

***Steinernema* spp. (Steinernematidae, Rhabditida) infective juveniles in the soil of a mixed forest in Vladimir region, Russia: year-round observations**

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Summary. The population structure of *Steinernema* infective juveniles (Steinernematidae, Rhabditida) was studied in an experimental plot (10 × 10 m) over the course of a year. Soil samples from two layers (0–5 and 5–10 cm from the surface) were collected in a mixed forest (126 samples). Nematodes were extracted from soil by two centrifugations (water and MgSO₄ solution), and identified by the examination of morphology and selectively by ITS rDNA sequencing. Infective juveniles of entomopathogenic nematodes, along with plectids, were the dominant groups of soil nematodes. Two steinernematid species, *Steinernema kraussei* and *S. poinari*, were detected. Their abundance in the samples was negatively correlated. The steinernematids were distributed in an aggregated manner (the random factor ‘sample collection point’ explained 36% of the variation in abundance). In the majority of soil samples, their abundance averaged 0.02 ± 0.04 ind. g⁻¹ (mean ± standard deviation). However, in a few samples (10 out of 126), the abundance exceeded the background average by more than an order of magnitude (0.22–5.43 ind. g⁻¹). The effects of soil layer, sample weight, and sampling date (or soil temperature) on the abundance of steinernematids were not significant. Soil freezing from January to April did not affect nematode numbers. Active live juveniles were isolated from frozen soil even in February (soil temperature –1.5°C).

Keywords: abundance, extraction, entomopathogenic nematodes, freezing, juveniles, seasonal dynamics, soil nematodes, spatial distribution.

The entomopathogenic nematodes (EPN), representatives of two rhabditid families Steinernematidae and Heterorhabditidae, are natural pathogens of insects. Discovered in the early decades of the 20th century (Steiner, 1923; Filipjev, 1934), these nematodes attracted the attention of the scientific and agricultural community as possible biocontrol agents for insect pest control. After primary setbacks in the thirties of the 20th century, active studies of EPN were re-started in the sixties-seventies and resulted in the establishment of EPN production in some countries. Pest control formulations based on EPN are now available and the EPN applications have found their place in modern agriculture and horticulture. Despite intensive studies of the different aspects of EPN systematics, physiology and autecology, little is known about their life in nature.

Such a deficiency can be partially explained by the cryptic style of EPN life in soil. Only third stage juveniles (‘dauers’) are present freely in the soil. They do not feed, but can locate suitable insect hosts

and penetrate into their haemocoel. Further development and reproduction of nematodes in the insect is only possible after the establishment of the symbiotically associated bacteria. Bacteria release compounds that kill the insect, usually within 18–36 h. Products emitted by the bacteria protect the insect cadaver from other soil organisms, while these bacteria serve as food for the developing nematode colony (Poinar & Thomas, 1966). The depletion of food reserves and accumulation of metabolites result in the formation of non-feeding third-stage juveniles (IJ) and their migration back to the soil.

Important information about EPN in natural soils can be found in the pioneering work of Prosper Bovieen (1937), but only in a few more recent reports. In most of them (Amarasinghe *et al.*, 1994; Stuart & Gaugler, 1994; Campbell *et al.*, 1996; Jaffee & Strong, 2005; Mráček *et al.*, 2005; Půža & Mráček, 2005, 2010) the distribution and abundance of EPN were studied by baiting soil samples with *Galleria mellonella* L. (Bedding & Akhurst, 1975). This approach (an addition of living wax-moth

caterpillars to the soil samples) is widely used for EPN detection and isolation of new EPN strains. Being effective and not labour-intensive it does not allow observation of soil-inhabiting EPN IJ, but only their progeny in the insects; information on the biology of EPN IJ from soil is scarce (Spiridonov *et al.*, 2007). Direct extraction of living EPN IJ in soil by Spiridonov *et al.* (2007) provided novel data, such as their approximate age, the level of their food reserve depletion, and the presence of bacteria in the vesicle. This previous study was performed in a Belgian grassland, where two steinernematid species (*Steinernema affine* (Bovien, 1937) Wouts, Mráček, Gerdin & Bedding, 1982 and *S. feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982) coexisted. Both species demonstrated aggregated distribution. Throughout Europe, these two species are found mainly in cultivated land or grasslands, whereas in woodlands and forests, two other steinernematid species are common: *S. kraussei* (Steiner, 1923) Travassos, 1927 and *S. poinari* Mráček, Půža & Nermut, 2014. In this study, we provide new information about the distribution and seasonal dynamics of these latter two species in woodland soils. The following hypotheses were tested:

1. Steinernematid IJ are a prominent component of woodland soils in Russia, comparable in densities with the dominant groups of soil nematodes.
2. The abundance of steinernematids is higher in the upper soil layer (0-5 cm) than in the deeper layer (5-10 cm), likely due to a higher availability of potential arthropod hosts in the upper horizon.
3. Steinernematid density declines following the period of soil freezing (January-April, soil temperatures from -2 to 1°C).

MATERIAL AND METHODS

Study site and sampling procedure. A square plot of 10 × 10 m was chosen arbitrarily in the mixed forest (in descending order of prevalence: spruce, pine, birch) in the Vladimir region, on the territory of the municipal district of Pokrov (55.9631 N, 39.2159 E). Soil samples were collected in 2024-2025 with an auger – a stainless steel tube with an inner diameter of 35 mm and an outer diameter of 40 mm. In February 2025, when the soil was completely frozen, the samples were obtained with the use of a cordless hammer drill with appropriate nozzle. Positions for sampling were selected as points with randomly generated coordinates in the rectangular plot. Samples were collected nine times during the year (Supplement: Table S1) at the eight random points, except in

August and October 2024, when four and six points were sampled, respectively. At each point two samples were collected from depths of 0-5 cm and 5-10 cm. Soil cylinders were stored in a container with a tight screw cap and transported to the Laboratory of Systematics and Evolution of Parasites of the A.N. Severtsov Institute of Ecology and Evolution, RAS in Moscow, where they were stored in a fridge at 5°C for no more than 7 days until processing.

Sample processing, counting and identification of nematodes. The following processing of samples was similar to the procedure described earlier (Spiridonov *et al.*, 2007), with a few details modified: a centrifuge Biobase BKC-TH24RL (Jinan Biobase Medical Co. LTD, Jinan, China) was used at 1540 g with a centrifugation time of 7 min. The nematode suspension in the MgSO₄ solution was finally sieved through a 25-µm mesh and transferred into a 200-ml beaker with tap water. The sediment was examined under 20×-70× magnifications on a dissection microscope (Altami®, St. Petersburg, Russia) and nematode juveniles with a characteristic steinernematid appearance were picked alive with a needle and transferred into a drop of water for examination under a compound microscope.

Juvenile stages of nematodes with the features characteristic of the family Steinernematidae were selected under low magnifications and checked under a compound microscope. The size of the juveniles (body length 600-1000 µm), characteristic behaviour (speed of undulating movements of the body, appearance of body coils) and the shape of the tail (conical, tapering to the end but without thin filament) were the main selection criteria under the dissecting microscope. With the use of differential-interferential contrast (DIC) of the compound microscope under 40×-100× objectives four groups of morphological features were examined: the anterior end with a reduced and closed stomatal cavity; the bacterial vesicle in the anterior part of mid-gut; the lateral field structure; and the tail shape.

Light microscopy photographs and measurements were obtained using a NE910 microscope (Nexcope, Ningbo Yongxin Optics Co Ltd, Ningbo, China). Thin pads of 1% agarose were used to keep the juveniles in the lateral aspect (Spiridonov *et al.*, 2007). For seven samples, the identification based on morphological features was confirmed by the PCR amplification of ITS rDNA region and direct sequencing. The DNA from 6-11 juveniles was extracted with proteinase K digestion (Holterman *et al.*, 2006). The 'Curran's primers' TW81 (5'-GTT

TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') were used both for PCR and sequencing (Curran & Driver, 1994). PCR cycling parameters for these primers were: primary denaturation at 94°C for 3 min; 34 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, and post-amplification extension at 72°C for 5 min. The bands with PCR-product were excised from a preparative 0.8% agarose gel. The amplicon was extracted from the excised gel bands using a Cleanup Mini kit by Evrogen® (Evrogen Joint Stock Company, Moscow, Russia) and directly sequenced by Genotech LTD (Moscow, Russia). The obtained sequences were analysed with BLAST (Altschul *et al.*, 1990) against the NCBI GenBank database. The sequences obtained for the two discovered species were deposited in the NCBI GenBank database as PZ282522 for *S. kraussei* and PZ282523 for *S. poinari*.

Data analysis. In total, 126 samples were collected and analysed. However, six samples from the first sampling date (30 August 2024) were collected only from the upper horizon (0-5 cm) and were not weighed. Therefore, we excluded them from the quantitative data analysis.

Due to a high proportion of zero values (37%) in the nematode abundance data (Supplement: Table S1), we initially considered both standard negative binomial and zero-inflated generalised linear mixed models (GLMM) to investigate the effects of environmental factors. These initial models were fitted with the number of steinernematid IJ as the response variable, soil layer as a fixed factor, and soil weight (standardised *via* z-score transformation) as a continuous covariate. Sampling date and sampling point were originally included as random intercept factors.

Models were fitted using the glmmTMB package in R (Brooks *et al.*, 2017) and compared using the Akaike information criterion (AIC). The negative binomial model demonstrated a lower AIC value than the zero-inflated alternative (573 vs 575, respectively). To formally evaluate potential zero-inflation, we used the DHARMA package (Hartig, 2026). The simulation-based test confirmed no significant zero-inflation ($P = 0.536$), justifying the choice of the standard negative binomial model with a quadratic variance-mean relationship (nbinom2 family).

The random effect of sampling date did not explain a substantial part of the variance, so was excluded from the model. Replacing the sampling date with soil temperature as an alternative covariate yielded qualitatively identical results. Final selection of models and averaging of coefficients were

implemented using the MuMIn package (Bartoń, 2026). Models were ranked by AICc and those with $\Delta AICc < 2$ were used for model averaging (null model, model with soil layer, and model with soil weight).

To examine the effect of the freezing period on steinernematid abundance in the soil (hypothesis 3), we conducted a Kruskal-Wallis rank sum test, as the assumption of normality of residuals was not met. For this, the differences among three groups of samples were analysed: before freezing, during the freezing period (January-April, with soil temperatures ranging from -2 to 1°C), and after freezing (Supplement: Table S1).

In addition, we evaluated whether the probability of nematode occurrence differed between soil layers using Fisher's exact test. For this purpose, abundance data were transformed into a presence/absence format.

To analyse the association in the distribution of the two studied nematode species (*S. kraussei* and *S. poinari*), Spearman's ρ was calculated. We also fitted separate GLMM for the abundance of either *Steinernema* species, using the same fixed (soil layer, soil weight, and temperature) and random factors (sampling point).

RESULTS

Morphology and identification of studied species. The body length of the juvenile stages of nematodes primarily identified as steinernematids varied from 600 to 1000 µm. The juveniles with slightly flattened anterior end with a diameter of 5.6-6.1 µm; an 8-8.5 µm wide lateral field consisting of four central lines, prominent marginal lines and less prominent sub-marginal lines (with a total of eight recognisable lines); a thick-walled bacterial vesicle, often tapering toward the anterior end and touching the basal bulb; and a regularly conical tail end with 30-35 µm long hyaline part (Fig. 1) were identified as *S. kraussei*. The juveniles with rounded anterior end with a diameter of 6.2-6.5 µm; a 6-6.5 µm wide lateral field consisting of three prominent central lines and two marginal lines and rarely less prominent sub-marginal lines (total of five prominent lines); a thin-walled and, in some juveniles, quite long (up to 40 µm) bacterial vesicle, always located behind the basal bulb; and a 34-38 µm long hyaline part of the tail, usually with the dorsal or both sides slightly concave (Fig. 1) were identified as *S. poinari*.

An analysis of the ITS rDNA sequences obtained for two samples considered on the basis of morphological features as *S. poinari* and five

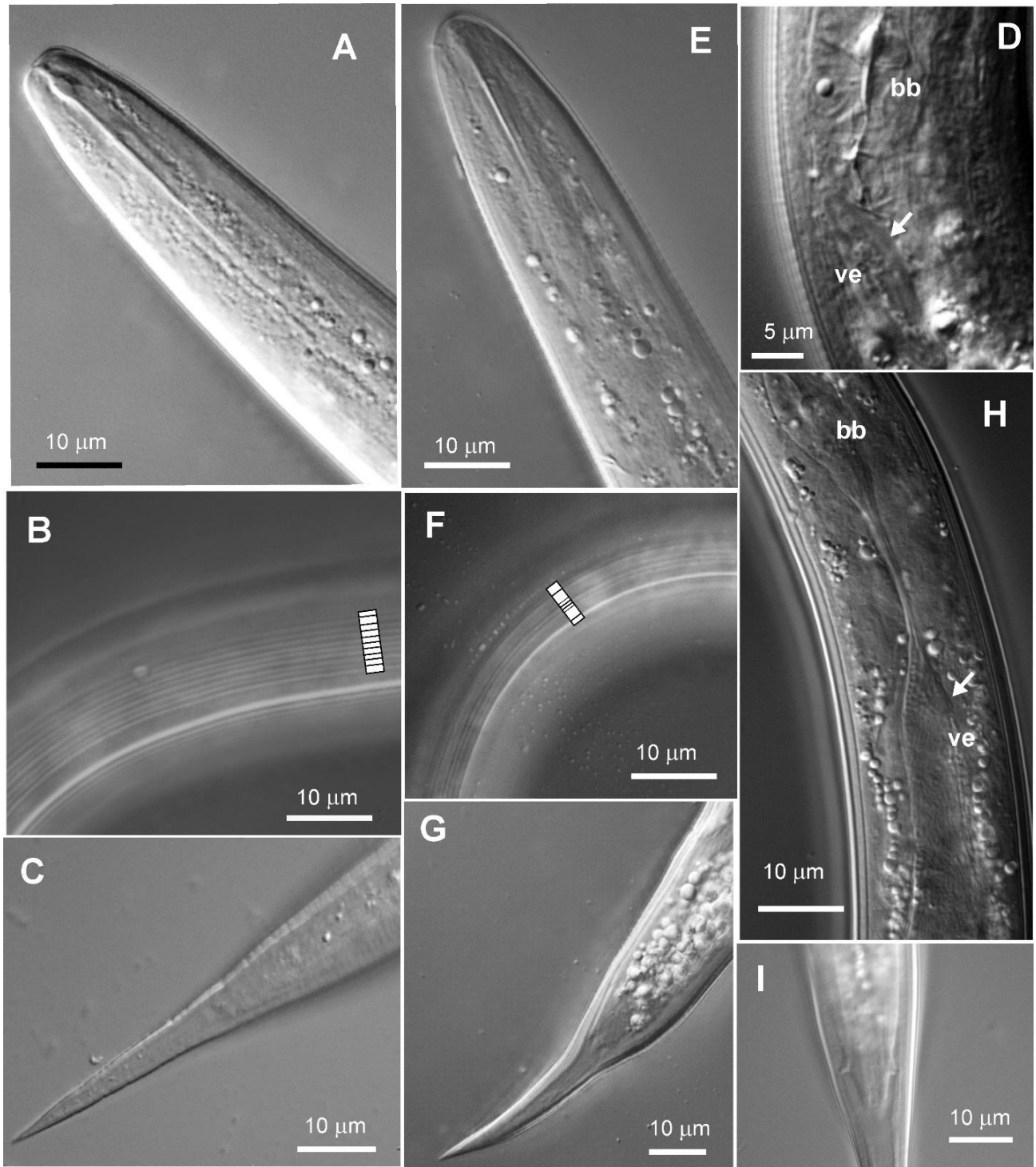


Fig. 1. Morphological differences between the infective juveniles of *Steinernema kraussei* (A-D) and *S. poinari* (E-I). A, E: Anterior end; B, F: Lateral field structure (the lines prominent under DIC are drawn in the white box); D, H: Bacterial vesicle (bb – pharynx basal bulb, ve – vesicle; arrow indicates vesicle wall); C, G, I: Hyaline part of tail (I – in ventral aspect, all other images in lateral aspect).

Table 1. Model selection table for the determinants of steinernematid abundance based on AICc.

Model	Fixed effects	df	logLik	AICc	Δ AICc	Weight
2	Layer	4	−281.414	571.2	0.00	0.334
1	Null model	3	−282.526	571.3	0.08	0.320
3	Scale (soil weight)	4	−281.799	571.9	0.77	0.227
4	Layer + scale (soil weight)	5	−281.350	573.2	2.05	0.120

Note: All models include a random intercept for site (60 groups) and a constant dispersion term (dispformula = ~1).

Table 2. Model-averaged coefficients (fixed effects) for the negative binomial GLMM of steinernematid abundance.

Parameter	Full average			Conditional average		
	Estimate	Adjusted SE	<i>P</i>	Estimate	Adjusted SE	<i>P</i>
Intercept	0.451	0.315	0.152	0.451	0.315	0.152
Layer (5–10 cm)	0.149	0.251	0.553	0.393	0.266	0.139
Scale (soil weight)	0.056	0.132	0.674	0.216	0.182	0.236

Note: Models with Δ AICc < 2 were used for model averaging.

samples considered as *S. kraussei* confirmed the primary species identification. The ITS rDNA sequence of *S. poinari* was completely identical to that of the type Czech population (KF241752 and KF241754) in the 855-bp long alignment, but differed in one nucleotide from the UK (AY171297) and Siberian (from the Altai mountains – AY171299) conspecific populations. The ITS rDNA sequence of *S. kraussei* found in the course of this study was identical in the 803-bp long alignment with four sequences in the NCBI GenBank: KC608621 and KC608624 deposited as *S. kraussei* and KU736743 and MG543846 deposited as *S. silvaticum*. These two species are very close to one another and can be distinguished by the structure of the lateral field. The former species is characterised by three central bands (four lines), prominent marginal and weaker submarginal lines. Such a lateral field pattern was observed in our study objects, and we consider them as *S. kraussei*.

Temperature conditions. Soil temperatures in the upper layers (0–10 cm) for each date are presented in the Supplement (Table S1). Comparatively low snowfall in January 2025 was followed by nearly complete thawing and vanishing of snow cover in February 2025 (Fig. 2), which was followed by a drop in temperatures and the freezing of the soil up to a 10–15 cm depth. Living juveniles of Steinernematidae were found in soil samples

throughout the year including completely frozen ones (e.g., February samples with a temperature −1.5°C).

Nematode abundances. Nematodes of the family Steinernematidae represented one of the dominant nematode groups in the soil samples, together with the family Plectidae, although the latter was not counted. Mean ($\pm$ standard deviation) numbers of IJ of the genus *Steinernema* for all samples were 0.16 ± 0.72 ind. g^{−1}. However, in most of the samples their abundance averaged ($\pm$ SD) 0.02 ± 0.04 ind. g^{−1} and only in a few (10 of 126 samples) the abundance was more than an order of magnitude higher (0.22 – 5.43 ind. g^{−1}). Such pronounced differences in nematode densities were observed even between sampling points and soil layers within a single day (Supplement: Table S1; Fig. 3), highlighting the patchy nature of nematode distribution.

The model of steinernematid abundance (negative binomial GLMM) included soil layer as a predictor, the weight of the soil sample as a covariate and sampling point as a random factor. Subsequent model selection (Table 1) and averaging (Table 2) revealed that neither soil layer nor the weight of the soil sample had a significant effect on nematode abundance ($P > 0.05$ for all coefficients in both full and conditional averages). The null model, containing only the random effect of sampling point,

was highly competitive ($\Delta AICc = 0.08$) and explained 36% of the variance, indicating that the fixed predictors (soil layer and weight) provided limited explanatory power. By contrast, the random effect of sampling point accounted for a large portion of the total variance ($\delta^2 = 2.88$, $SD = 1.70$, $N = 120$), suggesting that the observed variability in nematode abundance was primarily driven by strong spatial heterogeneity among the 60 sampling points. These results do not support our second hypothesis of an uneven vertical distribution of nematodes (with higher abundances in the upper soil layers), but rather indicate high heterogeneity in their horizontal distribution.

By contrast, Fisher's exact test showed that the probability of nematode occurrence (based on presence/absence data, independent of total abundance) was related to the soil layer. Nematodes occurred in the deeper layer (5-10 cm) with a higher probability than in the surface layer (0-5 cm) ($P = 0.037$).

There was no significant association between the

total abundance of nematode IJ and soil temperature (Spearman's $\rho = 0.059$, $N = 120$, $P = 0.521$). When testing hypothesis 3 (regarding a decrease in nematode numbers during the soil freezing period), we found no differences in nematode abundance among the periods before, during (January-April, soil temperatures from -2 to 1°C), and after soil freezing (Supplement: Table S1, Kruskal-Wallis test; $H = 0.077$, $df = 2$, $P = 0.962$).

A separate analysis of the distribution of each nematode species revealed no significant correlations with temperature for either *S. poinari* (Spearman's $\rho = -0.073$, $N = 120$, $P = 0.428$) or *S. kraussei* (Spearman's $\rho = 0.156$, $N = 120$, $P = 0.089$). However, the abundances of these two species within the samples negatively correlated with each other (Spearman's $\rho = -0.31$, $N = 120$, $P < 0.001$). Additional GLMM did not reveal significant effects of soil layer on the abundance of either *S. kraussei* (Estimate = -0.42 , $SE = 0.26$, $z = -1.64$, $P = 0.10$) or *S. poinari* (Estimate = 0.74 , $SE = 0.73$, $z = 1.00$, $P = 0.32$).

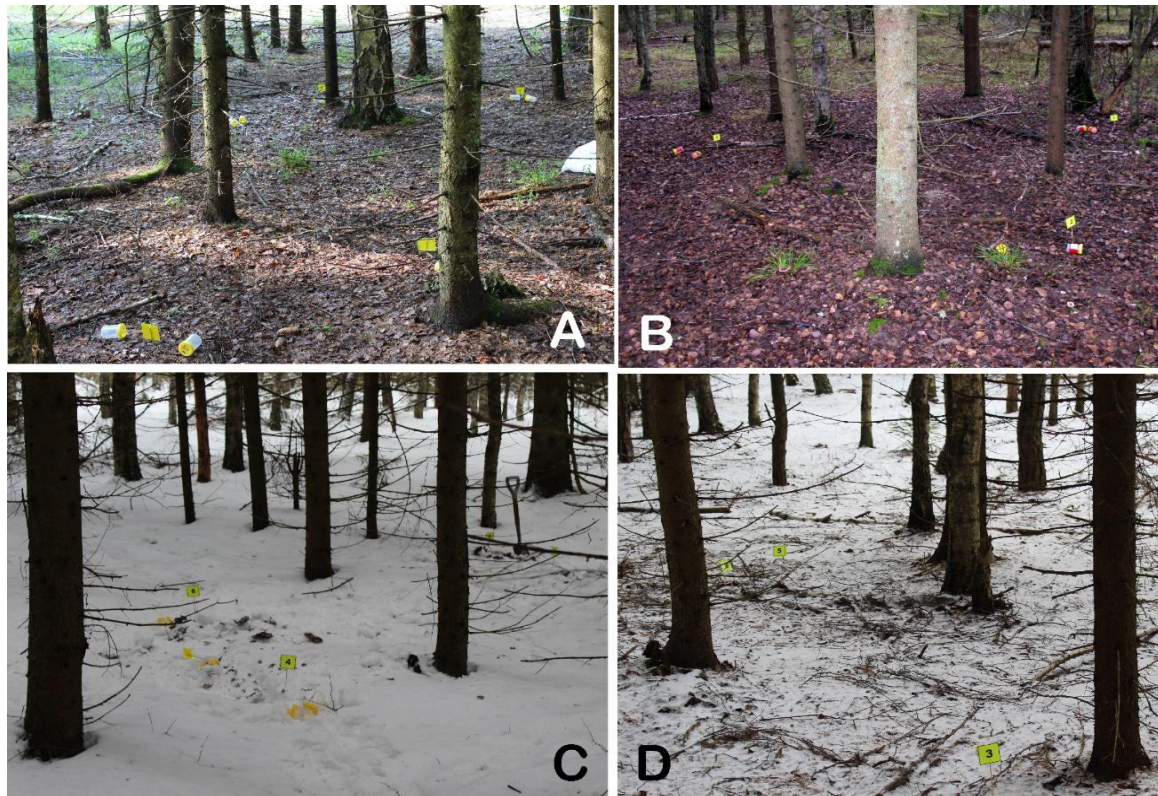


Fig. 2. General view of the plot throughout the year: A: August 2024 (screw-cap containers for each separate soil column are visible in the foreground); B: October 2024; C: January 2025; D: February 2025.

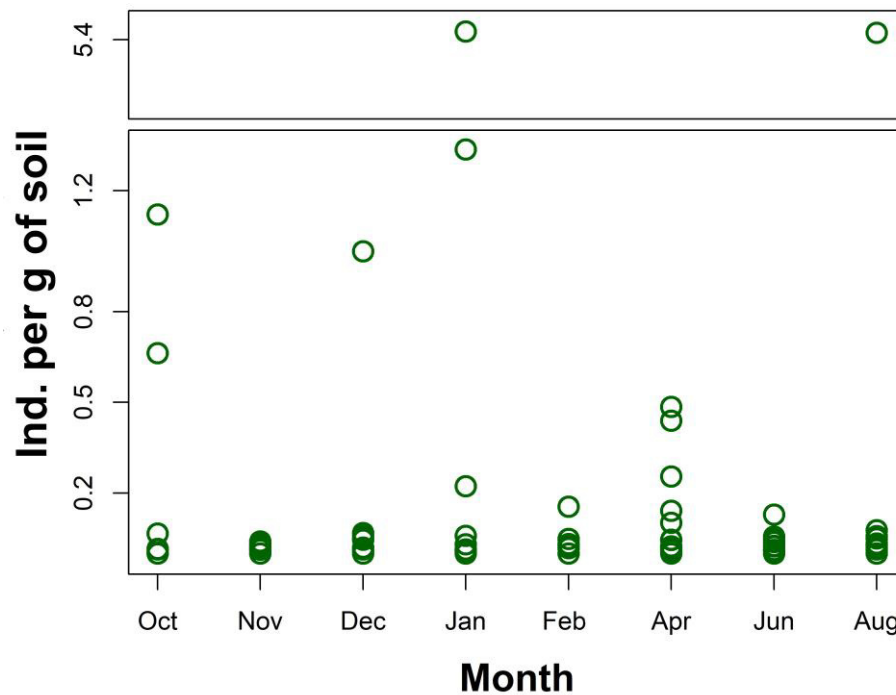


Fig. 3. Abundance of steinernematid nematodes at the study site in different seasons.

DISCUSSION

Since their discovery in 1923 by G. Steiner (1923), the nematodes of the genus *Steinernema* (Steinernematidae) have been considered as potential agents of biological control of insects. General interest in EPN has resulted over the last four decades in a wide variety of publications covering all aspects of biology, physiology and application of these soil nematodes. Field ecology of these organisms is a much less developed area of research.

Such neglect can be explained partially by the limitations of methods for detecting these nematodes in soil samples. Predominantly, the presence of steinernematids in soil is demonstrated with the *Galleria* baiting approach, where living insects (usually caterpillars of the greater wax moth *G. mellonella*) are added to the soil samples. The occurrence of *Steinernema* nematodes in the soil then becomes obvious after the development of adults with characteristic morphology. The application of real-time PCR proved to be helpful and informative for the description of complex EPN communities in soil (Jaffuel *et al.*, 2016). Direct extraction of EPN IJ from soil is also possible, and was used for the morphological description of EPN diversity in German soils (Sturhan, 1999). Wider application of the direct extraction of EPN juveniles from soil is compromised by the 'arduous task' of

'individual identification of all recovered nematodes' (Stuart & Gaugler, 1994). Nevertheless, direct extraction can be useful to solve some problems of EPN field ecology, including their distribution and occurrence in soil, relationships with insect hosts, and infectivity of juveniles, *etc.* A parallel application of direct extraction and real-time PCR-based quantification demonstrated that both methods provide similar estimations of EPN juvenile prevalence in the grassland and woodland soils of Scotland (Torr *et al.*, 2007). A study on EPN IJ distribution in the soil of a Belgian grassland (Spiridonov *et al.*, 2007) provided information on the abundance of *Steinernema* IJ within their soil aggregations. The approach used in the cited paper included the complete removal of soil monoliths, the extraction of nematodes from $5 \times 5 \times 5$ cm soil cubes, and the counting of steinernematid IJ in each separate cube.

The studies of EPN distribution in soil by all methods demonstrated 'patchiness occurred on a relatively small scale' (Stuart & Gaugler, 1994; Spiridonov *et al.*, 2007; Půža & Mráček, 2010). Also '...the low quantities were detected on average below 1 IJ (100 g)⁻¹ of dry soil' (Jaffuel *et al.*, 2016). Similarly, we found a patchy distribution of IJ in the woodland soil at our study site. According to the modelling results, differences among sampling points represented the main source of variation in total steinernematid abundance,

whereas other factors (soil layer, soil weight, and sampling date or temperature) had no significant effect. In studies of other steinernematid species, the soil layer significantly influenced the total abundance of IJ, which were more numerous in the upper layer (0-8 cm) than in 8-16 cm layer (Půža & Mráček, 2010). However, our study covered a smaller range of soil depths (0-5 and 5-10 cm were compared), which could explain why we did not find differences in the vertical distribution of steinernematids. Consistent with our results, temperature had no significant impact on steinernematid abundance in certain other habitats (Půža & Mráček, 2005). At the same time, seasonality can be either apparent (Půža & Mráček, 2005) or not (Campbell *et al.*, 1995; Mráček *et al.*, 2005), probably due to it being masked by high variation in IJ abundance. According to our results, even the soil-freezing period (January-April, soil temperatures between -2 and 1°C) did not affect the density of steinernematids, which were found to be active throughout the year. To our knowledge, these are the first available data on the population dynamics of these nematodes during winter months.

The IJ abundance in our plot was higher (mean $\pm$ SD 16 ± 72 ind. $(100\text{ g})^{-1}$) than that reported in earlier studies (Jaffuel *et al.*, 2016). Our results show that steinernematid IJ can represent one of the dominant nematode groups, together with plectids, in Russian woodland soils. The efficiency of the extraction method used (double centrifugation in water and magnesium sulfate) was estimated earlier as 65% (Spiridonov *et al.*, 2007). If we consider approximately 50% efficiency (each second juvenile extracted and counted), mean abundances of about 32-144 IJ $(100\text{ g})^{-1}$ soil are observed. In our opinion, the IJ quantities in aggregations are more important for the understanding of steinernematid life cycle in soil, as an indication of the size of a possible, as yet unknown, host (prey) of these nematodes.

An identification of IJ in the sample after the extraction procedure represents a key problem for the studies in EPN field ecology. The primary selection of nematodes with a morphology resembling that of steinernematids is based on their characteristic types of movement. Although it is difficult to describe the specificity of nematode movement, observing IJ under a dissecting microscope (at 20 $\times$ -70 $\times$ magnifications) provides the primary experience. Additional features, such as the shape of the tail end (conical, not rounded, but without a thin filament), and the appearance of the mid-gut (filled with globules of lipids, vacuolisation) are helpful. The nematodes with specific features of gross morphology such as

plectids, mononchids, dorylaimids, *etc.*, are usually excluded at this stage. Further study of nematodes under a compound microscope, desirably with the use of DIC, is sufficient to exclude all non-steinernematid specimens. Remarkably, these are mainly nematodes of the family Cephalobidae, which can be confused with EPN IJ under low magnifications. Further examination of the anterior end of the nematodes under higher magnifications excludes from consideration all the nematodes with an open buccal cavity, like Cephalobidae, Rhabditidae (including juveniles), and Diplogastridae, not to mention stylet-bearing tylenchids and aphelenchids. To distinguish between the two steinernematid species present in the plot soil, another set of features was used: body length, shape of the anterior and posterior ends, bacterial vesicle appearance, and lateral field structure. The latter character is the most decisive. All other features have limited applicability. Body length ranges overlap significantly in these two species (760-1050 μm in *S. kraussei* and 630-880 μm in *S. poinari* in our material). The shape of the anterior end (flattened vs rounded) is difficult to estimate, especially under different head positions. The bacterial vesicle is not seen in freshly migrated IJ. The shape of the tail is visibly different in these two species, but its appearance is highly dependent on the observation aspect (Fig. 2). Nevertheless, the appearance of the lateral field is clearly visible under DIC in IJ of all ages, especially at the level of the basal bulb-anterior part of the mid-gut (lower amount of lipid globules beneath). The number of visible lines and the width of the lateral field are clear and obvious features to distinguish the two steinernematid species in the soil of this plot. Our identification of steinernematids based on these morphological features was confirmed by the sequencing of ITS rDNA.

There are few reliable reports describing the distribution of particular EPN species in natural habitats. Probably the most extensive study is that on *Heterorhabditis marelatus* in the maritime lupine thickets of the Bodega Bay area of California (Strong *et al.*, 1996; Ram *et al.*, 2008), which reported temporal changes in population dynamics over at least two decades and investigated their possible causes. A survey of EPN in a Belgian grassland (Spiridonov *et al.*, 2007) revealed a strong association between the spatial distributions of *S. feltiae* and *S. affine*. In many European natural habitats, two *Steinernema* species co-occur, belonging to two distant phylogenetic clades within this genus (the '*feltiae-kraussei-oregonense*' and '*affine-intermedium*' clades; Spiridonov &

Subbotin, 2016). Such co-existence of phylogenetically distant steinernematids appears to be a stable and long-standing event, as despite the apparent specificity of their bacterial symbionts to the nematode host, the representatives of both clades share the same associated bacterial species (*Xenorhabdus bovienii*). A study of EPN IJ in Scotland revealed a habitat preference of *S. affine* for grasslands (Torr *et al.*, 2007). Such preference, however, does not seem to be very specific, as this species was also numerous in the soil of a Czech oak woodland (Půža & Mráček, 2010).

Our findings indicate that the abundance of the two dominant steinernematid species (*S. kraussei* and *S. poinari*) in the samples is negatively correlated, which could result from antagonistic relationships between these nematodes or different microhabitat preferences. Such a spatial relationship has not been reported for other steinernematid species, such as *S. feltiae* and *S. affine* (Spiridonov *et al.*, 2007), whereas some tendencies towards spatial segregation were observed for *S. affine* and *S. kraussei* (Půža & Mráček, 2010). According to the latter study, the probability of *S. kraussei* occurrence was slightly lower in samples containing *S. affine*. In addition, *S. affine* was more numerous in the upper soil layer (0-4 cm), while *S. kraussei* tended to occur in the lower layers (4-10 cm). Such differences in vertical distribution were assumed to be linked to the different foraging strategies of these species; for instance, the actively moving cruiser *S. kraussei* may reach patches unoccupied by the more common intermediate forager *S. affine* to avoid competition (Půža & Mráček, 2010). Simultaneous experimental infections of insect hosts with these species indicated that *S. affine* was more infective (Půža & Mráček, 2010). However, these results could also be explained by the specificity of the experimental arena used. Contrary to these findings, we did not find significant differences in the vertical distribution of *S. kraussei* and *S. poinari* at our study site, although *S. poinari* is likely to be similar to *S. affine* in its foraging strategy. Generally, the penetration of steinernematid juveniles into an insect host previously infected by the same or a different steinernematid species decreases significantly compared with non-infected hosts (Glazer, 1997; Wang & Ishibashi, 1998). Species of the genus *Steinernema* kill members of their own and other species using bacteriocins from their symbiotic bacteria and by directly injuring a competitor, or they benefit during exploitative competition (O'Callaghan *et al.*, 2014 and references therein). In such cases, the establishment of one steinernematid species in the colonised insect

host could prevent the spread of other species within the microhabitat.

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С.Э. Спиридонов и Е.И. Миронова. Инвазионные личинки *Steinernema* spp. (Steinernematidae, Rhabditida) в почве смешанного леса во Владимирской области: круглогодичные наблюдения.

Резюме. В течение одного года на экспериментальном участке (10 × 10 м) оценивали численность инвазионных личинок энтомопатогенных нематод рода *Steinernema* (Steinernematidae, Rhabditida). Образцы почвы из двух слоев (0-5 и 5-10 см от поверхности) были отобраны в смешанном лесу (126 образцов). Нематод извлекали из почвы с помощью двухэтапного центрифугирования (в воде и растворе MgSO₄). Определение нематод основывалось на особенностях их строения; для нескольких образцов оно было подтверждено секвенированием участка ITS рДНК. Инвазионные личинки этих энтомопатогенных нематод, наряду с плектидами, составляли одну из доминирующих групп почвенных нематод. Были обнаружены два вида *Steinernema*: *S. kraussei* и *S. poinari*. Их численность в образцах отрицательно коррелировала (коэффициент Спирмена, $\rho = -0.31$, $P < 0.001$). Личинки *Steinernema* были распределены агрегировано (случайный фактор «точка отбора проб» объяснял 36% вариации численности). В большинстве образцов почвы их численность в среднем составляла 0.02 ± 0.04 особей/г ($\pm$ стандартное отклонение). Однако в нескольких образцах (10 из 126) численность превышала фоновый средний уровень более чем на порядок (0.22-5.43 особей/г). Достоверного влияния слоя почвы, массы образца и даты отбора проб (или температуры почвы) на численность стейнернематид не обнаружено (GLMM). Промерзание почвы с января по апрель не влияло на численность нематод. Активные живые личинки были выделены из замерзшей почвы даже в феврале (температура почвы -1.5°C).

Table S1. Abundance of steinernematid IJs in the samples. Data for 30 Aug 2024 are not included (samples were not weighted, therefore, excluded from the quantitative data analysis).

Date	Soil T, °C	Season	Soil weight, g	Soil layer, cm	Sampling point	Total N, ind./sample	N unident., ind./sample	Total N, ind./g	N of <i>S. kraussei</i> , ind./g	N of <i>S. poinari</i> , ind./g
12.10.24	11	before freezing	61	0-5	7	4	4	0.066	0	0
12.10.24	11	before freezing	73	5-10	7	1	0	0.014	0	0.014
12.10.24	11	before freezing	62	0-5	8	0	0	0	0	0
12.10.24	11	before freezing	75	5-10	8	0	0	0	0	0
12.10.24	11	before freezing	74	0-5	9	83	77	1.122	0.068	0.014
12.10.24	11	before freezing	95	5-10	9	63	60	0.663	0.032	0
12.10.24	11	before freezing	65	0-5	10	1	0	0.015	0.015	0
12.10.24	11	before freezing	99	5-10	10	0	0	0	0	0
10.11.24	8	before freezing	70	0-5	11	0	0	0	0	0
10.11.24	8	before freezing	76	5-10	11	3	0	0.039	0.039	0
10.11.24	8	before freezing	73	0-5	12	0	0	0	0	0
10.11.24	8	before freezing	68	5-10	12	1	1	0.015	0	0
10.11.24	8	before freezing	62	0-5	13	1	0	0.016	0.016	0
10.11.24	8	before freezing	76	5-10	13	2	0	0.026	0.013	0.013
10.11.24	8	before freezing	68	0-5	14	2	0	0.029	0	0.029
10.11.24	8	before freezing	79	5-10	14	0	0	0	0	0
10.11.24	8	before freezing	72	0-5	15	0	0	0	0	0
10.11.24	8	before freezing	79	5-10	15	1	0	0.013	0.013	0
10.11.24	8	before freezing	68	0-5	16	0	0	0	0	0
10.11.24	8	before freezing	92	5-10	16	2	0	0.022	0	0.022
10.11.24	8	before freezing	65	0-5	17	0	0	0	0	0
10.11.24	8	before freezing	89	5-10	17	0	0	0	0	0
10.11.24	8	before freezing	64	0-5	18	1	0	0.016	0.016	0
10.11.24	8	before freezing	90	5-10	18	3	0	0.033	0	0.033
14.12.24	3	before freezing	61	0-5	19	3	0	0.049	0.049	0
14.12.24	3	before freezing	74	5-10	19	5	5	0.068	0	0
14.12.24	3	before freezing	63	0-5	20	3	0	0.048	0.048	0

14.12.24	3	before freezing	73	5-10	20	1	0	0.014	0.014	0
14.12.24	3	before freezing	51	0-5	21	1	0	0.020	0.020	0
14.12.24	3	before freezing	66	5-10	21	3	0	0.045	0	0.045
14.12.24	3	before freezing	51	0-5	22	0	0	0	0	0
14.12.24	3	before freezing	84	5-10	22	5	5	0.060	0	0
14.12.24	3	before freezing	90	0-5	23	6	0	0.067	0.067	0
14.12.24	3	before freezing	46	5-10	23	46	46	1.000	0	0
14.12.24	3	before freezing	46	0-5	24	0	0	0	0	0
14.12.24	3	before freezing	67	5-10	24	1	0	0.015	0.015	0
14.12.24	3	before freezing	60	0-5	25	0	0	0	0	0
14.12.24	3	before freezing	55	5-10	25	0	0	0	0	0
14.12.24	3	before freezing	79	0-5	26	0	0	0	0	0
14.12.24	3	before freezing	61	5-10	26	0	0	0	0	0
6.1.25	0	freezing	82	0-5	27	445	0	5.427	0	5.427
6.1.25	0	freezing	107	5-10	27	143	0	1.336	0	1.336
6.1.25	0	freezing	97	0-5	28	1	0	0.010	0.010	0
6.1.25	0	freezing	72	5-10	28	1	0	0.014	0.014	0
6.1.25	0	freezing	63	0-5	29	14	0	0.222	0.222	0
6.1.25	0	freezing	86	5-10	29	5	0	0.058	0.058	0
6.1.25	0	freezing	66	0-5	30	2	0	0.030	0.030	0
6.1.25	0	freezing	81	5-10	30	1	0	0.012	0.012	0
6.1.25	0	freezing	46	0-5	31	0	0	0	0	0
6.1.25	0	freezing	80	5-10	31	0	0	0	0	0
6.1.25	0	freezing	82	0-5	32	0	0	0	0.024	0
6.1.25	0	freezing	82	5-10	32	0	0	0	0	0
6.1.25	0	freezing	75	0-5	33	1	0	0.013	0.013	0
6.1.25	0	freezing	102	5-10	33	0	0	0	0	0
6.1.25	0	freezing	79	0-5	34	1	0	0.013	0	0.013
6.1.25	0	freezing	142	5-10	34	1	0	0.007	0	0.007
15.2.25	-2	freezing	57	0-5	35	1	0	0.018	0.018	0
15.2.25	-2	freezing	77	5-10	35	0	0	0	0	0
15.2.25	-2	freezing	75	0-5	36	0	0	0	0	0
15.2.25	-2	freezing	109	5-10	36	3	0	0.028	0	0.028

15.2.25	-2	freezing	42	0-5	37	0	0	0	0	0
15.2.25	-2	freezing	71	5-10	37	11	0	0.155	0	0.155
15.2.25	-2	freezing	78	0-5	38	0	0	0	0	0
15.2.25	-2	freezing	123	5-10	38	0	0	0	0	0
15.2.25	-2	freezing	73	0-5	39	0	0	0	0	0
15.2.25	-2	freezing	95	5-10	39	3	0	0.032	0	0.032
15.2.25	-2	freezing	83	0-5	40	2	2	0.024	0	0
15.2.25	-2	freezing	103	5-10	40	5	0	0.049	0	0.049
15.2.25	-2	freezing	75	0-5	41	0	0	0	0	0
15.2.25	-2	freezing	79	5-10	41	0	0	0	0	0
15.2.25	-2	freezing	72	0-5	42	0	0	0	0	0
15.2.25	-2	freezing	98	5-10	42	0	0	0	0	0
13.4.25	1	freezing	82	0-5	43	36	0	0.439	0	0.439
13.4.25	1	freezing	98	5-10	43	25	0	0.255	0	0.255
13.4.25	1	freezing	87	0-5	44	0	0	0	0	0
13.4.25	1	freezing	80	5-10	44	1	1	0.013	0	0
13.4.25	1	freezing	96	0-5	45	2	0	0.021	0.021	0
13.4.25	1	freezing	128	5-10	45	3	0	0.023	0.023	0
13.4.25	1	freezing	108	0-5	46	11	0	0.102	0.102	0
13.4.25	1	freezing	136	5-10	46	66	0	0.485	0.485	0
13.4.25	1	freezing	95	0-5	47	1	0	0.011	0	0.011
13.4.25	1	freezing	98	5-10	47	2	0	0.020	0.020	0
13.4.25	1	freezing	106	0-5	48	2	0	0.019	0.019	0
13.4.25	1	freezing	148	5-10	48	1	0	0.007	0	0.007
13.4.25	1	freezing	90	0-5	49	4	0	0.044	0	0.044
13.4.25	1	freezing	110	5-10	49	5	0	0.045	0	0.045
13.4.25	1	freezing	92	0-5	50	0	0	0	0	0
13.4.25	1	freezing	106	5-10	50	15	0	0.142	0	0.142
7.6.25	15	after freezing	78	0-5	51	10	0	0.128	0.128	0
7.6.25	15	after freezing	96	5-10	51	1	0	0.010	0.010	0
7.6.25	15	after freezing	52	0-5	52	0	0	0	0	0
7.6.25	15	after freezing	91	5-10	52	1	1	0.011	0	0
7.6.25	15	after freezing	42	0-5	53	2	0	0.048	0.048	0

7.6.25	15	after freezing	53	5-10	53	2	0	0.038	0.038	0
7.6.25	15	after freezing	67	0-5	54	0	0	0	0	0
7.6.25	15	after freezing	85	5-10	54	2	0	0.024	0.024	0
7.6.25	15	after freezing	54	0-5	55	3	0	0.056	0	0.056
7.6.25	15	after freezing	80	5-10	55	2	0	0.025	0	0.025
7.6.25	15	after freezing	53	0-5	56	2	0	0.038	0.038	0
7.6.25	15	after freezing	111	5-10	56	1	0	0.009	0	0.009
7.6.25	15	after freezing	57	0-5	57	0	0	0	0	0
7.6.25	15	after freezing	83	5-10	57	1	0	0.012	0	0.012
7.6.25	15	after freezing	78	0-5	58	0	0	0	0	0
7.6.25	15	after freezing	86	5-10	58	0	0	0	0	0
9.8.25	21	after freezing	42	0-5	59	0	0	0	0	0
9.8.25	21	after freezing	70	5-10	59	0	0	0	0	0
9.8.25	21	after freezing	52	0-5	60	282	0	5.423	0	5.423
9.8.25	21	after freezing	69	5-10	60	2	0	0.029	0	0.029
9.8.25	21	after freezing	35	0-5	61	2	0	0.057	0.057	0
9.8.25	21	after freezing	84	5-10	61	2	2	0.024	0	0
9.8.25	21	after freezing	76	0-5	62	1	0	0.013	0.013	0
9.8.25	21	after freezing	61	5-10	62	2	0	0.033	0.016	0.016
9.8.25	21	after freezing	66	0-5	63	0	0	0	0	0
9.8.25	21	after freezing	98	5-10	63	5	0	0.051	0.051	0
9.8.25	21	after freezing	65	0-5	64	5	0	0.077	0.077	0
9.8.25	21	after freezing	80	5-10	64	4	0	0.050	0.050	0
9.8.25	21	after freezing	47	0-5	65	0	0	0	0	0
9.8.25	21	after freezing	82	5-10	65	2	0	0.024	0.024	0
9.8.25	21	after freezing	35	0-5	66	0	0	0	0	0
9.8.25	21	after freezing	127	5-10	66	0	0	0	0.016	0