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RESEARCH PAPER

Innovative Approach of Asexual Reproduction Induction in *Holothuria polii* (Delle Chiaje, 1824)

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Abstract

The current study describes a new method for asexual reproduction induction by fission. It results in higher rates of growth, survival and gut regeneration as compared to the conventional technique. Prior to asexual reproduction induction, samples were exposed to chemical evisceration induction through injection of 0.5 ml of 0.45M KCl into perivisceral coelomic cavity. Fission was induced in eviscerated group and normal (non-eviscerated) group through tightening the rubber bands around animal's body at the middle. We compared the modes of fission and the rates of growth, survival and gut regeneration over five months between two groups. We propose a new asexual reproduction induction method in which sea cucumber chemically induced to eviscerate prior to fission induction.

Introduction

Sea cucumbers are among the most curious organisms having many peculiar morphogenetic reactions. They are known for autotomy that includes the loss of body parts by evisceration or asexual reproduction (fission) (Garcia-Araras *et al*., 1998). As well, they are capable to restore the lost organs after autotomy (Dolmatov, 2014).

Asexual reproduction (fission) is common for the class *Holothuroidea*. Successful asexual reproduction induction has different biological functions, it may offset the high mortality in the larval stages in hatchery operations (James, Gandhi, Palaniswamy, & Rodrigo,1994); produce seed stock for mariculture and grow-out operations (Reichenbach, Nishar, & Saeed, 1996); and double the population number resulting in acceleration the natural populations restoration (Purwati, Dwiono, Indriana, & Fahmi, 2009). The fission induction can potentially be applied to some non-fissiparous holothurian species (Purwati, Dwiono, Indriana, & Fahmi, 2009). It has been currently confirmed in 21 fissiparous holothurian; 13 aspidochirotida and 8 dendrochirotida (Dolmatov, 2014).

Despite the significance of evisceration and asexual reproduction phenomena to aquaculture, a few studies have evaluated the effects of them on the holothurian survival and regeneration rates. Previous studies on experimental fission induction were conducted in normal specimen including *Bohadshia marmorata* and *H. atra* (Laxminarayana, 2006), *H. atra* and *H. leucospilota* (Purwati and Dwiono, 2005), *H. atra* (Purwati and Dwiono, 2007; Purwati, Dwiono, Indriana, & Fahmi, 2009), *Actinopyga mauritiana*, *H. nobilis, H. fuscogilva* and *Stichopus variegarus* (Reichenbach, Nishar, & Saeed, 1996) but there is no reported study of fission induction in eviscerated specimen so far.

Some holothurian species are able to completely or partially regenerate after fission (Hyman, 1955) and evisceration (Garcı´a-Arrara´s and Greenberg, 2001). Knowledge of gut regeneration is necessary in particular for the holothurian culture as the information will provide the suitable time for feeding the regenerated individuals and the optimum releasing time to their natural habitats as suggested by Purwati and Dwiono (2007).

Holothuria polii is considered as the most common Mediterranean species among the targeted and overexploited sea cucumber species (Aydin and Erkan, 2015; Aydin, 2016) as well in Egyptian sea cucumber fishery. In Egypt, it was started in the Red Sea since 1990s (Lawrence *et al*., 2004). After depletion of commercially valuable sea cucumber stocks in many Red Sea areas as a result of over-exploitation, the holothurian fishery exploitation was directed to Egyptian Mediterranean Sea in 2000s (Abdel Razek, Abdel Rahman, Mona, El-Gamal, & Moussa, 2012). To counteract the expected damage and negative impacts of over-exploitation, necessary actions should be taken for sustainable development through restocking strategies and farming projects (Toscano and Cirino, 2018). Therefore, the present work evaluates for the first time the effect of chemical evisceration induction prior to inducing fission in enhancing the survival, regeneration and growth of the fissoned parts to be used for aquaculture and restocking strategies.

Materials and Methods

Samples Collection and Maintenance

Samples of adult *Holothuria polii* were collected through scuba diving at depth ranged from 5 to 12 m from Abu-Qir Bay (31.331° N, 30.0744° E), Alexandria, Egypt. The Abu-Qīr Bay is a spacious bay on the Mediterranean Sea, it is bounded to the northeast by the Rosetta mouth of the Nile. Samples collection was done in the morning and transported in plastic containers within two hours to the Invertebrate Aquaculture Laboratory in National Institute of Oceanography and Fisheries, Alexandria.

A number of 210 *H. polii* samples was collected and maintained in 300L fiberglass tanks with a thin layer of fine sand on the bottom. The tanks filled with aerated sea-water of 38 ppt and ambient temperature of 23±1°C. The water of the tanks was changed once daily. Before conducting experiments, samples were allowed to adapt for one week to the new environment. For each sample, total length (TL) was measured to the nearest 0.01 cm. Also, total wet weight (TW) was recorded with a digital electric balance to an accuracy of 0.01 g.

Induction of Asexual Reproduction in Eviscerated (EV) and Non-eviscerated (NEV) Samples

Samples were divided into three groups, control (30 samples), EV (90 samples), and NEV (90 samples) groups. Samples of the eviscerated group (EV) were induced to eviscerate by injection of 0.5 ml of 0.45M KCl into the perivisceral coelomic cavity as described by Byrne (1985). Samples of the non-eviscerated group (NEV) were not exposed to any treatment. Asexual reproduction "fission" was induced in both groups through tightening the rubber bands around animal's body in the middle. The daily check was done for rubber bands to be replaced if needed. Both of control, EV, and NEV were reared in small glass tanks filled with slowly aerated seawater. The water of the tanks was changed twice daily. No food addition was applied. The number of divided, undivided and dead samples was reported daily in each group. The percentages of fission rate were calculated as follow:

$$\text{Fission Rate} = D/N \times 100$$

Where,
D=number of divided samples
N=total number of samples

Survival rate was calculated as reported by Reichenbach and Holloway (1995) as follow:

$$\text{Survival Rate} = [(A+P)/2T] \times 100$$

Where,
A+P=number of the anterior parts (A) and posterior parts (P)
T=total number of specimens that had undergone fission

Survival, Growth and Gut Regeneration

After fission induction and wound healing period, divided parts were collected and hanged in nets in the open sea for five months. Temperature and pH were measured daily using multi-parameter "EXTECH" EC500. The rates of growth, and survival were weekly recorded. Dissection was performed weekly to follow the gut regeneration.

Data Analysis

Data were analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard deviation for normally distributed data. Comparisons between NEV and EV groups were done using independent t-test while results of fission and survival rates were analyzed by F-test (ANOVA) and compared using Post Hoc test (LSD).

Results

Description of Fission Process

Induced fission samples were ranged from

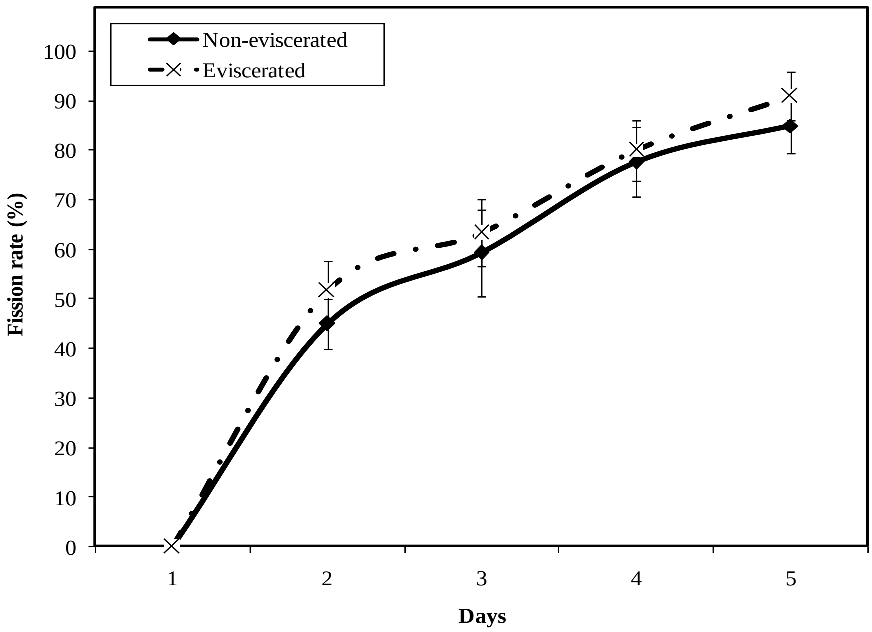

Figure 1. Fission rate of *Holothuria poli* in non-eviscerated (NEV) and eviscerated groups (EV).

Table 1. Survival rate ± SD of *Holothuria poli* anterior part (A) and posterior part (P) resulted from fission induction in non-eviscerated (NEV) and eviscerated groups (EV)

Part	Day	Survival rate (%)		t	p
		Non-eviscerated	Eviscerated		
A	2	100.0 ± 0.0	100.0±0.0	-	-
	3	81.0 ± 7.55	100.0±0.0	4.359*	0.012*
	4	72.0 ± 3.61	87.0±4.58	4.456*	0.011*
	5	63.0 ± 4.58	76.0 ± 7.0	2.691	0.055
	6	57.67 ± 8.50	63.0 ± 5.0	0.936	0.402
P	2	100.0 ± 0.0	100.0±0.0	-	-
	3	93.0 ± 4.0	100.0±0.0	3.031*	0.039*
	4	81.0 ± 4.58	92.0±3.6	3.267*	0.031*
	5	74.0 ± 5.29	89.0±6.0	3.248*	0.031*
	6	70.0 ± 3.61	85.0±7.0	3.300*	0.030*

t: Student t-test for comparing between non eviscerated and eviscerated group
*: Statistically significant at P≤ 0.05

29.7±1.2 to 49.28±1.9 g TW and 6 to 10.8 cm TL. Samples remained nearly stationary for little time on the glass tank floor, and then get swelling at posterior parts. Both A and P parts stretched and then separated. Divided parts were only connected to each other via the gut. The fission process lasted 5 days. After separation of A and P parts, the wounds healed and closed after 2-3 days. The fission process was the same in both NEV and EV except for the connection of two parts with gut; in EV, two parts were split after stretching and rotation.

Fission Rate

The fission rate was significantly increased by time within NEV (F=93.3) and EV (F=129.669). At the fifth day of the trial, fission was successful in 85%, and 91% of NEV and EV samples, respectively. The fission rate showed no significant difference between EV and NEV (Figure 1). On the other hand, no fission was observed in control group within the same period.

There was no recorded mortality of both A and P parts in EV and NEV until the 2nd and 3rd day of NEV and EV, respectively. Afterward, the survival rates decreased gradually until the 5th day. The survival rates of A in EV was significantly higher than those in NEV in 3rd day (*P*=0.012) and 4th day (*P*=0.011) (Table 1). Irrespective of survival rates of A and P parts, the survival rates were significantly declined by time in both NEV (F=25.96) and EV (F=13.61). By comparing the survival rates of NEV and EV, it was noticed that survival rates of EV were significantly higher than those

Table 2. Survival rate ± SD of *Holothuria poli* total divided parts resulted from fission induction in non-eviscerated (NEV) and eviscerated groups (EV)

Day	Survival rate (%)	
	Non-eviscerated	Eviscerated
2	$100.0^{a}\pm0.0$	$100.0^{a} \pm 0.0$
3	$87.0^{b} \pm8.51$	$100.0^{a} \pm 0.0$
4	$76.50^{c} \pm6.16$	$89.50^{b} \pm 4.59$
5	$68.50^{cd} \pm7.48$	$82.50^{bc} \pm 9.20$
6	$63.83^{d} \pm8.93$	$74.0^{c} \pm 13.22$
F	25.960^{*} ($<0.001^{*}$)	13.613^{*} ($<0.001^{*}$)

F: F test (ANOVA) for comparing between different days in each group
Significance between groups was done using Post Hoc Test (LSD)
*: Statistically significant at $P\leq 0.05$

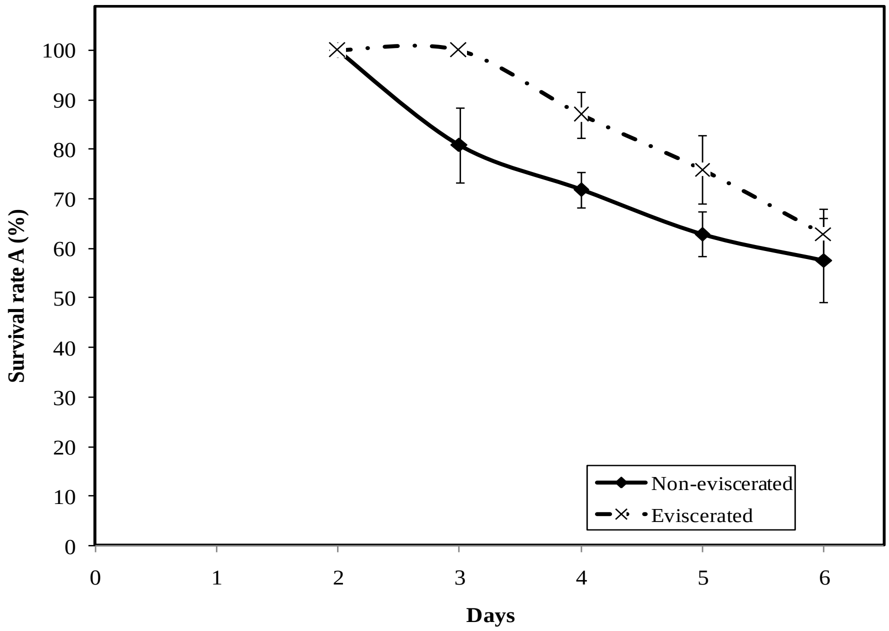

Figure 2. Survival rate of *Holothuria poli* anterior part (A) in non-eviscerated (NEV) and eviscerated groups (EV) after fission induction.

in NEV during the period from 2nd to 5th day (Table 2). Regardless of NEV and EV, P survival rates attained higher values than those of A.

Rates of Survival, Growth and Gut Regeneration

During the rearing experiment in open sea, the temperature values reached the lowest average value (17°C) and highest average pH value (8.3) in February. Afterward, the average temperature showed a gradual increase until reaching 25°C in June. However, the lowest pH average value of 8 was recorded in January then increased to 8.3 in June (Figure 2).

Over five month rearing period of divided parts, the mortality of A in both NEV and EV exceeded 90%, therefore, the survival and growth rates were calculated in the total remaining parts and not in A and P parts separately. The survival rates of divided parts showed a significant sharp decrease by time in NEV (F=152.678) and EV (F=30.330). The difference of survival rate between EV and NEV was significant ($P<0.05$). It was 12% and 51% in NEV and EV, respectively (Figure 3).

In both EV and NEV, weight was declined gradually after the fission induction, but thereafter, there was an increase in weight from 10th week in EV and from 15th week in NEV which correspond to April and June, respectively (Figure 4). No significant difference in weight gain was recorded between EV and NEV during the rearing period.

After fission, anterior parts of NEV kept esophagus, calcareous ring, Polian vesicle, gonad base without tubules, right respiratory tree and part of the intestine. However, A in EV was noticed to have esophagus, calcareous ring, part of right respiratory tree and gonad base without tubules. Regarding P, parts of the intestine, left respiratory tree and cloaca were kept in NEV, while EV was observed to have only part of right respiratory tree and cloaca.

Gut regeneration was monitored over 20 weeks in both divided parts. The regeneration process has taken longer period in NEV than in EV. The digestive tube regeneration was completed in the 8th week in EV while it was in the 12th week in NEV. Additionally, the mouth

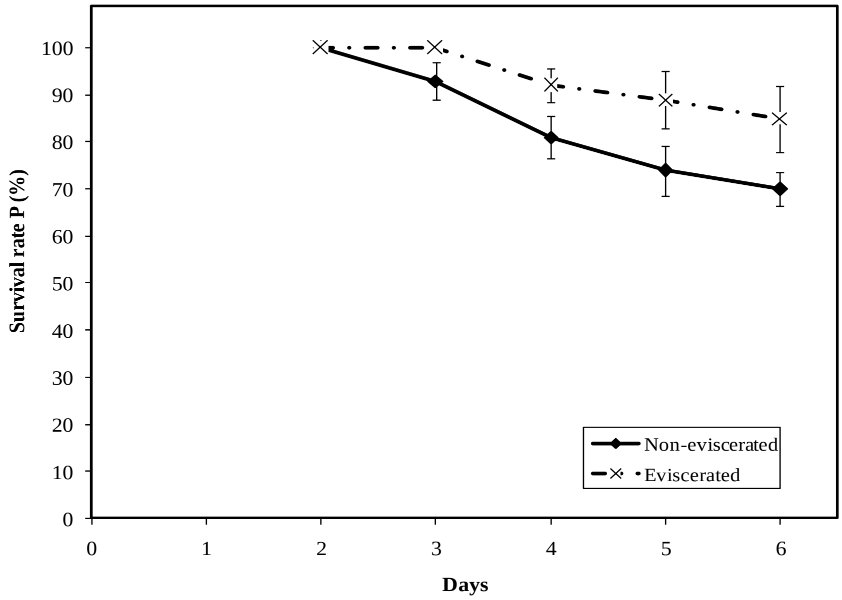

Figure 3. Survival rate of *Holothuria poli* posterior part (P) in non-eviscerated (NEV) and eviscerated groups (EV) after fission induction

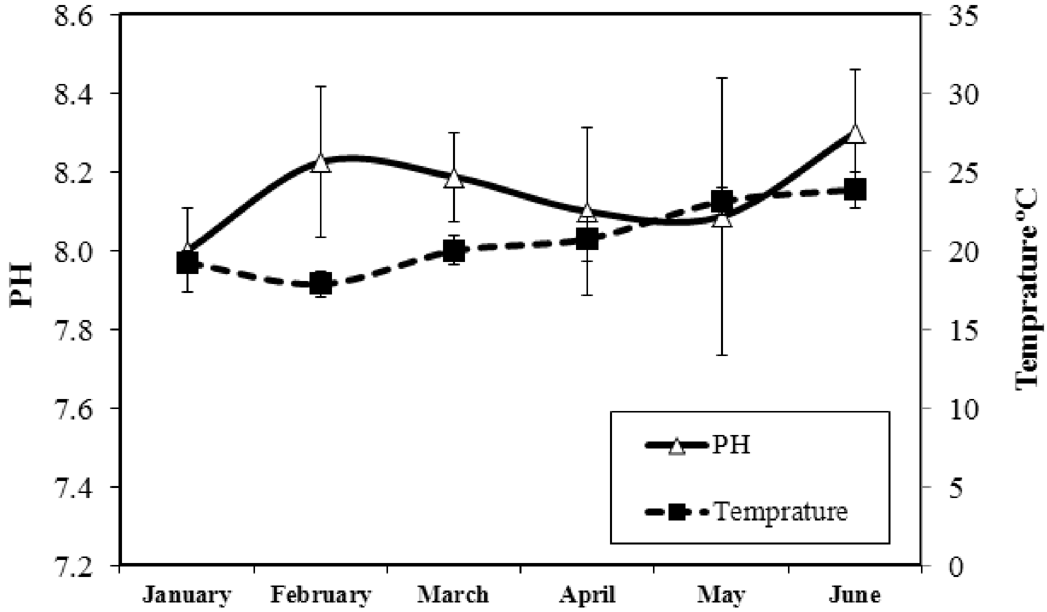

Figure. 4. Temperature and PH average values of rearing area during the experimental period.

was formed in P before anus in A in both EV and NEV. No regeneration of respiratory tree and gonads was noticed either in EV or NEV divided parts during the experimental period.

Discussion

On the face of notable holothurian power of fission, evisceration, and regeneration, the current study presents a new method of asexual reproduction (fission) induction in one of Egyptian Mediterranean holothurian species, *Holothuria polii*. The conventional method of fission induction in all previous studies was the fitting rubber bands or nylon string or cable tie (Purwati and Dwiono, 2005) or peristaltic silicone tubes (Toscano and Cirino, 2018) around the middle of the normal specimen. However; the present study induced samples of *H. polii* to chemically eviscerate as described by Byrne (1985) prior to fission induction by body fitting. Obtained results of the new method were represented by EV group while those for conventional method were by NEV. Rates of fission, survival, growth and gut regeneration were investigated and compared between the NEV and EV to illustrate the significance of the suggested method.

Nevertheless, *H. polii* is non-fissiparous; the present study was succeeded experimentally in inducing it to fission by 85% and 91% in NEV and EV, respectively. Regardless of the applied method, the duration taken by *H. polii* to attain the highest fission rate was 5 days which was significantly shorter than 11 days recorded by Toscano and Cirino (2018). The results of the survival rate of new method were significantly higher than those for conventional method during the period from 2nd to 5th day after fission induction. In both methods, survival rate of *H. polii* was similar to the recorded holothurian survival rates values after fission induction that varied but almost keep higher percentage for P than A such as *Actinopyga mauritiana, H. fuscogilva, S. variegatus* (O'Loughlin and O'Hara, 1992)*, Apostichopus japonicus, H. scabra* (Kille, 1942; Emson and Wilkie, 1980; Kohtsuka, Arai, & Ushimura, 2005), *H. atra* (Chao, Chen, & Alexander, 1993) and *Stichopus chloronotus* (Conand, Uthicke, & Hoareau, 2002). Similarly, total mortality of A was attained in *S. variegatus*, *H. fuscogilva* (Reichenbach, Nishar, & Saeed, 1996). By contrast, *Bohadshia marmorata* showed no mortality for both parts (Purwati and Dwiono, 2005) as well in experimentally fission induced *H. polii* by Toscano and Cirino (2018).

The remaining organs after fission induction in A of *H. polii* were similar to those of most holothurian species. Esophagus, calcareous ring, Polian vesicle, right respiratory tree, and part of intestine were mostly contained in A, whereas gonad basis mostly kept without tubules. However, gonad may be absent in A of some species such as *H. edulis* and *S. chloronotus* (Uthick, 1997). With regard to remaining organs of *H. polii* P, it was noticed to keep parts of the intestine, left respiratory tree and cloaca as reported in *H. edulis* and *S. chloronotus* (Uthick, 1997). However, *H. atra* contained both of respiratory trees, rete mirabile, and intestine (Uthick, 1997). This discrepancy of remaining organs was not only reported among holothurian species but also within the same species as observed in *H. leucospilota* (Purwati and Dwiono, 2007). Variations of remained organs of both divided parts were noticed in NEV and EV; A kept part of the right respiratory tree in EV, while A hold the whole right respiratory tree in NEV. In addition, P of NEV was observed with a left respiratory tree; however, P had part of the right respiratory tree in EV.

After five month rearing period of *H. polii* divided parts, the low survival rate of around 10% for A was recorded for both methods. This finding is in agreement with previous studies of non-fissiparous *H. arenicola* which attained survival percentage of 40% after 21 days (Raouf, Fatma, & Mohamed, 2000) and 0% after 75 days (Moussa, 2009). Low recorded values of A survival may be attributed to oxygen uptake deficiency as A obtains oxygen through diffusion across the body wall until complete regeneration of respiratory tree as reported by Reichenbach, Nishar, & Saeed (1996). Further, inability to restore the lost body fragments after fission induction may be another cause for low A survival (Emson and Wilkie, 1980; O'Loughlin and O'Hara, 1992; Kohtsuka, Arai, & Ushimura, 2005; Mashanov, Dolmatov, & Heinzeller, 2005; O'Loughlin *et al.*, 2009). Regardless of A and P parts, the overall survival rate showed a significant difference between two methods. It was 12% and 51 survival rate for the conventional and new method, respectively.

Studies of regeneration abilities are important to develop cultivation technologies and increase holothurian populations as reported by Dolmatov (2014). Regeneration of the internal organs after fission has been described in varying degrees of details for 6 holothurian species: *H. difficilis* (Deichmann, 1922), *H. parvula* (Emson and Mladenov, 1987), *H. atra* (Boyer, Cailasson, & Mairesse, 1995; Conand, 1996), *H. leucospilota* (Conand, Morel, & Mussard, 1997), *S. chloronotus* (Conand, Armand, Dijoux, & Garryer, 1998), and *C. schmeltzii* (Dolmatov, Khang, & Kamenev, 2012). Precedence of *H. polii* mouth formation indicated that P regeneration rate was faster than A. This finding is in similarity to findings of *H. parvula* (Kille, 1942), *S. variegates* (Reichenbach, Nishar, & Saeed,1996*), H. atra* (Purwati, Dwiono, Indriana, & Fahmi, 2009) and *H. impatiens* (Nugroho, Hartati, & Suprijanto, 2012). As pointed out in studies *H. parvula* (Kille, 1942) and *H. atra* (Conand, 1996), the late regeneration of reproductive system in *H. polii* was against early regeneration of digestive system.

More attention was directed in the current study toward gut regeneration for its evident importance in providing the suitable time for feeding the dividing individuals and the releasing time to their natural habitat. The new method showed a considerable effect on shortening the period of gut regeneration; gut was completed in the 8th week and 12th week in new and conventional methods, respectively. Gut regeneration in new method was completed in February at the lowest recorded average temperature of 17.8°C in the rearing period, while it was recorded in March for the conventional method at 20°C average temperature. Carnevali (2006) and Garcı´a-Arrara´s and Greenberg (2001) stated that regenerative potential can greatly differ among holothurian groups and ages. In the present study, regardless of the used method, the time of 56 - 84 days which were taken by *H. polii* to attain complete gut regeneration was almost longer than that recorded for *H. polii* by Toscano and Cirino (2018) (45-65). This variation may be also due to the difference in geographical locations besides differences in sample size, ambient temperature and rearing method. However, many holothurian species regenerate within shorter period such as *H. scabra* which required only 7 days (Bai, 1971); *H. glaberrima* with 20-30 days regeneration period (Garcı´a-Arrara´s *et al.,* 1998); and

H. atra, H. leucospilota, H. edulis, and *H. hilla* that completed the gut regeneration for both parts by 40-50 days post fission induction (Dolmatov, Khang, & Kamenev, 2012). On the other hand, the longest recorded period for gut regeneration was for *Stichopus mollis* which need 145 days (Dawbin, 1949). It is noteworthy that many authors indicated that regeneration is influenced by aquarium conditions, as well as by experimental variables (e.g. Reinschmidt, 1969; Garcı´a-Arrara´s and Greenberg, 2001). Most of the previous findings, however, were obtained with animals kept in the laboratory, whereas, the present study investigated the gut regeneration in the natural environment at 20-23˚C, and 8-8.3 pH.

Growth rate after fission induction was previously studied in *B. marmorata* and *H. atra* (Laxminarayana, 2006), *H. parvula* (Kille, 1942) and *Apostichopus japonicus* (Dolmatov and Mashanov, 2007). In the present study, weight loss over the rearing period of *H. polii* divided parts was observed in regeneration period. This reduce in weight was a consequence of non-feeding behavior during the regeneration period (Emson and Mladenov, 1987; Conand, Morel, & Mussard, 1997) which may lead to minimum metabolic expenditure (Uthick, 1997). Weight loss could be also attributed to remaining organ lysis (Emson and Mladenov, 1987; Conand, Morel, & Mussard, 1997; Dolmatov, 2014) that may provide energy for recovery and regeneration (Purwati and Dwiono, 2007). Further, many studies mentioned that holothurian tegument may be involved in feeding and respiration (Hyman, 1955). Observation of sand within the newly regenerated intestine indicates the feeding restarting and intestine functioning. Weight gain of *H. polii* divided parts observed at the 10th and 15th week in EV and NEV coinciding with March and April, respectively at a temperature range of 20-20.75°C.

In accordance to exploitation of Egyptian holothurian resources for the Asian market which has been developed by the year 2000 along the Egyptian Mediterranean coast on a large scale resulted in local extinction of high-value species in many localities (Abdel Razek, Abdel Rahman, Mona, El-Gamal, & Moussa, 2007; Moussa, 2009). Fission induction will acquire particular significance for commercially valuable species that are exposed to widespread overfishing as suggested by Purcell (2010). Undoubtedly, knowledge of asexual reproduction has a great influence on cultivation technologies and subsequently on enhancing the holothurian populations. Therefore, the findings from this study are valued to aquaculture specialists who produce individuals through fission.

References

Abdel Razek, F.A., Abdel Rahman, S.H., Mona, M.H., El-Gamal, M.M., & Moussa, R.M. (2007). An observation on the effect of environmental conditions on induced fission of the Mediterranean sand *sea cucumber, Holothuria arenicola* (Semper, 1868) in Egypt. SPC Beche-de-Mer Information Bulletin*, 26*, 33-34.

Aydin, M. (2016). Sea Cucumber (Holothuroidea) Species of Turkey. Turkish Journal of Maritime and Marine Science*, 2*, 49-58.

Aydin, M. & Erkan, S. (2015). Identification and some biological characteristics of commercial sea cucumber in the Turkey coast waters. International Journal of Fisheries and Aquatic Studies, *3*, 260-265.

Bai, M.M. (1971). Regeneration in the Holothurian, *Holothuria scraba* Jager. Indian Journal of Experimental Biology*, 19*, 467–471.

Boyer, C., Cailasson, S., & Mairesse, K. (1995). Asexual reproduction in *Holothuria atra* on a reef of Reunion Island in the Indian Ocean. SPC Beche-de-Mer Information Bulletin, *7*, 7–9.

Byrne, M. (1985). Evisceration behavior and the seasonal incidence of evisceration in the holothurian *Eupentacta quinquesemita* (selenka). *Ophelia*, 24, 75-90.

Carnevelio, M.D.C. (2006). Regeneration in Echinoderms: repair**,** regrowth, cloning. Invertebrate Survival Journal, *3*, 64–76.

Chao, S.M., Chen, C.P., & Alexander, P.S. (1993). Fission and its effect on population structure of *Holothuria atra* (Echinodermata: Holothuroidea) in Taiwan. Marine Biology, *116*, 109–115. https://dx.doi.org/10.1007/BF00350738

Conand, C. (1996). Asexual reproduction by fission in *Holothuria atra*: variability of some parameters in populations from the tropical Indo-Pacific. Oceanologica Acta, *19*(3-4), 209-216

Conand, C., Armand, J., Dijoux, N., & Garryer, J. (1998). Fission in a population of *Stichopus chloronotus* on Reunion Island, Indian Ocean. SPC Beche-de-Mer Information Bulletin, *10*, 15–23.

Conand, C., Morel, C., & Mussard, R. (1997). A new study of asexual reproduction in holothurian: fission in *Holothuria leucospilota* populations on Reunion Island in Indian Ocean. SPC Beche-de-Mer Information Bulletin*, 9*, 5–11.

Conand, C., Uthicke, S., & Hoareau, T. (2002). Sexual and asexual reproduction of the holothurian *Stichopus chloronotus* (Echinodermata): A comparison between La Réunion (Indian Ocean) and east Australia (Pacific Ocean). Invertebrate Reproduction and Development, *4*1, 235–242 https://dx.doi.org/10.1080/07924259.2002.9652756.

Dawbin, W.H. (1949). Auto-evisceration and the regeneration of viscera in the holothurian *Stichopus mollis* (Hutton). Transactions of the Royal Society of New Zealand, *7*, 497-523.

Deichmann, E. (1922). On some cases of multiplication by fission and of coalescence in Holothurians; with notes on the synonymy of *Actinopyga parvula.* Dr. Th. Mortensen's Pacific expedition, *4*,199–214.

Dolmatov, I.Y. (2014). Asexual reproduction in holothurians. Scientific World Journal*.* 2014, 13. https://dx.doi.org/ 10.1155/2014/527234.

Dolmatov, I.Y, Khang, N.A., & Kamenev, Y.O. (2012). Asexual reproduction, evisceration, and regeneration in holothurians (Holothuroidea) from Nha Trang Bay of the South China Sea. Russian Journal of Marine Biology, *38*(3), 243–252. https://dx.doi.org/10.1134/S1063074012030042.

Dolmatov, I.Y., & Mashanov, V.S. (2007). Regeneration in Holothurians. Vladivostok, Russia, Dalnauka Press., 212 pp.

Emson, R.H., & Mladenov, P.V. (1987). Studies of the fissiparous holothurian *Holothuria parvula* (Selenka) (Echinodermata: Holothuroidea). Journal of Experimental Marine Biology Ecology*, 111*(3), 195–211. https://dx.doi.org/10.1016/0022-0981(87)90028-1.

Emson, R.H., & Wilkie, I.C. (1980). Fission and Autotomy in Echinoderms, Oceanography and Marine Biology. Annual Review, *18*, 155–250.

Garcı´a-Arrara´s, J.E., Estrada-Rodgers, L., Santiago, R., Torres, I.I., Diaz-Miranda, L., & Torres-Avillan, I. (1998). Cellular mechanisms of intestine regeneration in the sea cucumber, *Holothuria glaberrima* Selenka (Holothuroidea: Echinodermata). Journal of Experimental Zoology, *281*, 288–304. https://dx.doi.org/10.1002/(SICI)1097-010X(19980701)281:4<288::AID JEZ5>3.0.CO;2-K·

Garcı´a-Arrara´s, J.E., & Greenberg, M. (2001). Visceral regeneration in holothurians. Microscopy Research and Technique, *55*,438-451. https://dx.doi.org/ 10.1002/jemt.1189.

Hyman, L.H. (1955). The invertebrates: Echinodermata (The coelomate Bilateria). New York, USA, Mc Graw-Hill Book Company., 1250 pp.

James, D.B., Gandhi, A.D., Palaniswamy, N., & Rodrigo, J.X. (1994). Hatchery techniques and culture of the sea cucumber *Holothuria scabra*. CMFRI Bulletin*, 48*,120–126.

Kille, F.R. (1942). Regeneration of the reproductive system following binary fission in the sea cucumber *Holothuria parvula* (Selenka). Biological Bulletin, *83*, 55–66. https://doi.org/10.2307/1538013.

Kohtsuka, H., Arai, S., & Ushimura, M. (2005). Observation of Asexual Reproduction by Natural Fission of *Stichopus horrens* Selenka in Okinawa Island, Japan. SPC Beche-de-Mer Information Bulletin, *22*, 23.

Laxminarayana, A. (2006*).* Induced spawning and larval rearing of the sea cucumbers*, Bohadschia marmorata* and_*Holothuria atra* in Mauritius. SPC Beche-de-Mer Information Bulletin, *22*, 48-51.

Lawrence, A.J., Ahmed, M., Hanafy, M., Gabr, H., Ibrahim, A., & Gab-Alla, A.A. (2004). Status of the sea cucumber fishery in the Red Sea - the Egyptian experience. In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hame, J.F., & Mercier, A. (eds). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper. *463*. 425 p.

Lee, J., Byrne, M., & Uthicke, S. (2006). To Split or Not to Split? A Study of Fission in Tropical Holothuroids and the Carrying Capacity of the Environment. SPC Beche-de-Mer Information Bulletin*, 23*, 41.

Mashanov, V.S., Dolmatov, I.Y., & Heinzeller, T, (2005) Transdifferentiation in Holothurian Gut Regeneration. Biological Bulletin, *209*,184–193. https://dx.doi.org/10.2307/3593108.

Moussa, R.M. (2009). Studies on reproductive biology of *Holothuria arenicola* (Echinodermata: Holothuroidea) in the Egyptian Mediterranean coast (PhD Thesis). Tanta University, Tanta, Egypt.

Nugroho, D., Hartati, R., & Suprijanto, J. (2012). Stimulation of Asexual Reproductive Fission of Sea Cucumber *Holothuria atra* and Sea Cucumber *Holothuria impatiens.* Journal of Marine Research, *1*(2), 161-166.

O'Loughlin, P.M., Eichler, J., Altoff, L., Falconer, A., Mackenzie, M., Whitfield, E., & Rowley, C. (2009). Observations of reproductive strategies for some dendrochirotid holothuroids (Echinodermata: Holothuroidea: Dendrochirotida). Memoirs of Museum Victoria, *66*(2), 215–220. https://dx.doi.org/10.24199/j.mmv.2009.66.19.

O'Loughlin, P.M., & O'Hara, T.D. (1992). New cucumariid holothurians (Echinodermata) from Southern Australia, including two brooding and one fissiparous species. Memoirs of Museum Victoria, *53*(2), 227–266. https://dx.doi.org/10.24199/j.mmv.1992.53.12.

Purcell, S.W. (2010). Managing sea cucumber fisheries with an ecosystem approach. FAO Fisheries and Aquaculture, *520*, 157.

Purwati, P., & Dwiono, S.A.P. (2005). Fission inducement in Indonesian holothurians. SPC Beche-de-Mer Information Bulletin, *22*, 11-13.

Purwati, P., & Dwion, S.A.P. (2007). Fission inducement in *H. atra*: changing in morphology and body weight. Marine Research of Indonesia, *32*(1), 1–6.

Purwati, P., Dwiono, S.A.P., Indriana, L.F., & Fahmi, V. (2009). Shifting the natural fission plane of *Holothuria atra* (Aspidochirotida, Holothuroidea, Echinodermata). SPC Beche-de-Mer Information Bulletin, *29*, 16-19.

Raouf, W.K., Fatma, A.A., & Mohamed, H.Y. (2000). Population growth and asexual reproduction of the sea cucumber *Holothuria arenicola* from the eastern Mediterranean. Egyptian Journal of Aquatic Biology and Fisheries, *4*(4), 119-135.

Reichenbach, N., & Holloway, S. (1995). Potential for asexual propagation of several commercially important species of tropical sea cucumber (Echinodermata). Journal of World Aquaculture Society, *26*, 272–278. https://dx.doi.org/10.1111/j.1749-7345.1995.tb00255.x.

Reichenbach, N., Nishar, Y., & Saeed, A. (1996). Species and size related trends in asexual propagation of commercially important species of tropical sea cucumbers (Holothuroidea). Journal of World Aquaculture Society, *27*(4), 475–482. https://dx.doi.org/10.1111/j.1749-7345.1996.tb00632.x.

Reinschmidt, D.C. (1969). Regeneration in the sea cucumber, *Thyonella gemmntn* (MSc Thesis), Florida State University, Florida, USA.

Toscano, A., & Cirino, P. (2018). First Evidence of Artificial Fission in two Mediterranean Species of Holothurians: *Holothuria tubulosa* and *Holothuria polii.* Turkish Journal of Fisheries and Aquatic Sciences, *18*, 1141-1145.

Uthicke, S. (1997). Seasonality of asexual reproduction in *Holothuria (Halodeima) atra, H. (H.) edulis* and *Stichopus chloronotus* (Holothuroidea: Aspidochirotida) on the Great Barrier Reef. Marine Biology, *129*(3), 435-441. https://dx.doi.org/10.1007/s002270050184.