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OLIGOCHAETES (OLIGOCHAETA): DERO FURCATA, SLUDGE WORM, ENCHYTRAEUS ALBIDUS AND GRINDAL WORMS AS VALUABLE FOOD OBJECTS IN FISH FARMING (REVIEW)

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Purpose. To analyze literature sources and summarize the obtained information on the vermiculture of such species of the subclass of Oligochaeta worms as: aulophorus (Dero furcata), sludge worm (Tubifex tubifex), potworm (Enchytraeus albidus), grindal worm (Enchytraeus buchhoizi). To present the achievements in modern biotechnology of these species. To examine the features of their cultivation using various methods, as well as the possibilities and prospects of their use as feed objects in aquaculture.

Findings. This work contains brief information on the biology of four most common in Europe species of Oligachaetes subclass and their rational use in fish farming.

The article provides recommendations on the specifics of purchasing and collecting the stock cultures from natural conditions, the best methods of their further cultivation, depending on the needs of farms, the basic requirements for the substrate and feeds. The data on the effective feeding of fish with these worms, as well as the technology of storage of the obtained biomass are presented.

A review of scientific papers showed that the above-mentioned species can be used as high-protein additives to the main feed and during the pre-spawning period, given their high caloric content. In fact, the latter indicator and poor mineral composition makes it impossible to feed fish with them on a regular basis.

Practical significance. An array of generalized information will be useful for scientists who are exploring the ways to optimize fish feeding. The presented technology of cultivation of Dero furcata, sludge worm, potworm and Grindal worms can be implemented in fish farms of various industrial capacities as well as in aquarium conditions.

Key words: Oligochaetes (Oligochaeta), aulophorus (Dero furcata), sludge worm (Tubifex tubifex), potworm (Enchytraeus albidus), Grindal worms (Enchytraeus buchhoizi), aquaculture, food organisms, fish feeding.

МАЛОЩЕТИНКОВІ ЧЕРВИ (*OLIGOCHAETA*): АУЛОФЛОРУС, ТРУБОЧНИК ЗВИЧАЙНИЙ, БІЛИЙ ЕНХІТРЕЙ ТА ГРИНДАЛЬ — ЦІННІ КОРМОВІ ОБ'ЄКТИ У РИБНИЦТВІ (ОГЛЯД)

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Мета. Проаналізувати масив спеціальної літератури та узагальнити отриману інформацію щодо вермикультури таких представників підкласу малощетинкових червів (Oligochaeta) як: аулофорус (Dero furcata), трубочник звичайний (Tubifex tubifex), білий енхітрей (Enchytraeus albidus), гриндаль (Enchytraeus buchhoizi). Представити досягнення сучасної біотехніки цих видів. Розглянути особливості їх культивування різними методами, а також можливості та перспективи використання в якості кормових об'єктів в аквакультурі.

Результати. Дана робота містить коротку інформацію з біології та раціонального застосування в рибництві чотирьох найпоширеніших в Європі представників підкласу малощетинкових червів.

У статті викладені рекомендації щодо специфіки закупівлі та заготівлі з природних умов маточної культури, оптимальні методи її подальшого культивування в залежності від потреб господарств, основні вимоги до субстрату й корму. Наведено дані стосовно ефективного згодовування рибам цих червів, а також технологію зберігання отриманої біомаси.

Огляд наукових праць виявив, що вищезазначені види доцільно використовувати в якості високобілкових добавок до основного корму та у переднерестовий період через їх високу калорійність. Втім, останній показник та бідний мінеральний склад унеможливлює годівлю ними риб на постійній основі.

Практична значимість. Масив узагальненої інформації буде корисним для науковців, які досліджують шляхи оптимізації годівлі риб. Представлена технологія культивування аулофоруса, трубочника звичайного, білого енхітрея й гриндаля можлива для впровадження як у рибницьких господарствах різної промислової потужності, так і акваріумістами.

Ключові слова: малощетинкові черви (Oligochaeta), аулофорус (Dero furcata), трубочник звичайний (Tubifex tubifex), білий енхітрей (Enchytraeus albidus), гриндаль (Enchytraeus buchhoizi), аквакультура, кормові організми, годівля риб.

МАЛОЩЕТИНКОВЫЕ ЧЕРВИ (*OLIGOCHAETA*): АУЛОФЛОРУС, ТРУБОЧНИК ОБЫКНОВЕННЫЙ, БЕЛЫЙ ЭНХИТРЕЙ И ГРИНДАЛЬ — ЦЕННЫЕ КОРМОВЫЕ ОБЪЕКТЫ В РЫБОВОДСТВЕ (ОБЗОР)

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Цель. Проанализировать массив специальной литературы и обобщить полученную информацию о вермикультуре таких представителей подкласса малощетинковых червей



(Oligochaeta) как: аулофорус (Dero furcata), трубочник обыкновенный (Tubifex tubifex), белый энхитрей (Enchytraeus albidus) и гриндаль (Enchytraeus buchhoizi). Представить достижения современной биотехнологии этих видов. Рассмотреть особенности их культивирования различными методами, а также возможности и перспективы использования в качестве кормовых объектов в аквакультуре.

Результаты. Данная работа содержит краткую информацию по биологии и рациональному применению в рыбоводстве четырех наиболее распространенных в Европе представителей подкласса малощетинковых червей.

В статье изложены рекомендации по специфике закупки и заготовке из естественных условий маточной культуры, оптимальные методы ее дальнейшего культивирования в зависимости от потребностей хозяйств, основные требования к субстрату и корму. Поданы данные касательно эффективного скармливания рыбам этих червей, а также технология хранения полученной биомассы.

Обзор научных работ обнаружил, что вышеупомянутые виды целесообразно использовать в качестве высокобелковых добавок к основному корму и в преднерестовый период, что обусловлено их высокой калорийностью. В то же время, последний показатель и бедный минеральный состав делает невозможным кормление ими рыб на постоянной основе.

Практическая значимость. Массив обобщенной информации будет полезным для ученых, исследующих пути оптимизации кормления рыб. Представленная технология культивирования аулофоруса, трубочника обыкновенного, белого энхитрея и гриндаля возможна к применению как в рыбоводных хозяйствах различной промышленной мощности, так и аквариумистами.

Ключевые слова: малощетинковые черви (Oligochaeta), аулофлорус (Dero furcata), трубочник обыкновенный (Tubifex tubifex), белый энхитрей (Enchytraeus albidus), гриндаль (Enchytraeus buchhoizi), аквакультура, кормовые организмы, кормление рыб.

PROBLEM STATEMENT AND ANALYSIS OF LAST ACHIEVEMENTS AND PUBLICATIONS

Live food for fish is not only a high-protein component of their diet, but also a necessary component of the formation of natural instincts under aquacultural conditions [1]. Thus, the motor activity of fish increases if they are fed live food, larvae begin the consumption of dry feed easier, fish fry develop search reflexes and increases appetite [2]. In addition, live food for aquarium fish of predatory species is mandatory, since in its absence they have disorders of the reproductive system [3]. In general, the rational use of living forage organisms helps to avoid contamination of water by uneaten feed residues when growing fish [4, 5].

Among living forage organisms that are appropriate to use in fish farming representatives of the subclass of Oligochaeta worms (*Oligochaeta*) are distinguished by their low maintenance under the conditions of cultivation and the ability to long-term survival in water [5, 6]. Following four species are widespread in Europe: aulophorus (*Dero furcata*), sludge worm (*Tubifex tubifex*), potworm (*Enchytraeus albidus*), Grindal worm (*Enchytraeus buchhoizi*): the biotechnology of their cultivating with the purpose of further feeding the fish is well-organized [1, 7].

Taxonomy of abobe mentioned species is following: the domain of the Eukaryotes (*Eukaryota*), the Kingdom of Animals (*Animalia*), a group of Bilaterians (*Bilateria*), the taxon of Protostome (*Protostomia*), the Superphylum of Lophotrochozoa (*Lophotrochozoa*), a type of Annelid worms (*Annelida*), the class of Clitellates (*Clitellata*) [8, 9].



Biologically, the representatives of this subclass of polychaetes (*Polychaeta*) are characterized in that the segments of their bodies never have paired outgrowtha, called parapodia. The chaeta of these worms are located directly on the surface of the segments, on the sides, two bundles on each side[10]. Along with the external, they also have internal segmentation. This results in the existence of special partitions between the segments, and even in segmentation of internal organs; there is the nerve center in each segment, the lateral protrusion of the intestines, the annular blood vessels, metanephridium and sex glands. The coelom is filled with a liquid where the phagocytic cells are suspended [11, 12].

All oligichaete worms have the ability to move by contracting and stretching the body. Under the epithelium they have a muscular system consisting of layers of circular and longitudinal muscles. This muscular structure allows them not only to bend from side to side, but also to significantly change the length of the body [8, 13, 14].

At the front end of the body they have a mouth opening, ending with a pharynx. The latter turns into a narrower gullet. It, in turn, expands and forms a goiter; after it there are one or more muscular stomachs. In the stomachs of oligochaetes food is only crushed, and it is digested in the intestine. Non-digestible residues are excreted into the external environment through the anus [13, 15].

The excretory system of these worms is represented by methanephridia of the coelomoduct type. They begin with funnel in coelom, and open outside in the next segment with the pores, and in the metanephridium tubules, the concentration of the metabolic products is taking place, and the fluid is thrown out again in the coelom (this is adaptation to life in the soil) [9, 11, 14, 16].

The nervous system is of the orthogon type (stem, that is, some nerve cells are collected in the nerve trunks, along with which the diffuse subcutaneous plexus is preserved). It is represented by the parapharyngeal nerve ring and the ventral nerve chain, which in each segment is slightly thickened, resulting in the formation of the ganglia of the corresponding segment [11, 14]. Numerous nerves come from segmental ganglia, some of which end in the skin sensitive receptors that respond to touch, light, temperature, chemical stimuli, etc. Paired supraesophageal ganglia, functioning as a brain are associated with the ventral nerve chain and is divided into three parts: protocerebrum, mesocerebral, deutocerebrum [15, 17].

The circulatory system is closed, there is no real heart, and the movement of blood through the vessels is carried out due to the pulsation of their walls. There are dorsal and ventral vessels connected by circular ridges (circular vessels) [8, 13]. Side vessels come from the main vessels, and they are strongly branched and eventually form a dense network of capillaries. High level of hemoglobin in the blood gives the sludge worm and aulophorus red body color. That is why when buying a stock culture of the sludge worm or aulophorus, it is necessary to take into account that healthy worms are elastic, red, and they are trying to gather in a dense lump. Not quality worms are dark, and there are light streaks of dead animals in the lump [10, 11, 17, 18].

All the oligochaete worms are hermaphrodites. Large mature individuals can have a thickening, a clitelium. There are concentrated special cells that secrete mucus, which, later, will be used for placing egg laying. The mucous cocoon protects eggs from drying out and is food for larvae at the very beginning of their development [14, 16, 17].

Below we will consider in more detail the methods of cultivation, methods of

feeding the fish and storage of biomass of aulophorus, sludge worm, potworm and Grindal worms.

STUDY RESULTS AND THEIR DISCUSSION

Aulophorus (*Dero furcata* or synonymous names — *Nais furcatus*, *Aulophorus furcatus*) is a freshwater representative of the family of saltwater worms *Naididae* [1, 10]. They have characteristic way of movement, they are writhing like snakes. Aulophorus is common in climatic zones from temperate to tropical; it prefers fresh water, rich in organic matter. It feeds on detritus as silt, bacteria, plant residues [19]. Its body is pink, up to 20 mm long and about 0.2 mm thick, divided into separate rings or segments (Fig. 1) [12, 20].

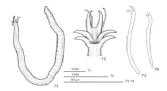


Fig. 1. Aulophorus (Aulophorus furcatus)

Sexual reproduction is not common for this worm, most often its reproduction is vegetative, when on the body of an adult (which has reached a length of 1-2 cm) individuals additional mouth openings are being formed through which new individuals are separated. Thus, most often the population of the aulophorus (especially in cultivation) is a mass of clones of a single ancestor. Its special feature is the presence of gills, which are located on the extension around the anus at the end of the body [14, 20]. Aulophorus is sensitive to lack of oxygen in the water; with its low content their colony in the form of "the ball" breaks down into individuals. The latter, float to the surface, where the oxygen content is higher and form a new colony. Usually, with a sufficient oxygen content in the water, they will form large colonies at the bottom, and with a decrease in this indicator they form smaller ones and in the higher layers of water [1, 21].

<u>Cultivation of the aulophorus</u> is based on its ability to form colonies on a nutrient substrate. The front part of the body it is immersed in the substrate, and the back one, with gills, is in the water. In the water tank worm is dispersed over the entire area, further concentrating in small colonies at the water surface, along the walls or at the bottom of the tank [1, 3, 20]. Thus, it is cultivated in wide low containers made of inert materials such as glass, plastic, clay. This species is not demanding of the water quality, so it is possible to use a settled or boiled water. General requirements for all methods of cultivation are:

- the presence of a cover with holes for air exchange;
- > shaded or dark place;
- > full water exchange once a day;
- ➤ water temperature 22-28°C (permissible t range from +15 to +32°C) [1].

The most common methods of cultivation of aulophorus are the following:

1) in the cuvettes: the best choice is the cuvettes with low sides, as the required water level is about 1.0-1.5 cm. The dimensions of the cuvette depend on necessary amound of the aulophorus culture. In any case, it is necessary to divide the colony into several cuvettes, then the whole culture will not suffer from negligence. Feed mixture is applied

to a flat piece of foam plastic, where worms are gathered. Instead of foam plastic, any sponge can be used (furniture, car, for washing dishes or for aquarium filters), but it needs to be prepared so that it sinks and does not retain air (for this it is boiled for a long time). The water in the cuvette should be poured so that the foam or sponge with food were partially immersed and the upper part was at its level. The amount of feed put into the cuvette is approximately equal to the biomass of worms, otherwise the water quickly deteriorates and the colony dies. Shortly before full eating of a forage it is necessary to bring the following portion. Water is replaced by opening the cuvette and gently pressing the sponge or foam a couple of times. This allows washing out dirt and waste products of aulophorus from its pores. After that, the foam or sponge is removed from the cuvette and all the water is poured out of it into a separate container for further decanting through a fine sieve, which will separate the worms;

- 2) in containers: the water level and dimensions of the containers are the same as in the case of cuvettes. The difference is that the feed is set on a nylon mesh No 64, stretched on a frame of expanded foam, which should float on the water surface in the container. The amount of feed should be no more than the mass of worms in the culture. Feed is given once every 2-3 days, as far as it is eaten;
- 3) in glass jars with a volume of 3l: 4/5 of the volume of water is replaced daily with fresh. Since these worms are very sensitive to lack of oxygen, stopping the aeration (closing a jar lid with no holes) cause them to rise to the surface of the water, where they are easily captured. This operation is carried out daily, selecting about 1/5 of the total weight of the colony, thereby stimulating their reproduction. Since the total mass of worms doubles in a few days, you can first settle several individuals into a jar. However, it is necessary to ensure that the total weight of the aulophorus does not exceed 100 g per jar;
- 4) in running water: requires a special system that provides a weak current of water from the aquarium with young fish into a container with aulophorus and back. In the presence of such a system, it is advisable to grow *Daphnia* simultaneously with the aulophorus to ensure full feeding of young fish. It is important to install a filter from a dense nylon sieve on the outlet hose to ensure the penetration of only young *Daphnia* [1, 3, 20, 22].

Feeding aulophorus is simple and cost effective; they are fed with vegetables (zucchini, carrots, cabbage, pumpkin) and fruits, herbs (nettle, clover, dandelion, oat flakes, oatmeal). However, it is necessary to avoid products that can cause clogging of the substrate; these are potatoes, tomatoes, apples, citrus and all kinds of berries. The addition of animal protein in the diet of aulophorus significantly accelerates the rate of their reproduction [22, 23].

As carrot is the most cheap and affordable vegetable, let us consider feeding it to aulophorus in more details. First, it must be peeled, cut into slices and frozen in the freezer. Before putting a vegetable into a container with a culture of aulophorus it is necessary to pour boiling water and withstand the day in aquarium water [3, 20]. The freezing and quick warm-up carrots with boiling water is required for the destruction of cells and softening the tissues, and the conditioning of the pieces in the aquarium water avoids bacterial outbreaks when feeding aulophorus. In common, the optimal feed mixture for feeding the aulophorus is:

- grass flour 500 cm³;
- giant clover -300 cm^3 ;



- carrots -100 cm³;
- brewers yeast -100 cm³;
- $-Daphnia 5 \text{ cm}^3$;
- mineral fertilizers -2 g;
- glucose (C₆H₁₂O₆) 5 pills [1].

All ingredients are dried, ground and mixed with a boiling water to a very thick dough consistency. The resulting feed mixture is dried or stored in a refrigerator [23].

Feeding the fish with aulophorus starts with capturing the worms from the bottom of the container or cuvette, or the water line in glass jars, using a net with a small mesh. Before feeding, thorough washing is not necessary. Larvae of fish that have just began the mixed feeding, need the individuals cut with a razor; thus feed particles similar in size to the *Artemia* nauplium are receive [1, 24]. It is important to remember that the aulophorus, after cutting into several parts, does not decompose, but regenerates, that is, each part eventually turns into a new individual [23].

The chemical composition of aulophorus in % of dry weight is: proteins 60%, fats 11%, carbohydrates 7.2%. It is used as a high-protein feed for larvae, fry and small fish [21].

As it is known, the effectiveness of any fodder culture is characterized not only by its density, but also the rate of reproduction. In this sense, aulophorus is one of the champions: every five days the number of worms doubles. Thus, in the presence of a colony of biomass of 100-200 g, without any damage, it is possible to withdraw 20-40 g of individuals for feeding fish daily [1, 16].

Sludge worm (*Tubifex tubifex*) was described by O. F. Mueller back in 1774. This is pinkish-red thread-like worm, up to 8 cm long and 0.6-0.7 mm thick. Each segment of the body has 4 chaeta [25, 26]. They are absent only on the two front segments in front of the mouth and on the mouth segment (Fig. 2).



Fig. 2. Sludge worm (Tubifex tubifex)

In the front third of the sludge worm's body, there is a noticeable thickening called clitelium, which contains many mucous glands. Each individual has both female and male genitals located in segments 10 and 11. However, sexual reproduction prevails; in autumn the female lays several eggs in cocoons, after hatching the youth winters in the silt until spring. The number of gonads, their location relative to each other and their shape are the special feature of species belonging to the genus *Tubifex* [9, 10, 12].

Sludge worm forms large clusters (up to several thousand individuals per 1 m² of bottom) in silt of rich in organic reservoirs, but also occurs on sandy and rocky soils, however, in small quantities. It lives in silt and slime tubular minks, that gave it its name. It puts over the surface of the ground back end of the body (with gills) from these tubes, which making oscillating movements provides breathing [27, 28]. In the event of the slightest danger, this part of the body is instantly hidden in the burrow. The front end of the body is constantly in the soil, at a depth of 5–10 cm. Using the front part of the body, sludge worm constantly swallows the sludge in amount, which far exceeds the weight of the body (Fig. 3).

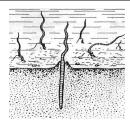


Fig. 3. Sludge worm in soil

Thus, these worms consume organic matter from silt, and excrement contain simple minerals, that is, contribute to the mineralization of the soil. The less organic matter is contained in the sludge, the more it has to be passed through the intestines [1, 15, 29, 30].

<u>Cultivation of the sludge worm</u> begins with the purchase of stock culture in a special store, which is desirable, or harvesting in natural conditions, which is quite risky, because it prefers contaminated waters. However, since this worm is quite common in Europe, we will consider in more detail the features of its harvesting from natural reservoirs. Thus, it is better to select a sludge worm at a distance of 3–4 m from the shore, preferably from sandy soil impregnated with organic residues. Moreover, if touching the ground, you feel a little depreciation (as from a piece of foam plastic or rubber), it is a sign of the accumulation of worms, and on solid soils the colony of worms are almost not found [17, 28–31].

The main methods of harvesting sludge worm from natural reservoirs:

- 1) using a shovel or scoop the top of the soil with worms is cut and put into a wash basin or bucket. Next, the silt can be washed under running water or, more simply, gauze is put on top, and the bottom of the basin or bucket is heated on low heat or poured with hot water. Worms, escaping from the heat will climb through the gauze up, where they are easy to collect;
- 2) using a sieve with a stainless steel mesh or fishing line; the diameter of the sieve should be approximately 20-25 cm, the height of its side 1.5 cm, and the mesh size $1\times1 1,5\times1,5$ mm. For convenience, it is necessary to attach a long handle to scoop silt from the bottom of reservoirs (Fig. 4). In addition, sludge worms are primary washed from silt in it. Large debris, such as leaves and branches are removed manually. A ball of worms that remains, is put into a clean container with a thin layer of water [1, 32].

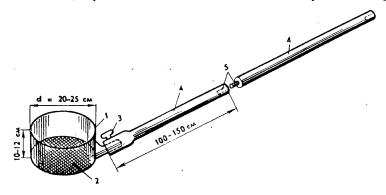


Fig. 4. Sieve with handle for selection of sludge worm stock culture from natural reservoirs



Transportation of the sludge worm is convenient to carry out without water in a specially made suitcase with built-in retractable wooden or plastic frames, covered with a grid. Frame made of wooden slats, should be treated with hot linseed oil. After the selection and washing of the stock culture from a natural reservoir, it is distributed over the frame in a uniform layer, 1 cm thick. With this method of transportation, the worms remain alive and active for several hours [1, 32].

Feed for the sludge worm is organic matter. Based on this, when it is cultivated, bread, dry fish food, manure or humus can be used. Fodder is given once per 3-4 weeks [17, 33].

The main methods of cultivating the sludge worms are:

- 1) in containers with a substrate from a mixture of 2.55 cm³ of garden soil or soil mixture for indoor flowers and powdered dry moss, which is watered with a decoction of wheat and rice. For the latter, it is needed 1/2 teaspoon of wheat and as much rice and it is boiled for 20 minutes in 0.5 liters of milk. The finished substrate is put for two days in a warm place where bacteria develop, which feeds the sludge worm. After two days of settling, 15–20 cm³ of soil and worm culture are added to the substrate. It is necessary to maintain a temperature of about 20°C and moderate lighting, full water exchange is carried out once a week;
- 2) in wooden boxes $50\times10\times12$ cm in size, in soil mixture of black earth and peat, with humidity not more than 40%. Using this method, up to 40 g of worms is collected from 1 m² of soil layer 10 cm deep. Feed is added in two or three grooves, sprinkled with soil on top;
- 3) in running water with use of rectangular container with a smooth bottom. A small slope of the bottom is required to allow water to flow downwards by gravity. Its level should be low, no more than 10 cm. The volume of containers is selected based on the needed amount of this forage organism. The presence of flow and sufficient oxygen content in the water are indispensable conditions for the life of worms during cultivation in running water. Therefore, it is necessary to use a compressor and a small pump. Moreover, the temperature should be not higher than 30°C (preferably normal room temperature) and weak or moderate lighting;
- 4) using a cascade of several plastic containers filled with water and connected by tubes that mimic an artificial stream. To embed 2 kg of worms by this method only 10 containers, with size of $100\times30\times20$ cm are necessary. On their bottom small (about 2 cm) a layer of expanded clay is put, and disinfected (boiling or UV radiation) sand in a layer of 4–5 cm is put above it. A layer of vegetable peelings is also put on it, which is covered with a layer of silt 4–5 cm high. The water level should not exceed 20 cm [1, 26, 28, 32, 34, 35].

<u>Feeding the fish with a sludge worm</u> requires mandatory aging, washing and cleaning of its culture. This is due to the fact, that when feeding fish with sludge worm from natural reservoirs, there is a risk of invasion with precursors such of diseases as parasitic invasions (the etiologic agent is the cestode *Caryophyllaeus laticeps*), ichthyophthiriasis (the etiologic agent is *Ichthyophthirius multifiliis*), chilodotiasis (the etiologic agent is the ciliate *Chilodonella cyprinid*), costiasis (the etiologic agent is *Costia necatrix*) and poisoning with accumulated polutants [36, 37].

The culture is settled and washed in two ways:



- 1) from 3 to 7 days: in a container with low sides and a layer of water at a height of 3-5 mm, at a temperature of 5-10°C. During this time, the culture is washed daily and separated from the dead individuals by a sieve with small mesh;
- 2) within 2–3 days: in the solution of acriflavine or its analogues (acriflavin, hydrochloric acid-3,6-diamino-10-methyl-acridine chloride) at the rate of 100 mg of preparation per 10 liters of water at a temperature of 10–12°C [1, 28, 32].

The main methods of cleaning the culture of the sludge worm are:

- 1) thick starch is poured into a bucket with worms, then a dense layer of worms at the top is formed, under it there is a layer of starch, and at dirt is on the bottom;
- 2) worms are tightly wrapped in gauze or nylon mesh bending the ends of the fabric and placing thus formed package in a container with a small layer of water, pouring hot water. Escaping from it, worms actively move through the cells of tissue and clean the intestines;
- 3) a jar with worms is tied with gauze and put it in hot water. Due to the extremely uncomfortable temperature, worms actively move through the tissue cells to the surface and clean the intestine (Fig. 5).



Fig. 5. Method of cleaning of sludge worm culture using water

After the above three operations, it is possible to feed worms to fish or keep it alive or frozen. Thus, it does not lose its useful properties for several months. Frozen sludge worm is easy to use, but the ice crystals significantly destroy its tissue, so it is advisable to freeze only large worms [1, 38].

To separate large individuals from small ones its inherent behavior in the water column is used; large worms sink to the bottom faster than small ones. Thus, worms are put at the bottom of the tank and filled with water (under slight pressure). First, the water is muddy, so after all worms are settled, it is carefully poured away. This procedure is repeated until the water becomes clear. Then the same procedure repeats, but allowing large individuals to reach the bottom, the top layer with small worms floating in it is removed into an empty container. Then, after worms sink to the bottom, the water will be also removed from this container. These operations repeats until the largest specimens will remain in the one container, and smaller ones, suitable for feeding young fish, in the other container [1, 17, 28, 32].

Sludge worm contains about 5% proteins and 1% lipids. It has a high level of amino acids, and many essential, but the profile of fatty acids does not meet the needs of fish, they are almost not absorbed by their body. Another disadvantage of live food like sludge worm is a small content of vitamins and a large amount of fat. Therefore, at a constant feeding fish with sludge worms it is observed obesity and infertility. After

that it is necessary to alternate with other types of food [1, 39, 40]. To improve the nutritional value of the sludge worm it is recommended to add to the culture complex vitamins in powder at the rate of 0.25 grams per 100 grams of worms biomass every week. It is also recommended to soak it in vitamin solutions (A, B1, E) at the rate of 250 mg of vitamins per 100 g of biomass [1, 3, 41].

Small fish are fed with a sludge worm using floating feeders with a mesh bottom, and for the young fish it is necessary to chop it with a razor. It is necessary to strictly monitor the survival of this forage organism, because not eaten worm immerses into the soil and begins active reproduction [7, 16, 39].

Potworm or *Enchytraeus albidus* has such name because it likes to live in flower pots. In natural conditions it lives in the soil and in fresh and salty waters, where it is found in detritus among the stones. The length of the body of potworm is 2 mm – 4 cm, and a thickness is up to 1 mm [1, 15]. The covers of the body are light and translucent, the filling of the digestive organs is visible through them (Fig.6).



Fig. 6. Potworm (Enchytraeus albidus)

The potworm is usually cultivated in the soil, although there are methods of breeding in other substrates: white brick, slag and gravel, between sheets of filter paper or layers of fabric as well as on the agar [1, 42].

The most favorable conditions for the cultivation of potworm are the following:

- ➤ temperature is 17–18°C (with possible fluctuations of 10–22°C);
- \geq 23–25 % of humidity;
- reutral or slightly acidic medium (alkaline reaction is contraindicated) [43].

The main methods of cultivation of potworm:

- 1) in Petri dishes on agar containing soil extract (22%) the surface of agar is covered with a small layer of soil where worms live, feeding on microflora developed on agar. To maintain normal humidity conditions, chambers with high humidity or special devices, regularly moistening the substrate are used;
- 2) in the soil with soft texture, high porosity and water-holding capacity. This soil can be taken from greenhouses, gardens, orchards, arable lands. The prepared soil is sifted through a grid with a mesh size of 3-4 mm, cleaned of impurities and moistened. Then it is poured on a layer of peat, in boxes with such characteristics: height of 10–15 cm and an area of 0.2–0.3 m². Worms culture is added to the soil to a depth of 3–4 cm, at the rate of 200–250 g/m². After that, 2–3 ditches with depth of 5 cm are made in the soil, where portions of food are put and thoroughly covered with soil. The surface of the soil is leveled and the box is closed with a glass or wooden lid. In a well-developing culture, worms are concentrated in the soil thickness near the forage clots. The large amount of the potworms on the surface of the soil or its accumulation near the cracks of the boxes indicates adverse conditions of cultivation [44–46].

Care for the culture of worms is reduced to systematic monitoring the soil,



population density and the presence of pests. Given that methods of dealing with pests (mites, fly larvae, collembolans and others) is not sufficiently developed, the culture must be guarded against their occurrence. That is why the surface of the boxes should be covered with clean covers, the food covered with soil, sour and mold particles of feed should be removed immediately. When flies appear, the outer surface of the lids, the doors and windows of the room are sprinkled with a solution of hexachloranum [45, 47].

With the growth of the biomass of the culture it is systematically singled out by removing some amount of worms (together with the ground or without it). To maximize the production of potworm distribution of culture is advantageously carried out when its biomass in a box with an area of 0.2 m² does not exceed 200–300 g. Use of culture of worms starts in the period of the maximum increase of their biomass, that is in 40–50 days from the moment of the cultivation beginning. Potworms are removed from the soil daily in amount of 35–420 g/m² (70 to 80 g from the box with an area of 0.2 m²). For this, in warm season sunlight is used and in a cool season – electric lamps set over cuvettes. Sometimes special electric heaters are used, which create 28–30°C temperature in the upper layers of the soil. Worms, avoiding light and heat, concentrate in deeper layers of the soil. Then the soil is removed layer by layer, getting to the bottom, where there is a accumulation of culture [1, 3, 44–46].

For the cultivation of potworms in significant amount these boxes are mounted on shelves in 8–10 levels. The passes between the shelves shall be 1–1.5 m. After about 6 months, the substrate should be changed and the culture should be transplanted in order to update [1, 48].

Products that are not conducive to the massive development of other soil inhabitants (springtails, mites, fly larvae, etc.) are used for feeding potworm. Basically these are different cereals, flour and bran, vegetables and roots, green herbaceous plants, berries, fruits, yeast. Before application to the soil, the feed is brewed and ground. When brewing separate products such proportions are followed: 100 g of bran -0.4 liters of water; 100 g of flour -0.5 liters of water; 0.7 liters of water is added to 100 g of potatoes for preparing mashed potatoes; 100 g of fodder yeast – 6.3 liters of water. Alternation of structural and non-structural feed is useful to maintain the structure of the soil. Feed is added once a week in an amount that is calculated for the increase in biomass of worms, taking into account the feed ratio. To obtain 1 g of biomass growth of oligochaetes it is needed 6 g of potatoes or 1 g of yeast. Maximum portions of feed, which are added to a box of 0.2 m², for one week are: flour or cereals - 180 g (dry weight), root crops, vegetables - 600 g (raw weight), herbaceous plants -750 g (raw weight), hydrolysed yeast – 50–60 g (dry weight). For the enrichment of worms biomass with vitamins liquid vitamin D, or such vitamin preparations as "Trivita" or "Tetravit" is added to the feed [1, 43–45, 49].

<u>Fish feeding with potworm</u> meets the need of the fish in vitamins, because it is rich in vitamins A (0,196 mg), B2 (0,134 mg) and E (0,058 mg). In addition, it contains 9.7% of dry matter, 70% of protein, 14% of fat and 10% of carbohydrates [48, 50].

Before feeding the fish, potworm should be cleaned from soil. This is achieved in several ways:

1) using a tray with a perforated fence, which is made in the form of a belt conveyor, with a light source;

2) using water; the soil with worms is placed in a container with water and, slightly crushing and leveling the surface, it is half lowered into a bucket with hot (50–60°C) water. Worms, creeping to the surface, forming dense tangles, which are transferred to cold water for final cleaning out the soil [1, 42, 43, 45].

The fish fry are effectively fed by small potworms, obtained 24–48 hours after giving the last portion of food. It is separated from larger individuals, placing in a glass of water. As water is carefully mixed, large worms sink to the bottom, and small ones remain in the water column. Washed worms are fed to fish using feeders or tweezers. When feeding fish with potworms, it is not necessary to worry about their sinking to the bottom, because they do not bury in the ground, and can live long in the water and be consumed by fish after a certain time, but for the day all not eaten residues should be carefully cleaned away with a siphon [23, 46, 50].

Grindal worm (*Enchytraeus buchhoizi*) is a small (0.5–12.0 mm) worm named after Mrs. Morton Grindal from Sweden, who was the first to cultivate it as a forage culture. It lives in moist soil areas rich in organic substances. The color of its body is white or yellowish, opaque (Fig. 7) [1, 15, 18].

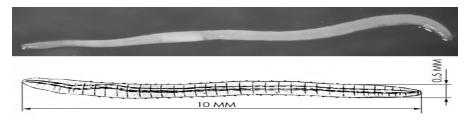


Fig. 7. Grindal worm (Enchytraeus buchhoizi)

It has a great reproductive ability; under optimal conditions it doubles its biomass in three days. It feeds on decomposing organic matter, bacteria and fungal hyphae. With mass reproduction, in the absence of competition and predators (for example, soil mites), grindal worm is able to damage the root system of plants. The advantages of grindal worm as an object of vermiculture include the ability to live and reproduce at room temperature (22–28°C), which is too high for other species of oligochaetes. The optimum temperature for the cultivation of grindal worm ranges is from 18 to 24°C. At a temperature of 14°C, it stops reproduction, but the temperature rise to 24–26°C increases the rate of reproduction. However, in this case reproduction of mites enhances, which often get into the culture. In this case, it is possible to separate the worms by putting them into the water; they will sink to the bottom, and the mites will be on the surface. After that, the container must be sterilized in boiling water, and the substrate destroyed [1, 15, 16, 51, 52].

Cultivation of Grindal worm is usually carried out in two main ways:

1) in a glass, plastic or wooden containers with a height of 8–10 cm, with a tight top lid to avoid passing insects. Between the lid and the substrate should be a space of about 1–2 cm. The best substrate are wet plates of fine foam plastic, as well as well-boiled or loose pasteurized peat, placed on the bottom of cubes, or a mixture of humus and peat, or treated with boiling water sphagnum moss. In the holes of wet peat or in the intervals between the plates, a nutrient substrate is placed: yeast mixed with ground oat flakes. Feed should be finely ground, it is good to add vitamin preparations. It is

necessary to carefully monitor that it does not sour, because it leads to the death of culture. The capture of worms for feeding fish is carried out by placing peat in a net of nylon sieve; when it is getting wet worms pass it, get into the water, washing the feed from the substrate and then they are collected with tweezers;

2) using synthetic batting as a substrate. The latter is folded into several layers, placed in a container and moistened with boiled water or water from the aquarium, 8–10 layers of network kapron cloth is put on a batting. The container is installed in a dark plastic bag. Forage is poured on a batting, under the bottom layer of a kapron cloth. A few days after the kapron canvas will gather a sufficient amount of worms, the top 5–6 layers of fabric can be removed and gently rinsed directly in the aquarium (with this method of cultivation, it is not necessary to wash the worms). For the cultivation of grindal worm it is more convenient to use large capacities, because in small ones even with a slight increase, feed begins to rot, which can lead to the death of the culture. The entire area of the cuvette is better to divide into 3–4 sectors and cover each with fabric, then feeding the fish, the fabric can be removed only from one sector. Grindal worms are fed not less than 1 time every 2 days; a new portion of food can not be given until the last was eaten [1, 53–57].

<u>Fish feeding with Grindal worms</u> is possible for all age groups. Its nutritional value depends on the composition of the worms's feed. The simplest way to enrich the biomass of worms with vitamins is to include vegetables in their diet. Long-term feeding fish with grindal worm is not advisable, because, despite the high protein content, it has little mineral salts and vitamins, which can lead to obesity, sterility, reduced immunity and other nutritional diseases. At the same time, the use of grindal worm in the diet of fancy fish species during preparation for spawning has a good effect. Given the above, it is recommended to feed fish with such high-calorie food as grindal worm no more than every other day, alternating it with other food. Living grindal biomass can be stored, almost without care, for three months in the fridge, at a temperature of $+ 4^{\circ}$ C [1, 52, 53, 58–60].

CONCLUSION AND PERSPECTIVES OF FURTHER DEVELOPMENT

The most common in Europe representatives of the subclass oligochaetes (Oligochaeta) worms such as aulophorus (Dero furcata), sludge worm (Tubifex tubifex), potworm (Enchytraeus albidus) and grindal worm (Enchytraeus buchhoizi) are appropriate to use in fish feeding, especially those that have just began exogenous nutrition under the conditions of fish farms and different age groups of the aquarium species.

As forage organisms, the above mentioned worms are characterized by high protein content and energy value, but they have a poor vitamin and mineral composition. However, their nutritional value can be increased by adding vitamin preparations to their feed. In any case, these species should be used as an additional high-protein feed, in certain periods of ontogenesis of fish (e.g., larval, or prespawning).

Biotechnics of cultivation of these species is economically advantageous and convenient to implement, and can be used in fish farms and by aquariumists. It involves the use of small-sized tanks and substrate, and is based on the ability of aulophorus, sludge worm, potworm and grindal worm to rapid increase in the biomass of a stock culture. The latter can be purchased in specialized companies, and captured from the wild.

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