Harnessing the Benefits of Breeding the Asian Medicinal Leech

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The Asian medicinal leech (*Hirudinaria manillensis* (Lesson, 1842)) can be found in freshwater environments including swamps and paddy fields. In Thailand, the Asian medicinal leech is most abundant in the Northeastern Provinces such as in Nakhon Phanom and Sakhon Nakhon. In Na Wa District, Nakhon Phanom Province for example, where the villagers have been capturing leeches in swamps during the rainy season (June-September) for more than 30 years as an alternative for rice farming. The people from Na Wa District also go to other provinces such as in Udon Thani, Nong Khai, and Khon Kaen to gather leeches. Wearing rubber boots, long pants, and long-sleeved shirts to protect themselves from leech attacks, the leech gatherers use scoop nets to capture the leeches attached in aquatic plants. Live leeches are sold to middlemen for THB 300-400/kg (400-500 leeches/kg) or about US$ 10-12/kg, while dried leeches are sold for THB 1,500-2,000/kg or about US$ 500-700/kg. For 30 years, Thailand exports dried *H. manillensis* to China, and in 2018 about 20 tons valued at THB 20 million or USD 590 thousand. The current price of powdered dried leech in the international market ranges between USD 10/kg and USD 200/kg. Currently, the only source of this medicinal leech in Thailand is from the wild and there are no leech farms to support the increasing demand. Consequently, *H. manillensis* population, like other populations of medicinal leeches, could be at risk of overexploitation and extinction. This study on the breeding and hatching of *H. manillensis* is therefore conducted with the aim of providing useful information on the possibility of establishing leech farms to increase the production and prevent the depletion of Asian medicinal leech population from the natural environment.

Many centuries ago, medicinal leeches have been used for phlebotomy (blood-letting) with records from ancient Egypt, Rome, and Greece. Leeching was one of several remedies used to restore the balance of the four humours (blood, phlegm, choler, melancholy). However in 1836, the renowned French physician, *Pierre Charles Alexandre Louis*, one of the earliest to assess statistically the value of various therapies, concluded that blood-letting was harmful rather than beneficial. Regardless of the negative effects of phlebotomy, medicinal leeches were continually used for other medical purposes such as counter-irritation, a treatment in which something was applied to irritate the skin or gut, and thereby counteract the effects of a disease. Also, these annelids are used to drain a hematoma (a collection of partially clotted blood) from a wound, the most obvious examples being a black eye, cauliflower ear, gum boils, and minor ulcers. Medicinal leeches are also used to remove post-operative occlusions to enhance the success of tissue transplants and the surgical joining of amputated appendages such as fingers and ears (Elliott & Kutschera, 2011). At present, many products are derived from leeches for pharmaceutical and medicinal purposes. For *H. medicinalis*, its saliva contains hirudin, the most powerful natural anti-coagulant, but the extraction of hirudin from whole *H. medicinalis* necessitates the destruction of large numbers of leeches and at least 12,000 kg of leeches are used for this purpose in Europe each year (Wells & Coombes, 1987).

Characteristics of *Hirudinaria manillensis*

Hirudinaria manillensis (Phylum: Annelida, Class: Hirudinea) is a tropical warm-water annelid which has been used in India and neighboring countries of Southeast Asia for medicinal purposes, thus it was named the "Asian medicinal leech" (Kutschera & Roth, 2006). This species was introduced from India to Europe and is now widely distributed in the Caribbean. These large, aggressive leeches arrived through ships that carried laborers from colonial India starting around 1845 and leeches were brought onboard for medicinal purposes (Sawyer *et al*., 1998).

Moreover, *H. manillensis* is proved to have close phylogenetic relationship with two of the most important European medicinal leeches *Hirudo medicinalis* and *Hirudo verbena* (Elliott & Kutschera, 2011). Adult specimens of *H. manillensis* can reach a body length of up to 18 cm (**Figure 1**). Some individuals of this species reach an enormous body length and therefore have been described as "buffalo leeches" (Kutschera & Roth, 2006). Leeches are hermaphrodites (**Figure 2**), *i.e.* they are bisexual with each mature individual producing both male and female gametes (Shain, 2009).

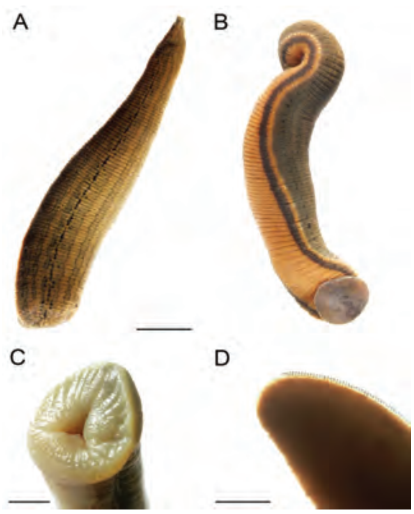

Figure 1. Live adult individual of *Hirudinaria manillensis*. Dorsal (A) and ventral (B) view; bar = 1 cm. The anterior sucker (mouth) of a preserved individual is characterized by a furrow in the upper lip (C); bar = 0.2 cm. Light micrograph of an isolated jaw, showing numerous monostichodont teeth (D); bar = 100 μm.

(Source: Kutschera & Roth, 2006)

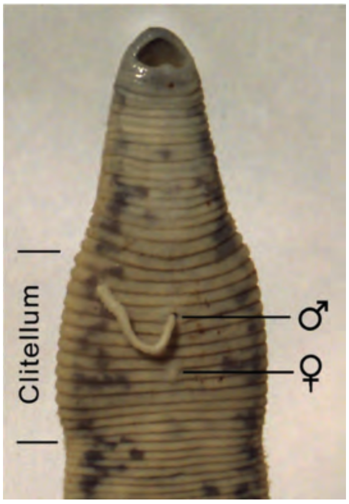

Figure 2. Ventral side of the head and clitellar region of an adult, alcohol-preserved *Hirudo verbana*. The male (♂) and female (♀) gonopores are visible, with the tube-like male copulatory organ outside of the body.

(Source: Elliott & Kutschera, 2011)

All leeches are predatory or parasitic carnivores, and their brain and sense organs combined with a flexible, muscular body enable them to actively pursue their prey, thus they have been described as "worms with character" (Kutschera & Elliott, 2010). In their natural habitat, these large blood-sucking leeches could be found attached to the belly and feet of cows, where they cause bleeding wounds and hence severely impairing the vitality of their hosts. Also, it was reported that humans are regularly attacked by *H. manillensis* (Elliott & Kutschera, 2011) and these leeches attach to and pierce the skin of humans in the laboratory (Kutschera & Roth, 2006).

Overexploitation and Reviving Leech Populations

During the first half of the 19th century, the trade in medicinal leeches (*H. medicinalis* and related species) became a major industry (Kutschera & Roth, 2006). European leech gatherers typically collected blood-sucking worms by wading in natural, shallow ponds and allowing the Hirudo-individuals to attach themselves to their legs. As many as 2,500 leeches per day could be harvested in this way so that the medicinal leech became almost extinct in Europe (Sawyer, 1986). As medicinal leeches became more difficult to find in Europe, the indigenous supply was supplemented by importations from abroad. Reduced populations *H. medicinalis* in Europe due to over-collection from the wild led to the need to import other species, especially the closely related *H. verbana* from Turkey and, more recently, the *H. manillensis*.

One way to combat the decline of the supply of wild leeches is the development of leech farming, particularly in France and Germany. In 1890, a leech farm in Germany was breeding 3-4 million leeches per year. Presently, culture and breeding of leeches in many countries are increasing in order to meet the demand for pharmaceutical and clinical use, Chinese traditional medicine, and for other scientific studies. Throughout Asia, many local leech farms (such as the Agro Medic Enterprise in Penang, Malaysia) are breeding and marketing large quantities of *H. manillensis* (Elliott & Kutschera, 2011). Thailand exports considerable quantities of dried *H. manillensis* (**Figure 3**) to China.

Figure 3. Dried *Hirudinaria manillensis* exported by Thailand to China (6 kg live leeches = 1 kg dried leeches)

Leech Culture in Thailand

Since leech farms are not yet established in Thailand, this study was conducted to gather information on the basic reproductive biology of *H. manillensis* at different broodstock densities. This study was conducted at the Nakhon Si Thammarat Inland Aquaculture Research and Development Center of the Department of Fisheries of Thailand from 1 September 2017 to 30 June 2018.

Leech Broodstock Management

Hirudinaria manillensis broodstock were gathered from the swamp in Nakhon Phanom Province in Northeastern Thailand, acclimatized in two-liter glass bottles with water (five leeches per bottle) on 1 September 2017, and kept in the laboratory at room temperature of 19-35 °C. The leeches were fed with animal blood (approximately five times the weight of leech) for four hours (9:00-13:00 h) every 15 days. The water in the bottle was changed after feeding the leeches.

On 1 October 2017, the broodstock leeches were selected randomly and transferred to plastic boxes (35.0 cm × 56.0 cm × 16.5 cm) for breeding. The broodstock densities (number of

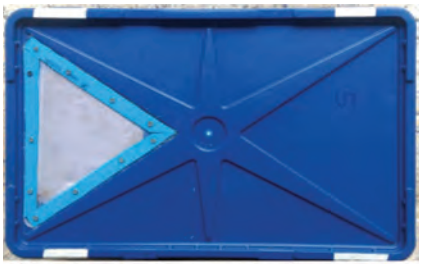

Figure 4. Breeding box for leech broodstock filled with clay loam soil and water (top) and lid (bottom)

leech per box) were two, three, and four leeches per box, and three replicates were set up for each density. Each breeding box (**Figure 4**) was filled with ten-centimeter thick clay loam soil sloping down to one side and five-centimeter deep water. The breeding box is covered with a lid that has a hole with screen for air ventilation. The body length and body weight of each leech were measured before putting them in the breeding box.

Leech Hatchery and Nursery

Cocoons (**Figure 5**) were deposited in the breeding boxes on 3 and 4 January 2018. The leech broodstock were kept in the breeding boxes until 30 June 2018 but no cocoons were deposited after January 2018. The cocoons were transferred to five-liter hatchery bottles with soil and water (one cocoon per bottle). The length, width, and wet weight of each cocoon were measured. The cocoons hatched on 18 and 19 January 2018 and the hatching rate was calculated using the formula below.

$$\text{Hatching rate} = \frac{(\text{no. of deposited cocoons} - \text{no. of dead cocoon}) \times 100}{\text{no. of deposited cocoons}}$$

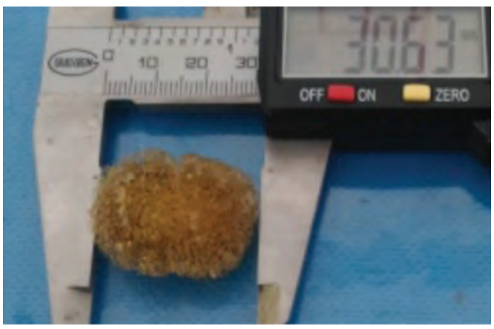

Figure 5. Spongy cocoon of *Hirudinaria manillensis*

The juvenile leeches were removed from hatchery bottles and cultured from 1 February to 2 May 2018 in nursery boxes similar to the breeding boxes described above. The densities (number of juvenile leech per box) were 10 and 20 juveniles per box. Animal blood (about three times of leech weight) was fed to juvenile leeches for four hours (9:00-13:00 h) every 15 days. The water in the nursery box was changed after feeding the leeches. The initial and final body length and body weight of each juvenile leech were measured. The specific growth rate and weight gain of each juvenile leech were calculated using the following formula.

$$\text{Specific growth rate} = \frac{(\ln \text{final weight} - \ln \text{initial weight}) \times 100}{\text{nursery period}}$$

$$\text{Weight gain} = \frac{(\text{final weight} - \text{initial weight}) \times 100}{\text{initial weight}}$$

Statistical Analysis

The Duncan New Multiple Range Test at 95 % confidence interval was used to analyze the cocoons (number, length, width, and wet weight) and leech offspring (number). Moreover, the T-test at 95 % confidence interval was used to analyze the offspring (body length, body weight, specific growth rate, and percentage weight gain).

Results and Discussion

For broodstock leeches (n = 27), the average body length was 6.46 ± 0.25 cm and the average body weight was 6.91 ± 0.10 g. The density of two leeches per box (n-6) had the highest average number of cocoons (1.33 ± 2.31). In terms of average length, width, and wet weight of cocoons, the results are almost similar among broodstock densities and there were no statistical differences (**Table 1**). The hatching rate was 100% for all densities of broodstock.

Zulhisyam *et al*. (2015) bred *H. manillensis* in different densities per tank (30 cm × 19 cm × 26 cm) and their results showed that the average number of cocoons developed in

Table 1. Cocoons and offspring produced by leech broodstock *Hirudinaria manillensis* after 94-95 days breeding

	Density (number of broodstock leech per box)		
	2	3	4
Number of cocoons per box	1.33 ± 2.31	0.67 ± 1.16	0.67 ± 1.16
Cocoon length (mm)	30.57 ± 0.07	30.43 ± 0.02	30.46 ± 0.13
Cocoon width (mm)	20.63 ± 0.19	20.54 ± 0.11	20.64 ± 0.21
Cocoon wet weight (g)	2.95 ± 0.06	2.96 ± 0.09	2.95 ± 0.05
Number of offspring per cocoon	13.50 ± 0.58	13.50 ± 0.71	13.00 ± 0.00

Note: Data in the table are mean and standard deviation (mean ± SD)

5, 10, 20 leeches per tank were 6.61 ± 1.00, 3.00 ± 1.00, and 1.33 ± 0.58, respectively. While in densities of 15, 25, and 30 leeches per tank, the average number of cocoons developed were 0.67 ± 1.16, 0.61 ± 1.26, and 0.67 ± 0.58, respectively; which are almost equal with the results of this study where the average number of cocoons developed in 2, 3, and 4 leeches per box (35.0 cm × 56.0 cm × 16.5 cm) were 1.33 ± 2.31, 0.67 ± 1.16, and 0.67 ± 1.16, respectively. Although the differences in leech densities were large between the two studies, the average number of cocoons that were developed was almost similar. Zhang *et al*. (2008) explained that the number of cocoons was influenced by leech density resulting to competition in food and space. The competition could stress the leeches and affect their reproductive behavior. Furthermore, feeding quantity and quality are other factors that could affect the number as well as length and weight of cocoons. The cocoon length and weight in low leech density were greater than in high leech density (Davies & McLoughlin, 1996; Elliott & Kutschera, 2011; Zulhisyam *et al*., 2015). For the number of leech offspring, the results of this study were the same with other studies using more than 10 offspring per cocoon (Zulhisyam *et al*., 2011; Ceylan *et al*., 2015; Davies & McLoughlin, 1996; Sawyer, 1986).

Table 2. Average initial and final body length and body weight, specific growth rate, and weight gain of juvenile leeches, *Hirudinaria manillensis* after 90 days of culture

	Density (number of juvenile leech per box)	
	10	20
Initial body length (cm)	1.12 ± 0.02	1.02 ± 0.00
Final body length (cm)	2.61 ± 0.04	2.59 ± 0.02
Initial body weight (g)	0.05 ± 0.00	0.05 ± 0.00
Final body weight (g)	0.59 ± 0.01	0.58 ± 0.00
Specific growth rate (%)	2.69 ± 0.04	2.68 ± 0.06
Weight gain (%)	1,024.98 ± 40.72	1,013.37 ± 62.10

Note: Data in the table are mean and standard deviation (mean ± SD)

The average initial and final body length and body weight of juvenile leeches are shown in **Table 2**. The values were comparable between densities of 10 and 20 juvenile leeches per box and did not show any statistical difference except in the initial average body length. The specific growth rate and weight gain of juvenile leeches were also similar between two densities. Within 90 days of culture, both densities of juvenile leeches had 100 % survival rate at the temperature of 24-27 °C, pH between 7.8-8.0, dissolved oxygen at 4.0-6.0 ppm, and total NH_3 at 0.0-0.5 ppm.

Different species of leeches have different body weights of juveniles. This study showed lower average body weight of *H. manillensis* juveniles than *H. medicinalis* juveniles but higher than *H. orientalis* and *H. verbena* juveniles (Ceylan *et al*., 2015; Petrauskienė, *et al*., 2011; Sawyer, 1986). The specific growth rates (2.69 ± 0.04 % and 2.68 ± 0.06 %) of *H. manillensis* in this study were lower than *Hirudinea* sp. (4.04 ± 0.03 %) (Zulhisyam *et al*., 2011). In this study, *H. manillensis* juvenile was fed with animal blood while in the study on *Hirudinea* sp., the juvenile was fed with blood of live eel (Zulhisyam *et al*., 2011). The differences in the specific growth rates could have been influenced by the nutritional values of the feed that affected the digestive system of the juvenile leeches.

Production Cost

Table 3 summarizes the production cost of breeding the Asian medicinal leech, *H. manillensis*. Among different densities, the breeding of two leeches per box (n = 6) which produced a total 54 offspring had the lowest production cost at THB 4.65 (USD 0.15) per leech offspring. This means that the lower the breeding density, the greater number of offspring is produced and the lower production cost is spent. Considering that labor

Table 3. Detailed production cost of breeding Asian medicinal leech, *Hirudinaria manillensis*

	Leech density (number of leech per box)		
	2	3	4
Total number of leech broodstock	6	9	12
Total number of leech offspring	54	27	26
Total number of box	3	3	3
Variable costs (THB)			
Feed[1]	11.2	16.8	22.4
Labor[2]	225	225	225
Opportunity cost[3]	2.02	2.09	2.16
Total variable cost	***244.22***	***252.89***	***261.56***
Fixed costs (THB)			
Opportunity cost[3]	0.06	0.06	0.06
Depreciation cost per box[4]	6.64	6.64	6.64
Total fixed cost	***6.70***	***6.70***	***6.70***
Total cost = total variable cost + total fixed cost (THB)	**250.92**	**259.59**	**268.26**
Production cost (total cost/leech offspring) (THB)	**4.65**	**9.61**	**10.32**
Production cost (excluding labor cost) (THB/leech)	**0.48**	**1.28**	**1.66**
Production cost (total cost/ leech offspring) (USD[5])	**0.15**	**0.31**	**0.33**
Production cost (excluding labor cost) (USD[5]/leech)	**0.02**	**0.04**	**0.05**

[1] THB 10 per 500 g of animal blood
[2] THB 300 per day or THB 37.50 per hour (minimum wage rate in Nakhon Si Thammarat for 8 hours per day), 1.5 h × THB 37.50 × 4 months = THB 225 for feeding and changing water every 15 days
[3] Interest rate of fixed deposit at the rate of 2.25 % of the Bank for Agriculture and Agricultural Cooperatives in 2017
[4] THB 200 per box, average lifespan of 10 years and used for four months; depreciation cost was calculated using straight-line method by setting the value to zero after the end of use
[5] USD 1.00 = THB 33.94, average exchange rate in 2017

cost (84-90 %) covers the highest percentage of the total cost, and if labor cost is excluded, the production cost would become much lower and ranges from THB 0.48 to THB 1.66 (USD 0.02-0.05) per leech offspring. Labor includes only the feeding and changing of water every 15 days, which can be easily done by the leech farmer and there is no need to hire a worker.

Conclusion and Way Forward

This study on the breeding, hatching, and culture of Asian medicinal leech, *H. manillensis* was conducted for the first time in Thailand. Because of its high hatching and survival rates, cheap production cost, high market value, and continuously increasing demand, medicinal leeches are excellent alternative for farmed aquatic animals. Through leech farming, the soaring market demand could be fulfilled without relying on leech stocks from the wild.

Therefore, the governments of the Southeast Asian countries, especially in the countries where *H. manillensis* and other species of medicinal leeches can be found, could utilize the results of this study to encourage and support stakeholders in establishing leech farms. Nonetheless, it is also recommended that further studies should be conducted, particularly on exploring other feed alternatives in order to enhance the growth rate of cultured juvenile leeches.

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