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PROPAGATION AND CULTURE SYSTEM OF LOCAL LEECHES

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Abstract: This particular research work was one of the few that have been reported on the basic requirements for culturing of local leeches including reproduction and also the behaviour of the animal. In this study, the effect of different cultures for leech-rearing in laboratory conditions on the reproductive performance of local leech was examined. After 91 days (3 months) of culturing, the number of cocoons produced was significantly different between the two conditions ($p \leq 0.05$). For 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 ($p \leq 0.05$). 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 the reproduction and the behaviour of the local leeches.

KEYWORDS: *Hirudinea* sp., leech, culture system

Introduction

Leeches (Phylum: Annelida, Class: Hirudinea) are widely distributed all over the world in a diversity of habitats, such as fresh water, seas, deserts, and oases (Gouda, 2006). Leech therapy is one of the most important and widely-practiced methods of regimental therapy used for local evacuation of morbid tumours (Ibn Sina, 1998). The therapy is known from the time of extreme antiquity, more than 2,500 years ago, and is still alive. This fact testifies to its efficiency in various health problems. In Malaysia, the entrepreneurs used leeches as trading animals and the stock are exported to meet the demand in countries such as Korea, Ukraine, China and Spain. Normally, the leech products are exported in the dried form. In some cases, traditional medicinal practitioners use leeches for therapeutic healing treatment locally called "bekam" and it is quickly gaining acceptance amongst the local folks. It is now becoming a thriving business.

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Leeches have a wide distribution in Southeast Asia, such as southern China, the Philippines, Thailand, Vietnam, and Malaysia. In Malaysia, this leeches are known as 'Lintah Kerbau' (406th Medical Lab. Special Report, 1968). There has been increasing collection of this species for medical purposes in the 20th century (Steiner *et al.,* 1990; Electricwala *et al.,* 1993; Singhal & Davies, 1996). The leech industry in this country was previously operated by the Department of Wildlife Protection and National Parks (PERHILITAN) and since 22 January 2009, the Department of Fisheries Malaysia has taken over the operation (Department of Fisheries Malaysia, 2009). The Fisheries Department have since identified the leech species used for commercial purposes. The taxonomic status of the local leech are as follows: Order: Hirudinea; Species: *Hirudinaria manillensis*; Local name: Buffalo leech or "Lintah kerbau".

However, it is not known or proven conclusively that the locally-named Buffalo Leech is not of *H. manillensis* although this has been confirmed by Department of Fisheries

Malaysia. In addition, local taxonomists have not been able to identify those species used for medicinal purposes (Personal communication[1]).

To meet the demand for clinical use, in Chinese traditional medicine and other scientific research, there has been growing interest in culturing and breeding of leeches in many countries (Yang, 1996; Trontelj & Utevsky, 2005). Several factors determine leech distribution in freshwater environments such as 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 (Sawyer, 1986). However, no study has been attempted to investigate the factors affecting growth and production of leeches in this country. Particularly lacking is the effect of water, temperature, dissolved oxygen, pH, and light intensity on growth performance of these leeches bred in a farm as well as their feeding requirements. Understanding the growth conditions required could help maximise the quality of leech-farming and those for medical purposes.

Leech farming was seen as a solution to the growing demand for the production of 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 special leech farms where they are subjected to severe quarantine requirements. Also, to exclude infection on to the patient, a leech is used only once and thrown away. In this country, there is no proper standard or guide on how leech farming should be carried out and this problem has led to the difficulty for entrepreneurs to handle breeding on a large scale, besides the fact that a few of them did not succeed in the leech industry as the leeches failed to reproduce well. This problem arose because the entrepreneurs are not aware of the basic practice that should be included in the breeding of leeches. Therefore, this study was carried out to ascertain the basic requirements for the culturing of leeches, their reproduction and the behaviour of the animal.

Materials and Methods

Origin of broodstock

Local leeches used in the study were provided by PT Dynamic Consultant Co., Kota Bharu, Kelantan. The leeches were cultured in concrete tanks (8 × 1 × 4 m) filled with non-chlorinated water source which was from a 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 was exposed to direct sunlight. 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 water (30cm depth, 600L), aerated and 50% of the water changed once every 3 days. The temperature, pH and light intensity were maintained at 27.12 ± 6.02 °C, 6.7 ± 0.5 and 100-150 lx, respectively. The leeches were fed once on live eel blood and once with an artificial booster in the preceding weeks before the proper study was initiated.

Experimental design

Two types of culture were used to test the mortality rate and reproductive efficiency of the local leeches. The leeches were cultured in aquarium tanks (30 x 19 x 26 cm) filled with non-chlorinated water the source of which was obtained from a river. The leeches were fed every month, once on live eel blood and once with an artificial booster. The booster diet was mixed with soil in the proportion of 1:4 (kg) before being fed to the leeches. The ingredients of experimental diet FT2 (Booster) are shown in Table 1. 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 were collected from the holding tanks, and randomly placed into the assigned experimental aquariums. For the first culture, the leeches were raised in tanks with 1cm depth of non-chlorinated water. Soil obtained from rice field with pH of about 4.5 to 6.5 was loaded into a hollow container and placed in the centre of the tanks. The height of soil was about 5cm. For the second culture, the leeches were raised in tanks with 15cm depth of non-chlorinated water and without soil. The non-chlorinated water tanks were aerated, with 50% of the water changed every 3 days. The experiment lasted three months and daily observations were made.

Table 1: Ingredients of the booster experimental diet.

Experimental diet	Ingredients
Booster (FT2)	Compost
	Molina
	Ziolite
	Allobohpora rosea
	Phosphorus
	Calcium

Data collection

Cocoon deposition number

At the end of the experiment, all the cocoons deposited by the broodstock were collected and counted and the average deposition number of cocoons for each culture system was calculated.

Hatching number and hatching rate

After the juveniles were released from the cocoons, they were collected and counted. The ratio of the numbers of juveniles to deposited cocoons was calculated for each culture system to obtain the average hatching number as given in Equation (1).

$$\text{Average hatching number} = \frac{\text{Number of juveniles}}{\text{Cocoons deposited}} \quad (1)$$

During the experiment, the abortion rate was high and the number of dead cocoons (juveniles failed to hatch) were counted and recorded. The ratio of dead to the deposited cocoons was then calculated as the average hatching rate for each culture system as given in Equation (2).

$$\text{Average hatching rate} = \frac{\text{Died of juveniles}}{\text{Cocoons deposited}} \quad (2)$$

Broodstock mortality

The number of dead broodstock leeches was recorded during the daily observation made.

Cocoon size and wet weight

All cocoons from each replicate tank were collected and their length and diameter measured. The wet weight of each cocoon was taken before hatching occurred.

Statistical analyses

Statistical analyses were conducted using the software SPSS 17.0 (Statistical Program for Social Sciences 17.0) to test the difference among the culture conditions and any differences obtained were considered significant at $p \leq 0.05$. The cocoon deposition number of broodstock leeches, hatching number and hatching rate of cocoons, survivorship of parent leeches, cocoon size and wet weight were analysed by one-way ANOVA.

Results and discussions

Determining optimum conditions 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 (Mann, 1957). The leeches used in the study were up to 15cm in length and green on the dorsal side and also had a thin darker stripe and, in this country, this species was used for "bekam" therapy (Personal communication[2]). For Culture 1, the temperature, pH, dissolved oxygen and light intensity were 27.30±0.167°C, 6.11±0.14, 7.54±0.16 mL^{-1} and 100-150 lx, respectively and for Culture 2, the temperature, pH, dissolved oxygen and light intensity were 27.3±0.21°C, 6.12±0.14, 7.55±0.19 mL^{-1} and 100-150 lx, respectively during the study period from April 1 2010 to 30 June 2010. The main purpose of this study was to ascertain the basic requirements for culturing leeches which would include those for reproduction and also the behaviour of the animal. In this study, the different culturing methods produced contrasting results on the number of cocoons deposited by the broodstock. Generally,

Table 2: Comparison of reproductive parameters obtained from two culture methods

Growth parameter	Culture 1	Culture 2
Cocoon deposition number	3.67±0.58	0.00±0.00
Mortality rate (%)	2.67±2.31	40±10.58
Cocoon length (mm)	13.47±1.24	0.00±0.00
Cocoon diameter (mm)	13.35±0.47	0.00±0.00
Cocoon wet weight (g)	1.5±0.29	0.00±0.00
Hatching rate (%)	98.86±0.43	0.00±0.00
Hatching number	6.22±1.11	0.00±0.00

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

in Culture 1 where the leeches were reared in tanks to 1cm depth of non-chlorinated water and with soil height of about 5cm, the number of cocoons deposited was higher with an average of 3.67±0.58 compared with that of Culture 2 where there was no production of cocoons by the broodstock (Table 2).

Fredric Govedich *et al.,* (2003) reported that cocoons that were produced by an adult *Barbronia weberi* were attached to a *Hydrilla verticillata* leaf but, in this study, it was found that each cocoon produced was laid on top of the soil displaying similar mechanism also as was found occurring naturally in rice fields. In contrast, Elliot & Mann (1979) reported that the cocoons of *H. medicinalis* were normally deposited in a damp place just above the water line on the shore or bank.

From observations made on mating, this usually involves the intertwining of the two leeches where each deposited sperm in the clitellar opening of the other, usually during night-time when leeches were most active. Eggs were deposited in a gelatinous cocoon containing nutrients, and further development of this cocoon depended on the species of parent leeches. With this species under study, the leeches either bury or attach their cocoons to soil surface and migrate away afterwards. According to Light & Siddall (1999) 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 offspring 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, the leeches produce the first cocoon within 1-3 days after copulation. 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 are 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 hardened, brown in colour 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. These local leeches showed that they do not take care of their offspring. On the contrary, Kutschera & Wirtz (2001) founded that *H. stagnalis* showed the most developed level of parental care to their offspring and some hatched larvae developed into juvenile leeches still attached to the ventral side of the parent leech for about three to four weeks. They also ventured that the young are frequently fed by the parent. With this high level of protection and consistent food supply, juveniles grow during the three weeks of post-embryonic parental care. So, for more effective method in the breeding of these species of leeches, the newly-hatched juveniles should be separated from the broodstock and fed manually as this particular leech species showed a low level of parental care or completely ignored them.

The cocoons of local leeches are lemon-shaped capsules that are characterised by two terminal plugs and the normal length of cocoon in this study was about 11 to 16 mm long. Kutschera & Wirtz (2001) found that *H. sanguisuga* also had a lemon-shaped cocoon but the difference with these local leeches was in their cocoon size where for *H. sanguisuga* the size was smaller, measuring only about 8 to 9 mm long. Terrestrial cocoon deposition was also documented for the two related jawed species, *H. medicinalis* and *L. nilotica* (Herter, 1968). According to Sawyer (1986b), some leeches deposit their cocoons, which have an outer coat of hardened froth, among moss, leaves or humus as protection from predators. With the local leeches it was evident that the formation of the cocoon was also related to that of the soil structure. After deposition, the cocoon was clearly visible as a globular structure encrusted with soil and the colour of soils act as a mechanism to protect the cocoon from aquatic predators. This 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, 1983. However, more detailed studies should be made on the cocoon structure of local leeches.

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. A similar habit was also reported by Fredric Govedich *et al.* (2003) where, in their study, *B. weberi* leeches 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.

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 that leeches kept in the Culture 1 were more active in movement as opposed to leeches cultured in the other culture.

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 during the course of the experiment which greatly influenced the survival rate and growth of the leeches. In the study conducted by Zhang *et al*., (2008), broodstock density had a significant influence on the survival rate of leeches and they showed that increasing 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 those that were imposed, namely water level which had a direct effect on mortality of the broodstock where an 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 might be other factors influencing it. More detailed studies should therefore be made on the factors that influence the mortality of this leech species.

Conclusions

This investigation has demonstrated that the different culturing methods significantly affected the reproductive efficiency and mortality of the local leech The results of this study shows that breeding leeches would do very well under the culture 1 for as long as three months as their mortality was minimal and cocoons number was highest when compared to that under culture 2. The results also indicated that the soil and water level are crucial according to the reproduction and survivorship of leeches. In breeding of leeches, culture 1 is recommended.

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