

Locomotor Activity Of *Ixodes Ricinus* Females In 900 Mhz Electromagnetic Field

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Abstract

Mobile telecommunications technologies have become an indispensable part of people's lives of all ages around the world. They affect personal life and social interactions and are a work tool in the work routine. The requirements for network availability and the quality of the Internet connection are constantly increasing, to which telecommunications providers are responding. Humans and wildlife live in an environment with the permanent presence of electromagnetic radiation with just a minor knowledge on the impact this radiation has. The aim of our study was to investigate the effect of 900 MHz electromagnetic field (EMF) on the exploratory behavior of female *Ixodes ricinus* ticks under laboratory conditions. Experiments were realized in the radiation-shielded tube (RST) test radiation-shielded circular open field arena, altogether 480 female *I. ricinus* ticks were tested. While in the RST arena we observed only a tendency to move towards the irradiated part of the arena, in the open field arena the time spent in the part exposed to EMF and the trajectory passed was significantly longer.

Introduction

Over the last decades the cell phone technology has rapidly changed. Nowadays, cell phones represent the core part of human life and cover personal family or social interactions and affect the working environment. However, there is limited data on the possible effects of electromagnetic field (EMF) exposure on living organisms. The number of mobile telecommunication devices and duration of usage is increasing at a rapid rate¹. The number of smartphone users today surpasses three billion worldwide and is predicted to grow by several hundred million in the next few years. China, India, and the United States have the highest number of smartphone users, with each country easily surpassing the 100 million user mark. Moreover, society claims to no longer be able to live without using such equipment, which functions by transmitting man-made electromagnetic waves². Global System for Mobile Communications (GSM) is a standard for mobile phones all over the world which operates in a number of carrier frequencies with most networks operating in the 900 MHz frequency. The GSM utilizes a cellular network provided by base-stations subsystem which provides radio coverage over a wide geographic area enabling wireless connection of devices³.

Living organisms are very sensitive to changes in their environment and react also to the presence or change of the electromagnetic field. Several authors have studied the impact of EMF on different types of organisms ranging from bacteria to invertebrates to vertebrates⁴⁻¹¹. Currently, it is a known fact that mobile phones affect living organisms⁶. Exposure of *Drosophila melanogaster* young adult insects to EMF radiation, emitted by an active GSM mobile phone handset during a usual "talk" operation for a few minutes daily for 2-5 days, revealed an impressive decrease (up to 57%) in reproductive capacity (fecundity)¹². Exposure of EMF from a mobile phone affected the generation time of *D. melanogaster*, although after several generations the generation time returned to normal levels¹³. A major field study on insect pollinators had evidence that electromagnetic smog may have important ecological and economic impacts on the pollination service that could significantly affect the maintenance of wild plant diversity,

crop production and human welfare¹⁴. Balmori⁴ proved that EMF has a significant effect on reducing the number of insects (pollinators) in nature. Together with climate change, invasive species, and other factors, this can lead to significant changes in agriculture⁴.

Ixodes ricinus (Acari:Ixodida) is an important vector of tick-borne pathogens¹⁵. *I. ricinus* is the most widespread hard tick species in Europe and feeds on a broad range of hosts, serving as reservoirs and vectors of many zoonotic pathogens, including viruses, bacteria, unicellular parasites, and helminths¹⁶. The influence of the electromagnetic field as a potential factor that ticks can sense was studied on the species *Dermacentor reticulatus* and *Hyalomma asiaticum*¹⁷⁻¹⁹. Only one study has investigated the effect of EMF on the behavior of *I. ricinus* ticks. Frątczak et al.²⁰ found that *I. ricinus* ticks were attracted to the irradiated area of the experimental arena. This effect was significantly stronger for ticks infected with *Rickettsia* spp., suggesting that pathogens can alter the ticks' response to environmental stimuli²⁰.

The increasing number of smartphones and their use in nature (cellphones, internet) is changing the local environment and affecting living organisms. In this study, we investigated the effect of 900 MHz RF EMF frequency on the locomotor behavior of female *I. ricinus* ticks in the radiation-shielded arena and in the circular open field arena under laboratory conditions.

Materials And Methods

Model organism

Ixodes ricinus females were used as experimental model organisms. In total, 620 adult female *I. ricinus* ticks were used. Ticks were collected in spring 2018 from vegetation by the flagging method with a white cotton blanket (1 m²). The ticks were collected in a forest park in Kosice city in eastern part of Slovakia (48,745 °N; 21,277 °E). In the laboratory, all collected ticks were determined to the species level according to Siuda and separated to individual tubes according to developmental stage and gender²¹.

All collected ticks were kept in polypropylene tubes under stable conditions in an environmental chamber at 16°C and 90% relative air humidity under 16:8-h light-dark regime. All ticks were maintained free from the influence of external stimuli, such as odor, that could affect their behavior.

Experimental setup

Radiation-shielded tube test

A total 280 female *I. ricinus* ticks were placed into the Radiation Shielded Tube test (RST), where females had a choice between a shielded and unshielded part. Altogether 140 female ticks were exposed to 900 MHz RF EMF in the RST arenas for 24 hours. A control group of *I. ricinus* ticks (140 individuals) were placed in RST tubes under the same conditions without RF - EMF exposure. For the detailed description of RST arenas see Vargová et al., 2018¹⁸.

Source of radiation for RST

We focused on the artificially emitted electromagnetic field with 900 MHz frequencies. These frequencies represent the range frequency limits that are used in common telecommunications services. The 900 MHz frequency band is used in the Global System for Mobile Communications (GSM).

A signal generator (N5183A, Agilent, MY) was used as a source of RF-EMF connected to the Double-Ridged Waveguide Horn Antenna HF907 (Rohde and Schwarz, Munich, Germany). The output power of the generator was calculated and placed at a distance 2 m from the antenna with a power flux density of 1 mW/m^2 which is 0.6 V/m on the electric field intensity. The temperature in the anechoic chamber was 22°C and the humidity was 60%, measured by using a LabQuest 2 unit (Vernier Software & Technology, Oregon, USA).

Experimental procedure of RST

In a single RST test, 10 female ticks *I. ricinus* were placed directly in the middle of the test tube. 14 tubes were used to conduct an irradiation or control sample tests. The total number of females used in the irradiation experiment was 140. They were exposed to 900 MHz RF-EMF. The control group consisted of 140 ticks and they were not exposed to RF-EMF. The experiments lasted for 24 hours. Finally, the total number of ticks in both individual arms of RST was collected. The experiments were performed in the darkness, to exclude the influence of light to individual's behavior.

Radiation-shielded circular open field arena

In the specifically modified behavioral arena, 2 zones with different experimental conditions: 1. with EMF and 2. EMF free were established (Fig. 1). The arena is of a circular base with a diameter of 100 cm and a height of 50 cm made of white opaque polypropylene (PP), in a position 1 meter above the floor. Copper foil 35 Micron Double-sided on 1.6 mm epoxy glass laminate desk (DSCED in short) served as EMF shielding. The DSCED semicircle attached to the bottom of the arena half-circular and perpendicular to the base the rectangle shape divided the space of the interest into shielded/unshielded environments. There was no barrier in the arena between the two halves so that the ticks could move freely. The cell phone was used as an EMF source. It was placed on the unshielded side, 0.2 m from the center of circular projection to the floor (in Fig. 1 depicted as a dashed line).

Source of radiation for circular arena

As a source of RF-EMF common cell phone registered in the cellular telecommunications network was used. 900 MHz frequency RF - EMF emission was provided by an ongoing call on the cell phone (model Samsung J5 2016, The South Korea). The 900 MHz mode was performed by changing the phone settings by selecting the network mode 2G only. The phone operation at maximum power was achieved by limiting the intensity of the GSM base station external signal to reach the lowest received signal strength. The availability of the external GSM signal was controlled inside the chamber simply by the

position of the electromagnetic shielding on the entrance door. The lowest achieved operating intensity of the signal was 0.037 V/m as measured by a signal analyzer (Spectran HF 60105, Aaronia, DE). Generated by the cell phone during a call, the maximum intensity of EMF in the unshielded part of the arena was 0.61 V/m, while in the shielded one it was 0.09 V/m, both measured in situ by the signal analyzer.

Experimental procedure of circular arena

Ten individual female *I. ricinus* ticks were placed on the centerline of the circular arena, where ticks had a choice between a shielded and unshielded part. The experiment lasted 120 min, after this time the emission on EMF was stopped and ticks were counted in the shielded and unshielded part and collected into polypropylene tubes with 70% ethanol. Altogether 20 experiments were performed.

The movement of the ticks in the arena was recorded by a CCD camera (model Panasonic HC-X920 Japan). The camera was equipped with a wide-angle 29.8 mm lens and the aperture F1.5 was used. The recording was made in Full HD resolution. The recording rate of 1 frame per second was established experimentally. It provided sufficiently detailed sequences for further tick movement analysis. Up to 12x optical zoom and 5-axis image stabilization were used. The individual experimental observations were recorded and stored in a separate record.

Anechoic chamber

The experimental arena was placed in the anechoic chamber (model 1710 – 100, Comtest, The Netherlands) which ensured no external electromagnetic fields to be used to violate the experiment. All experiments were performed in an anechoic chamber. Temperature in the anechoic chamber was 22°C and air humidity was 60%, measured by using a LabQuest 2 unit (Vernier Software & Technology, Oregon, USA). The interior was illuminated by the pair of low power EMF free lights placed in two opposite corners of the ceiling of the chamber.

Feature extraction and statistical analysis

We used a camera-based method for quantifying track length and the time spent in irradiated or shielded part of the arena. To analyze recorded tick's movement sequences the software tool has been used, discussed elsewhere. The recorded area was 1920x1080 pixels, out of which a circular arena was extracted.

Both horizontal and vertical neighbor pixels' displacement represents approx. 1 mm distance in real measure in these directions.

Ticks in the arena were tracked and digitized information saved in the data file, i.e. tick's identity and x,y-coordinates, too. The coordinates represented the center of the tick's body (center of the gravity). The tracking performance is depicted in Fig. 2. Shadowed area means EMF shielded part of the arena.

The spatio-temporal evidence of ticks inside the arena during the experiment was analyzed. The arena digital representation was divided into the shielded and unshielded parts. The analysis consists of the registration of the tick's digitized coordinate in that specific part where it belongs. It was realized in a cycle over all digitized coordinates.

The trial time was divided to 10-minute periods. Digitized path data of 160 individuals were used to calculate several parameters in these time parts: (1) the total track length (TTL) as the sum of track length contributions walked by ticks included in the experiment; (2) the total track length in the irradiated zone (TTL_I); (3) the total track length in the shielded zone (TTL_S) zone. Similarly, during the temporal observation individuals' evidence in the shielded and unshielded parts was performed. Additional parameters were calculated: (4) percentage of the time spent in the arena (PIZ); (5) percentage of the time spent in the irradiated zone (PIZ_I); (6) in the shielded zone (PIZ_S). The descriptive statistics was calculated and compared.

Results

Radiation-shielded tube test

In total, we performed tube tests with 420 *I. ricinus* females. When analyzing the impact of 900 MHz, in total 59 ticks moved toward the irradiated part of the tube and 81 reached the shielded part of the tube. In the control group, out of the total number of tested females, 69 moved toward the unshielded part and 71 moved toward the shielded part of RSTs.

Fisher's exact test did not show significant differences ($p = 0.2803$) in preference of irradiated parts of experimental modules. However, the tendency of movement toward the irradiated part with 900 MHz frequency is evident (Table 1, Fig. 3).

Table 1

The indication of EMF (900 MHz) irradiated zone preference by *Ixodes ricinus* females during 14 trials in the RST arena without significance established. The preference is indicated by differences between control and test populations ($n = 140$) in arena zones.

RST Arena	Median Occurrence per trial	Occurrence Sum
Irradiated zone	Control: 5.5	Control: 69
	Test: 4.0	Test: 59
Shielded zone	Control: 4.5	Control: 71
	Test: 6.0	Test: 81

Radiation-shielded circular open field arena

After ticks were released in the center of the circular arena, they immediately started to explore the arena. No evidence to escape was observed. In the first ten minutes of locomotion activity was the highest, the

total length they travelled was largest and it subsequently decreased gradually with the experimental time, Fig. 4.

When comparing TTL in the irradiated (TTL_I) and shielded (TTL_S) part of the arena we observed similar movement in the first 30 minutes of the experiment and after this time TTL was higher in the irradiated part. After one hour of exposure, the length travelled in the irradiated zone is significantly longer (Fig. 5).

Differences were observed also in the time spent in parts of the arena. The percentage span spent in the irradiated part (PIZ_I) ranged from 50.5 to 66.7% while in the shielded part from 33.3 to 49.5% (Fig. 6).

Individuals also walked a significantly different when radiation took place. It was reflected in changed movement dynamics in both irradiated and shielded zones. It is indicated by median comparison of absolute distance records between two following positions of the individuals, i.e. walked distance per sample (WDS) in (mm/s), as recorded in both zones and whole arena. One sample Wilcoxon test for 10, 60 and 120 minute periods in irradiated and shielded zones was done against the median of the WDS parameter calculated in whole arena in the corresponding periods, Table 2. The significance level was 0.05 (Table 2).

Table 2

The effects of cell phone radiation on *Ixodes ricinus* females walking dynamics change. One sample Wilcoxon Signed Rank Test at significance level 0.05.

<i>Trial time (min)</i>	<i>WDS Median in whole arena</i>	<i>zone</i>	<i>WDS Median</i>	<i>p-value</i>	<i>Significance</i>
10	1.306	Irradiated	1.359	< 0.001	significant
		Shielded	1.250	0.384	not significant
60	1.232	Irradiated	1.208	0.183	not significant
		Shielded	1.262	< 0.001	significant
120	0.508	Irradiated	0.619	< 0.001	significant
		Shielded	0.073	< 0.001	significant

Discussion

The electromagnetic field is a phenomenon that is present in the ecosystem, and all living organisms evolved in the presence of weak, however omnipresent geomagnetic fields. Over the last decades also artificial sources of this EMF are expanding rapidly and are affecting all living and nonliving systems²². Man-made EMF, which are new, unknown for organisms, could interfere and disturb normal physiological functions¹⁰. The question of this disturbance carries information that transforms into a subsequent reaction or causes damage or disorientation is not answered yet. Studying the impact of artificial EMF on

living organisms, as a relatively new phenomenon that has appeared in nature in connection to human activity, is important to clarify what EMF causes and how it affects nature and organisms.

In our study, we performed two types of experiments where *Ixodes ricinus* was a model organism and 900 MHz was selected frequency. The first type of experiments were realized in RTS modules what is a modified T maze for testing the preference of area with EMF versus shielded area and EMF was emitted by generator and antenna. In the second type of experiment, the exploratory behavior of ticks was monitored in an open field arena and the source of EMF was an ongoing call on the cell phone registered in the GSM network.

In the RST test we observed the tendency to move toward the 900 MHz irradiated part of the experimental module. The results of testing of *D. reticulatus* ticks in the same RST modules revealed that ticks were attracted significantly more to a frequency of 900 MHz RF-EMF frequency in comparison to 5000 MHz RF-EMF frequency and control. Moreover, the reaction of *D. reticulatus* to the presence of the EMF was more prominent than in our experiment with *I. ricinus*, which gives us the presumption that there could be differences in the reaction to the EMF in different tick species. The movement toward the source of artificial EMF in natural habitat was observed also in wild bees and bee flies¹⁴.

Based on our knowledge, until now, no study has been carried out with call in progress as the source of 900 MHz EMF. Our data clearly indicate that *I. ricinus* females moved faster, walked longer distances in the arena and stayed longer in the irradiated zone of the arena in the presence of 900 MHz frequency EMF originated from the cell phone during a call. Several studies have focused on the walking and questing behavior of ticks in dry versus humid conditions, as air humidity is the key factor for tick activity and survival^{23,24}. Few authors have focused on the effect of extremely low EMF frequencies on insects walking²⁵⁻²⁷. Wyszkovska et al. showed that exposure to relatively high levels of extremely low frequencies of EMF, present around overhead power transmission lines, and affect the behavior, neuronal and muscular responses and levels of heat shock protein in the locust. Extremely low frequencies EMFs significantly alter the normal walking behavior of locusts, with distance travelled being reduced following exposure to EMF²⁶. However, our findings suggest that *I. ricinus* females walk significantly faster. Moreover, our results indicate that ticks walked faster at the beginning of the experiment and gradually decreased, which may be due to either exploratory behavior where ticks habituated to a new environment or, they gradually adapted to the presence of the field. Favre and Johansson (2020) described that the EMF disturbed the colony of honeybees which produced strong worker piping signals. Such signals are typically produced shortly before take-off of a swarm, or as the sign of a disturbed colony²⁸.

After one hour of a phone call there were significantly longer travelled distances observed in the irradiated zone. Buczek et al., described the locomotor activity of *D. reticulatus* ticks in their natural environment. Questing, *D. reticulatus* females walked a greater distance than males²⁹. Wyszkovska et al. discovered that EMF reduces distance walked in locusts²⁶. Jacskon et al. investigated the effect of the static electric field on the locomotion activity of cockroaches and explained significant changes in locomotion by covering less distance, walking slowly and turning more often³⁰. We can assume that the 900 MHz

frequency used in our experiment has the opposite effect of inducing a longer distance travelled in ticks of the *I. ricinus* ticks.

In conclusion, we found that ticks react to the presence of man-made EMF and there is change in the *I. ricinus* female tick locomotor behavior when exposed to 900 MHz frequency. The study indicates that the usage of cell phones which is an exponentially expanding phenomenon introducing electromagnetic load in the environment which inevitably has a biological effect on all living organisms not excluding tick which are epidemiologically very important parasites.

Declarations

Acknowledgments

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Author Contributions

J.K. and I.M. made the conceptualization; J.K., I.M., V.M., R.C., P.T. developed methodology and performed software, validation, formal analysis, and investigation; B.V., J.Z. collected resources; data curation; B.V. performed writing—original draft preparation, V.M., J.K., I.M., P.T. writing—review and editing; R.C., J.K. and V.M. conducted supervision, project administration, funding.

Competing interest Statement

Authors declare any competing financial and/or non-financial interests exist.

Availability of Data and Materials

The datasets used and/or analysed during the current study is available from the corresponding author on reasonable request.

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Figures

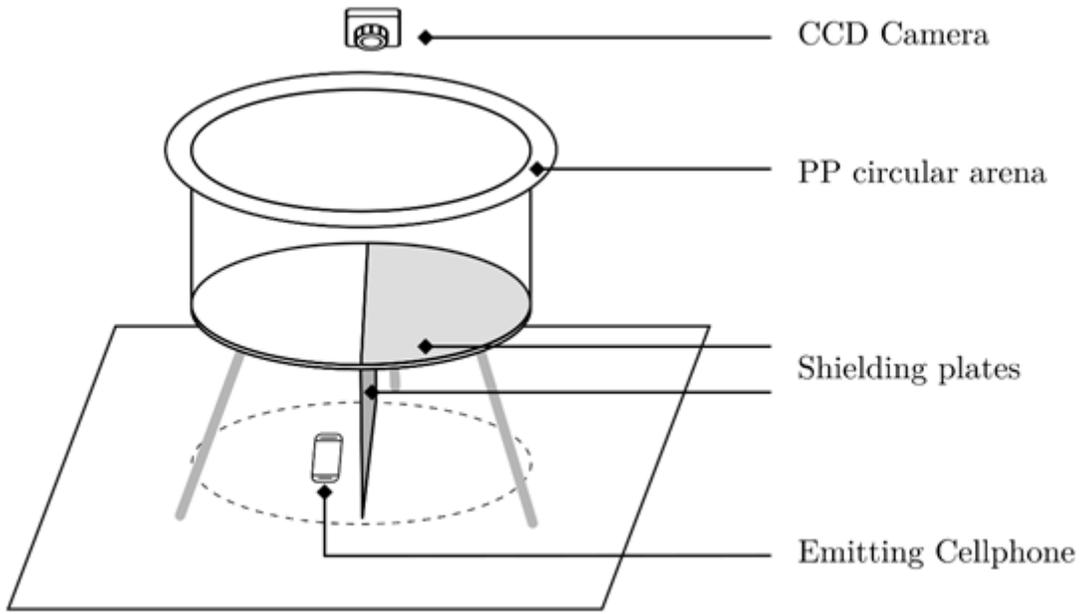


Figure 1

Experimental setup of radiation-shielded circular open field polypropylene (PP) arena with CCD (charge-coupled device) camera for recording movements of ticks (not to scale)

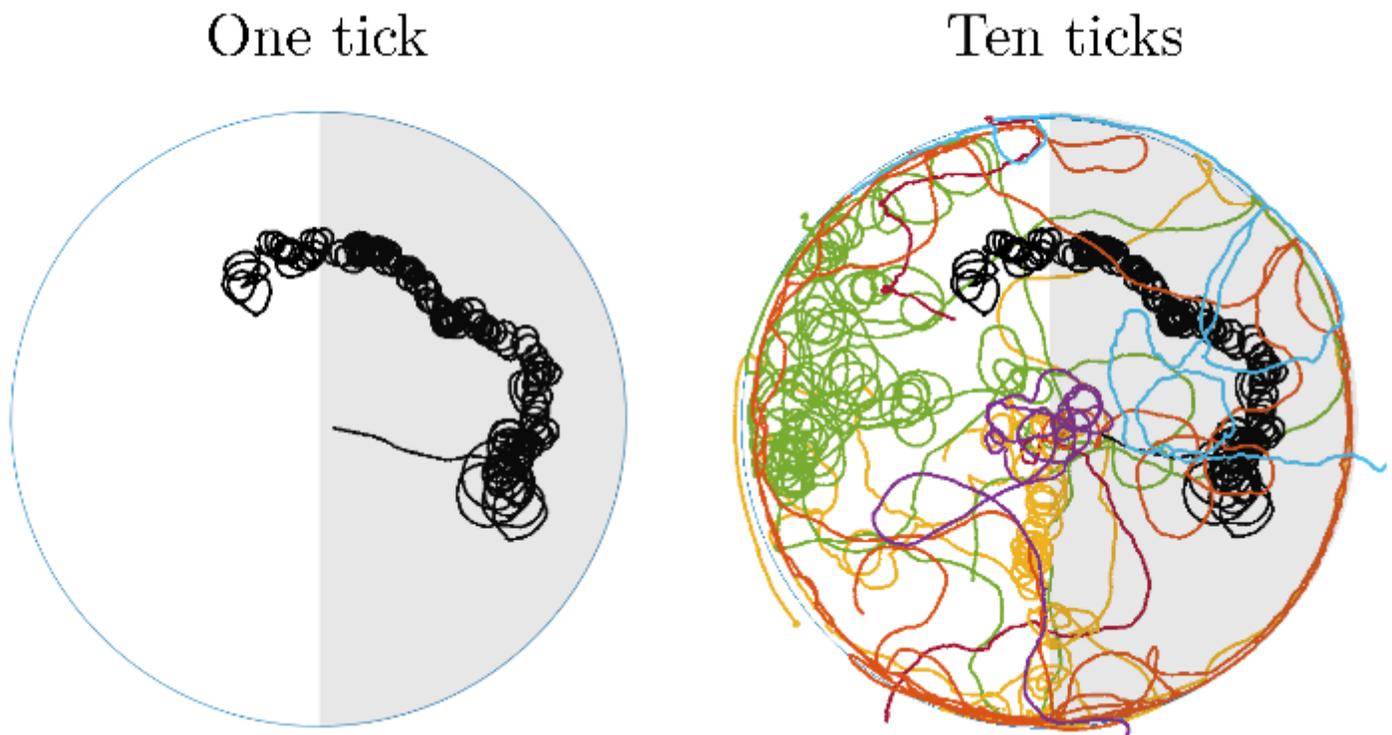


Figure 2

Example of tracking of tick movement in the circular arena.

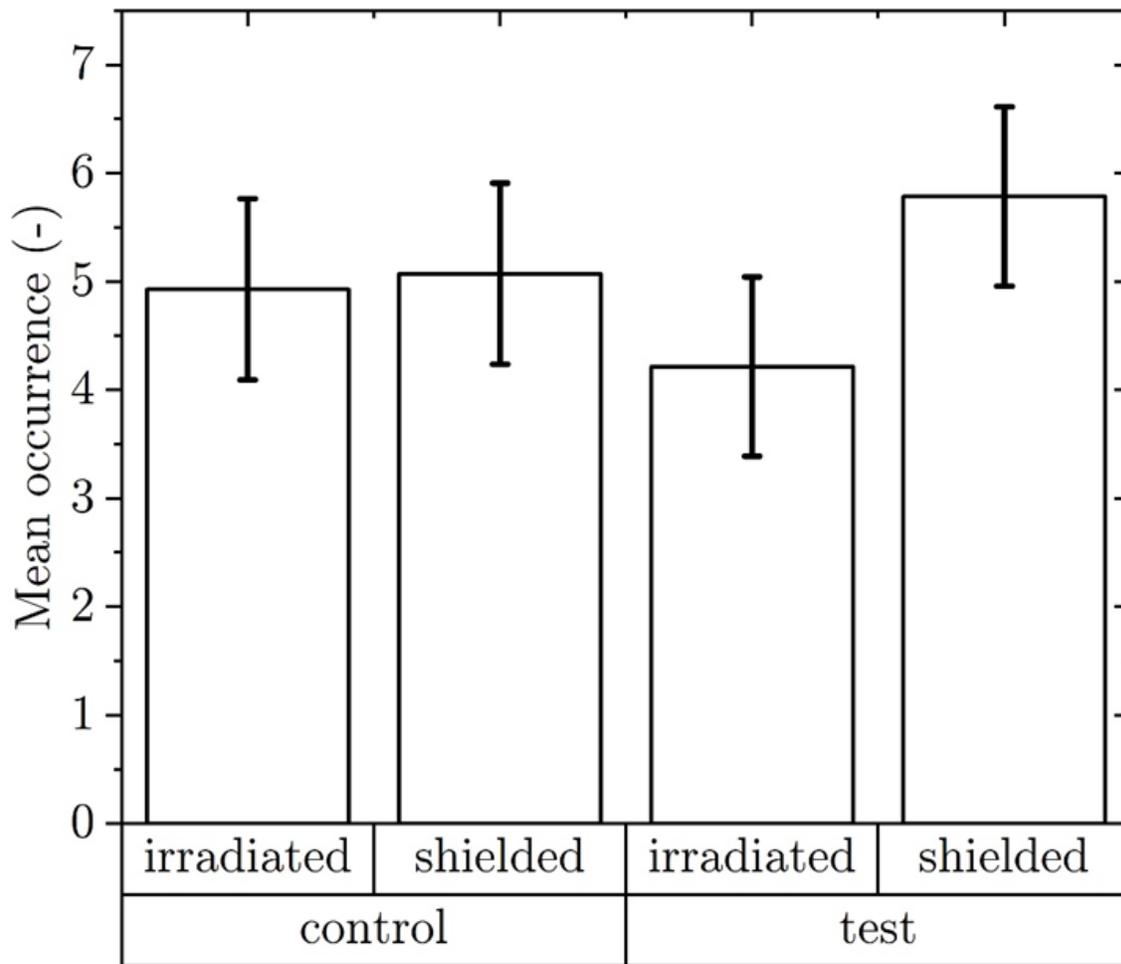


Figure 3

Mean occurrence of *Ixodes ricinus* females per trial in the RST arena in arena zones irradiated or shielded (n=140, trials=14).

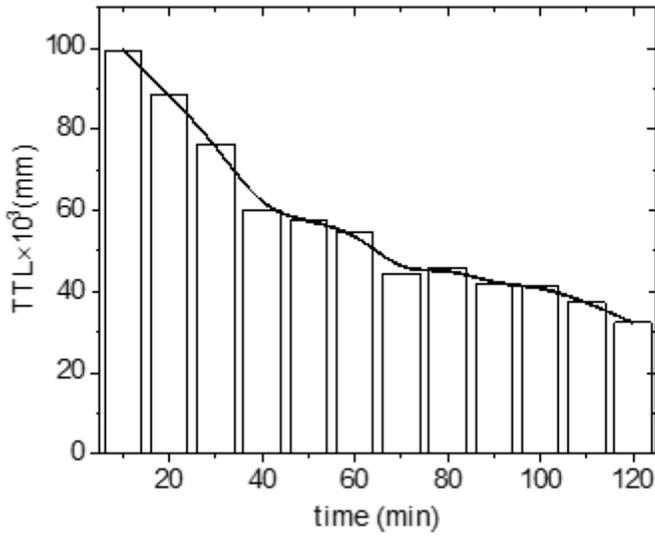


Figure 4

The total travelled length (TTL) by *Ixodes ricinus* females during 120 minutes of the experimental trials. Boxes are sums of a single track lengths (n = 160).

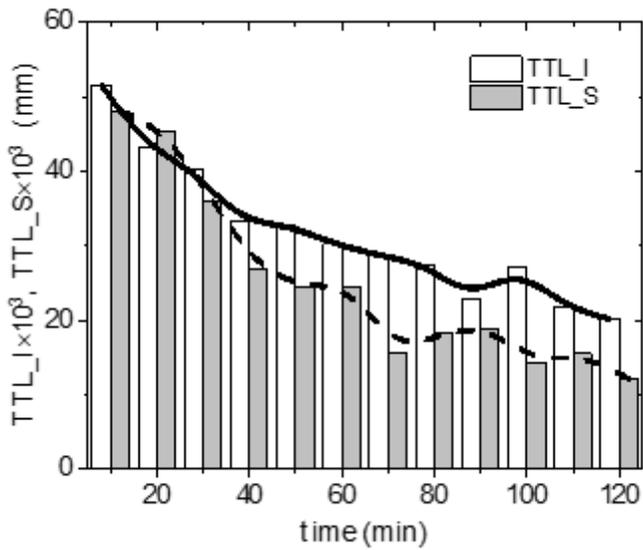


Figure 5

The changes of total travelled length by *Ixodes ricinus* females in irradiated (TTL_I) or shielded (TTL_S) arena zones during 120 minutes of the experimental trials. Boxes are sums of track lengths (n = 160).

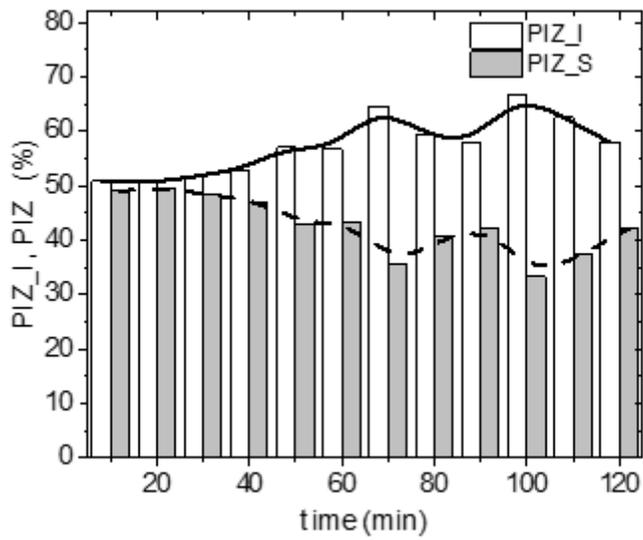


Figure 6

The percentage of time spent by *Ixodes ricinus* females in irradiated (PIZ_I) or shielded (PIZ_S) arena zones during 120 minutes of the experimental trials (n = 160).