Odrednice ponašanja i habituacija u testu otvorenog polja kod dvije vrste gušterica, primorske (Podarcis siculus) i krške gušterice (Podarcis melisellesis)

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Bahaviour profile and habituation in open field test of two species of lizard, the Italian wall lizard, *Podarcis siculus* and the Dalmatian wall lizard, *Podarcis melisellesis*

Graduation thesis

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This thesis is created in the laboratory on the Institute for Animal Physiology of the Faculty of Science, University of Zagreb, under the mentorship of Dr. Duje Lisičić, Asst. Prof.. The thesis was handed over to the Department of Biology of the Faculty of Science, University of Zagreb for evaluation in order to acquire the title Master of Experimental Biology.

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Graduation Thesis

BAHAVIOUR PROFILE AND HABITUATION IN OPEN FIELD TEST OF TWO SPECIES OF LIZARD, THE ITALIAN WALL LIZARD, *PODARCIS SICULUS* AND THE DALMATIAN WALL LIZARD, *PODARCIS MELISELLESIS*

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Abstract: The Italian wall lizard, *Podarcis siculus* and the Dalmatian wall lizard, *Podarcis melisellesis* are two lizards from the family of Lacertidae. When this two species share same habitat, *P. siculus* overpowers *P. melisellesis* as a dominant competitor, usually leading to extinction of *P. melisellesis*. In this Graduation thesis I was trying to establish the behaviour of these two species of lizards, *Podarcis siculus* and *Podarcis melisellesis* in a new environment. In order to do so, we observed habituation period in open field test and 8-arm radial maze. Habituation is an extremely simple form of learning, in which an animal, after a period of exposure to a stimulus, stops responding. We had 28 specimens of each species, 14 females and 14 males. Each experiment lasted 15min or maximum of 23 minutes. We had 3 trials and then compare first and third trial. Parameters of interest were latency time, time spent in the central vs. marginal area and returning into hiding place. We tested for the behavioural differences between the species and sexes.

(59 pages, 45 figures, 17 tables, 48 references, original in: English)

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Key words: Habituation, *Podarcis siculus*, *Podarcis melisellesis*, open field test, 8-arm radial maze

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ODREDNICE PONAŠANJA I HABITUACIJA U TESTU OTVORENOG POLJA KOD DVIJE VRSTE GUŠTERICA, PRIMORSKE (*PODARCIS SICULUS*) I KRŠKE GUŠTERICE (*PODARCIS MELISELLESIS*)

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Sažetak: Talijanska zidna gušterica, *Podarcis Siculus* i Dalmatinska zidna gušterica, *Podarcis melisellesis* su dvije vrste guštera iz porodice Lacertidae. Kada ove dvije vrste dijele isti habitat, *P. Siculus* nadjačava *P. melisellesis* kao dominantna vrsta, što obično dovodi do izumiranja *P. melisellesis*. U ovom diplomskom radu sam pokušavala utvrditi ponašanje tih vrsta guštera, *Podarcis Siculus* i *Podarcis melisellesis* u novom okruženju. Da bi se to postiglo, gledali smo razdoblje navikavanja u otvorenom prostoru i u radijalnom labirintu. Habituacija je vrlo jednostavan oblik učenja, u kojem je životinja, nakon razdoblja izloženosti poticaju, prestaje reagirati. Imali smo 14 uzoraka svake vrste, 7 ženki i 7 muškaraca. Svaki eksperiment je trajao 15 minuta. Imali smo 3 pokusa, a zatim usporedili prvi i treći pokus. Parametri interesa su vrijeme, vrijeme provedeno u središnjem odnosno vanjskim površinam i povratak u mjesto za skrivanje. Testirali smo za razlike u ponašanju između vrste i spolova.

(59 stranica, 45 slika, 17 tablica, 48 literaturnih navoda, jezik izvornika: engleski)

Rad je pohranjen u Središnjoj biološkoj knjižnici

Ključne riječi: Habituacija, *Podarcis siculus, Podarcis melisellesis,* test otvorenog prostora, radijalni labirint sa 8 krakovima

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Table of Contents

1. INTRODUCTION

1.1 Ecology and interspecific relations between species

The Italian wall lizard, *Podarcis siculus* Rafinesque, 1810 is a species of lizard from the family of Lacertidae that ranges throughout Italy, south of the Alps (including Sicily, Sardinia, and many other islands in the Tyrrhenian Sea), in southern part of Switzerland, Corsica (France), and along the Adriatic coastal area from southwestern Slovenia, through western and southern Croatia, southern Bosnia and Herzegovina to Kotor, Montenegro. It also occurs as isolated introduced populations in southern France, the Iberian Peninsula (Spain and Portugal), Menorca in the Balearic Islands (Spain), on both sides of the Bosporus in Turkey, in Tunisia, and Lampedusa (Italy). It has been introduced to a number of sites in the United States, and might have been introduced to Libya. It ranges from sea level up to 2,200 m asl. It is not an endangered species and its population is currently increasing. It is considered an invasive species in some areas and it is found in grassy areas, roadside verges, hedgerows, scrubland, woodland edges, in pine plantations, vineyards, orchards, meadows, coastal dunes, parkland, urban areas, and on stone walls and buildings. In the northern part of its range, it mainly lives in riparian or coastal areas. It thrives in habitats disturbed by human activities in the southern part of its range. In the northern part it is threatened by small scale agricultural conversion of its riverine habitats. Localized or insular populations may be vulnerable by predation of cats. Because it is locally common, this species is collected as food for pet snakes (Arnlods, 2003).

The Dalmatian wall lizard, *Podarcis melisellensis* Braun, 1877 occurs in Mediterranean and sub-Mediterranean zones from extreme north-eastern Italy through southwestern Slovenia, Croatia, southern Bosnia-Herzegovina, southern Montenegro to north-western Albania. It is present on many Adriatic islands. The species ranges from sea level up to 1,400 m asl. It is found in dry open woodland, scrub, pastures and overgrown areas. It can be found on cliffs, rocks and stone walls. Some distinct island populations may be threatened by the introduction of cats and other predators, such as the Indian mongoose on some Adriatic islands (Arnold, 2003).

When the two species live in the same habitat, the *Podarcis siculus* seems to be the dominant competitor, which usually leads to the extinction of the *Podarcis melisellensis* from that area. In this graduation thesis we were interested to know why. We focused on the ability of those two species to adapt to the new environment. In other words, our main focus was habituation (Downes et al. 2002).

1.2 Intra- and interspecific competition

When discussing competition, one of the most important, basic concepts is that of the niche. The niche is the functional relationship of an organisms to its physical and biological environment. Niches are multidimensional in that they include a wide variety of aspects of the environment which must be considered. Normally, when defining a niche we look at 3 sets of parameters:

- Range of physical factors for survival and reproduction: temperature, humidity, pH, soil, sunlight, etc.
- Biological factors: predators, prey, parasites, competitors, etc.
- Behaviour: seasonality, diurnal patterns, movement, social organization, etc.

The ecological definition of niche includes a place where an organism lives (its habitat) as well as all the aspects defined above.

Competition is a biological interaction among organisms of the same or different species, associated with the need for common resources that occur in a limited supply relative to demand. In other words, competition occurs when the capability of the environment to supply resources is smaller than the potential biological requirement and organisms interfere with each other (Zug, 1993).

Intraspecific competition occurs when individuals of the same species compete for access to essential resources. Stresses associated with competition are said to be symmetric if they involve organisms of similar size and/or abilities to utilize resources. Competition is asymmetric when there are substantial differences in these abilities.
Individuals of the same species have virtually identical resource requirements. Therefore, whenever populations of a species are crowded, intraspecific competition is intense.
Intraspecific competition in dense populations results in a process known as self-thinning, which is characterized by mortality of less-capable individuals and relative

success by more competitive individuals. In such situations, intraspecific competition is an important regulator of population size. Moreover, because individual organisms vary in their reproductive success, intraspecific competition can be a selective factor in evolution (Pough et al. 2001).

Interspecific competition refers to the competition between two or more species for some limiting resource. This limiting resource can be food or nutrients, space, mates, nesting sites- anything for which demand is greater than supply. When one species is a better competitor, interspecific competition negatively influences the other species by reducing population sizes and/or growth rates, which in turn affects the population dynamics of the competitor. Competitive interactions between organisms can have a great deal of influence on species evolution, the structuring of communities (which species coexist, which don't, relative abundances, etc.), and the distributions of species.

Whatever the type of competition, it will be strongest at high population densities. The more organisms there are, the more strongly they will compete for the remaining resources (Zug, 1993).

Competition shapes the natural world to a great extent. One principle of ecology is called **competitive exclusion**, which states that two species competing for the same limiting resource in an area cannot coexist. This means that it is rare indeed to find two very closely related species in the same area. If they are closely related they will compete for almost all of the same things, and this will mean that inevitably they will compete for some item that is in short supply (the limiting resource). One species or the other will be better at the competition and will displace the other. If two similar species are found in the same place, careful examination often finds that they differ in the way they use the resources is some critical, but often not apparent way.

Not all environments are resource limited, and in such situations competition is not a very important process. There are two generic types of non-competitive environments - recently disturbed and environmentally stressed. In habitats that have recently been subjected to a catastrophic disturbance, the populations and biomass of organisms is relatively small, and the biological demand for resources is correspondingly not very intense. Species that are specialized to take advantage of the resource-rich and competition-free conditions of recent disturbances are known as ruderals. These species are adapted to rapidly colonizing disturbed sites where they can grow freely and are highly fecund. However, within several years the ruderals are usually reduced in abundance or eliminated from the community by slower growing, but more competitive species that eventually take over the site and its resources and dominate later successional stages (http://w3.marietta.edu/~biol/biomes/competition.htm: 9.2.2017; 17:01; https://www.khanacademy.org/science/biology/ecology/community-ecosystem-ecology/a/niches-competition; 10.2.2017; 10:18; Dowes et al. 2002)

1.3 Study of interspecific relations with behavioural tests

Habituation is an extremely simple form of learning, in which an animal, after a period of exposure to a stimulus, stops responding. The most interesting thing about habituation is that it can occur at different levels in the nervous system. Sensory systems may stop sending signals to the brain in response to a continuously present or often-repeated stimulus (Cohen et al. 1997). Habituation to a complex stimuli may occur at the level of the brain; the stimulus is still perceived, but the animal has simply "decided" to no longer pay attention (Rose and Rankin 2001). Habituation is important in filtering the large amounts of information received from the surrounding environment. By habituating to less important signals, an animal can focus its attention on the most important features of its environment. A good example of this is species that rely on alarm calls to convey information about predators. In this case animals stop giving alarm calls when they become familiar with other species in their environment that turn out not to be predators. (http://www.animalbehavioronline.com/habituation.html; 17.1.2017; 13:21).

1.4 Theme explanation

To learn mora about the process of habituation in our two species of lizard, we executed simple experiments. The tests we used were open field test and 8-arm radial maze. Our parameters of interest were latency time, time spent in the central vs. marginal area, returning into hiding place, food eaten, laterality, time needed to get lizard back into the tube, lifting on hind legs, mean velocity, distance moved, meandering, mobility state, maximum alternations in 8-arm maze, direct revisits, indirect revisits, and time chasing. We tested for the behavioural differences between the species and sexes.

The objective of this graduation thesis was to establish whether there is a significant difference between behaviour of two species, *Podarcis siculus* and *Podarcis melisellensis* in the new environment. Our hypothesis is that these two species act different in the new environment and that there is also a significant behavioural difference between sexes within the species, which consequently leads to the different survival success of the species.

2. MATERIALS AND METHODS

To achieve our objectives, we decide to use the following methods and materials.

2.1 Description of the species

2.1.1 Italian wall lizard, *Podarcis siculus*:

We collected 28 animals (14 males and 14 females) of the species *Podarcis siculus*, family Lacertidae, order Squamata, class Reptilia from the Sinjsko polje, Croatia, with the accordance to permit of the category UP/I-612-07/16-48/142, no. 517-07-1-1-1-16-4.

The length of this lizard is up to 9 cm. Pattern and coloration of the dorsal side may vary from green (which is the usual coloration) to grey-brown with mixed dark pattern. The abdominal side is whitish or reddish, without dark pattern. It prefers rocky-stony places and rough stone walls, seen on garden walls or in cemeteries. It feeds on insects. Female lays 3-12 eggs. (http://www.cabi.org/isc/datasheet/68192; 12.2.2017; 18:17; Arnold 2003)

Figure 1: Photograph of *Podarcis siculus*

2.1.2 Dalmatian wall lizard, *Podarcis melisellensis*:

We collected 28 animals (14 males and 14 females) of species *Podarcis melisellensis*, family Lacertidae, order Squamata, class Reptilia from the Sinjsko polje, Croatia, with the accordance to permit of the category UP/I-612-07/16-48/142, no. 517-07-1-1-1-16-4.

Dalmatian wall lizards grow up to 6.5 cm in length. Tail is about twice as long as the body. Female lizards lay 2–8 eggs. After hatching, the juveniles are about 2.5 cm in lenght.

These lizards display three ventral color morphs: from white and yellow to orange. A male of orange colour is seen as more dominant male than any other morph in intrasexual competition, since the orange color displays the lizard as more aggressive. Orange morph lizards have a larger size and bite force. In this species of lizards, the females tends to prefer the orange males since they are bigger and percieved to be healthier and can give a female's offspring better benefits. Even though females prefer to mate with orange morphs, they will still mate with yellow morphs. Yellow morph lizards give females more direct benefits like protection and small territory. Meanwhile, white males are only able to mate by intruding another male's territory and mating with their females. (Arnold, 2003; Huyghe et al. 2007)

Figure 2: Podarcis melisellensis, photograph taken fromhttp://www.bio-foto.com/displayimage-4187.html

2.2 General procedure

The animals were kept in the room in Department of Animal Physiology, modified with air conditioning apparatus in order to sustain colony of lizards for a few weeks. Animals were held individually in the plastic terrariums, with ad libitum access to food and water and a plastic tube in each terrarium for a hiding place and transfer of a lizards from and into the terrarium. The light was equivalent to the outside circadian rhythm and the temperature ranged from 28 – 29°C during the day and it was falling at 22°C during night. When the experiment started, the lizards were put in a test box/maze using the plastic tube that each lizard was familiar with. The experiment lasted 15 min, unless the lizard did not came out of the tube in the first 10 min, in that case, the experiment was prolonged for 8 minutes, which means that the experiment lasted maximum of 23 minutes. We conducted the experiment four times. Two times in July 2016 and two times in September 2016.We marked the lizards according to species, sex and number from 1 to 14. For example, we marked the lizards of the species *Podarcis siculus* with letters PSF (female) or PSM (male) and numbers 1-14 (PSF1-PSF14 and PSM1-PSM14) and *Podarcis melisellensis* with PMF or PMM 1-14 (PMF1-PMF14 and PMM1-PMM14). In July we used the first 28 lizard of each species, 14 lizards of each sex (on 4.- 6.7.2016 and again on 19.- 21.7.2016) and in September we used other 14 specimens of each species and sex (on 5. - 7.9.2016 and 20. - 9.2016). We conducted the experiment each day of the trial about 6 hours per day, from approximately 9 am to 15 pm and the order in which the lizards were put through the experiment was randomized to prevent lizards from memorising a certain setup. In the first set of trials we put *Podarcis melisellensis* in the radial maze and *Podarcis siculus* in the open field test and in the second we switched that order. All lizards were recorded with camera in all of the trials, videos were then saved on the PC and cut for each individual lizard in each individual trial. There were some differences between open field and radial maze. There are different zones in the open field and radial maze. In the open field we have only two zones – central and peripheral zone and in the radial maze we have central zone, arm1, arm 2, arm 3, arm 4, arm 5, arm 6, arm 7, arm 8, goal zone 1, goal zone 2, goal zone 3, goal zone 4, goal zone 4, goal zone 5, goal zone 6, goal zone 7 and goal zone 8. We also did not checked for attempts of escape in the radial maze and we did not put food in the open field, so we did not have the dependant variable food eaten and group visits to certain zones in the open field.

2.3 Open filed test

2.3.1 Equipment

For this experiment a cubic box (50*50*50 cm) is used. It is made of transparent Plexiglas and there is a grid of squares on the bottom of the box. A plastic tube is also used in this experiment, to put in the lizard and insert it into the box and at the end, the lizard is chased back into this tube and removed from the open field test.

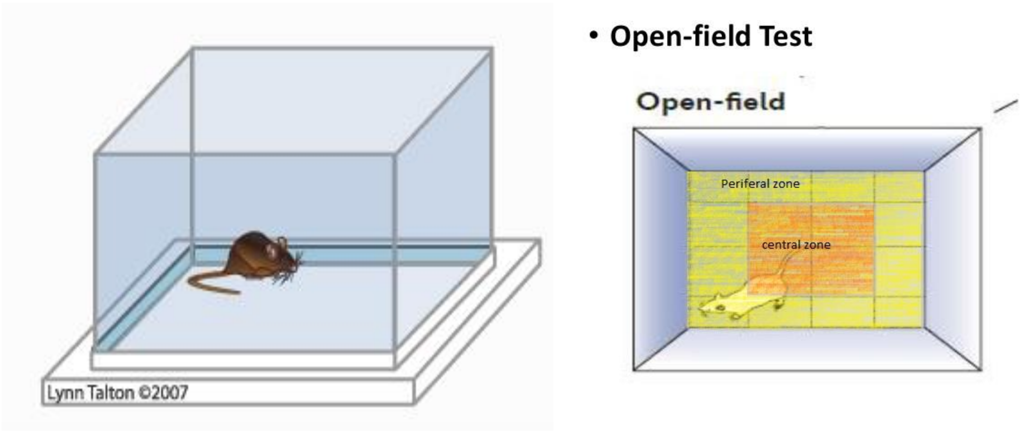

Figure 3: The illustration of open field test apparatus, taken from http://btc.psych.ucla.edu/openfield.htm andhttp://www.slideshare.net/AdvaithaMv/screening-of-anxiolytics-44529278

2.3.2 Procedure

The experiment begun when the tube with the lizard was inserted into the test box. To prevent lizards from memorising certain direction, we used research randomizer program, with the help of which we turned the plastic tube and the box in a different direction each time. From that moment on, we started to measure time. The experiment was over after 15 minutes or maximum 23 minutes, when the lizard eithers returns to the tube by itself or is chased into the tube. Once the lizard is out of the open field test, the box is disinfected, and wiped and ready for the next lizard.

2.4 8-arm radial maze

2.4.1 Equipment

For this experiment the 8-arm radial maze was used. It is divided into central zone, 8 arms and 8 goal zones, which are at the end of each arm. The diameter of this maze is 74.5 cm.

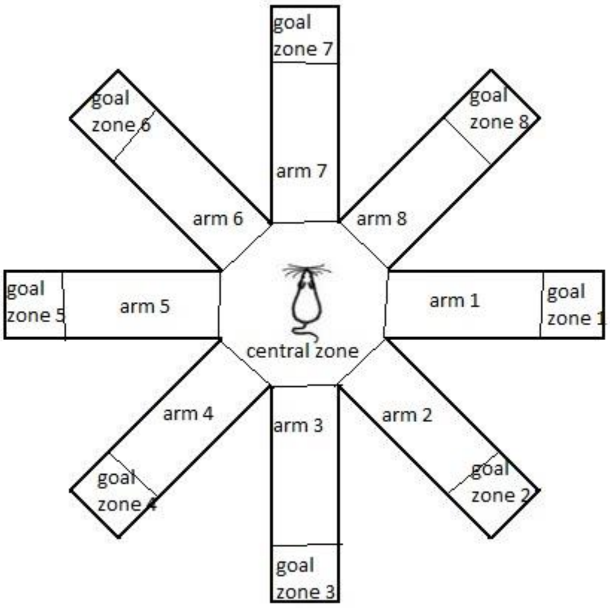

Figure 4: 8-arm radial maze

2.4.2 Procedure

For the first set of trials (4.7.2016 and 6.7.2016), the *Podarcis melisellensis* were put into the radial maze. Each trial we changed the position of the first arm and the orientation of the tube with the help of research randomiser program. The experiment begun when the tube with the lizard was inserted into the maze. From that moment on, we started to measure time. When the lizard leaves the tube, the tube is removed from the maze. After 15 minutes or maximum 23 minutes, the experiment was over. The lizard was chased into the tube or it returned to the tube by itself. After the lizard was in the tube, it was put back into his terrarium. The maze was disinfected dried and prepared for the next lizard.

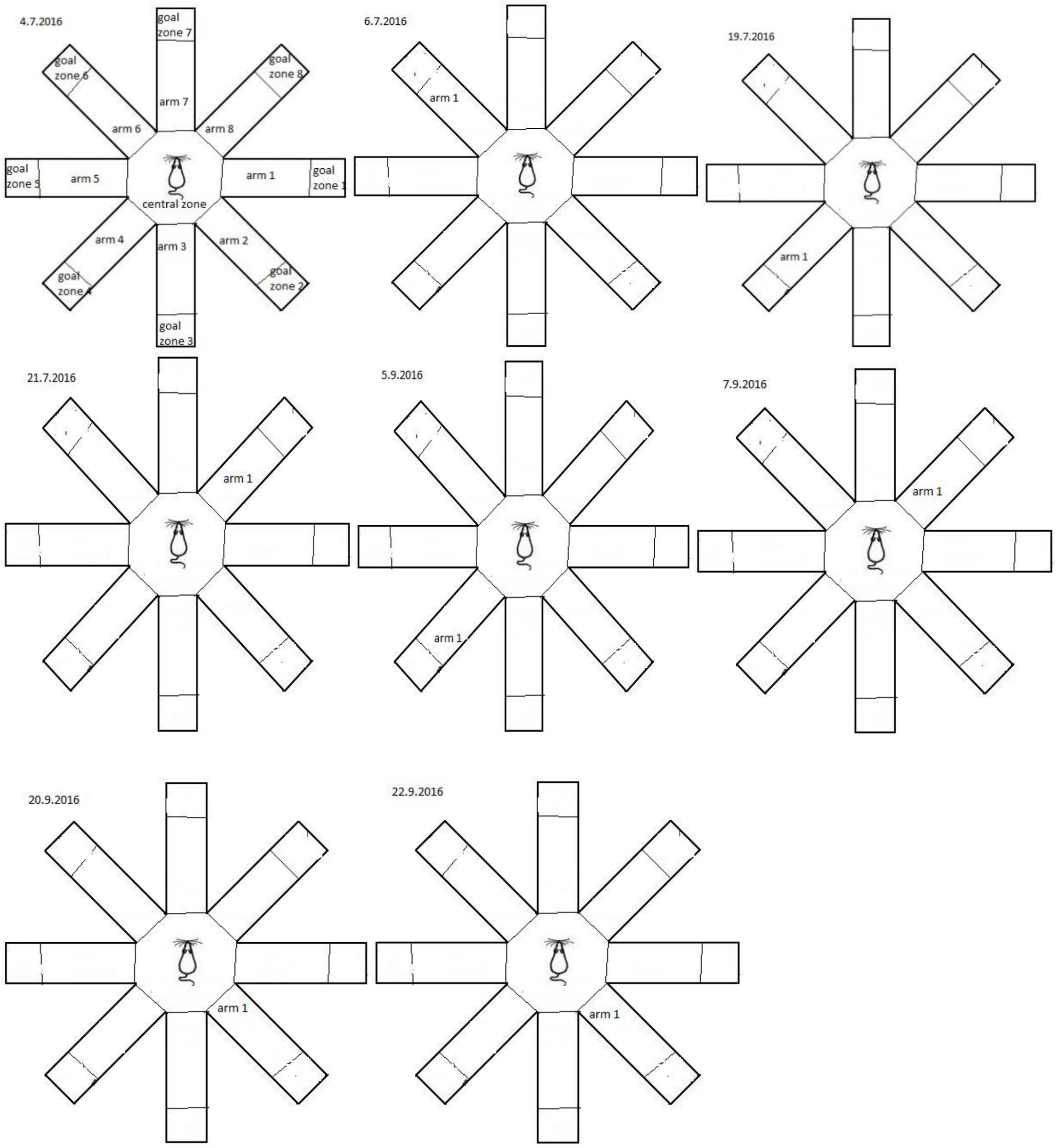

Figure 5: The changing of the position of the first arm in radial maze through trials

2.5 Data processing

We processed information, acquired in open field test and 8-arm radial maze in two separate steps. First step was the acquisition of data with the computer program Noldus Ethovision XT 12, which is a computer program that either monitors the animal in a new environment directly, or, like in our case, analyses the video of the experiment and gives certain information, needed for further statistical analysis (Step two) in the computer program Statistica 13.1.

2.6 Noldus Ethovision XT 12

Noldus EthoVision XT 12 is a computer program that traces the animal in the maze and then analyse and calculate the parameters needed.

2.6.1 Procedure

When you open the program, you have to choose first if you want to track animal live or acquire data from the video file. Then you have to choose from several options to create the right settings for the current experiment. For our experiment, we used the following protocols for open field test and 8-arm radial maze.

2.6.1.1 Open field protocol:

NEW EXPERIMENT - New from template -Apply predefined template - From video file (Browse) – change in all files-enter

EXPERIMENT SETTINGS: subject rodents; other, Arena template (open field square); Zone template (No zone template) - Track features: Centre point, Darker - Sample rate: 2 samples/sec – Finish

MANUAL SCORING SETTINGS: delete predefined behaviour-add behaviour (time spent in tube (key: x-x; lifting on hind legs key: c-c)-validate.

ARENA SETTINGS: grab video-adjust aspect ratio (640:360)-draw scale (50cm)-draw arena-add zone group (2 row of squares to the inside), (outer square, as arena)-label new zones-validate.

DETECTION SETTINGS: advanced: forward till the animal is not near the tube-dynamic subtraction-minimum-maximum (watch the animal for a certain period of time to get data about its size)-if the program is not tracking the animal near the tube, try frame weight or brightness and darkness-save.

TRIAL LIST: add variable (ID of the animal)-add trial (14 trials for 14 animals)-add video-add arena settings-add detection settings-add ID of the animal.

ACQUISITION: forward till the animal leaves the tube-press rec when the animal is out of the tube with its hips-(press key if needed (manual scoring))-play video until the end.

2.6.1.2 8-arm radial maze protocol

NEW EXPERIMENT - New from template -Apply predefined template - From video file (Browse) – change in all files-next.

EXPERIMENT SETTINGS: subject rodents; other, Arena template (8-arm radial maze); Zone template (No zone template) - Track features: Centre point, Darker - Sample rate: 2 samples/sec – Finish.

ARENA SETTINGS: arena settings 1-n; grab video -draw scale (74,5cm)-draw arena-add zone group (central zone)- - add zone group- 8 squares that represent arms of the maze-- add zone group- 8 goal zones – at the end of the arms, they are as longs as the lizard itself without the tail)-add hidden zone(*)-label new zones-- add zone group - add new zones (food in every arm 1-8; radius big as snout - centre point distance)-label new points-validate. **goal and food zones are included into arm zones.

DETECTION SETTINGS: advanced: forward till the animal is not near the tube-dynamic subtraction-minimum-maximum (watch the animal for a certain period of time to get data about its size)-if the program is not tracking the animal near the tube, try frame weight or brightness and darkness-save.

TRIAL LIST: add variable (ID of the animal)-add trial (14 trials for 14 animals)-add video-add arena settings-add detection settings-add ID of the animal (the animal ID is the same as in the video title).

ACQUISITION: forward till the animal leaves the tube-press rec when the animal is out of the tube with its hips-(press key if needed (manual scoring))-play video till the end.

*If the animal leaves the tube and then returns into it, then we have to define hidden zone, which represents the tube and entry zone in front of it.

2.7 Statistical analysis

We used computer program Statistica 13.1 for descriptive statistics and statistical significance between independent variables. All statistical significance were set at $p = 0.05$. We used independent variables: species, trial and gender and dependant variables, which we divided into groups to simplified the data analysis. Groups were the same for both mazes, but the variables within group differed in some groups because of the specifics of the mazes. In both mazes we had groups Movement (distance moved, mean velocity, time moving, mean meandering), Boldness and anxiety (latency snout, latency exit, angular velocity, highly mobile cumulative time and frequency, mobile, peripheral zone, central zone, time in tube frequency and cumulative duration, immobile time), Learning (time of chasing in both mazes and also food eaten in the radial maze) and Laterality. In open field we also added group called Attempts to escape (Lifting on hind legs, frequency and cumulative time) and in the radial maze we added group Visits to certain zones, which includes maximum alternations, direct revisits and indirect revisits.

2.7.1 Procedure

For our experiment we used:

- Descriptive statistics: We used one way and breakdown ANOVA to compare dependant variables latency – snout, latency – exit and time of chasing. We tested these dependent variables in relation to independent variables: maze, species, trial and gender. We got mean values, standard deviations and minimums and maximums for each dependant variable. This is a basic descriptive statistics that tells us more about the range of dependent variables.
- Parametric statistic: Factorial ANOVA for distance moved, velocity, meander, latency to snout, latency to exit, angular velocity, mobility state, peripheral zone, central zone, time in tube, lifting on hind legs, time chasing to tube. The independent variables were species, gender and trial. We tried to established if the difference of dependant variables are significant different in relation to independent variables.
- Nonparametric: Mann-Whitney test for laterality and food eaten and frequency in the zone. The independent variables in this test were also species, gender and trial.

3. RESULTS

All of the results that were statistically significant, are written in bold writing and only graphs that show significant difference are displayed. If this is not the case, there is an explanation in the discussion. The results were divided according to test used: open field and radial maze. Then we subdivided the results according to groups of related dependant variables. The groups were movement, boldness and anxiety, attempts of escape, learning, visits to certain zones and laterality. The description of those parameters is located in table 1.

Table 1: the description of dependant variables according to groups.

CATEGORY	BEHAVIOUR	DESCRIPTION
MOVEMENT	*Distance moved*	Distance, walked by the individual lizard in the maze in the time of the experiment.
	Mean velocity	Mean velocity of the individual lizard in the time of experiment for each maze.
	Time moving	The period of time when the individual lizard was not standing completely still.
	Mean meandering	The amount of meandering that the individual lizard did during the experiment.
BOLDNESS AND ANXIETY	*Latency - snout*	Time needed for the each individual lizard to put their snout out of the tube.
	Latency - exit	Time needed for each individual lizard to leave the tube in the maze.
	Angular velocity	Velocity, with which each individual lizard is changing angles and directions.
	Highly mobile – frequency	Frequency, with which each individual lizard is in highly mobile state.
	Highly mobile - cumulative duration	Total duration of highly mobile state of each lizard and individually for every trial and maze.
	Mobile - frequency	Frequency, with which each individual lizard is in mobile state.
	Mobile – cumulative duration	Total duration of mobile state of each lizard and individually for every trial and maze.
	Immobile - frequency	Frequency, with which each individual lizard is in immobile state.
	Immobile – cumulative	Total duration of immobile

	duration	state of each lizard and individually for every trial and maze.
	Peripheral zone - *frequency*	Frequency, with which each lizards visits peripheral zone of the open field, calculated for each trial individually.
	Peripheral zone – cumulative duration	Total time that each lizard spent in peripheral zone in each trial of the open field.
	Central zone - *frequency*	Frequency, with which each lizards visits central zone of the open field, calculated for each trial individually.
	Central zone – cumulative duration	Total time that each lizard spent in central zone in each trial of the open field.
	Time in tube - *frequency*	Frequency, with which each lizards enters the tube, in the open field, calculated for each trial individually.
	Time in tube – cumulative duration	Total time that each lizard spent in the tube in each trial of the open field.
	Arm 1	First zone in the radial maze.
	Arm 2	Second zone in the radial maze.
	Arm 3	Third zone in the radial maze.
	Arm 4	Fourth zone in the radial maze.
	Arm 5	Fifth zone in the radial maze.
	Arm 6	Sixth zone in the radial maze.
	Arm 7	Seventh zone in the radial maze.
	Arm 8	Eighth zone in the radial maze.
	Goal zone 1	Zone at the end of the first arm in the radial maze, it is a length of a lizard.
	Goal zone 2	Zone at the end of the second arm in the radial maze, it is a length of a lizard.
	Goal zone 3	Zone at the end of the third arm in the radial maze, it is a length of a lizard.
	Goal zone 4	Zone at the end of the fourth arm in the radial maze, it is a length of a lizard.
	Goal zone 5	Zone at the end of the fifth arm in the radial maze, it is a length of a lizard.
	Goal zone 6	Zone at the end of the sixth arm in the radial maze, it is

		a length of a lizard.
	Goal zone 7	Zone at the end of the seventh arm in the radial maze, it is a length of a lizard.
	Goal zone 8	Zone at the end of the eighth arm in the radial maze, it is a length of a lizard.
ATTEMPTS OF ESCAPE	*Lifting on hind legs* **-** *frequency*	Frequency, with which each lizards lifted on his back legs, in the open field, calculated for each trial individually.
	Lifting on hind legs – cumulative duration	Total time that each lizard spent on its back legs in each trial of the open field.
LEARNING	*Time of chasing*	Time needed to get each lizard back into the tube
	Food eaten	The quantity of food, eaten by each lizard until the end of experiment in the radial maze
	Time in zone	Time each individual lizards spent in certain zone in the radial maze.
	Maximum alternations	Maximum number of time, when each lizard change its position from one zone to the next in the radial maze.
	Direct revisits	Times when lizard came out from one zone and went right back into the same zone.
	Indirect revisits	Times when lizard came from one zone, into the other and then into the first one again.
LATERALITY		The direction in which the lizards positioned their body, right after they left the tube for the first time. There were 3 possible directions – straight, left and right. We marked those directions with numbers 1, 2 and 3.

3.1 Basic statistic

First, we analysed general dependant variables, which are common to both mazes – open field and radial maze in relation to independent variables maze, species, gender and trial. This was done for a better review of the differences between independent variables. For this purpose we used basic statistics – One way and breakdown ANOVA.

3.1.1 Mazes

We tested dependant variables according to the maze, open field or radial maze and compare values for species, gender and trial, for every dependant variable – laterality, latency to snout, latency to exit and time of chasing.

Table 2: Mean values of dependant variables laterality, latency snout, latency exit and time of chasing according to maze, species, trial and gender.

Maze	*Species*	*trial*	*gender*	*Laterality (s)* Mean	Std. dev.	*latency – snout (s)* mean	std. dev.	*latency – exit (s)* mean	std. dev.	*time of chasing (s)* mean	Std. dev.
open field	*Podarcis siculus*	1	F	1.64	0.74	127.57	116.58	258.64	235.70	33.18	29.71
		1	M	1.43	0.76	161.64	205.73	361.36	338.70	26.75	23.66
		3	F	1.79	0.97	108.29	66.76	185.26	89.09	42.14	39.36
		3	M	1.62	0.96	100.23	104.24	279.38	294.67	21.00	19.42
	Podarcis melisellensis	1	F	1.57	0.76	94.14	54.07	177.21	110.96	34.79	20.90
		1	M	1.29	0.61	72.81	75.55	207.32	235.10	25.29	16.42
		3	F	1.14	0.36	103.57	191.05	140.00	164.28	21.62	11.61
		3	M	1.00	0.00	108.85	94.76	230.14	253.85	23.64	17.75
radial maze	*Podarcis siculus*	1	F	1.93	0.83	95.00	73.49	165.50	101.72	22.07	14.62
		1	M	1.71	0.83	98.31	74.34	279.79	284.63	26.93	24.91
		3	F	1.71	0.83	144.14	191.11	175.71	187.96	30.86	17.23
		3	M	1.79	0.80	113.71	180.51	214.93	285.23	25.31	15.85
	Podarcis melisellensis	1	F	2.00	0.96	80.79	116.86	231.57	266.13	46.08	20.80
		1	M	1.86	0.77	180.67	184.70	284.64	172.34	25.90	20.17
		3	F	1.71	0.83	124.70	119.64	270.21	250.25	23.00	11.52
		3	M	1.86	0.77	112.38	119.46	226.93	242.70	22.86	14.70
All groups				**1.63**	**0.79**	**113.78**	**131.05**	**230.32**	**229.49**	**27.78**	**20.27**

3.2 Open field

In the open field we tested four groups: Movement, the level of boldness and anxiety, attempts of escape and learning. For all of the variables we used the statistical method of factorial ANOVA.

3.2.1 Movement

Four dependent variables were tested in this group – distance moved, mean velocity, time moving and mean meandering. We decided to test those variables with factorial ANOVA, because with that method we can estimate interactions between all of the independent variables. The results of multivariate factorial ANOVA showed that only categories species and trial were significantly different.

Table 3: Results of a Factorial ANOVA of moving and independent variables species, gender and trial were categories (Bold indicates significant difference).

EFFECT	Value	F	Effect df	Error df	p
Intercept	0.13	168.86	4	100	0.00
species	**0.76**	**7.91**	**4**	**100**	**0.00**
trial	**0.08**	**5.94**	**4**	**100**	**>0.01**
gender	0.97	0.90	4	100	0.47
species*trial	0.93	1.99	4	100	0.10
species*gender	0.97	0.66	4	100	0.62
trial*gender	0.97	0.69	4	100	0,61
species*trial*gender	0.98	0.52	4	100	0.72

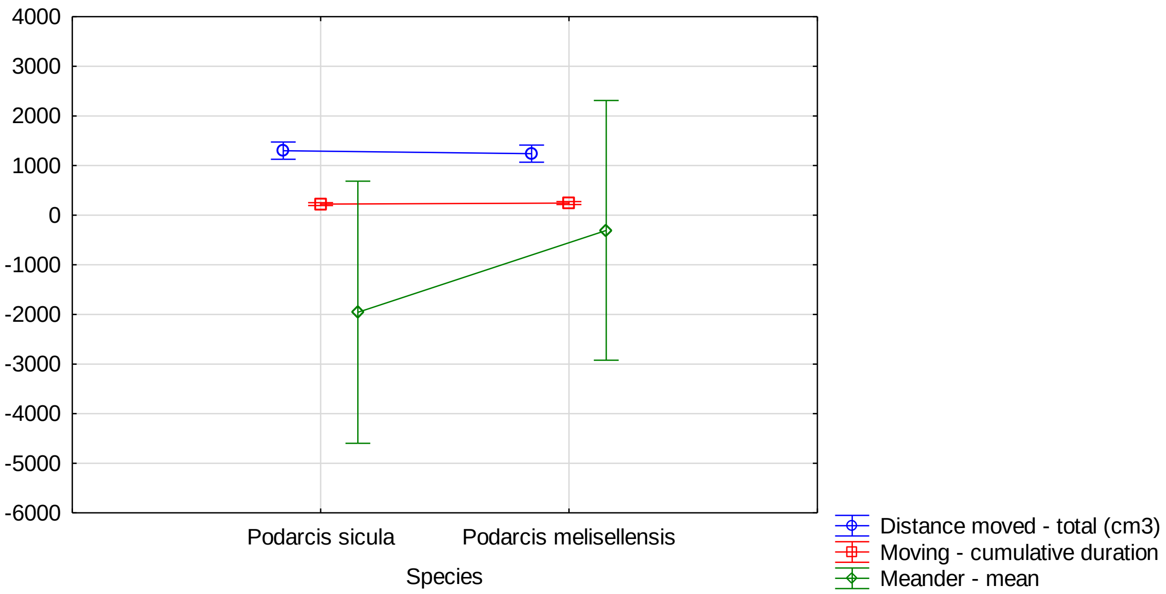

Figure 6: Graph of parameters of movement, (mean velocity is omitted from graph) of the lizards in the open field according to species. Vertical bars denote 0,95 confidence intervals.

This graph (Figure 6) show us the parameters of movement according to species, where multivariate test showed significant difference, with p=0.00006. We can see that the species *Podarcis siculus* meanders far less than *Podarcis melisellensis.*

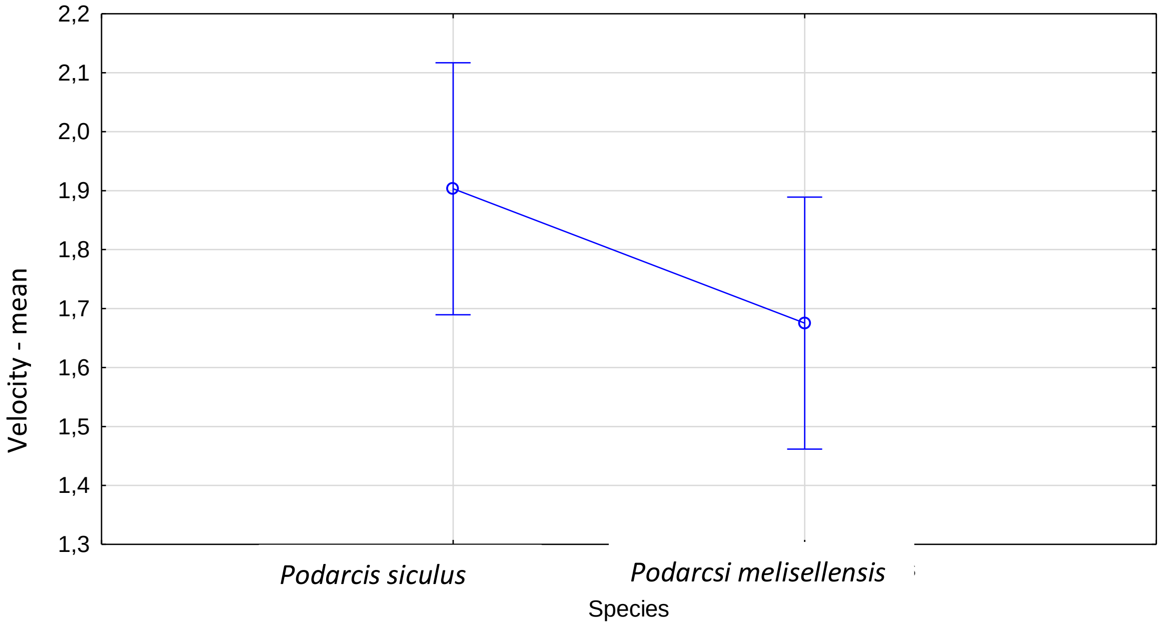

Figure 7: The graph of mean velocity of the lizards in the open field, according to species. Vertical bars denote 95% confidence intervals.

The subsequent univariate ANOVA showed that there was no significant difference of the variable mean velocity in any category – species, trial or gender (Figure 7).

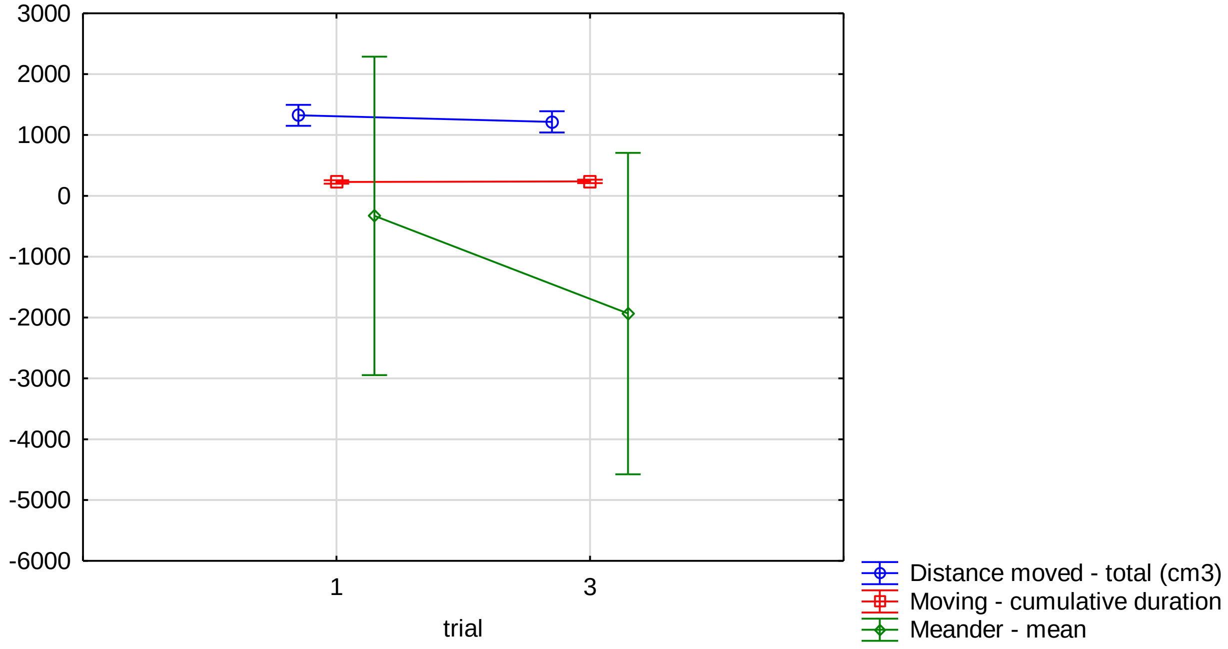

Figure 8: Graph of parameters of movement, except mean velocity of the lizards in the open field according to trial, vertical bars denote 95% confidence intervals.

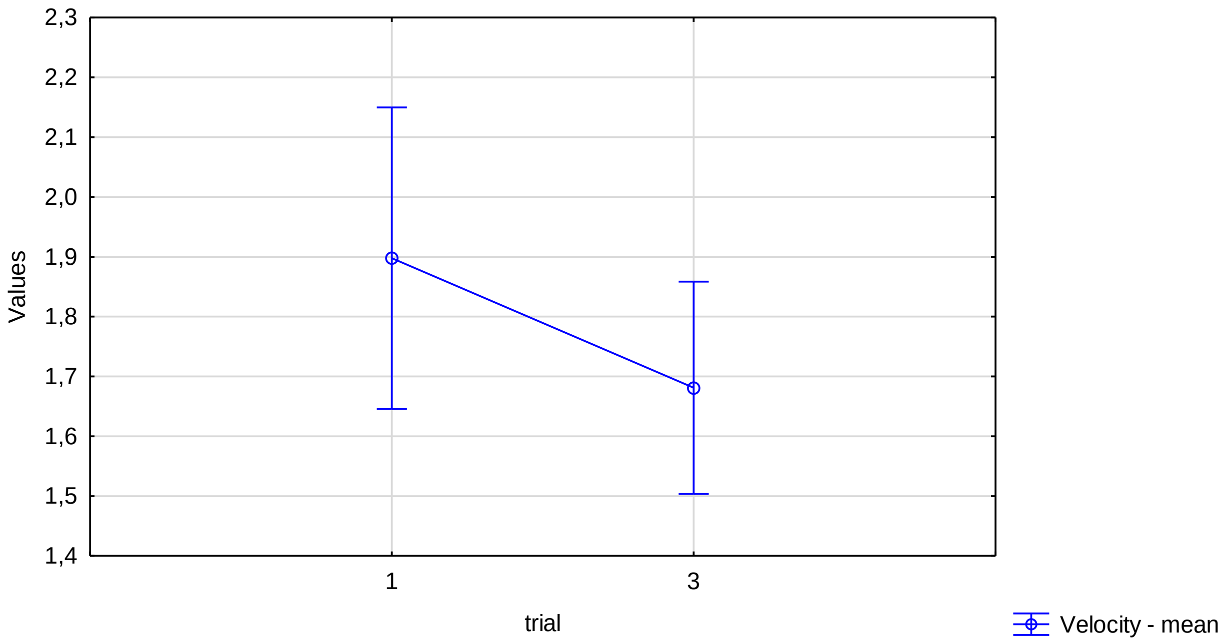

Figure 9: Graph of mean velocity of the lizards in the open field according to trial. Plot of Means and Confidence Intervals 95%.

This graphs (Figure 8, Figure 9) shows us the parameters of movement according to trial, where multivariate test showed significant difference, with p=>0.001. From the results we can conclude that the lizards were meandering significantly more in the first trial.

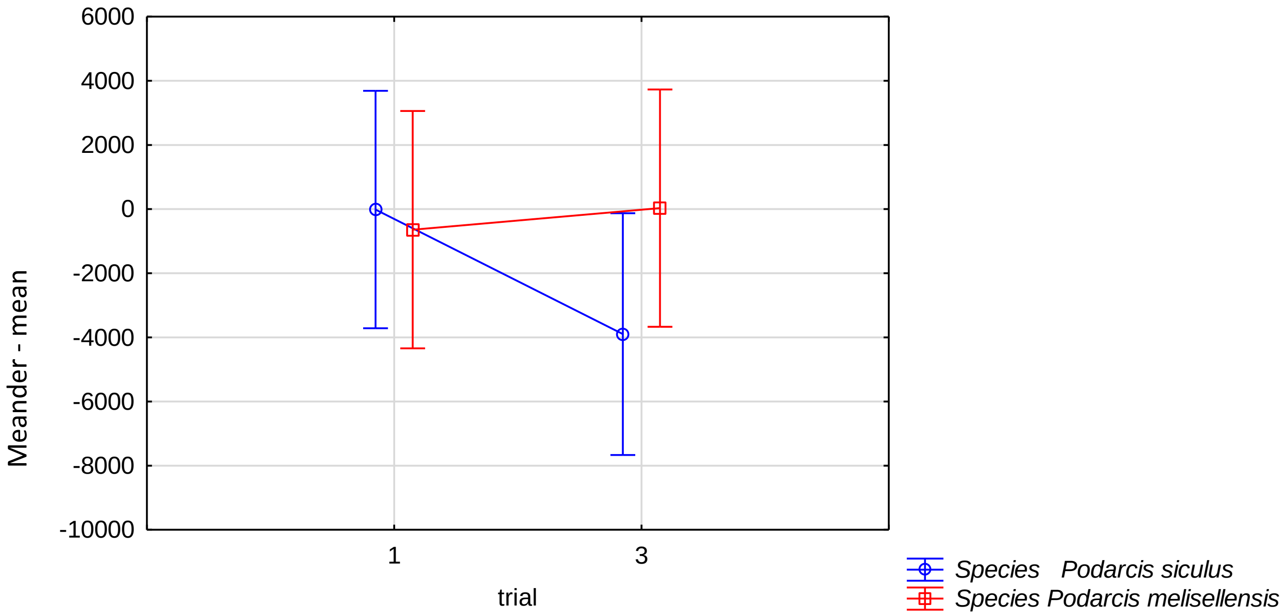

Figure 10: Graph of mean meandering of lizards in the open field according to species and trial, vertical bars denote 95% confidence intervals.

Graph (Figure 10) shows the dependant variable of mean meandering. The results suggest that there is a significant difference between trial 1 and 3 in species *Podarcis siculus*, but not in *Podarcis melisellensis.*

3.2.2 The level of boldness and anxiety

In this group of variables, we tested for boldness/skittishness and level of anxiety (see Table 1) of the lizards in the open filed test.

Table 4: Results of a Factorial ANOVA for the boldness/skittishness and level of anxiety done by species, trail and gender.

EFFECT

	Value	F	Effect	Error	p
Intercept	0.01	557.58	15	85	0.00
species	**0.49**	**5.96**	**15**	**85**	**0.00**
trial	**0.76**	**1.84**	**15**	**85**	**0.04**
gender	0.82	1.27	15	85	0.24
species*trial	**0.66**	**2.95**	**15**	**85**	**>0.01**
species*gender	**0.73**	**2.09**	**15**	**85**	**0.02**
trial*gender	0.77	1.68	15	85	0.07
species*trial*gender	0.84	1.09	15	85	0.38

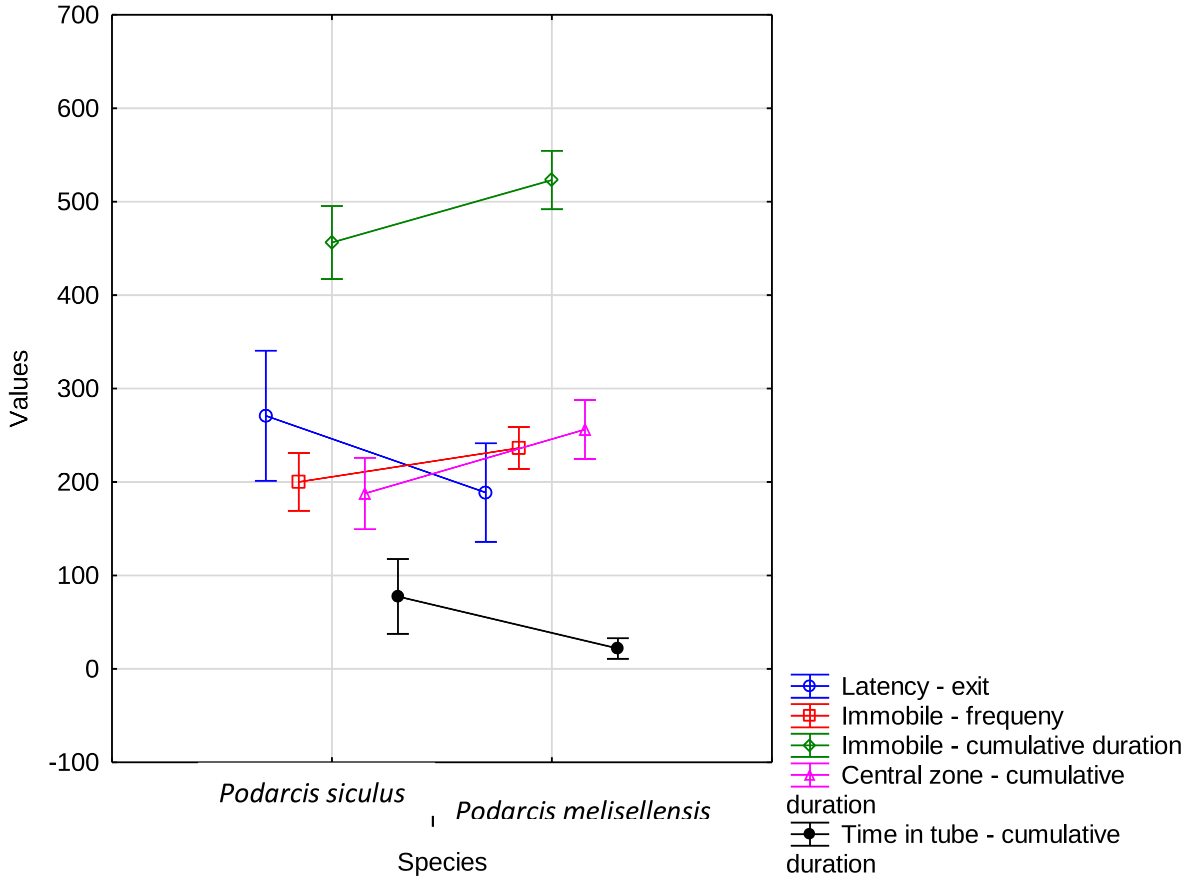

Figure 11: Graph of parameters, connected with boldness and anxiety of the lizards in the open field, according to species, vertical bars denote 95% confidence intervals.

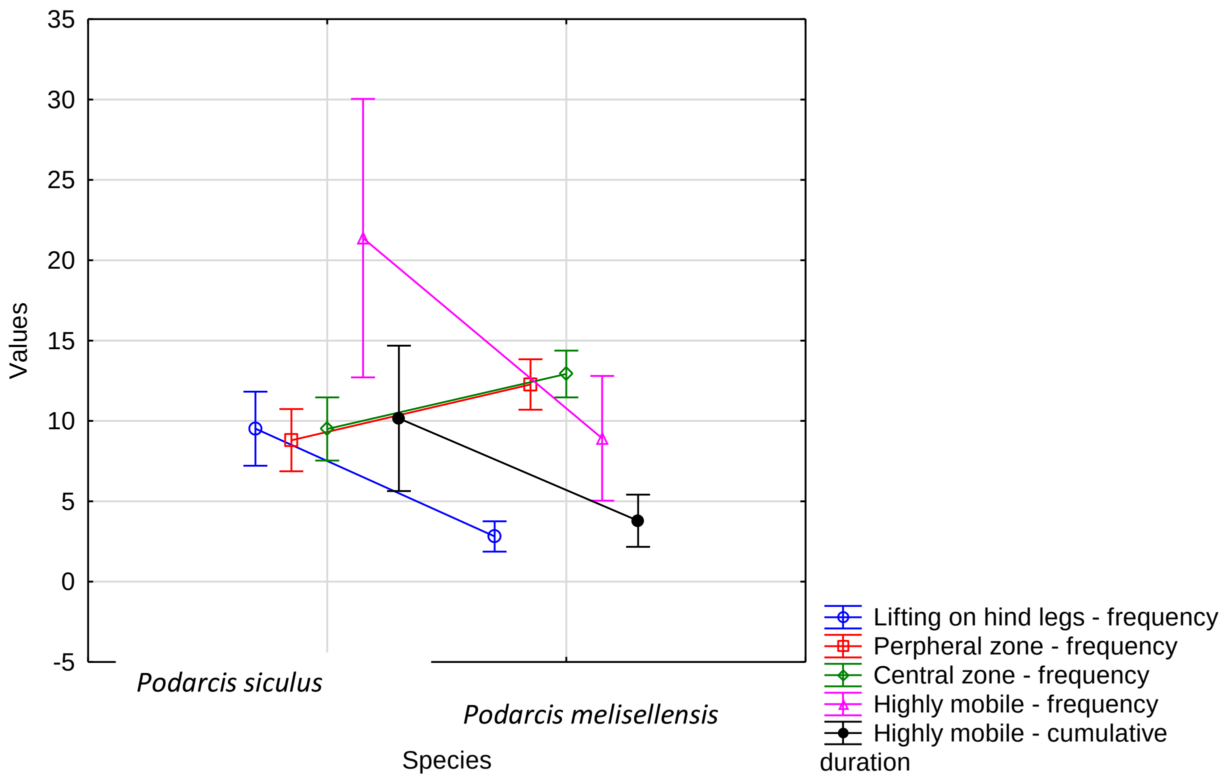

Figure 12: Graph of highly mobile – frequency, highly mobile – cumulative duration, peripheral zone – frequency and central zone – frequency, according to species. Vertical bars denote 95% confidence intervals.

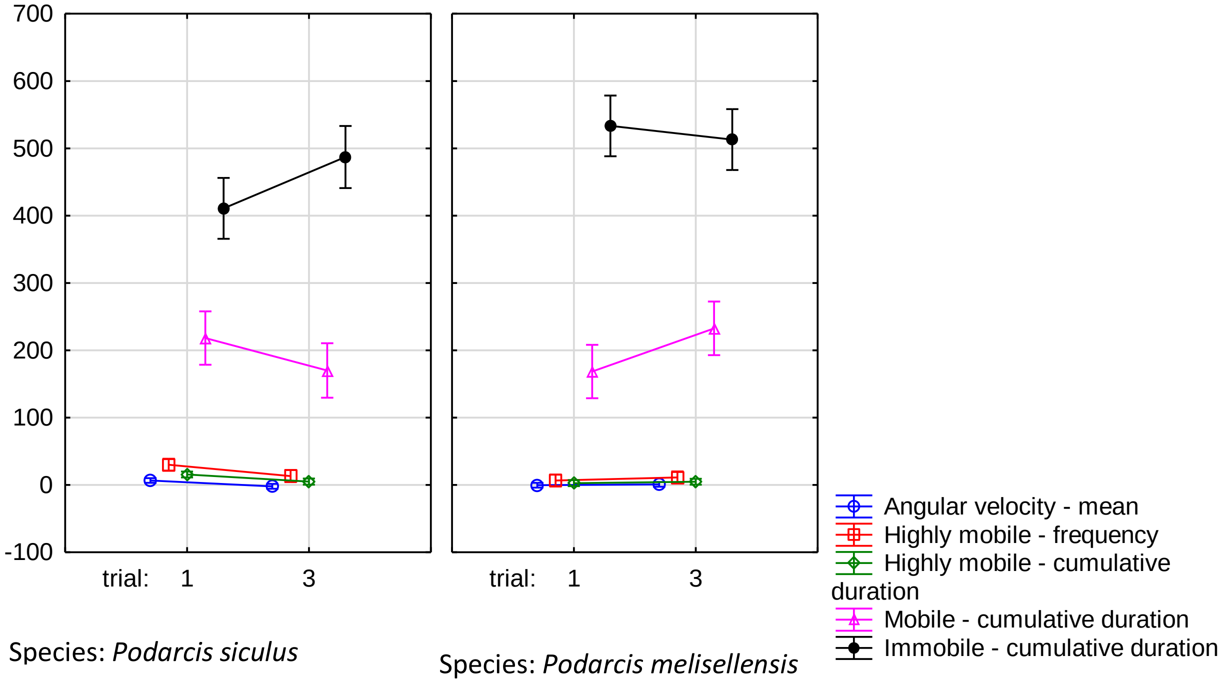

Figure 13: Graph of parameters with significant difference according to interaction of species and trial. Vertical bars denote 95% confidence intervals.

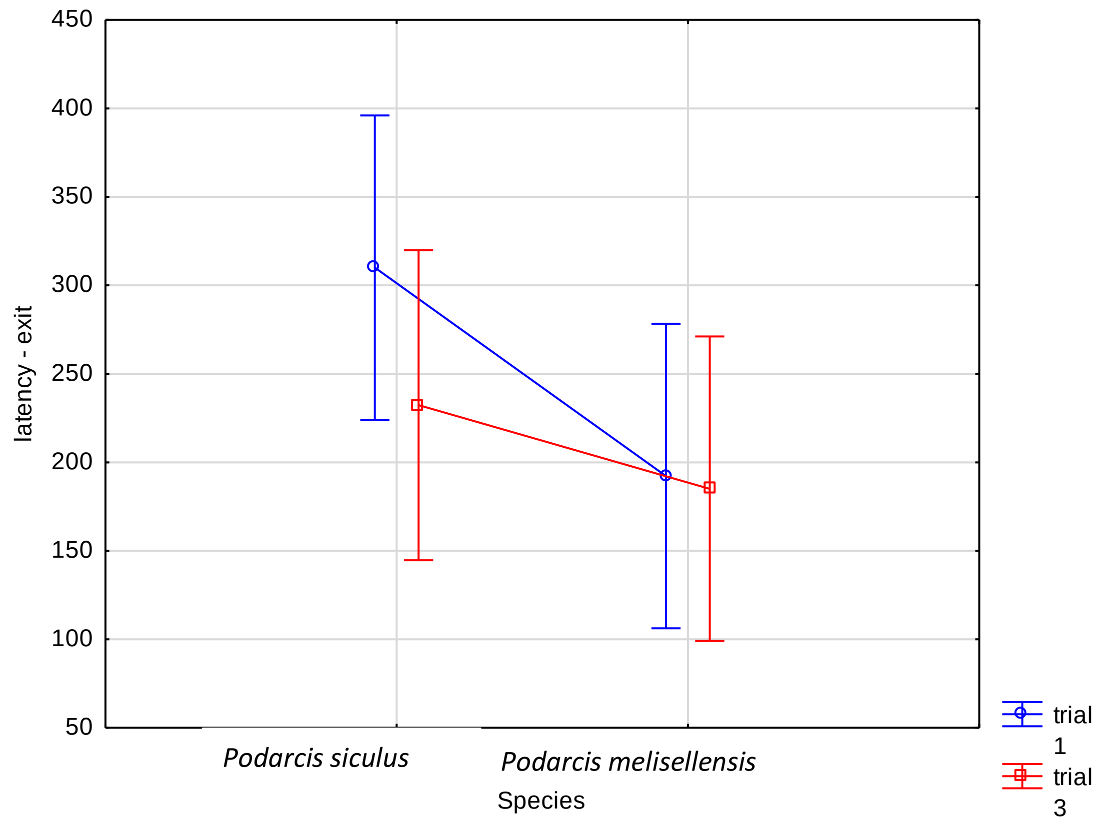

Figure 14: Graph of latency – exit , according to species and trial. Vertical bars denote 95% confidence intervals

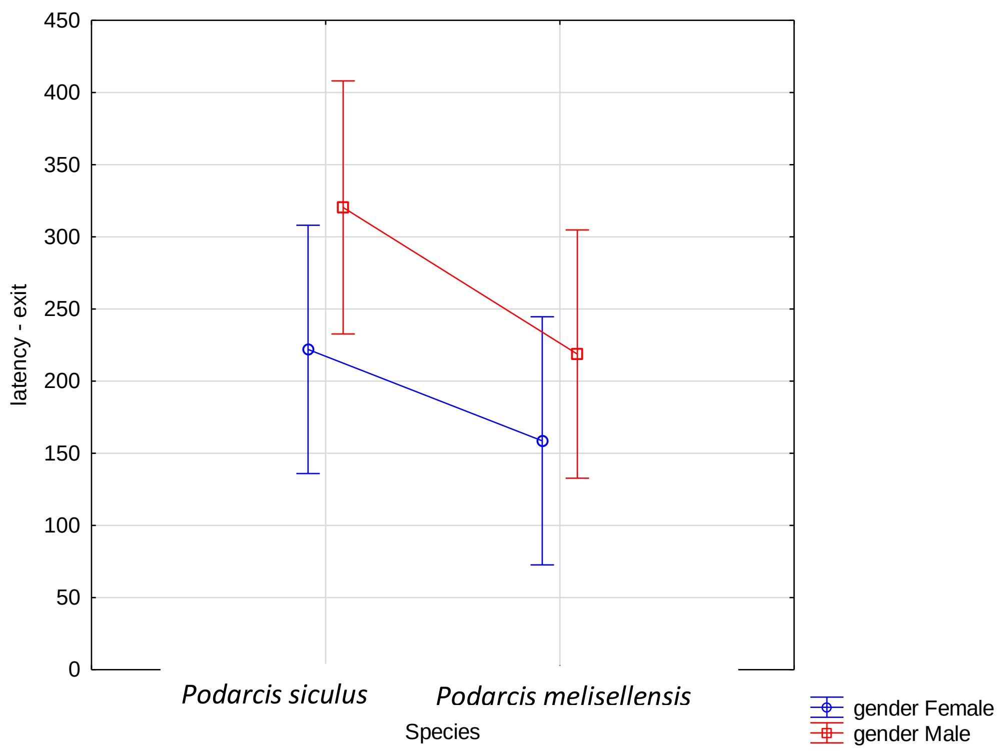

Figure 15: Graph of latency until the lizard has left the tube, according to species and gender; Vertical bars denote 95% confidence intervals.

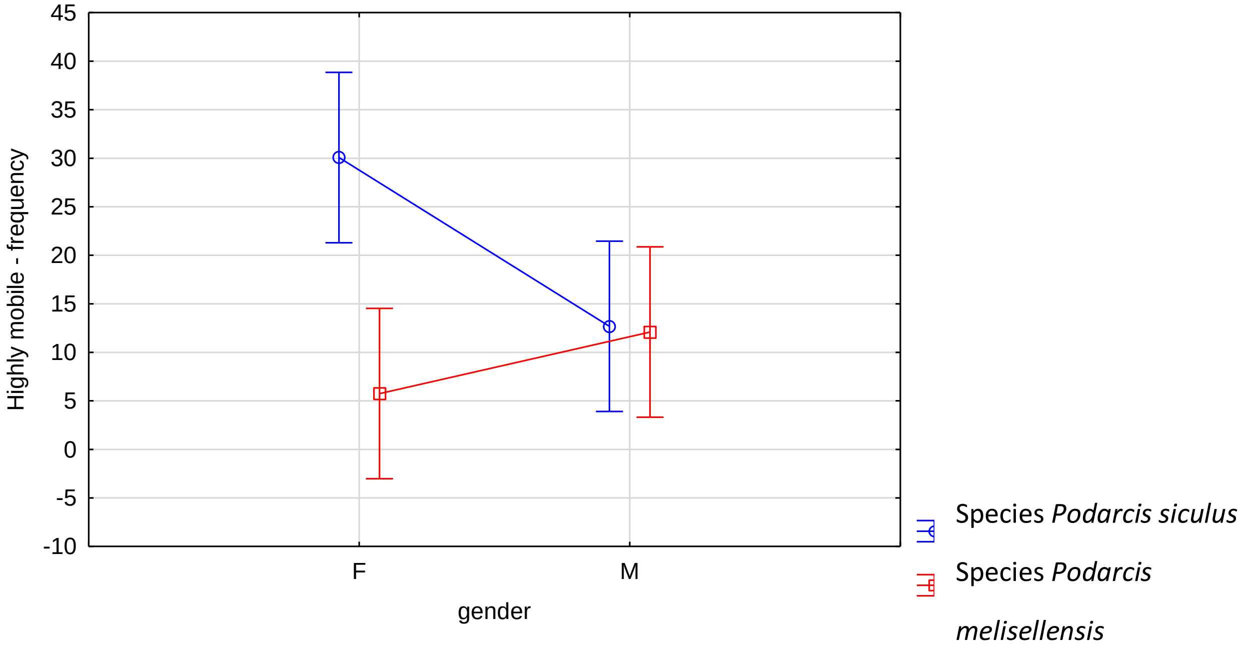

Figure 16: Graph of highly mobile state of lizards in the open field taking into account species and gender. Vertical bars denote 95% confidence intervals.

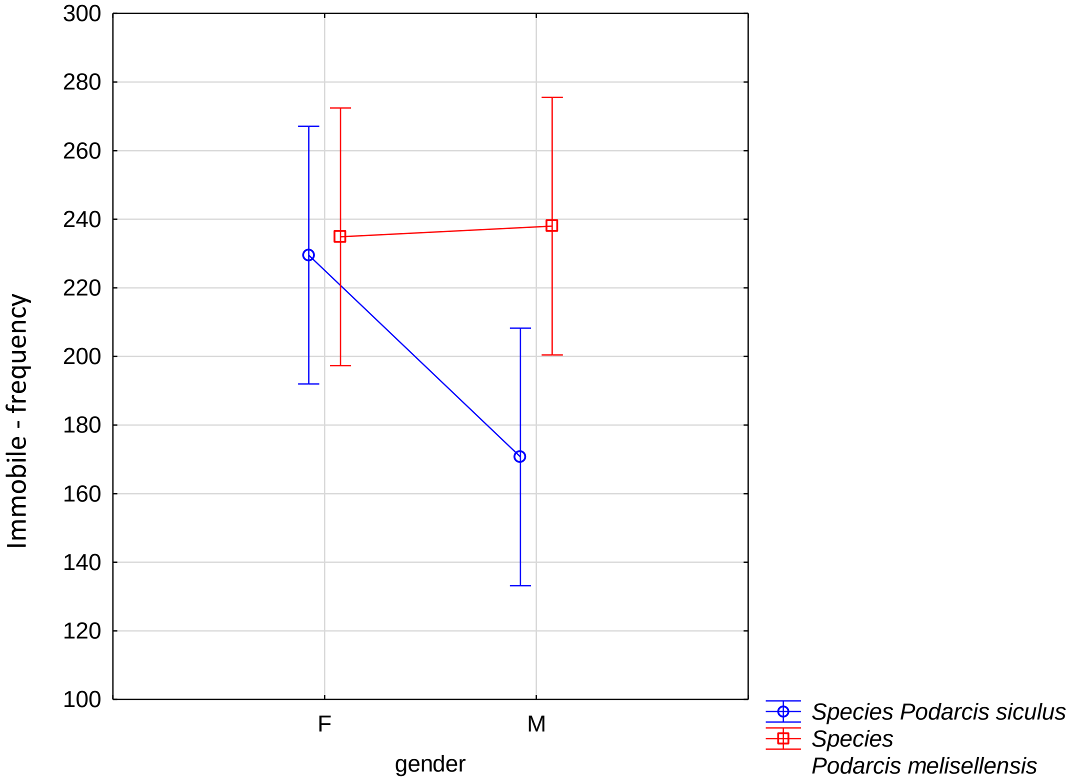

Figure 17: Graph of immobile state of the lizards in the open field, taking into account species and gender. Vertical bars denote 95% confidence intervals.

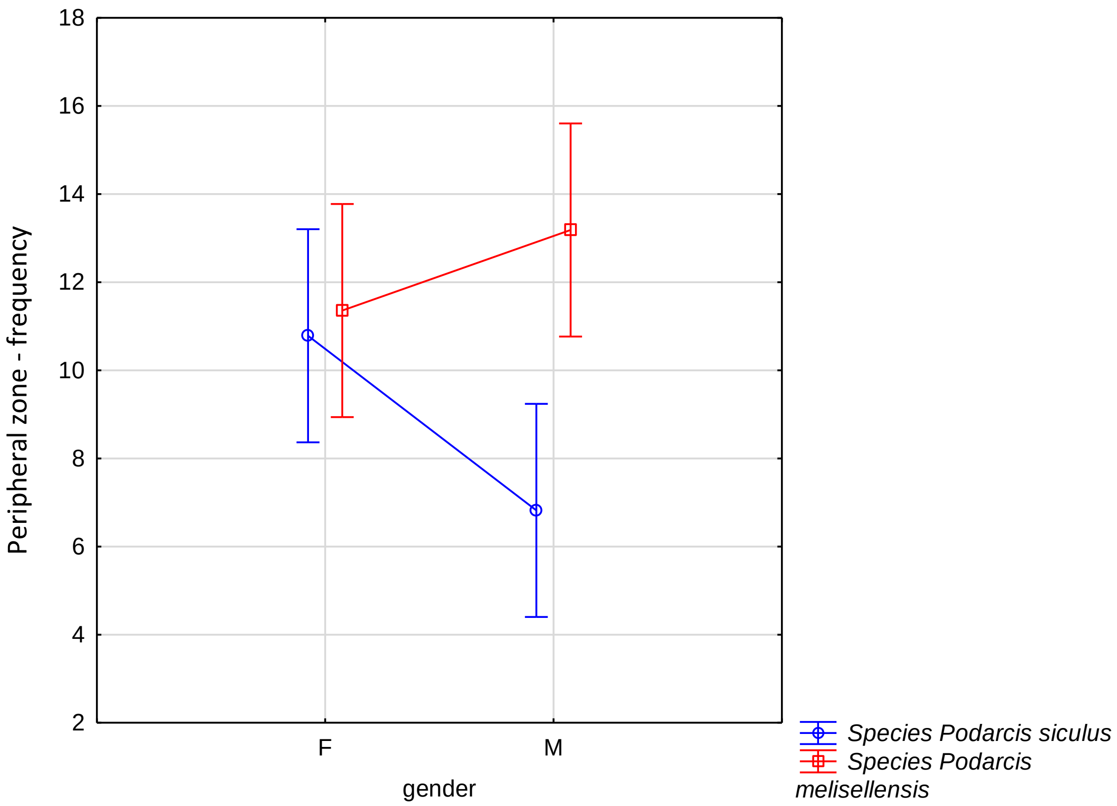

Figure 18: Graph of the frequency with which the lizards visited peripheral zone of the open field, taking into account species and gender. Vertical bars denote 95% confidence intervals.

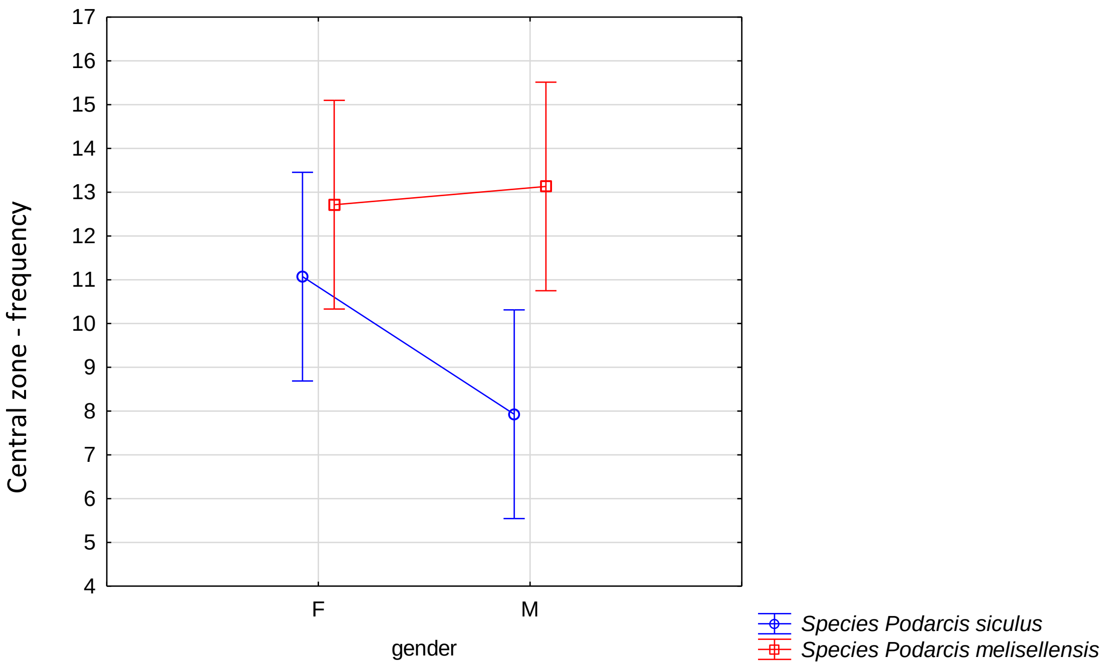

Figure 19: Graph of the frequency with which the lizards visited central zone in the open field test, taking into account species and gender. Vertical bars denote 95% confidence intervals.

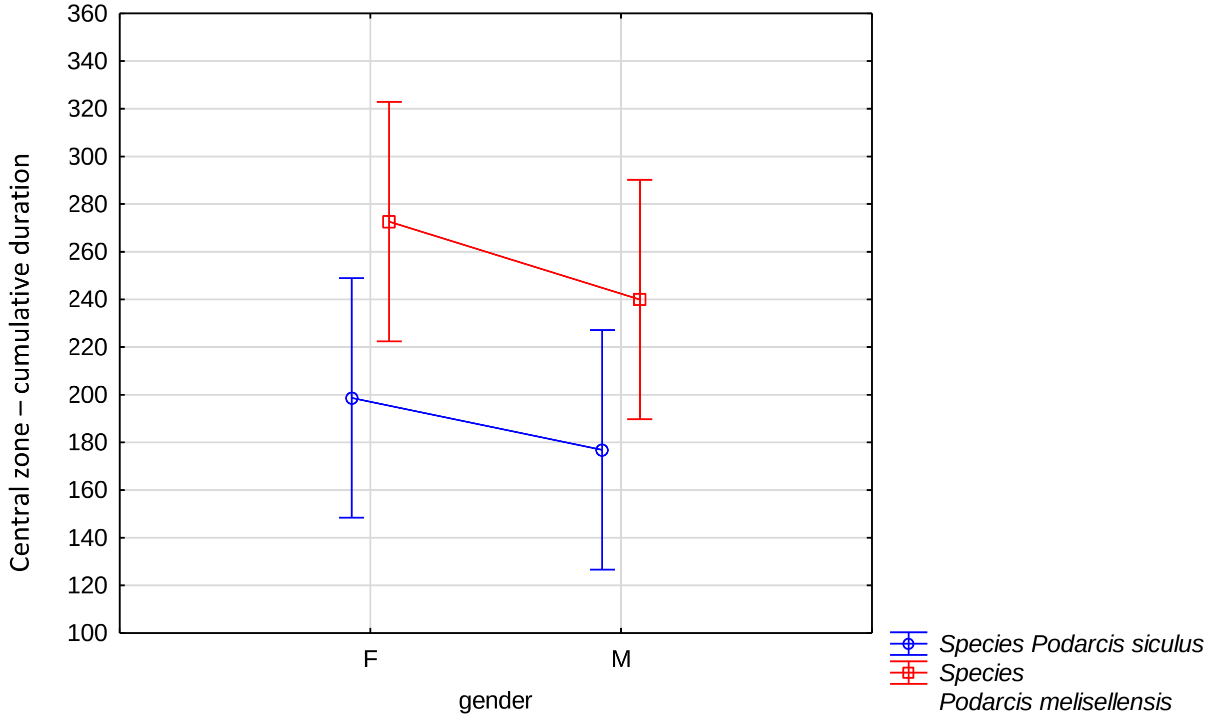

Figure 20: Graph of total time that the lizards spent in the central zone of the open field, taking into account species and gender. Vertical bars denote 95% confidence intervals.

The results of multivariate test factorial ANOVA (Table 4) showed significant difference in this group of dependant variables in categories species, trial, interaction of species and trial and species and gender (Figure 11 and Figure 12). Further univariate factorial ANOVA performed on this group of dependant variables indicated significant differences in the category species in the variables latency – exit (p= >0.01) (Figure 13), highly mobile – frequency (p= >0.01) (Figure 12), highly mobile – cumulative duration (p= >0.01) (Figure 12), immobile – frequency (p=0.02) (Figure 11), immobile - cumulative duration (p=>0.01)(Figure 11), peripheral zone – frequency (p=>0.01) (Figure 12), central zone – frequency (p=>0.01) (Figure 12), central zone – cumulative duration (p= 0.0004) (Figure 11), time in tube – cumulative duration (p=>0.01) (Figure 11). In the category of interaction of species and trial an univariate factorial ANOVA showed significant difference in the following variables (Figure 13): angular velocity (p=>0.01), Highly mobile - frequency (p=0.02), Highly mobile – cumulative duration (p=>0.01), Mobile – cumulative duration (p=>0.01), Immobile – cumulative duration (p=0.05). In the category of interaction of species and gender: Highly mobile - frequency (p=>0.01) (Figure 16), Highly mobile – cumulative duration (p=0.05), mobile – frequency (p=>0.01), Mobile – cumulative duration (p=>0.01), Immobile – frequency (p=0.01)(Figure 17), peripheral zone – frequency (p=>0.01) (Figure 18), central zone – frequency (p=0.04) (Figure 19) and time in tube – cumulative duration (p=>0.01) (Figure 20).

3.2.3 Attempts of escapes

In this group of variables we tested how many times and for how long, the lizards try to escape during the experiment in the open filed (see Table 1). The attempts of escape indicates the weariness of the lizards.

Table 5: Results of a Factorial ANOVA, in which lifting on hind legs was a dependant variable and species, gender and trial were categories.

EFFECT

	Value	F	Effect	Error	p
Intercept	0.5	47.42	2	103	0.00
species	**0.79**	**13.98**	**2**	**103**	**>0.00**
trial	0.97	1.47	2	103	0.24
gender	0.99	0.71	2	103	0.49
species*trial	0.99	0.08	2	103	0.92
species*gender	0.99	0.18	2	103	0.84
trial*gender	0.97	1.67	2	103	0.19
species*trial*gender	0.99	0.33	2	103	0.72

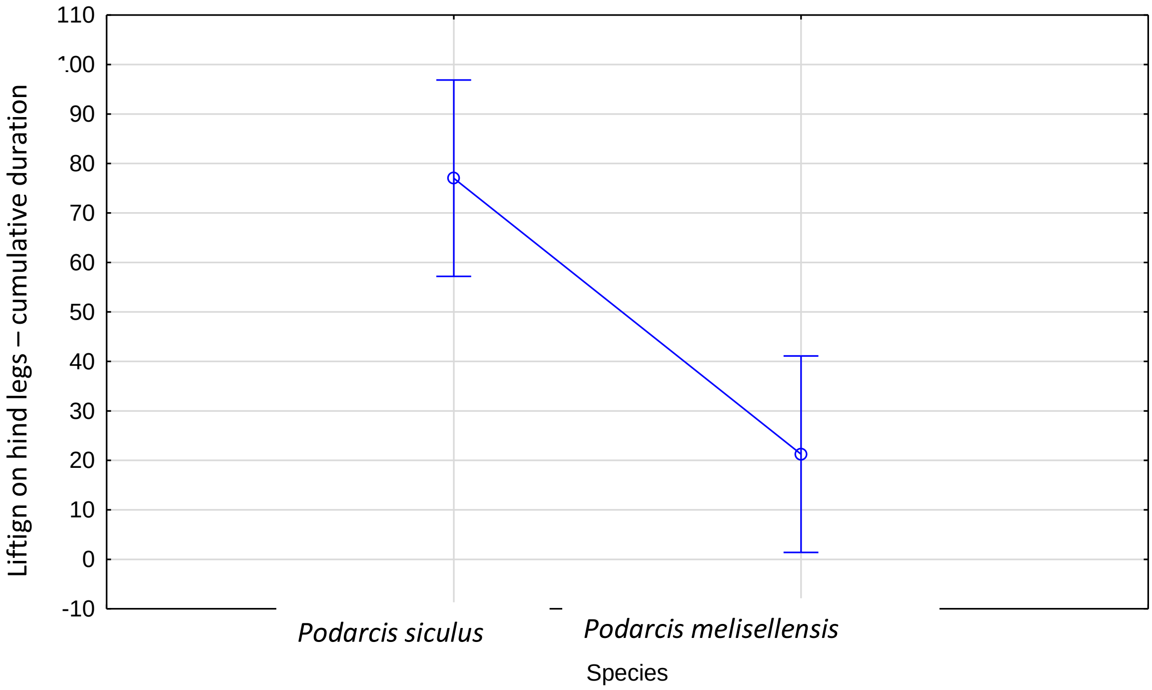

Figure 21: Graph of the parameter lifting on hind legs – frequency in the open filed, according to species. Vertical bars denote 95% confidence intervals.

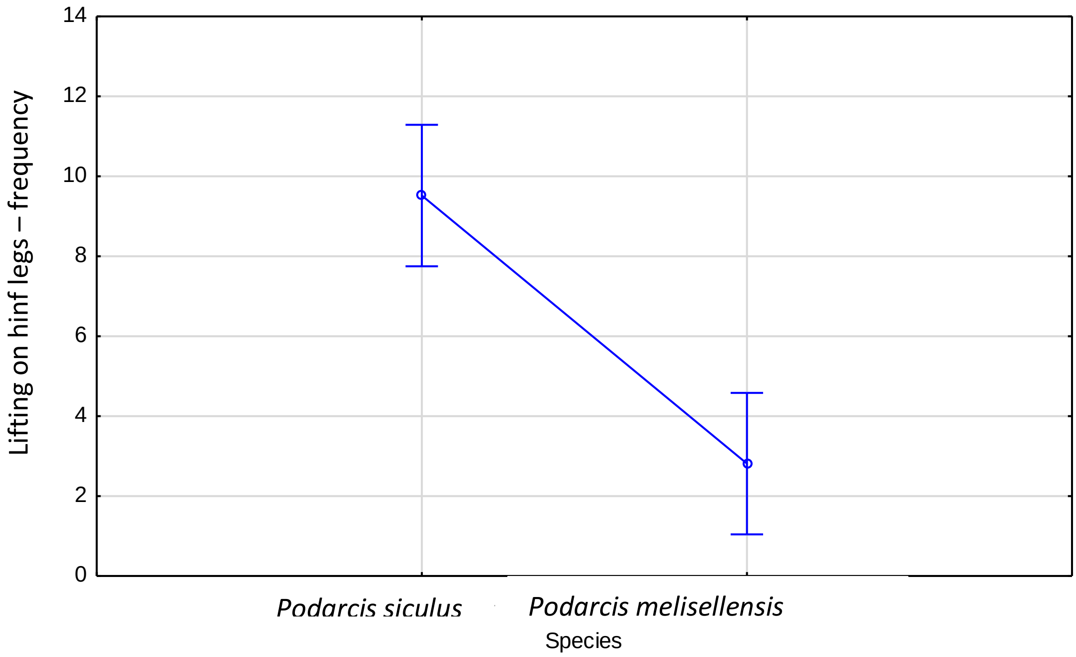

Figure 22: Graph of total time that lizards spent on their hind legs in order to escape from the open field test, sorted by species. Vertical bars denote 95% confidence intervals.

The results of multivariate ANOVA (Table 5) showed that there is significant difference in the group of variable in the category of species. Further univariate results showed that there is a significant difference in both variables lifting on hind legs – frequency (Figure 21) and lifting on hind legs – cumulative duration (Figure 22).

3.2.4 Learning

Table 6: Results of a univariate factorial ANOVA, in which time of chasing was a dependant variable and species, gender and trial were categories.

EFFECT	SS	Degr. of	MS	F	p
Intercept	68544.86	1	68544.8 6	140.69	0.00
species	413.73	1	413.73	0.85	0.36
trial	176,. 7	1	176.87	0.36	0.55
gender	1613.89	1	1613.89	3.31	0.08
species*trial	426.85	1	426.85	0.88	0.35
species*gende r	530.95	1	530.95	1.09	0.30
trial*gender	13.32	1	13.32	0.03	0.87
species*trial* gender	904.57	1	904.57	1.86	0.18
error	39951.50	82	487.21		

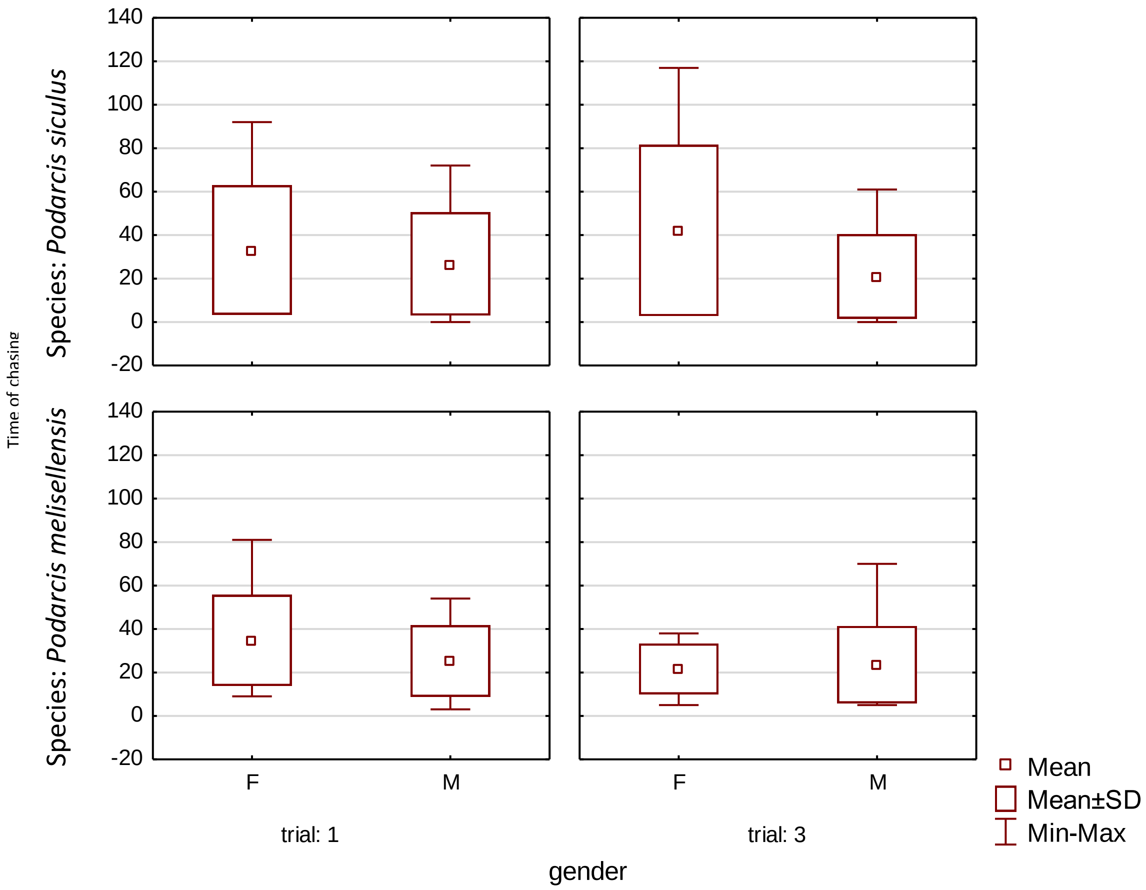

Figure 23: Graph that represents time needed to get the lizards back into the tube after the experiment was over. It is sorted by species, gender and trial. Vertical bars denote 95% confidence intervals.

The results of univariate ANOVA (Table 6) showed that there is no significant difference when it comes to time of chasing between species, gender or trials (Figure 23).

3.3 Radial maze

3.3.1 Movement

For the analysis of movement (see Table 1) of the lizards in the radial maze we used the same variables as in open field and Factorial ANOVA to test for differences between species, trail and gender.

Table 7: Results of a Factorial ANOVA, in which the distance, velocity, meander and time moving were dependant variables and species, gender and trial.

EFFECT

	Value	F	Effect	Error	p
Intercept	0.07	348.09	4	100	0.00
species	**0.76**	**7.86**	**4**	**100**	**>0.01**
trial	**0.83**	**4.96**	**4**	**100**	**>0.01**
gender	0.99	0.38	4	100	0.82
species*trial	0.96	0.96	4	100	0.43
species*gender	0.98	0.46	4	100	0.77
trial*gender	0.95	1.39	4	100	0.24
species*trial*gender	0.96	1.17	4	100	0.33

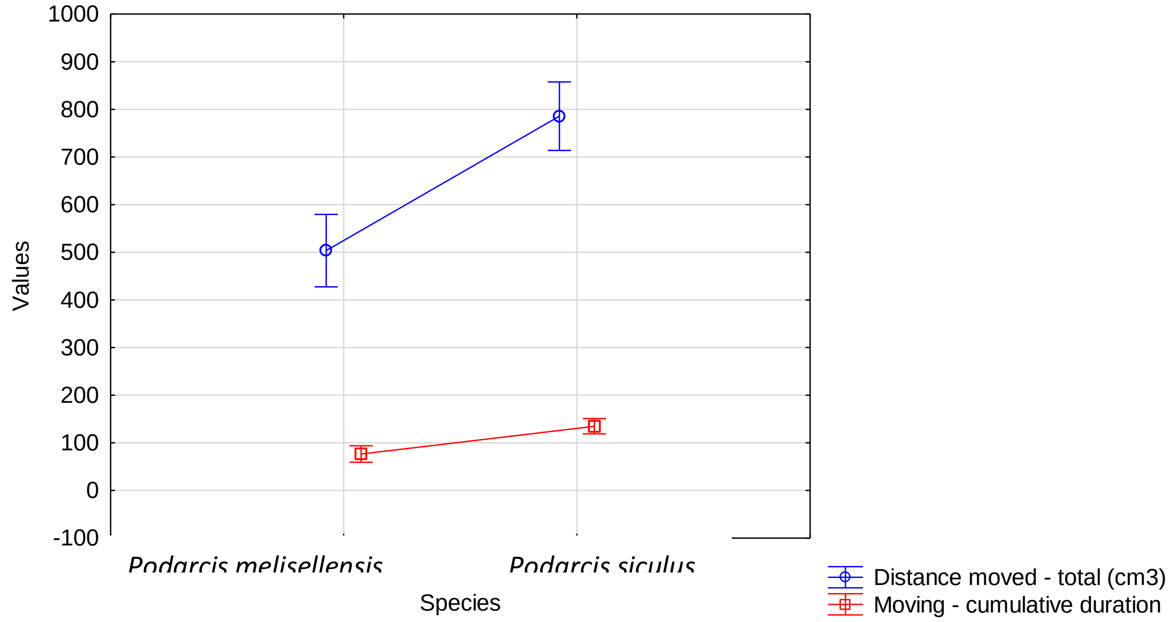

Figure 24: Graph of parameters of distance moved and moving – cumulative duration, according to species. Vertical bars denote 95% confidence intervals.

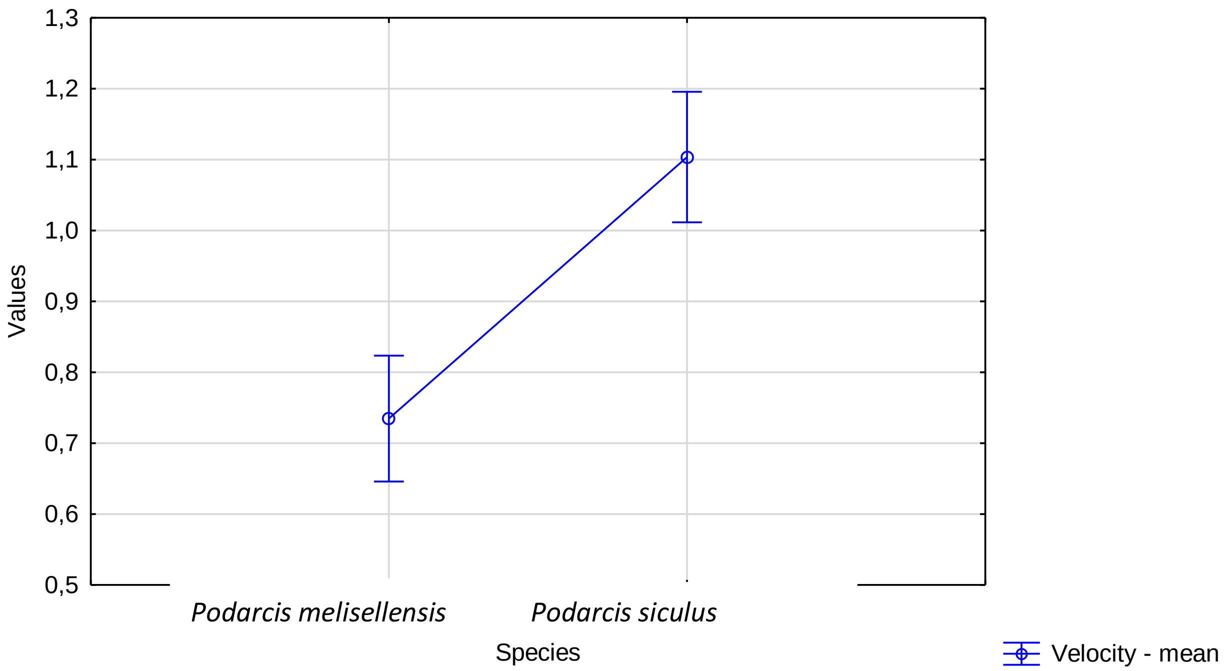

Figure 25: Graph of mean velocity of the lizards in the radial maze, sorted by species. Vertical bars denote 95% confidence intervals.

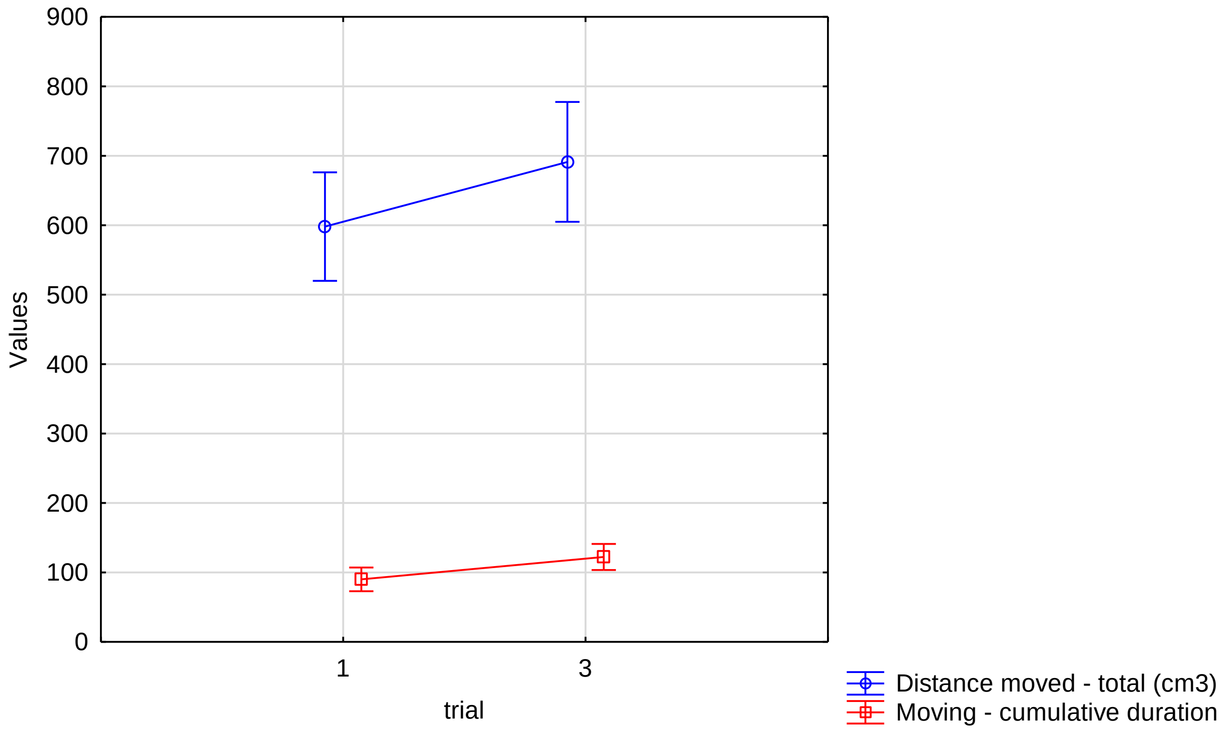

Figure 26: Graph of parameters distance moved and moving – cumulative duration in the radial maze, according to trial. Vertical bars denote 95% confidence intervals.

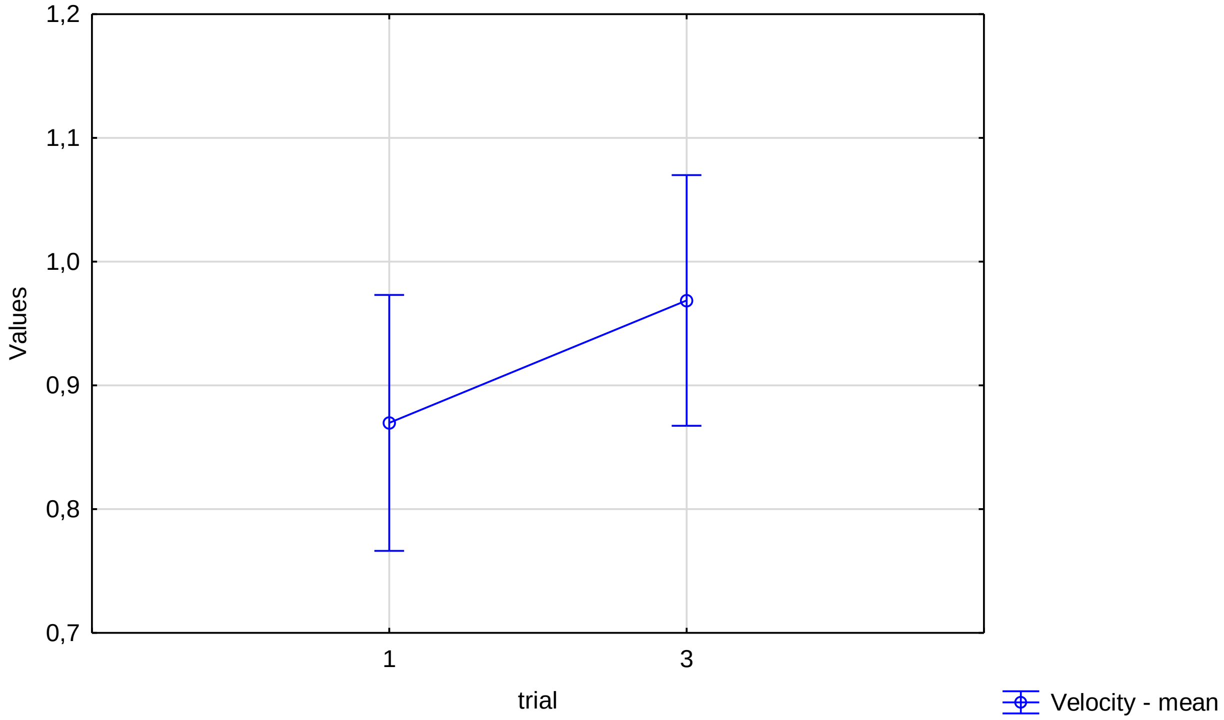

Figure 27: Graph of total time that lizards in the radial maze have been moving, sorted by trial. Vertical bars denote 95% confidence intervals.

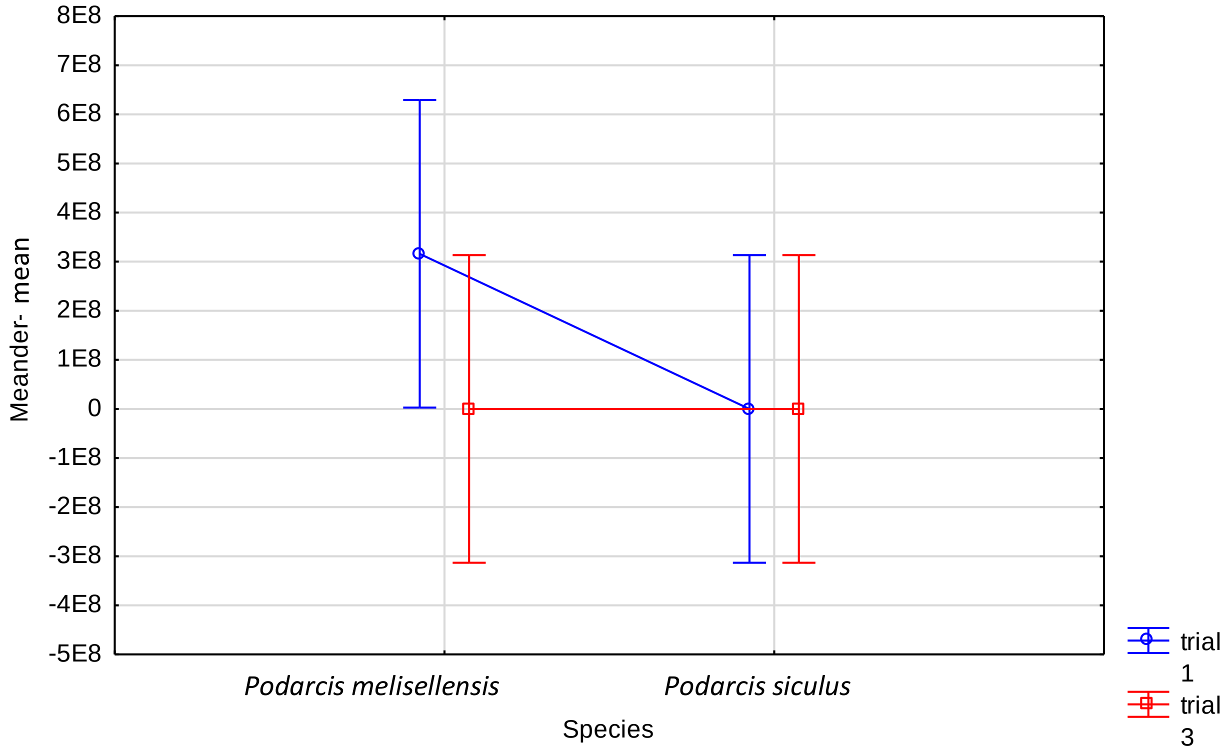

Figure 28: Graph of mean meandering in the radial maze according to species and trial. Vertical bars denote 95% confidence intervals.

The multivariate factorial ANOVA (Table 7) showed that the categories species and trial are significantly different. In the category species variables distance moved (p=>0.01) (Figure 24), mean velocity (p=0.00) (Figure 25) and moving – cumulative duration (p=>0.01) (Figure 24) were significantly different and in category trial, moving – cumulative duration (p=>0.01) (Figure 26) was significant. Mean velocity (Figure 27) and meandering was proven not to be significantly different (Figure 28).

3.3.2 The level of boldness and anxiety

In this group of parameters we again tested parameters in related to level of boldness/skittishness and anxiety (see Table 1), this time in the radial maze.

Table 8: Results of a Factorial ANOVA, parameters, related to boldness and anxiety were dependant variables and species, gender and trial were categories.

EFFECT

	Value	F	Effect	Error	p
Intercept	0.01	859.52	9	91	0.00
species	0.90	1.16	9	91	0.33
trial	**0.78**	**2.80**	**9**	**91**	**>0.01**
gender	0.91	1.02	9	91	0.43
species*trial	0.90	1.05	9	91	0.41
species*gender	**0.83**	**2.10**	**9**	**91**	**0.03**
trial*gender	0.93	0.7728	9	91	0.64
species*trial*gender	0.84	1.9360	9	91	0.05

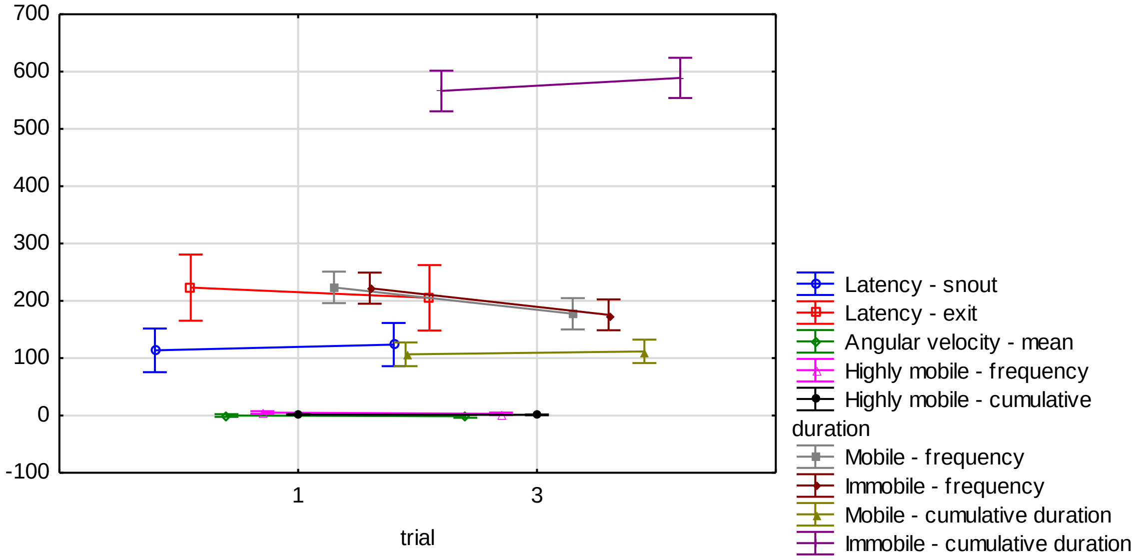

Figure 29: Graph of parameters that represent boldness and anxiety level of the lizards in the radial maze, according to trial. Vertical bars denote 95% confidence intervals

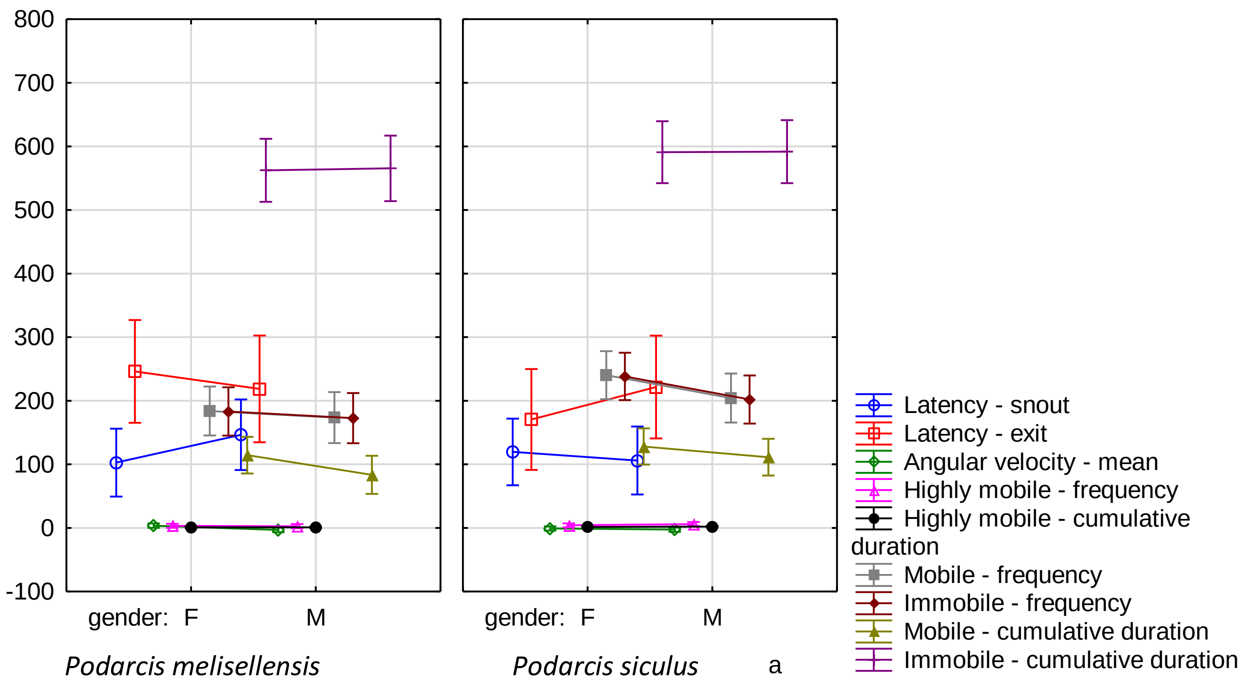

Figure 30: Graph of parameters that represent boldness and anxiety level of the lizards in the radial maze, according to interaction of species and trial. Vertical bars denote 95% confidence intervals.

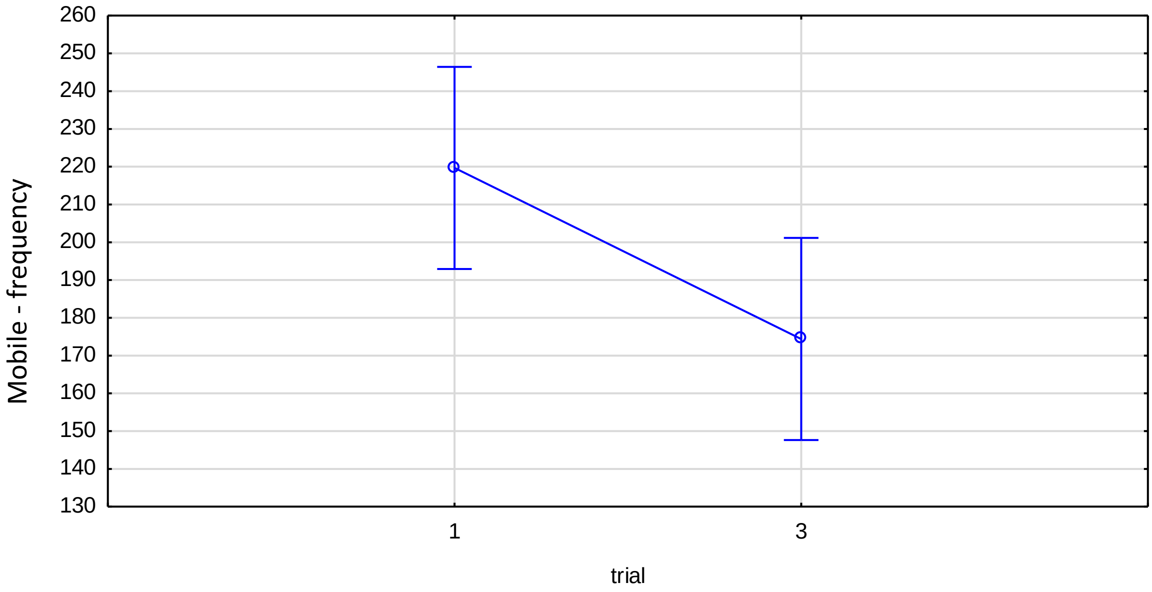

Figure 31: Graph of frequency of the mobile state of the lizards in the radial maze according to trial. Vertical bars denote 95% confidence intervals.

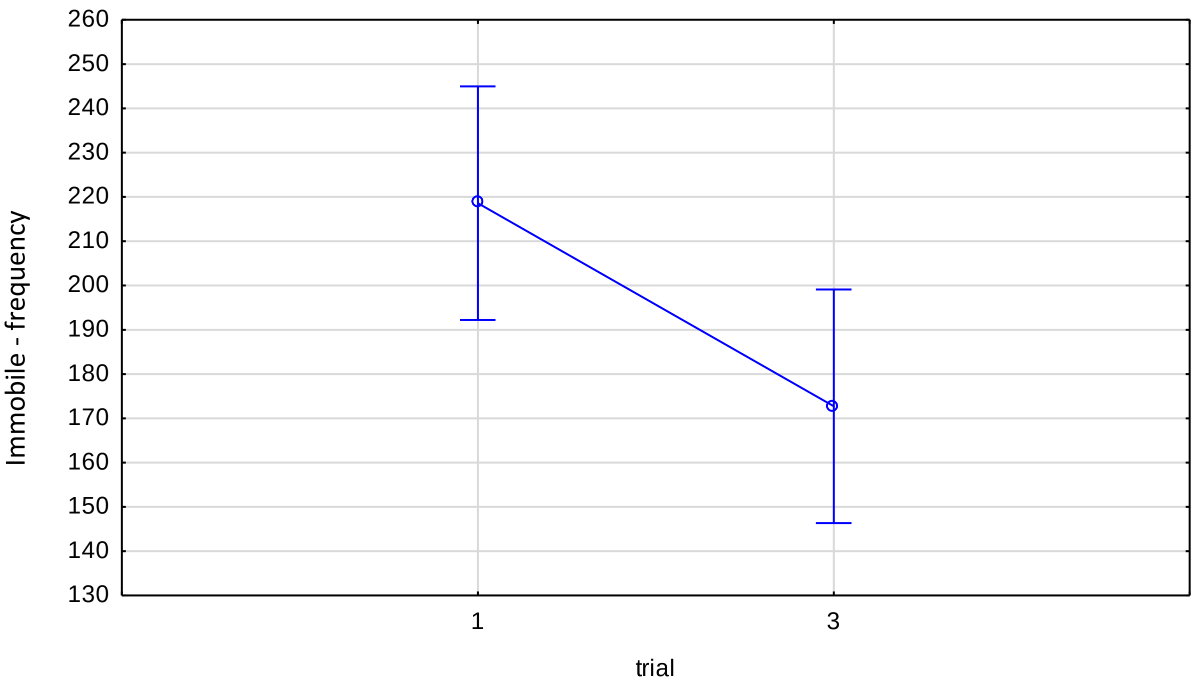

Figure 32: Graph of frequency of the immobile state of the lizards in the radial maze according to trial. Vertical bars denote 95% confidence intervals.

Multivariate statistical ANOVA showed significant difference in categories trial and in the interaction of species and trial (Table 8). Further univariate ANOVA revealed that the variables, significantly different in the category of trial are mobile state – frequency (p=0.02) (Figure 31) and immobile state frequency (p=0.02) (Figure 32).

3.3.4 Learning

In the group of learning we tested two variables – food eaten and time of chasing in three categories – species, gender and trial.

3.3.4.1 Food eaten

This variable was tested with non-parametric statistic, Mann-Whitney test with two sided p value. The result showed that there is significant difference of the parameter in the category of species (p=>0.0001) (Figure 33), but it was not significant in the categories gender (p=0.91) and trial (p=0.15) (Figure 34).

Figure 33: Graph of food eaten by the lizards in the radial maze, according to species.

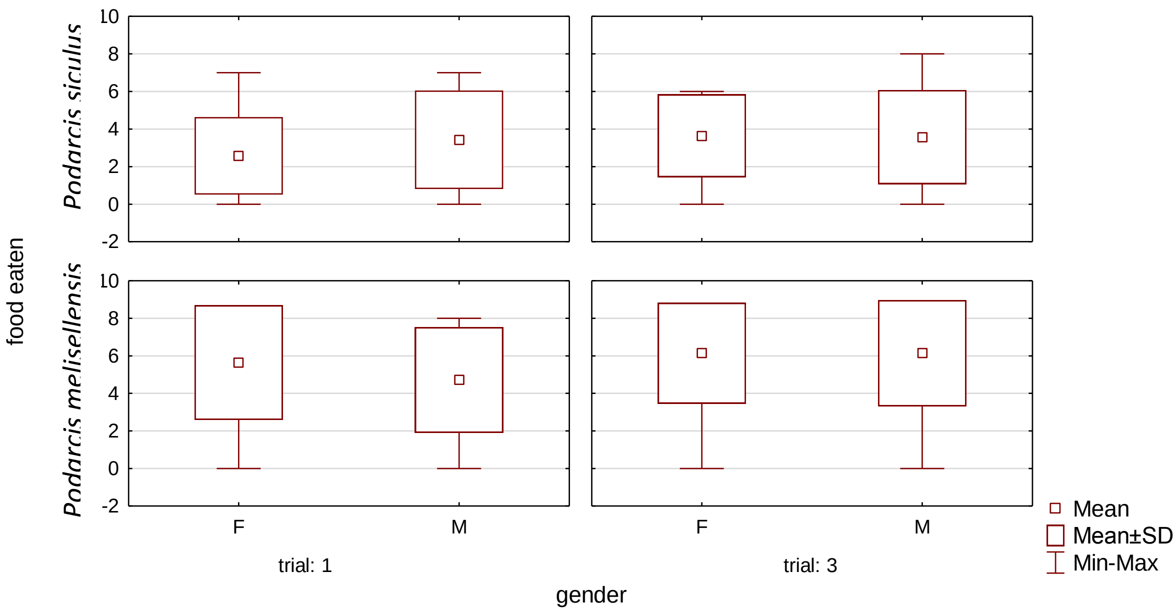

Figure 34: Graph of food eaten by the lizards in the radial maze according to species, gender and trial.

3.3.4.2 Time of chasing

This means the time that each individual lizards needed to go back into the tube. The lizard either went into the tube or chased into the tube by a stick.

Table 12: Results of a Factorial ANOVA, in which time of chasing was a dependant variable and species, gender and trial were categories.

EFFECT

	SS	Degr. of	MS	F	p
Intercept	80549.77	1	80549.77	254.69	0.00
species	260.24	1	260.24	0.82	0.37
trial	582.33	1	582.33	1.84	0.18
gender	715.55	1	715.55	2.26	0.14
species*trial	**1795.10**	**1**	**1795.10**	**5.68**	**0.02**
species*gender	624.38	1	624.38	1.97	0.16
trial*gender	150.33	1	150.33	0.48	0.49
species*trial*gender	**1501.50**	**1**	**1501.50**	**4.75**	**0.03**
error	30677.87	97	316.27		

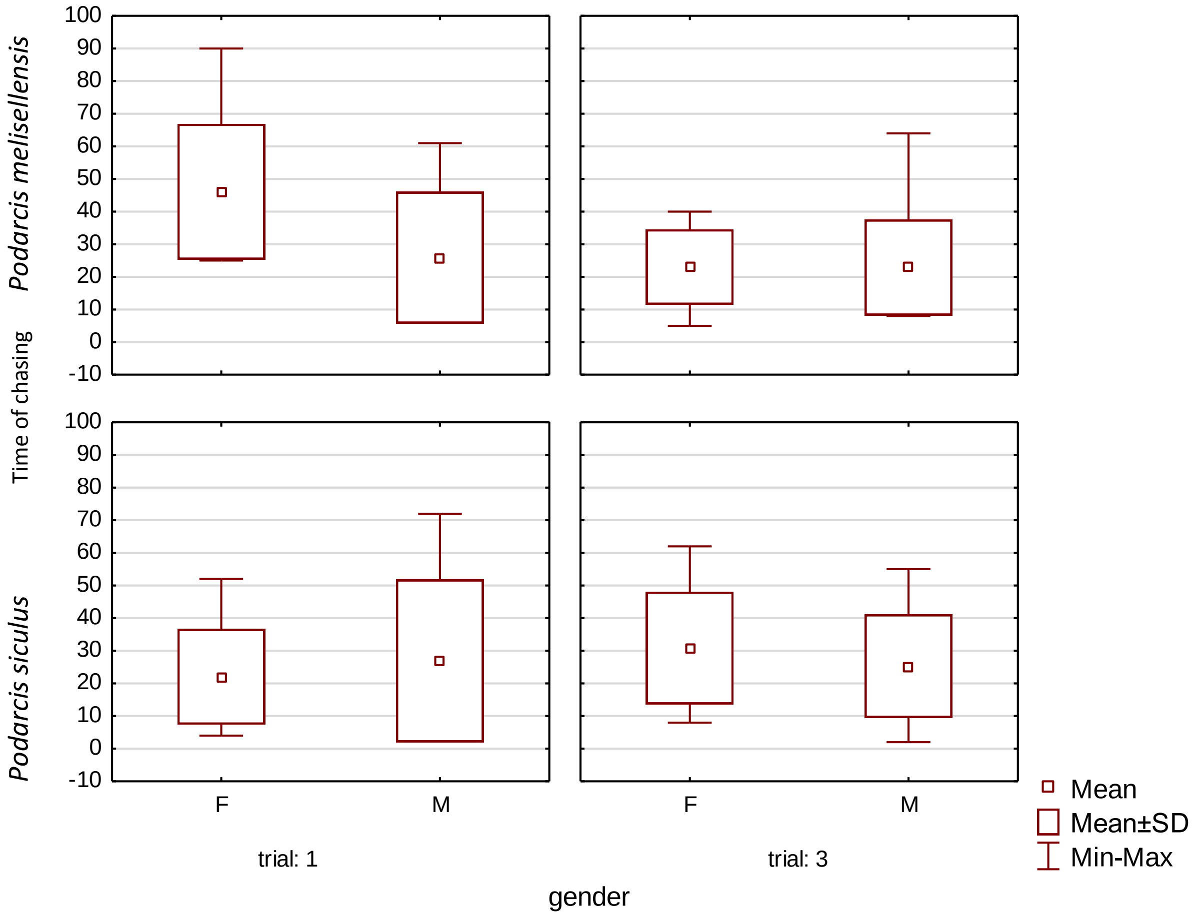

Figure 35: Graph of parameter – time of chasing – time that is needed to get lizard back into the tube after the end of the experiment in the radial maze, according to species*gender*trial.

The results of multivariate Factorial ANOVA (Table 12) showed that time of chasing is significantly different in categories of interaction of species and trial and species and gender and trial (Figure 35).

3.3.4.3 Visits to certain zones

In this group of variables we tested if the lizards have different approach to exploration of the new environment according to species, gender and trial.

3.3.4.3.1 Frequency

This means the number of times a certain lizard have visited each zone in the radial maze in the time of the experiment.

Table 13: Table of frequencies with which lizards visited certain zone in the radial maze according to species.

VARIABLE	U	Z	2*1 sided exact p
arm1/frequency	**1138.00**	**-2.50**	**0.01**
arm2 / frequency	**1167.50**	**-2.33**	**0.02**
arm3 / frequency	**1130.00**	**-2.55**	**0.01**
arm4 / frequency	1237.50	-1.92	0.05
arm5 /frequency	**1151.00**	**-2.42**	**0.01**
arm6 /frequency	1288.50	-1.62	0.10
arm7 /frequency	**1033.50**	**-3.11**	**0.00**
arm8 /frequency	**1047.00**	**-3.03**	**0.00**
central zone / frequency	**870.50**	**-4.06**	**0.00**
goal zone1 /frequency	**1117.50**	**-2.62**	**0.01**
goal zone2 /frequency	**1093.50**	**-2.76**	**0.01**
goal zone3 /frequency	**1120.50**	**-2.60**	**0.01**
goal zone4 /frequency	1287.00	-1.63	0.10
goal zone5 /frequency	1356.00	-1.23	0.21
goal zone6 /frequency	**1064.00**	**-2.92**	**0.00**
goal zone7 /frequency	**962.00**	**-3.52**	**0.00**
goal zone8 /frequency	**1010.50**	**-3.24**	**0.00**

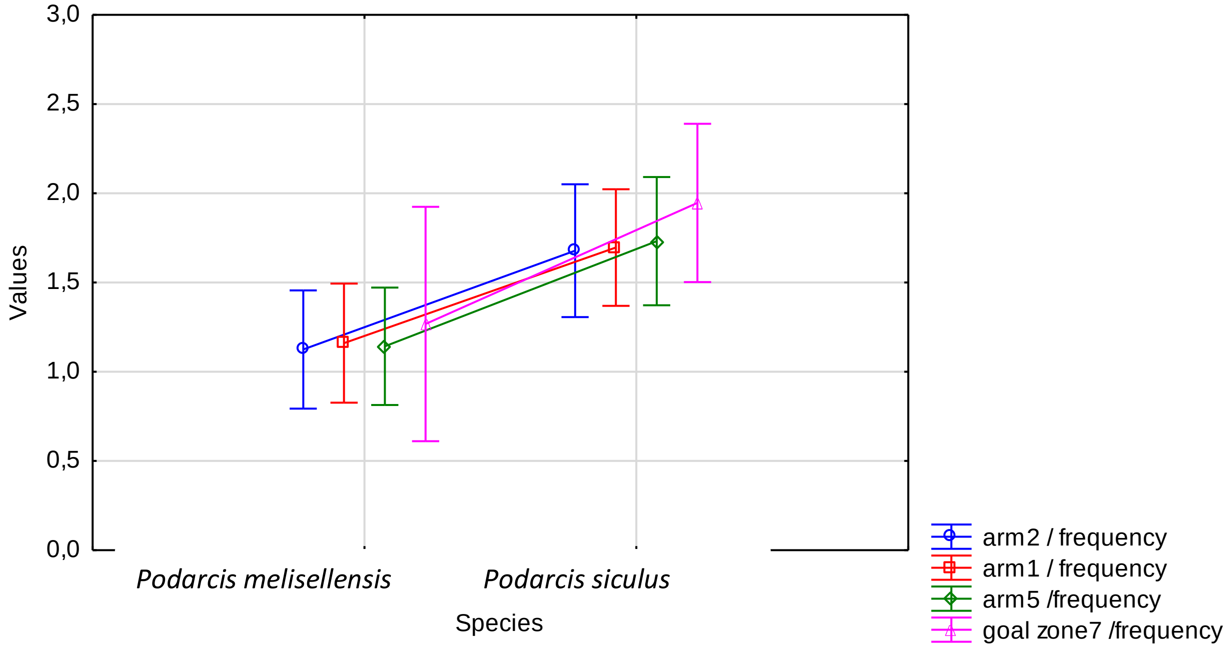

Figure 36: Graph of frequency, with which the lizards visited zones according to species. Plot of Means and Confidence Intervals 95%.

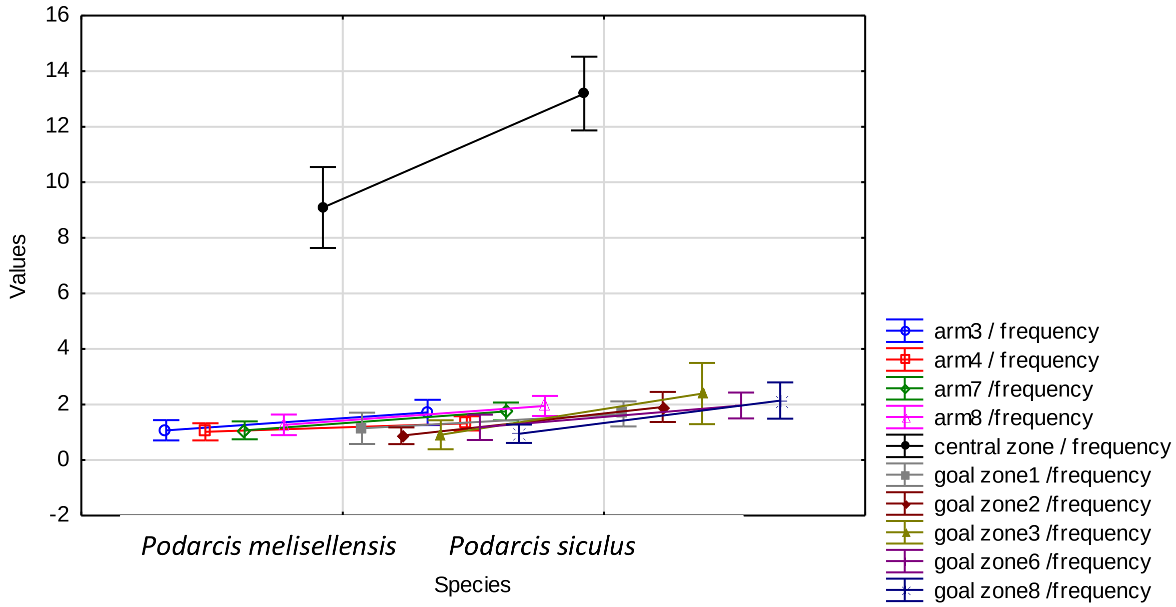

Figure 37: Graph of frequency, with which the lizards visited zones according to species. Plot of Means and Confidence Intervals 95%.

The Mann-Whitney test (Table 13) showed that there is a significant difference in the frequency of visiting of all zones, except arm 4, arm 6, goal zone 4 and goal zone 5 between the two species, *P. siculus* and *P. melisellensis* (Figure 36, Figure 37).

Table 14: Table of frequencies with which lizards visited certain zone in the radial maze according to gender.

VARIABLE

	U	**Z**	**2*1 sided p**
arm1 / frequency	1446.50	0.70	0.48
arm2 / frequency	1534.50	-0.19	0.85
arm3 / frequency	1469.50	-0.57	0.57
arm4 / frequency	1541.00	-0.15	0.88
arm5 /frequency	1542.50	-0.15	0.88
arm6 /frequency	1399.50	0.98	0.33
arm7 /frequency	1450.50	-0.68	0.50
arm8 /frequency	1545.00	0.13	0.90
central zone / frequency	1511.50	-0.33	0.74
goal zone1 /frequency	1538.50	-0.17	0.86
goal zone2 /frequency	1517.50	0.29	0.77
goal zone3 /frequency	1360.00	-1.21	0.22
goal zone4 /frequency	1551.50	-0.09	0.92
goal zone5 /frequency	1353.00	1.25	0.21
goal zone6 /frequency	1367.50	1.16	0.24
goal zone7 /frequency	1480.00	-0.51	0.61
goal zone8 /frequency	1375.00	-1.12	0.26

There is no significant difference in the visits of the individual zones according to gender (Table 14).

Table 15: Table of frequencies with which lizards visited certain zone in the radial maze according to trial.

VARIABLE

	U	Z	2*1 sided p
arm1 / frequency	**1093.00**	**-2.76**	**0.01**
arm2 / frequency	1247.00	-1.87	0.06
arm3 / frequency	1313.50	-1.48	0.14
arm4 / frequency	1494.00	-0.43	0.67
arm5 /frequency	**1134.00**	**-2.52**	**0.01**
arm6 /frequency	1311.00	-1.49	0.14
arm7 /frequency	1252.00	-1.84	0.07
arm8 /frequency	1482.50	-0.49	0.62
central zone / frequency	**1164.00**	**-2.35**	**0.02**
goal zone1 /frequency	1423.00	-0.84	0.40
goal zone2 /frequency	1368.50	-1.16	0.25
goal zone3 /frequency	1342.50	-1.31	0.19
goal zone4 /frequency	1349.50	-1.27	0.20
goal zone5 /frequency	**1172.50**	**-2.30**	**0.02**
goal zone6 /frequency	131.50	0.21	0.83
goal zone7 /frequency	1414.50	-0.89	0.37
goal zone8 /frequency	1518.50	-0.29	0.77

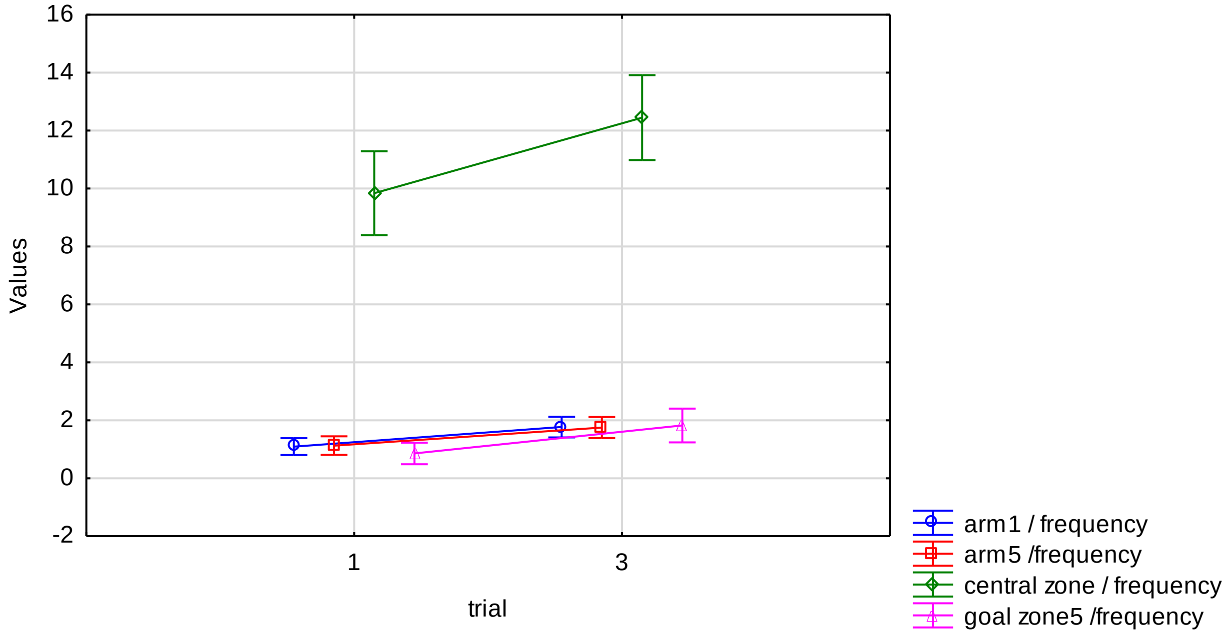

Figure 38: Graph of frequencies with which the individual lizards visited zone that are statistically significant. Plot of Means and Confidence Intervals 95%.

The nonparametric statistical method Mann-Whitney test (Table 15) showed significant difference in the frequency, with which lizards visited arm 1 (p=>0.01), arm 5 (p=>0.01), central zone (p=0.02) and goal zone 5 (0.01) (Figure 38).

3.3.4.3.2 Time in zones

Table 16: Total time spent in individual zone in the radial maze, according to species, gender and trial.

EFFECT	Value	F	Effect df	Error df	p
Intercept	0.03	186.24	17	88	0.00
species	0.75	1.68	17	88	0.06
trial	**0.66**	**2.70**	**17**	**88**	**>0.01**
gender	0.81	1.19	17	88	0.29
species*trial	0.82	1.13	17	88	0.34
species*gender	0.83	1.09	17	88	0.37
trial*gender	0.88	0.69	17	88	0.80
species*trial*gender	0.81	1.20	17	88	0.28

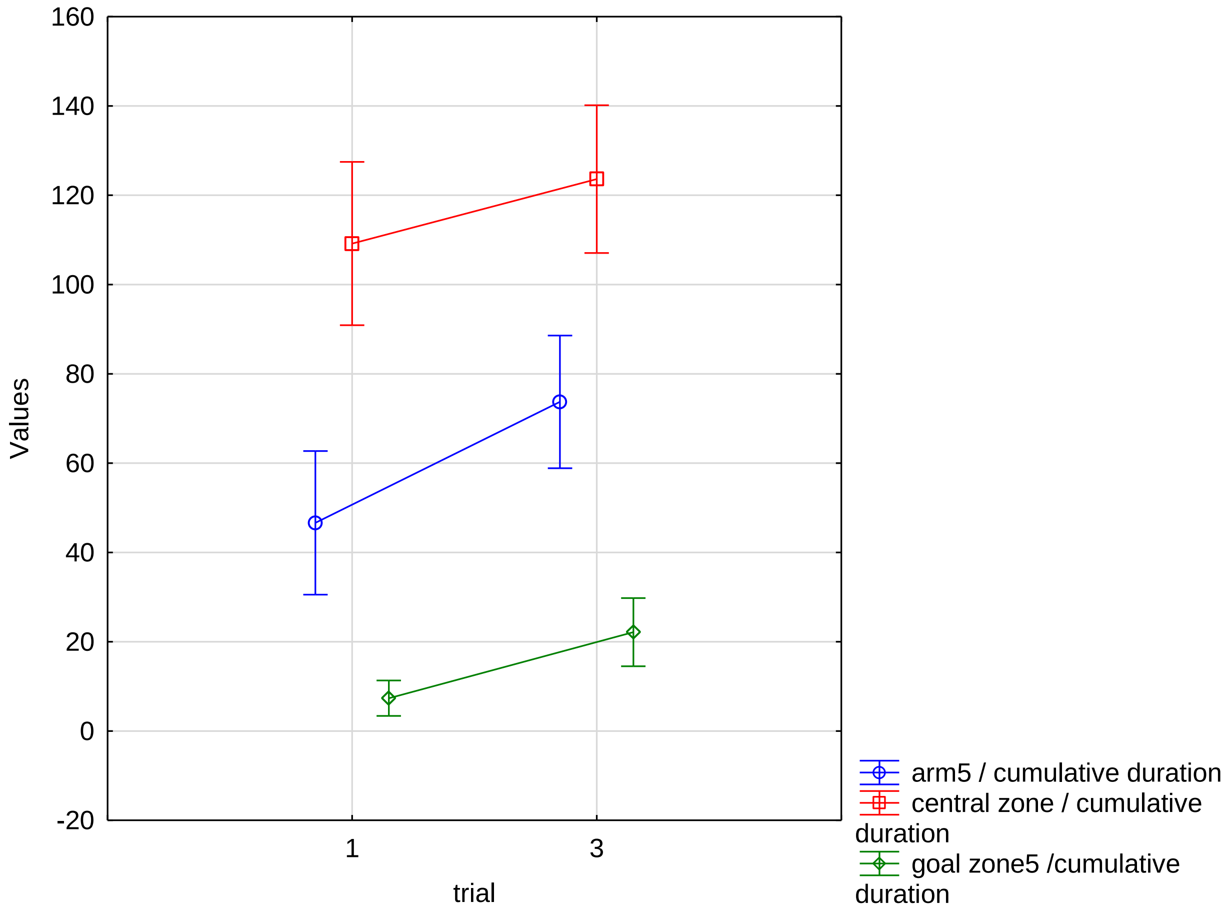

Figure 39: Graph of frequencies, with which the lizards visited the central zone in the radial maze, according to trial. Vertical bars denote 95% confidence intervals.

The multivariate Factorial ANOVA (Table 16) showed significant difference in the category of trial. Furthermore, univariate test showed that there was significant difference in variable central zone (p=>0.01), arm 5 (p = 0.01) and goal zone 5 (p=0.01) (Figure 39).

3.3.4.4.3 Zone alternations

In this group we put variables that shows maximum zone alternations, direct revisits to the zones and indirect revisits by the lizards in to the individual zones according to species, gender and trial.

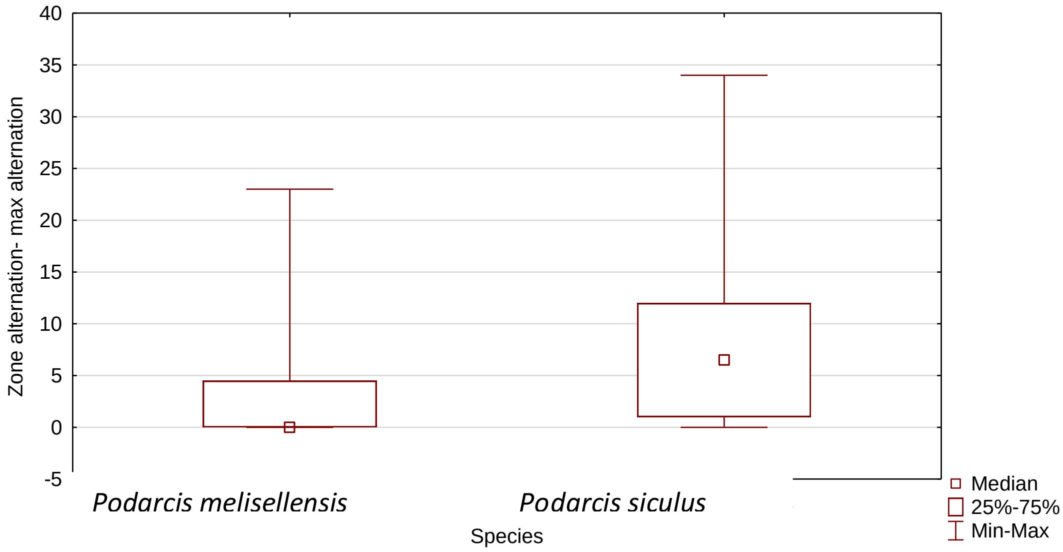

Figure 40: Graph of maximum zone alternations of the lizards in the radial maze, according to species.

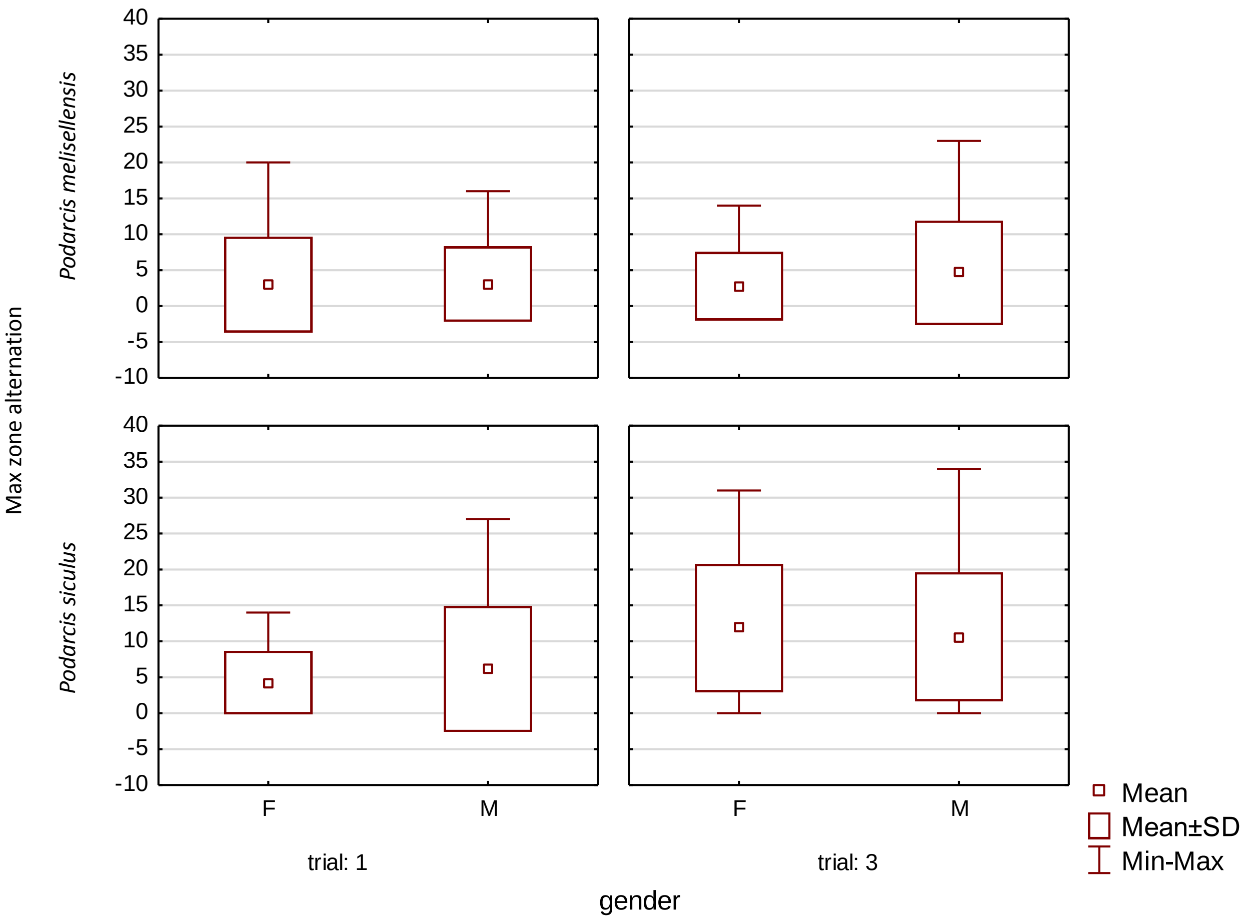

Figure 41: Graph of maximum zone alternations of the lizards in the radial maze, according to species, trial and gender.

The non-parametric method, Mann-Whitney test showed significant difference of variable maximum alternation in two out of three categories – species (p=>0.0001) (Table 17, Figure 40) and trial (p=0.04) (Table 19, Figure 41).

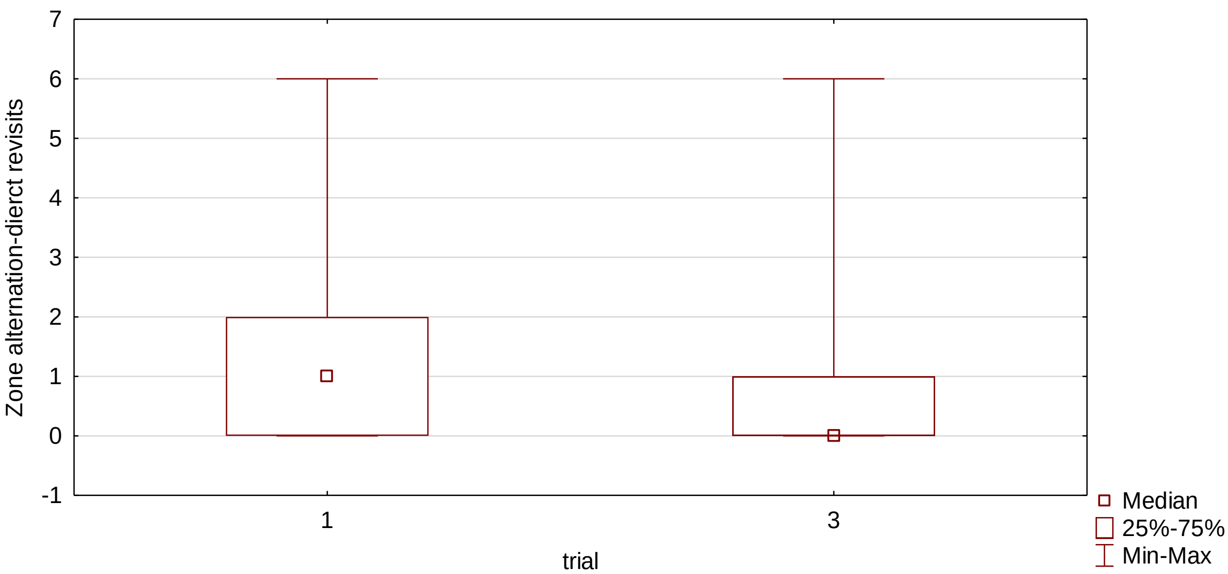

Figure 42: Graph of direct revisits to the zones in the radial maze, according to trial.

The Mann-Whitney test showed that variable direct revisits is significantly different only in the category of trial (p=0.01) (Table 22, Figure 42). The direct revisits were not statistically significant in the categories of species (0.28) and gender (0.37).

3.4 Laterality

We tested for the laterality, the direction in which the lizard turns his body, as soon as he leaves the tube.

3.4.1 Laterality according to the maze

We tested lizards of both species and then disregard the species, then try to establish if there is difference in laterality in different mazes.

Table 17: Results for laterality, the variable was maze.

EFFECT	SS	Degr. of	MS	F	p
Intercept	590.25	1	590.25	990.68	0.00
maze	8.44	1	8.44	14.16	**>0.001**
error	131.67	221	0.60		

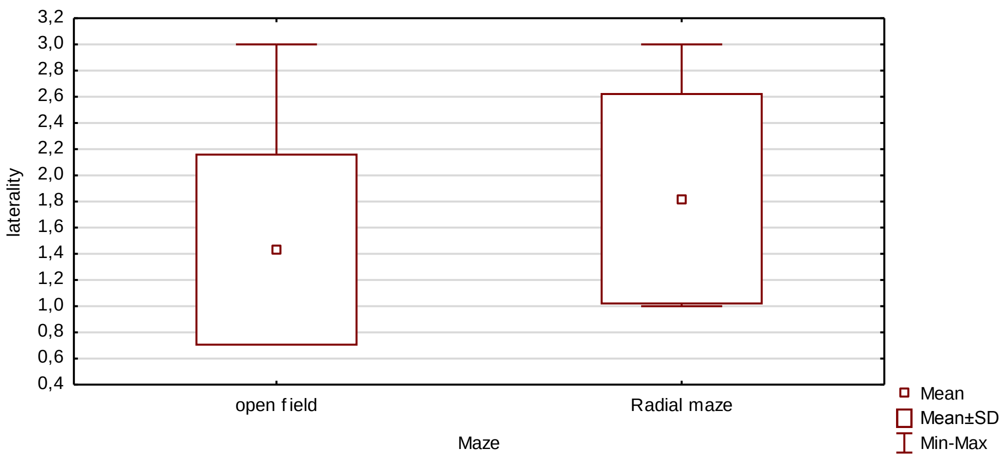

Figure 44: Graph of laterality of the lizards according to the maze.

The results shows there is significant difference between the laterality of the lizards, according to the maze. (Table 23, Figure 44).

3.4.2 Laterality according to the species in both mazes

We tested this variable because we were interested in the fact, whether the laterality is different according to the type of maze (p=>0.001).

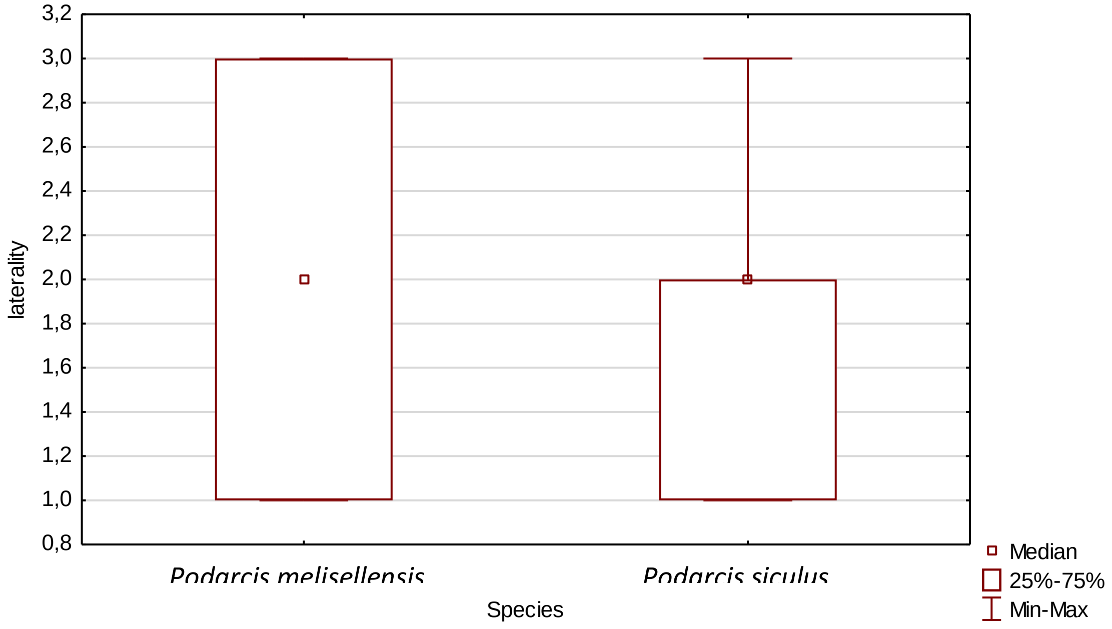

Figure 45: Graph of laterality of lizards in both mazes, sorted by species.

No significant difference was determined in the laterality of the lizards of each individual species (p=0.65) (Table 24, Figure 45).

4. DISCUSSION

There were previous studies done, that suggest competitive exclusion and dominance of *Podarcis siculus* over *Podarcis melisellensis* in the same habitat (Downes, 2002) and there were also a lot of studies that tested the behaviour of the animals in the new environment, open field and elevated plus maze, mostly done on mice (Holmes, 2000 and Carola, 2002). There were also experiments conducted that measured anxiety level of rats (Ramos 1997) and mice (Lipkind, 2004). We tried to combine all those tests and designed an experiment that would allow us to compare behaviour of *P. melisellensis* and *P. siculus*, when the two species are put in the same new environment with the same conditions separately and disclose reasons, why *P. siculus* is more successful (besides the aggression and bigger size) that *P. melisellensis.* Our results showed significant difference in almost all groups of parameters we tested. And all suggests that the results are in favour of *P. siculus.* We also wanted to proof the lizards learning abilities, which we succeed.

4.1 Movement

In the open field, in first group of parameters, movement, we established that there is significant difference in the categories of species and trial. In the category of species the results shows that *Podarcis sicula* moved greater distance in the time of the experiment and that it meandered less. There was no obvious difference in the parameter, moving – cumulative duration, which shows the period of time the lizards were in motion. In the category of trial, we can observe from the graphs that the time period, when the lizards were in motion is approximately the same in the third and first trial, lizards pass the smaller distance in the third trial and they meandered significantly less in the third trial. The mean velocity was also substantially lower in the third trial. In the interaction between species and trial the mean meandering was significantly different for *Podarcis siculus* – the lizards of this species meandered far less in the third trial, but there is no obvious difference in the meandering of the *Podarcis melisellensis* between first and third trial. The study on turtles (Senko et al., 2010) revealed that the East Pacific green turtles (*Chelonia mydas*), use meandering moves in the areas of high probability of gillnet bycatch and directed hunting, which could be the case also in our lizards – the usage of meandering moves to avoid being captured or eaten by the predators.

In the radial maze there is a significant difference in the group of movement in the categories species, trial and interaction between species and trial. In the category of species, there is a significant difference in parameters distance moved, *Podarcis siculus* moved far greater distance than *Podarcis melisellensis*, moving – cumulative duration – *Podarcis sicula* was in the movement much more time than *Podarcis melisellensis* and mean velocity – *Podarcis siculus* had far greater mean velocity than *Podarcis melisellensis*. In the category trial the parameter moving – cumulative duration was significantly different and it showed that the lizards were moving more in the third trial. From the graphs we can also conclude that greater distance was made by the lizards in the third trial, and the velocity was higher in the third trial, which is the exact opposite from the open filed. In the category interaction between species and trial the meandering showed no significant difference in the results, but it is clear from the graph that the *Podarcis melisellensis* meandered much less in the third trial, but there is no difference what so ever in the meandering of the *Podarcis siculus*. Less meandering could mean that the animals are learning about the environment and that is why there is no need to meander as much. A study was made on reintroduces voles (Banks et al 2002) that shows that low mobility is associated with high concentration of odour waste, which can attract the predators quicker. This could indicate that *P. siculus* is more successful, because he is mobile more frequently, moves through greater distances and is moving with higher speed than *P. melisellensis*, which could subsequently mean release of less odours into the environment for predators to smell and thus greater survival success. Another study, a study on colonisation and population recovery of stream fish (Albanese et al. 2009) has shown that population recovery and colonization are positively related with mobility rate. The species, which have higher mobility rates, recovered more quickly.

4.2 The level of boldness and anxiety

In the open filed, the group of parameters, related to boldness and anxiety, there was significant difference in the parameters in the categories species, trial, interaction between species and trial, interaction between species and gender. It was established that parameter latency to exit was significantly different in the categories species, trial, interaction between species and trial and interaction between species and gender. The time needed for the lizard to come out from the tube tells us a lot about his boldness – the bolder and more relaxed he is, the quicker he will come out of the tube. It is obvious from the graph that in the category species, *P. siculus* had higher values of latency to exit than *P. melisellensis*. In the category trial, the values of the parameter latency – exit were lower for the third trial. In the category interaction between species and trial there is a substantial difference in the values of *P. siculus* between first and third trial – the lizards of this species had far lower values in the third trial, whereas lizards of *P. melisellenis* had approximately the same values in both trial, which may indicate to the fact that *P. siculus* is learning quicker. Here, we can draw parallels with the results of study on boldness, exploration and sociability on habituation to predators in lizards (Rodríguez-Prieto et al. 2010), which discovered that exploration had a strong direct effect on habituation, with more exploratory individuals being able to habituate faster than less exploratory ones, probably because of their ability to assess risk better. In the category of interaction between species and gender there were also some interesting results, which in our study can suggest, that *P. siculus* is habituating faster. It is seen from the graph that females of both species had significantly lower values of latency to exit, which may indicate that the females are bolder and more fearless than males. In the category of species, these parameters are also significantly different: Immobile – frequency: *P. siculus* were more frequently immobile than *P. melisellensis*; Immobile – cumulative duration: *P. melisellensis* was immobile for longer period of time than *P. siculus*. Immobility is essentially connected with fear of predation, as shown in the previous studies (Henning et al. 1976; Edson et al. 1972), performed on the *Anolis carolinensis*. As indicated in the past studies, performed on mice (Lipkind et al. 2004), the central area of any experimental field is considered to be more threatening to the animals as the peripheral area. Consequently, the animal is considered bolder, if it spends more time in the central zone. This can be applied to our experiment - Central zone frequency: *P. melisellensis* was visiting central zone more often and *P. siculus*; Central zone – cumulative duration: *P. melisellensis* was in the central zone for longer period of time than *P. siculus*; Peripheral zone – frequency: *P. melisellensis* was in the peripheral zone more frequently than *P. siculus*. From the results, mentioned above and taking into consideration previous studies, we can conclude that *P. melisellensis* is bolder than *P. sicula*. Smilar conclusion can be drawn from the next parameter, Time in tube. Time in tube – cumulative duration: *P. siculus* spent significantly more time in tube than *P. melisellensis*; Lifting on hind legs*:* *P. siculus* lifted more frequently on its back legs than *P. melisellensis*; Highly mobile – frequency: *P. siculus* was in highly mobile state much more frequently than *P. melisellensis*; Highly mobile – cumulative duration: *P. siculus* was far more frequently highly mobile for far greater period of time than *P. melisellensis*. This can be also connected to the results of previously mentioned study (Banks et al. 2002), which suggests that animals, that are more mobile and faster, leave less traces for the predators. The graph, that represents latency to exit according to trial shows that there is no significant difference, but that the lizards needed less time to come out of the tube in the third trial than in the first trial. The graph of highly mobile state - frequency according interaction between species and gender shows that there is a significant difference in the category of gender and species *P. melisellensis,* females of this species were much more frequently in the highly mobile state than males of the same species, but there is not much difference between sex of the species *P. melisellensis,* where males were more frequently in a highly mobile state. There is also significant difference in the same category in the parameters: immobile – frequency: in the species *P. melisellensis*, the females were far more frequently immobile than males, whereas in *P. siculus* there is no obvious difference between the sexes (male were more frequently in an immobile state); Peripheral zone frequency: Females of *P. siculus* were visiting peripheral zone much more frequently than males and males of *P. melisellensis* were also visiting peripheral zone more frequently than females. There was no obvious difference between the females of both sexes, but very significant difference between males of the two species – males of *P. melisellensis* visited peripheral zone more than twice as much as males of *P. siculus*; Central zone – frequency: Females of *P. siculus* visited central zone much more frequently than males and males of *P. melisellensis* visited central zone slightly more often than females. Again, there is significant difference between males of both species – males of *P. melisellensis* visited central zone much more frequently than males of *P. siculus*; Central zone - cumulative duration: The results show that females of both species spent more time in the central zone. All of the results, connected with boldness and anxiety are consistent with the results of the previous mentioned study (Rodríguez-Prieto et al. 2010), made on Iberian wall lizards, *Podarcis hispanica* Steindachner, 1870, which showed that females habituate faster than males and with the study (Keely, 2015), indicating that females performed better in the fear related tasks in elevated plus maze.

In the radial maze in the group of parameters, related to the level of boldness and anxiety, the tests showed the significant difference in parameters in the categories of trial and interaction between species and trial. In the category trial, the following parameters were significantly different: Mobile – frequency: where results showed that the lizards were far less frequently in the mobile state in the third trial than in the first; and immobile – frequency, where results showed that the lizards were significantly less frequently in the immobile state. From the latter we can conclude that the lizards became bolder in the third trial (less “freezing effect” and less frequently in the mobile state, which means they spent more time in the highly mobile state). The difference in anti-predator behaviour and anxiety could also be related to different environment that the species lives in, as suggested in study (Sih et al. 2003), performed on larvae of two sister species of salamander. The results of this study showed that larvae of species of salamander, which lives in the stream, full of green sun fish (*Lepomis cyanellus*), have better anti-predator response than larvae of the species of salamander that live in the fishless pond. This could suggest that *P. melisellensis* has poorer anti-predator response that may indicate to lower survival success.

4.3 Learning

As it is shown in the previous studies (Fagan et al. 2013), the memory and movement are very closely connected. This suggests the majority of the results could also be in the group of parameters “learning”, because it is obvious that the lizards had to learn about new environment of the experiment and they adjusted their behaviour to this realisation (less “freezing”, lifting on hind legs, more time spent in the central zone, lower mean velocity, smaller distances walked,…). First variable in the group under the name of learning was food eaten. It was tested only in the radial maze and the results showed that, out of three tested categories, there is only significant difference in the category of species. The animals remembers that the visited certain location and even associates positives or negative events with that specific location, in our case, the lizard remembers that there was food in this arm before, therefore it could be again. The results showed that *P.siculus* ate a lot more food that *P. meslisellensis*, which is supported by the study (Capula, 2011) about the feeding habits of *P. siculus*, which confirms that *P. siculus* are very aggressive when it comes to feeding and were reported to attack smaller species of lizards, juvenile individuals of the same species and even small rodents. It is also consisting with the results of the study (Braid Day et al. 1999), performed on two species of lizards, *Acanthodactylus boskianus* and *Acanthodactylus scutellatus* that has two different foraging strategies. Another variable, which was tested in both mazes, was time of chasing. It was established that in the open field, there was no significant difference in any of the categories. In the radial maze, the difference was in the categories interaction between species and trial, where the results show that *P.*

melisellensis needed significantly less time to get back into the tube in the third trial in comparison to the first trial, which indicates that *P. melisellensis* is learning, while *P. siculus* needed a little more time to go back into the tube in the third trial. When we compare the two species, *P. melisellensis* needed far more time to get back into the tube than *P. siculus,* which could also suggest that *P. siculus* is learning quicker, which can be associated with the results, obtained in a study (Bouton, 1993), which is suggesting that time is an important context and that animal gain a sense of time of the experiment. This can be related to our results, which showed that some animals have a sense of time, they know when the experiment is over and go into the tube by themselves. In the next group of parameters, visits to certain zones, which we also tested only in radial maze, the results were following: The parameter frequency of visiting certain zone was significantly different in the categories species, where the results showed that *P. siculus* was visiting all the zones with higher frequency than *P. melisellensis*. The results of testing in the category of trial showed that lizards visited zones: arm1, arm5, central zone and goal zone 5 more frequently in the third trial than in the first. When we tested for variable time spent in individual zone, we established that the parameter is significantly different only in the category of trial. The results showed that the lizards spent more time in the third trial in zones arm5, central zone and goal zone 5. The variable maximum zone alternation was proven to be significant in two categories, species and trial. In the category species, *P. siculus* had much higher maximum alternations than *P. melisellensis* and in the category of trial there was established that maximum alternation were higher in the third trial. The parameter direct revisits was significantly different only in the category of trial, the lizards had more direct visits in the third trial, which also suggests that they memorized were the food was. All of this is consisting with the study (Pullum et al.), which implies that animals remember certain patterns and stringset classes. And last parameter we tested, the laterality. We did not found any significant difference in the laterality between the species, gender or trial, but we discovered that there is a significant difference in the category of maze. We tested laterality according to the maze and established that lizards prefer to go straight ahead in the open field and to the left in the radial maze. The study performed on mice (Groleau, 2016) suggests that the decision about the direction, which the animal choose is based on cascade of neural signals that culminate in a single decision that prompts the animal to choose one direction over another. This could be the case in our experiment, because the animal seemed to pause and "think", before they decided to go in either way.

5. CONCLUSIONS

Both species of lizards, *P. siculus* and *P. melisellensis* were learning through trials, which can be seen from the food eaten, the time needed for lizards to come out of the tube or the time needed to get lizards back into the tube once the experiment was over or. It is also showed in the amount of time that they spent in the central area of the maze, in the quantity of movement (smaller distances travelled), less freezing, less meandering. This means that they learned about the new environment and they felt more relaxed in roaming freely through the whole space available.

Podarcis siculus is more agile than *Podarcis melisellensis*. *Podarcis siculus* is more successful since it meandered less (Senko et al., 2010), walked significantly more than *P. melisellensis* and had much higher mean velocity in both mazes (Banks et al 2002) .

P. melisellensis is bolder than *P. siculus*. *P. melisellensis* spent much more time in the central zone of the open field and significantly less time in the tube than *P.siculus*. *P. siculus* also spent a significantly longer time on its hind legs, which suggests that they were more careful and that they tried to escape continuously.

P. siculus finds more food quicker in the radial maze than *P. melisellensis* (Capula, 2011, Braid Day et al. 1999), and had a wider range of zone alternations, which supports our theory that *P. siculus* is faster, more aggressive and vigilant, which can be the cause for their success.

It was also indicated that *P. siculus* learned quicker – the analysis showed that they needed more time to get out of the tube in general, but that they need much less time to get out of the tube in the third trial. Also, they had a significant more direct revisits to certain zones, which could indicate they memorised where the food was and went back to check if there is there is food there again.

There is a significant difference between males and females, especially in species *Podarcis siculus*. From our results we can conclude that the females of *P. siculus* are bolder than males. This is consistent with the results of study (Keely, 2015), which was performed on rats and suggest that females outperformed males in the contextual fear-conditioning task.

This was only a part of a greater study, so future researches in this directions are necessary.

6. LITERATURE

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7. CURRICULUM VITAE

My name is Tamara Gajšek and I was born on 19th of October 1988. I attended elementary school, called »Elementary school Martin Kores Podlehnik«. I finished elementary school in 2003 and enrolled in high school »Gimnazija Ptuj«. I graduated from high school in June of 2007 and in the fall of the same year I began to attend courses on the Faculty of Arts and Faculty of Natural Sciences and Mathematics in Maribor. My majors were translation and interpretation of English language and Biology. In 2014 I graduated and earn the title Bachelor of translation and interpretation of English language and professor of Biology. The same year, I enrolled to University of Zagreb, program Experimental Biology, module zoology. Besides the formal education, I always try to learn something new. I have beginners course in Russian language, course for safe handling with firearms and I am a one star diver, according to CMAS. I my free time I like spending time in the nature and with my pets.