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STUDY TO INVESTIGATE INDUCED SPAWNING METHODS AND LARVAL REARING OF THE SEA CUCUMBER *Holothuria scabra* *)

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ABSTRACT

In order to increase natural population stocks, a sea cucumber cultivation project was attempted in the Karimunjawa Islands (Java Sea, Indonesia). Since continuous production of larvae is essential to such a project, this portion of the research focuses on methods of inducing the sea cucumber Holothuria scabra *to spawn and monitoring the development of resulting larvae. Four different types of environmental manipulation were used to try and induce spawning; artificial fertilization (using manually extracted eggs and sperm), thermal shock (raising the temperature of the spawning medium), desiccation (partially drying out) and treatment with potassium chloride (KCL) solutions of various concentrations. The development of the resulting larvae was carefully observed.*

Results indicate that thermal shock, desiccation and potassium chloride (KCL) are all effective in stimulating the sea cucumber Holothuria nobilis *to spawn. Thermal shock gave the best results with both male and female spawning and 90% larvae development. Using a stocking density of 300 larvae/liter in 10 liter aquaria, larvae were successfully raised up to pentactula stage. Regardless of the method used to induce spawning, fertilized eggs developed into auricularia larvae at 31 hours and 30 minutes. These larvae then metamorphosed into doliolaria and pentactula larvae at day 13 and 26. Mortality of all stocks at the pentactula stage was probably due to lack of provision of sttlement substrates.*

Keywords : Sea cucumber, induced spawing, larvae

I. INTRODUCTION

Five commercially important species of sea cucumbers, *Holothuria nobilis*, *H. vacabunda*, *H. vatiensis*, *H. marmorata*, and *H. scabra* have been reported from the Karimunjawa Islands. However the stocks of these species are in decline, and local abundance of species, especially *H. scabra* are in drastic decline, relative to previous observations (Pringgenies, 1994). The

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drop in stocks can be attributed to an increase in exploitation of local stocks by fishermen due to an increasing demand for sea cucumbers for export. Developing culture-based methodologies for restocking sea cucumber populations can mitigate this threat to the natural stocks of sea cucumbers. By involving local people in culture projects and in restocking, such endeavors also offer alternative livelihoods for members of coastal communities. An optimal culture program would be able to produce larvae continuously and would be able to minimise the loss of larvae during rearing.

A fundamental aspect of any culturing program is the ability to induce spawning in the study organism. For sea cucumbers, reported methods of inducing spawning have included the following; "killing method" or artificial fertilization (Maiyadeen, 1991), thermal shock, and desiccation (Anonymous, 1991; Notowinarto and Putro, 1992). The aim of this research is to compare the performance of these different methods of inducing spawning in sea cucumber *H. Scabra*, including one new methods, namely exposure to poetessum chloride (KCl) solution. After spawning, an additional goal is to monitor the development from egg to larvae, and to follow the development of the larvae through its various stages; auricularia larvae/early larvae, doliolaria/middle larvae, and pentactula larvae.

II. METHODOLOGY

Broodstock of the sea cucumber *H. scabra* were selected using the following criteria; minimum weight 400 grams, minimum length 20 cm, healthy and in good condition. The sea cucumbers used in this project came from Balai Budidaya Laut (Marine Culture Research Institution - Lampung), and had been reared in BBAP (Brackishwater Aquaculture Development Center, Jepara) for two months prior to the experiment. Selected individuals with mature gonads were acclimatized in broodstock tanks before being induced to spawn. Four methods of spawning were investigated as follows.

Method I. (Killing method"/artificial fertilization): The broodstock gonad (yellow egg sack in female and whitish sperm in male) was dissected from posterior to anterior, and placed together in a container of clean seawater. The egg sack and sperm sack were carefully opened and the contents mixed slowly. The water was then filtered using a fine cloth and the eggs placed in a larval rearing container.

Method II. (Thermal shock): This technique was originally developed in the Marine Culture Research Institution (Notowinarno, 1995). The temperature of the broodstock media was increased gradually from 27-27.5 degrees C to 31-32.5 degrees C. The broodstock were then returned to the spawning media at 27-27.5 degrees C. This drop in temperatures produces a thermal shock, which induces the sea cucumbers to spawn. In an adaptation of this method, broodstock were selected between 8:00-9:00 am and placed in plastic buckets of seawater hung in a brackish water pond (tambak). The temperature of the water and broodstock in the plastic bucket rose to match the temperature of the tambak during the day. At between 16:00-17:00 PM in the afternoon, the broodstock were washed and moved to the spawning tank.

Method III. (Desiccation): In the morning, selected broodstock were acclimatized in a fiberglass tank (volume 1 ton). In the evening at approximately 17:00 PM, the broodstock tank was drained and allowed to dry for 5 minutes. The desiccated broodstock were then sprayed with clean seawater for five minutes. The procedure of desiccating and spraying with seawater was repeated three times and the broodstock were then placed in the spawning tank.

Method IV. (Potassium chloride (KCl) stimulation): Selected broodstock were placed in an acclimatization tank, in a shaded location. At 15:00 PM, the broomstick were placed in an experimental media containing potassium chloride (KCl) at a concentration 0.25 gm/l and left for 30 minutes. The broodstock were subsequently moved to media containing concentrations of potassium chloride KCl of 0.5 gm/l, 0.75 gm/l and 1 gm/l, each time being left for thirty minutes. After the treatment with potassium chloride (KCl) solutions, the broodstock were placed in the spawning tank.

In the spawning tank, stocking density of broodstock was maintained at 8 individuals per tank. Due to difficulties in determining the sex of individuals, it was assumed that were male and female individuals in each tank. Past observations indicate that sea cucumbers tended to spawn between 20:00 - 21:00 pm with males expelling their sperm before the females release their eggs. For the purposes of this experiment, individuals that had undergone the treatments described above were observed in the spawning tank from 19:00 PM to 22:00 PM. After spawning eggs and sperm were mixed slowly in the tank, in order for fertilization to occur. Fertilized eggs were filtered, washed thoroughly to avoid polyspermy and moved to larval rearing container. Data was collected on the percentage of male and female spawning, the number of eggs fertilized and the percentage of successful larvae development.

An additional goal of this study was to attempt to raise the larvae to adult sea cucumbers. To achieve this, larvae were fed on *Isochrysis sp.* (auricularia larvae 20,000 cel/ml, doliolaria larvae 27,000 cel/ml) with a stocking density of 300 larvae/litre in 10 liter aquarium. Data was collected on the number of larvae that reached each stage in development, and the duration between each stage of development. For this part of the study, larvae from the different pawning methods were not differentiated.

III. RESULTS AND DISCUSSION

Patterns of reproduction in marine invertebrates are profoundly influenced by external environmental factors, such as temperature, day-length, food and specific chemistry of the animal's habitat (Barnes et al., 1994). Unlike freshwater and terrestrial invertebrates, marine invertebrates are able to discharge naked gametes into the surrounding medium where fertilization takes place. Externally fertilized eggs develop into mobile planktonic larvae, which in many instances, such as in Holothurians, transform into benthic organisms as they reach the juvenile stage. In tropical marine environments, temperatures remains relatively stimulating gonad development throughout the year (Tuwo and

Nessa, 1992), however for sea cucumber cultivation, it is necessary to provide artificial stimulation to induce spawning.

Using a variety of techniques, broodstock of *H. scabra* were induced to spawn under laboratory conditions. The 'killing method' resulted in the death of parent broodstock, and produced poor yields of larvae. Only a limited number of broodstock were available for these experiments, so it was decided not to replicate the 'killing method'. The main focus of this study is the three non-destructive methods of inducing spawning, thermal shock, desiccation and potassium chloride treatment, and for each of these, eight replicates were carried out. A summary of the overall percentage of male and female that spawned when exposed to the thermal shock, desiccation and potassium chloride (KCl) treatments are shown in Table 1. The percentage development of larvae from fertilized eggs for each experiment is shown in Table 2. Irrespective of the spawning technique, survivorship data was collected for all larvae, to provide basic information on rearing sea cucumbers. A summary of larval survival data for the five separate rearing tanks is given in Table 3.

Table 1. Percent Male and Female Spawned due to Treatment

Treatment					
Thermal Shock		Desiccation		Potassium Chloride	
Male	Female	Male	Female	Male	Female
100	80	60	30	80	60

Table 2. The percentage Development of Larvae According To the Method of Induced Spawning (%)

No. of Exp.	Treatment			
	Artificial Fertilz.	Thermal Shock	Desiccation	Potassium Chloride
1	12.37	89.86	86.48	87.30
2	-	91.55	87.83	83.12
3	-	91.67	87.50	86.41
4	-	91.58	-	83.91
5	-	89.09	-	88.10
6	-	90.70	-	85.71
7	-	89.83	-	-
8	-	88.04	-	-
Average	12.37	90.29	87.27	85.76

Table 3. Number of Larvae Alive and Survival Rate (SR, %) At the End of Experiment

Day	Tank					
	1	2	3	4	5	Average
0	3000	3000	3000	3000	3000	3000
5	2760	2735	2750	2810	2790	2769
10	2520	2460	2502	2470	2585	2507,4
15	1324	1470	1208	1374	1350	1345,2
30	328	311	207	392	359	319,4
SR (%)	10,93	10,37	6,90	13,07	11,97	10,65

Note : Day 0 : Auricularia stage, Day 13 : doliolaria stage
Day 26 : pentactula stage

The artificial or 'killing method' produced the lowest percentage of development of larvae of the four methods tested here (12.37%), and was the only method that resulted in the destruction of the broodstock. As such, it is not recommended as a method inducing sea cucumbers to spawn. The probable cause of the low success rate is that the gonads were not ready to spawn. Thermal shock produced the highest yield of larvae (90.29%, Table 2), and resulted in high numbers of males and females producing spawn (male 100%, female 80%). It appears that the males release sperm first, which in turn stimulate the females to release their eggs. Potassium chloride (KCl) stimulation gave the second best results in terms of inducing sea cucumbers to spawn (80% male, 60% female) with an average yield of 85.76% larvae development. Desiccation yielded slightly higher yields of larvae on average (87.27%) but was less successful in inducing sea cucumbers to spawn (male 60%, female 30%).

From these results, it appears that raising the temperature offers the optimal method of inducing spawning in sea cucumber culture programs. Raising the temperature can be done by a variety of methods such as exposing the sea cucumber broodstock to sunlight during the day, immersion in a water bath or supplying hot water. Previous work indicates that exposure to sunlight is the best method of raising the temperature, but this limits spawning activity to sunny days. Stimulating with potassium chloride (KC)l offers an alternative viable method of inducing spawning. Potassium chloride (KCl) has been successfully used to induce green mussel to spawn (Hartati and Pringgenies, 1993), and to induce sea urchins to spawn (John Pearse, pers. Comm., 1996). The results of this experiment indicate that the concentrations of potassium chloride (KCl) used to stimulate green mussels to spawn also produces spawning in sea cucumbers. Desiccation was the least effective method of inducing spawning, presum-

ably because this method stressed the broodstock. Although the environment never became completely dry, the sea cucumbers produced more slime and showed a more passive response than with the other methods, indicating high levels of stress.

There is little information on rearing sea cucumber larvae because few attempts have succeeded. In this study, successful rearing of sea cucumber larvae had led to a number of important observations on the embryonic development and larval growth of *H. scabra*. The following sequence of embryonic development was noted: 43-48 minutes - first cell division (2 cells), 48-53 minutes - second cell division (4 cells), 2 hours 30 minutes - third cell division (8 cells), 5 hours 40 minutes - blastula, 12 hours 20 minutes - hatching, 17 hours 40 minutes - gastrula, 31 hours - auricularia. Larvae metamorphose to doliolaria larvae at day 13 and to pentactula larvae at day 26 (Table 3). The survival rate of larvae for this stage of the experiment was 6.9 - 11.97% (average 10.65%). However all larvae/juveniles suffered sudden mortality at day 33. This was attributed to a change in life habit of the larvae from planktic to benthic. It is suggested that in future experiments, a screen be placed in the media providing a substrate for the larvae from the beginning of the pentactula stage.

IV. CONCLUSIONS

Environmental manipulation of sea cucumber *H. Scabra* by thermal shock, desiccation and addition of potassium chloride to the media, were shown to stimulate sea cucumbers to spawn. Thermal shock from heating by sunlight gave the best response with 100% male spawning, 90% female spawning and 90.29% larvae yield.

Fertilized eggs of the sea cucumber *H. Scabra* develop into auricularia larvae at 31 hours and 30 minutes. Auricularia larvae metamorphosed into doliolaria at day 13, and into pentactula larvae at day 26.

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