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ROTIFER BIOLOGY AND CULTIVATION

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This article reviews the biology of rotifers (*Rotatoria*), including their reproduction, structure, and ecological adaptations. The authors also provide an overview of culturing methods to increase biomass and its quality. The article summarizes the existing knowledge about the biology of rotifers and determines the optimal conditions for their successful cultivation in aquaculture.

Rotifers (*Rotatoria*) are among the most natural and available food for fry of almost any fish species. They are small multicellular animals, systematically related to the lower worms. At present, there are about 2,000 species that live in fresh water, seas, and hypersaline reservoirs. Most rotifers are herbivores eating *Chlorella*, scenedesmus, and other microalgae. There are rotifers that feed on bacteria and organic silt – detritus, but there are also predators, such as rotifers of the genus *Asplanchna*, as well as omnivorous forms. The vast majority of rotifers lay eggs. There are two types of reproduction. Unisexual and bisexual reproduction alternates depending on external conditions. Under sharp changes in environmental conditions and its physicochemical factors, such as temperature, pH, light, oxygen content, dissolved salts, as well as the quality and quantity of available food, rotifers switch to bisexuality reproduction.

The so-called “live dust”, i.e. the smallest plankton, carefully sorted through a sieve, is suitable for feeding the fry of most fish. “Live dust” includes infusoria, rotifers, juveniles of branchiopod crustaceans and naupliuses of paddlefish. Rotifers (*Rotatoria*) are also part of the so-called “live dust”.

In the article, the authors provide practical examples of breeding rotifers. The experiments have shown that *Brachionus calyciflorus*, which has a larva of 0.1-0.3 mm, breeds well in artificial conditions. The optimum water temperature for breeding is 22-30 °C. Females mature within 24 hours, adult life spans up to three weeks, and it can be cultivated at any time of the year and in the required quantity.

In our researches, this type of rotifers was used to feed producers of the European oyster *Ostrea edulis* to increase spawning conditions and occupied at least one third of the oysters' diet. Small size (0.15-0.35 mm), high nutritional value, undemanding environmental conditions, high reproduction rate made this rotifer one of the main food objects of mariculture.

Keywords: rotifers, biotechnology, cultivation, rotifer reproduction, adaptations to the environment, mariculture, bioproductivity.

Statement of the task. The aim of an article is to study and to analyze optimal conditions for rotifers' successful aquaculture production.

Analysis of recent research and publications. Among the most natural and available food for fry of almost any fish are rotifers (*Rotatoria*) – small multicellular animals, systematically belonging to the lower worms. At present, there are about 2000 species of them, which live in fresh waters, seas and hypergaline water bodies. The length of most rotifers does not exceed 0.15-0.30 mm [1], so they are available for fry of all fish from the first days of active feeding.

The body of rotifers is transparent and almost colorless. Its barely noticeable coloration depends on the contents of the digestive tract and some internal organs. In most cases, the body consists of a head, trunk and leg. At the anterior end of the head is the coelom (corona). It consists of two rings of fast and coordinated moving cilia, with the help of which rotifers can move in the water, catch food particles and direct them to the mouth. This is the most characteristic feature of rotifers, distinguishing them from all other small invertebrates.

The middle part of the body in some rotifers (they are called shells) is covered with a soft transparent sac-like shell. These include rotifers of the genus *Brachionus*, which are widespread in nature. Accordingly, not having a shell *Rotatoria* (a typical representative – *Philodina spp*.) are called respectively pancreaseless.

The rear part of the body – leg – is present in most rotifers, with its help they regulate the direction of movement. Thanks to special glands that secrete a sticky substance, rotifers can attach to the substrate.

Depending on their lifestyle, most rotifers belong to either planktonic or benthic. Planktonic species prefer to stay in the water column [2]. They are in continuous movement. The speed of movement in rotifers is noticeably slower than, for example, in infusoria, and is about 1mm/s.

Benthic rotifers spend most of their time in one place, attached to solid objects with their feet, and, making turns in different directions, filter food.

Most rotifers are herbivores, eating *Chlorella*, scenedesmus and other microalgae. There are rotifers that feed on bacteria and organic silt – detritus, but there are also predators, such as rotifers of the genus *Asplanchna,* as well as omnivorous forms. In the cultivation of most rotifers can be used various yeasts: baker's yeast, fodder yeast, etc.

Rotifers breathe with the entire surface of the body. Daily oxygen demand on average is 30-40mg per 1g of raw weight of organisms. At the same time, oxygen consumption of small, well-fed, as well as sexually mature rotifers increases 2-4 times. With increasing water temperature, this indicator also increases.

The vast majority of rotifer species lay eggs. There are two types of reproduction. The first type is only parthenogenetic (unisexual). In this case, the rotifer population consists exclusively of females. So, reproduce panceless rotifers of the genus *Philodina*.

Most species of rotifers are characterised by heterogony, and the worms themselves are called heterogeneous. In this case, unisexual and bisexual reproduction alternates depending on external conditions [3]. If the environmental conditions are favourable, the rotifer population consists of females incapable of fertilisation and are called amictic. They reproduce parthenogenetically by laying unfertilised amictic eggs. Under unchanged favourable conditions, this can continue indefinitely.

Under sharp fluctuations in environmental conditions associated with changes in such physical and chemical factors as temperature, pH, light, oxygen content, dissolved salts, as well as the quality and quantity of available food, rotifers switch to bipedal reproduction. In these cases, myctic females capable of fertilization begin to hatch from amictic (summer) eggs. If a myctic female is not fertilized, she lays eggs from which only males hatch; fertilized individuals lay resting (winter) eggs. The development of eggs (except winter eggs) lasts up to 24 hours depending on external conditions [4]. Resting eggs have a resting phase and are covered with a strong protective shell that allows them to withstand unfavorable external conditions, e.g. drying, freezing, etc.

The resting period may last several weeks, months, sometimes years. When favourable conditions occur, only amictic females hatch from eggs. Males in heterogeneous rotifers are always much smaller than females, have no digestive system and do not feed. They live only a few days and after fertilisation of the female usually immediately die.

When heterogeneous rotifers are cultured, females of both types are present in the population. The quantitative ratio between them depends on the culture conditions. To characterize the state of the population, the concept of 'mycticity of culture' is used, which is defined as the ratio of the number of myctic females to their total number. The more the environmental conditions meet the needs of a given species, the lower the mycticity of the culture, the more intensively the population develops, and vice versa. In the case of 100 per cent mycticity, the culture completely dies out in a few days, leaving only resting eggs behind.

The life span of rotifers depends largely on external conditions, mainly temperature, and varies from three to four days to one and a half months. Increase in temperature 'shortens' the life of rotifers.

The entire life cycle consists of three periods. The first is juvenile, from the moment of hatching from the egg to lay the first egg of their own.

In most rotifers, such organisms are called juvenile. After 0,5-1,5 days begins the main reproductive period, which lasts from the laying of the first to the laying of the last egg. Finally, the time of life after the laying of the last egg until the moment of natural death is called the senile period.

Rotifers can exist in a fairly wide range of temperatures – from 1-2 to 35-37 °C. In this case, there is a fairly clear division into thermophilic (heat-loving) species, found in nature mainly in summer at temperatures of 18-30 °C, and thermophobic (cold-loving), characteristic of autumn-winter zooplankton and living in colder water.

The thermophobic species, such as representatives of the genus *Brachionus,* are usually cultivated.

Materials and methods. The rotifer *Brachionus Plicatilis (Brachionus plicatilis*) has been used in our research – these are small rotifers – (0.1-0.3 mm). Saltwater rotifers are used as starter food for fry of spawning fish. Females live up to 2 weeks. The studies were carried out under the conditions of Culture blue (Lyngdal, Norway), a facility specializing in European oyster breeding.

One of the primary requirements for successful brachyonus culturing is temperature. In the available manuals on rotifer culturing, 24-26 °C is accepted as the optimum temperature for their cultivation. Taking into account the high tolerance of *Brachionus* to this parameter, in our work we used the temperature not lower than 25.0-26.5 °C, but not higher than 28.0 °C.

These limits, as confirmed by numerous works, the results of which were tested on pilot – production installations, turned out to be optimal. Naturally, the more intensive growth of brachyonus biomass with an increase in temperature in the cultivators by 1.5-2.0 °C required some adjustments in the feeding regime. Unfortunately, this important element of cultivation biotechniques has not been given due attention until recently. The examples given in the literature (introduction of nutrient media into cultivators with bred hydrobionts from once every 4-5 days to 12 times a day) are indicative in this respect.

The development of rotifer populations depends crucially on the quantity and quality of food used. In this case, the most important is the feeding regime, which is determined by the temperature of the medium, the concentration of food particles in it, and the population density, i.e., the number of individuals per unit volume of the medium. Increase in temperature increases the activity of rotifers and, consequently, their filtration capacity [5].

The concentration of food particles in the medium determines the productivity of rotifer culture. At its increase, the whole life cycle of rotifers proceeds faster, and fecundity increases.

If rotifers are kept at optimum temperature, an increase in food concentration leads to a decrease in the volume to be fished. At low feed concentrations, the filtering apparatus of rotifers works at maximum speed. The daily diet of animals in this case is less than 100 % of their body weight, and the digestibility of feed (part of the daily diet assimilated by the organism) is the highest – 80 %. In the range of feed concentration 5-50 mg/l daily volume of water filtered by rotifers decreases almost twice. In this case, the daily diet is 100-400 % of the organisms' own mass, but the digestibility of food decreases from 75 to 48 %. When the feed concentration increases to 0.5-1.0 g/l, the volume of filtered water decreases almost 10 times more, and the ration reaches maximum values – 500 % and more with digestibility of 40 %. Feed so quickly passes through the digestive apparatus of rotifers that it does not have time to fully digest. The productivity of the culture decreases. Further increase in concentration of feed particles depresses population development.

These patterns appear if the density of rotifer culture is constant and only the concentration of food particles changes. However, in the process of cultivation the density of rotifer culture also changes. Experience shows that a more complete trophic characteristic is not just the concentration of food particles, but the ratio of these particles and the number of rotifers in the culture. This indicator is called food supply, showing how many millions of food particles per rotifer. It depends on the rotifer species and the food. For example, for rotifers of the genus *Brachionus* when feeding them chlorella optimal food supply of 0.1 million kl/ex. Excessive or insufficient food supply in comparison with the optimal one depresses the growth of rotifer population.

In the process of cultivation in the medium gradually accumulate rotifer products, dead worms and uneaten food particles. During their decomposition, substances such as ammonia accumulate, depressing rotifer culture development. Accumulation of products of vital activity above the permissible level leads to death of animals.

To breed rotifers use a vessel – a cultivator, the volume of which depends on the productivity of the population and the required daily number of organisms. The rotifer culture obtained from resting eggs or from another cultivator is introduced into the cultivator. The parameters of the medium in the cultivator (temperature, salt composition, aeration, illumination, etc.) should be optimal [6].

The population develops in several stages. At the first stage (lag-phase) rotifers undergo a period of adaptation, adapting to the new environment. The lag phase usually lasts from several hours to one or two days. Then the phase of exponential population growth begins, when the density of the culture increases at a maximum rate. Further, as the number of rotifers increases and the accumulation of waste products in the medium, the growth rate of the culture gradually decreases, as deteriorating conditions depress rotifer reproduction and increase the number of dead organisms. Finally, a stationary phase occurs, during which the number of individuals born and killed per unit time is compared. During this period, the culture reaches maximum density, the value of which depends on rotifer species and specific culture conditions. If the culture is properly managed, the maximum population density can reach 300-1000 or more rotifers in one millilitre of medium, which corresponds to a biomass of 1-2 g/l. Subsequently, as a rule, there is a gradual die-off of the culture. Heterogeneous rotifers in this case in the mass pass to sexual reproduction. In the extreme case rotifers can disappear altogether, leaving resting eggs. If the initial density of the culture is 10 % of the maximum, it will take 3-4 days for productive species to reach the maximum density [7].

Feeding fry in their first stages of development is very difficult for aquarists and aquaculturists alike.

So-called ‘live dust’, i.e. the smallest plankton carefully sorted through a sieve, is suitable for feeding the fry of most fish. The best is ‘live dust’ taken from temporary puddles. ‘Live dust’ includes infusoria, rotifers, juveniles of branchiopod crustaceans and naupliuses of paddlefish.

Rotifers (*Rotatoria*) are also part of the so-called ‘living dust’. They can be found in almost every pond or puddle. They appear in large numbers in spring and live there until autumn.

Brachionus calyciflorus, which has a larva of 0.1-0.3 mm in size, breeds well in artificial conditions. Optimal water temperature for breeding is 22-30 °C. Females mature within a day, adult life spans up to three weeks. The female lays eggs every 12 hours. Feed for rotifers can be hydrolyzed or baker's yeast (1 g per 50 liters).

The brackish-water rotifer (*Brachionus plicatilis*) is cultivated for feeding marine and freshwater fish.

Result and discussion. In our studies, this species of rotifers has been used to feed European oyster *Ostrea edulis* to increase spawning condition. The rotifers occupied at least one third of the oyster diet.

Under artificial conditions it can be obtained at any time of the year and in the required quantity. Small size (0.15-0.35 mm), high nutritional value, undemanding environmental conditions, high reproduction rate made this rotifer one of the main food objects of mariculture.

For breeding rotifers you can take a variety of containers, from one liter and more, resistant to the action of salt solution (Fig. 1). Medium – solution of pharmacy sea salt (one full tablespoon per liter of water or 20g NaCI +6-10g Na2SO4). In our studies, sea water with a salinity of 32 ppm has used for incubation. The cultivation temperature was 24-26 °C.

Fig. 1. **Tank for rotifer maternal culture cultivation**

Standard aeration was provided. Feed – baker's or hydrolytic yeast (teaspoon per 20 litres of solution). After the introduction of feed, the medium is slightly cloudy. Its clarification – a signal to add a new portion.

Reproduction of rotifers, accumulation of biomass was significantly activated when adding baby food NAN to the feed.

The Rotatoria maternal culture was kept in a 160-liter aquarium. The density of the mother culture ranged from 600 to 1200 specimens per ml.

The Rotatoria maternal culture was regularly sampled and added to the columns with aging unicellular algae (Fig. 2).

Fig. 2. **Introduction of rotifers into an ageing culture of the microalgae *Isochrisis galbana***

In our case, the maternal culture was constantly renewed and showed no signs of senescence. But an ageing, declining culture can always be recovered from resting eggs. To do this, drain from the bottom of the sediment of the old solution (in it the resting eggs), dried and placed in the refrigerator for a long time. As needed, some eggs are taken and cultured [8].

The rotifers actively absorbed microalgae, fouling inside the columns and even detritus. Water supply to the column was stopped during incubation. The reproduction of rotifers in the columns is so rapid that in 2-4 days it was possible to obtain sufficient numbers for feeding oysters.

For this purpose, the water supply to the columns was resumed. The rotifers have been mixed with microalgae in the water flow and fed to the oysters through a pipeline.

The purpose of culturing can be either to obtain a large portion of rotifers at a time or to select a part of the population every day, which was the case in our case.

In the first case it makes sense to achieve maximum culture density, in the second case it is more advantageous to have maximum biomass growth per unit time at a culture density of 50-60 % of the maximum.

Conclusions. It should be noted that rotifer incubation is a rather simple and effective way to obtain complete feed for aquaculture facilities. Cultivation of rotifers is possible both in monoculture and in combination with other microscopic food objects, e.g. microalgae, infusorians or copepods.

In our studies, rotifer incubation was carried out to improve the quality of the sexual products of the European *Ostrea edulis* in preparation for spawning. It was found that rotifer incubation is easily incorporated into oyster reproductive biotechniques. Cultivation of microalgae for oyster feeding is cyclic in nature. After approximately four weeks of cultivation in plastic columns, the microalgae culture ages. Fouling and detritus appear in the columns. Addition of rotifers to such culture resulted in their rapid growth. At the same time, a significant amount of additional high-value food was obtained without additional costs.

БІОЛОГІЯ ТА КУЛЬТИВУВАННЯ КОЛОВОРОТКИ

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У статті розглядається біологія коловерток (*Rotatoria*), включаючи їх розмноження, будову та екологічні адаптації; проведено огляд методів її культивування для збільшення біомаси та якості; узагальнено існуючі знання про біологію коловерток та визначено оптимальні умови для їх успішного культивування в аквакультурі.

Коловертки (*Rotatoria*) є одними з найприродніших та найдоступніших видів їжі для мальків майже будь-якого виду риб. Це невеликі багатоклітинні тварини, що згідно систематизації відносяться до нижчих черв'яків. Наразі існує близько 2000 видів, які мешкають у прісній воді, морях та гіперсолоних водоймах.

Більшість коловерток є травоїдними, що харчуються хлорелою, сценедесмусом та іншими мікроводоростями. Є коловертки, які живляться бактеріями та органічним мулом – детритом, є хижаки, такі як коловертки роду *Asplanchna*, а також всеїдні форми. Переважна більшість коловерток відкладає яйця. Існує два типи розмноження. Одностатеве та двостатеве розмноження чергується залежно від зовнішніх умов. За різких змін умов навколишнього середовища та його фізико-хімічних факторів, таких як температура, pH, освітленість, вміст кисню, розчинені солі, а також якість і кількість доступної їжі, коловертки переходять до двостатевого розмноження.

Так званий «живий пил», тобто найдрібніший планктон, ретельно перебраний через сито, підходить для годування мальків більшості риб. «Живий пил» включає інфузорії, коловертки, молодь жаброногих ракоподібних та наупліуси веслоносів. Коловертки (*Rotatoria*) також входять до складу так званого «живого пилу».

У статті автори наводять практичні приклади розмноження коловерток. Експерименти показали, що *Brachionus calyciflorus*, личинка якого має розмір 0,1-0,3 мм, добре розмножується в штучних умовах. Оптимальна температура води для розмноження становить 22-30 °С. Самки дозрівають протягом 24 годин,

тривалість життя дорослої особини становить до трьох тижнів, її можна вирощувати в будь-який час року та в необхідній кількості.

У наших дослідженнях цей вид коловерток використовувався для годівлі виробників європейської устриці *Ostrea edulis* для покращення умов нересту та займав щонайменше третину раціону устриць.

Невеликий розмір (0,15-0,35 мм), висока харчова цінність, невибагливість до умов навколишнього середовища, високий коефіцієнт розмноження зробили цю коловертку однією з основних харчових об'єктів марикультури.

Ключові слова: коловертки, біотехнологія, культивування, розмноження коловерток, адаптації до навколишнього середовища, марикультура, біопродуктивність.

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