

## Basic Principles of Breeding of Medicinal Leech, *Hirudo verbana*

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Leeches are known as both therapeutic invertebrate live and parasites for people since ancient times. In this study, the basic principles of leech breeding were prepared by evaluating of the data obtained from studies carried out at Firat University, Faculty of Fisheries. Two soil pools that have total surface area 1500 m<sup>2</sup>, and five pieces 600 l recirculating tanks for the culture of leeches were used. The criteria were prepared to produce one million pieces of leeches with the knowledge accumulated on the breeding of medicinal leeches over many years. For the culture of *Hirudo verbana*, 6250 m<sup>2</sup> in the fully-controlled indoor system and 8500 m<sup>2</sup> in the semi-controlled outdoor system are required. It is sufficient 12-15 pieces of rootstock leeches per square meter. Water temperature, oxygen and pH in cultivation tanks should be set to 20-25°C, 3 mg/L and 7,6-8,5, respectively.

**Keywords:** Medicinal leech, *Hirudo verbana*, Breeding, Leech culture

### INTRODUCTION

Five million medicinal leeches were used by medical experts for treatment per year in Paris hospital of France in the 19th century. For a variety of reasons, this practice lost its popularity so that, in the 20th century, it became part of the folklore of traditional 'last century' medicine. However, because of their important salivary components, blood-sucking leeches such as *H. medicinalis* and other medicinal leech species have become of great interest for pharmaceutical companies seeking to expand their supply of various anticoagulants to prevent blood clotting in microsurgeries (Bednarek et al., 2010; Eldor et al., 1996; Electricwala et al., 1991; Godekmerdan et al., 2011; Kostromina et al., 2012; Kutschera and Wirtz, 2001; Lent, 1986; Markwardt, 1991; Markwardt, 1992; Munro et al., 1992; Weinfeld et al., 2000).

Aquaculture experiments were conducted on the Asian medical leech *Hirudinaria manillensis*. In these studies, the effects of bloodstocks density on cocoon productivity and reproduction biology of leech were investigated (Zhang et al., 2008; Zulhisyam et al., 2011). Some papers has been prepared on the reproduction and breeding, husbandry requirements, and feeding of medicinal leeches (Sağlam, 2000; Spencer and Jones, 2007).

The threat of extinction many populations of the medicinal leech (*Hirudo medicinalis* and *Hiruda verbana*) (IUCN, 1993), and because the extensive international trade in this species has been identified as a major threat to natural populations, they were added to Appendix II of the "Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)" in 1987. Molecular identification of *Hirudo* and

another leech species is important to reduce pressure of hunting and to trade within CITES rules. Therefore, the international CITES Secretariat wants to be constantly monitored of the populations status of medicinal leech species (CITES, 2006; Sağlam, 2011).

Turkey is a major source country for international trade of medicinal leeches and a very rich country in terms of leech diversity. But, recently the medicinal leeches have not been collected enough from nature due to the surface water pollution, extreme use of leeches, and the drying of wetlands. In addition, under the legislation of the FDA and the Turkish Ministry of Health, leeches should be taken from leech farms for use of human in the context of alternative and complementary medicine regulations. In this respect, medical leech breeding has gained great importance.

### MATERIALS AND METHODS

In this study, the basic criteria of medicinal leech breeding were determined according to data obtained from controlled indoor breeding system and semi-controlled outdoor system breeding systems in Firat University, Faculty of Fisheries for many years. These results were obtained by combining the results with incorporating of the results of the leech breeding attempts of various countries and own production unit. Broodstock medicinal leeches were provided collecting from natural area. Two soil pools that have total surface area 1500 m<sup>2</sup> in Fisheries Research Center, Faculty of Fisheries, Firat University, and five fiberglass recirculating tanks of size 80x75x90 cm in 540 L capacity in Aquaculture laboratory for the culture

of leeches were prepared. Each tank was filled with 250 L of dechlorinated freshwater until approximately half level and was placed special soft spawning soil for cocoon release of leeches. The water in the tank was aerated, and changed once every 2 days. In leech farming systems were performed water analysis as continuous, and checked hatching situation of leech every day and wrote records of results.

## RESULTS

### 1. Preparation of Leech Breeding Pools

The average 130 (between 120-160) leeches can be put into place of one square meters according to size. 180.000 leeches in 1000 m<sup>2</sup> place can be grown. The sufficient pond surface area is 6250-8500 m<sup>2</sup> for 1000 kg leech production. The size of the pools is determined by the terrain conditions. The floor of pools should be waterproof and should be put minimum 25 cm thickness special soil for spawning. The semi-controlled one farm for production of 1000000 leech should be totally 10000 square meters with pools (8500 m<sup>2</sup>), walking roads, parking and administrative building (200 m<sup>2</sup>) (Table 1).

### 2. Rootstock Needs of Medicinal Leech Farms

A medicinal leech can produce about 22 offspring leech per year. The number of offspring leech decreases to 12-14 in intensive stocking. For annual one-ton production were need to produce one million offspring leech. 1350000 offspring should produce because of 35% death rate of baby leeches. 1350000 pieces offspring leech can be obtained by 61364 rootstocks. 67500 rootstock leeches are needed to compensate the 10% death rate. The average weight of a rootstock medicinal leech is 2 g (1-3 g). Therefore, totally 135 kg of rootstock medicinal leech should be stocked.

### 3. Reproduction of Medicinal Leeches

Medicinal leeches lay their cocoons in average 9 (6-12) months. Baby leeches hatch in 30 days. There are approximately 6 months between the first and second spawning. One medicinal leech produces average 12 (1-41) cocoon during the life. One medicinal leech produces average 45 (13-97) baby leech during the life. One cocoon produces average 4 (0-14) baby leech. The lifetime of one leech is average 3 years (1.5-5 years) The death ratio baby leeches is 35% until the adult stage from egg.

### 4. Feeding of medicinal leeches

A leech is fed monthly with average 140 mg (between 70-190 mg) blood. One million leeches can consume monthly average 140 kg blood. One million leeches consume an annual average 1680 kg (840-2280 kg) blood. Annual average consumption

of blood in the medicinal leech farm is totally 1883 kg when rootstocks leech is added.

There are three methods for feeding of medicinal leeches. First method; feeding of leeches on the intestine with blood filled (feeding with blood sausage). Second methods; feeding of the leeches with placed sieve into a bow blood-filled. Third method; feeding of leech on alive frogs.

### 5. The water maintenance

The water that used in leech breeding should be clean spring water. City tap-water and treated bottled water is not suitable for breeding of leech. Disinfection of the spring water used by UV for the protection of leeches from diseases is important. The technical criteria of water used for the production of 1,000 kg medicinal leeches for controlled system (indoor system) and semi-controlled systems (outdoor system) are given in Table 1.

**Table1.** The technical criteria for breeding of 1 million leeches.

Criteria	Controlled Systems (indoor)	Semi-Controlled Systems (outdoor)
Breeding area	Pool/Tank	Earthen ponds
Total Pool / tank / ponds area	~6.250 m <sup>2</sup>	~8.500 m <sup>2</sup>
Water requirement	10 - 15 L/s	12-15 L/s
The water source	Natural water resources	Natural water resources
Water depth	30-50 cm	30-100 cm
Base soil depth	Minimum 25 cm special soil	minimum 25 cm special soft soil
Density of rootstock leeches	~14-16 pieces/m <sup>2</sup> /period	~12-14 pieces/m <sup>2</sup> /period
Rearing stock density	140-160 pieces/m <sup>2</sup> /period	120-140 pieces/m <sup>2</sup> /period
The number of annual breeding period	1-2 period	1-2 period
The change rate of pool water	Total water area / 24 hours	Total water area / 24 hours
Growth duration (month)	6-12 month	6-12 month
Vital water temperatures	Min: 1°C - Max: 35°C	Min: 1°C - Max: 35°C
Reproduction optimal water temperature	20-25°C	20-25°C
Growth water temperature	20-25°C	20-25°C
PH	7,6 – 8,5	7,6 – 8,5
Oxygen requirement	>3 mg/L	>3 mg/L
Ammonia	<0.005	<0.005
Nitrite	<0.1	<0.1
Nitrate	<0.1	<0.1
Chlorine	0	0

### 6. The soil conditions for medicinal leeches

Floor and side walls of the soil pool for leech culture should be covered with 25 cm of special soft soil to get a good cocoon. Also, aquatic plants should be planted in this pool to easy movement and preservation of leeches. The moss and peat

combination for leaving eggs and reproduction of Turkey medicinal leeches. The special soft soil provide protection to leeches in pool bottom with bury themselves whenever it gets hot or cold. The aquatic plants provide oxygen to the water in leech pools and protect the leeches by hiding among them.

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## **Tibb zəlisi *Hirudo Verbana*-nın Yetiştirilməsinin Əsas Prinsipləri**

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Qədim dövrlərdən zəlilər onurğazıslar və insanların parazitləri kimi tanınırlar. Təqdim olunan işdə zəlilərin seçilməsinin əsas prinsipləri Fırat Universitetinin Su Heyvanları Fakültəsində aparılmış tədqiqatlar nəticəsində əldə olunmuş nəticələrin qiymətləndirilməsi ilə hazırlanmışdır. Zəlilərin yetişdirilməsində ümumi sahəsi 1500 m<sup>2</sup> olan iki hovuz və hər birinin həcmi 600 litr olan beş rezervuardan istifadə edilmişdir. Tibb zəlilərin artırılması prinsipi - uzun illər ərzində yığılmış məlumatlara əsaslanaraq, bir milyon zəli istehsalına hazırlıq idi. *Hirudo verbana*-nın becərilməsi məqsədilə idarə olunan daxili sistemdə 6250 m<sup>2</sup> və yarım-idarə olunan açıq sistemdə 8500 m<sup>2</sup> sahə tələb olunur. Hər kvadrat metrə 12-15 zəli kifayətdir. Kultivasiya rezervuarlarında suyun temperaturu, oksigeni və pH müvafiq olaraq 20-25 °C, 3 mg / l və 7,6 - 8,5 səviyyəsində olmalıdır.

*Açar sözlər: tibb zəlisi, yetişdirmə, zəlilərin kulturaları*

## **Основные Принципы Разведения Медицинской Пиявки, *Hirudo verbana***

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С древних времен пиявки известны как живые терапевтические беспозвоночные и паразиты людей. В этом исследовании основные принципы селекции пиявок были подготовлены путем оценки данных, полученных в исследованиях, проведенных в Фыратском университете, на факультете Рыболовства. Использовались два почвенных бассейна общей площадью 1500 м<sup>2</sup> и пять рециркуляционных резервуаров для культивирования пиявок объемом 600 л. Принципом было готовка к производству одного миллиона пиявок, опираясь на накопленные на протяжении многих лет знания о разведении медицинских пиявок. Для культивирования *Hirudo verbana* требуется площадь 6250 м<sup>2</sup> в полностью контролируемой внутренней системе и 8500 м<sup>2</sup> - в полуконтролируемой наружной системе. Достаточно 12-15 пиявок на квадратный метр. Температура воды, кислорода и pH в культивационных резервуарах должны быть установлена на 20-25 °C, 3 мг / л и 7,6 - 8,5 соответственно.

*Ключевые слова: медицинская пиявка, разведение, культура пиявок*