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THE CULTIVATION OF THE NEMATODES *PANAGRELLUS, TURBATRIX* (*ANGUILLULA*) AND *RHABDITIS* FOR USING IN FISH FEEDING

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Purpose. *To analyze professional literature and summarize the information on the cultivation of the representatives of three nematode (Nematoda) genera. To highlight the optimal biotechnology of the cultivation of banana nematode (Panagrellus redivivus), vinegar eelworm (Turbatrix aceti) and rhabditis (Rhabditis). To review the biological characteristics of these species along with the possibilities for their use as food objects in aquaculture.*

Results. *This work contains brief information on the biology and cultivation features of the above-mentioned worms. It presents the most cost-effective and simple mass production biotechnology of the cultivation of banana and vinegar nematodes and rhabditis. It can be used under different conditions due to its simplicity and cost-effectiveness.*

The data regarding the use of nematodes in feeding fish juveniles in general and ornamental fish culture in particular are presented. The chemical composition and nutritional value of these worms, as well as their behavior in aquariums are described.

Practical significance. *The summarized information will be useful for researchers who are exploring the ways to optimize fish feeding as well as for aquarists. The presented technology of cultivation of banana and vinegar nematodes and rhabditis can be used by a broad range of stakeholders.*

Key words: *live food cultivation, fish fry, nematodes (Nematoda), banana nematode (Panagrellus redivivus), vinegar eelworm (Turbatrix aceti), rhabditis (Rhabditis), aquarium husbandry.*

КУЛЬТИВУВАННЯ НЕМАТОД З РОДІВ *PANAGRELLUS, TURBATRIX* (*ANGUILLULA*) І *RHABDITIS* ДЛЯ ВИКОРИСТАННЯ В ГОДІВЛІ РИБ

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Мета. *Проаналізувати масив фахових літературних джерел та узагальнити отриману інформацію з культивування представників трьох родів круглих хробаків нематод (Nematoda). Викласти оптимальну біотехнологію культивування бананової нематоди (Panagrellus redivivus), оцтової нематоди (Turbatrix aceti) та рабдитисів (Rhabditis). Розглянути біологічні особливості цих видів, а також можливості й перспективи використання в якості кормових об'єктів у аквакультурі.*

Результати. *Дана робота містить коротку інформацію з біології та специфіки культивування вищезазначених хробаків. Представлені найбільш економічно вигідні та в доступні у впровадженні до виробництва біотехнології культивування бананової й оцтової нематоди, а також рабдитисів. Вони можливі для застосування в різних умовах за рахунок своєї простоти та невибагливості.*

Наведені дані стосовно специфіки використання нематод в годівлі молоді риб в загалі, й в акваріумістиці зокрема. Описано хімічний склад і поживну цінність цих хробаків, а також їх поведінку в умовах акваріумів.

Практична значимість. *Масив узагальненої інформації буде корисним для науковців, які досліджують шляхи оптимізації годівлі риб, а також акваріумістів. Представлена технологія культивування бананової і оцтової нематоди, а також рабдитисів можлива для використання широким колом осіб.*

Ключові слова: *культивування живих кормів, молодь риб, нематоди (Nematoda), бананова нематода (Panagrellus redivivus), оцтова нематода (Turbatrix aceti), рабдитиси (Rhabditis), акваріумістика.*

КУЛЬТИВИРОВАНИЕ НЕМАТОД ИЗ РОДОВ *PANAGRELLUS*, *TURBATRIX (ANGUILLULA)* И *RHABDITIS* ДЛЯ ИСПОЛЬЗОВАНИЯ В КОРМЛЕНИИ РЫБ

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Цель. *Проанализировать массив специальной литературы и обобщить полученную информацию по культивированию представителей трех родов круглых червей нематод (Nematoda). Изложить оптимальную биотехнологию культивирования банановой нематоды (Panagrellus redivivus), уксусной нематоды (Turbatrix aceti) и рабдитисов (Rhabditis). Рассмотреть биологические особенности этих видов, а также возможности и перспективы использования в качестве кормовых объектов в аквакультуре.*

Результаты. *Данная работа содержит краткую информацию по биологии и специфике культивирования вышеупомянутых червей. Представлены наиболее экономически выгодные и в легкие во внедрении в производство биотехнологии культивирования банановой и уксусной нематоды, а также рабдитисов. Они возможны для применения в различных условиях за счет своей простоты и непритязательности.*

Приведенные данные применительно к специфике использования нематод в кормлении молоди рыб в целом и аквариумистике в частности. Описаны химический состав и питательную ценность этих червей, а также их поведение в условиях аквариума.

Практическая значимость. *Массив обобщенной информации будет полезным для ученых, исследующих пути оптимизации кормления рыб, а также аквариумистов. Представленная технология культивирования банановой и уксусной нематоды, а также* *рабдитисов возможна для использования широким кругом лиц.*

Ключевые слова: *культивирование живых кормов, молодь рыб, нематоды (Nematoda), банановая нематода (Panagrellus redivivus), уксусная нематода (Turbatrix aceti), рабдитисы (Rhabditis), аквариумистика.*

PROBLEM STATEMENT AND ANALYSIS OF LAST ACHIEVEMENTS AND PUBLICATIONS

High reproductive capacity, short life cycle and tolerance to changes in environmental conditions cause a constant demand for brood cultures of free-living nematodes among aquarists [1, 2]. The most popular of them are representatives of the *Panagrellus, Turbatrix (Anguillula)* and *Rhabditis* genera, which are known among aquarists as "Micra" [3, 4].

These nematodes are valuable forage organisms for juvenile fish, because they contain about 76% of water, 24% of dry matter consisting of 40% of protein and 19.5% of fat [5, 6]. Compared to other invertebrates, the fat content in the body of nematodes is very high, thus, they should be alternated with other feeds [7, 8]. However, they can withstand salinity up to 40‰ for 8 hours, which is important for marine aquariums [9, 10]. It should be noted that the nematodes could live in aquarium water for several days [11, 12].

Nematodes have not yet been widely used in production, but with further improvement of the cultivation technology, they can be widely used as starting live feeds for fish larvae [13-15]. For this reason, we review in detail the main links of the cultivation biotechnology for the most common representatives of the above genera for the needs of fish farming.

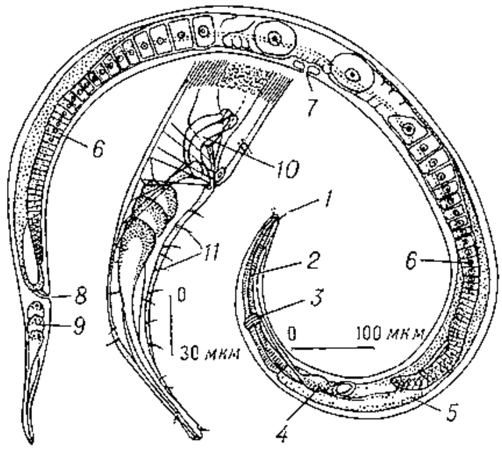
STUDY RESULTS AND THEIR DISCUSSION

Taxonomically, banana *(Panagrellus redivivus)* and acetic nematodes *(Turbatrix aceti)*, and rhabditis *(Rhabditis)*, according to the classification of 2011 are related to the domain: eukaryote (*Eukaryota*), Kingdom: animals (*Animalia*), group: Bilaterians (*Bilateria*), Taxon: Protostomes (*Protostomia*), superphylum: (*Lophotrochozoa*), phylum: nematodes (*Nematoda* Rudolphi, 1808), class: *Chromadorea* (Inglis, 1932), infraclass: *Plectia* (Hodda, 2007) [5, 16, 17].

Nematodes appeared in the Upper Carbonic, the last period of the Paleozoic era, which lasted about 360 – 300 million years ago [18, 19]. Today these worms exist in all of the known habitats [5, 20, 21]. First of all, they are often found in bottom deposits of reservoirs of all types of salinity, from the North to the South poles of the planet [17, 22, 23]. However, in addition to free-living nematodes, this class includes widespread parasites of plants, fish, birds, mammals and humans [16, 24, 25].

The total number of nematode species has not yet been determined by science. Thus, currently, more than 24 thousand species of parasitic and free-living nematodes have been described, but estimates of real diversity, based on the rate of description of new species (especially specialized insect parasites), give reason to believe that the total number of species is near one million [5, 18, 26, 27].

The body of nematodes is elongated, typical for worms, filamentous or elongated and fusoid, round on the cross section [28, 29]. It is covered with a strong and elastic cuticle [5, 17]. Under it there is the muscular system with only by longitudinal fibers that do not form a full composite [21, 24]. They form four strands (lateral, dorsal and abdominal), divided into epithelial rolls. In the middle of the side rolls there are organs [16, 23]. Nematodes do not have circular muscular fibres, due to the appearance of a primary body cavity in these animals, which acts as a hydrostatic skeleton[10, 27].



A B

Fig. 1. **Structure of free-living nematodes: A — General view of the female; B — the tail end of the male body; 1 — head bristles; 2 — esophagus; 3 — nerve ring; 4 — cervical gland; 5 —intestine; 6 —sex glands; 7 — female genital opening; 8 —anus; 9 —tail glands; 10 — spicules; 11 — tail bristles.**

A special feature of nematodes is that their muscle cells are elongated and oriented in one direction, and also form filamentous processes [16]. They approach the nerve cords and thus synchronize the processes of contraction and relaxation, allowing more complex movements [18]. Directly under the muscular system there is the archicoel, filled with liquid, without its own tissue lining (in contrast to the coelom) [23, 24]. All internal organs and their systems are in it [28].

The main part of nervous system of nematodes is nerve cords; abdominal cords are more developed and spinal ones are less developed; they are connected by semicircular webs or commissures [24]. In front of the body of these worms, at the head end, there is a circumpharyngeal nerve ring, which covers the front of the digestive tube (Fig. 4) [23, 28].

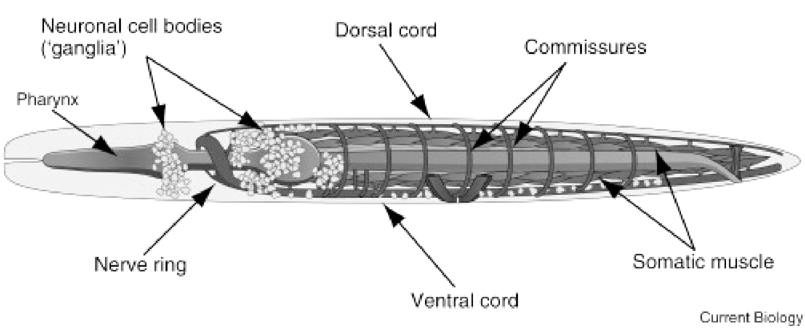


Fig. 4. **Nervous system of free-living nematodes**

Sense organs of nematodes are poorly developed [5, 29]. First of all, they include tactile tubercles or papillomas, which are located around the oral and anal openings in males, and help them navigating during mating [26]. In addition, at the head end of the body of nematodes organs of chemical senses or amphids, and primitive eyes in the form of pigment spots are concentrated [18, 29].

The digestive system of nematodes is a relatively complex structure. Thus, in their intestines there are three parts: anterior, middle and posterior. Anterior and posterior parts have ectodermal origin, middle one is endodermal [5, 16]. The anterior part or pharynx includes the oral cavity and esophagus [24, 30]. The mouth opening is surrounded by three lips and is located on the front end of the body. The cells of the esophagus secrete enzymes, which help in digesting food. The middle part of the intestine corresponds to the middle intestine, and the posterior part corresponds to the posterior intestine, which opens with the anus [23, 27].

The circulatory and respiratory systems are absent. The absorption of oxygen is carried out by the entire surface of the body. The epithelial cells contain hemoglobin [28, 30].

The excretory system is represented by lateral channels or rennet glands located in the epidermal side rolls. These channels merge in the front part of the body into one excretory duct opening behind the head end on the abdominal side [10, 24]. In addition, there are epidermal glands associated with the lateral excretory channels, first of all – cervical, and phagocytic cells or pseudocoelomocytes, which contact chamber fluid. In addition, ammonia can be released from the nematode body by diffusion through the body wall [17, 27].

The reproductive system of nematodes has a number of features. Thus, they are dioecious, and some species have sexual dimorphism [3, 9]. Among free-living nematodes there are both oviparous and viviparous species. The genitals of nematodes are paired tubes, while in females the bigeminy is preserved, and in males one of the tubes is reduced [23, 30]. Thus, the male reproductive system in the nematode is represented by a single seminal gland. The spermaducts are connected to the seminal bladder, which passes into the spermatic duct, opening into the posterior intestine. It contains a copulative bag with two bristles [24]. During mating, the bristles push the vaginal lumen apart, which facilitates the fertilization process. The female reproductive system is represented by two ovaries, merging into the oviducts, which, expanding, form two tubes of the uterus. They group together, resulting in a vagina that opens with an unpaired sexual opening on the abdominal side (Fig. 5) [2, 9, 23, 30].

Free-living nematodes retain mobility at temperatures from 10 degrees below zero to 30 degrees Celsius, but the optimal temperatures for the growth of their population are +22 – 23 °C [31, 32]. They prefer acidic reactions (pH 3.4 to 4.2) of the medium. They prefer the high level of humidity, but are able to withstand prolonged droughts, restoring their livelihoods after moistening. [33-35].

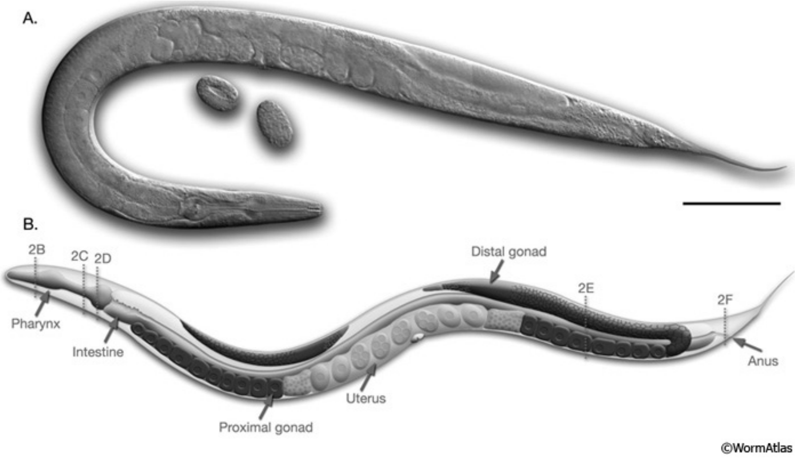


Fig. 5. **Reproductive system of free-living nematodes, A – General view (next to the eggs) under a microscope; B – view in longitudinal section.**

Banana nematode (*Panagrellus redivivus* or *Panagrellus nepenthicola*) is a small, 1.5 − 2.5 mm long, whitish, very motile nematode (Fig. 6) [5, 36].

Fig. 6. **Banana nematode (*Panagrellus redivivus*)**

It is characterized by sexual dimorphism; males are smaller than females, have a coiled tail [1, 3, 37]. It inhabits areas with excessive humidity: landfills, among decaying vegetation, in substrates with fermentation and even in the culture of other worms, such as pot worms (*Enchytraeidae*) [6, 7, 38].

<u>Cultivation of banana nematode.</u> Usually culture has 1 male per 4 females. At extinction of culture, there are only 2 females per 1 male. The average fecundity of *P. redivivus* females is 12 − 40 eggs. Until age 20 − 25 days they produce about 15 generations, approximately 300 individuals in each. At a temperature of 20°C, intrauterine development of eggs in banana nematodes lasts for 2 − 2.5 days [4, 39-42].

The substrate for the cultivation of *P. redivivus* is prepared from oatmeal or barley, cooked as porridge, or boiled vegetables. In both cases, it is desirable to add a little milk, however, even without it the culture of banana nematodes turns out to be quite hardy [10, 30, 31].

The nutritional substrate for *P. redivivus* should be thick. In this case, its surface is quickly populated by banana nematodes, this rate prevents the development of concomitant microflora. In contrast, the liquid substrate is covered with a bacterial film at the end of the first day and the rate of worm culture development reduces [8, 11, 28]. The nutrient substrate of the desired consistency is obtained when brewing of one of the following products in 1 liter of boiling water: oatmeal (150 g), oat flour (200 g), feed oat (300 g) barley flour (250 g). All these products have to be boiled for 7-10 minutes, except for feed oats, which should be boiled for 40-50 minutes. In addition, boiled vegetables with the addition of a small amount of milk, soy peptone, casein, liver extract, vitamins can be used. The nutrient substrate is used cooled and thoroughly mixed [5, 32, 36, 38].

Prepared nutrient substrate should be put in a clean, dry cuvette with a layer thickness of 1-1.5 cm. After that, the stock culture of banana nematodes with an initial stocking density of 300 ind./cm^2 or 400 thousand individuals per cuvette is applied to its surface, which corresponds to 350-400 mg of pure culture per cuvette [4, 6, 31]. It should be noted that the rate of maturation and general state of culture of *P. redivivus* largely depends on the initial number of individuals. If the initial number of worms is small, a significant part of the nutrient substrate is quickly populated by various groups of microorganisms that liquefy and ferment it [43, 44].

Banana nematodes inhabit mainly the upper 5 mm layer of the substrate, during the period of active population growth it can be reduced to 2-3 mm. It was shown that a layer of nutrient substrate with a thickness of 10-15 mm is sufficient for the successful growth of *P. redivivus* (Fig. 7). When using a smaller layer, the substrate dries rapidly and it becomes necessary to transplant the banana nematode culture more often [7, 8, 45, 46].

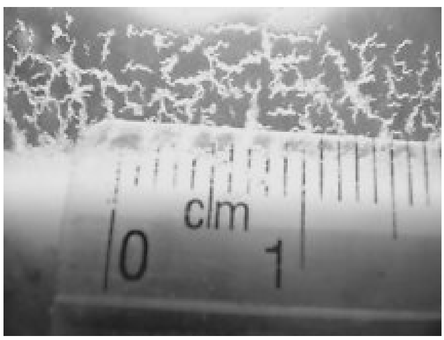


Fig. 7. **The culture of banana nematodes (*Panagrellus redivivus*)**

Such reservoirs as a cuvette, made of any material (wood, glass, enameled, plastic and so on) can serve as reservoirs for cultivation of *P. redivivus* [11, 12]. Thus, for the mass breeding of banana nematodes, enameled cuvettes with a size of 30x40 cm, with a wall height of 4 cm are used. Each cuvette of this size requires 1.5–2 liters of nutrient substrate [7, 8, 10]. These cuvettes are covered with glass; a wet sponge is attached to its inner side. Glass protects the culture from drying out, and a wet sponge creates the necessary moisture. Cuvettes are placed on wooden racks (Fig. 8) [5, 36, 38].

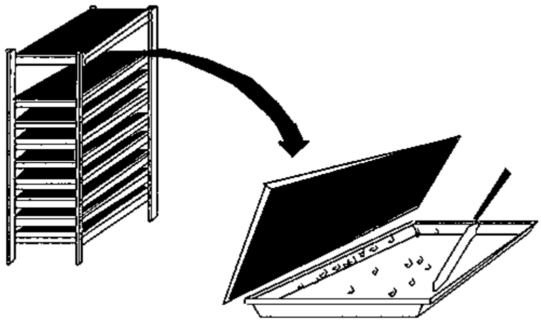


Fig 8. **Scheme of a cultivator for nematodes**

Cultivation of banana nematode is usually carried out at a room temperature (20 – 25°C), but the best results are obtained at lower temperatures (7 – 10°C). In the latter case, biomass growth is somewhat slower, but *P. redivivus* culture remains in good state longer [46, 47]. This is explained by the fact that under such conditions there is no rapid development of microorganisms that liquefy the nutrient substrate after 15 days [28, 31].

The pH should be in the range of 3.5–4.5 (up to a maximum of 7.5) [30].

The maturing time of *P. redivivus* culture is easily determined by the appearance of clusters of banana nematodes on the walls of the cuvette and the glass covering it [12, 48].

Vinegar eelworm (*Turbatrix aceti* or *Anguillula aceti*) got its name because it is most common in unpasteurized vinegar. They feed on acetic acid bacteria. Nematodes can live in the acidic environment, pH of which ranges from 1.6 to 1.1. Generally, these nematodes live and breed under conditions of vinegar fermentation [5, 30, 48, 49]. The size of an adult is 1 − 2 mm (fig 9). The length of the esophagus is 1/9 the length of the whole body in females and 1/7 in males [50].

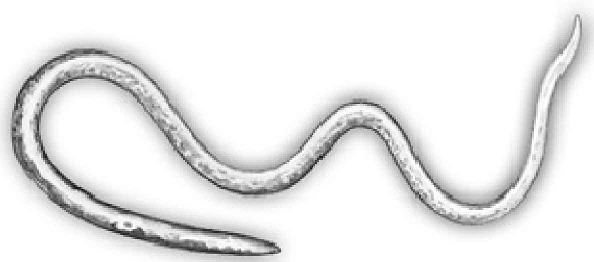


Fig. 3. **Vinegar nematode (*Turbatrix aceti*)**

Vinegar nematodes are dioecious, viviparous. Under a microscope, young individuals twisted in a spiral are clearly visible inside the females of *T. aceti*; their development lasts about 8 days [51, 52].

Cultivation of the vinegar nematode is quite simple, and can occur in two ways:

1) in cuvettes. The nutrient substrate is porridge-like oatmeal, oat flakes, soaked bread, grated boiled carrots, etc. [35, 37]. For the cultivation of *T. aceti*, enameled, glass or plastic cuvettes with a lid are used, which should be sterilized before use [53, 54]. On

the inner surface of their covers, a piece of sponge is attached, which is moistened in water, thus creating a moist environment in the cuvette. The cuvette is set in an inclined position, placing a plate under one corner [2, 8]. After that, in the lower part of the cuvette a nutrient substrate is added. Stock culture of vinegar nematodes is added to the surface of the nutrient substrate. After 2 – 4 days at a temperature of 20 – 22°C, these worms crawl out on wet areas of the walls and the substrate-free part of the bottom of the cuvette [3, 4, 55, 56]. There they are removed with a brush and placed in a glass of water for washing from the substrate. As the vinegar nematode breeds (after about 1 – 3 months), the nutrient substrate becomes liquid, which indicates the need to replace it [2, 5, 7];

2) in 3 L glass jars. One jar of this volume will need 1.5 liters of distilled water, 1.5 liters of vinegar, a small opaque plastic bag, a small apple. Vinegar should not be discolored or flavored [57, 58]. Distilled water and vinegar should be mixed to obtain 3 liters of 50/50 solution. Peeled and cut apple is added to the obtained solution. It is better to use a dense, not loose apple, so that its particles sink to the bottom, and do not float on the surface. After that, the jar is placed under bright lighting and after one–two weeks its content becomes cloudy from bacteria, which are the food for the vinegar nematode [35, 37]. At this stage, the stock culture of vinegar nematode is added to the jar and it is wrapped in a plastic bag, so that light does not get it. After 3 − 4 weeks of development at a room temperature in a shaded area, the culture will be suitable for use as food for juvenile fish. Such a culture can be used for many months without any care, except for darkness and, occasionally, adding an additional piece of apple [6, 58-60].

Rhabditis (*Rhabditis*) is a very interesting genus biologically, as it is a good example of heterogony [30]. Namely, one generation of its members is dioecious and have dimorphism (i.e. males differ from females in appearance), it is free-living and was described under the name of *Rhabditis* and the other in the next generation, is hermaphroditic and leads a parasitic way of life. In the past, the latter has been described as an independent genus called *Rhabdonema* [61, 62].

Cultivation of rhabditis is carried out by two main methods:

1) With the substrate – in containers filled with garden soil or a mixture of chernozem soil (50%), sand (30%), peat and moss. On the surface of the substrate, a nutrient medium and the original culture are added, and then covered with glass. Worms are fed several times a week [4, 9, 59];

2) Without substrate − rhabditis are cultivated directly in medium without substrate [1, 2].

Densely cooked oatmeal, soaked bread, oatmeal, grated carrots, moistened flour, yogurt can serve as the nutrient medium [3, 6, 7].

With any recipe, sterility should be maintained, since the appearance of fungi makes the culture of rhabditis die [5, 60].

CONCLUSION AND PERSPECTIVES OF FURTHER DEVELOPMENT

Based on the above, it can be argued that the use of nematodes of genuses *Panagrellus, Turbatrix (Anguillula)* and *Rhabditis* in fish farming is promising, in particular in feeding juveniles. This is due to the fact that they are not only a source of animal protein (100 g of nematode biomass contains 10.1 protein and 19.5 g of fat), but also due to their size (they are much smaller than Artemia nauplii and Cyclops), allowing completely abandon the cultivation of ciliates. In addition, the use of nematodes in aquarium husbandry is especially convenient because they are able to live in water of different salinity up to 4 days and not reproduce. However, it should be noted that not all fish species react in the same way on nematodes in the aquarium – the best reaction is observed in such genuses of the family *Poeciliidae* as guppies (*Poecilia reticulata*), moonfishes (*Xiphophorus*), mollie (*Poecilia sphenops*) and bellyfish (*Poecilia*).

Regarding the cultivation of the above-mentioned roundworms, it is quite simple and cost-effective, nematode cultures almost do not get sick and give a high increase in biomass under a wide range of environmental conditions. The basis of cultivation is the use of plastic cuvettes and a small amount of vegetables or cereals at room temperature and moderate lighting.

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