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REPRODUCTION OF *Hirudo orientalis* IN LABORATORY AND NATURAL CONDITIONS

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РАЗМНОЖЕНИЕ *Hirudo orientalis* В ЛАБОРАТОРНЫХ И ЕСТЕСТВЕННЫХ УСЛОВИЯХ

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Abstract. The medicinal leech is included in Azerbaijan's "Red Book". For this reason, research has been conducted to increase leeches to protect the hirudofauna of our country. Three methods were used to breed it in the laboratory. The laboratory research was conducted at the Institute of Zoology. Studies have been conducted in natural conditions At the Mingachevir Scientific-Experimental Laboratory. In laboratory conditions, leeches were fed once a month, while those selected for reproduction were fed twice a month with the blood, spleen, and liver of large livestock. Based on our research, we can conclude that the optimal temperature range for the survival of *Hirudo orientalis* in laboratory conditions is 20–25°C.

Аннотация. Медицинская пиявка занесена в «Красную книгу» Азербайджана. По этой причине были проведены исследования по увеличению численности пиявок для защиты гирудофауны Азербайджана. Для ее разведения в лабораторных условиях использовались три метода. Лабораторные исследования проводились в Институте зоологии и в Мингячевирской научно-экспериментальной лаборатории. В лабораторных условиях пиявок кормили один раз в месяц, а отобранных для размножения — два раза в месяц кровью, селезенкой и печенью крупного рогатого скота. На основании исследований можно сделать вывод, что оптимальный температурный диапазон для выживания *Hirudo orientalis* в лабораторных условиях составляет 20–25°C.

Keywords: Hirudo orientalis, laboratory condition, breeding, hermaphroditic, heparin, hirudofauna.

Ключевые слова: *Hirudo orientalis*, лабораторные условия, разведение, гермафродит, гепарин, гирудофауна.

The medicinal leech is an ectoparasitic invertebrate representative of the aquatic ecosystem, feeding by sucking blood. The most widespread species of the class *Hirudinea* include *Hirudo orientalis*, *H. verbana*, *H. medicinalis*, and *H. sulukii*. The species characteristic of Azerbaijan is *H. orientalis*, which differs from other species in terms of its morphological and biological features. As a representative of annelid worms, the medicinal leech has an approximate length of 12 cm, possesses three jaws surrounded by 270 teeth, and has five pairs of eyes. Despite feeding on the blood of animals and humans, it can survive in a food-deprived environment for up to six months and is capable of consuming an amount of blood seven times its body weight. In general, literature sources indicate that leeches can endure starvation for up to two years [1].

Due to the uncontrolled commercial use of this species over many years, its population has declined sharply, leading to its inclusion in Azerbaijan's "Red Book" in 1984 and 2023 [2].

Currently, *H. orientalis* can be found for sale in Baku and various cities and districts of the country, with the main collection sites being local water bodies. As a result, a significant decrease in the population of medicinal leeches in these aquatic ecosystems has been observed. Additionally, factors such as the drainage of lakes where leeches are found and changes in their usage purposes have contributed to this decline. The harvesting and utilization of medicinal leeches in Azerbaijan began in the 1870s. According to Q. K. Qafarov (1979), approximately 1.55 million medicinal leeches were harvested and exported to various republics of the Soviet Union. In the 1970s, six artificial lakes were constructed over a 15-hectare area near Yanardag in the Absheron region for leech cultivation.

The medicinal leech is a hermaphroditic species that, while reaching sexual maturity in its third year under natural conditions, is capable of reproducing during different seasons under laboratory conditions. Although its reproductive system contains both male and female gonopores, fertilization occurs through cross-fertilization, meaning that both male and female individuals must be present in the same environment for reproduction to occur. The species has a weak regeneration capacity. The cocoon is formed by secretions from the skin glands located in the 9th-11th segments of the body [1-4]. These segments correspond to the clitellum of the leech. Leeches deposit their cocoons at the bottom of water bodies, on algae, or on moist soil along the shoreline. *H. orientalis* produces 1-8 cocoons per reproductive cycle, with each cocoon containing up to 33 embryos that develop into mature individuals without undergoing metamorphosis [3].

Material and Methodology

The reproductive characteristics of *Hirudo orientalis* were first studied by Petrauskienė et al. (2011) [4]. In many countries, medicinal leeches are bred exclusively in biofactories. For reproduction, leeches are initially fed and grown, after which 8–10 individuals are placed in 3-liter jars and kept undisturbed for two months. If cocoon-laying behavior is observed, such individuals are transferred to an environment suitable for cocoon deposition. A single leech can lay between 2 and 10 cocoons. The next generation develops inside the cocoon and, after a certain period, breaks through its wall and enters the water.

According to some experiments, newly hatched leeches are fed with calf blood heated up to 36°C. Special membranes resembling human skin are used for feeding. The leeches cut through the membrane with their jaws and feed slowly. Over time, they undergo significant growth. After feeding, they are washed with clean water. Before being sent to pharmacies, the leeches are starved for 3–4 months and then prepared for sale [5].

From 2017 to 2020, research was conducted under laboratory conditions to reproduce medicinal leeches. Medicinal leeches were collected from natural water bodies in the Masalli region using hydrobiological methods and then brought to the laboratory for classification. During laboratory experiments, various leech breeding techniques developed by experts were considered. While grouping leeches in jars, particular attention was given to weight (2–5 g, 5–10 g, >10 g). Aquariums designed for different leech groups were equipped with river stones, aquatic plants, a temperature regulator, and an oxygen supply system. Each aquarium contained 8–10 individuals.

In laboratory conditions, leeches were fed once a month, while those selected for reproduction were fed twice a month with the blood, spleen, and liver of large livestock. During blood feeding, heparin was used to prevent coagulation. Specifically, 0.833 ml of heparin was added to 1 liter of blood, and each leech was fed for 2–3 minutes.

Our research revealed that the heparin solution often resulted in leech mortality or rupture of the lacunar system. For this reason, liver and blood clots were used as an alternative food source. Leeches that exhibited signs of disease were treated with an Amoxicillin-based antibiotic preparation. One tablet was dissolved in 1 liter of water, and the affected leeches were kept in this solution for 1 hour before being transferred to separate containers. Deceased individuals were preserved in 95% alcohol in specialized laboratory containers. Some of the dead leeches were examined under a microscope to determine the cause of death, with most cases linked to ruptures in the lacunar system.

For leech reproduction under laboratory conditions, the following ways were employed:

First way: Leeches were placed in containers with peat and moss (5–6 individuals per container) and kept in a dark environment for an extended period.

Second way: Leeches were placed in an aquarium equipped with various river stones. On top of these stones, special plastic containers filled with moss and soil were placed to facilitate cocoon deposition. To prevent the moss from drying out, it was sprayed with water daily. The presence of cocoons was regularly checked, and the aquarium water was periodically changed.

Third way: The entire aquarium containing leeches was placed inside a thermostat with a stable temperature regime.

Among these methods, the second method proved to be the most efficient, as it resulted in a significantly higher reproduction rate while minimizing mortality and disease cases among leeches.

The identified cocoons were transferred to separate containers, where special conditions were created to ensure the safe and healthy emergence of juvenile leeches. In some cases, small openings were made in the cocoons to facilitate the release of the juveniles. Upon hatching, the weight and number of leeches were recorded, and they were placed in small-sized jars.

For their first feeding, the juveniles were provided with frog blood. Subsequently, they were fed with spleen, liver, and blood, similar to adult individuals. The further development and survival of the parent leeches in cocoon-designated aquariums were also monitored. During the winter months, various heating devices were used to maintain a stable room temperature.

Juvenile leeches that had emerged from cocoons were transferred to artificially created ponds in natural environments, where their growth characteristics were observed. At the Mingachevir Scientific-Experimental Laboratory, special earthen ponds were constructed to facilitate leech development. Water supply to these ponds was ensured through the Mingachevir Reservoir. During periods of high water availability, the water depth in the ponds ranged between 1.2 to 1.5 meters.

For research purposes, approximately 800 leeches were released into the earthen ponds at different times. To simplify cocoon collection in these environments, floating platforms (measuring 48m² and 78m²) were constructed (Figure 1).

For the construction of the floating platforms, foam plastic (penoplast) and clay were used, and the interior was filled with peat (3 cm thick), wheat sprouts, and moss. The water and air temperature were measured twice a day (morning and evening).

Leeches were regularly fed, primarily with blood, fresh liver, and spleen. To positively influence reproduction, frogs, turtles, and water snakes were also introduced into the ponds [6].

Conclusion

Based on our research, we can conclude that the optimal temperature range for the survival of *Hirudo orientalis* in laboratory conditions is 20–25°C. Deviations from this range significantly reduce leech activity, and temperatures below 15°C or above 32°C lead to high mortality rates.

In laboratory conditions, leeches kept at 18–22°C in winter and 24–27°C in summer were able to produce cocoons every 6–8 months. Each cocoon typically contained 15–20 embryos (most commonly 8–15). The cocoons had an average length of 20 mm, a width of 16 mm**,** and were spherical gray in color.

Figure 1. Artificial "Floating Platforms" Created for Cocoon Deposition

At the Mingachevir Scientific-Experimental Laboratory, the highest leech activity was observed at 20°C. In the artificially created ponds, cocoons were regularly collected, with each cocoon producing 6–12 juveniles. The June–September period provided the most favorable conditions, with air temperatures recorded at 23–25°C in the morning (9:00) and 22–26°C in the evening (18:00), while water temperature ranged between 24–26°C.

Leeches primarily deposited their cocoons in the artificially created floating platforms, especially among the moss layers. However, in platforms containing wheat sprouts, almost no cocoon deposition was observed. The cocoons in these environments had an average length of 1.5 cm and a width of 0.6 cm. In some years, cocoon deposition was occasionally observed even in October.

Figure 2. Cocoons of *Hirudo orientalis*

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To provide initial feeding for the juvenile leeches emerging from the cocoons, frogs were placed in the same environment to facilitate their nourishment.

Although the research was conducted throughout the year, no results were obtained during the months of November to April. To protect the leeches from cold temperatures during this period, special coverings were used, which resulted in a lower mortality rate during the winter months (15%). In other months, cocoon production was successfully achieved.

The number, length, and weight of each leech emerging from the cocoon were measured. The monthly distribution of cocoons laid over a four-year period is presented in the following diagram (Figure 3).

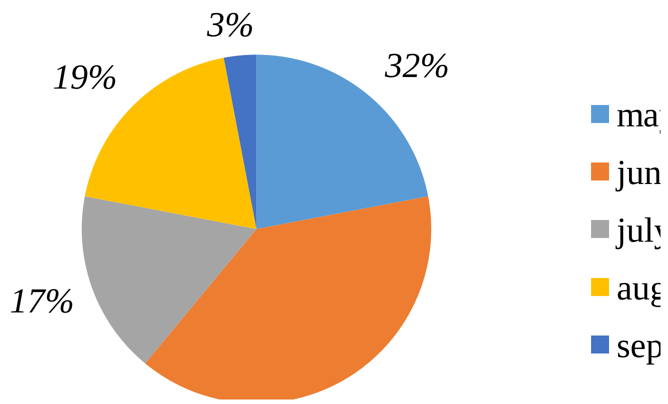

Figure 3. Percentage of Medicinal Leech Cocoons Laid by Month (2017-2020)

According to the diagram, the highest percentage of cocoons laid over the four-year period was recorded in June (39%).

The results obtained during the research can be utilized in medicine, traditional healing practices, and cosmetology for the production of sterile medicinal leeches. Additionally, these findings can be applied in conservation efforts aimed at preventing the extinction of medicinal leech populations.

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