Induced spawning in the sea cucumber *Holothuria atra* from the Egyptian coast of the Red Sea.

Mohammed I. Ahmed
Marine Science Department, Faculty of Science Suez Canal University, Egypt

ABSTRACT

In hatcheries the induction of spawning in sea cucumbers has been typically carried out using different techniques and combinations of mixed induced spawning techniques, for the *Holothuria atra* for the first time combination of heat and cold chock were used to induce spawn samples collected from the Red Sea. Combination of rising water temperature by 10º C and then reduced by 10º C provoked animals to take the (Copra position) the reproductive position in sea cucumber. Gametes shedding have started about 60 min and 80 min after placing the animals back in normal sea temperature, and was completed almost simultaneously in the two sexes during 2 hours. Different other treatments were used including spirulina bath; exposure to water jet, draying and injection with Serotonin, however heat chock was the only successful technique. Larvae were fed on unicellular algae and *Sargassum* extract. Early juveniles were obtained on day 35. Survival rate was 4% to the juvenile stage.

Keywords: Sea cucumber, *Holothuria atra*, induced spawning, heat stress, Red Sea.

INTRODUCTION

The processed Beche-de-mer of *Holothuria* species is a source of income for the coastal communities of the Northern coast of the Red Sea. However, severe overfish-ing has led to a significant decrease in the natural sea cucumber population. This continuous over-exploitation is likely to have a huge impact on the ecosystem and marine environment as a whole (Conand 2004). Research on the feasibility of restocking and stock enhancement of tropical sea cucumber have been conducted in many tropical countries (Purcell *et al*., 2011, 2014; Eriksson *et al*., 2014; Watanabe *et al*., 2014).

There is also an urgent need for restocking and stock enhancement in Egypt. The Sand fish sea cucumber *Holothurian atra* is the most common species in the Egyptian coast of the Red Sea (Ahmed and Lawrence, 2007). *Holothuria atra* is the third class commercial species and has been exploited as part of the Bech-de-mer trade in Egypt since 2002. Fishing pressure is increasing mainly because of a growing demand for export to China. Therefore, the sustainable production of the sea cucumber is vital for conservation of species in the Red Sea and the sustainability of the industry. Sea cucumber was not considered as a culture species and no induced spawning were never conducted on these species (Ahmed and Lawrence, 2007). The current situation concerning sea cucumber fisheries makes this activity more important than ever. In hatcheries, the induction of spawning in sea cucumbers is typically carried out by regulation of rearing conditions such as temperature, water exchange, and light intensity (Battaglene *et al*., 2002). However, these methods never been tested on Red Sea species and the rate of spawning is unpredictable. Therefore, the main aim of this paper is to select the most effective method to induce spawning of *Holothurian atra*.

MATERIALS AND METHODS

Broodstock collection

Healthy *H. atra* weighing between 100 and 200 g were collected by snorkeling in depths of 0.5–3 m at low tide along the northern coast of the Red Sea from the city of Hurghada during March 2014. Broodstock collected from the nearby coastal area were carefully transported to the hatchery in tubes of seawater. The broodstock was transferred by car to the National Institute of fisheries and Oceanography. At the hatchery, the work was conducted following the methods outlined in Agudo (2006). Fifteen sea cucumbers were maintained in a 500-L tank with an 8-cm layer of sand on the floor. The tank was filled with 1-mm filtered and UV-sterilised seawater that was changed daily.

Induced spawning

We used three methods to induce spawning: thermal stimulation, combination of heat pressure and thermal shock and algal stimulation. Thermal stimulation was found to be the better method and was used thereafter.

Heat and cold shock treatment.

Water temperature in the spawning tank was raised by 10°C (by adding warmed seawater) from the ambient temperature of 27–28°C and the broodstock was held under these conditions for 1 hour. Temperature were then reduced by 10°C (by adding cold seawater) and the Broodstock was held under these conditions for 1 hour. Animals were then placed in normal sea temperature. A cover was placed on the Broodstock tank and spawning later occurred.

Combination of water pressure and heat shock treatment.

Animals were left in the tank at a depth of 2 cm for 40 minutes, and then subjected to a powerful jet of seawater for 20 minutes. Subsequently, the water temperature was raised by 5°C for 1 hour.

Algal stimulation

For algal stimulation, 100 g of dried *Spirulina* was mixed with seawater and dissolved in a one-litre spawning tank. Additional dried *Spirulina* was added if the concentration was still insufficient to induce spawning. Thirty to 40 sea cucumbers were introduced into the tank and left for an hour to spawn.

Larval rearing

After spawning of the females, the broodstock tank was left for 1 hour to allow the eggs to be fertilized by sperm from the males. Spawned eggs were gently siphoned onto an 80-mm sieve into the bucket. The collected eggs were transferred carefully into clean 10-L buckets. To estimate egg density, the water in the buckets was gently stirred to distribute the eggs uniformly. Three 1-mL subsamples were taken from the bucket and eggs were counted under a microscope using a counting cell. Finally, the average density was calculated. The hatching rate was later estimated from the number of early auricularia divided by the number of eggs. The larvae were stocked in a 1,000 L larval rearing tank with a stocking density of 200 to 250 larvae per liter. The tank was filled with 1 µm UV sterilised seawater at a temperature between 26 and 30°C. Salinity was maintained between 32 and 36 ppt, and pH between 8.0 and 8.2. The larvae were checked every day for changes in shape, size and stage, as well for the presence of bacteria and protozoans.

Feeding had been started on day 3 with *Isochrysis* sp. Subsequently, a mixture of algae (*Chaetoceros muelleri*, *C. calcitrans*, *Tetraselmis* sp.) was fed in a density that was gradually increased from 20,000 to 40,000 cells ml^{-1}.

The water in the larval tank was changing every second day by siphoning the water through a sieve in a bucket. Growth and survival data were recorded every second day, when siphoning the larvae onto the sieve finished, by taking three ml^{-1} subsamples. To induce doliolaria to metamorphose into settled pentactula larvae. S*argassum* sp. extract was added daily to water in which the plates were immersed (in a separated tank) so that the plates became coated with a fine layer of algal material. These settlement plates were then placed into the larval tank. After two days, none of the (non-feeding) doliolaria larvae were seen on a sieve during a complete water change. Within three days, pentactula larvae were formed. At this stage, the larvae were fed daily on *Sargassum* extract.

RESULTS

Results from Table 1 and Figure 1 show that *Holothurian atra* were only induced to spawn by using heat and cold water shock treatment. After 1 hour of treatment, the males took the copra position released their sperm; subsequently, 1 female spawned after 10 minutes, releasing about 400,000 eggs. The hatching rate was 70%. The amount of time taken for larvae to reach the juvenile stage is shown in Table 2.

Table 1: Number of eggs produced from different types of spawning method of *Holothuria atra*.

Treatment	**No. of eggs**	**No. of Broodstock**
Heat and cold stress	40000	15
Water jet and heat stress	0	15
Algal treatment	0	15

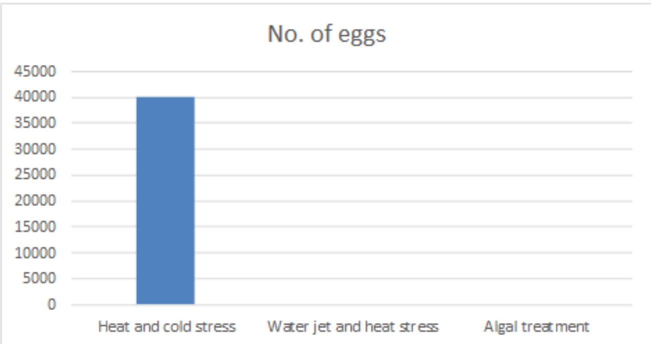

Fig. 1: Number of eggs produced from different spawning method of *Holothuria atra*.

Table 2: Development of *Holothuria atra* from fertilization to 1mm juveniles.

Development stage	**Time**
Fertilization	1 d
Late gastrula	3d
Early auricularia	4-10d
Late auricularia	11-20d
Doliolaria	22-25d
Settlement	30d
Juvenile, 1mm	35d

The percentage of larval hatch was calculated on the appearance of auricularia larvae; approximately 48 hours after fertilization. From the three spawning trials, 92% of auricularia larvae were hatched. Auricularia is the start of the feeding stage, and during this stage the larvae were fed on 20,000 cells ml^{-1} of microalgae. The feed was subsequently increased by the larval demand. Auricularia sizes range from about 425 to 450 µm. Middle auricularia were bigger, with a size range between 750 and 950 µm. After 11 days, about 38 % auricularia larvae metamorphosed into the non-feeding stage, doliolaria; a very active non-feeding, fast moving larva between 450 and 650 µm.

Doliolaria has been transformed into creeping stage pentactula on the 18th day. They have been seen on the settlement plates, on the bottom and on the wall of the tanks as they search for suitable substratum to settle on. The pentactula larvae size ranges from 350 to 750 µm. The survival rate of *H. scabra* larvae was 4.25% (Table 3 & Fig. 2)

Table 3: larval survival rate of *Holothuria atra* with days

days	**No. of larvae**	**Survival percentage**
0	400000	100
5	300000	75
10	180000	45
15	150000	37.5
20	100000	25
25	70000	17.5
30	35000	8.75
35	17000	4.25

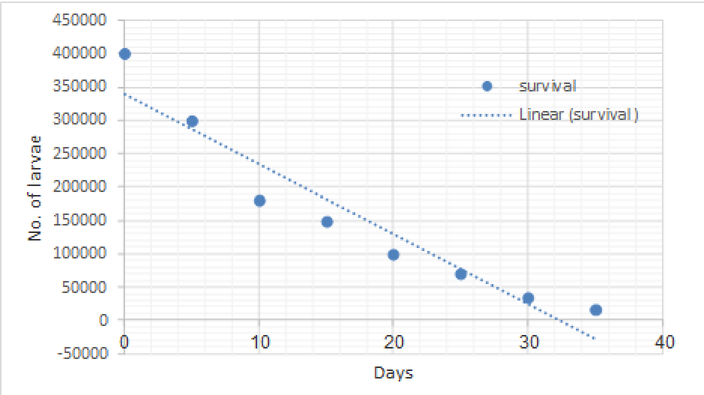

Fig. 3: Survival rate of larvae of *Holothuria atra* with time

DISCUSSION

Restoration of depleted natural stocks through restocking, reseeding is seen as a way of rebuilding after overfishing of certain marine species. Many countries have started their own stock enhancement programme by doing research into the feasibility of tropical sea cucumber breeding (Purcell *et al*., 2011, 2014; Eriksson *et al*., 2014; Watanabe *et al*., 2014). The development of three commercial sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana* was studied by Ramofafia *et al*. (2003). Hamel *et al*. (2003) reported larval development of *Isostichopus fuscus*. James (2004) conducted a breeding trial of *H. scabra* in India. Spawning and larval rearing of *H. atra* were also reported from Mauritius (Laxminarayana, 2005). However, the only tropical species that can be mass-produced in hatcheries is *H. scabra* (Battaglene and Bell, 1999). *H. scabra* culture started in 1988 in India for farming purposes, although the possibility for stock enhancement was suggested (James *et al*., 1988; James, 1996). This species appears capable of spawning all year long without the influence of lunar periodicity (Ong Che and Gomez, 1985; Conand, 1993). They are dioecious, highly fecund and are broadcast spawners. Unfortunately *H.scabra* was overfished in the Red Sea and in our research we could not find enough animals to use them in spawning experiments.

Successful breeding by induced spawning has been widely reported and most of the authors claimed that thermal induction is the best method for sea cucumber spawning (Battaglene *et al*., 2002; Laxminarayana, 2005; Ivy and Giraspy, 2006; Eeckhaut *et al*., 2012). There are other methods for induced spawning, such as powerful water jets on drying individuals (James, 1996; Liu *et al*., 2004; Wang and Yuan, 2004) and blended gonad as stimulant (Battaglene, 1999). *Stichopus* sp. (curry fish) was induced to spawn by open-air drying followed by flow-through seawater stimulation.

In this study, three methods were compared and thermal induction gave a better result than algal stimulation and combination of water jet and thermal induction. Laxminarayana (2005) reported that *H. atra* larvae that were reared at a salinity of 34–36 ppt reached the pentacula stage by day 20. In the present study, with larvae reared at 37‰, doliolaria larvae were only obtained by day 22 to 25. The low growth rate was probably due to high salinity conditions, which prevail in the Red Sea. The survival rate of *H. atra* from this study was 4.25%. The poor survival rate of tropical sea cucumber species has been reported by Purcell *et al*. (2012). Ivy and Giraspy (2006) reported that the survival rate for *H. scabra versicolor* was 1.12% in 2004 and 4.53% in 2005. The survival rates for *B. marmorata* and *H. atra* were 12.5% and 6.4% respectively (Laxminarayana, 2005).

Decline in the percentage of the survival rate occured during the metamorphosis stage in our study. From 92% hatched larvae, only 38% of auricularia larvae morphed into doliolaria. The rate further declined to 4.25% when they transformed into pentactula larvae. In this experiment, the mortality rate was up to 96%; from fertilized eggs to the formation of the pentactula larvae. Battaglene (1999) reported that the highest mortality occurred at the first feeding and settlement. This also may be due to the food demands and the induction of the larval metamorphosis. The presence of bacteria and Protozoaans in the tank also affects the mortality and survival rate. Furthermore, it may be also related to the anatomy and physiology of the larvae of the sea cucumber species. In summary this work was conducted for the first time in the Red Sea and the results need to be enhanced in the future by trying other commercial species and further optimization of breeding conditions.

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ARABIC SUMMARY

تحفيز الأخصاب لنوع من انواع خيار البحر (هولوثوريا اترا) من السواحل المصريه للبحر الاحمر

محمد اسماعيل احمد
كليه العلوم جامعه قناة السويس, مصر

علي مستوي العالم في مفرخات خيار البحر يتم تحفيز الاخصاب وانتاج البيض عن طريق تقنيات مختلفه حسب النوع المستهدف. ولم يتم تحفيز الاخصاب وانتاج البيض في مصر من قبل لانواع خيار البحر وفي هذه الدراسه تم لاول مره تحفيز الاخصاب تحت ظروف معمليه باستخدام خليط من رفع درجه الحراره لدرجه عشر درجات سيلزيه ثم وضع الكائن في ماء بارد برجه حراره منخفضه عشر درجات عن الطبيعي وفي النهايه وضع الكائن في درجه حراره الماء الطبيعي. وقد نجحت الطريقه في تحفيز كائنات خيار البحر لأخذ وضع الكوبرا وهو وضع القاء البيض والحيوانات المنويه وقامت الكائنات بعض وضعها في درجه الحراره الطبيعيه بالقاء البيض بعد حوالي 60 دقيقه. وتم تجربه طرق اخري مثل التنشيف او استخدام تيار مياه قوي ولكن هذه الطرق رغم نجاحها في دول اخري مع خيار البحر لم تحفز الكائن للتكاثر في مصر. تم تغذيه اليرقات باستخدام خلايا احاديه من الطحالب ومستخلص من السرجاسم. ولم تتعدي نسبه نجاه اليرقات 4% وهو ما يعتبر نسبه جيده لخيار البحر حيث ان المعدل العالمي يتراوح ما بين 8%- 1% نجاه لليرقات.