

Report on the outcomes of a Short-Term Scientific Mission¹

Action number: CA18221

Grantee name: Miruna-Gabriela Vizireanu

Details of the STSM

Title: Feasibility of ecological mesocosms to environmental risk assessment of pesticides on reptiles. A pilot test with wall lizard juveniles.

Start and end date: 01/08/2022 to 15/09/2022

Description of the work carried out during the STSM

The present STSM is a continuation of a previous pilot study on wall lizards, which was conducted by Geanina Fanaru (STSM CA18221). The model species used in the study was *Podarcis bocagei*. This species can be found in agroenvironments and, thus, is frequently exposed to agrochemicals.

The main objective of the present study was to test the viability of the mesocosm environments on **juvenile** wall lizards and identify and test the improvements required for a functional experimental system that will allow to evaluate the impacts of pesticides on this species in the future manipulation of the mesocosm environment.

Every 18 days, the adult lizards from the mesocosms were collected to take measurements of snout-vent length (SVL) and body mass (BM). We analysed the BM change over time per mesocosm, sex and individual. Also, we checked if lizards had scars as a sign of copulation or pregnancy. If the later, females were extracted from the mesocosms and kept in indoor terraria to obtain clutches, which were incubated under controlled conditions of humidity and temperature till hatching.

Both juveniles hatched in the incubator and in the mesocosms, were kept in the lab. Each individual was put in a separate box with bioactive substrate, a small bowl filled with water and 2 terracotta bricks. Every day for one hour, we treated the juveniles with UV and IR lights to give them the opportunity to thermoregulate. The lights were kept at 1 meter distance from the juvenile boxes. The environmental temperature in the lab was set at 26°C and 55% humidity.

Feeding was done every day using *Tenebrio molitor* larvae. We followed a feeding scheme according to their size and changed based on their growth rate. After a while, we added to their diet *Acheta domesticus*.

¹ This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

At the age of 4 days, we took the first measurements of SVL and BM. After this, we measured the juveniles every 18 days. We analysed BM change over time since hatching for every individual.

Juveniles

From the previous STSM, 36 eggs were kept in the incubator. Only 8 of them successfully hatched in the end. The unsuccessful 28 eggs were removed.

There were 27 juveniles in total. 8 hatched in incubator, and 19 were found in mesocosms. Out of 27 juveniles, only 1 survived by the end of the present STSM. The death cause of the other 26 juveniles is unknown.

During this time, we continued to monitor daily the juveniles, the mesocosms, the incubator and the irrigation system, as per the previous STSM protocol.

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Description of the STSM main achievements and planned follow-up activities

Adults

In the previous STSM, 5 adult females and 2 adult males died due to unknown causes. During the present STSM, another adult female died. Dead animals were replaced with other lizards collected from the same area.

Between the first measurement of BM by Fanaru and the last one, by us, females showed a decreasing trend with an average difference of $-0.53 \text{ grams} \pm 0.12$. For males, there was an initial decreasing trend and afterwards it grows and stabilises, likely in parallel with seasonal changes in the field, with an average difference of $-0.01 \text{ grams} \pm 0.03$.

For 9 juveniles we measured only once, because they died after. For 9 juveniles we measured twice and for 8 juveniles we measured 3 times. The average BM in juveniles at first measurement was 0.2883 grams (n=26). BM average in second measurement was 0.3102 grams (n=17) and in third measurement was 0.3908 grams (n=8). More statistical analysis have no significant result because of the small sample size left.

Regarding the death of the juveniles, we hypothesize that lack of food diversification is the biggest factor. This remains an issue to be solved in subsequent breeding seasons.

The 16 mesocosms that were prepared in the previous STSM by Fanaru were improved in the present study as such:

- we added more terracotta bricks for increased substrate support;
- we added raffia fabric nails on a wood platform with holes in it to drain excess water
- we improved the arrangement of terracotta bricks position to have repeatability in mesocosm environment

To conclude, mesocosm environments in experiments using adult lizards are feasible for a long period, breeding under environmental conditions in mesocosms is possible and this mesocosm design is suitable for future modification by manipulating biotic and abiotic factors. We did not achieve a similar success with raising juveniles. Nevertheless, juvenile care in mesocosm and lab conditions is possible, but because of their fragility, more factors have to be taken into consideration.

The next step is to analyse the data collected and discuss about the possible solutions to the problems encountered and establish the protocol for future experiments. This way, experimental design is prepared for future experiments studying the impact of pesticides on adult and juvenile lizards.

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