Evidence-based Protection of Sea Turtle Eggs and Hatchery Practices

Short title: Evidence-based protection of sea turtle eggs

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Abstract

It is important for emerging conservationists and wildlife managers to gain experience in the use of evidence-based conservation, by way of critical analysis and understanding of the context and application of conservation actions. We developed a teaching case and activity for undergraduate and graduate courses in conservation biology, wildlife management etc., although it could also be adopted for upper-level high school classes. The case is based on a recent study that assesses hatchery practices in India. Hatcheries are commonly established to protect sea turtle eggs and hatchlings from threats at the nesting beach. Guidelines for sea turtle hatcheries have been widely available and followed by sea turtle conservationists for decades, and their use has potentially contributed to successful conservation of some sea turtle populations worldwide. However, best practices in the collection, transport, and incubation of eggs, and holding and release of hatchlings should be followed to ensure hatchling production and fitness exceeds that of unprotected nests. The teaching activity builds conservation science literacy as students identify studies describing methods to assess risks to *in situ* clutches and mitigate threats including tidal inundation, depredation, and illegal take. A practical exercise asks students to assess threats at multiple nesting beaches on an island and propose which protection strategy (protect *in situ*, relocate to safer individual location on the beach, or relocate to a hatchery) would be most appropriate at different locations.

Introduction

Hatcheries are a common *ex situ* conservation strategy to protect sea turtle eggs and hatchlings from abiotic (e.g., tidal inundation, light pollution) and biotic (e.g., depredation, illegal take) threats. Eggs are collected from the nesting beach, transported to a protected area (the hatchery) located on or close to the beach, and buried in artificial nests for incubation. When hatchlings emerge from the nest, they are released to the sea to complete the sea turtle life cycle.

Guidelines for sea turtle hatcheries have been widely followed by sea turtle conservationists for decades (Mortimer 1999), and their use has potentially contributed to successful conservation of some sea turtle populations worldwide (Mazaris et al. 2017). However, the conservation value of hatcheries has long been debated (e.g., Mrosovsky and Yntema 1980; Pritchard 1980; Mrosovsky 1983; Mrosovsky 2006). Unless best practices in the collection, transport, and incubation of eggs, and holding and release of hatchlings are followed (Table 1), relocation of clutches to hatcheries may result in lower hatchling production (e.g., Limpus et al. 1979; Eckert and Eckert 1990; Wyneken et al. 1998; Pintus et al. 2009; Revuelta et al. 2015), reduced hatchling fitness (e.g., Pilcher and Enderby 2001; Maulany et al. 2012b; Rusli et al. 2015), and/or skewed sex ratios (e.g., van de Merwe et al. 2005; Sieg et al. 2011; Maulany et al. 2012a; Revuelta et al. 2015; Sari and Kaska 2017).

Sea turtle hatcheries should achieve a hatching success (the proportion of eggs in the clutch which produce hatchlings that completely exit the eggshell) and emergence success (the proportion of eggs in the clutch which produce hatchlings that successfully exit the nest; Miller 1999) higher than that of unprotected *in situ* nests. The sex ratio and fitness of hatchlings produced from clutches of eggs incubated in hatcheries should also be similar to those emerging from nests left undisturbed on the nesting beach. Hence, designing and operating a hatchery requires an understanding of sea turtle reproductive biology, nesting behaviour, nest environment, embryo development, hatching and hatchling emergence, and hatchling energetics and survival (see Supporting Information), and the use of evidence-based best practices.

In locations where clutches are heavily threatened, Mortimer (1999) recommended that at least 70% of eggs should be protected to facilitate successful hatching. However, hatcheries are not the only strategy for protecting sea turtle eggs and hatchlings; relocating clutches to individual, protected locations (Pike 2008) and *in situ* protection structures (reviewed by Phillott 2020) can be viable alternatives.

Case Examination

This case study compares the practices of 36 hatcheries in India with accepted best practices (Table 1), and the hatching success of clutches protected in hatcheries with that of unprotected nests incubated *in situ* (Phillott et al. 2021). Due to the risks and costs of *ex situ* conservation interventions, it is important to assess if conservation strategies follow best practices to achieve their objectives (Pullin and Knight 2001, 2003; Sutherland et al. 2004, 2009).

Use of Best Hatchery Practices

The primary goal of nearly all hatcheries in this study was to protect sea turtle eggs from depredation and illegal take. Personnel at most hatcheries reported that they had received training in evidence-based procedures for collecting, handling, transporting, and incubating eggs, and holding and releasing hatchlings (Phillott et al. 2021). All hatcheries were either temporary structures that were moved annually, or permanent structures with their substrate replaced annually to biannually to minimize the accumulation of organic matter and microbial load. The nest density was $<1/m^2$ in the majority of hatcheries (74%), thereby minimizing the impact of adjacent clutches on incubation temperature and respiratory gas availability. Nests were marked and labeled appropriately (86% of hatcheries) to facilitate accurate estimations of incubation period and hatching success (however, see concerns about caging nests below) (Phillott et al. 2021).

Hatchery Practices of Concern

The relocation interval- the time between oviposition and egg collection before reburial of eggs in the hatchery- was often within preferred (<3 hr) or maximum (<6 hr) period in 74% of hatcheries. Long relocation intervals might have resulted from the practice by some hatcheries of collecting eggs from multiple beaches (up to 14 beaches; some 78 km from the hatchery). Transporting eggs in soft-sided containers and incubating clutches at nest depths shallower than the average for the species, could reduce hatching success. Moving eggs in soft-sided containers allows movement that can result in embryo mortality (Maulany et al. 2012b), while incubating eggs in comparatively shallow nests increases the likelihood of experiencing temperatures at the upper lethal limit for sea turtle embryos (Valverde et al. 2010) and deeper than average nests can require hatchlings to expend additional energy during prolonged digging and potentially reduce emergence success and hatchling survival (e.g., Dial 1987; Rusli et al. 2016).

Of the hatcheries that used shading or watering nests to mitigate high temperatures, only 56% also monitored nest temperatures. High nest temperatures can be lethal to sea turtle embryos or result in feminization of populations as sea turtles have temperature-dependent sex determination (see Mrosovsky and Yntema 1980; Morreale et al. 1982; Pintus et al. 2009; Sieg et al. 2011), but cooling nests unnecessarily may lengthen incubation periods, skew sex ratios (van de Merwe et al. 2005; Sieg et al. 2011; Maulany et al. 2012a; Revuelta et al. 2015; Sari and Kaska 2017), and/or have unforeseen impacts (Santridián Tomillo et al. 2021). As a new best practice, hatcheries should monitor their nest temperatures to determine if and when shading and/or watering might be required throughout the nesting season and assess the impact of temperature mitigation measures (Phillott et al. 2021). Accurate estimations of incubation period and hatching success would be improved if more hatcheries (57% in this study) caged their nests (Phillott et al. 2021).

Hatchlings were released within 30 min of emergence from the nest by only 44% of hatcheries. The remaining hatcheries held hatchlings for hours to days or months, often in water (42% of hatcheries) (Phillott et al. 2021). Holding hatchlings in these conditions will reduce their energy reserves for crawling and swimming activities required to traverse the beach and coastal waters quickly to avoid predators (Pilcher and Enderby 2001).

Comparative Hatchling Production

The hatching success of clutches incubated *in situ* throughout the northern Indian Ocean region (n = 14, mean 76% ± SD 11 (range 44-87)) was higher than that of clutches incubated in hatcheries in India (n = 10; 67% ± 21 (21-95) and in other countries in the region (n = 6; 59% ± 28 (26-92)), but not significantly so ($P = 0.457$) (Phillott et al. 2021).

Conclusion

Hatcheries in India did not always use best practices, and clutches incubated in hatcheries demonstrated a hatching success comparable to that of unprotected *in situ* clutches when a higher hatching success should result from conservation effort. Findings of this study indicated that some hatcheries may be limited in their potential effectiveness as an *ex situ* conservation strategy, with implications for hatchling production and fitness. It is recommended that 1) hatchery personnel have access to regular capacity building opportunities to ensure ongoing understanding of sea turtle biology in relation to best hatchery practices and resources to ensure best practices can be implemented, and 2) hatcheries conduct a periodic self-assessment of their hatchling production and revise their practices if needed. Prior to relocating nests, hatcheries must also consider if, based on the locations, *in situ* protection of nests would result in better productivity than *ex situ* strategy.

Case Study Questions

Following the evidence-based best practices for sea turtle hatcheries (Table 1) maximizes the likelihood of a high hatching success and recruitment of hatchlings to the population. Hatchery personnel may be faced with the following questions and tasks when assessing threats to clutches of sea turtle eggs and deciding on the best conservation strategy if needed. Consult Table 1 and published literature in databases available to you, such as Google Scholar, Web of Science, Scopus etc., to do the following:

1. Describe tools and/or methods that could assess the risk to *in situ* clutches of sea turtle eggs or hatchlings by the following threats:
   a. Inundation of the nest by wave run-up or groundwater intrusion.
   b. Depredation of eggs.
   c. Illegal take of eggs.
   d. Light pollution resulting in hatchling disorientation (crawling in random directions) or misorientation (crawling away from the sea towards the artificial light) and potentially resulting in mortality.

2. Propose alternative nest and/or beach management strategies to relocating threatened sea turtle eggs to a hatchery if *in situ* clutches of sea turtle eggs or hatchlings are threatened by:
   a. Inundation of the nest by wave run-up or groundwater intrusion.
   b. Depredation of eggs.
   c. Illegal take of eggs.
   d. High nest temperatures.
   e. Dry nest substrate.
   f. Light pollution resulting in hatchling disorientation or misorientation.

3. What data or other evidence are needed to assess if the management strategies you suggested in Question 2 are more likely to improve hatching success in comparison to clutches relocated to a hatchery? How should this information be collected and recorded?

4. You are spending the summer monitoring sea turtles on a remote island (Figure 1). Nesting occurs at different densities on five beaches; unprotected clutches are at risk of depredation by monitor lizards and wild pigs, and nests may also be inundated during extreme high tides resulting from seasonal storms and cyclones (Table 2). The average time from a turtle emerging from the water and finishing oviposition is 1.5 hr.

With only enough resources to establish a maximum of two hatcheries, and given the limitations of time to move between different locations using the pre-existing tracks (Table 2):

a. On which beach/es would you locate the hatchery/hatcheries and why?
b. How many clutches of eggs could you relocate to the hatchery/hatcheries each year and from which beaches?
c. How big (in square metres) would the hatchery/hatcheries need to be? Why?
d. Should clutches on some beaches remain *in situ* and unprotected and/or be protected *in situ* and, if so, using what type of structure?

5. What data or other evidence are needed to demonstrate if relocating eggs from different beaches on the island results in a higher hatching success in comparison to clutches left to incubate *in situ*, either protected or unprotected? How should this information be collected and recorded?

6. Identify evidence-based method/s of extending the maximum interval between collection of eggs and reburial in a hatchery without increasing the rate of embryo mortality. Can you predict any factors which might limit the use of these methods in different locations and/or by different stakeholders?

7. Some hatcheries hold hatchlings for periods of time ranging from minutes to hours or far longer after they emerge from the nest. Reasons for this include hatchling emergence at an inopportune time for immediate release (e.g., daytime experiencing high temperatures), or to display hatchlings and growing turtles to raise revenue for hatchery operation, educational purposes, and/or to increase community awareness. Increasing education and awareness can be important objectives for hatcheries but holding hatchlings after emergence from the nest decreases their fitness and chance of survival when released. Suggest alternative strategies that hatcheries could use to achieve the same objective. How would you assess the impact of these strategies?

8. What factors may limit the abilities and opportunities for those operating sea turtle hatcheries to use evidence-based practices? How can these challenges be overcome?

Author Contributions

ADP was responsible for the conceptualization of this manuscript. ADP and NK prepared portions of the manuscript. Both authors contributed to revising the final manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

Supporting Information

Teaching notes on relevant sea turtle biology-docx file.

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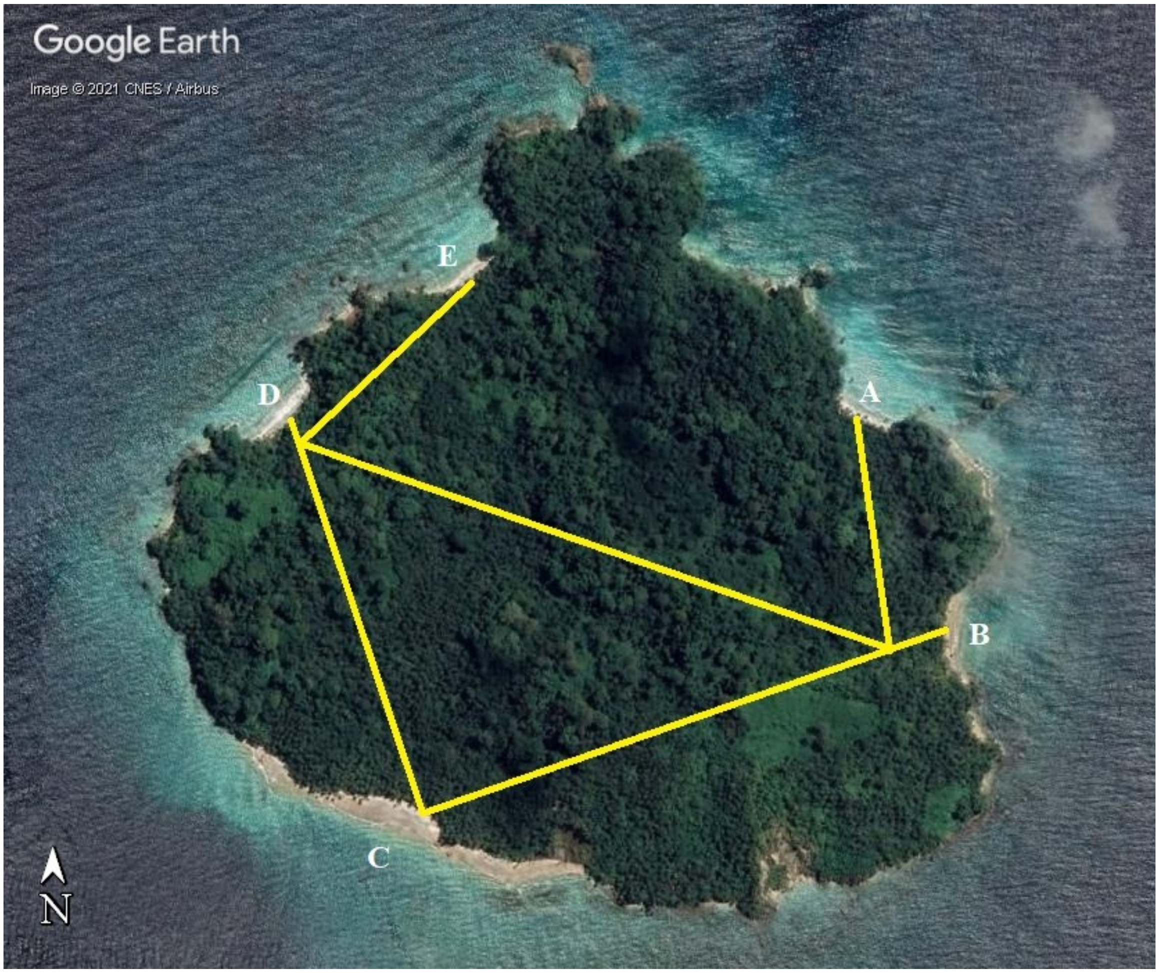
Figures and Figure Legends

Figure 1. Remote island with sea turtles nesting on five beaches with inter-connecting paths. Beaches are subject to different threats.



Tables

Table 1. Best practices for sea turtle hatcheries. (Adapted from Phillott and Shanker 2018 and Phillott et al. 2021).

Best Practice and Justification		Supporting Literature
Personnel	• Train hatchery employees and volunteers in sea turtle biology, conservation, and hatchery management techniques. • Ensure access to evidence-based information about sea turtle biology and hatchery practices.	Shenoy et al. 2011
Hatchery location and construction	• Ensure diverse nest microhabitats (e.g., shade, slope) within the hatchery to mimic conditions on the nesting beach and avoid potentially skewing sex ratios of hatchlings. • Change location of hatchery annually to avoid accumulation of organic material and subsequent high microbial load.	Mortimer 1999; Spanier 2010; Shenoy et al. 2011; Maulany et al. 2012a,b
Egg handling and transport	• Use rigid containers during egg transport to minimize rotation. • Ensure eggs remain in their original orientation, without vertical or horizontal rotation, if transporting eggs >3 h after oviposition. • Rebury eggs in hatchery within 3 h (preferable) to 6 h (maximum) of oviposition to minimize embryo mortality. • Use low-temperature or hypoxic environments to maintain embryo viability if eggs require long distance and/or long travel.	Limpus et al. 1979; Parmenter 1980; Harry and Limpus 1989; Williamson et al. 2017
Incubation conditions	• Construct hatchery nest with dimensions that closely match those of the natural nest, including depth and shape. • Rebury nests at a density of 1 nest/m^2 or less to reduce the effects of adjacent nests on temperature and respiratory gas availability. • Avoid 'pouring' eggs from a bucket or bag, and instead put each egg into the hatchery nest individually. • Retain moist sand removed during nest construction and reuse it to cover eggs to avoid the risk of desiccation caused by dry sand. • Ensure that all eggs from a single clutch are incubated in the same hatchery nest. Avoid dividing or splitting up of a single clutch or combining separate clutches in a single nest. • Consider partial shading of the hatchery to reduce the risk of lethal nest temperatures, especially late in incubation. Record nest temperatures using data loggers to determine incubation temperatures and avoid skewing hatchling sex ratios.	Mortimer 1999; van de Merwe et al. 2005; Maulany et al. 2012a,b; Rusli and Booth 2016
Nest enclosures	• Insert markers to indicate the location of hatchery nest along with a label showing the date of nest, estimated emergence date and number of eggs for monitoring. • Protect each nest using individual cages made of rigid material about 60 cm diameter to reduce depredation and energy expenditure by hatchlings crawling throughout hatchery. Avoid metal wire as it may interfere with later geomagnetic orientation during natal homing.	Mortimer 1999; Shenoy et al. 2011
Hatching release	• Calculate emergence date, approximately at 45-55 days after oviposition. A soon-to-emerge nest is often characterised by a subtle 'caving-in' of sand at the surface. • Inspect enclosures every 30-60mins for signs of hatchling	Mortimer 1999; Wyneken 2000; Pilcher and Enderby 2001;

	emergence, mainly from afternoon to dawn (e.g., on overcast days and after rain) around the predicted emergence date. • Release hatchlings as soon as possible after emergence to avoid risk of predation, exhaustion, desiccation, loss of vigor or possible injury. • Ensure that all hatchlings are not released at the same site and the different release sites are sufficiently spaced to avoid creating feeding stations for predators in the sea. • Release hatchlings that have emerged around the same time and day in groups, if possible, to reduce the loss in vigor due to extended holding period and to improve survival probability. • Ensure that hatchlings crawl across the beach width and enter the water without assistance to facilitate imprinting on the nesting beach. • Inform observers to keep an appropriate distance from released hatchlings to reduce the chances of injuries or obstructed progress into the sea for hatchlings. Draw parallel lines about 10m away on either side of the site of hatchling release for observers to stand behind while hatchlings crawl between lines. • Reduce hatchling disorientation during emergence and release by reducing the effect of artificial lights on or around the beach. • Keep emerged hatchlings in a waterless container placed in a cool, dark place away from heat and when immediate release is not feasible. Do not use water while holding hatchlings as swimming will deplete their energy reserves required to avoid depredation in the open ocean and hinder the 'swimming frenzy' stage.	Shenoy et al. 2011; van de Merwe et al. 2013
Hatchery records	• Note data on date of oviposition, clutch size, date of emergence, number of hatchlings, and (if possible) weight and carapace length.	Mortimer 1999; Schäuble et al. 2002; Shenoy et al. 2011
Monitoring and evaluation	• Record the incubation period as the number of days between oviposition and emergence. • Calculate hatching and emergence success by excavating nest 2-3 days after the most hatchlings have emerged. a) Hatching Success = (Number of hatched eggs/Total number of eggs) × 100 b) Emergence Success = (Number of naturally emerged hatchlings/Total number of eggs) × 100 • Collect data on nest temperature and hatchling sex ratio from a statistically valid proportion of nests in hatchery and compare with data from the natural beach/es for your population of sea turtles.	Mortimer 1999; Schäuble et al. 2002; Shenoy et al. 2011

Table 2. Characteristics of beaches and their connecting paths. Patrol time- time taken to walk one length of the beach, uninterrupted.

				Connecting Paths	
Beach	**# Nests/ Year**	**Threats to Eggs**	**Patrol Time**	**Beaches**	**Travel Time**
A	30	Tidal inundation	7 min	A-B	30 min
B	30	Monitor lizards	15 min	A-C	100 min
C	150	Wild pigs, monitor lizards	60 min	A-D	170 min
D	30	Monitor lizards	15 min	A-E	125 min
E	30	Monitor lizards	15 min	B-C	75 min
				B-D	90 min
				B-E	115 min
				C-D	70 min
				C-E	95 min
				D-E	30 min