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Effects of single and combined seaweeds diets on growth performance and ammonia-nitrogen production of the sea cucumber *Holothuria impatiens* Forskaal, 1775

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ABSTRACT
This study investigates the effects of single and combined seaweeds diets on growth performance and ammonia-nitrogen production of the sea cucumber *Holothuria impatiens* under controlled laboratory conditions. Three naturally available seaweeds *Caulerpa racemosa*, *Cystoseira indica* and *Jania rubens* were used to feed sea cucumber juveniles, which averaged 6.09 g in weight at the beginning of the experiments. Generally, growth rate were faster in combined algae than in single algae diets. Specific growth rate (SGR) and relative weight gain (RWG) of sea cucumber fed the combined diet *C. indica* and *C. racemosa* (0.7%d^{-1} and 5.9 %w^{-1} respectively) were significantly higher than that of sea cucumber fed the other diets (P<0.05). The lowest SGR and relative weight gain (RWG) were observed in sea cucumber fed the *J. rubens* diet. The highest value of ammonia-nitrogen production was 1.6 mg/L in *H. impatiens* fed *J. rubens* diet but the lowest value was 0.56 mg/L in *C. indica* and *C. racemosa* diet. Therefore, this study indicates that *C. indica* and *C. racemosa* are optimum seaweeds for use in the culture of the commercially valuable sea cucumber *H. impatiens*.

INTRODUCTION

There has been enormous commercial interest in culturing sea cucumbers in countries where sea cucumber populations have been overexploited. The production and rearing of sea cucumber juveniles in the hatcheries require suitable conditions feeds to maximize survival and growth rates (An *et al.* 2007; Mahmoud *et al.* 2018). Most sea cucumbers are deposit-feeders that ingest organic and inorganic matter with sediment (Michio *et al.* 2003; Li *et al.* 2015; Shi *et al.* 2015a,b), and their gut contents are dominated by decaying materials from macro-algae and seaweeds, shell fragments from mollusks, crustaceans and barnacles, echinoderm ossicles, many pelagic and benthic foraminifera and diatoms (Zhang *et al.* 1995). Some studies have also shown that sea cucumbers ingest faeces of marine animals (Yuan *et al.* 2006; Zhou *et al.* 2006) and even their own faeces (Liu *et al.* 2009).

Holothuria impatiens (Family: Holothuriidae) is a cryptic species. It lives in shallow coral reef habitats, it can be found under rocks in shallow waters, however, it can be observed up to 30 m depth. This species is harvested in artisanal fisheries, by hand collection in shallow waters. (Sun *et al.* 2007). Tropical sea cucumber mariculture has the potential to become a commercial industry and contribute towards natural population restocking (Purcell *et al.* 2012).

Technology for commercial-scale aquaculture has been established for several of the overfished tropical species, subsequently, culture methods are beginning to develop for several other tropical species. Thus, mariculture may also provide a pathway to the reconstruction of wild stocks via culture-based restocking or as a by-product of sea ranching (Ivy and Giraspy, 2006; Bell *et al.* 2008). Sea cucumber aquaculture has the priority in the development plans of numerous Indo-Pacific countries (Jimmy *et al.* 2012; Mills *et al.* 2012).

In recent years, bivalve faeces and powdered algae have been used as the food sources of holothurians; however few studies have been reported on the nutritional requirements of sea cucumbers in the Red Sea. Zhu *et al.* (2005) have reported the optimum dietary protein and lipid requirements of juvenile sea cucumbers to be about 18 to 24% and 5%, respectively. Since microalgae and organic materials in the sediment are the main food sources for sea cucumbers, sea mud, a mixture of microalgae and decaying organic matter, is provided as the main component of formulated feeds (Mou *et al.* 2000). The suitable proportion of dietary sea mud is 20 to 50% for juveniles and 60% for adult sea cucumbers (Song, 2003; Yuan, 2005). Yuan *et al.* (2006) indicated that growth of sea cucumbers fed a diet containing 75% dried faeces and 25% powdered algae was higher than other treatments. However, there is no agreement on the optimal proportion of sea mud in diets for sea cucumbers.

Several studies showed that juvenile sea cucumbers fed commercially available dried powdered seaweeds and sea mud exhibited significant growth (Battaglene *et al.* 1999; Zhu *et al.* 2007; Liu *et al.* 2010). An important step in optimizing the sea cucumber culture system is to identify the most suitable seaweeds to use in their artificial diet, which could be used to improve sea cucumber production and to provide suggestions for seaweeds culture.

This study investigated the effect of seven different diets made from single and combined powdered seaweeds on the growth of sea cucumber *H. impatiens* and thus determine the proper diet for land-based intensive culture of this species.

MATERIALS AND METHODS

Collection of animals

Juvenile sea cucumbers of similar size were collected from Hurghada City northern the Red Sea by snorkelling during May of 2014. The depth of the sampling location was up to one meter beneath rocks. Sea cucumbers were moved to the wet laboratory at the National Institute of Oceanography and Fisheries, the Red Sea branch, Hurghada. They were placed in 1.0 m^3 capacity cylindrical fiberglass tank for 2 weeks prior to the experiment and fed with a mixture of algae powder for acclimation. To ensure the gut content were evacuated, they were left for one day without feed for starvation. Then, each juvenile was drained for one hour in a dry bench before they were weighed to ensure weight stability.

Experimental setup and diets

The diets were single and mixtures of powdered seaweeds. Each diet contained sea mud and one or more kinds of the following seaweeds: *Caulerpa racemosa*, *Cystoseira indica*, and *Jania rubens.* The three types of fresh seaweeds and sea mud were collected from an intertidal zone from the Red Sea, in front of Hurghada City. Fresh seaweeds were dried in the sun for 48 hours and then pulverized into ultra-fine pieces (<100µm). Sea mud was dried at 70 °C for 48 h, sieved using a 0.20 mm mesh. Each type of diet contained 20% powdered seaweed and 80% sea mud, according to the ratio used by Liu *et al.* (2009), which has been shown to produce the best growth performance for sea cucumbers beside the starvation test. The slurry was slightly stirred, extruded by a grinder to cylindrical form, then dried at 70 °C for 48 h and kept at 4 °C until used. The diets sank easily and kept good form in the water for a long time.

The eight experimental diets tested were seaweed powder that each contained sea mud and a single or combined of the following seaweeds (Table 1): (A) no seaweeds (starvation), B) *J. rubens,* C) *C. racemosa,* D) *C. indica,* E) equal portion of *C. racemosa* and *C. indica*, F) equal portion of *J. rubens* and *C. racemosa,* G) equal portion of *J. rubens* and *C. indica*, H) equal portion of *J. rubens, C. racemosa* and *C. indica.* After 24 h of starvation, one hundred and ninety-two sea cucumbers with initial wet body weights of 6.1±0.6 g were randomly selected from the acclimatized animals and each eight sea cucumbers placed into 24 plastic aquaria (50x40x30 cm with water volume of 40 L) to form eight groups in triplicate. The last seven groups were fed the different diets (A, B, C, D, E, F, G, and H) once a day at about 12:00 h. Feeding rate was 6% of their initial body weight after every two weeks until the end of the experiments. The feed was mixed with 30% seawater before used (Xia *et al.* 2012).

Environmental conditions and cleaning

Environmental conditions were monitored daily in the aquaria using YSI professional multiparameter. Sea water was continuously aerated, and the mean water quality parameters during the experiments times were: temperature 24.2 ± 0.6 °C, salinity 40.9 ± 0.7 ‰, pH 8.1 ± 0.4 and the dissolved oxygen was kept above 5.0 mg L^{-1}. About one half of the seawater (15 L) was exchanged daily using fresh natural seawater. Seawater used in the experiment was filtered using a 6.0 µm mesh size filters and sand filter.

Fecal matter and uneaten algae were siphoned from the tanks daily from each tank and separated from each other gently and were rinsed in distilled water to remove the salt. Uneaten feed was dried at 90 °C to a constant weight to calculate how much feed had been consumed then weighed. Fecal matter was rinsed very gently in distilled water to remove the salt dried at 90 °C to a constant weight then stored at -20 °C for analysis. Every two weeks, all of the sea cucumbers in each tank were separately weighed.

Weight measurement

The weight of the sea cucumbers was measured to the nearest milligram using an analytical digital balance with 4 decimal places on the start of the experiments and every two weeks over ten weeks. In the start of the experiments, there was no significant difference in the initial body weight among all treatments. Mean specific growth rate (SGR), relative weight gain (RWG), ingestion rate (IR), faeces production ratio (FPR), feed conversion ratio (FCR) and apparent digestive ratio (ADR) of the sea cucumbers for each feed treatment were calculated according to the following equations:

$$SGR(\%d^{-1}) = \frac{lnW_f - lnW_i}{T} \times 100$$

$$RWG(\%) = \frac{W_f - W_i}{W_i} \times 100$$

$$IR(gg^{-1}d^{-1}) = I/(\frac{W_f + W_i}{2}) \times T$$

$$FPR(gg^{-1}d^{-1}) = F/(\frac{W_f + W_i}{2}) \times T$$

$$FCR\ (\%) = \frac{W_f - W_i}{I} \times 100$$

where, W_i and W_f are initial and final combined dry weights of all 8 sea cucumbers in each aquarium; T is the duration of the experiment; I is the dry weight of feed ingested, and F is the dry weight of faeces.

Ammonia-nitrogen production

Ammonia-nitrogen production was analyzed after 24 h from changing the water at 12:00h and before placing different kinds of diets in the tanks. One tank without any sea cucumber was used as a control. To measure the concentration of ammonia-nitrogen in each tank, a 50 ml water sample was removed from each tank and analyzed using Nessler's reagent method (Krug *et al.* 1979).

$$ANP(mg\ (g\ diet)^{-1}) = \frac{[fi\ ANC - i\ ANC]\ x\ V}{I}$$

Where, ANP is Ammonia-Nitrogen production; fi ANC is Final Ammonia-Nitrogen Content; i ANC is initial Ammonia-Nitrogen Content, I is the dry weight of feed ingested and V is the water volume

Statistical analyses

Statistical analysis was performed using SPSS *V.* 18.0 for Windows. Data (mean ± S.E.) 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 LSD (least significant difference) test. The statistical significance level was set at 0.05.

RESULTS

Daily Specific growth rates (DSGR)

There were no significant differences in body weights of juvenile sea cucumber *H. impatiens* among diet treatments at the beginning of the experiments (6.09 ± 0.06 g $P > 0.05$). There was a significant difference in growth performance parameters of sea cucumber *H. impatiens* fed single and combined seaweed diets over ten weeks (Table 1 & Fig. 1). Generally, growth rate was faster in combined algae than in single algae diets. Moreover in single diets growth was significantly high in *C. indica* than *C. racemosa* and *J. rubens* diets, but in combined diets growth was significantly high in *C. indica* and *C. racemosa* diet and the lowest growth rate was in *J. rubens* and *C. racemosa* diet (F=12.55; $P < 0.05$). The highest SGR was (0.7±0.05% d^{-1}) shown in the combined diet brown algae *C. indica* and green algae *C. racemosa* but the lowest values were (0.41±0.2% d^{-1}) in single diet red algae *J. rubens*, however, the expected lowest growth rate was (0.13±0.01% d^{-1}) in the starvation test.

Relative weight gained (RWG)

Relative weight gained of the juveniles sea cucumbers *H. impatiens* (Table 1 & Fig. 2) showed significant differences among single and combined seaweed diets over ten weeks (F=4.37; $P < 0.05$). The highest RWG value in single diets was 4.4% w^{-1} in sea cucumber fed in *C. racemosa* but the lowest value was 3.01% w^{-1} in *J. rubens* diet. In combined algae diets, the highest value of RWG was 5.9% w^{-1} in sea cucumber fed in *C. indica* and *C. racemosa* diet but the lowest value was 3.86% w^{-1} in *C. racemosa* and *J. rubens* diet.

Table 1: Effect of single and combined seaweed diets on growth performance of *H. impatiens* and ammonia-nitrogen production (mean ± SE).

Parameters	Diet treatments								df	MS	*F*	*P*
	A	B	C	D	E	F	G	H				
SGR($\%d^{-1}$)	0.41±0.04^{c}	0.41±0.04^{b}	0.51±0.04^{a,b}	0.57±0.03^{a,b}	0.49±0.06^{a,b}	0.70±0.05^{a}	0.58±0.05^{a,b}	0.67±0.05^{a,b}	7	0.24	12.55	0.000
RWG(%)	n.d.	3.01± 0.36^{b}	4.40± 0.30^{a,b}	4.36± 0.37^{a,b}	3.86± 0.52^{b}	5.90± 0.28^{a}	4.56± 0.51^{a,b}	4.29± 0.49^{a,b}	6	5.97	4.37	0.001
IR($g\,g^{-1}d^{-1}$)	n.d.	0.95 ± 0.05^{a}	0.85± 0.10^{a,b}	0.71± 0.05^{c}	0.76± 0.19^{b}	0.66± 0.10^{c}	0.70± 0.02^{c}	0.65± 0.02^{d}	6	0.44	11.08	0.000
FPR($g\,g^{-1}d^{-1}$)	n.d.	0.83± 0.04^{a}	0.73± 0.05^{a,b}	0.63± 0.03^{c}	0.69± 0.06^{b}	0.61± 0.05^{c}	0.64± 0.08^{c}	0.55± 0.07^{d}	6	0.36	57.35	0.000
FCR (%)	n.d.	1.45± 0.22^{b}	1.54± 0.27^{b}	2.13± 0.16^{a,b}	2.06± 0.18^{a,b}	2.72± 0.14^{a}	2.96± 0.27^{a}	2.99± 0.35^{a}	6	3.28	7.30	0.000
ANP($mg\,(g\,diet)^{-1}$)	0.35 ±0.01^{g}	1.6± 0.08^{a}	1.31± 0.07^{c}	0.82± 0.06^{e}	9.51± 0.04^{b}	0.56± 0.04^{f}	1.22± 0.03^{d}	1.14± 0.02^{d}	7	0.79	56.62	0.000

A) no seaweeds (the starvation test), B) *J. rubens*, C) *C. racemosa*, D) *C. indica*, E) equal portions of *J. rubens* and *C. indica*, F) equal portions of *C. indica* and *C. racemosa*, G) equal portion of *J. rubens* and *C. indica*, H) equal portion of *J. rubens*, *C. racemosa* and *C. indica*., df (degree of freedom), MS (Mean Square), *F* (F value) and *p* (*P* value) for the effect of test diets between groups. Values in the same row with different superscripts differ significantly ($P < 0.05$). n.d. (not detected).

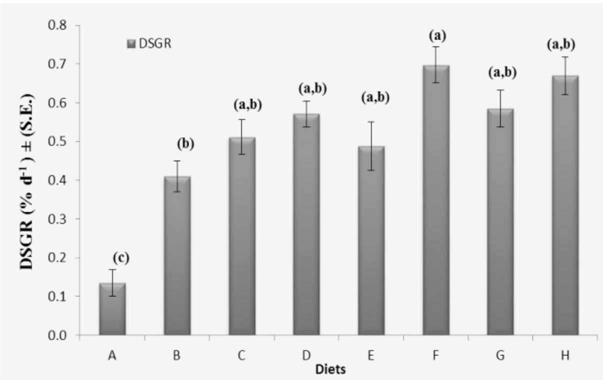

Fig. 1: Specific growth rates (SGR) of *H. impatiens* fed different single and combined seaweed diets. Different letters indicate significant differences (ANOVA, $P < 0.05$) between treatments within the same group, and bars represent standard errors. A) no seaweeds (starvation), B) *J. rubens,* C) *C. racemosa,* D) *C. indica,* E) equal portions of *J. rubens* and *C. indica*, F) equal portions of *C. indica* and *C. racemosa,* G) equal portions of *J. rubens* and *C. indica,* H) equal portions of *J. rubens, C. racemosa* and *C. indica.*

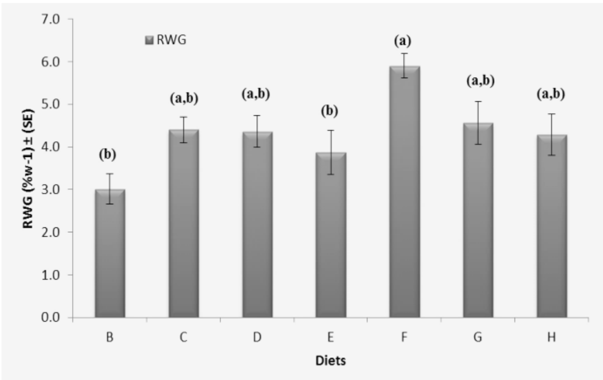

Fig. 2: Weekly relative weight gain (RWG) of *H. impatiens* fed different single and combined seaweed diets. Different letters indicate significant differences (ANOVA, $P < 0.05$) between treatments within the same group, and bars represent standard errors. B) *J. rubens,* C) *C. racemosa,* D) *C. indica,* E) equal portions of *J. rubens* and *C. indica,* F) equal portions of *C. indica* and *C. racemosa,* G) equal portions of *J. rubens* and *C. indica,* H) equal portions of *J. rubens, C. racemosa* and *C. indica.*

Ingestion rate (IR)

Ingestion rate of the juveniles sea cucumbers *H. impatiens* (Table 1 & Fig. 3) showed significant differences among single and combined seaweed diets over ten weeks (F=11.08; $P < 0.05$). The highest IR value in single diets was 0.95 g $g^{-1}d^{-1}$ in sea cucumber fed in *J. rubens* but the lowest value was 0.71 g $g^{-1}d^{-1}$ in *C. indica* diet. In combined algae diets, the highest value of IR was 0.70 g $g^{-1}d^{-1}$ in sea cucumber fed in *C. indica* and *C. racemosa* diet but the lowest value was 0.65 g $g^{-1}d^{-1}$ in *J. rubens*, *C. racemosa* and *C. indica* diet.

Faeces production efficiency (FPE)

Faeces production ratio of the juveniles' sea cucumbers *H. impatiens* was presented in Table (1) and Fig. (4). There were significant differences among different single and combined seaweed diets (F=57.35; $P < 0.05$). The highest FPR value in single diets was 0.83 g $g^{-1}d^{-1}$ in sea cucumber fed with *J. rubens* but the lowest value was 0.63 g $g^{-1}d^{-1}$ with *C. indica* diet. In combined algae diets, the highest value of IR was 0.64 g $g^{-1}d^{-1}$ in sea cucumber fed with *C. indica* and *C. racemosa* diet but the lowest value was 0.55 g $g^{-1}d^{-1}$ with *J. rubens*, *C. racemosa* and *C. indica* diet.

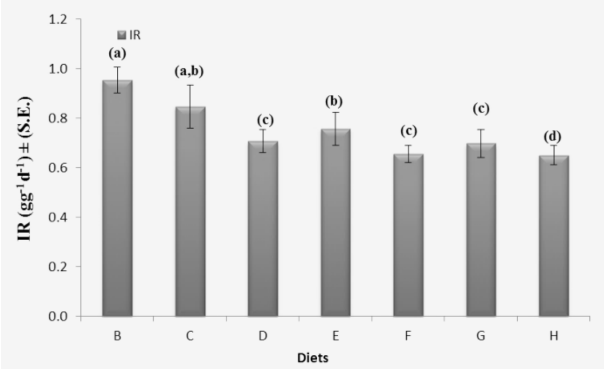

Fig. 3: Ingestion rates (IR) of *H. impatiens* fed different single and combined seaweed diets. Different letters indicate significant differences (ANOVA, $P < 0.05$) between treatments within the same group, and bars represent standard errors. B) *J. rubens,* C) *C. racemosa,* D) *C. indica,* E) equal portions of *J. rubens* and *C. indica,* F) equal portions of *C. indica* and *C. racemosa,* G) equal portions of *J. rubens* and *C. indica,* H) equal portions of *J. rubens, C. racemosa* and *C. indica.*

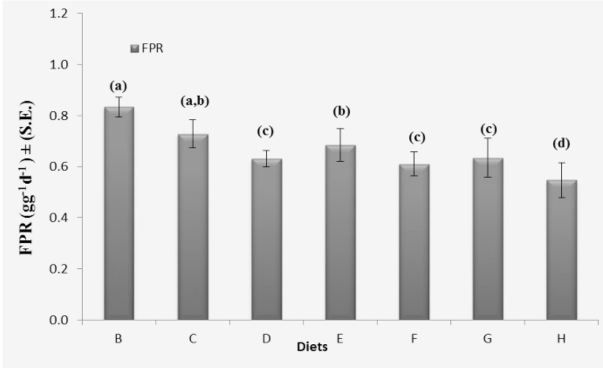

Fig. 4: Faeces production Ratio (FPR) of *H impatiens* fed different single and combined seaweed diets. Different letters indicate significant differences (ANOVA, $P < 0.05$) between treatments within the same group, and bars represent standard errors. B) *J. rubens,* C) *C. racemosa,* D) *C. indica,* E) equal portions of *J. rubens* and *C. indica,* F) equal portions of *C. indica* and *C. racemosa,* G) equal portions of *J. rubens* and *C. indica,* H) equal portions of *J. rubens, C. racemosa* and *C. indica.*

Feed conversion ratio (FCR)

Food conversion ratio of sea cucumbers juveniles *H. impatiens* are illustrated (Table 1 and Fig. 5). There were significant differences among different single and combined seaweed diets (F=7.3; $P < 0.05$). The highest FPR value in single diets was 2.13 % d^{-1} in sea cucumber fed with *C. indica* but the lowest value was 1.45 % d^{-1} with *J. rubens* diet. In combined algae diets, the highest value of FPR was 2.99 % d^{-1} in sea cucumber fed with *C. indica, C. racemosa* and *J. rubens* diet but the lowest value was 2.06 % d^{-1} with *C. racemosa* and *J. rubens* diet.

Ammonia-nitrogen production (ANP)

Ammonia-nitrogen production of juvenile sea cucumbers *H. impatiens* (Table 1 and Fig. 6) showed significant differences among different seaweed diets treatments (F= 56.62; $P < 0.05$). The highest value in single diets of ammonia-nitrogen production was 1.6 mg l^{-1} in *H. impatiens* fed *J. rubens* diet but the lowest value was 0.82 mg l^{-1} in *H. impatiens* fed *C. indica*. In combined algae diets, the highest value of ANP was 1.51 mg l^{-1} in sea cucumber fed with *C. racemosa* and *J. rubens* diet but the lowest value was 0.56 mg l^{-1} in *C. indica* and *C. racemosa* diet.

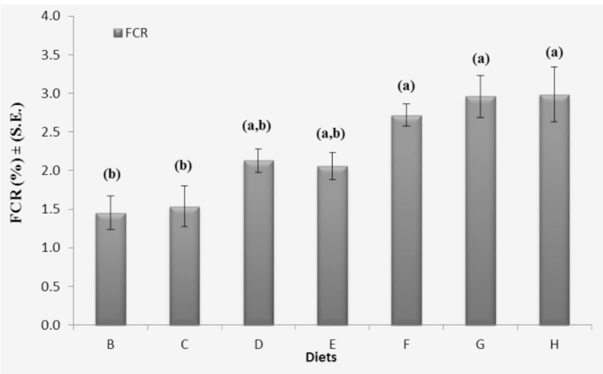

Fig. 5: Feed conversion ratio (FCR) of *H impatiens* fed different single and combined seaweed diets. Different letters indicate significant differences (ANOVA, $P < 0.05$) between treatments within the same group, and bars represent standard errors. B) *J. rubens,* C) *C. racemosa,* D) *C. indica,* E) equal portions of *J. rubens* and *C. indica,* F) equal portions of *C. indica* and *C. racemosa,* G) equal portions of *J. rubens* and *C. indica,* H) equal portions of *J. rubens, C. racemosa* and *C. indica.*

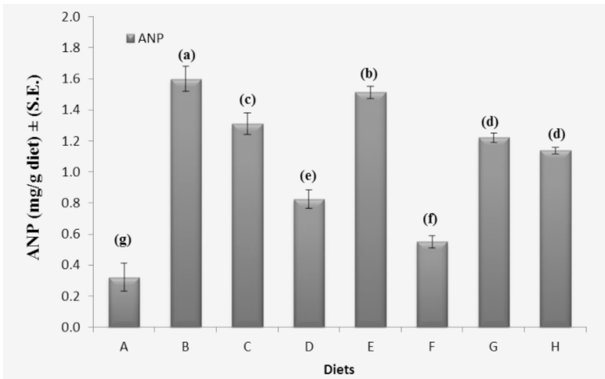

Fig. 6: Ammonia-nitrogen production (ANP) of *H impatiens* fed different single and combined seaweed diets. Different letters indicate significant differences (ANOVA, $P < 0.05$) between treatments within the same group, and bars represent standard errors. A) no seaweeds (starvation), B) *J. rubens,* C) *C. racemosa,* D) *C. indica,* E) equal portions of *J. rubens* and *C. indica,* F) equal portions of *C. indica* and *C. racemosa,* G) equal portion of *J. rubens* and *C. indica,* H) equal portion of *J. rubens, C. racemosa* and *C. indica.*

DISCUSSION

Deposit-feeding holothurians ingest sediment bearing organic matter, including bacteria, protozoa, diatoms, and detritus of plants or animals (Zhang *et al.* 1995). In the practice of hatchery-produced juveniles, newly settled larvae were commonly fed with diatoms and then in nursery tanks, powdered algae were added for holothurian juveniles (Sui, 1988; Battaglene *et al.* 1999). Powdered algae are an essential ingredient in the artificial diet of the sea cucumber in aquaculture systems (Xia *et al.* 2012).

Several research studies have used different types of seaweeds, such as (*Sargassum thunbergii*, *Sargassum polycystum*, *Zostera marina*, *Ulva lactuca*, *Laminaria japonica*, *Spirulina platensis*, and *Undaria pinnatifida* to study sea cucumber nutrition requirements (Chen *et al.* 2010; Liu *et al.* 2009; Seo and Lee, 2011; Slater *et al.* 2009; Xia *et al.* 2012; Yuan *et al.* 2006).

In the present study, the effect of single and combined seaweeds in growth performance and ammonia-nitrogen production has been investigated by using three common marine seaweeds in seven different diets besides a starvation test. *Cystoseira indica*, *C. racemosa* and *J. rubens* were used as a single and equal combined ratio between each other.

Generally, the results showed that the highest values of specific growth rate (SGR) (Fig. 1), and relative weight gain (RWG) (Fig. 2) were in sea cucumbers' juveniles *H. impatiens* fed combined algae than single algae especially diets that contained a ratio of *C. indica*. Specific growth rate and RWG of the experiment sea cucumbers varied in different diet treatments and showed a descending order; diet F > diet H > diet G > diet D > diet C > diet E > diet B > diet A. The significantly lowest SGR occurred in diet A. While, no significant differences in SGR were found among treatments of diet C, diet D, diet E, diet G and diet H, but all were significantly different from the treatment of diet F ($P < 0.05$). Moreover, the lowest values of SGR were 0.41% day^{-1} in single diets *J. rubens* but the lowest value was 0.13% day^{-1} in starvation treatment (Fig. 1). Tukey's multiple range tests showed that there were no significant differences in IR and FPR among all treatments ($P > 0.05$).

Ingestion rate (IR) (Fig. 3) and faeces production rate (FPR) (Fig. 4) showed the same descending trend as diet B > diet C > diet E > diet D > diet G > diet F > diet H. Tukey's multiple range tests showed that there were no significant differences in IR and FPR among all treatments of diet ($P > 0.05$). Two strategies have been suggested for deposit feeders; the first strategy is optimal foraging suggesting that animal increases food uptake rate with increasing food quality (Taghon 1982); and the second strategy is the 'compensatory' feeding, which suggests the converse by increasing feeding with decreased food quality (Cammen 1980; Lopez and Cheng 1983). Selective feeding has been shown in some tropical and abyssal aspidochirotes sea cucumber (Hudson *et al.* 2005).

Foraging models predict that consumers will feed in a way that optimizes their intake of energy, or limiting nutrients, to maximize their fitness (Perry and Pianka, 1997). Thus, compensatory feeding may be a main factor contributing to the low feeding rate of sea cucumbers fed on *C. indica* diet but feeding rates were higher in case of adding any sea weeds with *C. indica* diets.

The result showed that the quality of diets also affected FCR in *H. impatiens.* The highest values of food conversion ratio (FCR) (Fig. 5) were in juveniles sea cucumbers *H. impatiens* fed combined algae than single algae, especially diets containing a ratio of *C. indica*. Moreover, the lowest value of FCR was in single diets *J. rubens* but the highest value of FCR was in sea cucumber fed *J. rubens, C. racemosa* and *C. indica* diet. Consequently, the growth rate of the animal was affected by different diets. These results are in complete agreement with the results of Yuan *et al.* (2006) in sea cucumber *Apostichopus japonicas.*

Because ammonia-nitrogen is the principal end product of nitrogen metabolism for most aquatic animals and thus can indicate how much protein is deposited in the body, it can be an effective additional indicator for evaluating the nutritional quality of feed. Furthermore, ammonia-nitrogen excretion must be considered in the process of designing recirculating aquaculture systems (Eding *et al.* 2006). The present study was conducted to evaluate which of the seven types of seaweed diets is the most suitable for use in sea cucumber aquaculture or which types of seaweed diets significantly influenced ammonia-nitrogen production of *H impatiens.* The ammonia-nitrogen production of sea cucumbers fed *C. indica* and *C. racemosa* diet was much lower than that of sea cucumbers fed the other diets except the starvation test (Fig. 6). In addition, the sea cucumbers fed *C. indica* and *C. racemosa* grew faster and most of the protein was deposited in the body, so the ammonia-nitrogen production in this treatment was the lowest value. On the other hand, the highest value of ammonia-nitrogen production was in *J. rubens*. The present study agreed with the finding of Xia *et al.* (2012) for the sea cucumbers *A. japonicus* which preferred the diet containing *L. japonica* or *U. lactuca*.

CONCLUSION

There was a clear difference in growth performance parameters of juveniles sea cucumber *H. impatiens* fed different seaweed diets over ten weeks. The present study showed that growth was significantly faster in combined algae diets than in single algae diets. The highest growth rate was in juveniles sea cucumber *H. impatiens* fed combined *C. indica* and *C. racemosa* diet. The lowest value of ammonia-nitrogen production was in juveniles fed *C. indica* and *C. racemosa*. Therefore, this study recommends *C. indica* and *C. racemosa* as an optimal dry food for rearing and cultivating juveniles' sea cucumber *H. impatiens*.

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