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Captive Spawning of *Holothuria arenicola* (Semper, 1868) from Egyptian Mediterranean Coast

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ABSTRACT

In Egypt, *Holothuria arenicola* is distributed along the Alexandrian Mediterranean coast. Its natural stock has decreased significantly, having been over fished in the last years. This study provides an overview of different spawning induction methods for mature *H. arenicola*. Thermal stress through elevating the water temperature by 5°C is effective more than 8°C. The current attempts demonstrated that good spawning rate can be achieved by injection of Perivisceral Coelomic Fluid (PCF) even in specimens collected before the spawning season (by month) and held for 45 days before inducing experiment. Using stripping method, proved that this method mostly effective specially within the active spawning months June and July with a high rate of fertilization. Chemical stimulation by injecting 3 mL of 10% $MgCl_2$ or 3 mL of 5% $CaCl_2$ induced spawning but in small numbers. No induction was noticed with applying drying or addition of diatoms and micro algae methods. Injection with PCF in *H. arenicola* is recommended due to its efficient and quick response of egg release as well as its positive response with samples collected and remained in the hatchery for a time before spawning.

Key words: Spawning, induction methods, sea cucumber, *Holothuria arenicola*, Egyptian Mediterranean waters

INTRODUCTION

In 1998, small scale sea cucumber fishery began in Egypt in the southern part of the Red Sea. Later in the year of 2000, the sea cucumber fishery excelled greatly as a result of the high demand for the Beche-de-mer (sea cucumber) due to its consumption as a food newly introduced in Egypt and for exportation purposes. During those years Egypt has become one of the most important countries supplying the Beche-de-mer (Ahmed, 2006), the sea cucumber production has increased sharply from 20 tons in 2000 to 2310 tons in 2002 and then dramatically decreased to 6 in 2006. In 2003, as a result of depletion of stocks in the Red Sea as a whole for the most commercial valuable species (Lawrence *et al*., 2004), the exploitation of holothurian fishery is directed to Mediterranean Sea shore for the dominant species *Holothuria arenicola*. Abdel Razek *et al*. (2010) reported that decreased size of *H. arenicola* reflects the intensive fishing activity in the last years which supports the necessity of rapid management.

Recovery of over-fished sea cucumber stocks is a lengthy process, taking several years (Silva, 2001). It can take over 50 years for isolated and heavily harvested stocks to recover

(Battaglene, 1999b). Additionally, breeding and recruitment success of sea cucumber become low when population density is reduced below a critical level. This is because they are broadcast spawners and rely on the proximity of other animals for successful fertilization (Uthicke and Conand, 2005). Both of increased demand for beche-de-mer and worldwide declines in stocks of tropical sea cucumbers has encouraged aquaculture, stock restoration and enhancement programs for holothurians (Conand and Byrne, 1993; Battaglene and Bell, 1998).

A worldwide declines in stocks of tropical sea cucumbers have encouraged many countries to adopt aquaculture and stock restoration trends. In facing the over-exploitation of *H. arenicola* in Egyptian Mediterranean coast, these trends are urgently needed to be adopted. The present study aimed to demonstrate different spawning induction methods in order to determine their rate of efficiency with its practical value.

MATERIALS AND METHODS

Samples were collected from Abu-Qir Bay in the Egyptian Mediterranean coast at the eastern Alexandrian coast 31°16.5'N and 30°07'E in the spawning season (June and July 2008). Samples were gently cleaned and washed to remove sediments and other small organisms attached to their body. Samples were maintained in stock tank (1 tone) filled with 1 μm filtered sea water at ambient temperature of ±27°C. Samples exposed to different spawning induction methods within two weeks after collection time. While one sample collected in April (2008) was kept for 45 days prior to spawning induction to investigate the effect holding period prior to conducting different spawning induction methods. Each induction method was repeated for 3-5 times with new individuals. However, if the trial (in any induction method) failed to stimulate samples to spawn, the same method would be reapplied for up to 4 days. The following spawning induction methods were studied:

Thermal stress method: Two groups were given thermal shocks. One group of ten samples was transferred from brood stock tank (at ambient temperature 25°C) into spawning tank at temperature of 30°C, while the other group was transferred to spawning tank at 33°C. Control samples were remained in the spawning tank with ambient temperature. Temperatures were raised by the use of aquarium heaters. The thermal stress period was two hours daily. Samples were changed between two different temperatures (25 and 30°C) and (25 and 33°C) every 15 min during the period of thermal stress.

Chemical method: The injection of two different chemicals at different concentrations was examined as a method for spawning induction. Two groups of ten samples were subjected to injection of 3 mL of 10% $MgCl_2$ and 3 mL of 5% $CaCl_2$, respectively, through the body wall on the last third of the animal.

Injection of perivisceral coelomic fluid (PCF) method

Preparation of PCF: Ten milliliter of sea water filtered on 1 μm mesh was injected through body wall into the coelomic cavity with 20 mL syringe. About 10 mL of air was also infused to facilitate the collection of PCF afterwards without injuring the animals, as the air present in the cavity facilitated the collection of liquid and minimized the chance of siphoning the internal organs during the process. Sampling of PCF was performed after the water/air injection. The sampling spot was on the last third of the animal near the cloaca. The whole procedure was conducted within one

minute and in a single spot to avoid loss of liquid to the environment. PCF was collected only once from each individual. The collected PCF sample was filtered on 1 μm mesh to remove most of the suspended matter (Mercier and Hamel, 2002).

Injection of PCF: Three milliliter of PCF was injected through the body wall on the last third of another animal. Samples that failed to spawn spontaneously overnight under PCF injection were injected the second day with 1 mL PCF.

Stripping method: Ovary and testis were taken out and dried in the shade. The ovary was then placed in a container filled with sea water, the eggs were filtered through plankton net of mesh size of 50 μm. Testis is placed in another container filled with sea water and cut into pieces, when the sperms swim out into the sea water. The sea water with eggs is then poured into one with sperms for the eggs to be fertilized, the ratio of spermatozoa is 1-5 per ovum and only one animal for each trial is used. They left for one hour to ensure the fertilization (James, 1996) only one animal for each trial is used.

Drying method: All the water in the brood stock tank was removed and the samples were dried in the shade for about half an hour. Then the samples were subjected to powerful jet of sea water for five minutes. Afterward, the samples were put back into the tank with 1 μm filtered sea water at ambient temperature of 27±°C. The drying stress was conducted three times in each trial.

Mixture of diatoms and microalgae: Mixture of diatoms (*Chaetoceros* sp. and *Skeletonema* sp.) and microalgae (*Tetraselmis* sp.) at a concentration of 150,000 cell mL^{-1} for each species, were added to samples in a static tank for 3 h. Afterward, samples were put back into spawning tank filled with 1 μm filtered and aerated sea water. This method was repeated for three times in each trial.

RESULTS

Thermal stress: Thermal stress by rising of 5 and 8°C above the ambient temperature showed a positive effect on spawning induction. The range number (30-500×10^3) of spawned eggs released in trials of 5°C rise is considerably higher than that (18-100×10^3) in trials of 8°C (Table 1). By comparing the number of spawned eggs in different trials, it was found that the rise of 5°C stimulate the ripe females to release larger number of eggs than the rise of 8°C. As the period of keeping samples before spawning induction increased, more times of repeating thermal stress was needed to stimulate samples to spawn.

Table 1: Trials of spawning induction of *Holothuria arenicola* using thermal stress method

				Thermal stress			
Month	Days after collection	Animal (No.)	Ambient temp. (°C)	Amount (°C)	Period (days)	Times	Spawned eggs (×10^3)
June, 2007	6	12	25	5	5	5	30
June, 2007	6	11	25	8	5	5	18
July, 2007	1	10	26	5	1	1	500
July, 2007	1	10	26	8	1	1	30
July, 2007	2	10	26	5	1	1	160
July, 2007	2	10	26	8	3	3	100
June, 2008	45	10	25	5	3	3	0

Chemically: Groups injected with 10% $MgCl_2$ stimulate relatively higher number of eggs ($6\text{-}10\times10^3$) than that of 5% $CaCl_2$ ($4\text{-}8\times10^3$) (Table 2). The samples in each group showed response to the induction stimuli (injection) after once. The number of spawned eggs was relatively lower than that recorded in other spawning induction.

Injection of perivisceral coelomic fluid (PCF): Injection of Perivisceral Coelomic Fluid (PCF) showed the best results as compared to the other studied spawning induction methods. The samples began to spawn after 4-12 h after PCF injection. This method was the fastest method among others with releasing egg number at range of $6\text{-}210\times10^3$ (Table 3). For samples collected before the spawning season (by month) and held for 45 days prior to experiment, they stimulated to spawn after injection of 3 mL PCF (in the 1st day) and 1 mL PCF (in the 2nd day).

Stripping: Stripping method could be used to obtain larvae from artificial external fertilization. Fertilization rate was in range between 10-53% with a ratio of spermatozoa (1-5) per one ovum. No significant effect was observed for the period between collection time and the experiment time on the fertilization rate and the number of fertilized eggs (Table 4), using this method during June and July gives a good results for *H. arenicola*.

Table 2: Trials of spawning induction of *Holothuria arenicola* using injection of 10 $MgCl_2$ or 5% $CaCl_2$

				Chemically		
						Spawned
Month	Days after collection	Animal (No.)	Ambient temp. (°C)	Injected conc.	Times	eggs ($\times10^3$)
June, 2008	5	10	25	10% $MgCl_2$	1	10
June, 2008	5	10	25	5% $CaCl_2$	1	8
June, 2008	11	10	25	10% $MgCl_2$	1	5
June, 2008	11	10	26	5% $CaCl_2$	1	6
June, 2008	14	10	26	10% $MgCl_2$	1	6
June, 2008	14	10	26	5% $CaCl_2$	1	4

Table 3: Trials of spawning induction of *Holothuria arenicola* using injection of perivisceral coelomic fluid (PCF) method

				PCF injection		
						Spawned
Month	Days after collection	Animal (No.)	Ambient temp. (°C)	Injected amount (mL)	Times	eggs ($\times10^3$)
June, 2008	13	10	26	3	1	60
June, 2008	17	10	25	3+1	2	20
June, 2008	15	10	25	3	1	210
June, 2008	45	10	25	3+1	2	6

Table 4: Trials of obtaining *Holothuria arenicola* fertilized egg by using stripping method

			Stripping		
Month	Days after collection	Ambient temp. (°C)	Eggs (No. $\times10^3$)	Fertilized egg (No. $\times10^3$)	Fertilization (%)
July, 2007	1	26	1400	210	15
July, 2007	8	26	135	14	10
June, 2008	1	24	600	320	53
June, 2008	13	27	600	84	14
June, 2008	13	27	640	140	21
June, 2008	13	27	1500	300	20

Drying: The drying method was repeated three times in two spawning seasons (2007 and 2008) with no response.

Addition of blend mixture of diatoms and microalgae: No response was recorded with applying this method for longer time or with repeating it for three times in each trial.

DISCUSSION

The spawning of sea cucumbers is related to the interaction of environmental cues and reproductive maturity (Morgan, 2009). The Chinese and the Japanese are the pioneers in the seed production of the sea cucumbers. Thermal stress is a well-known practice used to stimulate spawning in sea cucumbers (James *et al.*, 1988; Morgan, 2000; Battaglene, 1999a,b; Giraspy and Ivy, 2005). The current attempts to spawn *H. arenicola* in a mature condition demonstrated that spawning can be induced by a simple method of heat stress. The rise of 5°C above ambient sea water temperature is better range for spawning induction in *H. arenicola* than 8°C. As wise, both of Battaglene *et al.* (1998) and Morgan (2009) reported that *H. scabra* and *Australostichopus mollis* can be spawned in the hatchery during heat-shock trials conducted 3-5°C above ambient seawater temperature, respectively. Similarly, Dabbagh *et al.* (2011) demonstrated that *Holothuria leucospilota* has been induced to spawn by combining two methods: water pressure and rasing temperature by 5°C. While thermal stimulation proved less effective with *H. fuscogilva* (Battaglene *et al.*, 2002).

Chemical stimuli by injecting 10% $MgCl_2$ or 5% $CaCl_2$ in the posterior part of the body wall of *H. arenicola* induce the egg releasing. Previously, neither isotonic potassium chloride injection nor addition of various levels of Cystine concentrations in water medium provides any results (James *et al.*, 1994) as well as potassium chloride according to James (1996). Therefore, the present result can be considered one of the promised treatment can be used with *H. arenicola*. Recently, Cubifrin-L has been shown to be potent substances that induce reproductive behaviors and gamete shedding in mature *Apostichopus japonicus* (Fujiwara *et al.*, 2010).

The second important recorded result in this study is the success of *H. arenicola* spawning induction through injection of PCF even in specimens collected before the spawning season (by month) and held for 45 days before experiment. It was observed that the spawning response took from 4-12 h after PCF injection, while Mercier and Hamel (2002) reported that it took between 30 min and 1 h after the PCF injection, suggesting that the substance needs this time to stimulate potential receptors located on the gonadal tissues or elsewhere. The action of PCF can be attributed to a substance or a combination of substances, implicated in the initiation and progression of spawning behavior and gamete broadcasting in holothurians which were transmitted via it. Also, Mercier and Hamel (2002) suggested that messages sent via the PCF could help holothurians synchronize and propagate spawning in the field. This might explain the epidemic spawning observed in numerous holothurians, as well as in other echinoderms and marine invertebrates.

The success of stripping method which yielded 10-53% fertilization rate in *H. arenicola* in considered one effective methods used during the maturing time. While, James (1996) reported that stripping method yield low rate of fertilization as 20% and the number of deformed individuals was large.

No spawning response was noted using drying and addition mixture of diatoms and microalgae methods in *H. arenicola*. However, James (1996) reported drying method as an effective method

for spawning induction of *H. scabra*. This may be due to the characteristics of its natural habitat which *H. arenicola* collected from in comparison with the other species inhabited Red sea with tropical characters and richest fauna.

Collection timing is very important; it is desirable to collect the brood stock material during the spawning peaks because the chances of inducing spawning will be more since most of specimens have ripe gonads ready for release (James, 1996).

CONCLUSION

However, in the present study, *H. arenicola* showed a unique spawning induction response both in specimens collected before the spawning season and in specimens held for 45 days before spawning induction. These responses are noticed with applying the effective method which is PCF. While, there was no recorded response of spawning induction of *H. arenicola* using thermal stress in specimens collected out of spawning season. In addition, it is observed that as the period from collection to conducting experiment is longer, the number of released eggs is lower.

From practical point of view, PCF injection and thermal stimulation can be used as effective inducers of spawning in *H. arenicola*. The stripping method is recommended for obtaining fertilized eggs, especially within the period spawning activity period in June and July.

For future work, it is recommended to explore more species especially in the Red Sea, where there's a variety of the most commercial desirable species of sea cucumber. As well to test the efficacy of the induction methods achieved in this study and whether they succeed with other sea cucumber species or there are other methods that would work best.

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