Songklanakarin J. Sci. Technol.
37 (5), 581-585, Sep. - Oct. 2015

http://www.sjst.psu.ac.th

Original Article

Effect of broodstock density on reproduction and juvenile culture of green buffalo leech, *Hirudinea manillensis*

Zulhisyam Abdul Kari*, Mohd Hafiz Jamaludin, Lee Seong Wei, Yusrina Andu, Wan Ahmad Izuddin Wan Ibrahim, and Sarijan Shazani

Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Locked Bag No.100, 17600, Jeli, Kelantan, Malaysia.

Received: 9 July 2014; Accepted: 16 May 2015

Abstract

In this study, the effect of the broodstock density on reproduction and juvenile culture of green buffalo leech, *Hirudinea manillensis* was examined with six different densities (5, 10, 15, 20, 25, and 30 ind tank^{-1}). The number of cocoons produced was significantly different among the different densities (p = 0.00) after three months of culture. Although hatching rate was not (p = 0.354), the average number of hatchings per cocoon was also significantly different ($p \leq 0.05$) with 5 ind tank^{-1} gave the highest number (6.61±1). The 25 ind tank^{-1} produced the highest mortality of parent leeches (67±13.16%). According to their length and diameter, the sizes of the cocoons were not significantly different among the treatments, with the 5 ind tank^{-1} having the largest cocoon of 22.19±0.92 mm and 13.25±0.07 mm, respectively. The wet weight of cocoons was significantly different at $p \leq 0.05$ with the 5 ind tank^{-1} producing the heaviest cocoons of 1.26±0.11g compared to the 25 ind tank^{-1}producing the lightest cocoons of 0.22±0.38 g.

Keywords: buffalo leech, hirudinea manillensis, density, growth, survivorship, reproduction

1. Introduction

Leeches (Phylum: Annelida, Class: Hirudinea) are widely distributed all over the world in a diversity of habitats, such as freshwater, seas, deserts, and oases (Gouda, 2006). They can be predators, vectors of parasites, as well as prey of other aquatic animals (Saywer, 1986a; Keim, 1993). 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 (Sawyer, 1981; Elliot and Tullett, 1984; Wells and Coombes, 1987; Petrauskiene, 2003; Trontelj and Utevsky, 2005). Today, there are many products which have been derived from leeches for pharmaceutical and medicinal purposes, globally. In order to meet the demand for clinical use, Chinese traditional medicine and other scientific researches, the culturing and breeding of leeches in many countries are increasing (Yang, 1996; Trontelj and Utevsky, 2005).

Leech farming is still perceived as a solution to the growing demand for the production of leeches throughout the world. Modern leech therapy differs from conventional method because now wild leeches are no longer widely been used. The leeches are grown at special leech farms where they are subjected to severe quarantine requirements. This is also to exclude infection on the patient as a leech is only used once and thrown away. In Malaysia, large-sized leeches are usually used for breeding and production while small-sized leeches (2-3 weeks juveniles) are used for “cupping” therapy. As it is widely known, up until now the leech stock is always insufficient. The shortages of leech supply caused

* Corresponding author.
Email address: zulhisyam.a@umk.edu.my

many entrepreneurs to discontinue their business for a temporary period of time due to lack of leeches. In order to address this problem, optimizing their growing condition is strongly needed.

2. Materials and Methods

2.1 Origin of broodstock

Local leeches *(Hirudinea manillensis)* 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 resource which were obtained from a river, well, and rain until a depth of 25 cm. The concrete tanks were divided into four compartments. Sand was placed in the concrete tanks at a height of 12 cm. Approximately 1,000 leeches were cultured in every compartment, and directly exposed to sunlight without aeration. Water hyacinth was placed in the concrete tanks. The leeches were fed once every week on live eel blood and once a month with an artificial booster.

2.2 Experimental design

Six broodstocks density treatments at 5, 10, 15, 20, 25 and 30 ind $tank^{-1}$ were used to test the effect of breeding densities on the reproductive efficiency. Each treatment consisted of three replicates with a total of 18 aquarium tanks each measuring 30 × 19 × 26 cm in size with different densities of leeches introduced in each replicated tank. Approximately 315 leeches were collected from the holding tank and randomly placed into the assigned experimental aquariums. Soil up to 5 cm depth was provided as substrate in each aquarium. Daily observations were made in three months period.

2.3 Cocoon deposition number

At the end of the experiment, all cocoons deposited by the broodstock were collected and counted. The average deposition number of cocoons for each growth condition was also calculated.

2.4 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 growth condition to obtain the average hatching number as follows (Zhang *et al.*, 2008):

$$\text{Average hatching number} = \frac{\text{Number of juveniles}}{\text{Cocoon deposited}}$$

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 cocoons to the deposited cocoons was also calculated as the average hatching rate for each treatment as follows (Zhang *et al.*, 2008):

$$\text{Average hatching rate} = \frac{\text{Dead juveniles}}{\text{Cocoon deposited}}$$

2.5 Broodstock mortality

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

2.6 Cocoon size and wet weight

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

2.7 Statistical analyses

Statistical analyses were conducted using the software SPSS 17.0 (Statistical Program for Social Sciences 17.0) to test the differences among the growth conditions and any other 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 analyzed by using a one-way ANOVA and where this effect was significant. Duncan Multiple Range Test was performed to compare between the treatments.

3. Results

The comparison of reproductive parameters, which are cocoon number, hatching number, hatching rate, cocoon length and cocoon diameter, cocoon wet weight and mortality rate under 6 different densities is shown in Table 1.

3.1 Cocoon deposition number

The changes in the deposition of cocoons under different densities are shown in Table 1. The results of present study showed that the number of cocoon deposited by the broodstock leeches was significantly different among the different growth conditions ($p = 0.00$). The average number of cocoons was highest in the 5 ind $tank^{-1}$ (6.61±1), followed by 10 ind $tank^{-1}$ (3±1). Both treatments were significantly different from the rest of the different densities (15, 20, 25, and 30 ind $tank^{-1}$). However, among themselves, they were not significantly different from each other.

3.2 Hatching number

The changes in the hatching number with different densities are shown in Table 1. The results showed that the hatching number was significantly different under different growth conditions ($p \leq 0.05$). The condition under 5 ind $tank^{-1}$ had the highest hatching number (8.33±0.15), while

Table 1. Comparison of reproductive parameters of the local leeches under different densities: cocoon number, hatching number, hatching rate (%), mortality rate of parent leeches (%), cocoon standard length (mm), diameter (mm) and cocoon wet weight (g).

Parameters	Breeding density (ind tank^{-1})					
	5	10	15	20	25	30
Cocoon number ($p = 0.00$)	6.61±1^{a}#	3±1^{b}	0.67±1.16^{c}	1.33±0.58^{c}	0.61±1.26^{c}	0.67±0.58^{c}
Hatching number ($p \leq 0.05$)	8.33±0.15^{a}	3.14±1.27^{b}	0.5±0.87^{b}	2.83±2.47^{b}	2.17±0.29^{b}	2.33±2.08^{b}
Hatching rate (%) ($p \geq 0.05$)	96.23±8.26^{a}	62.77±25.64^{ab}	16.67±28.87^{b}	69±51.96^{ab}	75±17.46^{ab}	66.67±57.74^{ab}
Cocoon length (mm) ($p \geq 0.05$)	22.19±0.92^{a}	19.27±0.19^{ab}	5.58±9.67^{bc}	16.6±0.39^{c}	4.74±8.22^{c}	3.38±8.21^{c}
Cocoon diameter (mm) ($p = 0.113$)	13.25±0.07^{a}	10.19±0.13^{ab}	3.89±5.87^{b}	11.27±0.3^{ab}	4.14±5.78^{b}	3.7±5.8^{ab}
Cocoon wet weight (g) ($p \leq 0.05$)	1.26±0.11^{a}	1.05±0.01^{a}	0.23±0.4^{b}	1.11±0.02^{a}	0.22±0.38^{b}	0.52±0.41^{ab}
Mortality rate (%) ($p = 0.00$)	2.57±2.31^{a}	13.11±2.31^{a}	42±4^{d}	31.3±1.3^{bc}	67±13.16^{e}	28.33±8.13^{b}

#Data in the table was mean and standard deviation (mean ± S.D). Means with the same letter within the same column are not different at the 5% of significant level as determined by Duncan test.

under the 15 ind tank^{-1} conditions the lowest hatching number was obtained (0.5±0.87). Hatching numbers of cocoons in the 10, 20, 25, and 30 ind tank^{-1} were not significantly different ($p > 0.05$).

3.3 Hatching rate

The changes in the hatching rate (%) with different culture conditions are shown in Table 1. The results here showed that the hatching rates of cocoons under different conditions were not significantly different ($p \geq 0.05$). Although the 5 ind tank^{-1} had the highest hatching rate (96.23±8.26%), however, no significant differences were shown (p>0.05).

3.4 Cocoon size

The effect of different densities did not significantly influence the standard length and diameter of the cocoons produced ($p \geq 0.05$, p = 0.113). The 5 ind tank^{-1} culture provided the largest size of cocoon, indicating with the length and diameter (22.19±0.92 mm and 13.25±0.07 mm, respectively), whereas the 30 ind tank^{-1} treatment gave the smallest cocoon size (3.38±8.21 mm and 3.7±5.8 mm, respectively). There was no difference shown in cocoon standard length and diameter obtained from those cultures under 10, 15, 20, and 25 ind tank^{-1} ($p > 0.05$).

3.5 Cocoon wet weight

In this study, the results obtained showed that the different densities imposed significantly influenced cocoon wet weight ($p = 0$). Broodstock in the 5 ind tank-^{1} produced the heaviest cocoons (1.26±0.11 g), whereas the 25 ind tank^{-1} treatment provided the lightest cocoon wet weight (0.22± 0.38 g). Cocoon wet weight under the 10, 15, 20, and 25 ind tank^{-1} system were not significantly different ($p > 0.05$).

3.6 Mortality rate

The change in the mortality rate (%) under different densities is shown in Table 1. The results obtained showed that the mortalities of broodstock leeches differed significantly under different growth conditions ($p = 0$). The 5 ind tank^{-1} had the lowest mortality rate (2.57±2.31%), while 5 ind tank^{-1} gave the highest mortality rate (67±13.16%). Mortality under other conditions also gave significant differences ($p > 0.05$).

4. Discussion

The main purpose of this study was to optimize the growth condition for the culture of leeches. In this study, it was found out that the increasing of the density gave a negative effect on the number of cocoons produced by the broodstock. Under the condition where the density was 5 ind tank^{-1} cocoon deposition number was optimal depositing an average of 6.61±1 cocoons per replicate which gives a higher number if compared with the previous study. This is in agreement with Zhang *et al.* (2008), reporting the same phenomenon where an amount of 5 leeches per tank was optimal for cocoon deposition, with an average of 3.84±0.12 in cocoons numbers. Zhang *et al.* (2008) also stated that the low cocoon numbers of broodstock leeches under high density appeared to be related to competition for food and space among the leeches, creating a stressful condition which directly affects the natural reproductive behavior. Fredric *et al.* (2003) reported that cocoons that were produced by an adult *Barbronia weberi* are attached to *Hydrilla verticillata* leaf. However, in this study, it was found that each cocoon produced was laid on top of the soil displaying similar mechanism as is also found occurring naturally in rice fields. In contrast, Elliot and 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.

Each of the cocoons that was produced was laid on top of the soil, although the number was lower but it is still within the range of 4 to 8.15 cocoons deposited obtained from *Haemadispa hainana* (Tan *et al.*, 1992) and *N. obscura* (average of 8.33±0.68 cocoons deposited) (Collins and Holmstrand, 1984).

Eggs were deposited in a gelatinous cocoon containing nutrients, and further development of this cocoon depends on the species of parent. In this study, we found that the leech was either bury or attach their cocoons to soil surface and migrate away afterwards. According to Light and Siddall (1999) leeches of the family *Glossiphoniidae* showed a high level of parental care. They also stated that leeches brood their eggs until hatch and carry their young under their dorsoventrally flattened bodies. The offspring stay attached until they reach a certain size. In our observation, some leeches can survive on their own after their first meal 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 and colorless bag that usually contains an average of 10 tiny 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 harden, brown in color 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 and Wirtz (2001) found 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 for about three to four weeks. They also observed 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. Thus, 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.

In this study, broodstock density significantly influenced the size and wet weight of the cocoons that were produced, with the largest cocoons produced at the lowest density (5 ind tank^{-1}). Cocoon sizes are approximately equal to the size of about 22 mm in mean length and 13 mm in mean diameter as also reported in Tan *et al.* (2002). Generally, the wet weight of leech cocoons can be substantially different between leech species, e.g. 1.6-2.0 g in *Whitmaniapigra* (Shi *et al.*, 2006a) and 0.15-0.18 g in *H. hainana* (Tan *et al.*, 1992). Here, the wet weight of the cocoons ranged from 1.26±0.11g in the 5 ind tank^{-1} treatment to 0.22±0.38g in the 25 ind tank^{-1} treatment.

Water quality, temperature and parasitism are known to significantly influence the survivorship of the leech species (Sawyer, 1970; Tan, 2005; Shi *et al.*, 2006b). Life-span of leech was also one of the key impediments for the leech culture (Mann, 1957). In the present study, we found that the broodstock density also could significantly influence the survival of the parent leeches, where high broodstock density led to a high mortality, probably due to competition for living space and water quality. Majority, the leeches reared at the higher density treatments mostly died due to the fighting among themselves. Zulhisyam A.K. (2011) stated that a growth condition at a temperature of 25-28 °C and zero light intensity fed with fresh eel blood is recommended for the commercial breeding of this species which gave low mortality rate.

The cocoons of local leech are lemon-shaped capsules that are characterized by two terminal plugs and the normal length of cocoon in this study was about 11 to 16 mm long. Kutschera and Wirtz (2001) found that *H.sanguisuga* also had a lemon-shaped cocoon but the difference with local leeches was in their cocoon size. Here, *H.sanguisuga* size was smaller measuring at 8 to 9 mm long. In Herter's study (as cited in Elliott and Tullett, 1984), terrestrial cocoon deposition was also documented for the two related jawed species, *H.medicinalis* and *L.nilotica*. According to Sawyer (1986b), some leeches deposit their cocoons, which have outer coat of hardened froth, among moss, leaves or humus as protection from predators. In our study, it was evident that the formation of cocoon was also related to that of the soil structure. After deposition, the cocoon was clearly visible as globular structure encrusted with soil and the color of soils act as a mechanism to protect the cocoon from aquatic predators and 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. However, more detailed studies should be made on the cocoon structure of local leeches.

In conclusion, this investigation has demonstrated that broodstock density significantly affects the reproductive efficiency of the buffalo leech. A broodstock density of 5-10 parent leeches per tank (30 × 19 × 26 cm) is recommended for the commercial culture of this leech species. These local leeches exhibited their lack or total absence of parental care for their offspring. To ensure effective breeding of these leech species, the newly hatched juveniles should be separated from the broodstock and fed manually with eel blood as it has been observed that this leech species showed a low level or complete absence of parental care.

Acknowledgements

The author is grateful to the Faculty of Agro-based Industry (FIAT) for providing space and facilities to carry out the study. This study was funded by the University Malaysia

Kelantan and supported by PT Dynamic Consultant Co., Kota Bharu, Kelantan and Freshwater Fisheries Research Centred, GelamiLemi, Jelebu, Negeri Sembilan.

References

Bin, Z., Lin, Q., Lin, J., Chu, X. and Lu, J. 2008. Effects of broodstock density and diet on reproduction and juvenile culture of leech, *Hirudinariamanillensis* Lesson, 1842. Journal of Aquaqulture. 276, 198-204.

Collins, H.L. and Holmstrand, L.L. 1984. Indicators of sexual maturity in the leech, *Nephelopsis obscura* (Annelida: *Hirudinea*). American Midland Naturalist. 112(1), 91-94.

Elliot, J.M. and Mann, K.H. 1979. A key to the British freshwater leeches with notes on their life cycles and ecology, Freshwater Biological Association Scientific publications, No. 40.

Elliott, J.M. and Tullett, P.A. 1984. The status of the medicinal leech *Hirudomedicinallis* in Europe and especially in the British Isles. Biological Conservation. 29(1), 15-26.

Gouda, H.A. 2006. The effect of peritrich ciliates on some freshwater leeches from Assiut, Egypt. Journal of Invertebrate Pathology. 93, 143-149.

Keim, A. 1993. Studies on the host specificity of the medicinal blood leech *Hirudomedicinalis L.* Parasitology Research. 79, 251-255.

Kutschera, U. and Wirtz, P. 2001. The Evolution of parental care in freshwater leeches. Theory Bioscience. 120, 115-137.

Light, J.E. and Siddall, M.E. 1999. Phylogeny of the leech family Glossiphoniidae based on mitochondrial gene sequences and morphological data. Journal of Parasitology. 85, 815-823.

Mann, K.H. 1957. The breeding, growth and age structure of a population of the leech *Helobdella stagnalis*. Journal of Animal Ecology. 26(1), 171-177.

Mason, T.A., McIlroy, P.J. and Shain, D.H. (2004, March). A cysteine-rich protein in the Theromyzon (Annelida: Hirudinea) cocoon membrane. FEBS Letters. 561(1-3), 167-172.

Petrauskienë, L. 2003. Water and sediment toxicity assessment by use of behavioural responses of medicinal leeches. Environment International. 28, 729-736.

Sawyer, R.T. 1970. Observations on the natural history and behavior of *Erpobdella punctata* (Leidy) (Annelida: *Hirudinea*). American Midland Naturalist. 83(1), 65-83.

Sawyer, R.T. 1986a. Leech Biology and Behavior. Oxford University Press, New York, U.S.A.

Sawyer, R.T. 1986b. Leech biology and behavior II. Feeding Biology, Ecology and Systematics. Oxford: Clarendon Press, U.K.

Sawyer, R.T. 1981. Why we need to save the medicinal leech. The International Journal of Conservation (Oryx). 16, 165-168.

Shi, H.Z., Liu, F. and Guo, Q.S. 2006a. Studies on impact of temperature and weight in *Whitmaniapigra* bred. China Journal of Chinese Material Medical. 31(24), 2030-2032.

Sineva, M.V. 1941. Zoology Pollution. 25, 163-196.

Tan, E.G., Huang, L.Y., Guan, Y. and Wang, B. 2002.Studies on growth and reproduction of *Hiruninariamanillensis* in Guangdong province. Chinese Traditional and Herbal Drugs. 33(9), 837-840.

Tan, E.G., Chen, J. and Qian, Y.T. 1992. Growth, feeding and reproduction of the leech *Haemadispa hainana*. Natural Science Journal of Hainan University. 10(1), 16-23.

Tan, E.G. 2005. The response of *Haemadip sahainana* to temperature, relative humidity and light. Chinese Traditional and Herbal Drugs. 36(9), 1409-1411.

Trontelj, P. and Utevsky, S.Y. 2005. Celebrity with a neglected taxonomy: molecular systematics of the medicinal leech (genus *Hirudo*). Molecular Phylogenetics and Evolution. 34, 616-624.

Wells, S. and Coombes, W. 1987. The status of and trade in the medicinal leech. Traffic Bulletin. 8(4), 64-69.

Wilkialis, J. 1975. Zoology Pollution. 25, 163-196.

Yang, T. 1996. Fauna sinica (*Annelida Hirudinea*) (pp. 117-129), Beijing: Science Press, China.

Zulhisyam A.K., A. Anwar Ismail and Ibrahim Che Omar (2011). Optimization of growth conditions of *Hirudinea* sp. Australian Journal of Basic and Applied Science. 5(3), 268-275.