

Influence of the gregarine *Stenophora julipusilli* (Eugregarinorida, Stenophoridae) on the trophic activity of *Rossiulus kessleri* (Diplopoda, Julidae)

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Abstract

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Analysis of the contents of the intestines of *Rossiulus kessleri* (Lohmander, 1927) revealed presence of gamonts of *Stenophora daulphinia* Watson, 1916, *S. juli* (Frantzius, 1846) Labbe, 1899 and *S. julipusilli* (Leidy, 1853) Crawley, 1903. For the analysis of the consumption of leaves of *Acer negundo* L. in the course of a two week laboratory experiment 122 specimens of *R. kessleri* infested with *S. julipusilli*, and 32 specimens not infested with gregarines were investigated. The initial and final body weight of each millipede was determined as well as the mass of food consumed, the amount of excrement produced and the extent of infestation with gamonts of *S. julipusilli*. No relationship between the amount of food consumed and initial weight of *R. kessleri* specimens was found. Nor was any dependence between the amount of excrement, amount of food consumed and change in body mass established. *S. julipusilli* gamonts and syzygys were found in 79.2% of millipede specimens. The infestation with gamonts of younger *R. kessleri* specimens was significantly lower than with average to large specimens of millipedes. The sex of the host was not found to influence food consumption, change in body mass or in mass of faeces. The extent of infestation with gamonts of *S. julipusilli* did not significantly differ between males and females of *R. kessleri*. In specimens not infested with gregarine gamonts the food consumption during the two-week experiment was at a high level, in those with a slight infestation (1–8 gamonts) it increased by 29%, in those with average and high extent of infestation it decreased by 8% compared with uninfested individuals. During the experiment influence of the number of *S. julipusilli* gamonts upon change in body mass and production of excrement by *R. kessleri* was not observed.

Keywords

change in body mass, gregarine, litter consumption, saprophagous invertebrate, *Stenophora*

Introduction

Up to now no attempt has been made to form a quantitative assessment of the influence of gregarines upon the trophic activity of millipedes. The scattered studies which have been made of gregarines which parasitize insects (SIVA-JOTHY and PLAISTOW, 1999; CANALES-LAZCANO et al., 2005; RODRIGUEZ et al., 2007) show a weakly negative impact of the parasites upon the organisms of their hosts. However, the capacity of many gregarine species to achieve high populations wi-

thin certain host individuals excludes the possibility of making a clear unambiguous assessment of their impact upon the metabolic activity of a macroorganism.

The ecological peculiarities of diplopod gregarines have been studied in India (BHAMARE et al., 2014), Taiwan (CHANG et al., 2004), Japan (HOSHIDE et al., 1970), Poland (LIPA, 1967), Germany, Yugoslavia (GEUS, 1969), Slovakia (VALIGUROVÁ and MATIS, 2001) and some other countries. Diplopod gregarines belonging to the Ukrainian fauna have remained unstudied until now.

The species of the *Stenophora* genus are included in a well delineated group – the Stenophoridae family (GEUS, 1969). A systematic revision of gregarines (CLOPTON, 2002, 2009) raised them to the rank of superfamily Stenophoricae. The species of the *Stenophora* genus parasitize centipedes (Myriapoda: Chilopoda) and millipedes (Myriapoda: Diplopoda). The ecological peculiarities of the species of this genus remain unstudied. The literature dedicated to morphology and description of gregarines of this genus (DA COSTA and BARTH, 1967; LIPA, 1967; GEUS, 1969; HOSHIDE et al., 1970; VALIGUROVÁ and MATIS, 2001) has made only fragmentary references to the peculiarities of the origins of certain development stages of *Stenophora* species.

One of the most common diplopod species of the steppe and forest-steppe zones of Ukraine, Belarus and Russian Federation is *Rossiulus kessleri* (Lohmander, 1927). Scientists have long considered it a convenient object for bioindication and morphological researches (PRISHUTOVA, 2001a; POKHYLENKO, 2014). Studies have been made of its distribution (CHERNYI and GOLOVACH, 1993), development cycle (STRIGANOVA, 1996; PRISHUTOVA, 1988a, 2001b; BRYGADYRENKO, 2004), habitat preferences (POKARZHEVSKIJ, 1983; PRISHUTOVA, 1988b; POKARZHEVSKIJ et al., 1989), temperature conditions (STRIGANOVA, 1972) and range of diet (STRIGANOVA and PRISHUTOVA, 1990; KULBACHKO and DIDUR, 2012; SVYRYDCHENKO and BRYGADYRENKO, 2014). The gregarine fauna inhabiting the intestines of this species have been little studied up to now. GEUS (1969) reported the discovery in the intestines of this millipede species of *Stenophora juli* (Frantzius, 1846) Labbe, 1899, *S. julipusilli* (Leidy, 1853) Crawley, 1903 and *S. daulphinia* Watson, 1916. Unfortunately, the nomenclature of diplopod species suffers from a confusing range of synonyms, and *R. kessleri* has been described by more than 15 different names (CHERNYI and GOLOVACH, 1993). That is why even within a single publication (GEUS, 1969) it is designated by a variety of different names.

At the beginning of the research we held a null hypothesis that gregarines in low, average and high numbers have an equally low impact upon their hosts' food consumption. Besides this, the objective of this research is to examine the following hypotheses: (1) maximum increase in body mass will be seen in the millipede specimens that have maximum levels of food consumption, (2) at different development stages diplopods have different levels of gregarine infestation, the level of infestation increasing with the age of the host, (3) the number of gregarines in the intestines will influence changes in body mass and the tempo of faecal formation, the higher the number of gregarines the lower the body mass of the host on account of rapid formation of excrement.

Materials and methods

Objects of the experiment

Specimens of *R. kessleri* were taken manually on 22 May 2014 from the litter and soil surface of a wind-break forest plantation of *Acer negundo* L. near Aleksandrovka village (48°46'N, 34°56'E), Magdalinovka district, Dnipropetrovsk region, Ukraine. 160 *R. kessleri* specimens were used in this study. Green leaves of a single *A. negundo* tree were collected in the middle of May at 2 meters height from the ground. They were collected from the same plantation where the *R. kessleri* were collected. The leaves were air-dried over a week, without exposure to direct sunlight. Then they were sorted (leaf stalks, deformed leaf laminae, leaves damaged by insects, and by bacterial and fungal diseases were removed). The remaining leaves, which were uniform in size and condition were dried over two hours at a temperature of +60 °C and 20% humidity, weighed to an accuracy of 0.5 mg and placed ($1,550 \pm 268$ mg) in separate containers for the millipedes. The containers were 0.5 l polystyrene cups.

Experimental technique

Before the experiment each millipede was placed in a small separate container without food for no more than 3–4 hours. For preventing infestation of leaves and containers by gregarine gametocytes or oocysts, bacterial spores, microspora, fungal spores, viral polyhedrons, this task was performed with scrupulous care and cleanliness (TARASEVICH, 1975). Then each *R. kessleri* individual was weighed and put in a separate experimental container with previously weighed leaves of *A. negundo*. After the experiment the leaves in the 160 experimental and 10 control (leaves without millipedes) containers were moistened from a sprayer, and then covered with clean sheets of standard Xerox paper for preventing moisture evaporation. Each morning the control cups and cups with *R. kessleri* were moistened from a sprayer with equal amounts of distilled water.

Determination of body weight of the millipedes

The weight of the millipedes, their faeces and the litter was determined with the use of a torsion balance (to an accuracy of 0.5 mg). Throughout the study, a consistent temperature of +25 to +27 °C and air humidity of 70–90% was maintained in the laboratory. Food consumption (C_f^*) was determined with a modified formula of DAVID (1998): $C_f^* = (M_0 - M_0D - M_n) / (1 - D)^{1/2}$, where M_0 – initial food mass (dry mass) offered to each millipede for consumption, M_n – food mass (dry mass) not consumed by a millipede by the end of the exper-

periment, D – coefficient of reduction of food mass as a result of its microbiological decomposition calculated with the use of control set of experiments ($n = 10$) in identical containers without millipedes ($D = (M'_0 - M'_n) / M'_0$, where M'_0 and M'_n – dry mass of food at the beginning and at the end of control experiment without the presence of millipedes). Microbiological decomposition of leaves, which were moistened in the same way as the containers with millipedes, for two weeks, was $3.5 \pm 1.7\%$.

Identification of gregarines

After the experiment the millipedes were taken out of the containers, transferred into clean containers without food and during two days were analysed to define the extent of infestation of their intestines with gregarines. The observations were made using microscope with $\times 5$, $\times 10$ and $\times 40$ planapochromatic objectives. For controlling the counts of the number of gamonts 10% of specimens were checked a second time. In the control count the coefficient of variation in the number of gamonts was 2–14%. The gregarines were identified using GEUS (1969) and CLOPTON (2002). Additional sources used in identification and morphology of *Stenophora* species were the works of DA COSTA and BARTH (1967), LIPA (1967), HOSHIDE et al. (1970), VALIGUROVÁ and MATIS (2001), BHAMARE et al. (2014). For identification of the species of the genus *Stenophora* we used total length, length and width of the protomerite, and length and width of the deutomerite. In addition to this we used the ratios of length of protomerite to total length and width of protomerite to width of deutomerite. Our morphological data of *S. julipusilli* corresponded very closely to the measurements presented in GEUS (1969).

Statistical data processing

Exploratory data analysis (EDA) was performed in the MS Excel software package. The rate of microbial decay, rate of consumption of leaf litter, changes in body weight and daily formation of faeces were calculated for individual specimens of *R. kessleri* for each specific container. Statistical data analysis was performed in Statistica 8.0 software package (HILL and LEWICKI, 2007). To characterise particular samples the following characteristics were provided: mean \pm SD, minimum and maximum and median. Samples were compared using one-way analysis of variance (ANOVA). Differences between sample means were detected by the Tukey test and considered significant at $P < 0.05$.

Results

Our analysis of the content of the intestines of *R. kessleri* showed gamonts of *Stenophora daulphinia* Watson,

1916, *S. juli* (Frantzius, 1846) Labbe, 1899 and *S. julipusilli* (Leidy, 1853) Crawley, 1903. The first two species comprised no more than 2% of the total number of gregarines in the intestines of the millipede individuals studied in the experiment (taken from Magdalinovka District of Dnipropetrovsk region). The numbers of *S. julipusilli* usually reached 100% of all studied gregarines in the intestine of a single millipede. *S. daulphinia* and *S. juli* were found in the intestines of 6 out of 160 studied specimens. For the sake of the accuracy of the experiment 6 millipede specimens with counts of *S. daulphinia* and *S. juli* under 30% of the total number of gregarines in their intestines were excluded from the subsequent analysis. Thus, 154 millipede specimens infested with *S. julipusilli* were used for analysing trophic activity.

Linear dependence between the amount of food consumed and the body mass of *R. kessleri* was not found (Fig. 1a). There was also no such dependence between the amount of excrement and food consumption (Fig. 1c), and change in body mass (Fig. 1d). An increase in size of millipedes in the experiment also did not bring a statistically significant change in one of the most important ratios, which reflects the efficiency of food processing (the ratio of change in body mass to mass of food consumed, Fig. 1b).

The initial body mass of *R. kessleri* in the experiment was 452 ± 130 (122–692) mg. The males on average weighed 20% less than the females (404 ± 125 and 510 ± 113 mg respectively, $F = 29.84$, $F_{0.05} = 3.90$, $df = 1, 152$, $P < 0.001$). The asymmetry values ($As = -0.19$) and excess values ($Ex = -0.72$) do not exceed critical values for $P = 0.05$ (0.321 and 0.834 respectively). Thus, the distribution of individuals according to weight in the *R. kessleri* population studied may be considered normal. This normal distribution of size classes of *R. kessleri* is typical for three populations of the species which we have studied (in Dnipropetrovsk, Magdalinovka, and Novomoskovsk districts of Dnipropetrovsk region). After the two week experiment the module of asymmetry increased ($As = -0.32$), and the module of excess decreased ($Ex = -0.44$): the distribution pattern became less marked by sharp peaks, though larger individuals gained weight more intensively than smaller ones. This led to a shift of distribution peak to the right. Nonetheless the distribution of the animals according to weight remained normal.

The decrease in body mass of *R. kessleri* (mg/individual) during the two-week experiment when fed on leaves of *A. negundo* (Table 1) was on average 15.6 ± 34.2 (from -136 to $+81$) mg. A statistically significant negative asymmetry ($As = -0.36$, $P < 0.05$) and positive excess ($Ex = 0.89$, $P < 0.01$) were registered – i.e. if millipedes gained weight, they significantly increased in size, if they lost weight they only increased in size slightly. No millipedes died in the experiment. During the experiment there were no statistically significant differences in changes in body mass in different size classes of *R. kessleri*. The size classes with

minimal and maximum body mass (less than 250 and more than 550 mg) were characterised by minimum variability in body mass.

The millipedes' food consumption during the two-week experiment varied from 1 to 277 mg, on average 113 ± 65 (median – 101) mg. A statistically significant

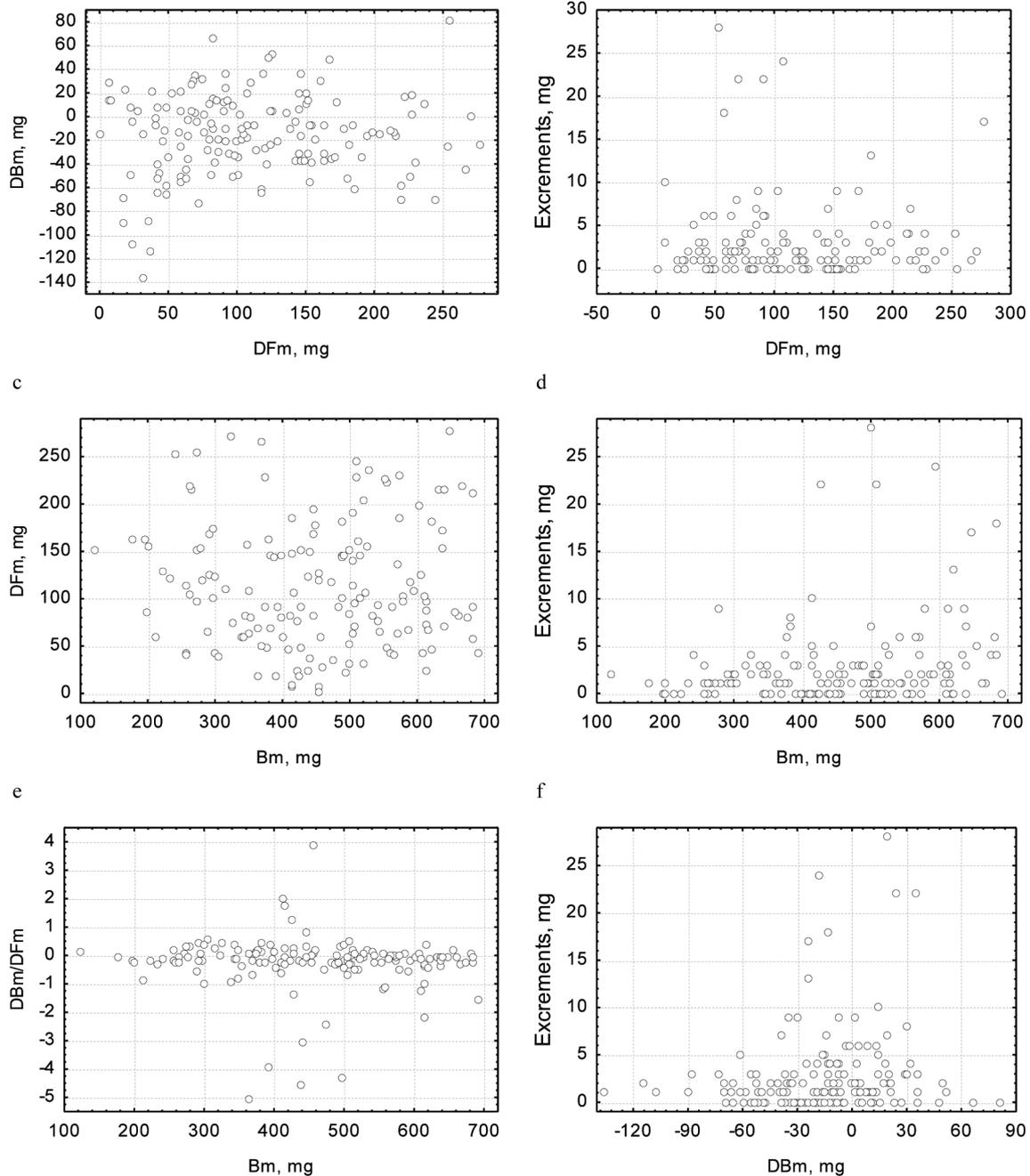


Fig. 1. The characteristics of trophic activity of *R. kessleri* individuals fed on the leaves of *A. negundo* during two weeks: a – relationship between change in body mass (ordinate, DBm, mg) and amount of food consumed (abscissa, DFm, mg); b – relationship between mass of excrements (ordinate, mg) and mass of food consumed (abscissa, DFm, mg); c – relationship between mass of food consumed (abscissa, DFm, mg) and body mass (abscissa, Bm, mg); d – relationship between mass of excrements (ordinate, mg) and the body mass (abscissa, Bm, mg); e – relationship between efficiency of food consumption (ordinate, DBm/DFm) and body mass (abscissa, Bm, mg); f – relationship between mass of excrements (ordinate, F, mg) and the millipedes' body mass (abscissa, Bm, mg).

asymmetry ($As = 0.526$, $P < 0.01$) and statistically insignificant negative excess ($Ex = -0.453$, $P > 0.05$) were registered, i.e. food consumption by most of the individuals make up smaller values than the average. No differences in amount of leaves consumed for different size classes of millipedes were registered (Table 1). During two weeks some *R. kessleri* individuals consumed an amount of food that exceeded their body weight (Fig. 1e).

On average during two weeks a single *R. kessleri* individual produced 2.8 ± 4.6 (from 0 to 28, median – 1.0) mg of excrement. Within the distribution of produced excrement (Fig. 1f) a statistically significant positive asymmetry is seen ($As = 3.28$, $P < 0.001$). The median quantity of excrement increases from 1 to 4 mg, though no statistically significant relationship between increase in the quantity of excrement and increase in an individual's body mass was observed (Table 1).

S. julipusilli gamonts and syzygy were found in 122 of the 154 millipedes individuals used in the experiment (79.2%). On average 17 ± 24 *S. julipusilli* gamonts were found in a single *R. kessleri* individual (median – 8). The distribution of parasites in the selection is asymmetric (2.23 , $P < 0.001$), with a notable excess (5.73 , $P < 0.001$), which indicates an average and a low rate of infestation of most *R. kessleri* individuals. Although our research concentrates on the weight of the

millipedes, it is interesting to note that infestation by gregarine gamonts of second and third year *R. kessleri* individuals (up to 350 mg) is significantly lower than in fourth and fifth year millipedes (Table 1).

No influence of the host's sex was found upon consumption of the food plant (males – 106 ± 69 mg, females – 121 ± 59 mg, $F = 1.91$, $F_{0.05} = 3.90$, $df = 1, 152$, $P = 0.169$), changes in body mass (males: -15 ± 38 mg; females: -17 ± 29 mg; $F = 0.09$, $F_{0.05} = 3.90$, $df = 1, 152$, $P = 0.761$) and mass of faeces produced by the millipedes (males: 3.4 ± 5.4 mg; females: 2.1 ± 3.2 mg; $F = 3.07$, $F_{0.05} = 3.90$, $df = 1, 152$, $P = 0.082$). Also no statistically significant differences were found between males and females of the *R. kessleri* specimens in the level of infestation with *S. julipusilli* gamonts (males: 14 ± 22 specimens; females: 21 ± 25 specimens; $F = 2.67$, $F_{0.05} = 3.90$, $df = 1, 152$, $P = 0.105$).

According to the results of the experiment it can be stated that food consumption of *R. kessleri* decreased insignificantly when there was an increase in the number of *S. julipusilli* gamonts in their intestines (Table 2). In the absence of gregarine gamonts food consumption was at a quite high level (121 ± 66 , median – 109 mg), with slight level of invasion (1–8 gamonts) it increased by 29% (136 ± 69 , median – 141 mg), with a high level of invasion (33–133 gamonts) it decreased by 8% (108

Table 1. Change in body mass, food consumption, production of excrement by *R. kessleri* during the two-week experiment and number of *S. julipusilli* gamonts in *R. kessleri* intestines

Characteristics	Body mass, mg	n	$\bar{x} \pm SD$	Min	Max	Median	$F (F_{0.05} = 2.28, df = 5, 148)$	P
Change in body mass of <i>R. kessleri</i> , mg/individual	<250	12	-20.1 ± 20.5	-51	20	-23	1.53	0.183
	250–349	25	0.1 ± 36.2	-58	81	0		
	350–449	37	-14.1 ± 42.1	-114	66	-12		
	450–549	40	-18.2 ± 33.5	-136	35	-15		
	550–649	26	-23.1 ± 27.1	-73	27	-20		
	>650	14	-22.3 ± 25.3	-70	14	-14		
<i>R. kessleri</i> food consumption, mg/individual	<250	12	123.4 ± 60.9	41	253	125	1.33	0.255
	250–349	25	125.5 ± 64.2	39	271	110		
	350–449	37	95.6 ± 64.8	7	267	82		
	450–549	40	110.6 ± 63.1	1	245	103		
	550–649	26	108.6 ± 62.4	23	229	98		
	>650	14	140.7 ± 76.6	42	277	122		
Production of excrement by <i>R. kessleri</i> , mg/individuals	<250	12	1.1 ± 1.3	0	4	1	1.93	0.093
	250–349	25	1.7 ± 1.9	0	9	1		
	350–449	37	2.5 ± 4.2	0	22	1		
	450–549	40	2.8 ± 5.5	0	28	1		
	550–649	26	3.6 ± 5.3	0	24	2		
	>650	14	5.6 ± 5.7	0	18	4		
Number of <i>S. julipusilli</i> gamonts in <i>R. kessleri</i> intestines, mg/individual	<250	12	$5.5 \pm 9.7a$	0	32	1	3.99	0.002
	250–349	25	$2.6 \pm 4.9a$	0	19	0		
	350–449	37	$21.2 \pm 30.5b$	0	133	10		
	450–549	40	$24.3 \pm 23.1b$	0	88	18		
	550–649	26	$15.8 \pm 21.7b$	0	107	9		
	>650	14	$24.2 \pm 25.3b$	0	88	19		

± 67 , median – 100 mg) compared to uninfested *R. kessleri* specimens.

No influence was noted of the number of *S. julipusilli* gamonts upon the change in *R. kessleri* body mass and amount of excrement produced when fed on leaves of *A. negundo* (Table 2). Thus, with a low rate gregarine infestation the millipedes, despite an increase in food consumption, produce the same amount of excrement and did not gain weight compared to healthy individuals. An average or a high-rate of gregarine invasion did not lead to a statistically significant increase in the amount of food consumed, body weight and amount of excrement.

If gregarines have a low impact upon the characteristics of trophic and metabolic activities of their hosts, then it is interesting to follow the inverse dependence: how do tempi of food consumption, formation of excrement and changes in body mass affect the numbers of *S. julipusilli*. No statistically significant influence was found of rates of food consumption (Fig. 2), rates of excrement formation (Fig. 2b) and changes in body mass (Fig. 2c) upon the numbers of gregarines ($F = 0.85$, $F_{0.05} = 2.16$, $df = 6, 147$, $P = 0.533$ for food consumption, $F = 0.97$, $F_{0.05} = 2.43$, $df = 4$,

149 , $P = 0.424$ for mass of excrement and $F = 1.12$, $F_{0.05} = 2.16$, $df = 6, 147$, $P = 0.351$ for change in body mass).

Discussion

The consumption of diplopods is not regulated by the regularities general for most invertebrates. The standard ratio for calculating the efficiency of conversion of food consumed into biomass of the body is the ratio of change in body mass (DBm) to mass of food consumed (DFm). If the duration of an experiment is at least 2–3 cycles of filling and voiding the intestine (for most invertebrates that is several days, for a human, in general, – 1.5 days) the DBm/DFm ratio becomes a valuable indicator of an extent of food fixation, its biological significance for an animal. However, for large species of Julidae, for example *R. kessleri*, we have observed rhythmicity in the digestion processes (SVRYDCHENKO and BRYGADYRENKO, 2014). Over several days diplopods can intensively consume food, increasing their body mass by 20–80%. After that they do not eat for

Table 2. Influence of number of *S. julipusilli* gamonts in intestines upon food consumption, changes in body mass and mass of excrement of *R. kessleri* during the two-week experiment

Characteristics	Number of gamonts in a single <i>R. kessleri</i> individual	<i>n</i>	$\bar{x} \pm SD$	Min	Max	Median	$F (F_{0.05} = 2.07, df = 7, 146)$	<i>P</i>
Food consumption of <i>R. kessleri</i> , mg	0	32	121.1 \pm 65.5a	1.2	270.9	109.2	2.67	0.013
	1–2	22	127.4 \pm 73.9a	8.1	266.5	116.6		
	3–4	12	160.6 \pm 67.1b	71.1	255.2	172.6		
	5–8	12	126.8 \pm 60.5a	45.7	222.2	124.2		
	9–16	22	83.4 \pm 44.7a	7.5	181.2	79.6		
	17–32	23	90.2 \pm 54.5a	17.6	214.8	91.3		
	33–64	23	115.2 \pm 71.2a	7.0	277.3	117.8		
	65–133	8	85.3 \pm 50.4a	24.2	180.6	81.2		
Changes in body mass of <i>R. kessleri</i> , mg	0	32	–13.1 \pm 36.8	–88	66	–16	0.66	0.705
	1–2	22	–16.3 \pm 29.9	–70	48	–21		
	3–4	12	–3.4 \pm 32.7	–58	81	–13		
	5–8	12	–7.5 \pm 29.7	–61	32	–3		
	9–16	22	–13.9 \pm 30.2	–66	36	–15		
	17–32	23	–25.1 \pm 42.2	–136	36	–14		
	33–64	23	–18.6 \pm 35.8	–114	30	–4		
	65–133	8	–22.4 \pm 27.2	–53	15	–22		
Mass of <i>R. kessleri</i> excrement, mg	0	32	1.1 \pm 1.3	0	5	1	1.86	0.080
	1–2	22	3.6 \pm 5.3	0	22	2		
	3–4	12	1.8 \pm 1.4	0	4	1		
	5–8	12	5.1 \pm 5.9	0	22	4		
	9–16	22	3.1 \pm 4.6	0	18	2		
	17–32	23	2.4 \pm 2.3	0	7	2		
	33–64	23	4.4 \pm 7.7	0	28	2		
	65–133	8	1.6 \pm 2.4	0	6	0		

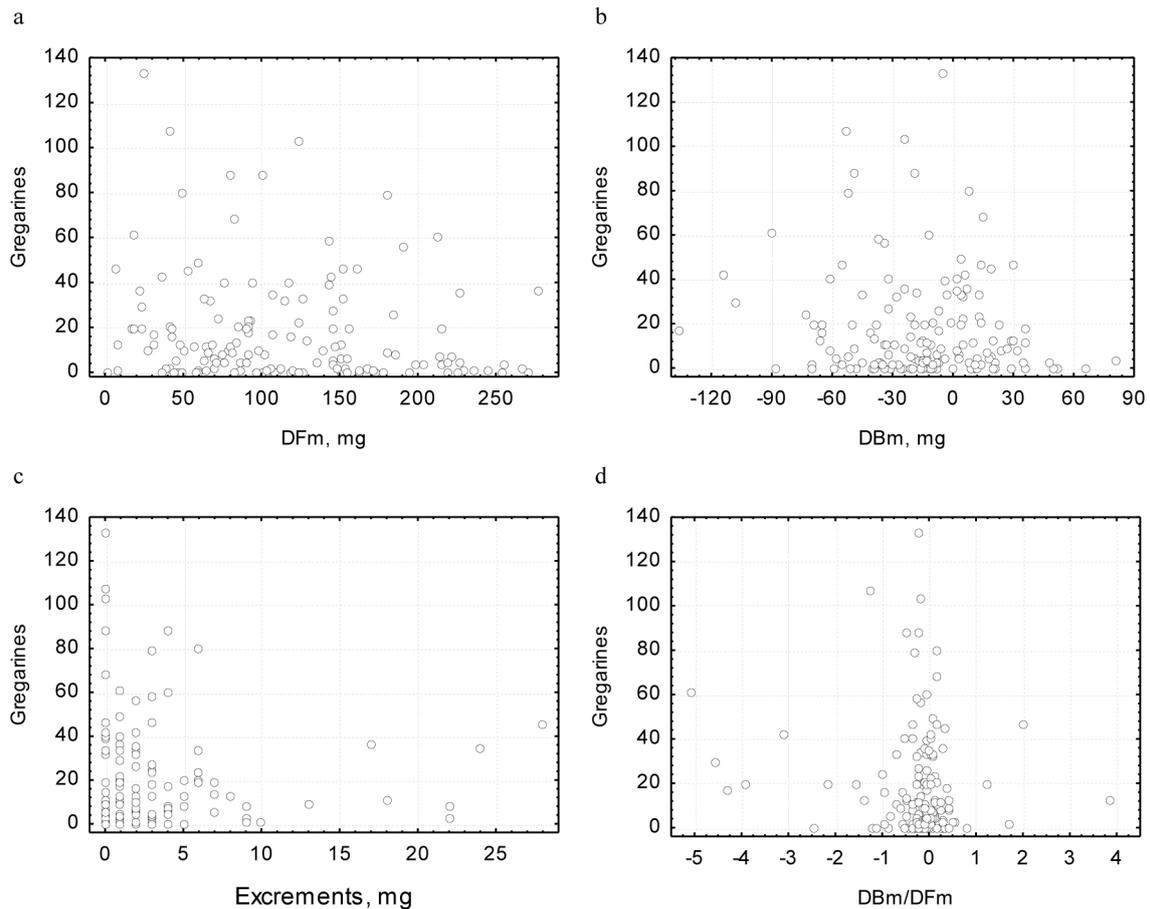


Fig. 2. The relationship between of the number of *S. julipusilli* gamonts in the studied *R. kessleri* specimens on the amount of food consumed (a), change in body mass (b), mass of excrement (c) and the ratio of change in body mass to mass of food consumed (d).

one-two weeks. Intestinal voiding can occur either during the consumption of a new intake of food, and also during the periods between intensive feeding. That is why for conducting laboratory experiments it is important to take into account the rhythmicity of the feeding cycle of *R. kessleri* feeding, its life expectancy, which can exceed 5 years, and the fact that the periods without feeding activities can exceed couple of months (PRISHUTOVA, 2001b; BRYGADYRENKO, 2004).

When analyzing diplopods' trophic activities we have encountered numerous fluctuations in the parameters of trophic activity for certain Julidae individuals (BRYGADYRENKO and IVANYSHIN, 2014; SVYRYDCHENKO and BRYGADYRENKO, 2014). Experiments with 10 or 12 *R. kessleri* specimens did not provide the desired accuracy in evaluating averages. That is why we set as our objective the evaluation of the overall variability of the diplopods' trophic activity, and assess the influence of gregarines living in the intestines of *R. kessleri*. A necessary stage for such an evaluation is the description of general variability in food consumption, the change in body mass and rates of excrement formation, which is given in the first part of the results of this article. It

was discovered that there is no clear relationship between the above-mentioned parameters and the sizes of the animals in the experiment: two to five year old *R. kessleri* specimens might in several weeks only consume food, only void their intestines, do both things at the same time, or do neither. This quite unexpected result makes it more difficult to conduct further experiments on the feeding of *R. kessleri*. The question "Do gregarines influence the basic parameters of the trophic activity of *R. kessleri*?" was the second component of the research.

Data from the literature indicate that gregarines have a pathogenic effect on their hosts during the early stages of their development, as trophozoites attached to the host's epithelium (VALIGUROVÁ, 2012). Our research, however, did not take into account the relationship between the age of the gregarines and the physiological activities of the hosts.

The influence of gregarines upon their hosts can be classified as negative, positive and neutral. Cases of negative impact can manifest themselves as (1) extension of duration of the entire ontogenesis or a certain developmental stage of the invertebrate host (HARRY, 1970;

BOUWMA et al., 2005; LORD and OMOTO, 2012), (2) increase in mortality at certain stages of development (ZUK, 1987b; BOLLATTI and CEBALLOS, 2014), (3) increase in mortality through predation, other parasites or diseases of the hosts infested with gregarines, or through damage to their normal intestinal microflora (BYZOV et al., 1996; MARAUN et al., 2003; BYZOV, 2006), (4) the impact upon the population through mechanisms of sexual selection (ZUK, 1987a). It is probable that in the near future other manifestations of the negative impact of gregarines upon multicellular organisms will be identified, though the mechanisms involved in the alteration of the physiological processes of invertebrate-hosts will be studied for a long time to come.

Hypothetically, the positive impact of gregarines upon the carrier (in this case it would not be correct to use such terms as “parasite” or “host”) could be realized through of (1) optimization of intestinal microorganism communities, (2) protection against other parasite species entering the intestine and thence other body cavities, (3) extraction by gregarines in the intestine of metabolites, which participate in exchange of matter by the host (4) consumption of chemical compounds present in the intestines unfavorable to the host, etc. However, there are very few examples of research in which these mechanisms have been found. This area of research is only at an early stage.

Many authors have emphasised that a negative impact of certain species of intestinal gregarines only occurs in cases of intensive, “massive” invasion – through mechanical congestion of the intestine (ZUK, 1987). Low and average numbers of intestinal gregarines usually do not affect their hosts. This means that a type of interaction which can be termed a “dose – effect” operates, which appears as a manifestation of the philosophical principle of conversion of quantity into quality. Theoretically a variant is possible, when a low number of gregarines is advantageous for a host (which would have to have mechanisms of optimizing the number of gregarines, for example, by the way of changing the diet), and high number of gregarines is harmful. Most likely, this type of interaction of hosts with high extensiveness and low to medium intensity of gregarine infestation can be found in infestation of diplopods.

Gregarines, probably, evolved synchronically with the organisms of their hosts (GEUS, 1969; CLOPTON, 2002, 2009). At least, their exceedingly wide-range distribution among invertebrates indicates the possibility that these protists evolved from parasitism to mutualism. In this case there is observed a manifestation of a general rule concerning the parasite’s impact upon the host: the earlier the connection “parasite – host” appears within phylogenesis, the weaker becomes the negative impact upon the host’s organism (BULAKHOV and PAKHMOV, 2010). In the process of time phylogenetically young “parasite – host” pairs become stable,

mutually advantageous, mutually interacting pairs of species. Diplopoda is one of the oldest, one of the first groups of invertebrate-saprophages to have evolved on land (HOPKIN and READ, 1992). Thus, the interaction between representatives of Julidae and Stenophoridae has every chance to become neutralist or even mutual, to lose all the features of parasitism, which is supported by the results of our research. The classification of interaction between species’ as “parasitism – neutralism – mutualism” is quite relative and is defined by the intensity of the interaction of a macroorganism with its environment. In unfavorable conditions, which were observed in our laboratory experiment (limitation of space, only a single type of plant food, no possibility of feeding on plant detritus rich in microorganisms) the intensity of the “*S. julipusilli* – *R. kessleri*” interaction would be expected to sharpen. Perhaps in natural conditions, in order to prevent massive development of gregarines in the intestines, and to speeding-up the movement of food through the intestine *R. kessleri* uses a wide food base (fallen leaves from trees and shrubs, herbaceous plants, excrement of different vertebrates). As with humans the diet of *R. kessleri* should include certain “laxative” and “fixative” types of plant food, whose effect based on secondary metabolites (terpenes, alkaloids, flavonoids, etc.) present in plant remains, and combinations of microorganisms that develop at certain stages of leaf litter decomposition (SVYRYDCHENKO and BRYGADYRENKO, 2014). Indirect confirmation of this is the increase in *R. kessleri* numbers in ecotones conditions (at the borders of forest and steppe, forest and meadow ecosystems), and also in mixed forests, where within a small territory leaf litter of a maximum number of plant species accumulates and where there are different types of elementary soil processes formation (POKARZHEVSKIJ, 1983; POKARZHEVSKIJ et al., 1989; BRYGADYRENKO, 2004, 2006).

Gregarine infestation of males and females in some invertebrates can significantly vary (ABRO, 1971; HECKER et al., 2002). Perhaps, this is connected with the manifestation of sexual selection: the female chooses the brightest, biggest and strongest males for fertilization. Perhaps resistance to parasites is also a factor in sexual selection (ZUK, 1987b). *R. kessleri* males are smaller than females. The literature contains no information covering the sexual selection of this species of diplopod. This is probably why during our research we did not find any differences in the range of gamont infestation of *S. julipusilli*. This might also indicate that there is little difference in the feeding ecology of males and females of this diplopod species.

The possibility of gregarine infestation increases proportionally with the amount of food consumed by a certain individual (proportional to the life expectancy of a certain host specimen). According to the data of LOCKLIN and VODOPICH (2010), heavier specimens of dragonflies (older ones) have statistically significant-

ly higher levels of gregarine infestation. On the other hand, the results of this two-year experiment clearly prove that the intensity of dragonflies' infestation increases from spring to autumn, i.e. with the increase in dragonfly imagos' life expectancy. In our experiment the larger specimens of *R. kessleri* were infested with a higher number of gamonts. Perhaps the results of the experiment were also affected by the high-capacity of the intestines of the larger specimens of *R. kessleri*, which can include a larger of *S. julipusilli* gamonts without causing damage to the host.

Laboratory experiments studying the feeding of diplopods show a significant variability in the consumption of leaf litter depending upon the temperature, moisture, stage of leaves decomposition, species composition of the litter and a number of other factors (GERE, 1956; KONDEVA, 1980; DANGERFIELD and MILNER, 1993; COUTEAUX et al., 2002; ASHWINI and SRIDHAR, 2005; ROY and JOY, 2009). Perhaps a significant contribution to the results of studying trophic activity may be made by study of diplopods' infestation with parasites, and the most common among them – gregarines.

Studying the interaction of diplopods and parasites is a promising research area from an evolutionary perspective, which is important for understanding the processes of regulation of biological populations in natural ecosystems, study of the ways these processes are damaged in anthropogenically affected ecosystems (KÖHLER and ALBERTI, 1992; KÖHLER et al., 1992; KULBACHKO and DIDUR, 2012; BRYGADYRENKO and IVANYSHIN, 2014; POKHYLENKO, 2014; SOUZA et al., 2014) – pollution with heavy metals, radio-nuclides, persistent organic pollutants, pesticides.

The hypotheses formulated in the introduction to this article have partly proved right, partly wrong. Different numbers of *S. julipusilli* gamonts can decrease or increase *R. kessleri*'s food consumption by 25–29%. (1) The hypothesis that maximum increase in the body weight will be observed with specimens which had consumed maximum levels of food proved incorrect. (2) The hypothesis that the extent of infestation with *S. julipusilli* varied at different stages of ontogenesis is proved correct. (3) The hypothesis that the number of gregarines affects changes in *R. kessleri* body weight and rates of faecal formation proved incorrect. The results of this investigation should be useful for better understanding of the relationship between parasites and hosts.

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