions in commercially valuable sea cucumbers[17,18].

Several studies showed that juvenile sea cucumbers fed commercially available dried powdered macroalgae (*U. lactuca*, *Laminaria japonica*, *Sargassum thunbergii*, *Sargassum polycystum*) exhibited significant growth[18-21].

Asha and Muthiah[22] studied the effects of five single microalgae species and their mixture on the larval growth, development and survival of sea cucumber *Holothuria spinifera* to explore the most feeding preference. They found that *Chaetoceros calcitrans* itself or in combination with *Isochrysis galbana* is the effective feed for the larvae of *H. spinifera*.

In aquaculture practice, to meet the food requirement of cultured sea cucumber with higher stocking density relative to that under natural conditions, artificial feed is generally supplemented to the sea cucumber and powder of macroalgae such as *Thallus laminariae*, *Sargassum thunbergii* and *Sargassum fusiforme* is the main ingredient of the formulated diet used in the intensive sea cucumber cultivation[17,23]. Brown algae are the most favorite macroalgae for the manufacture of sea cucumber feed[24].

An understanding of juvenile habitat preferences may help scientists to determine accurately the ultimate carrying capacity of a given sea cucumber habitat (while acknowledging the space occupied by juveniles) and additionally, enable the eventual release of juveniles to appropriate habitats with enhanced probability of survival[25,26].

Several studies on artificial diets have focused on juveniles of the temperate species[8,17,27-30]. A few studies have also investigated artificial feeds for growing juvenile of other tropical sea cucumbers[19,24,31,32], but no research has examined the most effective feed to promote growth and survival in *H. impatient* juveniles. This study will provide requires knowledge of feed intake behavior and dietary requirements.

The present study is the first work in *H. impatient* juveniles in Egypt. This experimental work aimed to study the effect of different ration of some different local macroalgae species diets on the significant growth rate and the survival rate of *H. impatient* juveniles to provide a preview for the large-scale sea cucumber aquaculture.

## MATERIALS AND METHODS

**Collection of animals:** Juvenile sea cucumbers of a similar size (8.04$\pm$0.08 g) were collected from the Sea in Hurghada city northern Red Sea by snorkeling during March, 2014. The depth of the sampling location was up to 2 m beneath rocks and died coral. The sea cucumbers were moved to the wet laboratory at National Institute of Oceanography and Fisheries, Red Sea branch at Hurghada.

They were placed in 1 $m^3$ capacity cylindrical fiberglass tank for 2 weeks prior to the experiment and fed with mixture algae powder for acclimation. To ensure the gut content were evacuated they were lived for 2 days without feed for starvation. Each juvenile was drained then one hour in a dry bench before they weighed to ensure weight stability. No mortalities or animals eviscerated were found during the acclimation period.

**Experimental set up:** The ten experimental diets tested were 1.0, 2.0 and 3.0% of the sea cucumbers body weight of three seaweeds, (a) *Jania rubens* (red algae), (b) *Caulerpa racemosa* (green algae) and (c) *Cystoseira indica* (brown algae) separately as a powdered, beside the starvation test.

Seaweeds were collected from concrete shore, a one meter depth on the Red Sea in Hurghada City, Egypt in February, 2014, washed carefully with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes, dried in the sun and then pulverized into ultra-fine pieces by mortar grinder (<100 µm). One hundred and fifty sea cucumber juveniles were used in the experiments as fifteen in every aquarium (50×40×30 cm$^3$, L×W×H) to form ten groups. Seawater used in the experiment was filtered using a sand filter. About two third of the sea water (10 L) was exchanged daily using fresh natural seawater.

The environmental conditions were monitored daily on the aquaria using multi parameter. Sea water was continuously aerated and mean water quality parameters during the experiments times (from April to July) were: Temperature 23.5±0.7°C, salinity 40.7±0.8%, pH 8.1±0.3 and the dissolved oxygen was kept above 5.0 mg L$^{-1}$.

**Feeding and cleaning:** The daily feed ration of powdered algae "1.0, 2.0 and 3.0% of the sea cucumbers body weight" was replenished daily. Fecal matter and uneaten algae were siphoned from each tank daily and separated from each other gently and were rinsed in distilled water to remove the salt. Uneaten feed were dried at 90°C to a constant weight to calculate how much feed had been consumed then weighed. Fecal matter was dried at 90°C to a constant weight then stored at -20°C for analysis. Growth rate of the juvenile sea cucumber were estimated every 2 weeks from the weight gain.

The weight of the sea cucumbers was measured to the nearest milligram using an analytical digital balance with 4 decimal places on start of the experiments and every 2 weeks over 12 weeks. There was no significant difference in the initial body weight among all treatments.

Mean daily Specific Growth Rate (SGR) Ingestion Rate (IR) and Feed Conversion Ratio (FCR)[33] of the sea cucumbers for each feed treatment were calculated according to the following equations:

$$\text{SGR } (\%\text{ day}^{-1}) = \frac{\ln(W_2)\text{-}\ln(W_1)}{T} \times 100 \quad (1)$$

$$\text{IR } (\text{gg}^{-1}\text{ day}^{-1}) = \frac{W_1+W_2}{2} \times C/T \quad (2)$$

$$\text{FCR } (\%) = \frac{W_2\text{-}W_1}{C} \quad (3)$$

where, $W_1$ is the initial body weight of sea cucumbers, $W_2$ is the final body weight of sea cucumbers, T is the duration of the experiment in days and C is the dry weight of feed consumed.

**Ammonia-nitrogen production:** Ammonia-nitrogen concentration was estimated daily before the addition of feed. One tank without any sea cucumber was used as a control. Production was calculated as follows.

To measure the concentration of ammonia-nitrogen in each tank, a 50 mL water sample was siphoned out from each tank and analyzed using Nessler's reagent method[34].

Production was calculated as follows:

$$\text{ANP (mg (g diet)}^{-1}) = \frac{\left[\text{fi ANC (mg L}^{-1}) - \text{i ANC (mg L}^{-1})\right] \times \text{Water volume (L)}}{\text{Feed intake (g)}} \quad (4)$$

where, ANP is ammonia-nitrogen production, fi ANC is final ammonia-nitrogen content and i ANC is initial ammonia-nitrogen content.

**Statistical analyses:** Statistical analysis was performed using SPSS 18.0 for Windows. Data (Mean±SE) were analyzed with a one-way ANOVA, using Tukey *post hoc* comparison of means to identify differences among groups. The means of treatment effects were compared using the Least Significant Difference (LSD) test. The statistical significance level was set at 0.05[35].

## RESULTS

### Growth performance

**Specific Growth Rates (SGR):** There was an obvious difference in growth performance parameters of sea cucumber *H. impatiens* fed different seaweed diets over 12 weeks (Fig. 1). Generally, growth significantly faster in brown algae *C. indica* than green algae *C. racemosa* and red algae *J. rubens*, respectively (F = 25.5, p<0.05). The highest SGR was 0.50% day$^{-1}$ for a diet 2.0 and 3.0% ratio diet of *C. indica*, but the smallest values was 0.21% day$^{-1}$ for a diets 2.0% of *J. rubens*. The SGR did not differ significantly among the 1.0% of *C. indica*, 2.0 and 3.0% of *C. racemosa* and 2.0 and 3.0% of *J. rubens* (p<0.05), the expected smallest growth rate was (0.12% day$^{-1}$) in starvation.

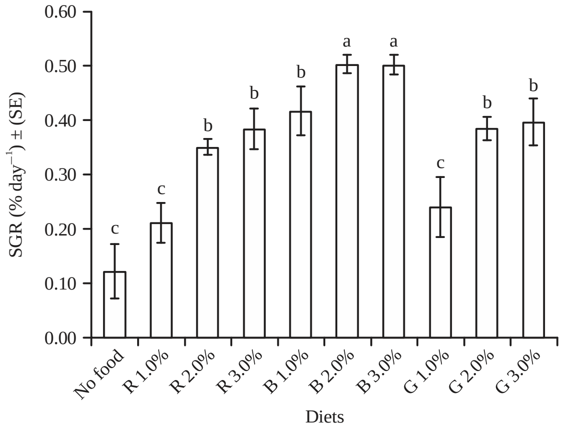


Fig. 1: Specific Growth Rates (SGR) of *H. impatiens* fed different diets ratio of macroalgae (n = 6)

R: Red algae *J. rubens*, B: Brown algae *C. indica* and G: Green algae *C. racemosa*. Different letters indicate significant differences ($p<0.05$) between treatments within the same group and bars represent standard errors

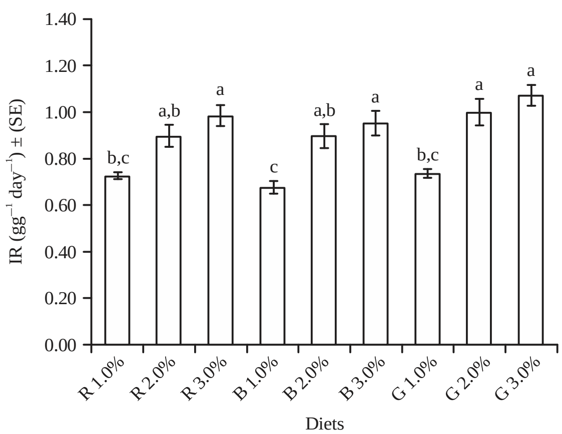


Fig. 2: Ingestion Rates (IR) of *H. impatiens* fed different diets ratio of macroalgae (n = 6)

R: Red algae *J. rubens*, B: Brown algae *C. indica* and G: Green algae *C. racemosa*. Different letters indicate significant differences ($p<0.05$) between treatments within the same group and bars represent standard errors

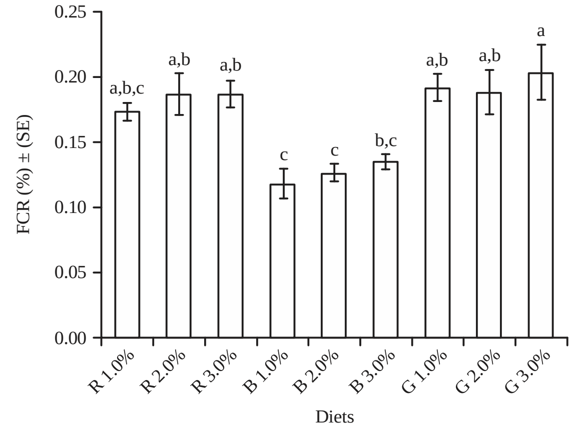


Fig. 3: Food Conversion Ratio (FCR) of *H. impatiens* fed different diets ratio of macroalgae (n = 6)

R: Red algae *J. rubens*, B: Brown algae *C. indica* and G: Green algae *C. racemosa*. Different letters indicate significant differences ($p<0.05$) between treatments within the same group and bars represent standard errors

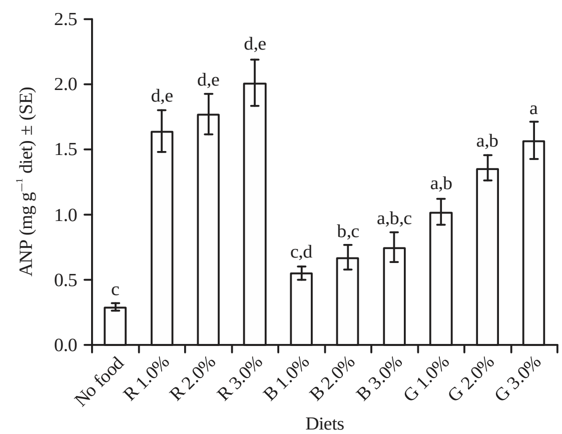


Fig. 4: Ammonia-Nitrogen Production (ANP) of *H. impatians* fed different diets ratio of macroalgae (n = 6)

R: Red algae *J. rubens*, B: Brown algae *C. indica* and G: Green algae *C. racemosa*. Different letters indicate significant differences ($p<0.05$) between treatments within the same group and bars represent standard errors

**Ingestion Rate (IR):** Ingestion rate of the sea cucumbers juveniles *H. impatiens* (Fig. 2) showed significant differences among different seaweed ratio treatments ($F = 32.1$, $p< 0.05$). Generally IR in sea cucumber juveniles increases with food ratio increasing, the highest value was 1.08 gg$^{-1}$ day$^{-1}$ in sea cucumber fed in 3% of *C. racemosa* but the lowest value was 0.68 gg$^{-1}$ day$^{-1}$ in 1% *C. indica*.

**Food Conversion Ratio (FCR):** The FCR of the juveniles sea cucumbers juveniles *H. impatiens* are presented in Fig. 3. There were significant differences among different seaweed ratio treatments ($F = 4.54$, $p<0.05$). Generally, FCR in sea cucumber juveniles increases with food ratio increasing, the highest value was 0.20% day$^{-1}$ in sea cucumber fed in 3% of *C. racemosa* but the lowest value was 0.12% day$^{-1}$ in 1% *C. indica*.

**Ammonia-Nitrogen Production (ANP):** Ammonia-nitrogen production of sea cucumbers *H. impatiens* (Fig. 4) showed significant differences among different seaweed ratio treatments ($F = 23.5$, $p<0.05$).

Generally, the highest values of ammonia-nitrogen production was 1.64, 1.77 and 2.01 in *H. impatiens* fed 1, 2 and 3% *J. rubens*, respectively but the lowest values was 0.55, 0.67 and 0.75 in *H. impatiens* fed 1, 2 and 3% *C. indica*.

## DISCUSSION

In this study the effect of various seaweeds in SGR were been investigated by using three common marine seaweeds in three different diets ration besides a starvation test. *Cystoseira indica* (brown algae), *Caulerparacemosa* (green algae) and *Janiarubens* (red algae) were used in 1, 2 and 3% diets ration separately. To date, no study has compared these different seaweeds for use in sea cucumber diet together.

The results showed that the highest values of SGR was in sea cucumbers *H. impatiens* fed 2 and 3% *C. indica* (Fig. 1), these values lowed in case of 1% *C. indica*, 2 and 3% C. *racemosa* and 2 and 3% of *J. rubens* as a food but the lowest values of SGR was 0.21% $day^{-1}$ for a diets 1.0% in *H. impatiens* fed *J. rubens* but the most lower value was 0.12% $day^{-1}$ in starvation.

The results showed that the estimated values of SGR were in agreement with Zhu *et al.*[20], who reported that the SGR of sea cucumbers *Apostichopusjaponicus* decreased when they were fed *L japonica* (green algae) and increased when they were fed *S. Polycystum* or *S.thunbergii* (brown algae) but disagreed with Xia *et al.*[18], who showed that the SGR was much lower in sea cucumbers fed *S. polycystum* or *S. thunbergii* (brown algae) compared to *U. lactuca* or *L. japonica* (green algae).

The results showed that the IR was higher for *C. racemosa* than *J. rubens* and *C. indica*, furthermore the ammonia-nitrogen production of sea cucumber fed *J. rubens* and *C. racemosa* was higher than those fed *C. indica* in any ration indicating that Sea cucumber *H. impatiens* preferred the diet containing *C. indica* and it's the most benefit for *H. impatiens* (Fig. 1). Hence, sea cucumber *H. impatiens* eating *C. indica* diet grew much faster than those feeding on *C. resemosa* and *J. rubens*.

In general, ammonia is the most common toxic form of nitrogenous waste in aquaculture environments. Ammonia nitrogen accumulates from the waste excretion of aquatic animals and uneaten feed decomposition. The un-ionized ammonia nitrogen form ($NH_3$) is toxic and more harmful than the ionized ammonia or ammonium form ($NH_4^+$) because of its higher permeability through membranes. The proportion between these two forms of ammonia nitrogen varies and the proportion of the un-ionized form increases with increasing of pH and temperature and declines slightly with increasing salinity[36].

Many studies showed that the high concentration of ammonia will disrupt growth leading to stress and death for animals[37]. Boyd[38] reported that an increase in $NH_3$ concentration in the water may result in an accumulation of $NH_3$ in the blood and leading to physiological stress or death of aquatic animals.

The present study showed that different types and ratios of seaweed diets significantly influenced ammonia-nitrogen production of sea cucumber *H. impatiens*. The ammonia-nitrogen production of sea cucumbers *H. impatiens* fed *C. indica* seaweed diets in any ratio was much lower than that of sea cucumbers fed the other diets. This means that most of the protein was deposited in the body and ammonia-nitrogen production in the water tanks was low.

Early juvenile rearing fundamental is important to hatcheries that rear sea cucumbers because sea cucumbers juveniles are more sensitive[7]. Powdered macroalgae are used as the main component of formulated feeds because detritus of macroalgae in the sediment are the main food sources for sea cucumbers. Also, juvenile sea cucumbers that fed commercially available dried powdered macroalgae exhibited significant growth[18,21].

In the hatchery production of sea cucumber juveniles, powdered algae have been used since the late 1980s in China, Japan and India[7,19]. In China *Sargassum thunbergii* and *Sargassum polycystum* (red algae) traditionally were considered to be the best choices in the formulated feed for sea cucumber culture industry grew so these two macroalgae species were overexploited and they became very expensive[21,39].

Therefore, this study recommends the use of brown algae as a food for juvenile seacucumbers and then use green algae but does not recommend the use of red algae as a single food. This study also recommends further doing more studies, especially in which a mixture of different algae is made, to reach the maximum benefit for sea cucumber juveniles growth.

## CONCLUSION

In the present study, there was an obvious difference in growth performance parameters of sea cucumber fed different seaweed diets over 12 weeks. Generally growth significantly faster in brown algae *C. indica* than green algae *C. racemosa* and red algae *J. rubens*, respectively. The IR and FCR ratio in sea cucumber juveniles increase with food ratio increasing, the highest IR value was sea cucumber fed in 3% of *C. racemosa* but the lowest value was in 1% *C. indica*, the highest FCR value was in sea cucumber fed in 3% of *C. racemosa* but the lowest value was 1% *C. indica*. The highest values of ammonia-nitrogen production was in juveniles fed 1, 2 and 3% *J. rubens*, respectively but the lowest values was in juveniles fed 1, 2 and 3% *C. indica*, respectively.

## SIGNIFICANCE STATEMENT

This study discovers the effect of different ration of some different local macroalgae species diets on the significant growth rate and the survival rate of *H. impatiens* juveniles. This study will help the researcher to provide a preview for the large-scale sea cucumber aquaculture. Hence, the suitable food forsea cucumber aquaculture may be known.

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