Chapter 16
Use of the Simulation Model for the Analysis of the Impact of Parasites on the Dynamics of Abundance, Reproduction, and the Transition to Diapause in the Mass Rotifers *Brachionus calyciflorus* Pallas of the Volga Delta

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Abstract Rotifers are one of the most important groups of zooplankton, and they play an important role in aquatic ecosystems as initial energy converters of primary and bacterial products. In the course of monitoring studies of the state of ecosystems, many questions arise, the solution of which is necessary for correct interpretation of the data obtained. When considering the dynamics of the abundance of zooplankton and its constituent groups, the main focus is on the state of temperature and oxygen regimes, feed conditions, competitive relationships, and predator consumption, and too little attention is paid to the effects of parasitic organisms on plankton. The question of rotifer infection and the possible influence of this factor on their population dynamics is still poorly studied. Simulating of these processes with the help of mathematical model is described.

Keywords Dormancy, Zooplankton, Population dynamics, Resting egg production, Disease in aquatic invertebrates

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16.1 Introduction

In Russia, N. V. Voronkov was the first to pay attention to the infection of rotifers (rotifer as host) by parasitic organisms; the work examined small temporary water bodies in the vicinity of Lake Glubokoe (Voronkov 1910).

Later, N. K. Decksbach noted the infection of four species of rotifers with the same type of parasite in the waters of Moscow neighborhoods (Decksbach 1928). In reservoirs outside of Russia, a number of researchers have indicated infection of rotifers by parasitic organisms. A. Ruttner-Kolisko (1977) noted a strong infection of the *Conochilus unicornis* Rousselet 1892 population by microsporidia in Lake Lunts (Austria) and its influence on rotifer population dynamics. In another body (Lake Banyoles, Spain), M. R. Miracle (1977) showed a strong influence of parasites on the development of two populations of rotifer species.

Infection of rotifers has not been previously studied in Volga delta-associated water bodies. The Volga delta, however, is a unique natural complex of great economic importance. Effective management of its resources and preservation of its ecosystems require an understanding of all of the natural components and their functions, including rotifers. The available data on infection of rotifers raises questions regarding parasite impacts on this important group of zooplankton. Abundance and other indicators of population dynamics including diapause (the most important seasonal and biotopic adaptation of aquatic invertebrates) are worth consideration (Alekseev et al. 2007).

The purpose of the research was to study the infection dynamics of predominant rotifer species in natural ecosystems and to quantify the impact of infection on population dynamics, productivity, and seasonal adaptations in *Brachionus calyciflorus* Pallas rotifers based on the data obtained. Direct assessment of infection's impact on rotifer population dynamics by field observations alone is quite difficult due to short rotifer life cycles. Assessments of this kind performed under controlled laboratory conditions, however, raise fair questions about the validity of generalizing such experimental infection data to natural populations. In this situation, an ideal compromise is one in which an experimental model is used that takes into consideration knowledge of a species' life cycle parameters and verifies any data obtained by examining (measuring, estimating, extrapolating, etc.) data regarding the states of real populations under natural conditions.

Modern simulation methods allow researchers to reproduce and trace the seasonal course of selected population parameters in model "populations" with a high level of approximation to reality. They make it possible to quantify indicators of population dynamics and seasonal adaptation which are difficult to obtain by direct observation. These include mortality, productivity, and population growth rate (Mnatsakanova and Polishuk 1996). Important indicators of the effectiveness of rotifer seasonal adaptations are the time of appearance and the proportion in the population of mictic females (which have transitioned from parthenogenesis to bisexual reproduction) and the production of diapausing (resting) eggs sufficient to maintain the population. Considering the multifactorial nature and complexity of

parasite impacts on host population dynamics, a simulation model was developed to handle the problem, and this study is devoted to analysis of the model's behavior.

16.2 Materials and Methods

In order to quantify the impact of parasites on rotifer productivity and life cycle parameters, a population dynamics model featuring *Brachionus calyciflorus* Pallas was developed in collaboration with researchers from Germany. To create and verify this model, experiments were performed to evaluate the duration of embryonic development, postembryonic development, and other life cycle parameters of the species, and field studies were carried out. The main study materials were standard "quantity samples" (more than 700), as well as live and fixed "quality samples" of zooplankton collected in water bodies of the Volga delta at the Damchiksky site of the Astrakhan State Nature Biosphere Reserve in 2001–2005.

Samples were collected by filtration of 100 liters of water through Apstein plankton nets made from number 73 mill screen. Immediately after collection, samples were adjusted to a volume of 100 ml and fixed with 4% formalin. Collection of quality materials was carried out by hand towing of plankton nets. A 20 μm mesh plankton net was used for more effective collection for small rotifer species. When taking samples, water depths and temperatures were measured simultaneously. To characterize water temperature changes in the Bystraya channel, data from the "Damchik" pheno-hydrometeorological station were used (Fig. 16.1). Counting, viewing, and identification of rotifers were carried out using light microscopy

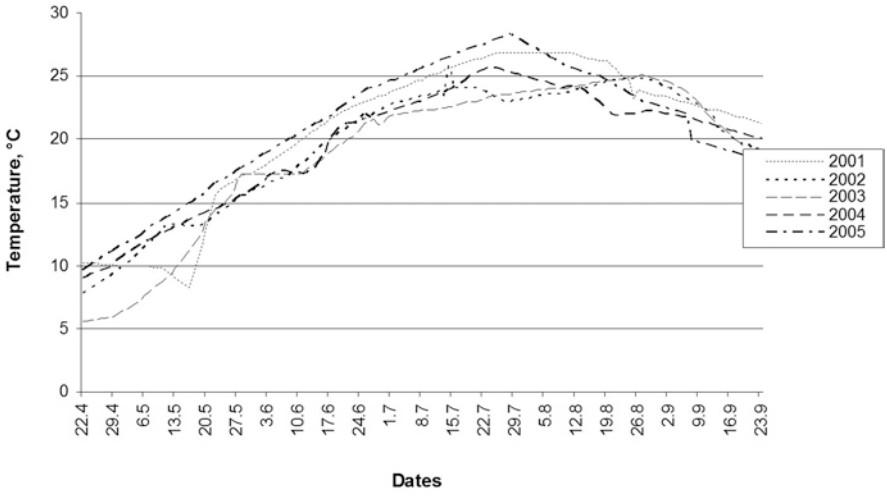

Fig. 16.1 Seasonal water temperature variations in the Volga delta Bystraya channel, by year and model reproduction, based on field measurements

(MBS-9 and MBI-1). To photograph infected rotifers in a living state, a custom microphotography apparatus was constructed; it is a refinement of earlier flash microphotography methods (Gorbunov 1979). The latest version uses a digital camera with a resolution of 8 million pixels, and the arrangement permitted us to effectively process both live and fixed materials.

For proper modeling and study of parasite involvement, it is necessary to establish baseline biological parameters of the living host rotifer (*Brachionus calyciflorus*). Therefore, laboratory manipulation and monitoring of samples were as follows: live rotifers from natural populations were placed in micro-aquariums with volumes of 1–50 ml and filled with water from the sampling site. Micro-aquarium water was changed several times a day. For visualization and photography, rotifers were pipetted onto slides and covered with coverslips featuring plasticine or wax legs. After photographing, rotifers were returned to micro-aquariums, where they continued their development.

16.3 Model Description

The model was developed in the C++ software environment and includes the following elements: a numerical model of *B. calyciflorus* population dynamics with step-by-step display of results (1 step = 1 day); and the ability to change infection levels, and other indicators, via macros. This makes it very convenient, both for simulation of natural conditions and also for quantifying the possible impact of parasites at specified infectiousness values.

The dynamics of the total number of rotifers, by age, on a specific date *N[d,k]* was approximated by the formula:

$$N[d,k] = N[d-1,k-1]*(1-M)*(1-I) \tag{16.1}$$

where

N is the initial number of rotifers at the beginning of the population's development
d is the day number from the beginning of observations (discrete time)
k is the age group of rotifers, in days
M is the coefficient of death
I is the coefficient of infection

The dynamics of the number of infected rotifers specimens *(Ninf [d, k])* was estimated by the formula:

$$Ninf[d,k] = Ninf[d-1,k-1]*(1-M) + N[d,k]*I \tag{16.2}$$

If (degree-days [d, k > 0]) and (degree-days [d, k]) $\leq$ 60), then:

$$Ninf[d] = Ninf[d] + Ninf[d,k]$$

Rotifer age was determined in degree-days, with the following groups identified:

1. Parthenogenetic eggs: 20 degree-days (development time 24 h uniformly at 20 °C with the possibility of correction for the actual temperature)
1. Young females before the start of reproduction: 40 degree-days
2. Mature parthenogenetic females: life span up to 140 degree-days
3. Adult parasite-infected females: life span up to 60 degree-days

The dynamics of degree-day accrual (degree-days [d, k]), by each age stage, was carried out at a frequency of once per day according to the formula:

$$\text{degree days}[d,k] = \text{degree days}[d-1,k-1] + t^{\circ}C[d] \tag{16.3}$$

$t^{\circ}C[d]$ actual measured water temperature

The transition to the formation of males and diapausing eggs (seasonal adaptations) corresponded with conditions in which increasing numbers of rotifers were under the influence of a combination of two signal factors: temperature (natural data) and photoperiod (calendar data). Collectively, these determine the value of the diapause coefficient (K_D), which ranges from 0 to 1.

The number of mictic eggs (for males and females capable of laying diapausing eggs) was determined in this case by the formula:

$$N_{\text{stad}_{3[d,k]}} = N_{\text{stad}_{3[d,k]}} * \left(1 - K_D\right) \tag{16.4}$$

The model took into account the possibility of diapause reversibility (the return of some rotifers to parthenogenetic reproduction) in the case of high floods; this has been noted in cases in which water temperatures have decreased by no less than 5 degrees during 3–10 days (Alekseev 1978).

$$N_{\text{stad}_{3[d,k]}} = N_{\text{stad}_{3[d,k]}} + N_{\text{diap}[d,k]} * E_D \tag{16.5}$$

E_D fraction in diapause exit

The process of diapause exit lasted no more than 10 days during such temperature drops.

Population fecundity (Eggs[d]) was determined by the formula:

$$\text{Eggs}[d] = N_{\text{stad}_{3[d,k]}} * F \tag{16.6}$$

F average number of eggs laid by the female (determined by sampling).

The model reproduces *B. calyciflorus* seasonal dynamics and includes the following elements:

1. Parthenogenetic eggs, development time 24 h (uniformly at 20 °C with the possibility of correction for actual temperature)
1. Mature parthenogenetic females
2. Mictic eggs for males (mictic eggs from which males hatch)

3. Sexually reproducing mictic females
4. Diapausing (resting) eggs (as a result of fertilization)

Key population parameters used for simulation, including signal temperature and photoperiod, were based on empirical data. Specifically, data on the conditions surrounding development of natural Volga delta *B. calyciflorus* populations (2001–2005) was used. *Brachionus calyciflorus* production was estimated from the number of eggs produced (Edmondson 1960; Galkovskaya 1963). The level of infection of the *B. calyciflorus* population was reproduced in accordance with the results of real estimates following years of observations. In the model, it is taken into account with the help of the infection coefficient, which ranges from 0.01 to 0.05.

A few trends can be noted regarding the coefficient of infection in the model. Of the estimated infection parameters, the infection coefficient correlated best with the percentage of infection at peak population size. The average level of infection was less correlated with the infection coefficient over the population's entire existence. The maximum level of infection had almost no correlation to the coefficient of infection. The latter (max. inf.) usually occurred at the end of a population's existence (when their abundance is low). The coefficient of natural mortality during the years studied ranged from 0.12 to 0.26, and this range was determined by adjustment of model parameters until computed estimates matched observations.

16.4 Results of Model Analysis

To assess the impact of infection (infection of rotifer by bacteria, fungi, protozoan parasites, etc.) in a model that reproduces the population observed under natural conditions, population dynamic characteristics under conditions of natural infection were restored (0-variant). It was assumed that food was not a limiting factor during population growth. Accordingly, the increasing values of quantity, productivity, fertility, etc., seen in the model, after taking into account infection, reflect, in our opinion, the real potential of the population, although it is somewhat hindered by the negative impact of parasitism/disease (Fig. 16.2). As such, comparison of population development parameters without infection (the 0-variant) and with infection (the I-variant) allowed us to obtain a quantitative assessment of parasite/disease influence on *B. calyciflorus.* Infection is an external environmental factor, and its influence on population parameters is shown (Fig. 16.3). The parasite factor's role in changing *B. calyciflorus* seasonal adaptations was assessed by calculation of the accumulation of diapausing eggs by the population during the season. It is known that the outcome of competition between species, especially in temporary reservoirs, depends on the sizes of their diapausing egg banks (Mnatsakanova and Polishuk 1996). Therefore, the impact of parasites and diseases on the accumulation of diapausing eggs can also be considered as an environmental factor that changes the competitive relationship between species in subsequent years. As such, in order to quantify the impact of infection on *B. calyciflorus* population dynamics and

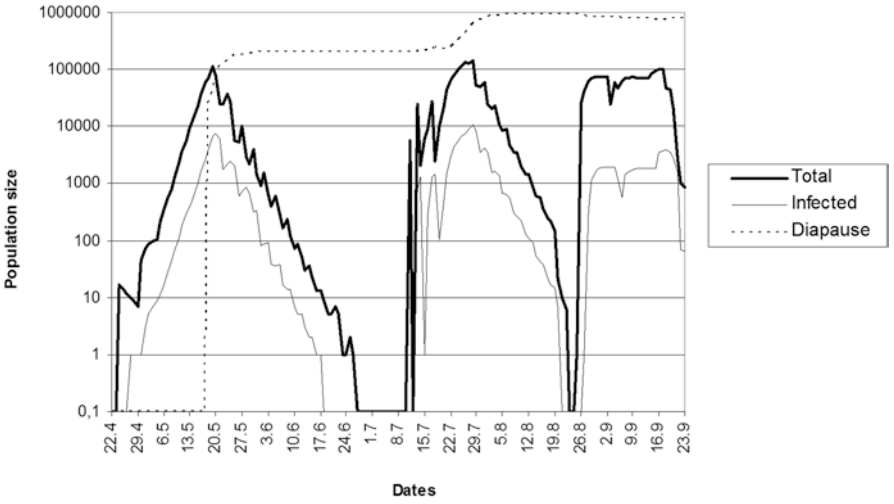

Fig. 16.2 Population dynamics (total number, number of infected individuals, and stock of diapausing eggs) of *Brachionus calyciflorus* Pallas, featuring an average infection level of 3.86%, by model for 2005 (thick line = total number; thin line = number of infected individuals; dotted line = stock of diapausing eggs)

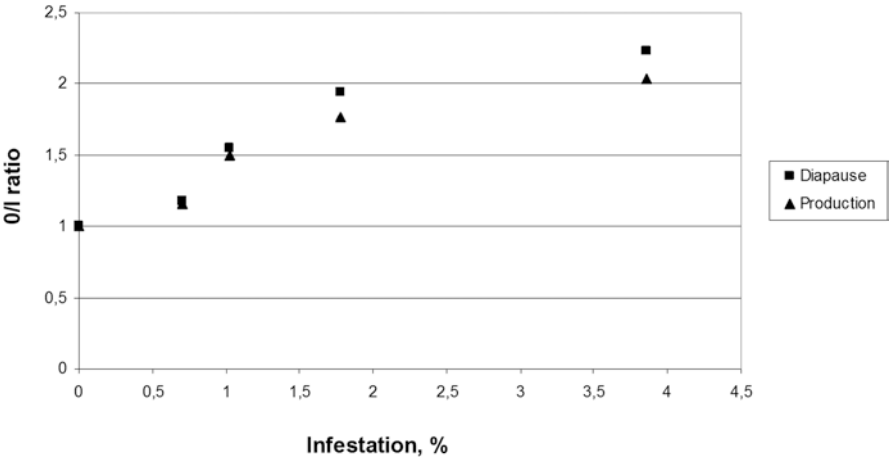

Fig. 16.3 0/I ratio of egg production and diapausing eggs in "clean" and infected *Brachionus calyciflorus* populations at different levels of infection close to those observed in nature, by model (triangles = 0/I ratio of egg production in "clean" and infected populations; squares = 0/I ratio of diapausing eggs in "clean" and infected populations)

seasonal adaptation, we selected the following rotifer life cycle parameters: maximum total population size at their first and second peaks, average daily fertility per season, egg production per season, and total number of resting eggs by the end

of the season. The results of these comparisons are summarized in the following final tables, by year (Tables 16.1, 16.2, 16.3, 16.4 and 16.5).

In 2001, the average infection rate was 5.9% in the model population and 9% in the natural (field data) population. The difference in these estimates is primarily due to different sample sizes for these quantities. In the model population, the average value was obtained from 152 points, while in natural conditions, it was obtained from only 6. Closer data regarding infection in the model and under natural conditions were obtained by comparing them at the population maxima: 7% (model) versus 7.9% (nature) (Table 16.1).

The average infection value, as estimated by the model, for 2002 was 0.7%; this almost coincides with field observations (1%). Infection values at the first population peak in the two systems were also very close: 1.2% (model) and 1% (nature) (Table 16.2).

Thus, *Brachionus calyciflorus* infection levels in 2002 were minimal in relation to all of the variants considered. Nevertheless, in this year, all of the indices clearly indicated a negative effect of even a weak infection on population parameters. The O/I ratios at first and second pop. peaks were 1.16 and 1.11.

In comparison with the previous year, 2001s first population peak was not as significantly suppressed by the infection. Yet, average fertility in 2001 was even more sensitive to infection than before, and it was 1.3-fold lower in the infected

Table 16.1 Simulation modeled *B. calyciflorus* population dynamics parameters reproducing the 2001 natural population

Parameters	0-variant	I-variant	0/I ratio
Maximum quantities at			
1st pop. peak	105,792	36,243	2.919
2nd pop. peak	144,290	119,736	1.205
Average seasonal daily fertility, eggs/ind.	1.158	1.09	1.16
Number of eggs, eggs/season	3,325,128	2,166,443	1.53
Stock of diapausing eggs	2,085,654	1,250,747	1.668

0-variant, population size without the influence of the infectious factor; I-variant, population size under the influence of the infectious factor

Table 16.2 Simulation modeled *B. calyciflorus* population dynamics parameters reproducing the 2002 natural population

Parameters	0-variant	I-variant	0/I ratio
Maximum quantities at			
1st pop. peak	96,737	83,178	1.163
2nd pop. peak	1,142	1,027	1.112
Average seasonal daily fertility, eggs/ind.	1.349	1.037	1.3
Number of eggs, eggs/season	319,981	276,023	1.159
Stock of diapausing eggs	185,109	156,436	1.18

0-variant, population size without the influence of the infectious factor; I-variant, population size under the influence of the infectious factor

Table 16.3 Simulation modeled *B. calyciflorus* population dynamics parameters reproducing the 2003 natural population

Parameters	0-variant	I-variant	0/I ratio
Maximum quantities at			
1st pop. peak	78,074	51,465	1.52
2nd pop. peak	169	107	1.58
Average seasonal daily fertility, eggs/ind.	1.273	1.317	0.966
Number of eggs, eggs/season	257,723	172,253	1.5
Stock of diapausing eggs	128,421	83,050	1.55

0-variant, population size without the influence of the infectious factor; I-variant, population size under the influence of the infectious factor

Table 16.4 Simulation modeled *B. calyciflorus* population dynamics parameters reproducing the 2004 natural population

Parameters	0-variant	I-variant	0/I ratio
Maximum quantities at			
1st pop. peak	19,377	12,496	1.55
2nd pop. peak	2,145	1,142	1.878
Average seasonal daily fertility, eggs/ind.	1.076	0.921	1.17
Number of eggs, eggs/season	139,955	78,905	1.774
Stock of diapausing eggs	77,018	39,614	1.94

0-variant, population size without the influence of the infectious factor; I-variant, population size under the influence of the infectious factor

Table 16.5 Simulation modeled *B. calyciflorus* population dynamics parameters reproducing the 2005 natural population

Parameters	0-variant	I-variant	0/I ratio
Maximum quantities at			
1st pop. peak	123,793	62,961	1.966
2nd pop. peak	138,970	62,772	2.213
Average seasonal daily fertility, eggs/ind.	0.617	0.6099	1.01
Number of eggs, eggs/season	613,362	299,358	2.049
Stock of diapausing eggs	433,822	194,429	2.231

0-variant, population size without the influence of the infectious factor; I-variant, population size under the influence of the infectious factor

variant than in the uninfected one. Differences in egg production during the season and in the stocks of diapausing eggs turned out to be small, 16% lower and 18% lower, respectively, in the infected population.

The average fraction of the population infected, in 2003, was 1.02% in the model; this is markedly different from the 4.73% level of the natural population (Table 16.3). At the same time, estimates of infection at peak population size were almost identical between the model and natural populations, amounting to 1.7 and 2%, respectively.

The maximum total population sizes for the first and second 2003 quantity peaks were almost the same for both variants. Their O/I ratios at first and second pop. peaks were1.52 and 1.58, respectively. The average fertility, over the entire 2003 observation period, was found to be only slightly lower in the uninfected population. The total egg production was 1.5 times lower in the infected population, and the stock of resting eggs was almost the same (Table 16.3).

Brachionus calyciflorus population dynamics parameters, reproduced by the model simulating 2004s natural population, are presented in Table 16.4.

In 2004, the average size of infection, according to the model, was 1.78%, and this is almost threefold different from field observations (5.1%). In contrast, the infection values at the first population peak were significantly closer between model and nature variants: 3.1% and 2%, respectively. The reason behind the differences in some of these parameters is likely related to size differences between the modeled data and field data. Of the variants considered, infection of *B. calyciflorus* in 2004 was intermediate between the extreme values of 2001 and 2002. Nevertheless, a number of indicators for 2004 indicated a significant negative effect of this average infection on population parameters. The O/I ratios at first and second pop. peaks were 1.55 and 1.88, respectively.

In 2005, the (population max.) first and second O/I ratios (0-variant/infected variant) were 1.97 and 2.2, respectively. The overall infection maximum was 3.86% in the studied populations. Average fertility values were almost identical between variants. Total egg production was more than twofold lower in the infected population, and the stock of diapausing eggs was even lower (Table 16.5).

Analysis of the changes in production and accumulation of diapausing eggs showed a definite correlation between these important *B. calyciflorus* parameters and infection levels (see Fig. 16.3). It is easy to see that the point at which infection begins to have a significant *impact on the population is at 0.5% infection (*defined as declines of 20% or more, in comparison with uninfected). Therefore, even 1% infection of rotifers (a moderate seasonal infection level) can be considered an important environmental factor that significantly influences their population dynamics and seasonal adaptations.

16.5 Conclusion

Thus, by comparing data on the effects of infection in populations of *Brachionus calyciflorus* from several years, including mean infection levels, we were able to model population features. It should be pointed out that the 2002–2005 model variants are in good agreement with field (nature) observations. The 2001 variant falls significantly outside of the general pattern. It is possible that in that year some unaccounted for external factor exerted an additional influence on population dynamics. It may appear as though the model produces a relatively high number of dormant eggs for the conditions, but it should be noted that the model focuses on relatively short time frames in which *Brachionus calyciflorus*' main survival/adaptive

behavior emphasizes production of parthenogenetic eggs over dormant. In habitable, longer-term water bodies, different mechanisms regulate the balance between production of parthenogenetic eggs and dormant; the latter situation is also analogous to that which is seen in crustaceans (Alekseev 1978).

Examination of the simulation model shows that even an insignificant seasonal infection of the *Brachionus calyciflorus* population with parasitic and/or bacterial infections (starting at 1%) leads to a twofold or greater reduction in the population's production and diapausing (resting) egg stock values. This significantly impacts populations' adaptive and competitive capabilities. Collectively, these results indicate that infection is an important environmental factor, and work related to rotifers and other hydrobionts, such as monitoring and general environmental studies, must take it into account.

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References

Alekseev VR (1978) *Microcyclops gracilis* (Lill.) and *M. varicans* (Sars) in temporary water bodies of the Volga delta. Hydrobiol Mag 14b(4):29–34

Alekseev VR. De Stasio B, Gilbert JJ (2007) Diapause in aquatic invertebrates. theory and human use, Springer, 257 pp.

Decksbach NK (1928) Zur Kenntnis der Parasiten der Radertiere. Zoolog Anz 78:274–278

Edmondson WT (1960) Reproductive rates of rotifers in natural populations. Mem Ist ital Idrobiol 12:21–77

Galkovskaya GA (1963) About the use of food for growth and the conditions for the maximum output production of rotifers *Brachionus calyciflorus* Pallas. Zool Mag 43(4):506–512

Gorbunov AK (1979) A method for rapid intravital measurement of microscopic animals using flash backlight microphotography. Hydrobiol Mag 6:114–115

Miracle MR (1977) Epidemiology in rotifers. Arch Hydrobiol Beih Ergebn Limnol H 8:138–141

Mnatsakanova EA, Polishuk LV (1996) Role of parthenogenetic natality and emergence from diapausing eggs in the dynamics of some rotifer populations. Diapause in the Crustacea. Dev Hydrobiol 114:169–178

Ruttner-Kolisko A (1977) The effect of the microsporid *Plistophora asperospora* on *Conochilus unicornis* in Lunzer Untersee (LUS). Arch Hydrobiol Beih Ergebn Limnob Stuttgart 8:135–137

Voronkov NV (1910) On the question of the development cycle of the sporozoa *Ascosporidium asperosporum*. Transactions of the Hydrobiological station on the Glubokoe lake 3:219–222