

Evaluating Environmental DNA for Monitoring Siamese Crocodiles

Applying novel eDNA approaches for monitoring of a critically endangered top predator to support reintroduction efforts

Project Lead
FISHBIO

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Project Contact

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Collaborators

Angkor Wildlife and Aquarium, Fauna and Flora International (FFI), NatureMetrics, Jonah Ventures, Rising Phoenix, Young Eco Ambassadors (YEA)



FISHBIO staff collecting a DNA sample from a Siamese crocodile at Angkor Wildlife and Aquarium.

Why Are We Sharing This?

As multiple efforts are working to reintroduce Siamese crocodiles throughout their historic range in Cambodia, effective monitoring will be critical for evaluating the persistence and distribution of reintroduced populations. The novel eDNA approaches being developed and tested by this study are being evaluated as a potential tool for organizations working to conserve and recover this keystone predator.

Project Background

Siem Pang Wildlife Sanctuary is a protected area that encompasses over 130 thousand hectares in northeastern Cambodia. Historically, the wetland pools and streams of this region were home to a population of Siamese crocodiles, but over the twentieth century, populations of this species in Cambodia dwindled due to hunting and human disturbance. By the 1990s it was believed Siamese crocodiles were extirpated from Cambodia, and it wasn't until the year 2000 that Siamese crocodiles were confirmed to have persisted in the country. However, surveys from 2000–2014 yielded only sporadic confirmed sightings in the Siem Pang region. As of 2022, some researchers believe that crocodiles have been fully extirpated from Siem Pang.

FFI is leading the effort to establish a Siamese crocodile reintroduction program in Cambodia, and they recently completed their largest release of captive-reared Siamese crocodiles into the wild in the Cardamom Mountains. Simultaneously, FFI has been working with the U.K.-based laboratory NatureMetrics to develop eDNA methodology for the detection of Siamese crocodiles, and they have been implementing eDNA sampling in their release sites. In an effort to expand reintroduction, FFI has shared their expertise and experience with other organizations also working to conserve the species. One of these organizations is Rising Phoenix, which released 15 Siamese crocodiles into a “soft-release” enclosure in a wetland pool in Siem Pang in March of 2022. This release enclosure is intended to contain the crocodiles for the remainder of the dry season, and help them to acclimate to the new habitat before rising water levels in the wet season will allow them to disperse from the pond.

In an effort funded by the Wonders of the Mekong Project, FISHBIO has collaborated with the Rising Phoenix ranger team to collect eDNA samples from locations throughout the Siem Pang Wildlife Sanctuary where extant crocodile populations may have persisted, as well as from the release site. Collecting samples from the release site at varying distances from the enclosure and over an extended period of time provides a means of evaluating the likelihood of crocodile eDNA detection, and is intended to inform the development of best practices to maximize the likelihood of detection in natural habitats.

Key Points

- This project seeks to **apply eDNA technology to detect and monitor** rare Siamese crocodile populations
- Reintroduction efforts in Siem Pang Wildlife Sanctuary have provided **an opportunity to test new eDNA primers** to evaluate their effectiveness at detecting Siamese crocodiles
- Collaboration and data sharing with FFI is **extending evaluations of Siamese crocodile eDNA methodologies throughout the species' range** in Cambodia, including the Tonle Sap and Cardamom Mountain regions
- Initial results for positive control samples collected within a release enclosure suggest **Siamese crocodiles may shed too little DNA into their environment** to be readily detected with eDNA methodologies
- Project partners will work together to interpret results and help **improve understanding of the tools that may be most effective for monitoring** of extant and reintroduced Siamese crocodile populations

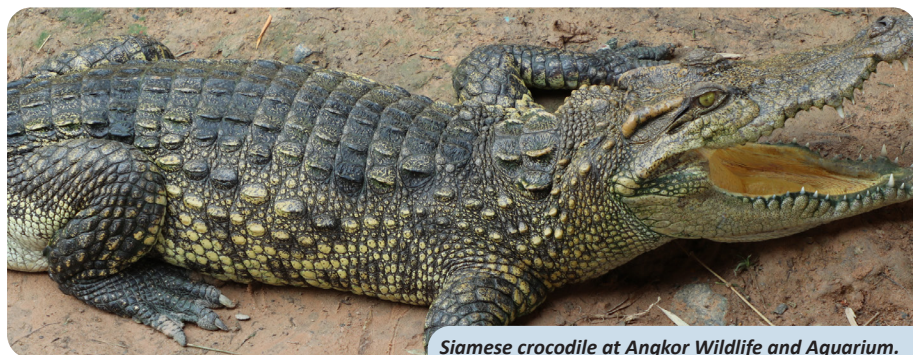
Objectives

The objectives of this study are to 1) evaluate the effectiveness of newly developed Siamese crocodile eDNA primers by collecting samples at varying distances from a soft-release enclosure containing 15 Siamese crocodiles, and 2) to sample the watershed in Siem Pang for Siamese crocodile and thereby test for the presence of an extant population in the Wildlife Sanctuary.

Methods

In the spring of 2022, Rising Phoenix rangers collected eDNA samples from streams throughout Siem Pang Wildlife Sanctuary. The streams that were sampled in the region were the O'Khampha, O'Tanel, and Tin Hiang rivers, all of which are tributaries of the Sekong River. Water samples were collected, processed through 5µm filters, and preserved with a specially formulated solution using kits provided by U.S.-based laboratory Jonah Ventures. In total, 26 samples were collected from various locations around the Sanctuary (Table 1). Following the introduction of 15 Siamese crocodiles to a soft-release enclosure in a wetland pool in the Sanctuary, an additional 14 samples were collected to serve as positive controls (Table 1). These samples were collected both immediately adjacent to the enclosure and at the furthest downstream point of the pool over a period of three weeks following crocodile introduction. The intention of this sampling is to develop an understanding of how long crocodiles must be in a location for their eDNA signal to be detectable, and how close to the crocodiles the samples must be collected in order to achieve effective detection. Data on both of these factors will help to improve best practice for eDNA sample collection in future studies.

Next steps for the application of Siamese crocodile eDNA methodologies will be developed collaboratively, and the feasibility of including eDNA in long-term monitoring associated with ongoing reintroduction efforts will be considered.



Siamese crocodile at Angkor Wildlife and Aquarium.

Table 1. The locations of eDNA samples collected in the spring of 2022. Initial results found no Siamese crocodile DNA in any of the samples, even those collected within and adjacent to the soft-release enclosure in Boueng Nava, which contained a total of 15 Siamese crocodiles.

Location	Samples Collected
O'Tanel River	2
O'Khampha River	16
Tin Hiang River	8
Soft-Release Enclosure (positive control experiment)	14
Total	40

Initial Results

The collected samples recently underwent analysis at the Jonah Ventures eDNA laboratory, and initial results indicate that more work is needed to understand the utility of eDNA for Siamese crocodile monitoring. Although the genetic primers effectively detected known DNA samples collected from captive Siamese crocodiles at Angkor Wildlife and Aquarium, none of the environmental samples collected from the wild or from the release enclosure resulted in detection of crocodile eDNA. This may be due to the species' keratinized scales or infrequent defecation, which consequently lead to very low DNA shedding rates of crocodiles, a challenge that has been noted in eDNA studies of other crocodilian species. Further research is necessary to evaluate potential means of improving detection (such as filtering larger volumes of water).



The release enclosure while under construction in Boueng Nava in Siem Pang Wildlife Sanctuary.



Preparing to collect DNA from a Siamese crocodile.