Requirements for culture media of local leech breeding

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ABSTRACT

Leeches (Phylum: Annelida, Class: Hirudinea) are widely distributed all over the world in various habitats, such as freshwater, seas, desert, and oases [1]. In this study, the effect of different culture for leech rearing on the reproductive efficiency and mortality rate of local leech was examined. In culture 1, the leeches were reared in a tank with a 1cm depth of non-chlorinated water. The soil obtained from a rice field was loaded into a hollow container and placed in the center of the tanks. In culture 2, the leeches were cultured in a tank with a 15cm depth of non-chlorinated water and without soil. After 3 months of culturing, the number of cocoons produced was significantly different between the two conditions (p=0.01). The leeches cultured in the tank with 1cm depth of non-chlorinated water with soil height of about 5cm, cocoon deposition number was optimal depositing an average of 3.67±0.58 compared with culture 2 where there was no cocoons produced by broodstock. Mortalities of broodstock leeches differed significantly under the two culturing methods (p=0.04). The culture 1 gave the lowest mortality rate with mean and standard deviation of 2.67±2.31% compared with the second culture which gave the highest mortality rate of 40±10.58%. The study also discussed about the reproduction, growth and development of this local leech.

Keywords: *Hirudinea* sp., survivorship, reproduction, leech behavior, leech culturing method

1. INTRODUCTION

Leeches are distributed all over the world in a variety of habitats; in freshwaters, seas, deserts, and oases. They are important components in food chains; as predators, vectors of parasites, preys of aquatic animals [2]. They occur in habitats that range from terrestrial to aquatic (both marine and freshwater) environments and are found on all continents. Leech was used by toxicologists and pharmacologists as a convenient tool for various investigations [3; 4; 5] in the past when its natural resources were boundless. In recent years, some leech populations have declined dramatically due to over-exploitation for fishing bait and medicinal purposes (particularly in Europe and Asia), and due to pollution [6; 7; 8; 9; 10].

Leech is a sanguivorous (haemopagic), freshwater leech, with a wide distribution in Southeast Asia, such as in southern China, the Philippines, Thailand, Vietnam and Malaysia. In Malaysia, these leeches are known as 'Lintah Kerbau' [11]. Traditionally, leeches are widely used as a model animal in toxicological, physiological, neurobiological, biochemical, histological and many other studies [3; 12; 13; 6; 14; 15; 16; 17; 18]. There has been an increasing harvest of this species for medical purposes in the 20th century [19; 20; 21] and so is in Malaysia. In this country, it is not known or proven conclusively that the locally named Buffalo Leech is not of *H. manillensis* although it has been confirmed by Department of Fisheries Malaysia and local taxonomists have not been able to identify the species used those for medical purposes.

Leech are prohibited (Haram) to consumption as a food, but it recommended (Sunnah) in cosmetic and medical products [22, June 10, 2009]. Nowadays, the breeding of leeches for medical purposes has bright commercial potential and of late many entrepreneurs have embarked on the farming of leeches. Besides that, Malaysian National Fatwa Council of Islamic Affairs that met for the 81st time on the 31st March 2007 has decided that the usage of worms and leech should be encouraged. Trade of these two animals for the intention of mutual benefit is recommended in Islam [23].

During the reproductive process, parent leeches secrete cocoons that protect and often nurture the developing eggs during the critical stages of early development [24; 25]. Components of the cocoons are released from specialized glands situated within the clitellar sex segments, forming a sheath around the clitellum into which fertilized eggs are deposited. The cocoon membrane is then passed over the head and sealed at both ends forming "plugs" at either end [26]. Embryos are dependent upon cocoon fluid contained in hard-shelled cocoons, while embryos from membranous cocoons can develop independently of the cocoon [27].

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The breeding of leeches for medical purposes has bright commercial potential and of late many entrepreneurs have embarked on the farming of leeches. Leech culture was seen as a solution to growing demand for leeches throughout the world. Modern leech therapy differs from that of an ancient one because now wild leeches are not used. The leeches are grown at a special leech farms where they are in severe quarantine. Also, to exclude infection of the patient a leech is used only once. In order to meet the demand from clinical use, Chinese traditional medicine and other scientific research, there has been growing interest in culturing and breeding leeches in many countries [25; 10;].The factors which determine leech distribution in freshwater environments are, in approximate order of significance, availability of food organisms; nature of the substrate; depth of water; presence of water currents; size and nature of the body of water; hardness and pH; temperature of the water; dissolved oxygen; siltation and turbidity; and salinity [5]. In this country, there is no standard culturing system available for breeding of leeches. The aim of the present study is to evaluate two different methods of culturing local leech for a large scale production based on their reproductively and mortality rate. This study also contribute to the some extent limited research concerning of reproduction, growth and development of the leeches.

2. MATERIAL AND METHODS

2.1 Sample preparation

Leech used in the study was provided by PT Dynamic Consultant Co., Kota Bharu, Kelantan. The leeches were cultured in concrete tanks (20 ×10 × 20 m) filled with non-chlorinated water source which were from river, well and rain to a depth of 25 cm. The concrete tanks were divided into four compartments. Approximately 1000 leeches were cultured in every compartment. The water in the concrete tanks was not aerated and exposed to direct sun light. Water hyacinth was placed in the concrete tanks and the leeches were fed once on live eel blood every week and once with an artificial booster every month. Sand was placed in the concrete tank to a height of 12 cm. Before the start of the experiment, leeches were cultured for 1 week in an indoor aquarium filled with non-chlorinated freshwater (30cm depth, 600L),aerated and 50% of the water changed once every 3 days. The temperature, pH and light intensity were maintained at 27.92 ± 6.62 °C, 6.7 ± 0.5 and 100-150 lx, respectively. The leech was fed once on live eel blood and once with an artificial booster in the preceding week before the proper study was initiated.

2.2 Experimental treatments

Two types of culture were used to test the mortality rate and reproductive efficiency. The leeches were cultured in aquarium tanks (30 x 19 x 26 cm) filled with non-chlorinated water source which was obtained from river. The temperature, pH and light intensity were maintained at 27.62 ± 5.62 °C, 6.8 ± 0.9 and 100-150 lx, respectively. The leech was fed every month once on live eel blood and once with an artificial booster. Three replicates were run for each treatment with a total of 6 aquarium tanks (30 x 19 x 26 cm). Each replicate contained 25 leeches. Approximately 150 leeches was collected from the holding tank, and randomly placed into the assigned experimental aquariums. For the first culture, the leeches were raised in tank with 1cm depth of non-chlorinated water. The soil obtained from rice field was loaded into a hollow container and placed in the center of the tanks. The height of soil was about 5cm. For the second culture, the leeches were raised in tank with 15cm depth of non-chlorinated water and without soil. The experiment lasted three months and daily observations were made.

2.2 Data collection and statistical analysis

At the end of the experiment, all the cocoons deposited by the broodstock were collected, counted and the average deposition number of cocoons for each culture method was calculated. The number of dead broodstock leeches was recorded daily.

Statistical analyses were conducted using the software SPSS 17.0 (Statistical Program for Social Sciences 17.0) to test the difference between the culture methods and any differences obtained were considered significant at $p \leq 0.05$. The cocoon deposition number of broodstock leeches and survivorship of parent leeches were analyzed by independent sample t-test.

3. Results

Table 1
Comparison of reproductive features and mortality rate obtained from two culture methods

Growth parameters	Culture 1	Culture 2
Cocoon deposition number (p=0.00)	3.67±0.58	0.00±0.00
Mortality rate (%) (p=0.04)	2.67±2.31	40±10.58

Data in the table were means and standard deviations (mean±S.D.)

Table 1 shows changes in the cocoon deposition number and mortality rate with two different culture methods. The results of present study showed that the number of cocoon deposited by the broodstock leeches was significantly different among the different densities (p=0.01). The cocoon deposition number was highest in the culture 1 with mean and standard deviation of 3.67±0.58 compared with culture 2 where no cocoon produced after 3 months culture.

The mortalities of broodstock leeches also differed significantly under different culture methods (p=0.00). The culture 1 had the lowest mortality rate with mean and standard deviation of 2.67±2.31% compared with culture 2 which gave the highest mortality rate of 40±10.58%.

4. Discussion

Determining optimum condition is a key factor for successful leech culture and reproduction. For example, mortality of the leech *Hellobdella stagnalis* is influenced by broodstock density and the density of their offspring [28]. The main purpose of this study was to examine and explore the basic needs of the leech culture methods, inclusive of reproduction, growth and development of the leeches. In this study, the different culture methods produced contrasting results on the number of cocoons deposited by the broodstock. Generally, in culture 1 where the leeches reared in tank to 1cm depth of non-chlorinated water and with soil height of about 5cm, cocoon number was greater depositing an average of 3.67±0.58 compared with culture 2 where there was no cocoon produced by the broodstock. The number of cocoon deposited by the broodstock leeches in culture 1 was highly significant different with that of culture 2 (p=0.00) (Table 1).

Reference [29] reported that cocoon that was produced by an adult *Barbronia weberi* was attached to a *Hydrilla verticillata* leaf. In the present study, it was found that each cocoon produced was laid on top of the soil (Figure 1) and displaying similar mechanism also was found occurring naturally in rice fields. However, Elliot & Mann [30] reported that the cocoons of *H. medicinalis* are normally deposited in a damp place just above the water line on the shore or bank.

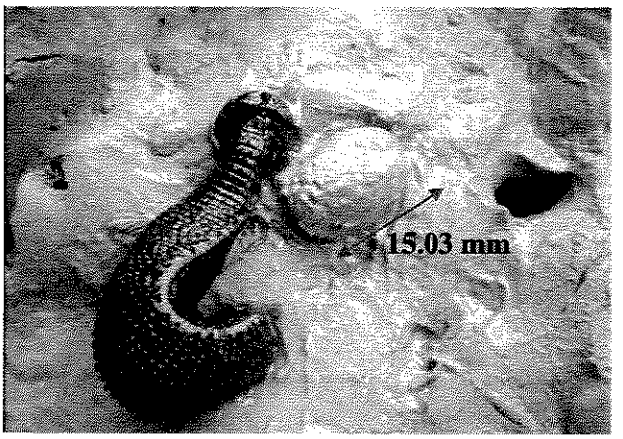

Figure 1: Individual cocoon produced by an adult local leech laid on top of the soil and they were fully hardened about 4 days after deposition. The normal size (length) of cocoon in this study was about 14 mm to 16 mm. Cocoon is clearly visible due to soil colour and structure that surrounding the cocoon structure.

From the observation, mating usually involves the intertwining of the two leeches where each deposits sperm in the clitellar opening of the other usually during night time when leeches are most active. Eggs are deposited in a gelatinous cocoon containing nutrients, further treatment of this cocoon depends on the parent leech species. For this species either bury or attach their cocoons to a soil and leave them afterwards. According to Light & Siddall [31] leeches of the family Glossiphoniidae show a high level of parental care. He also stated that they brood their eggs until the eggs hatch and carry their young under their dorsoventrally flattened bodies. The offsprings stay attached until they reach a certain size. After their first meal some can survive on their own while others still stay attached for some time. In the present study, within 1-3 days after copulation the leeches produce the first cocoon. The leech places its clitellum over the soil and secretes the cocoon. After deposition, the cocoon is a soft, translucent, colourless bag that usually contains on average 10 very small eggs that embedded into a viscous nutrient solution. After deposition, the parent begins to ventilate the fresh cocoon but leaves after about 15 minutes. Within a few hours, the surface of the cocoon becomes hard, brown and almost opaque. Two to three weeks later, the young hatch through the holes, which are created after plugs are broken off, and the cocoon become dry as shown in Figure 2 (a) and Figure 2 (b). These local leeches showed that they do not take care of their offsprings. On the contrary, Kutschera & Wirtz [32] stated that *H. stagnalis* shows the most developed level of parental care to their offsprings and some hatched larvae develop into juvenile leeches still attached to the ventral side of the parent leech for about three to four weeks. He also stated that the young are frequently fed by the parent. With this high level of protection and consistent food, juveniles grow during the three weeks of post-embryonic parental care. So, for more effectively method in the breeding for these leeches species, the newly hatched juveniles should be separated from the broodstock and they will be fed manually because this leech species shows a low level of parental care or they do not take care of their offsprings.

This local leech cocoons are lemon-shaped capsules that are characterized by two terminal plugs and the normal length of cocoon in this study was about 14 mm to 16 mm long. From the study conducted by Kutschera & Wirtz [32], he stated that H. *sanguisuga* also have a lemon-shaped cocoon but the differences with this local leech were their cocoon size where the H. *sanguisuga* cocoon size was only about 8 mm-9 mm long compared with this local leech was about 14 mm to 16 mm long. Terrestrial cocoon deposition was also documented for the two related jawed species, H. *medicinalis* and L. *nilotica* [4]. According to Sawyer [33], some leeches deposit their cocoons, which have outer coat of hardened froth, among moss, leaves or humus. The present study shows that it was evidence that the formation of cocoon also related to that soil structure. After deposition, cocoon is clearly visible due to soil colour and the soil media that surrounding the cocoon structure and this mechanism is meant to protect the cocoon from aquatic predators and it can be interpreted as parental investment. In nature, these unprotected fresh cocoons are in danger of being eaten by many potential predators and even after hardening the cocoon structure can be attacked by water snails. Formation and fixation of the cocoon has been described in detail by Kutschera [34]. However, more detailed

studies should be made on this local leech according to their cocoon's structure.

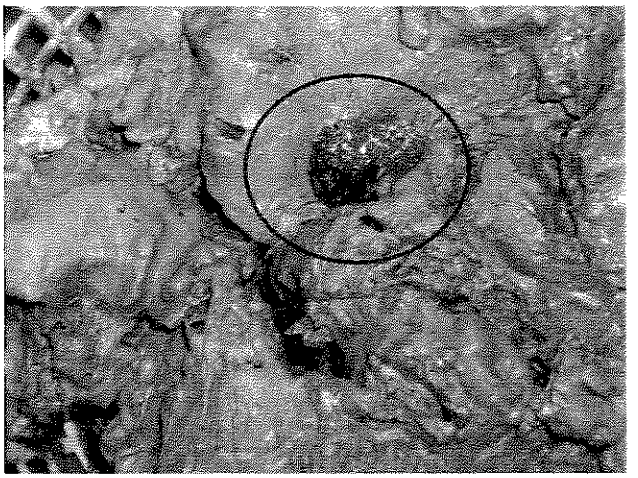

Figure 2(a): A dry cocoon that laid on the soil structure after 2 weeks deposition.

Figure 2(b): A dry cocoon structure. Cocoon will become dry usually one week after the hatching process. The structure of dry cocoon was totally destroyed after 1 month without preserved.

When the leeches were placed into an aquarium with a soil base, individual leeches were observed to penetrate soil substrate, making them virtually undetectable. Leeches will make a hole on the soil surface to allow their body to enter into the soil. In this study, the depth of the hole made by leeches was usually about 3-4cm from the surface (Figure 3). A similar habit was also reported by Fredric R. Govedich [29] where in their study, *B. weberi* leech when placed into an aquarium with gravel base, were observed to burrow into the gravel, making them also virtually undetectable. However, the adult *B. weberi* were most commonly found attached to *Hydrilla verticillata*, a weedy aquatic plant that has been known to invade freshwater ecosystems. Individual leeches were usually found attached near the base of the leaf whorls or along the stem making them difficult to observe. Although most of the individual cocoons were found attached to the bottom or sides of the container, some of the cocoons were laid on individual leaves.

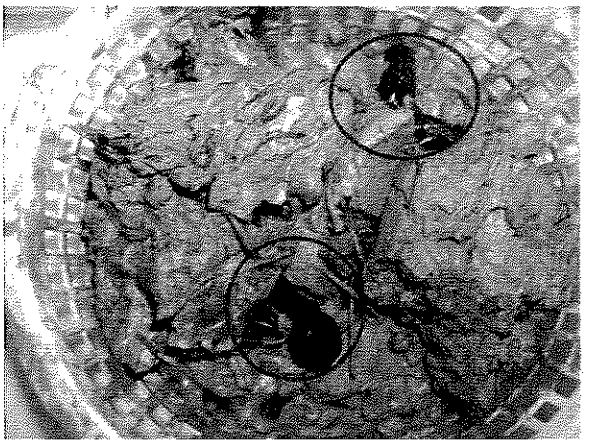

Figure 3: A burrowing hole made by leeches to enable them to hide in the soil.

The leeches that were cultured in an aquarium with 15cm depth of non-chlorinated water and without soil produced no cocoon after three months of rearing indicating that the leeches needed soil as a medium to lay their cocoons. In addition, the observation showed leeches that were kept in the culture one was more active in movement as opposed to leeches cultured in the second method.

Mortalities of broodstock leeches differed significantly between the two culturing methods (p=0.04). In culture 1, mortality was lowest with mean and standard deviation of 2.67±2.31% compared with culture 2 which gave the highest mortality rate of 40±10.58%. In this study, many leeches were found to be infected by parasitic protozoans and flatworms in culture 2 method during the course of the experiment which greatly influenced the survival rate and growth of the leeches. In the study conducted by B. Zhang et al., [35] broodstock density had a significant influence on the survival rate of leeches and they showed that increasing of the broodstock density during the course of the experiment led to high mortality. In the present study though, the broodstock density was constant throughout with 25 leeches for each treatment thus ruling out other factors except that was imposed namely water level which had a direct effect on mortality of the broodstock where increase in water level had a negative effect. However, these reasons may still not be sufficient to provide a comprehensive explanation of the mortalities of broodstock

and there is might be other factors influence it. Therefore, more detailed studies should be made on this local leech due to their factors that influence the mortality of this leech species.

5. Conclusion and recommendations

This investigation has demonstrated that the different of culturing methods were significantly affect the reproductive efficiency and mortality of the local leech The results of this study shows that for breeding leeches would do very well under the culture one for as long as three months as their mortality was minimal and cocoons number was highest when compared to that under a culture 2. The results also indicated that the soil and water level are crucial according to their reproductive and survivorship of leeches. In breeding of leeches culture one is recommended.

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