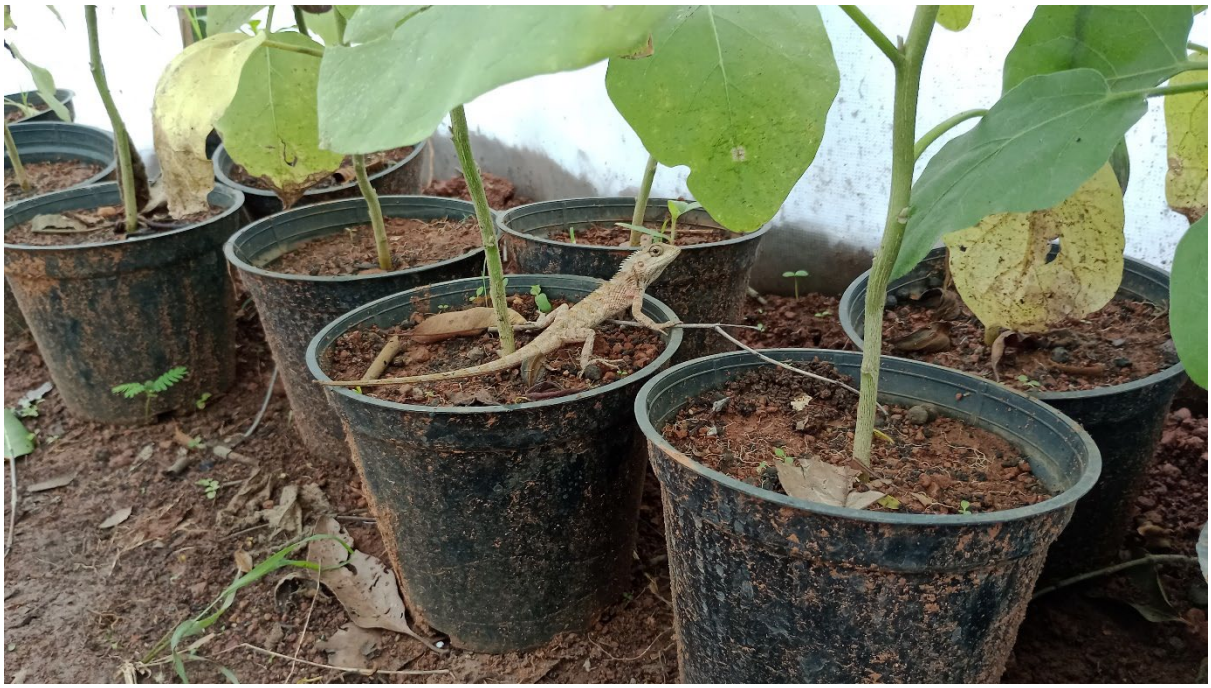
Project Update: May 2022

Assessing reptile cognition and its role in biological pest control amidst future climate warming in a tropical agroforest landscape in India



Lizard on a brinjal plant in a crop patch with crop pest: Learning about foraging patch based on colour and positional cue.

Background of the project

Reptiles are being increasingly appreciated for their cognitive abilities. Though recently there has been an increase in research focusing on the wide range of cognition reptiles exhibit yet such studies suffer from ecological relevance. This project is aimed at understanding how cognition can be used to understand the ecological services delivered by reptiles. The focus of this project is to investigate biological pest regulation by reptiles. Pest regulation studies by natural enemies to date have focused on the reduction in pest abundance and keeping the pest population below the threshold level however such information suffers from efficiency as it does not address the cognition the bioregulator uses to target the crop pest.

In this context, our work is the first attempt of its kind where we are focusing on cognition to re-examine the bioregulatory role in reptiles using wild *Calotes versicolor* as a model.

The main objectives and timeline that we proposed –

Activities and Timescale	**Proposed Time**	**Accomplished**	**Sections for reference**
A. Collection of Specimens	June to September 2021	November – 2021 March- 2022	Sec. 1

Permission Obtained *		January 2022-May 2022 from both the institutes- i. Ashoka Trust for Research in Ecology and the Environment, India, Bangalore ii. University of Lincoln, England	Sec. 2
B. Animal Housing	June to August 2021	November- ongoing	Sec.3
Preparing thermally regulated enclosure			
Egg incubation			
Habituation of the juveniles	Till November		
C. Preparing Experimental Set-up	August 2021 to October 2021	February 2022- April 2022	Sec. 4
D. Experiments	November 2021 to December 2022		
Learning task	November 2021-January 2022	April 2022- **ongoing**	Sec. 5*
Preference task	February 2022		
Cue conflict	March to April 2022		
Translating results to the natural habitat to develop a conservation strategy	July 2022-December 2022		
E. Analyses of results and Publication	from May 2022		
F. Conservation and awareness building	July 2022 through December 2022		
*Indicate new activity added to the project			

- **Section 1**

Collection of specimens

This phase included reptile collection from the wild and housing them. The first batch of nine reptiles was collected on the 4th of November 2021 and maintained till March 13th, 2022 (Fig 1).

The second batch of reptiles was collected on the 23rd of March which is being marinated by the team for all the experiments.

They were collected 6 km away from the field site, from human settlement on the outskirts of the city. *Calotes versicolor* is a diurnal species. To prevent stressing the animals they were collected at night when they are often found resting on small bushes and grass blades. They were picked by hand and kept in a 20 l bucket. To reduce anxiety and stress in the reptiles they were transferred to the enclosure on the same night.

Nevertheless, field collection was delayed due to the delay in the transfer of the fund to the host institute ATREE by when the breeding season was over. The second batch of reptiles collected did not start breeding. Therefore, we had to refine the objective of the proposal while keeping the main aim unaltered.

The third field visit was made on 25th May where we collected only 1 gravid female.

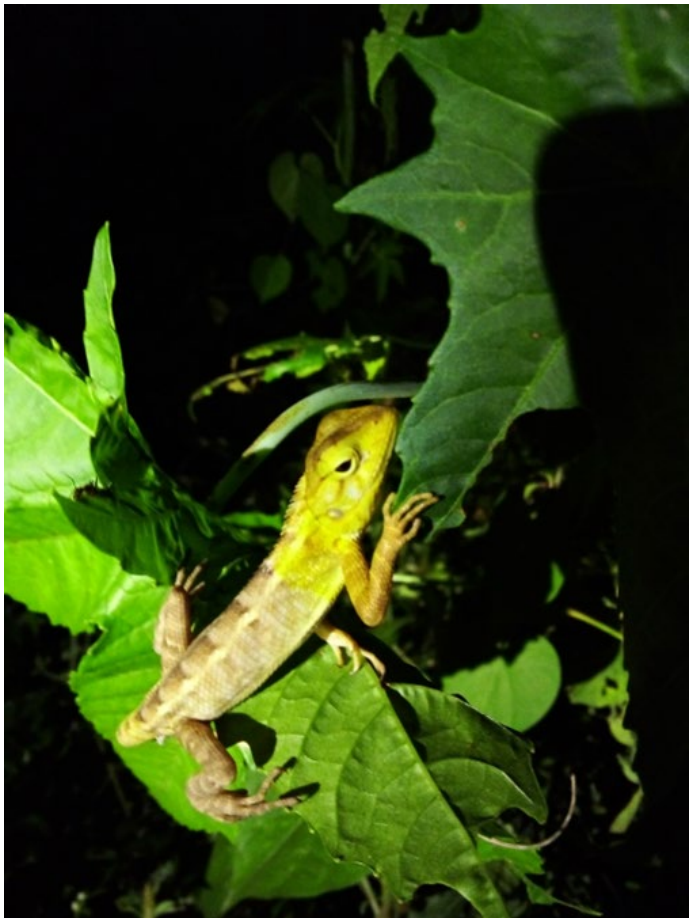


Fig 1: Lizard collected at night from their resting spot

- **Section 2 Permission Obtained ***

The study required obtaining ethical clearances from two institutes which the team has already received.

- **Section 3 Animal Housing**

The field site is Gandhi Krishi Vigyana Kendra, Bangalore, India (Fig 2).

This species has never been studied for cognitive purposes. We did not have any prior knowledge about housing them in the wild and hence we had no reference to the time required for habituation and whether they can at all make a good research model.

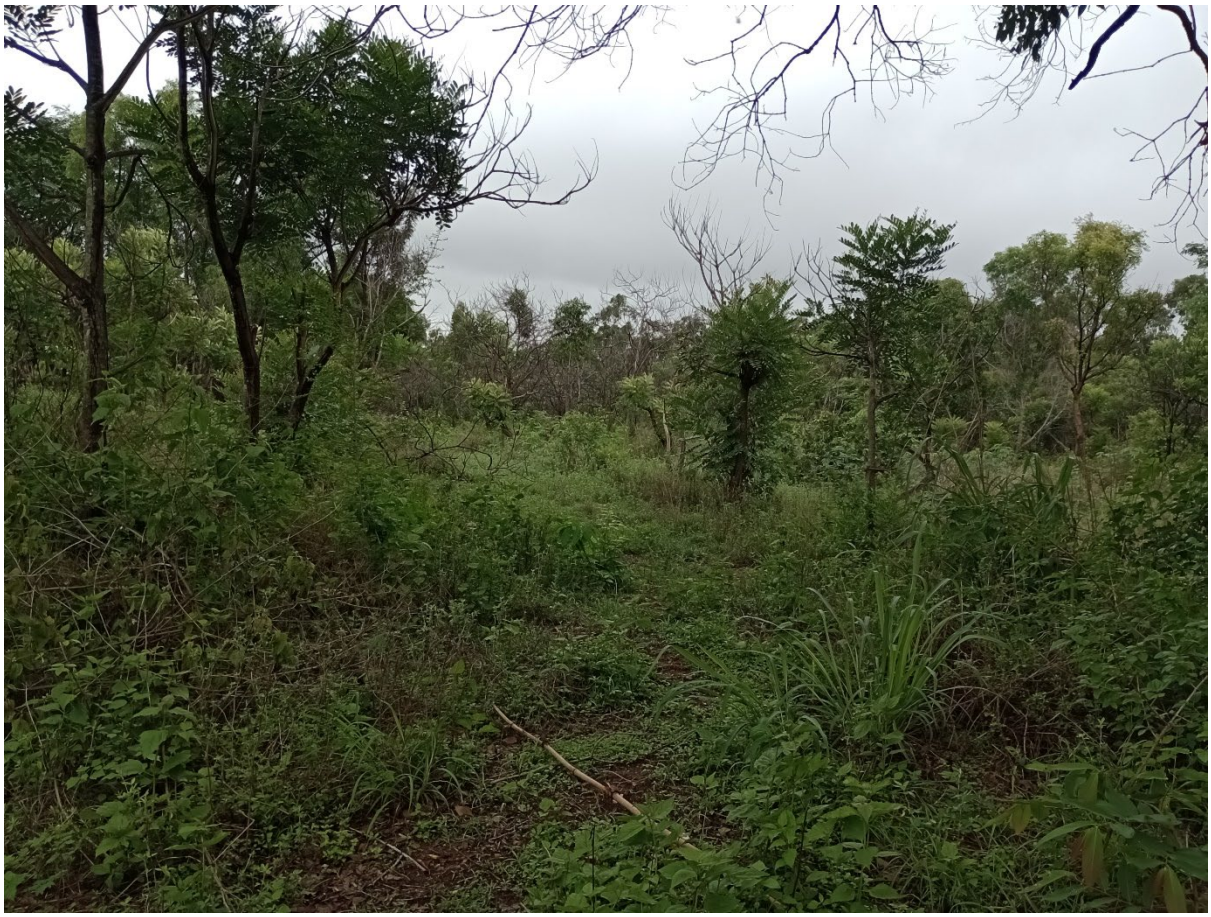


Fig 2: The study location in GKVK

Phase 1

The reptile enclosure was 1 X 2 m in size and was secured by a mesh net on all sides (Fig 3,4). Reptiles were maintained in a group.

Reptiles were maintained on live grasshoppers and moths collected every day from the wild using a sweep net. One to two grasshoppers were fed to each individual. The enclosure was supplemented with enrichments like twigs and branches that allowed them to perch and bask in the sun. A water bowl was provided at one corner of the enclosure, and it was replenished with fresh water every alternate day.

Fig 3: First trial enclosure

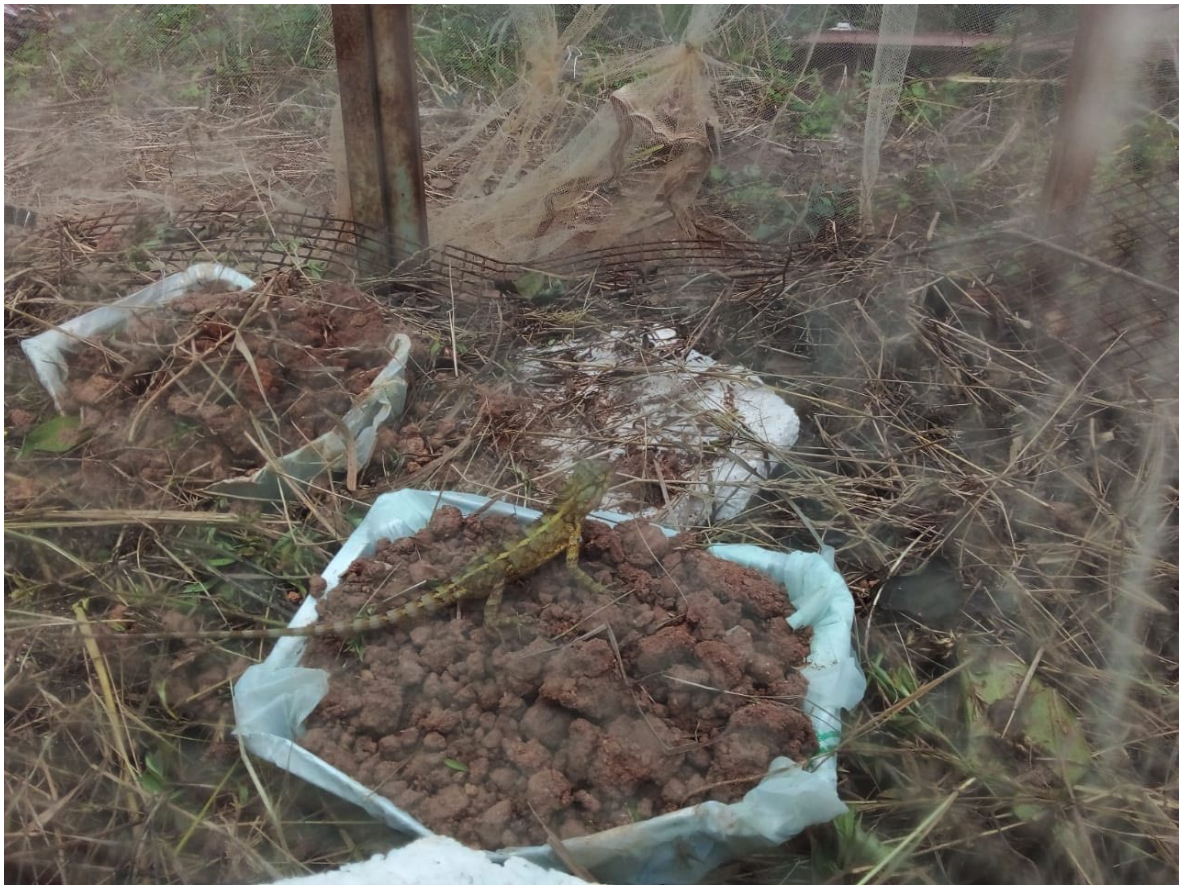


Fig 4: Experimental lizard

Practical Challenges in developing outdoor reptile enclosure

Weather
We intended to maintain the lizards outdoors where they can experience normal day and night cycles and weather that they are used to in the wild. We didn't want to tamper with the natural experience of the lizards.

Predatory threat
Though it's still easier to execute the above-mentioned plan however maintaining them in the wild increases the risk of predation. Since GKVK has a natural forest patch it was challenging to avoid predation from wild rats, squirrels, peacocks, and other birds, dogs, cats and civets. Though we could successfully maintain the batch till March, yet we encountered predation two times during the first phase of housing. The second time we lost the batch probably because reptiles escaped due to the mesh net being torn by wild animals. We anticipate this to be correlated to forest clearance due to forest fire which brought the predators closer to our enclosure and piqued their curiosity.

Disease
Lizards were closely monitored, and we did not find any occurrence or outbreak of disease.

Phase 2: Reptile Housing Enclosure
To ensure safety we had to go through trials and errors. Since the work had no previous literature to refer to it has to go through continuous scrutiny and refinement. We build

an enclosure of 3 X 3 m with aluminium mesh. The top was covered by aluminium mesh as well. This took almost 3 months to finish. The initial plan was to release lizards inside this enclosure as it was increasingly difficult to catch them due to the area being large and the lizards being most active in the daytime. Also, for cognitive study least animal handling is recommended to prevent stress and anxiety. This can seriously impact the experiments and lower the life expectancy of the captive reptiles.

We had to house them in individual plastic boxes of 18 X 12 X 8 inches and covered the top with mesh. This allowed air to circulate and secured the enclosure. These boxes were then put inside the mesh enclosure and left in the field.

This arrangement benefitted by securing the lizards, preventing any sort of predatory threats yet allowing animals to experience normal day and night cycles and outdoor weather conditions.

We supplemented the enclosures with paper rolls and a water bowl which were changed every alternate day to keep the enclosure clean and dry. The paper rolls allowed grip and ease their movement inside the enclosure.

On days when there was a prediction of rain, we shifted the lizards inside the SEC laboratory with proper ventilation. In such cases, lizards were kept outside for 30 min every alternate day especially when they had to perform a task. This prevented lower temperatures from influencing their performance.

Feeding Routine
Lizards are maintained on mealworms, crickets, and grasshoppers (collected by sweep net from the wild habitat).

- **Section 4 Preparing Experimental Set-up**

i. Enclosure
The enclosure is of 165 X 129 X 140 cm (Fig 5), made of polymer sheets on three sides, leaving the front open to serve as an entrance to the crop patch. The front face is secured by a mesh net fixed on the sides of the polymer sheet by velcro. The top of the enclosure is left open. The end of the polymer sheets is buried into the soil sealing all probable escape routes.

ii. Crop patches
We selected brinjal plant (Family Solanaceae) as the main crop. We procured 60 brinjal saplings from IAIM health care centre, Bangalore, and maintained them in our field site (Fig 6). The saplings were arranged in two rows separated by 30cm of patch.

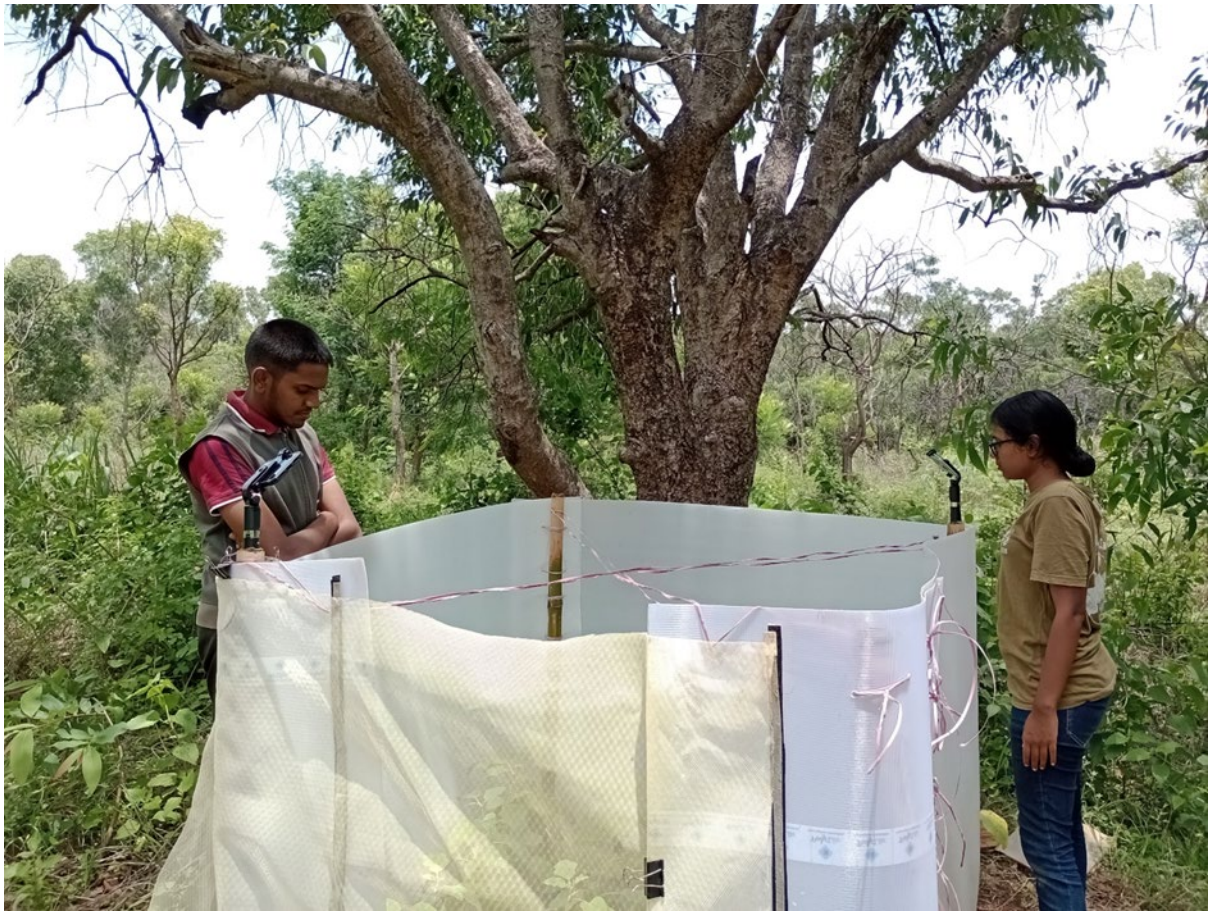


Fig 5 Experimental arena and set-up: crop patch


Fig 6: Brinjal plant

- **Section 5 Experiments: Learning task***

The following are the new secondary objectives that we have explored.
Total sample size = 9

i. *Colour Association

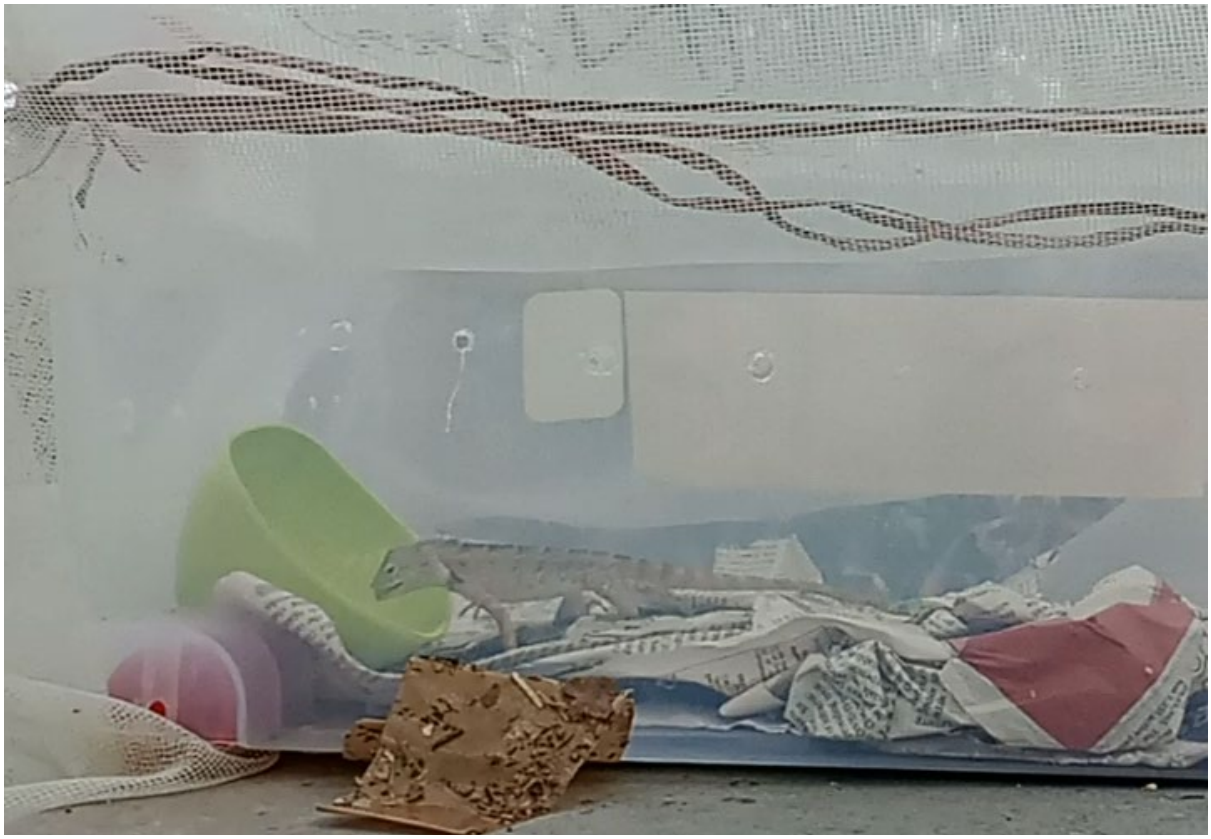


Fig 7: Lizards learn to take food from green bowl: Association trials

Here we wanted to understand if wild caught reptiles can associate colour to food and whether they can discriminate between colour (Fig 7). Trials included associating them with colour and food

ii. *Task: Food procuring

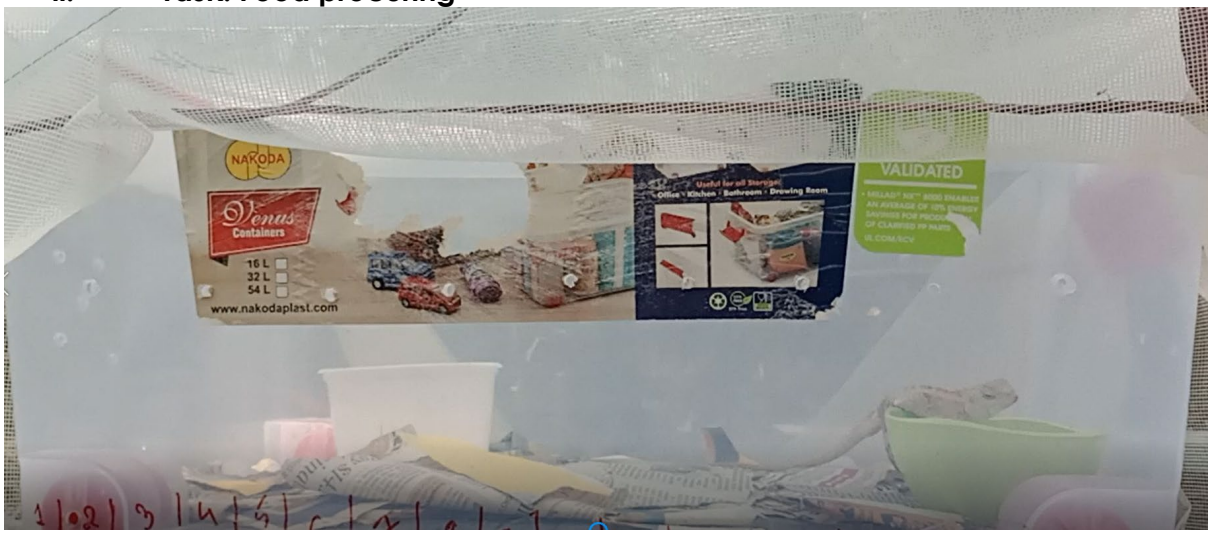


Fig 8. Lizard undertaking food procuring task

If they search for food based on their experience on associating food with colour (Fig 8)

iii. *Colour Discrimination

If they can discriminate between colour (Fig 9)
Total trials conducted so far

i. Association test 10*9=90
ii. Test- Searching food based on colour learning – 10*9=90
iii. Test- Colour Discrimination- 6*9=54

This objective has been completed. We are still left with data analysis.

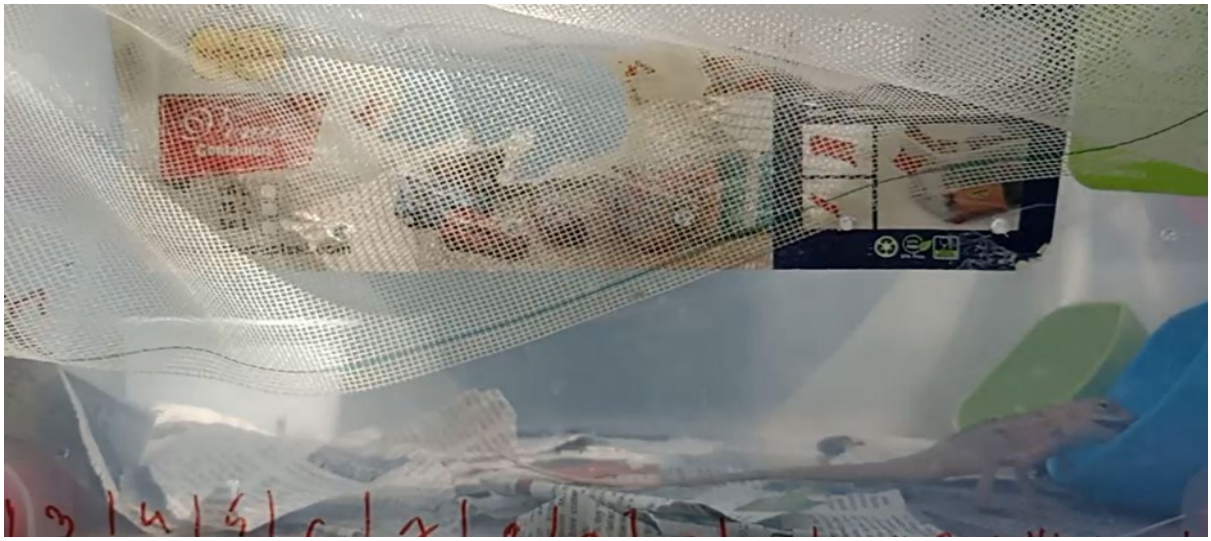


Fig 9 Lizard undertaking colour discrimination test

iv. *Novelty test

We are currently exploring how wild-caught reptiles respond to novel objects added into their enclosure (Fig 10)

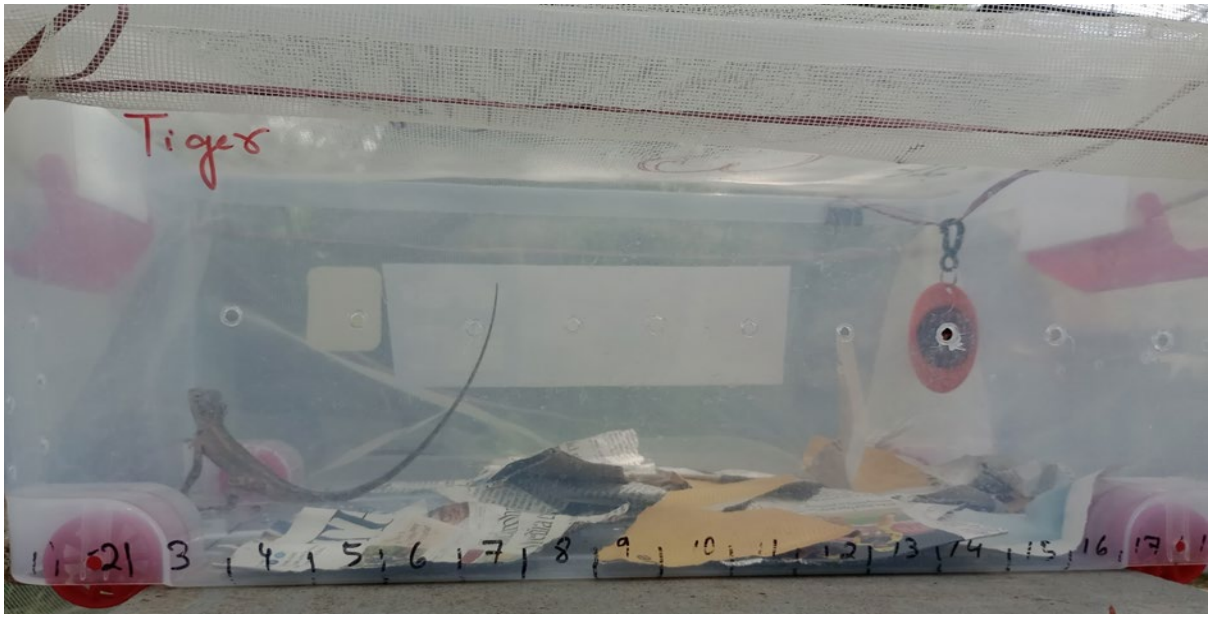


Fig 10: Response to novel object test

v. **Repeatability of colour discrimination test- June 4th onwards**

We are going to conduct a discrimination test on the same reptiles after a hiatus of 1 month where they do not experience any colour association for food.

We will be conducting a colour discrimination test again

vi. **Learning colour cue and positionality of crop patch: learning and remembering rewarding food patch influencing their biological pest regulation efficiency**

At present we are conducting trials where lizards have to learn about their foraging patch. We have completed exploration trials/habituation trials for 10 lizards and currently, we are conducting learning trails (Fig 11, 12). We selected crickets (*Teleogryllus mitratus*) as the crop pest. This is a defoliator species and is one of the important pests to crop plants.

We have conducted 36 trials till now, including 3 exploration trials.

Fig 11: Cricket (*Teleogryllus mitratus*); crop pest

Way forward

- I anticipate completing the learning colour cue and positionality of crop patch by July end.
- Impact of climate change on cognition-July onwards.
- Cue conflict – in August.
- Outreach – from August onwards.

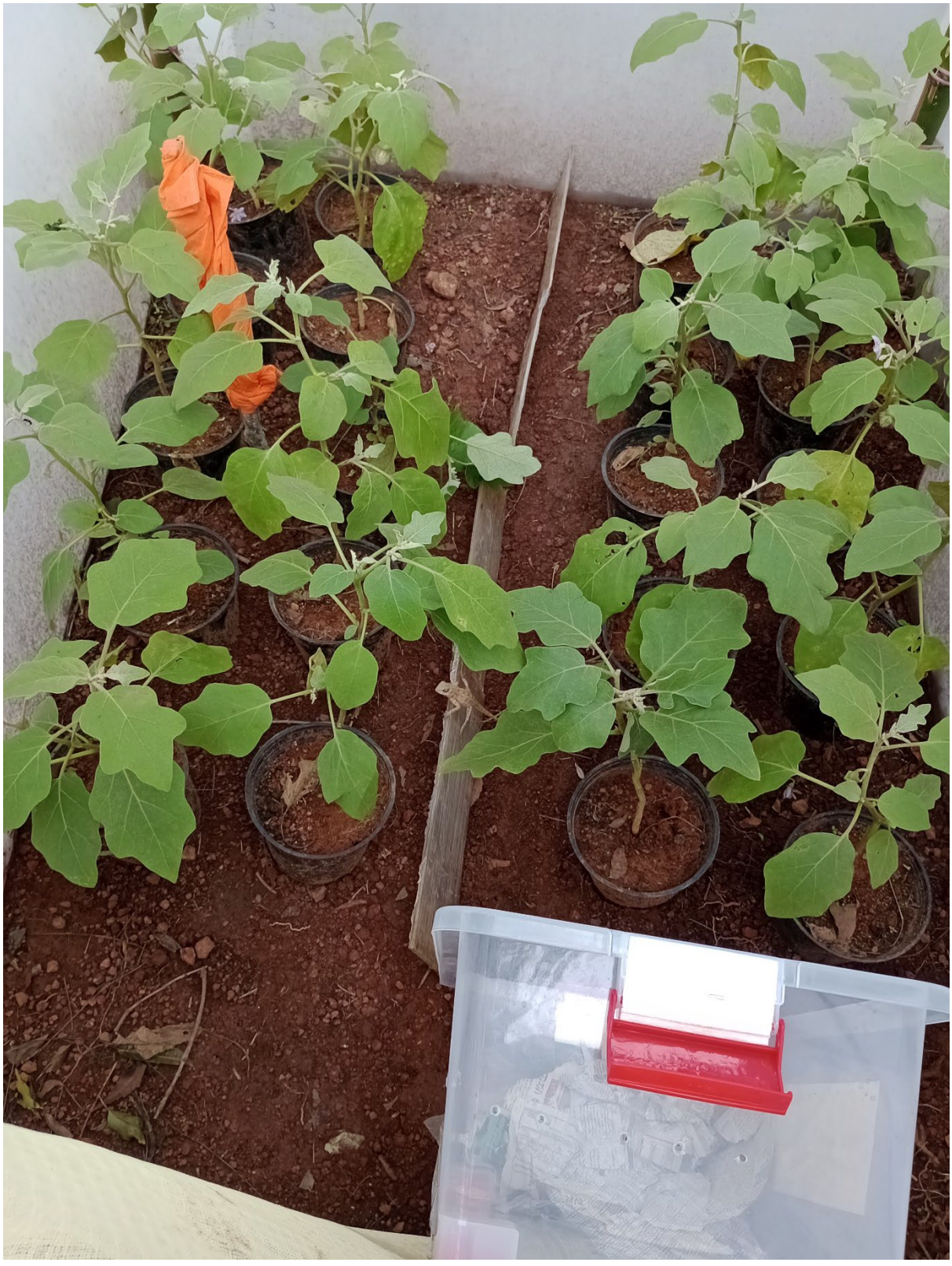


Fig 12. Lizard performing positional (left patch) and colour cue (orange flag) learning trials in crop patch: Using cues to navigate to crop patch with crop pest (left patch)
Summary

The work got delayed due to Covid-19 situations, fund release process, paperwork from the host institute, and procuring ethical clearance from both institutes. Also, unforeseen field conditions slowed down the pace of the work. Nevertheless, progress made so far is satisfactory and the project should be able to abide by the timeline proposed.

The main aim of the proposed project is to link the cognition of reptiles with the biological pest regulation role. The findings can be translated to other endangered species eventually conserving their service provisioning.

The last fieldwork (25.5.2022) yielded only a single gravid female. Therefore, instead of starting with the question of how climate change could be impacting cognition and pest regulation, we started exploring if learning and cognition in reptiles help in biocontrol. It is important to know the role of cognition in pest regulation before we can explore the impact of climate warming.

Therefore, we have a few new objectives which though not proposed in the project still are pertinent from the perspective of biocontrol by reptiles.

The secondary objectives are- associating food with colour, the task of finding food (depending on their learning of colour association with food), colour discrimination, response to novel objects, and novel environment- all of which can be interpreted from a pest regulation perspective.

Data analysis
We have started analysing the data and can be shared in the next report.

Challenges
This work is novel and there is no reference to conducting experiments or housing the animals. It is also elaborate and requires resources and hence is time-consuming. The whole study and the set-up required has to go through several trials and errors before we can decide on any specific method. The work may deviate from the proposed methods as field-realistic situations could not be perceived unless fieldwork is initiated. However, the main goal of the work is to look at pest regulation through their cognition which remains unaltered.

Considering all these, the work has immense impact and I believe the project is running smoothly and can be anticipated to generate good quality data. This is the first time we are looking at reptile cognition from a pest regulation perspective.

Special Mention
Considering the effort and the challenge this project has, the following team members need a special mention- Dr. Vikram Aditya, Postdoctoral fellow, ATREE has been looking after the feasibility, scientific component, field travel, accommodation, and conducting the fieldwork along with me. He has been managing the whole project since the very beginning and is an integral part of this project.

Important contributors
Dr. A Madhyastha- Senior fellow ATREE who provided the plot for experiment and constant refinement to the fieldwork

Dr. Uma Shaankar- Professor, College of Agriculture- who gave access to the land in School of Ecology and Conservation, GKVK

Prof. Wilkinson, University of Lincoln for time-to-time supervision and refinement of the project

Amarthya Chandar and Raksha More- extremely motivated interns who have been working in the project for the last two months

Field collection: assisted by Mr. S Narayanan, Mr. R Sharma, Mr. A Kanan, and Mr. A. Chandar

Field support- School of Ecology and Conservation laboratory members

Details on the findings will be shared in the final report