

Final Evaluation Report

Your Details	
Full Name	Brinky Desai
Project Title	Monitoring stress physiology of mugger crocodiles (<i>Crocodylus palustris</i>) within a human-dominated landscape of central Gujarat, India
Application ID	36494-1
Date of this Report	December 2023

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
Development, validation, and standardization of a non-invasive method to measure faecal glucocorticoid metabolite (fGCM) concentrations in mugger crocodiles				We used 'restrain and capture' technique for biological validation of the selected hormone assay system (11-oxoetiocholanone enzyme immunoassay) for measuring faecal glucocorticoid metabolites (fGCM). We collected scats from a focal enclosure (20 females and two males) of captive crocodiles at Madras Crocodile Bank trust, Chennai, India for 15 days before the day of capture and for 15 days after the day of capture of the mugger crocodiles. There was an 11-fold increase in fGCM concentrations between pre- and post-capture samples, which was evident by the fGCM concentration as measured by the selected assay system. Thus, the assay was validated for measuring fGCM in mugger crocodile species.
Application of the developed tool to monitor the well-being of muggers, living under free-ranging conditions across diverse habitats within central Gujarat, India				The standardised and validated assay was then applied to the free-ranging populations of muggers during both the breeding and the non-breeding seasons. We sampled different mugger populations living across three diverse habitats or zones. Zone A (N=5 sampling sites; Figure 1) within Charotar area of Gujarat, a rural area with local people having a high tolerance towards co-existing muggers, which mostly live in ponds or lakes and face low or no incidents of HMC; zone B (N=4 sampling sites; Fig 1), an urban belt within Vadodara with high HMC and having several chemical industries disposing of sewage in the rivers (for example, Vishwamitri river)

				that are predominantly mugger habitats; and zone C (N=4 sampling sites; Figure 1), a rural part within Vadodara having mostly agricultural lands with a high dependency of local people on adjoining rivers (for example, Vishwamitri river) that hold a significant mugger population (Fig. 1), and thus, having high HMC. Vishwamitri River cuts across both zones B (upstream sites) and C (downstream sites) (Figure 1), and is a predominant mugger habitat

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

a). Standardisation and validation of a non-invasive method to measure fGCM concentrations in mugger crocodiles: Our research project biologically validated an assay, targeting glucocorticoid metabolites having 5 β -3-ol-11-one structure (11-oxoetiocholanolone EIA), for mugger crocodiles by showing an 11-fold in fGCM levels between the pre-and the post-capture phases of the captive muggers (Figure 2).

b). Application of the validated assay to free-ranging populations across diverse habitats: Our study also demonstrated that fGCM levels were significantly different between Charotar (Zone A) and Vadodara (Zone B and C), and this contrast in physiological stress levels between the two habitats was similar during both the breeding and non-breeding seasons of the mugger crocodiles (Figure 3).

c). Compare and contrast results between captive versus free-ranging populations: Our study showed that the fGCM levels of pre-capture (captive) and Charotar region (Zone A) were similar. The Vadodara (Zone B and C) region showed four times higher fGCM levels in comparison to pre-capture as well as the Charotar region.

Looking at the results we speculate that the immediate local environment here plays an upper hand in influencing fGCM levels than the reproductive biology of the individuals. Moreover, there were no differences in fGCM levels between pre-capture and Charotar region, thus, indicating that the Charotar habitat might be less challenging than Vadodara and have conditions similar to captivity, which reflect a controlled environment providing good nourishment to the mugger individuals.

This is the first study that has non-invasively monitored fGCM levels in a free-ranging crocodilian species. Further, we showed that levels of fGCM, the stress indicating hormone, is contrastingly different in two habitats (Charotar and Vadodara, which are just 40 km away from each other), possibly due to differences in several ecological and environmental conditions between Charotar and Vadodara.

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

During our biological validation we could not collect any scats from male individuals this was due to the skewed sex ratio in the enclosure and moreover, one of the males never came out of the water during our study period.

We had proposed to collect data for breeding as well as non-breeding seasons across the three zones. Non-breeding season in our study area coincides with monsoon time, which made the majority of the scat collection sites inaccessible due to flooding. Hence, we have a smaller sample size for the non-breeding season when compared to the breeding season data.

One of the methods that we had proposed was to measure scat diameter to avoid pseudoreplication. However, this could not be achieved as the muggers already squashed the majority of the scats at the sampling sites. So as an alternative, we tugged the sample, post-collection, with sand to avoid confusion during the next round of sampling at the same site.

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

We employed a local student on a monthly stipend, and the student was based in the Charotar region. We trained him in data collection and fieldwork on mugger crocodiles. Apart from this, we met with all the village heads across all the sampling sites to inform them about our research project and gave them regular updates on the progress of the project.

5. Are there any plans to continue this work?

Yes, we plan to do long-term research work focusing on focal individuals using behaviour (reproductive and basking behaviours) and physiological (testosterone, progesterone, and thyroid hormone) readouts to better understand the impact of different habitats on the biology, and in turn, on the adaptation strategies of the mugger crocodiles. As a part of the future project, we also plan to share our research work with local schools and colleges in our study area through developing focused awareness programs about the mugger crocodile species, which live in close association with the human settlements in most parts of our study area.

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

We have submitted our work as a research article in the Conservation Physiology Journal, Oxford University Press, which will be shared with local non-profit organizations and the forest department.

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

As mentioned, the next step will be to expand the project and involve more readout including abiotic factors (water and soil quality analysis), behavioural (basking behaviour) and nutritional (thyroid hormone concentrations) measures and quantifying anthropogenic disturbances to have a more in-depth understanding of the impact of habitats on the behaviour and physiology of the mugger crocodiles.

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?

Yes, while applying for permissions to the forest department and sharing our regular updates with them we have mentioned our funding and support from The Rufford Foundation

We have also acknowledged The Rufford Foundation in the article that originated from the work and is now submitted to the Conservation Physiology Journal (CONPHYS-2023-120).

Desai B, Bhowmik T, Srinivasan R, Whitaker N, Ghosal R. Monitoring stress physiology of free-ranging mugger crocodiles (*Crocodylus palustris*) across diverse habitats within central Gujarat, India, Manuscript under review, Conservation Physiology Journal.

Apart from this, the results of the project have been presented as oral presentations at two international conferences (Figure 4):

Desai B, Ghosal R. Understanding ecological adaptation of a large reptilian species, marsh crocodiles, through a multidisciplinary lens. 59th Annual Meeting of the Association for Tropical Biology and Conservation, 2-6 July 2023, Coimbatore, India.

Desai B, Bhowmik T, Srinivasan R, Whitaker N, Ghosal R. Non-invasive monitoring of stress-indicating hormone in mugger crocodiles (*Crocodylus palustris*) across diverse habitats. 8th Conference of the International Society of Wildlife Endocrinology, 6-10 November 2023, Jim Corbett National Park, India.

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

Team member and their roles:

Dr. Ratna Ghosal (Supervisor): Co-executed the project and provided all the supervision required during the process. Further, a separate grant, which was awarded to her, was used to support the additional costs during the project.

Tathagata Bhowmik (Ph.D. student): Assisted with free-ranging data collection, lab work, and paper writing.

Nirali Panchal (master's student): Contributed during the initial phase of proposal writing and acquiring lab material.

Rohith Srinivasan (master's student): Assisted with free-ranging data collection.

Nikhil Whitaker (Zoo curator): Assisted with captive work at the zoo.

Ronak Trivedi (master's student from Charotar): Assisted with free-ranging data collection.

10. Any other comments?

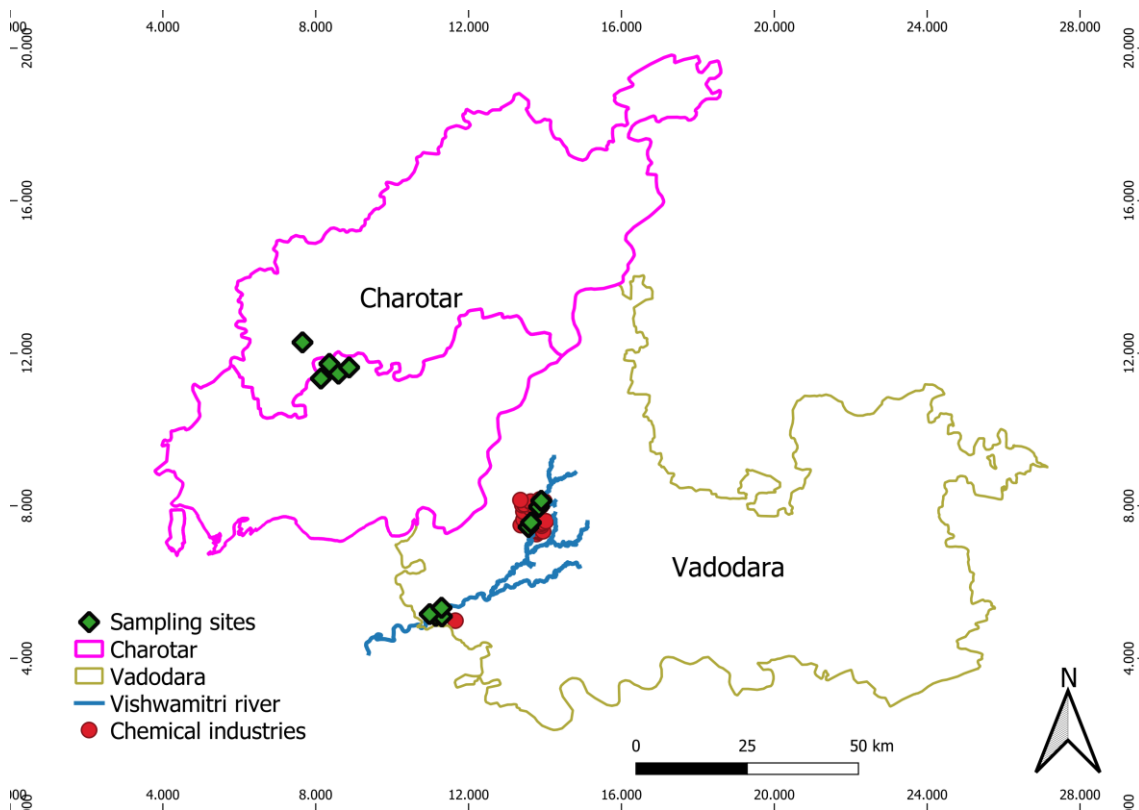


Figure 1: Map represents Charotar (with pink border) and Vadodara (with brown border) regions within the state of Gujarat; zones A, B and C within the Charotar and Vadodara regions, and the sampling sites within the three zones, and the locations of chemical industries within 5 km radius of each of the sampling sites.

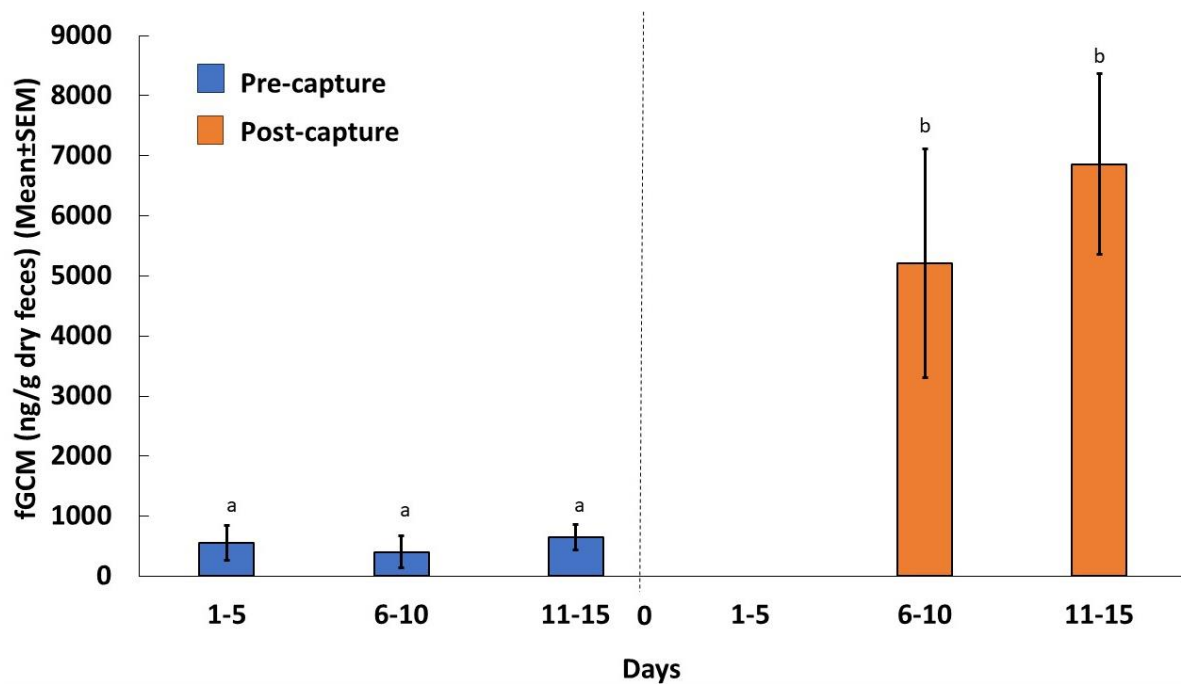


Figure 2: Levels of fGCM (Mean±SEM, ng/g dry faeces) in captive muggers during the biological validation study. Different alphabets represent significant differences ($P \leq 0.05$) (We considered day 0 being the day of capture and plotted the fGCM concentrations for 1-5 days (N=3 scats), 6-10 days (N=3 scats) and 11-15 days (N=5 scats) before capture. We followed a similar interval for the post-capture phase, however, we could not collect any scats for the 1–5-day time period, whereas 6-10 day and 11–15-day time periods had a sample size of 4 and 7 scats.)

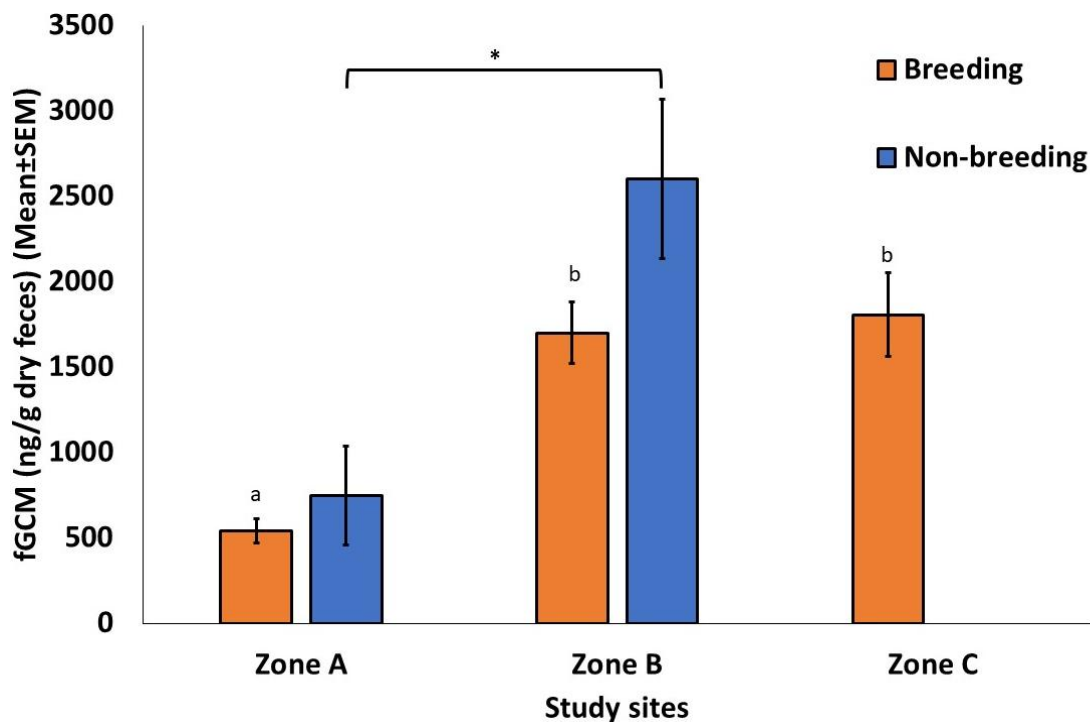


Figure 3: fGCM concentrations (Mean±SEM, ng/g dry faeces) of free-ranging mugger crocodiles during breeding and non-breeding seasons across the three

zones, A, B and C. Different alphabets and asterisks indicate significant differences ($P \leq 0.05$) across or between zones for breeding and non-breeding seasons, respectively.



8th Conference of the International Society of Wildlife Endocrinology



59th Annual Meeting of the Association for Tropical Biology and Conservation

Figure 4: Conference presentations.