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Parameters of ontogeny and population dynamics modeling of *Panagrolaimus detritophagus* (Nematoda: Rhabditida) *in vitro*

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ABSTRACT

The parameters of individual development and population cycle in *in vitro* nematodes *Panagrolaimus detritophagus* were revealed. The nematodes are bacterial feeders and commensals of the cerambycid *Monochamus galloprovincialis* from the pine *Pinus sylvestris*; nematodes use beetles as vectors. Mean development time (T) from egg to juvenile is 1–2 days for J2, 3–4 days for J3, and 4–7 days for J4; to adults (G, generation) 7 (6–8) days. *In vitro* the population cycle is equal to 4 generations and ends with 90% of survival juveniles (J3, day 34). In the growth phase of the population, the proportion of eggs exceeds the proportion of other stages of the developmental cycle: 39±11% for 7 days; 53±10% for 21 days. The average oviposition rate of females is 4.5±1.3/day and only 56±12% of eggs proceed to immediate development (hatching and molting of juveniles). The remaining mass of eggs enter development only after 27 days (4 individual generations). This feature may be considered as a form of delay or a brief diapause at the egg stage. Individual females may accumulate up to 4 synchronous eggs in the body and lay them simultaneously. The average life span of an adult female is 13–20 days. Formulas for the exponential growth of the number of females and the total nematode population have been developed.

Key words: agar culture, biological model, detrital horticulture, diapause, exponential growth formula, fecundity, food web, life cycle, microbiome, oviposition, population dynamics

Параметры онтогенеза и модель динамики численности популяции *Panagrolaimus detritophagus* (Nematoda: Rhabditida) *in vitro*

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РЕЗЮМЕ

Выявлены параметры онтогенеза и цикла популяции *in vitro* нематод бактериофагов *Panagrolaimus detritophagus*, комменсалов усача *Monochamus galloprovincialis* из сосны *Pinus sylvestris;* нематоды используют жуков в качестве переносчиков. Среднее время развития от яйца до личинок (T): J2 – 1–2 дня, до J3 – 3–4 дня; до J4 – 4–7 дней; до половозрелой особи (G, генерация) – 7 (6–8) дней. В лабораторных культурах популяционный цикл равен 4 поколениям и завершается с 90% долей диапаузирующих личинок J3 (34 день). В фазе роста популяции доля яиц превышает доли других стадий цикла развития: 39±11% – 7 дней; 53±10% – 21 день. Средняя скорость яйцекладки самки 4.5±1.3/сут, из них только

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56±12% яиц приступают к немедленному развитию. Остальные яйца вступают в развитие после 27 дней (4 индивидуальных генераций), что можно рассматривать как форму задержки или краткой диапаузы. Отдельные самки могут накапливать в теле до 4 синхронных яиц и откладывать их единовременно. Средняя продолжительность жизни самки в половозрелом состоянии 13–20 дней. Разработаны формулы экспоненциального роста числа самок и общей популяции нематод.

Ключевые слова: агаровая культура, модельный объект, детритное садоводство, диапауза, экспоненциальная модель, плодовитость, пищевая сеть, жизненный цикл, микробиом, яйцекладка, фазы роста популяции

INTRODUCTION

Nematodes play an important role in the detritus food web; in the phylogenetic tree of the phylum Nematoda the lineages of advanced parasitic taxa contain detritus feeders in basic clades (Holterman et al. 2006; Megen et al. 2009). During colonization of the substrates stressed by chemical pollution, salinity, drought or crop removal, the R-strategists of the first and second succession guilds are bacterial and fungal feeding nematodes; commonly they use detritivorous insects as vectors of their transmissive (dauer) juveniles (Ferris et al. 2001; Ferris 2010).

Many families of saproxylic nematodes are associated with bark and longhorn beetles in their life-cycles. Beetles act as vectors of nematode dauers, or the nematodes play the role of their commensals or parasites (Rühm 1956; Polyanina et al. 2019). A peculiar feature of the detritus food web is that the detritivorous animals do not feed on detritus matter itself. However, these animals use the detritus-inhabiting microorganisms, such as fungi and bacteria, as their foods. The dispersal stages (propagules) of their food microorganisms are carried by detritivores to populate the dead organic matter in their new habitats. Bacteria and fungi in these substrates multiply faster than their hosts and provide the latter with food. This phenomenon has been denoted as the detritivorous horticulture (Odum 1983).

We use the hypothesis of sufficiency of the natural nematode ectosymbionts, such as the unidentified bacteria to provide a nematode population with nutrition for the rapid reproduction. We decided to test this working hypothesis, and in the case of success, to study the development of the nematode population on agar medium *in vitro*, without using other laboratory microorganisms as an artificial food for nematodes. For this purpose, we used the bacterial-feeding nematodes *Panagrolaimus detritophagus* Fuchs, 1930, which inhabit the tree bark and use the beetles *Monochamus galloprovincialis* (Olivier, 1795) as vectors.

The goals of the present study were: (1) To calculate the main parameters of ontogeny of the saproxylic nematode *P. detritophagus*; (2) To construct a population dynamics model as a function of time, under conditions of initial food abundance and the inoculum of mature adults, males and females; (3) To combine the ontogenetic parameters with the key timing and stage pattern data of the population cycle, and thus to determine the number of generations and the final population structure when food resources are exhausted.

MATERIAL AND METHODS

Panagrolaimus detritophagus

Isolate origin: Russia, Nizhny Novgorod region, town Vyxa. GIS: 55.319100, 42.212830. Wood and bark of pine *Pinus sylvestris* L. Imagoes *Monochamus galloprovincialis* (Oliv., 1795). Under elytrae and in folds between head and protorax. Sampling: July 2015; the laboratory nematode isolate was maintained in the Collection of living cultures of the Zoological Institute of the Russian Academy of Sciences (ZIN RAS) in sterile potato sugar agar medium (PSA) with an annual passage via multiplication in branches of the pine *P. sylvestris*.

Preparation of 2% potato sugar agar (PSA) medium

Forty g of peeled potatoes were cut into 1 cm^3 cubes, boiled for 40 min in 200 ml of distilled water, mashed with a press; the solution was filtered through two layers of gauze, poured into a heat-resistant container. Ten ml of glycerol, 4 g of sucrose and 4 g of agar were added to the hot solution, stirred until the reagents were dissolved, the volume of the solution was completed up to 200 ml, and sterilized for 40 min in an autoclave at 110°C and pressure of 3.5 atm. The

Table 1. *Panagrolaimus detritophagus.* Measurements (in µm) and indices of developmental stages. Values are expressed as mean ± standard deviation (minimum-maximum). J1–J4 are juveniles with their stage numbers.

Character / stage	J2	J3	J4	Male	Female
n	10	30	30	20	20
L	262±18 (235–277)	491±40 (369–607)	714±54 (586–823)	1005±70 (904–1065)	929±65 (836–984)
Body width	13±1 (12–14)	22.0±1.7 (20–26)	23.7±1.8 (20–27)	42.5±3.0 (38–45)	36.9±2.6 (33–39)
Genital primordium, length (GPL)	12.7±0.9 (11-14)	27.4±2.4 (18–36)	157.6±12.3 (126–186)	584.7±40.9 (526–620)	546.6±38.3 (492–579)
Genital primordium, width (GPW)	4.8±0.3 (4.5-5.0)	7.7±0.6 (6–9)	9.8±0.9 (6–14)	33.9±2.4 (30–36)	16.1±1.1 (15–17)
a	20.2±2.2 (16–24.6)	22.3±2 (18.2–26.4)	30.1±3 (24.0–36.2)	23.6±2.6 (18.4–28.8)	25.2±3 (19.1–31.2)
GPL/GPW	2.7±0.2 (2.4–2.8)	3.6±0.4 (2.1–5.4)	18.8±0.7 (9.6–28.4)	17.3±1.2 (15.5–18.3)	33.9±2.4 (30.5–35.9)
GPL/L, %	5.0±0.3 (4–5)	5.6±0.4 (4–5)	22.5±2 (16.2–29.1)	58±4 (52–62)	59±4 (53–62)

autoclaved solution was poured at 80°C into 6 cm Petri dishes in a sterile microbiological box. The agar layer should be very thin (1–2 mm) for subsequent observation of nematodes.

Observations were carried out in a 1–2 mm PSA layer at room temperature of 21–23 (22)°C. The juvenile stages (2–4) of nematodes were identified by size (Table 1). The morphology of juveniles and their molts was preliminarily studied; it was found that the first molt occurs in the egg shell, with three subsequent molts after hatching in the PSA medium. In addition to differences in body length, the juvenile stages differ in the size of the sexual primordium. The mature individuals were the largest, with external copulatory organs that were absent in juveniles: the spicules in males and the vulval structures in females.

Inoculation

Fifty most motile adults (females and males at a ratio of 30:20) were individually picked up with an entomological needle and transferred into a 20 µl drop of distilled water. They were washed twice in distilled water. In the sterile agar layer, a truncate pipette tip was used to make three depressions in the center of the Petri dish, then a 20 µl nematode suspension was introduced in these depressions. After 24 hours, bacteria from the surface of the nematode bodies multiplied and spread, forming white 1–3 mm spots on the surface of the agar medium; the nematodes fed at the periphery of the spots.

Observation of ontogenetic stages (from the egg)

After 1 to 3 days, the nematodes laid multiple eggs. Twenty eggs were pipetted into a new Petri dish with the PSA medium to monitor the course of individual development of juveniles and adult individuals. The experiment was performed in 10 replicates. When adult females were found, they were transferred to a new PSA medium to determine the timing of oviposition by new-generation females.

Observations on population dynamics

During the initial period (first generation), it is not difficult to count all nematodes and group them according to age-size stages, as well as to count the number of eggs in a thin layer of agar, for the entire Petri dish surface. However, when the nematode number increased significantly, accurate counting of subsequent generations becomes impossible. An alternative way to estimate the nematode densities using nematode extraction and counting in a sample of small partial volumes (see below) would disturb the microbiome conditions of copulation and egg production. Therefore, we used nematode counting in

a random sample of 15 microscopic light fields, 3 cm^2 in size (10x microscope objective Mikmed-6 var-7., LOMO-Microsystems, https://lomo-microsystems.ru). Counting was performed through a thin layer of agar on the bottom lid, turning the Petri dish upside down. Thus we avoid opening the dish and disturbing the microbiome. The distribution of nematodes across the dish surface was characterized by aggregation around the spots of bacterial colonies. We delineated the areas of nematode concentration with a marker under a stereomicroscope Micromed MS-5-ZOOM LED and then counted nematodes in randomly selected 3 mm^2 light fields within those areas. The pattern of marked areas was photographed using a smartphone, and the total square value of the sum of areas was calculated using ImageJ 1.53e (https://imagej.nih.gov/ij/; National Institutes of Health, USA). Nematode numbers grouped by ontogenetic stage (eggs, J2, J3, J4, females and males) were summed for 15 randomly selected microscopic light fields and then these values were recalculated for the total surface of marked areas of aggregated nematode distribution. Population dynamics were traced in 13 Petri dishes at the following times starting from the moment of inoculation: 1, 2, 3, (4 or 5), 6, 7, 8, 9, 11, 14, 17, 21, 27 and 34 days.

Observations during the first generation

Introducing an inoculum synchronized to the adult stage and with a known number of females (30) makes it possible to calculate the time of one generation by the date of the first noticeable increase in the number of adult individuals. We denote the doubled number of adult females as an indicator of the end of one generation period; it means that all females of the inoculum, on average, laid at least one egg from which a female of a subsequent generation was developed. The date preceding the moment of adulthood of the subsequent generation makes it possible to calculate the total number of eggs laid for a single generation, an average egg production rate, as well as the proportion of eggs that proceed to hatching and a series of molts without delay (diapause).

Formulas for the day before the appearance of females of the second generation (G-1)

The following formulas were used:

F(egg + jj) = [N(egg) + N(J2) + N(J3) + N(J4)]/dd, where F (egg + jj) – mean fecundity of female per day; N(egg) – number of eggs on a counting date; N(J2)... N(J4) – number of juveniles of 2,3 and 4 stages on a counting date; dd – number of days from the moment of inoculation until the counting date (dd = G-1).

% Egg-Dev = [N(J2) + N(J3) + N(J4)]/[N(egg) + N(J2) + N(J3) + N(J4)], where % Egg-Dev – proportion of eggs, immediately starting to molt without a pause.

% Egg (diapause) = 1 – % Egg-Dev, where % Egg (diapause) – proportion of eggs with the arrested development (diapause).

Determination of the time of the end of the population cycle

When the number of females ceased to grow and the number of eggs decreased to rare finds, we considered that the population cycle was completed due to the exhaustion of food resources by the increased population. After counting in the light fields, we flushed the Petri dish with distilled water five times and poured the nematode suspension from a Petri dish into a 1.5 ml Eppendorf tube, then centrifuged the tube in a Microspin FV-2400 centrifuge-vortex (Biosan) at 2800 rpm; the supernatant was withdrawn with a syringe, the nematode suspension sediment was washed 5 times with distilled water and centrifuged in a vortex as described above, then fixed with hot TAF (4% formaldehyde with the addition of 2 ml triethanolamine per 100 ml solution as a buffer; in water bath with boiled water for 30 min) (Ryss 2017a).

Two days after fixation date (time of hardening of the morphological structures of fixed worms), nematodes grouped by stage were counted in 10 drops of 20 μl volume to control the last count in the light fields in a Petri dish. The number of nematodes detected in 20 μl drop was recalculated to the entire 1.5 ml microtube volume, which corresponded to the number of nematodes in the Petri dish at the last count. Permanent collection slides were made from the nematode suspension by the express method of Ryss (2017b).

Statistical calculations were performed using the MS Excel software. Calculations of the mean and standard deviation of the sample were used; Student's test (module "Student.test") was used to assess the statistical significance values of difference between the samples.

Parameters and indices

Indices of individual development. G (egg-egg) is the duration of ontogeny (generation), time of

Table 2. *Panagrolaimus detritophagus.* Duration of ontogenetic stages (days). Values are expressed as: mean ± standard deviation (minimum-maximum). J1–J4 – juveniles with their stage numbers. G – number of days in one generation; left column – after the moment of egg laying (individual observation, n = number of eggs); right column – timing of population development after inoculation of 50 individuals (30 females and 20 males; n = number of Petri dishes during monitoring). F(1d) and F(G) – fecundity, i.e. the number of eggs per day laid by one female during the first day after inoculation of adults and the mean fecundity calculated for the whole period of the first generation, respectively. Proportion of eggs without diapause (% Egg-Dev) is the ratio of the number of all juveniles to the total number of juveniles and eggs laid by females during the whole period of the first generation.

	Individual studies (from a group of 20 eggs)	Population studies
n	20	13
Egg laying start	–	1.1±0.3 (0–2)
J2	1	1.8±0.4 (1–2)
J3	3	3.8±0.4 (3–4)
J4	5.6±0.5 (5–6)	5.0±1.0 (4–7)
G-Female	6.7±0.5 (6–7)	7.3±0.8 (6–8)
G-Male	6.7±0.5 (6–7)	7.3±0.8 (6–8)
F (1d)	–	7.8±2.7 (6–11)
F(G)	–	4.5±1.3 (3–6)
% Egg-Dev, proportion of eggs without diapause	–	56±12 (42–75)

individual development from egg to egg. Due to the developmental arrest of some (diapausing) eggs, this value varies greatly. Therefore, an equivalent parameter may be calculated: the average developmental time from a female of the previous generation to a female of a subsequent generation, G (fem-fem) (see below). T(J2), T (J3), T(J4), G-Female and G-Male is the timing of development from the egg to the corresponding subsequent stages: second, third, fourth stage juvenile and the nematode adult.

Indices of population cycle. Inoculum is the number of active adult females and males used to start the experimental culture in agar medium. The start of oviposition is the time (day) of the first egg laying by a group of inoculated females. H (J2..J4) is the time when the number of juveniles of the J2, J3 or J4 stage becomes equal to or exceeds the number of inoculated females. G (fem–fem) is the day when the number of adult females exceeds at least twice the number of inoculated females. Pre-adult phase of the population cycle is the maximum period from the moment of inoculation when the number of females in the population is still equal to their number in the inoculum, but there are already numerous juveniles of different stages of the subsequent generation. Numbers of juveniles at this time were used to calculate the mean number of eggs produced by a female per day during the first generation as well as the percentage of eggs hatched without a developmental delay. Percentage of eggs proceeding to immediate reproduction (% Egg-Dev) and the residual index of % Egg-Dia = 1 – % Egg-Dev is the proportion of diapausing eggs.

RESULTS

Parameters of ontogenesis

For population observations, the correspondence of juvenile stages and size groups was established (Table 1). The first molt occurred in the eggshell, J2 hatched from the eggshell, further transforming in a series of molts into J3, J4 and adult male and female. The latter proceeded to oviposition of the subsequent generation. All observations in this study were based on the correspondence of the size groups to the developmental juvenile stages.

On average, the development from egg to new generation (G) egg was completed in 7 days (Table 2). However, the G values obtained in two series of experiments differ by approximately 0.5 days. There were (1) observations of the population synchronized

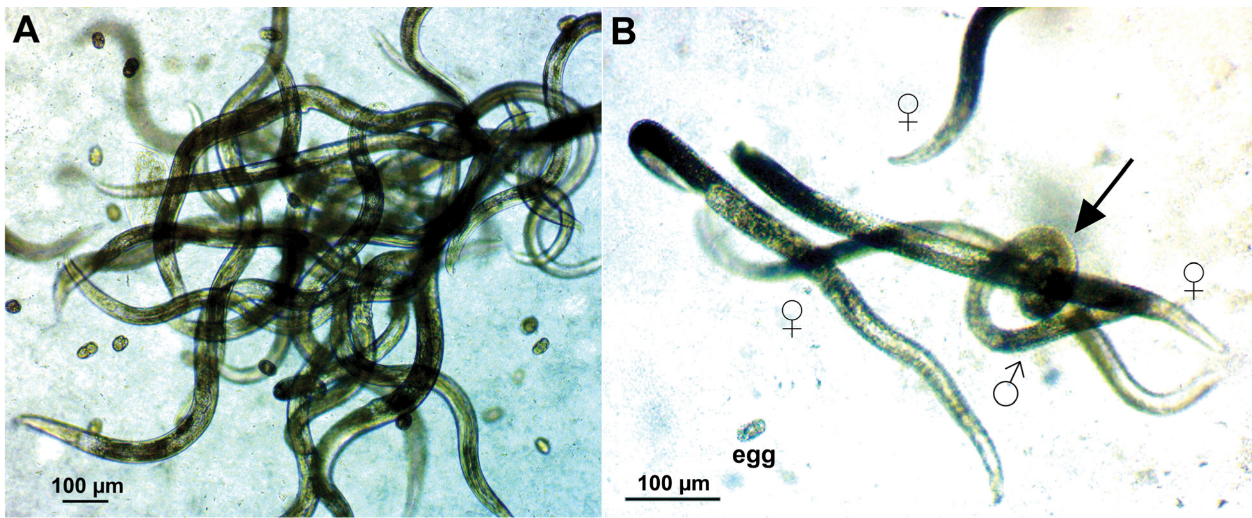

Fig. 1. *Panagrolaimus detritophagus.* A – swarming of adult males and females. B – copulation. Arrows indicate the copulation pose: male captures the vulval area of female with his tail, forming a ring around her body. Male, female and egg are marked by signs.

at the egg stage (n = 20) and (2) observations of the population synchronized at the adult stage (females and males, 30 and 20, respectively; n = 13; cultures studied in Petri dishes). This difference is happened because females in the second series of experiments must first lay eggs, which takes time ranging from 0 to 1 day (Table 2).

Although the ranges of development time of different stages overlap, they are significantly different at $\alpha < 0.05$ (t-test). Females initially lay an excessive number of eggs, which are accumulated in the population, and only slightly more than half (56±12%) of eggs immediately proceeds to subsequent molts, the rest being delayed in a temporary diapause. The average fecundity per female also differs by two different estimates; F = 7.8±2.7 when counted on the first day for inoculated adult females *vs.* F = 4.5±1.3 calculated for the whole period of the first generation. These differences were expected, because the largest and most active adults were selected for the inoculum; the inoculated females contained the synchronous eggs in their bodies. In contrast, the breeding population of the first generation was characterized by slower oviposition rates as the nematode food source are bacteria from the nematode surface coat; they need some time to be multiplied on agar medium.

Population dynamics

When assessing the population size, the high aggregation of mature individuals and preadult J4 juvenile is striking. Nematodes form mobile swarming clusters in which female nematodes copulate with males and lay eggs (Fig. 1). The nematode swarming clusters were located at the periphery of bacterial colonies, and the surface of the agar medium in these zones was destroyed almost to the bottom of Petri dishes. In these clusters (harems), a single male fertilized 3–5 females, or there were groups of two males and 5–8 females. Single females of the population actively moved towards the swarming clusters. As bacteria were eaten away, the black patches of nematode excretions surrounded by clutches of 30–300 eggs in each microscopic light field remained in the places of former swarming zones.

The most important parameter of population cycle is the number of females, because their fecundity ensures the growth of the whole population. After the period of the first generation (G = 7), an exponential increase in the number of females was observed until the 17th day, after which the female adults' number reached its maximum level and then decreased, first slowly (21–27 days) and then rapidly (27–34 days) (Fig. 2). It can be concluded from the diagram that there are no more than four generations in the population cycle (27:7); the decrease in the number of females after 27 days can be explained by the limited lifespan of most females. Since the number of first-generation females is a small portion of the total population, the female lifespan has to be 7 days (G) shorter than the time of population decline

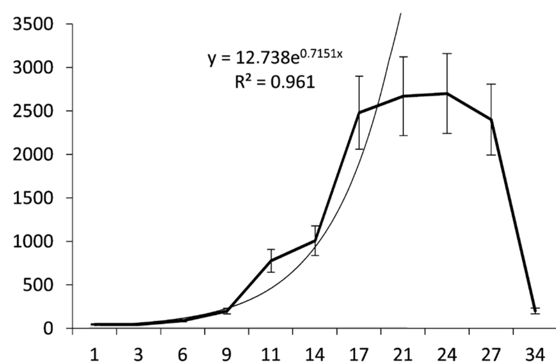

Fig. 2. *Panagrolaimus detritophagus.* Number of females in the population cycle in a laboratory culture. Ordinate axis: abundance, abscissa axis: number of days after inoculation with 50 adult nematodes. Error bars show standard deviation; the smooth curve serves as an approximation of the exponential growth in the period up to 21 days after inoculation.

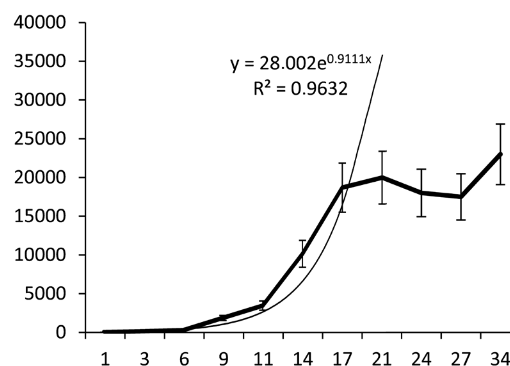

Fig. 3. *Panagrolaimus detritophagus.* Changes in the total abundance of worm stages (juveniles, males, and females) during the population cycle in a laboratory culture. Ordinate axis: abundance, abscissa axis: number of days after inoculation with 50 adult nematodes. Error bars show standard deviation; the smooth curve serves as an approximation of the exponential growth in the period up to 21 days after inoculation.

(20–27 days). Consequently, the female lifespan in the sexually mature phase is 13–20 days at 22°C.

The exponential growth formula for the number of females as a function of time (from the 1st to 21st day): $y = 12.738e^{0,7151x}$; $R^2 = 0.961$. For the same period the exponential growth formula of the whole population of worm-like individuals (except eggs): $y = 28.002e^{0,9111x}$; $R^2 = 0.9632$ (Fig. 3). A similar increase up to 21–27 days is characteristic of the number of eggs (Fig. 4), followed by a rapid decline to the lowest values due to an increase in the number of J3 juveniles by the end of the population cycle.

In the dynamics of relative abundances of juvenile stages, starting from the second (i.e., the first post-inoculum) generation, there is predominance of eggs in the population until the population number maximum at the 21st day. At the end of population cycle (day 34), over 90% of the population were the J3 survival juveniles, which indicated late hatching of most eggs (Table 3).

DISCUSSION AND CONCLUSIONS

(1) Mean development time (T), from egg to J2 was 1–2 days; to J3, 3–4 days; to J4, 4–7 days; and to adults, 6–8 days. The total duration of ontogeny is about 7 days on average, which agrees with the previous studies of the *Panagrolaimus* spp. (Honnens et al. 2013).

(2) The new approach resulted in the actual direct calculation of the number of generations (4) for *in vitro* conditions; changes in the densities of specimens belonged to different ontogenetic phases were examined across the whole population cycle, from the inoculation with synchronous adult stages up to the final multiplication standstill with the prevalence of survival stages (J3). A new contribution to the population model is the formulas for the exponential growth of the number of females and the total number of worm-like stages.

(3) In *Panagrolaimus*, abundant oviposition does not mean an immediate hatching from eggs and start of larval development. A mass of eggs is accumulated, of which only 56±12% proceed to immediate development. The remaining eggs entered development after 27 days (4 individual generations), which may be regarded as a form of delay or a brief diapause. This diapause is temporary because by the end of the population cycle (34 days) almost all eggs produced the juveniles, which developed to J3 stage. This is a new feature of the panagrolaimid cycle, previously unknown and distinguishing them from other rhabditid nematodes. The adaptive significance is obvious: in a short period of time, nematodes need to fully populate an unstable food substrate with the survival stages, which in the case of abrupt desiccation would not be able to ensure the stable development of the worm-like stages.

(4) The average lifespan of the adult *Panagrolaimus* female is between 13 and 20 days, after which the number of females sharply decreases and numerous dead individuals are seen in the culture. This lifespan

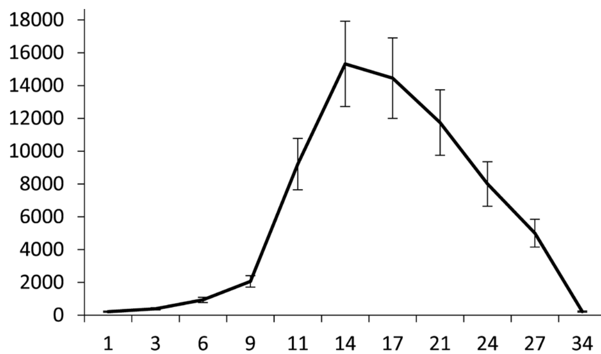

Fig. 4. *Panagrolaimus detritophagus*. Changes in the number of eggs during population cycle in a laboratory culture. Ordinate axis: abundance, abscissa axis: number of days after inoculation with 50 adult nematodes. Error bars show the values of standard deviation from mean.

duration is close to the values previously reported for the *Panagrolaimus* spp. (Honnens et al. 2013). If we add the developmental time from egg to female, the total lifespan of the female nematode individual can be 20–27 days.

(5) The population cycle completed with 90% of J3 juveniles that are probably the main survival stages. Then the number of eggs and female adults sharply decreased. Probably, it was caused by the overconsumption of the bacterial film, which was the source of nematode nutrition. Completion of the cycle by a sharp increase in the number of survival juveniles was revealed earlier for *Panagrolaimus* spp. and *Ditylenchus* spp. (Perry 1977, 1999; Perry and Moens 2013).

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REFERENCES

Ferris H. 2010. Contribution of nematodes to the structure and function of the soil food web. *Journal of Nematology*, **42**(1): 63–67.

Ferris H., Bongers T. and Goede De R.G.M. 2001. A framework for soil food web diagnostics: Extension of the nematode faunal analysis concept. *Applied Soil Ecology*, **18**: 13–29. https://doi.org/10.1016/S0929-1393(01)00152-4

Holterman M., Van Der Wurff A., Van Den Elsen S., Van Megen H., Bongers T., Holovachov O., Bakker J. and Helder J. 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution*, **23**: 1792–1800. https://doi.org/10.1093/molbev/msl044

Honnens H., Assheuer T. and Ehlers R. 2013. Desiccation and storage of *Panagrolaimus* sp. (strain NFS-24-5). *Nematology*, **15**(5): 557–566. https://doi.org/10.1163/15685411-00002701

Megen H.V., Van Den Elsen S., Holterman M., Karssen G., Mooyman P., Bongers T., Holovachov O., Bakker J. and Helder J. 2009. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology*, **11**: 927–950. https://doi.org/10.1163/156854109x456862

Odum E.P. 1983. Basic Ecology: Fundamentals of Ecology. Philadelphia, Saunders College Pub., 325 p.

Perry R.N. 1977. The water dynamics of stages of *Ditylenchus dipsaci* and *D. myceliophagus* during desiccation and rehydration. *Parasitology*, **75**: 45–70. https://doi.org/10.1017/S0031182000048320

Perry R.N. 1999. Desiccation survival of parasitic nematodes. *Parasitology*, **119**: 19–30. https://doi.org/10.1017/S0031182000084626

Perry R.N. and Moens M. (Eds). 2013. Plant nematology. Second edition. CABI Publishing, Wallingford, Oxfordshire, Boston, 536 p. https://doi.org/10.1079/9781780641515.0000

Polyanina K.S., Mandelshtam M.Y. and Ryss A.Y. 2019. Brief review of the associations of xylobiont nematodes with bark beetles (Coleoptera, Curculionidae: Scolytinae). *Entomological Review*, **99**: 598–614. https://doi.org/10.1134/S0013873819050038

Rühm W. 1956. Die Nematoden der Ipiden. *Parasitologische Schriftenreihe*, **6**: 1–435.

Ryss A.Y. 2017a. The simplest “field” methods for extraction of nematodes from plants, wood, insects and soil with additional description how to keep extracted nematodes alive for a long time. *Parazitologiya*, **51** (1): 57–67.

Ryss A.Y. 2017b. A simple express technique to process nematodes for collection slide mounts. *Journal of Nematology*, **49**(1): 27–32. https://doi.org/10.21307/jofnem-2017-043