

## *Research Notes...*

# Seychelles and its Key Role in Developing Novel Methods for Identifying Causes of Hatching Failure in International Turtle and Tortoise Species (working title)

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## Purpose of the project

The global decline of turtle and tortoise species is alarming: The International Union for Conservation of Nature (IUCN) Red List of Threatened Species™ ([www.iucnredlist.org](http://www.iucnredlist.org)) classified nearly 60% as ‘threatened’ (i.e., vulnerable to endangerment in the near future), of which 20% are ‘critically endangered’ (Rhodin et al., 2018). Despite many populations benefiting from dedicated conservation programmes, reproductive failure is a significant obstacle to population recovery. Many populations suffer failure at the egg phase of reproduction, where high levels of hatching failure have been observed (e.g., Bell et al., 2003; Sinaei and Bolouki, 2017; Lovich et al., 2018). Hatching failure can result from (a) fertilisation failure or (b) embryo death – two underlying issues with very different causes.

Seychellois master’s degree student, Alessia Lavigne, became fascinated by the problem of hatching failure in turtles and tortoises during her undergraduate study at the University of Sheffield. One of her lecturers, Dr Nicola Hemmings, had developed microscopy-based methods to discriminate between fertilization failure and embryo death in bird eggs, even at the earliest stages of development (Assersohn et al., 2021). From her background in turtle conservation in Seychelles, Alessia already knew how challenging it was to identify the causes of hatching failure in undeveloped turtle and tortoise eggs and recognized this could be an important tool for management. Seeing the value of this work, she suggested adapting the methods for turtles and tortoises, and this was how an international master’s research project began.

Dr Hemmings is now Alessia’s supervisor, and they have set out to develop new methods for analysing failed, unhatched eggs that allow them to accurately diagnose true fertility problems (e.g., transfer failure or sperm production) and issues with early embryonic survival (e.g., unfavourable incubation conditions or parental genetic incompatibilities) in turtles and tortoises. Understanding the causes of hatching failure using these methods will empower

conservation programmes, providing vital information on specific reproductive issues in managed populations. It will also help improve the efficiency of management interventions in both cost and time – both of which can be very limited in the world of conservation.

## International collaboration and recognition

The potential of this innovative study has been recognized by several conservation programmes on an international scale. The project is supported by the British and Irish Association of Zoos and Aquariums (BIAZA), of which four members are contributing samples from their UK captive breeding programmes. Unhatched eggs will be collected from ZSL Whipsnade Zoo (Spiny turtle – *Heosemys spinosa*); Crocodiles of the World (Galapagos tortoise (exact species to be confirmed) and Red-footed tortoise – *Geochelone carbonaria*); Birmingham Wildlife Conservation Park (African spurred tortoise – *Centrochelys sulcata*); Durrell Wildlife Conservation Trust (Radiated tortoise – *Astrochelys radiata*). An additional three conservation bodies in the Seychelles islands (Nature Seychelles, Save Our Seas Foundation and the Fregate Island conservation team) will also be contributing failed eggs from wild populations of Hawksbill turtles (*Eretmochelys imbricata*).

## Seychelles' role and benefits from the study

Seychelles has some of the world's longest sea turtle monitoring programmes, such as that on Cousin Island which has been in operation for the last 50 years. Moreover, Seychelles has documented a consistent recovery pattern for both hawksbill and green turtles (*Chelonia mydas*), with up to an 800% increase in clutches over 40 years of protection (Mortimer et al., 2020). This means that a relatively large sample of turtle nests are expected to be available for the study.

During the decades of turtle protection in Seychelles, tagging of turtles has also been part of the conservation efforts, enabling studies to observe inter-island nesting and nesting frequency of individuals per island (Allen et al., 2010). This information has guided the choice of islands on which to study turtle hatching failure and allows the researchers to account for confounding factors such as non-independence of eggs from the same mother. Additionally, Seychelles partners are crucial to the study as a source of wild samples for comparison against captive zoo samples from the BIAZA partners. Seychelles is an exceptionally good site for Hawksbills as it hosts one of the five largest known populations in the world, and has islands such as Cousin which has long been known as one of the most important rookeries on the planet (Hitchins et al., 2004)

As well as being a key contributor, Seychelles will also benefit from the study. Comparisons can be made between the Inner and Outer Islands of Seychelles with respect to fertility rates, to understand whether human activity could be a significant factor driving egg failure. Since tourism is very active in Seychelles, it is important to understand these possible issues and strategize immediate solutions. The Seychelles Hawksbill population stands to benefit greatly from these new methods; a recent study by Gane et al. (2020) has reported negative trends such as decreasing clutch sizes and increased infertility rates. In Gane et al.'s study, eggs were classified as infertile when the 'contents contain no visible embryo of any size', but development of the embryo might have ceased before it was visible. Therefore, this method of defining infertility (often used both in Seychelles and globally) is likely to overestimate the number of infertile eggs, as it cannot distinguish the earliest stages of embryo development from fertilization failure. This is where this new project comes in. Being able to discriminate between unfertilized eggs and fertilized, undeveloped eggs is essential for making more efficient and informed management decisions to mitigate hatching failure. Time and money can be saved by investing in solutions to known reproductive problems, rather than basing decisions around assumptions. Also, conservation bodies searching for funding to address hatching failure issues can build a convincing case to possible investors, backed by scientific evidence as to what is going wrong and what is needed to address the problem.

To conclude, the Seychelles' extensive long-term turtle and tortoise population records, relatively healthy turtle clutch numbers, and strong existing conservation management programmes make it an ideal location for studying hatching failure in the wild populations of the critically endangered Hawksbill turtle. The inclusion of Seychelles in this international partnership-based project will also provide a unique opportunity to share insights from a variety of species and locations, ensuring wider applicability of the techniques and the opportunity to highlight Seychelles' scientific potential at an international level (e.g., BIAZA research conference). Furthermore, the Seychelles' samples will enable important comparisons between captive and wild populations.

## Expected duration and outcomes

Collections of eggs are taking place as they become available during 2021/2022. Turtle egg collection kits with detailed protocols have been sent to conservation partners, and once the samples have been collected, they will be shipped to the UK for laboratory examination at the University of Sheffield. The egg examinations and a complete report on the project findings are due to be completed in August 2022, and data on fertility and sperm numbers obtained from analyses will be returned to the relevant conservation managers for their records. This report will form part of Alessia Lavigne's masters project and will be developed into a paper suitable for submission to a scientific journal in due course.

Alessia and Nicola hope to extend this research to a PhD project and are actively seeking and welcoming funding opportunities to enable this. Several of the current conservation partners have also expressed their interest not only to continue their collaboration, but to expand the array of turtle/tortoise species and even include other reptile species. A PhD project would provide the extra time and resources required to optimize the methods over a wider range of species, thereby benefiting the management of global populations of reptiles in the wild and captivity. Additionally, the work would be expanded to include endangered green sea turtles and vulnerable Aldabra giant tortoises (*Aldabrachelys gigantean*) in Seychelles. Studying green sea turtles would provide particularly important benefits for the country due to the urgency in revitalizing the numbers of this species in the more populated Inner Islands. Green sea turtle population numbers have declined so severely from historic levels that they are now considered to be on the verge of local extinction (*Republic of Seychelles: Ministry of Agriculture, Climate Change and Environment*, 2017). Conservation bodies have also reported that some Aldabra giant tortoise populations in Seychelles are suffering a large proportion of unhatched eggs that appear to be unfertilized, highlighting the need to investigate the underlying reproductive problems more closely.

In summary, Seychelles, alongside BIAZA, is playing a crucial role in efforts to develop new methods of studying turtle/tortoise eggs that can help answer challenging questions concerning failed eggs – an increasingly worrying concern in many populations. Although there are several global monitoring and captive breeding programmes, the recovery of many species is being hindered at the egg phase of reproduction. The methods being developed by this ongoing project hold great potential for elucidating the causes of hatching failure and providing important information that can equip conservation bodies with the knowledge they need to address reproductive problems. With the use of this new method, scientifically backed decisions can be made that can increase the hatching success of turtle and tortoise species around the world, in both captive and wild conditions.

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