Final Evaluation Report

Your Details	
Full Name	Deyatima Ghosh
Project Title	Assessing Reptile Cognition and its Role in Biological Pest Control Amidst Future Climate Warming in a Tropical Agroforest Landscape in India
Application ID	34363-B
Date of this Report	22.1.2024

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
How temperature rise will impact their cognition(leaning) for foraging				In 18 months, we only procured a single gravid female. She was maintained in laboratory under favourable conditions for 4 months and was under the supervision of a vet. However, she did not lay eggs. Though this made the project far more challenging this behaviour is not surprising as female *Calotes* have been shown to arrest the development of eggs. But what was more surprising was the availability of only one female during our study period. This evidently indicated the threats to this species from anthropogenic pressure.
Translation into natural habitat: How learning in reptiles can be utilized in conserving their service provisioning				
*Taste vs colour discrimination in reptiles can impact pest regulation role				This objective has not been proposed in the project. However, during the course of our fieldwork we realised, this aspect of colour and taste learning will add significantly to the understanding of their role as bioregulators.

*New objective added during the course of the project

2. Describe the three most important outcomes of your project.

a) Reptiles can learn about the different locations with varying intensities of pest infestation. This was evident as reptiles in our study were found to make more significant correct choices of entering the patch with more crop pests (enhanced by adding 15 crickets) over the number of trials. This choice was significantly reflected in their pest regulation ability. In crop patches, the intensity of pest infestation varies, and reptiles can learn about the position of the enhanced crop patch. Further, reptiles give equal preference to both position and colour while choosing the correct patch. This is a novel finding and can have immense implications in the field of pest management (Fig 1, 2).

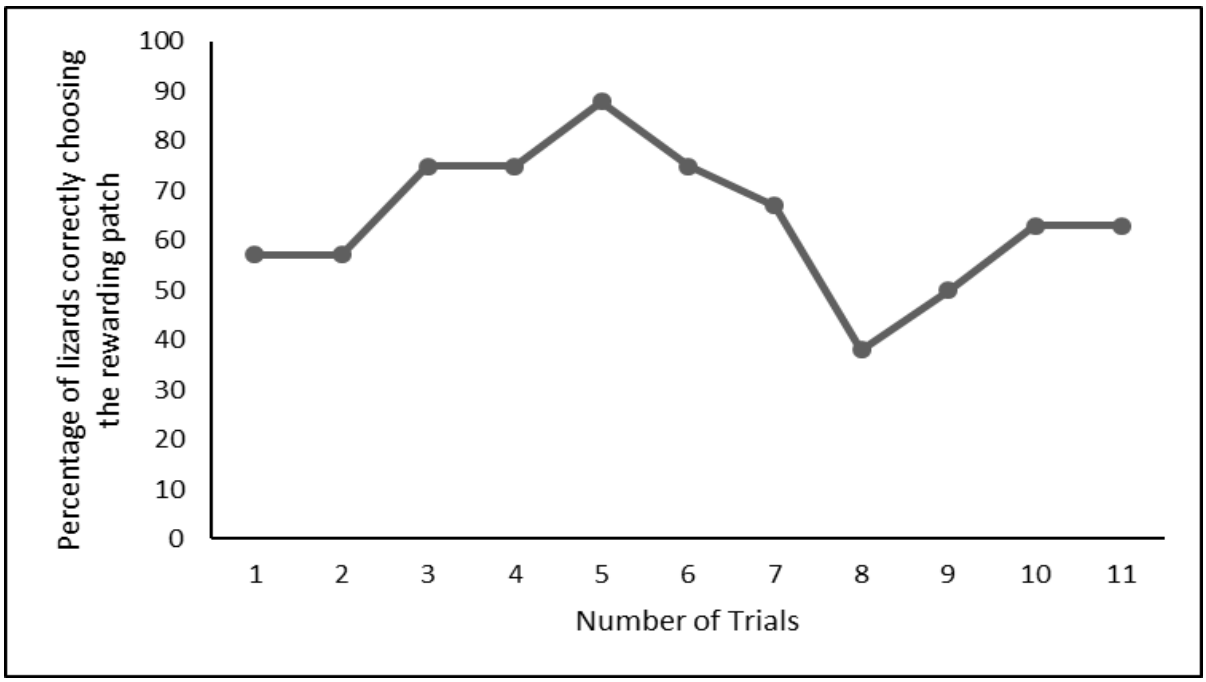


Figure 1. Percentage of correct choices made by the reptiles.

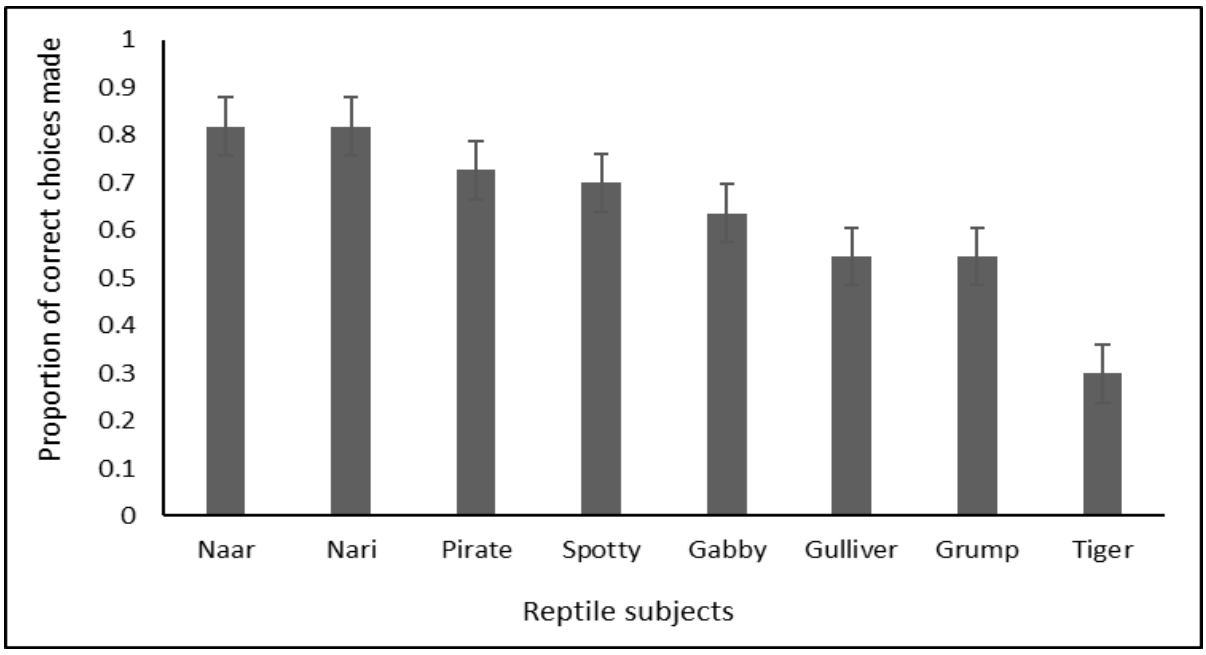


Figure 2. Individual performance of the reptiles while selecting the correct patch.

b) Reptiles spend more time in enhanced patches. Being able to choose the correct patch and spending more time reflected in their pest regulation ability. Pest regulation was double from heavily infested patches than non-enhanced patches (patches where pest number was not augmented). This is a novel finding and directly shows the role of animal cognition improving pest management (Fig 3, 4, 5).

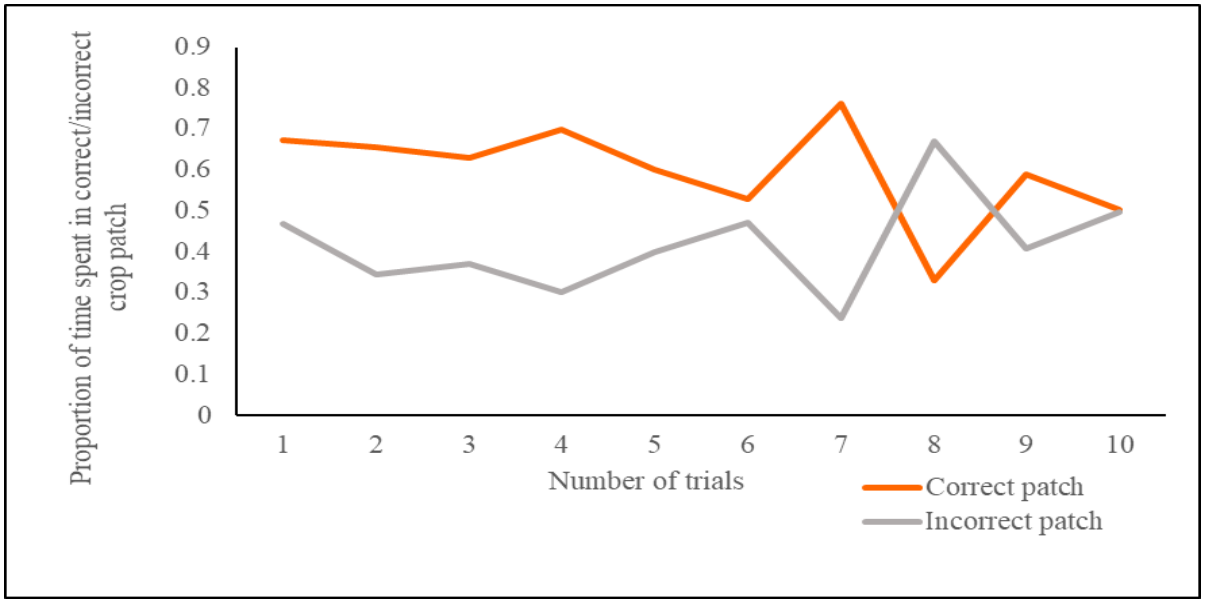


Figure 3. Proportion of time spent in enhanced (correct) and non-enhanced patch (incorrect)

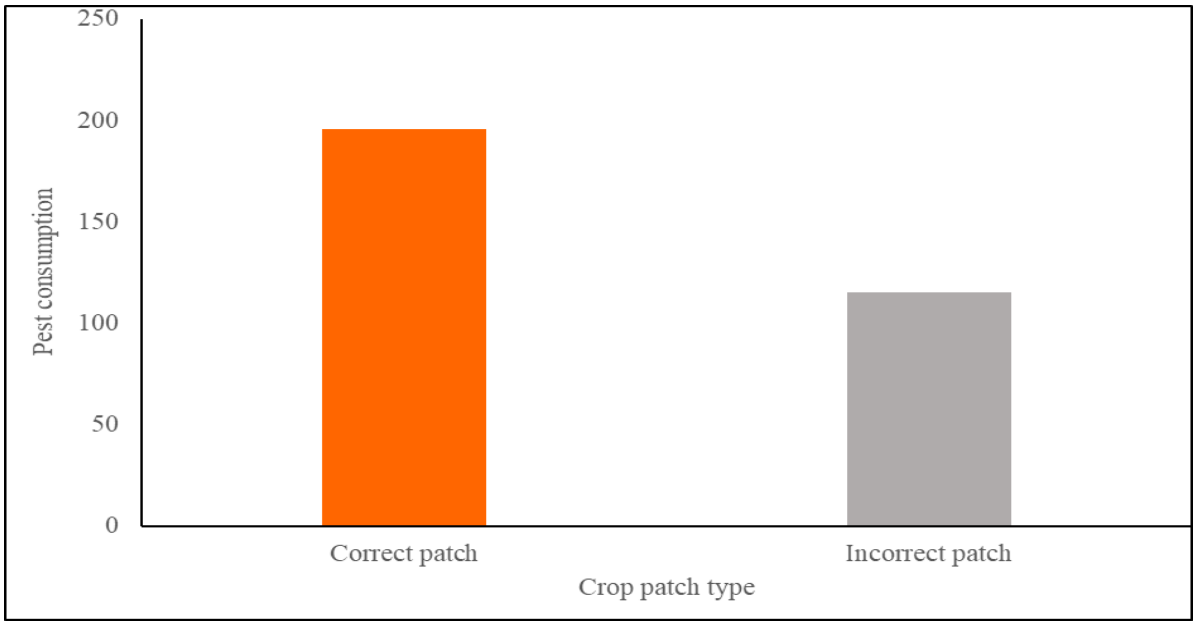


Figure 4. Amount of pest consumed from enhanced (correct) and non-enhanced patch (incorrect).

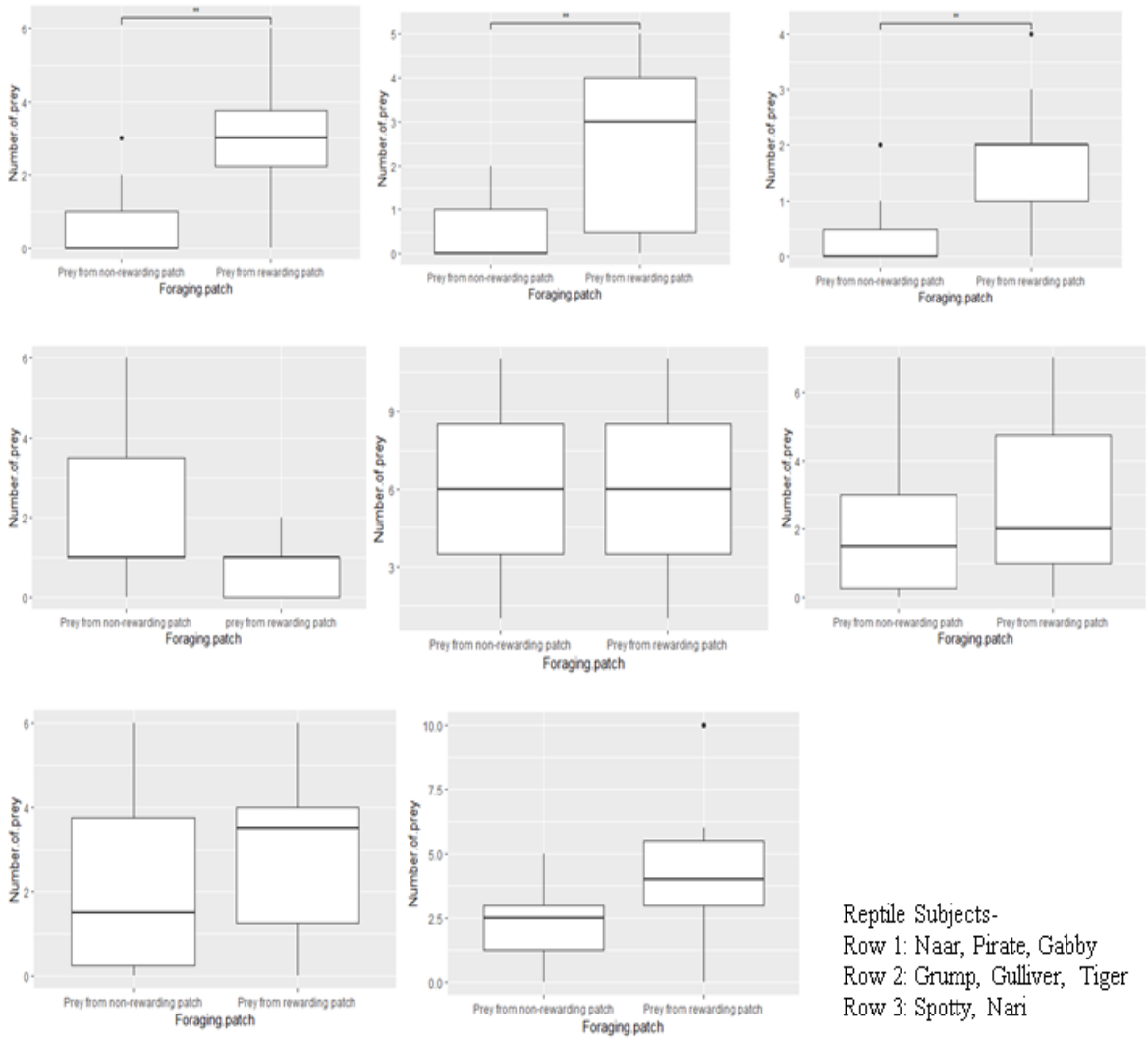
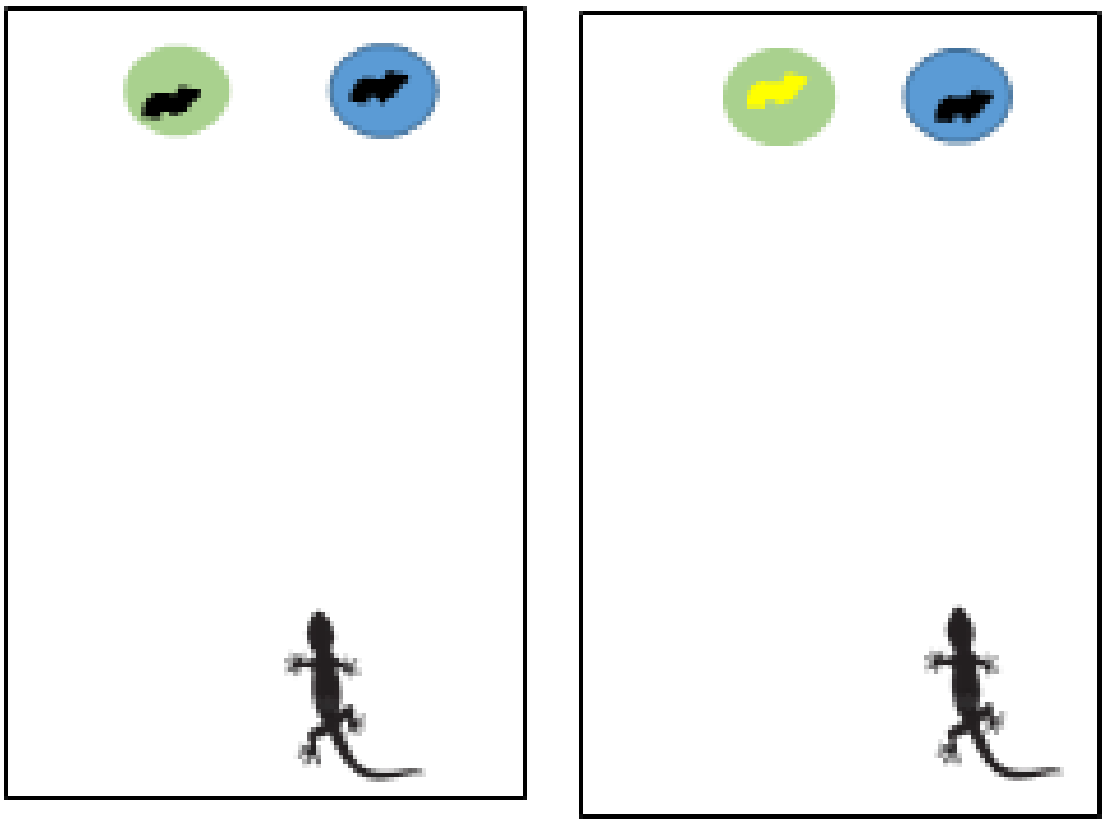


Figure 5. Individual performance of test subjects in reducing crop pests from high and low infested crop patches

c) Reptiles can associate taste with colour and show aversive learning. With non-conspicuous colours reptiles do not discriminate as long as the food quality is the same. However, if taste is manipulated, reptiles show rapid learning and start avoiding a colour that they have been conditioned to in previous trials. These results show the possibility of harnessing taste and colour association abilities in pest predators to modify prey preference thereby improving pest regulation efficiency (Fig 6, 7, 8).



Left: Colour association. **Right:** Taste discrimination. **Figure 6.** Schematic representation of colour and taste association. Lizards trained to eat food from green plate only were Left. made to choose food between green and unfamiliar blue coloured plate; Right. Familiar green colour plate offering unpalatable food altered colour association.

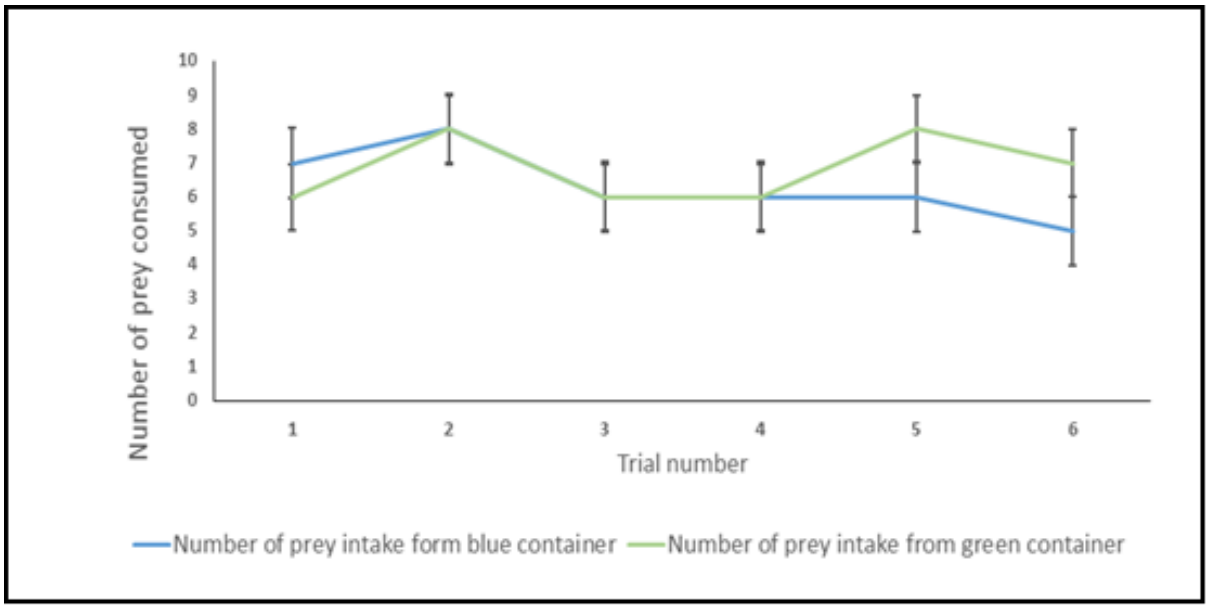


Figure 7. Graph showing more food is consumed from familiar green colour to which lizards were trained vera association take from blue coloured background.

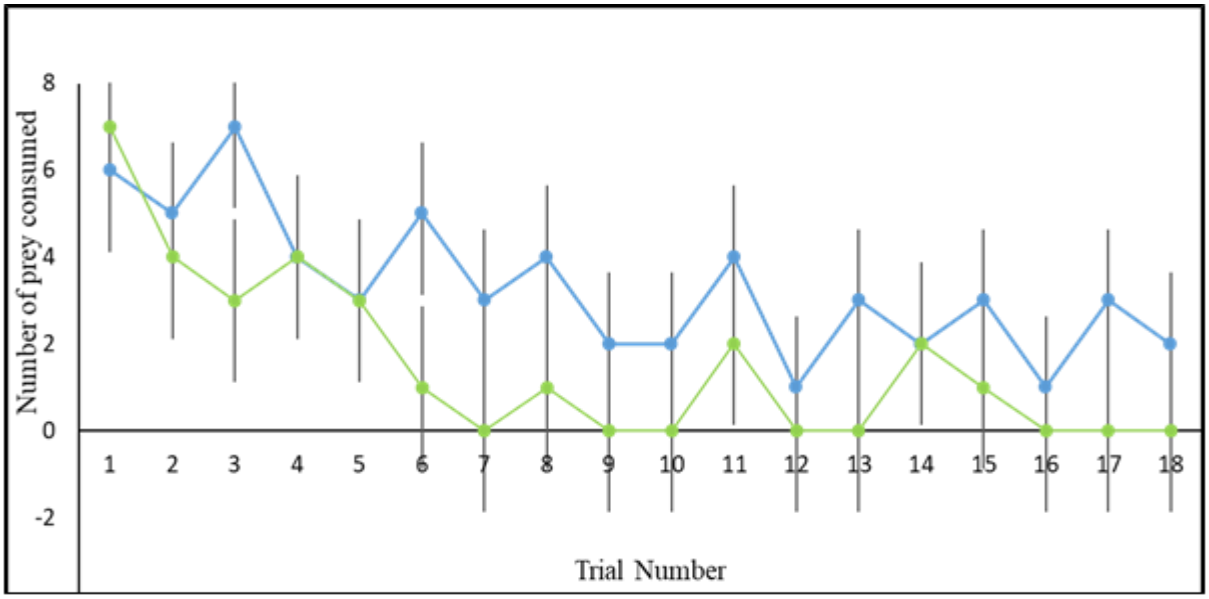


Figure 8. Graph showing altered colour association due to taste discrimination. *For general methods and statistics please refer to the detailed supplementary document.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

The project had a novel approach and is the first experiment conducted in vertebrates to explore possibilities of harnessing their cognitive abilities to improve their ecosystem service provisioning. The refinement of the methods and execution required a series of trial, testing and error before we could standardise the field-based protocol that can be replicated by other studies in future as well in our follow up projects. Most of the research done in the field of animal cognition specifically reptiles have been conducted in a laboratory set-up in controlled environments with captive-bred animals. Our project was the first ever attempt made to bring cognition to the wild in an implication-based study. The biggest challenges we faced were:

1. Collecting the animals from wild

This lizard is diurnal and extremely agile which presented the first difficulty of collecting them from the wild. We followed the **STRANGE** concept (proposed by Webster and Rutz, 2018) which is a new framework for animal behaviour research to help avoid sampling bias where STRANGE stands for **S**ociality, **T**rapability, **R**earing history, **A**cclimatisation, **N**atural history, **G**enetics and **E**xperience of the animals that can bias behavioural and cognitive studies.

Trapability ("T")

Stands for trapability and self-selection which could have biased our experiments due capturing animals which have trap bias. For example, the most used method for capturing diurnal reptiles like Oriental garden lizards is noosing. We did not use this method for the following issues which could definitely impact the psychology of the animals and their wellbeing: a. stressing the animals, b. incurring physical damage, and c. including those reptiles which are shy in nature rather than bold individuals. If these animals were included in our experiments the results would have been ambiguous. We had to use a unique method which allowed us to avoid the bias arising during trapping. We handpicked lizards while they were asleep on the grass blades at night. Though this method might give rise to other biases like thermal spot selection in individuals, still it was comparatively stress-free and did not incur any kind of physical damage to the animals.

2. Rearing history and Genetic makeup ("R" and "G" in STRANGE)

Another aspect that might have influenced the results is the lack of knowledge about their rearing history and genetic makeup. Since we worked with naïve reptiles collected from the wild, we do not have knowledge about their parental line or age. Age influences cognition. However, we tried to maintain a similar size class as in reptiles age positively correlates with size to negate the impact of age on their learning.

3. Acclimation and Habituation ("A" in STRANGE)

This was the biggest challenge while setting up the experiment. There is no record of its behaviour in captivity. Since our whole idea was to bring cognition study into the field unlike the laboratory-based work, we strongly aimed at maintaining the reptiles in outdoors. However, there is no record of space use and any antagonistic behaviour in social groups. We created a 1x1m arena and maintained five reptiles for 3 months as a trial period. However, with the clearance of the forest (to avoid forest fire) there was a high predation risk, and we lost the first captive batch.

4. Animal housing

We built a 3X3m arena of a height of 3ft from the ground to maintain the reptiles. The first issue was to recapture them for the experimental trials. This was stressful for the reptiles. Stress made them unwilling to perform in the task and impair their learning, food intake and disease resistance. The second issue was weather - unpredictable rains resulted in flooding of the arena. Hence, we had to maintain them in individual enclosures. This also prevented social learning in reptiles. We do not know yet if they learn from conspecifics, however, this might have impacted the outcomes of the project. They were habituated for minimum of 4-6 weeks. Habituation has an impact on learning and cognition. We allowed a standard time of 1-1.5 months to habituate to the housing conditions and during this time we provided food and water inside their enclosure. We made sure that they accepted food and water and were active. Every day they would get sunlight for 5 hours minimum. Since the whole project was to look at their pest regulation ability which essentially meant foraging, we needed the animals to stay active and willing to perform in the experiments. Below 1 month of habituation reptiles exhibited higher tendencies to escape which would influence their prey intake as their full energy was invested in finding an escape route. We suggest a hiatus of at least 1 month

before starting any experimental trials with the Oriental garden lizards. During the project reptile health and housing conditions were supervised by a veterinarian doctor (reports can be found in the appendix). We provided the lizards with diverse food items that included mealworms, crickets, and grasshoppers. The biggest challenge in food-related tasks is that animals lose the motivation to perform in captivity and if they are provisioned with the same food type. The floor of the enclosure was covered with paper to allow easy movement of the reptiles. Temperature of the housing area was maintained at 24 to 25.9 °C and humidity varied between 57 and 70%. In studies that deal with wild animals, bringing them to captivity and releasing them back to the wild, animal welfare should be kept as a priority. Our research, methods, and housing were approved by two ethical committees of two institutes, University of Lincoln and Ashoka Trust for Research in Ecology and the Environment.

5. Feasibility issue with the methodology

The other issue that we faced was to release the lizards in the experimental area and get them back again. These two events were stress-inducing which had a high probability of altering their behaviour and hence would have manipulated the results. While releasing them into the arena we left the enclosure open and allowed the animal to choose to enter the crop patches (built within the arena), the choice of which also is an outcome of our project. This reduced stress and improved their learning as they learned to choose a better vs poorer crop patch over time. If they did not enter the experimental arena on their own, we gently patted them below the head to orient them towards the crop patches. While getting them back we gently prodded them inside their enclosure. This was the most suitable and least stressful method which reduced handling time as well. The same challenge was faced during the experiment when we had to remove the crickets (crop pests from the arena).

6. Modifying the objective

During the whole course of the study, we could get only one gravid female (HOPE) from the wild. We maintained her in an enclosure with a flooring of vermiculite which would enable the female to lay eggs. However, after 4 months she did not lay eggs. Female Oriental garden lizards can store the sperm for 6 months only to fertilise the eggs in the next season. They can also cause embryonic diapause and prevent oviposition. The housing conditions were monitored by a vet. The whole idea of the project was to explore how climate warming can impact service provisioning by reptiles. We could not do the thermal manipulation as only one female was observed through the 1.5 years of the project and there was a lack of oviposition. However, we believe, irrespective of this difficulty, the project has addressed fundamental questions about the impact of cognition in the pest regulation role of reptiles and has generated several novel outcomes that have not been known thus far. The findings have given us food to think about for our future projects. It has advanced our knowledge, and the findings can be used to develop further projects on thermal manipulation and other aspects such as prey salient feature learning, discrimination, and climate warming could be affecting their learning. This study has been the first study that has brought out new and interesting aspects and generated more questions in cognition and conservation biology.

7. Loss of habitat for the species

We released all reptiles at the end of our study. All lizards were finally checked for parasite load or any other disease that they might have contracted while in captivity by a vet. Reptiles were marked with wearable non-toxic felt pens on the lateral sides of their body for post-release monitoring. However, we could not release the reptiles at their site of capture rather they were released to similar new sites close to their original habitats. The old sites were all claimed to be apartments and other human activities. This is a way to clearly indicate the amount of threat these lizards are faced with in semi-urban areas. We conducted a recce survey for potential release sites prior to release regarding the prey availability, predatory threats, and human disturbance (refer to the photographs in the detailed report). These sites were within 1 km of their original habitat and were similar in structure and composition.

4. Describe the involvement of local communities and how they have benefitted from the project.

The idea behind this was to integrate animal cognition into conservation of their ecosystem service provisioning. The main aim of the project was to introduce this concept to the scientific world. The outcome would be the acceptance and recognition of the concept. So far, our idea has been loved and appreciated. We received positive feedback from the farming community who will be the ultimate beneficiary from this study and other similar projects.

Participatory Rural Appraisal

Farmers' awareness campaign-

We organised a farmers' meeting in a community-based conservation centre of Ashoka Trust for Research in Ecology and the Environment. It was organised for 30 farmers from four villages Gorasane, Keeranahola, Konganuru and Haleyuru including "gram panchayat" (village head). The villagers belong to the Solega tribes.

The meeting was about how biodiversity in and around farmlands benefits us with a special focus on reptiles. We discussed some of the outcomes of our project where we show how reptiles discriminate between less and more pest-infested crop patches. This learning enables them to decide where to forage, and how long to forage which finally improves their pest consumption to two folds. My idea was also to pique their interest in the behaviour and cognition of reptiles their abilities and that animals should be conserved in their own rights. This is possible only if they see the benefit associated with an animal. Therefore, we started the meeting by discussing their thoughts and opinions.

Organised by – Deyatima Ghosh
Facilitated by – ATREE, Dr. Harish RP, Narayan Kumar
Native speaker- Dr. Harish RP (translated in Kannada)

The meeting started with engaging the farmers in an activity where we provided them with photos of arthropods, amphibians, reptiles, birds, and bats. Farmers had to

vote on which animal they believe is useful on the farm. This was an ice-breaking session where farmers interacted with each other and expressed themselves.

This was followed by a discussion where farmers were asked to explain their thoughts about the beneficial animals they chose to vote for. Farmers spoke about the bioregulatory role of animals and how that helps in reducing crop pests. Interestingly farmers voted amphibians as the most beneficial animal in farmlands.

We discussed with the farmers about the results and outcome of our project. We also suggested the economic benefit of having natural predators and why should we conserve them. We shared our experimental results. The photographs and content of the slides is attached in the supplementary document.

5. Are there any plans to continue this work?

Our work is the first-ever experiment done on vertebrate pest regulators in an attempt to harness their cognition to develop mitigation strategies for crop pests.
Now that we know reptiles are able to discriminate between different densities of crop patches and colour and taste can be important cues to alter prey specificity, we would carry this forward and explore avenues in which we can implement our findings. A follow-up project will also explore the remaining aspects of reptile cognition such as quality discrimination, discriminating between different salient features of crop pests such as colour, pattern, movement, and social learning. In the next phase, we will be training conspecifics, use robots and computerised visual cues to manipulate reptile cognition to influence and modify their role as pest predators.

6. How do you plan to share the results of your work with others?

A. Conference and talk at foreign university:
One of our outcomes from this project was selected by three international conferences where I have given talks online and in person. The conferences were:

i. Animal Behaviour Live.
ii. Association for Studies of Animal Behaviour (ASAB) winter meet.
iii. British Ecological Society.

1. The talk that I delivered at the Animal Behaviour live conference is available in the you tube and can be accessed through this link https://www.youtube.com/watch?v=GMDn8WNhSGU

Talk Title: Can biological pest regulation benefit from integrating predator cognition?

2. ASAB was an in-person conference in Edinburgh. My talk was selected among 150 talks across the whole of Europe. I received funding from ASAB of £2500 for the travel https://mailchi.mp/32622aed11bd/asab-newsletter-spring-2023?e=%5BUNIQID%5D

Talk Title: Exploring predator cognition to understand ecosystem service provisioning- a new approach to bioregulation of crop pests.

3. BES presentation has been appreciated by many behavioural ecologists and associate editors of a reputed journal who showed interest in our work and is willing to review the manuscript that was generated with the Rufford funding. I can anticipate that the networking I could do will result in potential collaborations in the future to advance our research ideas further.

Talk Title: Clever pest regulation? Patch learning can significantly improve pest consumption in wild *Calotes versicolor.*

4. I gave a talk at the University of Lincoln. The talk covered all the past three projects funded by Rufford and the current project. It was organised in the Department of Life Sciences, College of Sciences, University of Lincoln.

Organised by – Dr. Anna Wilkinson, Professor, University of Lincoln, UK.
Talk Title: Unsung heroes: Herps in tropical agroecosystems.

5. Invited talk. Unsung heroes: What do herps do for us?
Organised by - Cre'active

B. Publications:

Ghosh, D., John, E.A. & Wilkinson, A. Clever pest control? The role of cognition in biological pest regulation. Animal Cognition (2023). https://doi.org/10.1007/s10071-022-01731-4

Exploring cognition as a mitigation to crop pest. Commissioned article by Nature Communications (under prep. To be submitted)

Animal Behaviour Live: Conference abstract booklet

Deyatima Ghosh, Amarthya Chandar, Vikram Aditya 4, Aravind NA, Elizabeth A John, Anna Wilkinson. Can using the cognition of pest predators be the next step towards sustainable pest management? Submitted Npj Sustainable Agriculture.

Ready for submission:

Ghosh, D. et al. Bitter experience: Taste aversion improves non-conspicuous colour learning in reptiles.

Colour or position learning in reptiles and how that impacts pest regulation.

We expect a few more articles from the data we could collect-

1. Colour learning
2. Face recognition
3. Memory

Popular magazine articles-

Are we underestimating the reptiles in our farmlands? (Down to Earth, accepted for publication)

Understanding reptile intelligence can aid conservation and safeguard ecosystems (commentary): Mongabay

Killed in cold blood: Amphibians and reptiles are bearing the brunt of crop intensification: Down to Earth

Oriental Garden Lizards: Quick Learners or Bloodsuckers? Roundglass Sustain

C. Media coverage-

https://www.instagram.com/p/CoPUkozP_yE/?utm_source=ig_web_copy_link

https://www.instagram.com/p/CnoqbjLPZsV/?utm_source=ig_web_copy_link

https://www.instagram.com/p/CnDrjyNIcPk/?utm_source=ig_web_copy_link

Cre'Active: Invited talk: Unsung heroes: what do the herps do for us. https://www.youtube.com/watch?v=ycrN4Q3PxU4&t=1730s

Animals and their decisions 2023**–** Science talent search program, (live seminar) Organised by Karnataka Science and Technology Academy

Sakshi TV coverage

https://www.instagram.com/p/CnwdRu8veTl/?utm_source=ig_web_copy_link

https://www.linkedin.com/posts/atreeblr_reptiles-have-been-considered-to-be-less-activity-7022163340248440832-wi2o

D. Immersion programmes:

Students are an important part of any conservation programme. I have been delivering lectures on evolution of amphibians and reptiles and their role in the ecosystem as a part of my Teaching Assistantship for the course of "Biodiversity and Ecosystem Services". We organised a one day "immersion field visit" with the Masters' students in ATREE of the Conservation Practice course. They were exposed to animal cognition the underlying assumptions while we perform cognition study and how cognition in essential to conserve biodiversity and their service provisioning. They got trained hands-on on handling lizards and conducting experiments. We engaged them in discussion about the whole idea and gave them a brief of what we have been doing in the project which included our previous experiments on reptile colour learning, face recognition, memory etc.

7. Looking ahead, what do you feel are the important next steps?

The concept of utilising vertebrate cognition in pest management science is new. Biological pest regulation is heavily biased towards using insect pest predators. So far pest management has taken an ecological approach, and we suggest this field of science to be more inclusive. In the coming years we will be exploring non-invasive strategies based on manipulating cognitive traits of pest predators including birds, amphibians and bats.

We will harness social learning abilities in pest predators using model or robot conspecifics to attract individuals in the farmlands, use vibration to indicate location of pest infested crop lands, exploring decoy effect in manipulating the cognitive traits in pest predators, training conspecifics to a foraging technique or prey and releasing them back in the field to explore the diffusion of foraging strategies within the population.

A frustrating lack in up taking pest management strategy is a major reason why pest management doesn't have desired outcomes. As a remedy to this, we will be exploring non-invasive cost effect strategies that can be easily adapted by the farmers. We will encourage farmers to volunteer in our work and implement the pest management strategy.

A challenge in harnessing cognitive traits is the lack of information about the time of retention of any conditioning in animals and exploring avenues in which conditioning can have a longer persistence.

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

I have been attending international conferences in person as well as online and delivering talks at foreign Universities during the course of the project. In all talks, The Rufford Foundation logo has been used and Rufford has been acknowledged for the funding assistance not only for this project but the previous projects as well. In the farmers' meeting, The Rufford Foundation and the role it plays in conservation and community-based research was also explained. We also made our own merchandise in the form of t-shirts and caps for the interns and volunteers that had Rufford's logo.

9. Provide a full list of all the members of your team and their role in the project.

The following people have contributed to the project. Their names, current affiliations and their contributions are listed below.

Team leader- Deyatima Ghosh, Postdoctoral researcher
University of Lincoln
Ashoka Trust for Research in Ecology and the Environment

Collaborator- Anna Wilkinson, Professor
University of Lincoln
Anna has been involved in the conceptualisation phase of the project and refining the objectives over time.

Field work facilitated by-
1) Aravind N.A, Senior fellow (Professor)
Ashoka Trust for Research in Ecology and the Environment

2) UmaShankaar, Professor
School of Ecology and Conservation, GKVK

3) Vikram Aditya, Principal Scientist
Center for Wildlife Studies
Dr. Vikram, Dr. Aravind and Dr. Umashankaar helped with the land procuring, establishment of the field set up and maintaining the lizards.

Field work and data collection:
1) Amarthya Chnadar- Ashoka Trust for Research in Ecology and the Environment
2) Raksha More, Master student
3) Panchami Gaitonde, Master student
4) Chetana, Bachelors
5) Isha, Bachelors
6) Rajeev, Bachelors
7) Tushar, Bachelors
8) Arjun Kannan- Ashoka Trust for Research in Ecology and the Environment
9) Abhishek- School of Ecology and Conservation, GKVK
10) Harisha- School of Ecology and Conservation, GKVK
11) Chetana- School of Ecology and Conservation, GKVK

Crop and crop pest provisioning:
1) Kemparaju
Ashoka Trust for Research in Ecology and the Environment (rearing crop pests)

2) R Ganesh, Senior fellow (Professor)
Ashoka Trust for Research in Ecology and the Environment

3) Institute of Ayurveda & Integrative Medicine (I-AIM)

10. Any other comments?

Any work like ours needs to procure ethical clearance. This work has been conducted with ethical approval from the University of Lincoln and Ashoka Trust for Research in Ecology and the Environment. Animal safety and husbandry have always been given priority throughout all phases of the work including their release back into the wild. The study was conducted in the campus of Gandhi Krishi Vigyan Kendra (GKVK), in a plot that belonged to the School of Ecology and Conservation (SEC) Laboratory. Prof. Uma Shaankar, an Emeritus professor allowed us to use the plot and the whole team is grateful for his contribution to the fulfilment of the

project. Dr. Vikram Aditya has been crucial to the project and contributed significantly to the preparation of the plot construction of the enclosure and supporting the whole logistics of the experiment. Prof. Aravind Madhyastha has been involved in the refinement of the fieldwork. The whole team and I are grateful to all the people who volunteered for the work. This work is new, and every step has been a challenge especially due to the lack of any references on vertebrate cognition. The contribution of everyone including volunteers has been crucial to the completion of the project. The study generated more than 1000 videos that have all been coded by me and Amarthya Chandar using software BORIS over 7 months. This project is the baseline for any study and upcoming research intending to harness vertebrate cognition in pest management science. We thank The Rufford Foundation for funding this work, it contributed to developing the first steps towards a bigger project that we are conceiving towards refining our understanding and exploring the interface of animal cognition and ecology. We are keen to submit a second Booster grant project to explore other crucial aspects of cognition in reptiles that can add substantial information about pest management and strategies avenues in which farmers can be encouraged to volunteer.

General methods used for the study and the photographs could be found in the detailed supplementary document. Finally, the reports here are detailed and the work are either under review or under preparation.