

# Risk Assessment and Pathogen Surveillance in Endangered Freshwater Turtle Species in Hong Kong

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Beale's eyed turtles (*Sacalia bealei*)



Chinese three-striped box turtles (*Cuora trifasciata*)

(Photo Credit to Adam Francis; Hong Kong Snake ID)

## Background

Asia is a hotspot for freshwater turtles' biodiversity and endemism. They contribute to the continuity of the ecosystem through soil bioturbation, nutrient cycling, and seed dispersal. However, according to the IUCN Red List, over 60% of freshwater turtle species in the world are threatened with extinction [3]. This project studied the pathogen prevalence of captive and wild populations of two critically endangered turtle species endemic to south-eastern China: Beale's eyed turtle (*Sacalia bealei*) and the Chinese three-striped box turtles (*Cuora trifasciata*) in Hong Kong [3]. Both *S. bealei* and *C. trifasciata* are facing serious poaching activity and habitat loss in Hong Kong [2]. *S. bealei* can be hardly found in the wild and *C. trifasciata* is virtually extinct in the wild.

Two largest conservation organizations in Hong Kong, Ocean Park Conservation Foundation (OPC) and Kadoorie Farm & Botanic Garden (KFBG), aim to boost the wild populations of these two turtle species through ex-situ breeding programs. In 2021, the Zoological Society of London (ZSL) conducted a disease risk analysis on *C. trifasciata* and identified various infectious disease hazards which should be taken into consideration when planning a reintroduction [1]. Thus, this project aimed to evaluate the prevalences of four medium- and low-risk potential chelonian pathogens identified by ZSL, as well as animal and environmental factors, in captive populations which may contribute to future reintroduction and in wild populations.

## Objectives

- Objective 1:** Evaluate the prevalences of potential pathogens (Paramyxoviruses, Ferlavirus, *Entamoeba invadens*, *Chlamydia* spp., *Mycoplasma* spp.) in captive and wild populations of endangered freshwater turtles in Hong Kong
- Objective 2:** Evaluate the water quality of captive and wild locations.

## Hypotheses

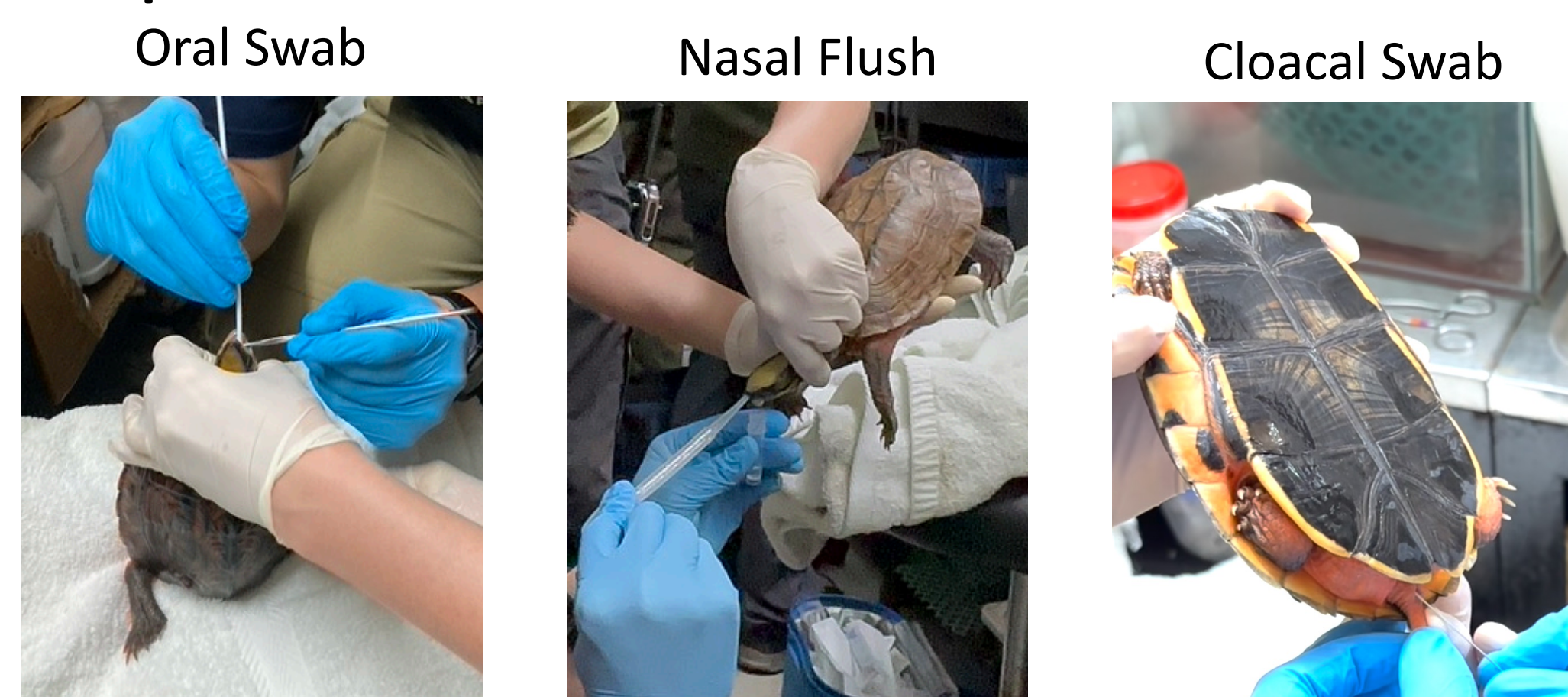
- Hypothesis 1:** Paramyxoviruses (including Ferlavirus) and *Entamoeba invadens* are absent in captive and wild populations of endangered freshwater turtles in Hong Kong.
- Hypothesis 2:** *Chlamydia* spp. and *Mycoplasma* spp. are present in captive and wild populations of endangered freshwater turtles in Hong Kong.

## Methods

### Locations

- Captive *C. trifasciata*: Kadoorie Farm & Botanic Garden (Lam Tsuen, Hong Kong)
- Captive *S. bealei*: Ocean Park Corporation (Aberdeen, Hong Kong)
- Two undisclosed wild locations surveyed by Dr. Yik-Hei Sung (Lingnan University)

### Sample Collection



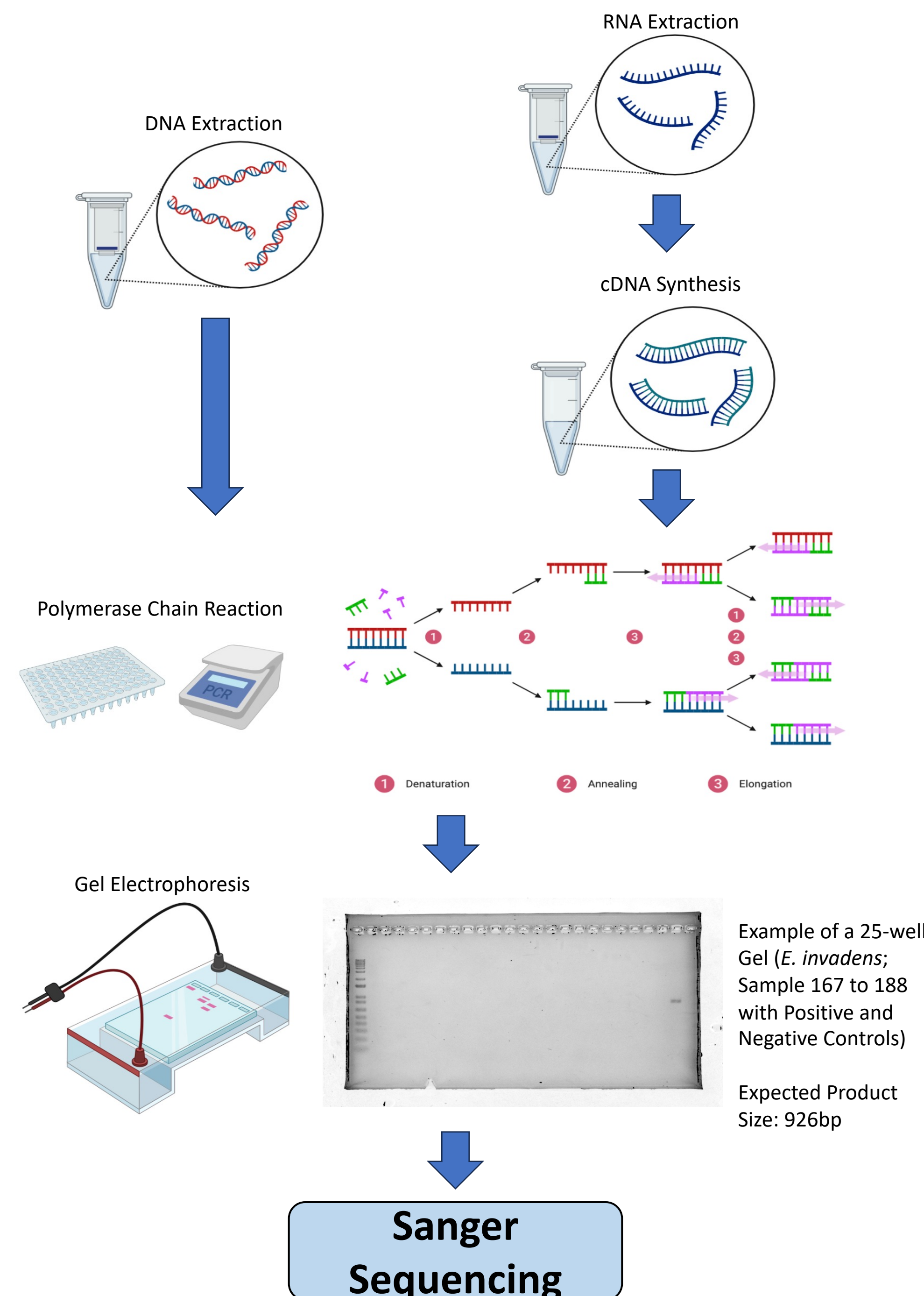
### Other Data Collected:

- Curved Carapace Length and Width (cm)
- Curved Plastron Length and Width (cm)
- Mass (g)
- Age (if known)/ Age Group
- Sex (if known)
- Health Condition
- Water Sample (250mL)

## Methods (continued)

### Laboratory Analysis

(University of Hong Kong, School of Public Health)



### Water Quality Analysis

(Environmental Laboratory, Ocean Park Corporation)

- pH
- Turbidity (NTU)
- Conductivity ( $\mu\text{S/cm}$ )
- Total Ammonia (ppm)
- Nitrite (ppm)
- Nitrate (ppm)

## Preliminary Results

### Sample Size (N): 95

- 60 Captive *C. trifasciata*
- 24 Captive *S. bealei*
- 7 Wild *S. bealei*
- 4 Wild *Platysternon megacephalum* (Big-headed turtle)



Photo of wild *P. megacephalum* (Photo Credit to Adam Francis; Hong Kong Snake ID)

Site	n	Age (Adult:Juv)	Sex (M:F:Unk)	Mean Juvenile Weight $\pm$ SE (g)	Mean Adult Weight $\pm$ SE (g)
KFBG	60	17:43	7:7:46	148.16 $\pm$ 23.43	926.35 $\pm$ 62.92
OPC	24	20:4	12:11:1	182.15 $\pm$ 29.86	362.62 $\pm$ 17.56
Wild	11	5:6	1:4:6	74.92 $\pm$ 12.26	279 $\pm$ 12.59

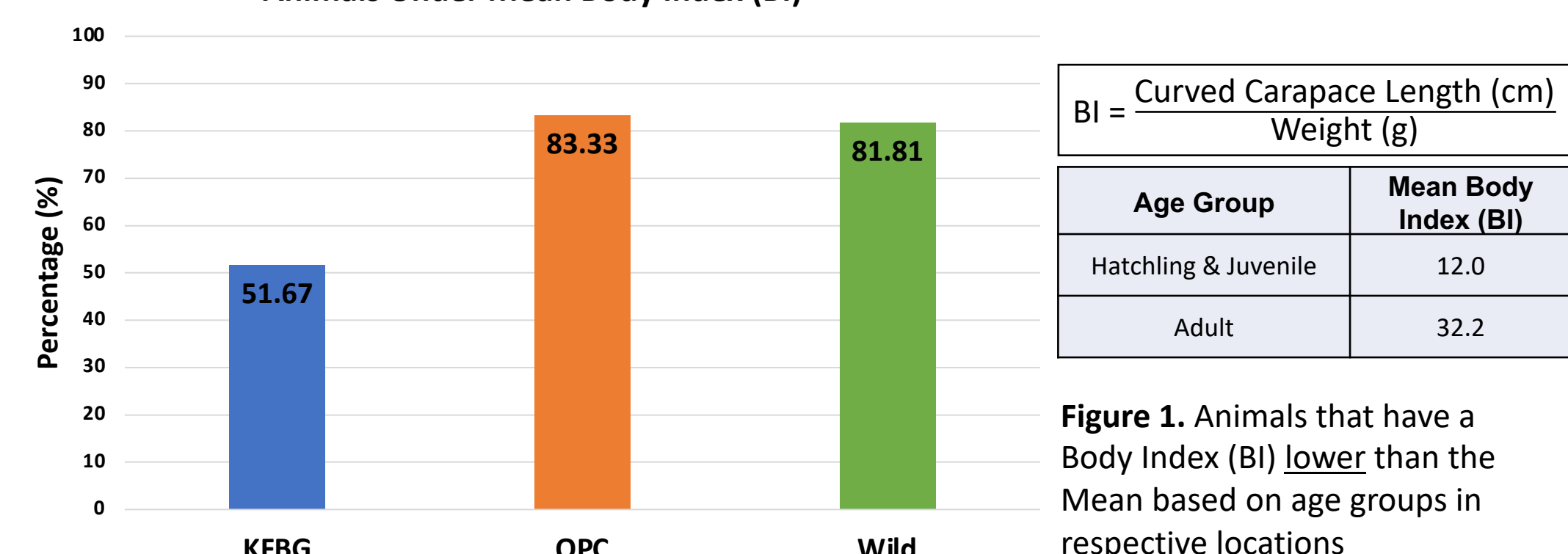
Table 1. Sample sizes, age ratio, sex ratio, and mean weight for turtles sampled at KFBG, OPC, and in the wild

Site	Age Group	Mean Body Index $\pm$ SE
KFBG	Juvenile	11.92 $\pm$ 1.19
	Adult	45.04 $\pm$ 2.61
OPC	Juvenile	14.9 $\pm$ 1.71
	Adult	23.93 $\pm$ 0.80
Wild	Juvenile	8.78 $\pm$ 1.29
	Adult	20.22 $\pm$ 1.49

Table 2. Mean body index based on age group in each location

### Body Index (BI) Analysis

Animals Under Mean Body Index (BI)



$$BI = \frac{\text{Curved Carapace Length (cm)}}{\text{Weight (g)}}$$

Age Group	Mean Body Index (BI)
Hatchling & Juvenile	12.0
Adult	32.2

Figure 1. Animals that have a Body Index (BI) lower than the Mean based on age groups in respective locations

### Potential Pathogens Results

- Paramyxoviridae: Negative
- Ferlavirus: Negative
- E. invadens*: Negative
- Chlamydia* spp.: Negative
- Mycoplasma* spp.: Pending

### Why is it still pending?

Primers used in initial PCR testing for *Mycoplasma* spp. were NOT specific enough to detect only *Mycoplasma* spp. Results from Sanger sequencing revealed multiple bacteria species were amplified.

## Preliminary Results (continued)

### Water Quality

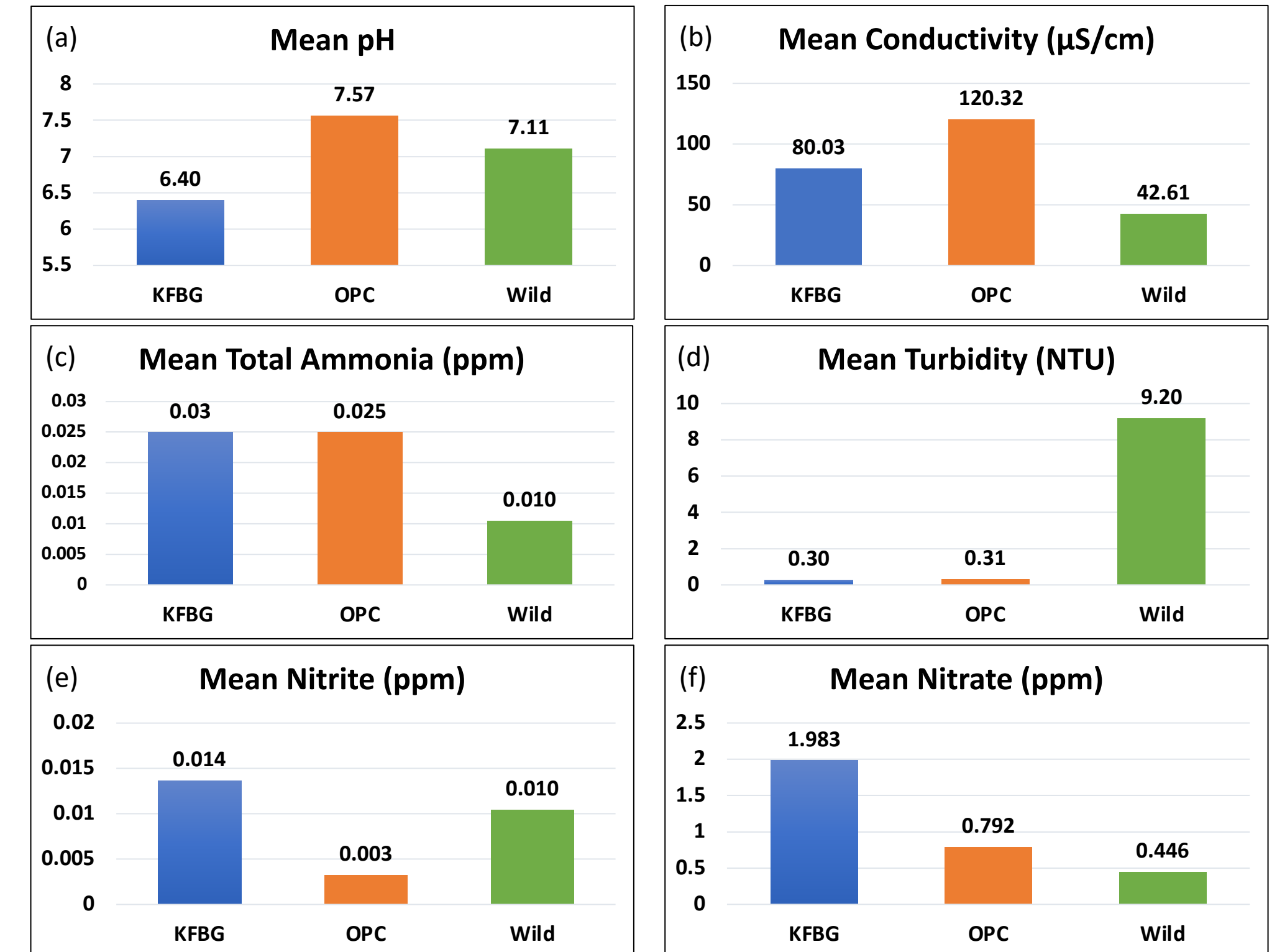


Figure 2. (a) Mean water pH in respective locations (b) Mean water conductivity in respective locations (c) Mean total ammonia in water from respective locations (d) Mean water turbidity in respective locations (e) Mean nitrite in water from respective locations (f) Mean nitrate in water from respective locations

## Discussions & Conclusions

- Paramyxoviridae, Ferlavirus, *Entamoeba invadens*, and *Chlamydia* spp. are not present in captive *C. trifasciata*, captive *S. bealei*, and two wild populations of freshwater turtles in Hong Kong.
- Most turtles appeared healthy at all sites. Some captive *C. trifasciata* have old tail injuries, missing digits, carapace deformities, and were seen more often in breeding animals.
- Water quality parameters (pH, conductivity, turbidity, nitrite, and nitrate) from the captive locations are significantly different than the wild locations ( $p < 0.05$ ; ANOVA-Single Factor) but the differences generally fall within recommended ranges and are not a concern for freshwater turtle health.
- Given that Paramyxoviridae, Ferlavirus, *Entamoeba invadens*, and *Chlamydia* spp. were not detected in turtles sampled, the risk of these potential pathogens to transmit from captive to wild populations is low at this time.

## Future Directions

Based on the data obtained from pathogen testing, clinical examination, and water quality analysis, reintroduction seems promising for these endangered turtle species (*Sacalia bealei* and *Cuora trifasciata*). Through this international collaboration, my understanding of wildlife conservation research and all the efforts that have been made to preserve these turtles has broadened. And the experiences that I gained from this project have solidified my goal to incorporate research into my future career.

Further data from the pending *Mycoplasma* spp. assay and another pathogen surveillance project (Herpesvirus, Ranavirus, *Hemogregarine* spp.) will further inform the decision regarding reintroduction. Overall, the risk assessment provides data to help decide the reintroduction of these turtle species from the two captive breeding programs. Future efforts will be focused on preparing the reintroduction and monitoring the population after reintroduction.

## References

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