

Marine Turtle Newsletter 151:16-21, © 2016

Marine Turtle Newsletter

Assessing the Impacts of Hatcheries on Green Turtle Hatchlings

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At present, six of the seven marine turtle species are globally classified as likely to become extinct in the near future by the International Union for Conservation of Nature (www.iucnredlist.org). One tool commonly utilized in sea turtle conservation is the translocation of eggs into hatcheries (Mortimer 1999). Hatcheries are widely perceived as being beneficial in protecting eggs from threats such as poachers, natural predators and environmental pressures (Mortimer 1999). Additionally, this strategy can be used to promote ecotourism and thereby provide financial income for local people (Rajakaruna *et al.* 2013). However, hatchery-based conservation programs have also provoked debate about their effectiveness because they may negatively affect turtle populations. Some of the potential dangers of hatcheries include: detrimental effects on embryonic development and hatching success (Pritchard 1980; Mortimer 1999); high rates of mortality caused by incorrect release methods (Mortimer 1999); skewed sex ratios due to the thermal effect of specific environmental conditions (Morreale *et al.* 1982; Mortimer 1999); and detrimental effects on hatchling energy and behavior when they are retained in artificial tanks (Pilcher & Enderby 2001; van de Merwe *et al.* 2013). Consequently, moving eggs to hatcheries is considered an option of last resort, when in situ conservation is not a viable option (IUCN 2005). Nevertheless, this does not mean that hatcheries cannot make a positive contribution, as their effectiveness relies on the way that they are managed (Tisdell & Wilson 2005).

In Sri Lanka, hatcheries have proliferated, primarily as an indirect consequence of the effects of the high human population density (Rajakaruna *et al.* 2013). There are few special protected areas for sea turtles in the country, which makes in situ conservation difficult (Hewavisenthi 1993). Under these circumstances hatcheries seem to offer the most suitable conservation strategy. Although it is claimed that the primary motive of most of the Sri Lankan hatchery owners is profit from tourism, there is also a general understanding on their part of the need for hatcheries in turtle conservation (Rajakaruna *et al.* 2013). According to Rajakaruna *et al.* (2013), the closure of hatcheries in Sri Lanka would be impractical, thus there is a need to improve the poor practices employed in most of them.

One of the hatchery practices identified as in need of improvement is the post-emergence handling of the hatchlings. In the wild, hatchlings emerge from the nest

and immediately crawl frenetically to reach the sea. Once in the sea, hatchlings swim continuously, in a state of energetic frenzy, in order to get away from the shore as quickly as they can. The frenzy period is characterized by rapid and effective power strokes interspersed by a less effective dog paddling swimming style and resting periods, which become more frequent as time passes (Wyneken & Salmon 1992). In most Sri Lankan hatcheries however, hatchlings are held for 1-7 days after emergence to provide a tourist attraction (Rajakaruna *et al.* 2013). This retention may result in a disturbance of their natural behavior so as to compromise their chances of survival by depleting some of the valuable energy reserves used to distance themselves from shore (Gyuris 1994).

The aim of this study was to examine how hatchling retention might affect survivability by assessing different quality parameters (body condition, crawling and swimming performance), in green turtles (*Chelonia mydas*). The results provide valuable information that can be used to improve practice in Sri Lanka and indeed, in hatcheries worldwide, in respect to their contribution to sea turtle conservation.

The study was conducted in May-June 2015 at the Kosgoda Sea Turtle Conservation Project (KSTCP; 6°N, 80°E), one of the seven hatcheries situated along the southwest coast of Sri Lanka (Rajakaruna *et al.* 2013). Kosgoda is Sri Lanka's second largest rookery, and is visited by five species of sea turtle, including the green turtle, which exhibits a year round high nesting frequency. The coastline of Kosgoda has a high presence of human activity, mainly due to beach tourism, which can be a cause of severe disturbance for in situ nests. Nests can also be affected by the presence of animal predators and tidal inundation (Ekanayake *et al.* 2010). In Kosgoda, local villagers collect the freshly laid eggs at night from the surrounding beaches and sell them to the hatchery owner to be reburied first thing in the morning in the incubation pens, where they are then protected until they produce hatchlings (Tisdell & Wilson 2005). In the case of KSTCP, hatchlings are kept in tanks for two or a maximum of three days. After this retention, tourists and volunteers release them at sunset at 5-10 m from the tideline so hatchlings can crawl down the beach and get to the sea.

During this study 10 ex situ green turtles nests were visually inspected for emergence and, before trials, all newly emerged hatchlings were captured and transported to seawater-filled holding tanks (160 cm long × 135 cm wide × 100 cm high) where they could freely swim. In order to assess crawling speed and swimming power stroke rate, hatchlings were divided into five groups according to the time since emergence: just emerged (0 hr), 6 hr, 12 hr, 24 hr and 48 hr after emergence, as hatchlings are usually kept for two days in KSTCP. Each group was comprised of 3 randomly selected hatchlings from each nest and they were marked on the carapace for identification and to avoid being selected twice. After each swimming and running trials, the hatchlings were weighed with an electric balance (± 0.01 g) and measured along their notch to tip straight carapace length (SCL) and straight carapace width (SCW) using a Vernier caliper (± 0.1 mm). An overall size

index, similar to that used by Ischer *et al.* (2009), was calculated by multiplying SCL by SCW. All procedures were carefully carried out while trying to diminish any procedural stress inflicted on study hatchlings. Hatchlings were not fed at any point during the first 48 hr after emergence and were eventually released at the discretion of the hatchery owner.

To test crawling speed, hatchlings were run along a 3 m raceway. The raceway (3 m × 0.5 m) was located outside with natural light conditions but permanently shaded and with a slight downward slope facing the sea in order to emulate natural conditions as much as possible. A dull light was also placed at the end of the raceway to add another stimulus for the hatchlings to run in the right direction; hatchlings naturally crawl towards the main light source they see (Pilcher *et al.* 2000). Crawling speed was calculated ($\text{speed [v]} = \text{distance [d]} / \text{time [t]}$) by timing how long each hatchling took to crawl the 3 m. As hatchlings were exposed to natural fluctuations in temperature, the air temperatures at the time of trials were obtained from the daily weather data recorded by *Freemeteo* <<http://freemeteo.com.lk>>.

Swimming performance in each group was measured using a method similar to the one described by Burgess *et al.* (2006). Hatchlings were allowed to swim individually for one hour in tanks (60 cm long × 42 cm wide × 36 cm high), filled with 30 cm of seawater at 30 °C (the average water temperature from the tanks of KSTCP during this season). They were fitted with a Velcro harness that provided resistance for the turtles to swim against, but did not impede motion, simulating the natural environment (Salmon & Wyneken 1987). The harness was connected to a monofilament nylon tied to another tense monofilament above in the center of the tank. To reduce visual stimuli and induce unidirectional oriented swimming (Salmon & Wyneken 1987), three sides of the enclosure were covered with black plastic and a dim light was placed at the remaining side. Hatchlings were allowed to swim freely in the tanks, but the nylon monofilament length prevented them from touching the sides or the bottom of the enclosure. After 1 min of acclimatization, hatchlings were videotaped for one minute at the beginning (0 min), middle (30 min) and end (60 min) of the hour trial. The videotapes were then played back at slow speed (25%) and the power strokes manually counted during the minute interval. Power stroke rate was calculated as the average of power strokes min⁻¹ of the three replicates.

IBM SPSS Statistics v22 was used to analyze the data. As the data did not conform to the assumptions of a normal distribution, the non-parametric Kruskal-Wallis H test (KW-H) was used to determine if there were differences in average power stroke rate, crawling speed and body measurements over time. In the cases where the KW-H test was significant a pairwise Mann-Whitney U test (MW-U) was executed to determine which groups exhibited significant differences. Relationship between variables of morphology, temperature and performance were investigated

using a Spearman's rank order correlation (S-rho). Statistical differences and rejection of the null hypothesis were assumed if $p < 0.05$.

Hatchling mass was correlated with hatchling size index in hatchlings from swimming (S-rho [rs]=0.761, $p=0.001$) and crawling ($rs = 0.654$, $p=0.001$) trials. However, the only morphology condition that showed a significant change during hours of retention was the size index (KW-H [H]=20.844, $p=0.001$) of hatchlings in swimming trials. Further comparisons (MW-U) showed between which periods of retention the significant differences were found (Table 1). For example, the first significant change (MW-U [U] =232, $p=0.001$) in size happened after 24 hr. As Fig. 1 indicates, there was an increase of the median size index between hours of retention. After 24 hr (Median [min, max]=1924 [1556, 2208] mm²), the median size index of the hatchlings was 5% greater than that of newly emerged hatchlings (Median [min, max]=1827 [1635, 2128] mm²). After 48 hr (Median [min, max]=1965 [1779, 2229] mm²), the median size index was 8% greater.

A total of 150 hatchlings completed the crawling trials. The KW-H test showed that crawling speed decreased significantly ($H=17.872$, $p=0.01$) when hatchlings were retained in the tanks for 48 hr. MW-U test (Table 2) specified the significant difference between particular groups, with the first one happening after 24 hr. According to the median speed of each group (Fig. 2), hatchlings assessed after 24 hr (Median [min, max] 0.072 [0.03, 0.169] m s⁻¹) ran 26% slower than newly emerged ones (Median [SE]=0.097 [0.041, 0.243] m s⁻¹) and up to 27% slower when assessed after 48 hr (Median [min, max]=0.071 [0.016, 0.118] m s⁻¹). In addition, minimum and maximum values in Fig. 2 denote a general tendency of the hatchlings to run slower with hours of retention. No correlation was detected between weight and crawling speed ($rs=-0.062$, $p=0.448$) or between size index and speed ($rs=-0.093$, $p=0.259$). Air temperature also was found to have no significant correlation ($rs=0.136$, $p=0.096$) with crawling performance.

Groups	0 hr	6 hr	12 hr	24 hr	48 hr
0 hr	1				
6 hr	0.564	1			
12 hr	0.668	0.976	1		
24 hr	0.001	0.005	0.048	1	
48 hr	0.001	0.001	0.015	0.478	1

Table 1. Pairwise MW-U test of hatchling size index between groups, using 95% confidence intervals, in swimming trials.

Groups	0 hr	6 hr	12 hr	24 hr	48 hr
0 hr	1				
6 hr	0.976	1			
12 hr	0.367	0.326	1		
24 hr	0.012	0.019	0.008	1	
48 hr	0.005	0.004	0.004	0.745	1

Table 2. Pairwise MW-U test of crawling speed between groups, using 95% confidence intervals.

Groups	0 hr	6 hr	12 hr	24 hr	48 hr
0 hr	1				
6 hr	0.128	1			
12 hr	0.004	0.188	1		
24 hr	0.002	0.121	0.859	1	
48 hr	0.001	0.010	0.371	0.478	1

Table 3. Pairwise MW-U test of power stroke rate between groups, using 95% confidence intervals.

Another 150 hatchlings participated in the swimming trials, where the application of the KW-H test to the resulting data indicated that power stroke rate decreased significantly ($H=19.538$, $p=0.01$) with increasing hours of retention. Subsequently, the MW-U test (Table 3) found that the differences were not significant between adjacent groups, suggesting a gradual change. According to the medians of the average power strokes for each group, there was a decrease in swimming performance through hours of retention (Fig. 2), e.g., after 48 hr (Median [min, max]=117 [57,149] strokes min⁻¹), the power stroke rate decreased up to 16%. However, retention between 0 (Median [min, max]=140 [99, 171] strokes min⁻¹) and 12 hr (Median [min, max]=126 ± [76, 163] strokes min⁻¹) was associated for a decrease of 10% in the median power stroke. Minimum and maximum values in Fig. 2 also denote a general tendency of the hatchlings to reduce their power stroke rate with hours of retention. The S-rho test revealed a weak, statistically significant, negative correlation between size index and power stroke rate ($r_s=-0.181$, $p=0.027$). No correlation was observed between weight and power stroke rate ($r_s=-0.035$, $p=0.673$).

The only body condition that recorded a significant change across hours of retention was the size index of hatchlings after swimming trials. Hatchlings slowly increased their size during time of retention with the first significant change after 24 hr. After 48 hr, hatchlings' median size index had increased 8%, from 1827-1965 mm². Since hatchlings were not fed at any point during the first 48 hr and the absorption of the residual yolk in reptiles is not likely to be directly involved in the

growth of the hatchlings (Kraemer & Bennet 1981; Radder *et al.* 2007), the most likely explanation for this increase in size is rehydration. Bennett *et al.* (1986) reported loggerhead hatchlings (*Caretta caretta*) losing 12% of their weight due to dehydration in the process of emergence. Hatchlings can rehydrate by drinking water once they enter the sea, but it takes from 10-15 days to recover their hatching weight (Bennett *et al.* 1986). In Sri Lankan hatcheries, hatchlings are placed in the tanks with marine water from the moment they are collected from the nests, despite suggestions from hatchery management guidelines (Mortimer 1999), which recommend that they should be kept inside a damp cloth sack in a cool dark quiet space. As hatchlings spent more time inside the tanks, they rehydrated, increased their weight and therefore their size, with hatchling mass showing a strong positive correlation with size. However, no significant change was shown in weight, which may be due to the fact that the increase in size was relatively small and a significant change in weight perhaps required more hours of rehydration. An increase in the body size of the hatchlings during retention might improve chances of survival, following the “bigger is better hypothesis” (Gyuris 2000). According to this hypothesis, larger hatchlings are less susceptible to predation as they can avoid gap-limited predators. However, only hatchlings from swimming trials showed a significant change in size, and although statistically significant, this change was relatively small and therefore unlikely to play a major role in regards to predation.

Crawling performance decreased with time of retention. Air temperatures during the speed trials ranged from 24-31°C, though the most frequent temperature was 29°C. Even though temperature has been reported to influence performance in turtles (Adams *et al.* 1989), in this case the time spent running in the trials, 12-18 s, was not long enough for temperature to influence hatchling crawling performance. In addition, body condition showed no correlation with hatchling crawling speed. Therefore, the observed change in crawling speed was most likely related to period of retention. In this study, the median hatchling speed was reduced by 26%, from 0.097-0.072 m s⁻¹, after 24 hr and 27%, from 0.097-0.071 m s⁻¹, after 48 hr of retention. This decrease in crawling speed was not gradual as the first significant change was observed after the first 24 hr. However, as hatching usually occurs immediately after sunset, if hatchlings are not released just after emergence, up to 24 hr will need to pass until the next release window, by which time the hatchlings will have already lost valuable running speed capacity. Releasing hatchlings during the morning is considered an improper method since it is likely to decrease their chances of survival (Mortimer 1999). A previous study by van de Merwe *et al.* (2013) also investigated the effect of time of retention in green turtle crawling performance. Although their results coincide in terms of speed reduction, in their study the decrease in swimming speed was greater; after just 6 hr of retention the hatchlings’ mean crawling speed decreased by 50%.

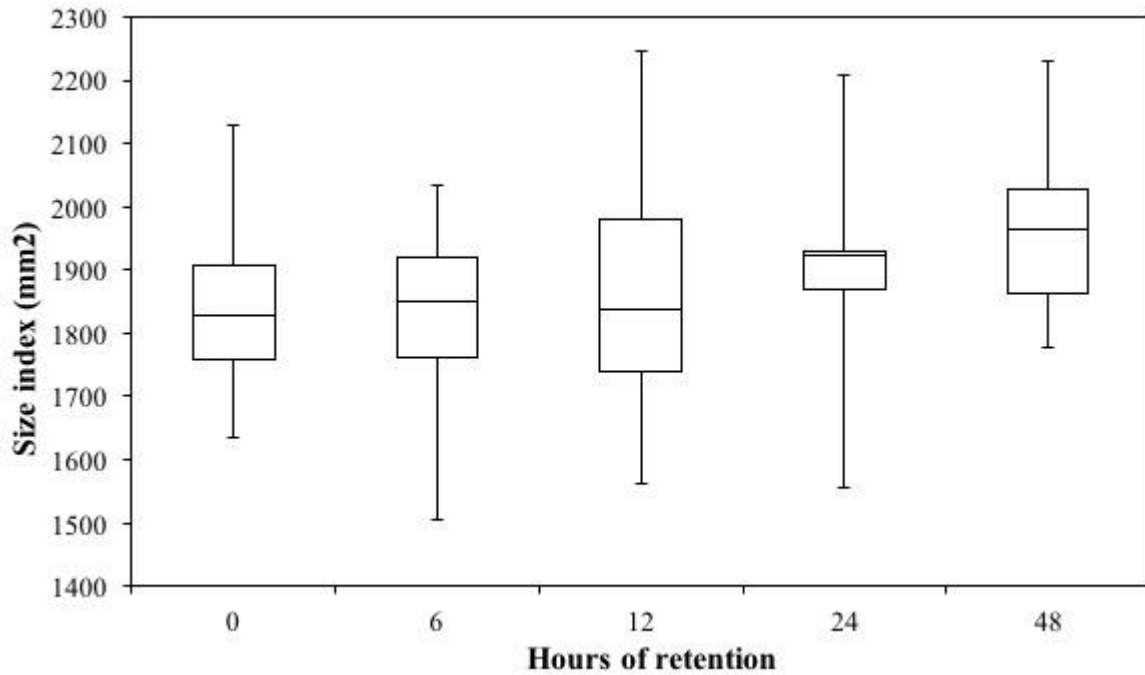


Figure 1. Body condition. Box and whiskers plot of the size index of green turtle hatchlings from swimming trials, during hours of retention (n=150). The boxes represent the 25%-75%, whiskers represent the minimum and maximum values, and lines in the box represent the median values of the distribution.

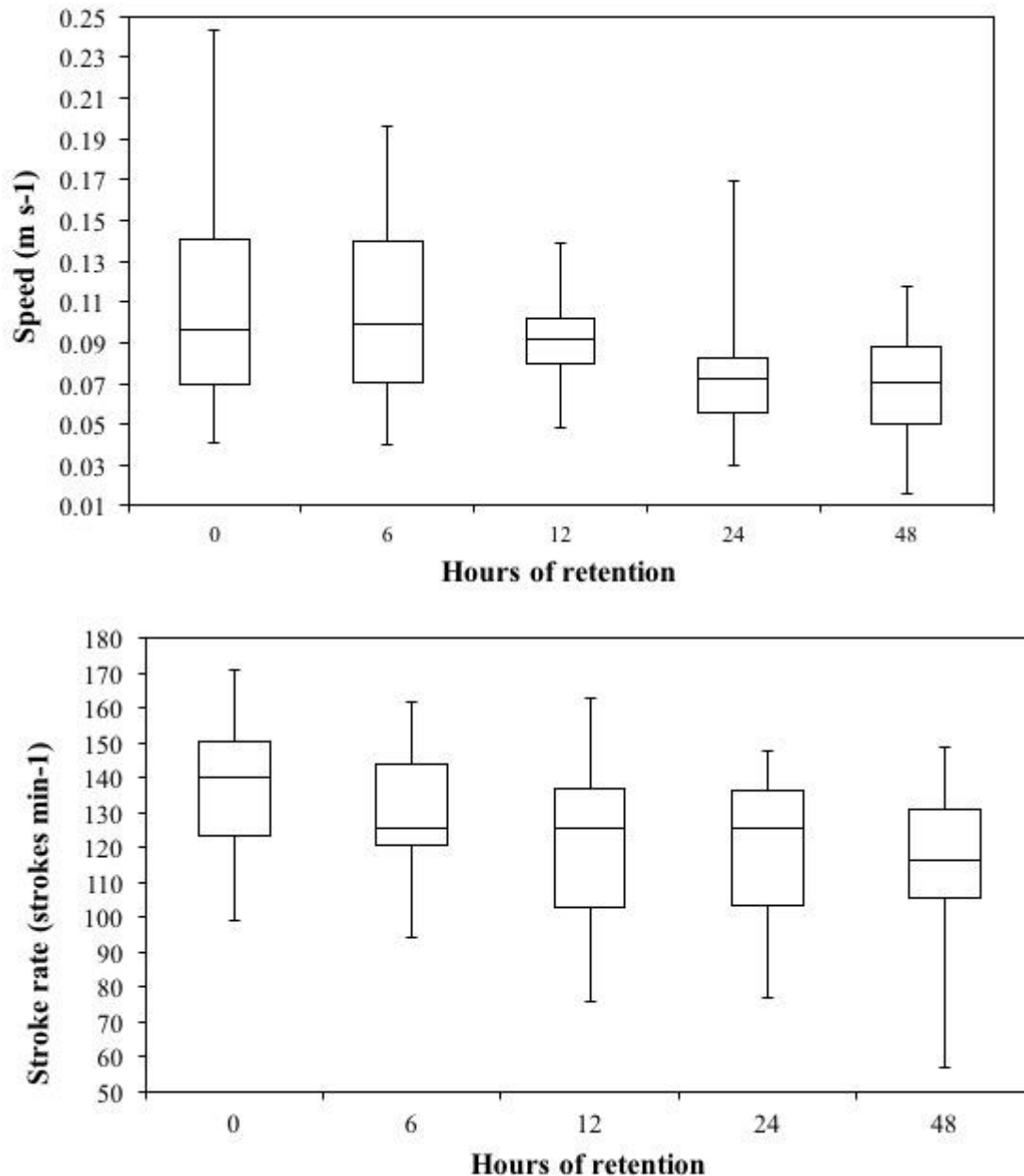


Figure 2. Locomotor performance. Box and whiskers plot of the crawling speed (upper panel) and power stroke rate (lower panel) of green turtle hatchlings during hours of retention (n=150). The boxes represent the 25%-75%, whiskers represent the minimum and maximum values, and lines in the box represent the median values of the distribution.

One plausible explanation for this difference from the current study is that retention conditions were not similar, as hatchlings were retained in nest netting during the entire period, thereby suffering dehydration (van de Merwe *et al.* 2013), whereas in this experiment they were kept in tanks. Rusli *et al.* (2015), who studied the effects of different incubation methods on locomotor performance, found that 48 hr retention in styrofoam boxes actually improved crawling speed. However,

this improved speed was approximately half that of hatchlings newly emerged from in situ and hatchery nests. So this alternative method of incubation and retention would likely not be effective for Sri Lankan hatcheries, where speed would be reduced in comparison with newly emerged hatchlings from ex situ nests.

In nature, hatchlings emerge from their nest and crawl rapidly towards the sea where they disperse into offshore waters (Wyneken & Salmon 1992). Their crawling performance is important as they are exposed to predators during the beach running stage. Nevertheless, in captivity some hatcheries offer protection when releasing the offspring (Mortimer 1999). In the observed case of KSTCP, volunteers, tourists and owners protected the hatchlings from predators on their way to the sea. However, this practice may not be followed by hatcheries worldwide, especially during the tourist off-season. In the case of hatchlings released and not protected after hours of retention, a decrease in crawling performance is likely to affect their chances of survival in the wild. And even if they are protected, retention is likely to affect their chances of being predated once they enter the sea.

Although some researchers have investigated swimming performance by employing direct measurements in the wild (e.g., Salmon & Wyneken 1987; Gyuris 1994; Pilcher *et al.* 2000), this is difficult in terms of logistics. In the present study, indirect measures of swimming performance were used following a model similar to the one described by Burgess *et al.* (2006); hatchlings were tethered in tanks instead of using a raceway system as other studies (e.g., Pilcher & Enderby 2001). It is important to consider that tethering the hatchlings might affect their swimming behavior and therefore the resulting data might not accurately reflect their behavior under natural conditions. However, results of previous studies with tethered hatchlings under experimental conditions recorded similar behavior to that found in their natural environment (Wyneken & Salmon 1992). Power stroke rate was assumed to be a valid parameter for assessing swimming performance, as power strokes generate the greatest swimming force and they have been found to be more than twice as effective as dog paddling (Ischer *et al.* 2009). In this study power stroke rate suffered a gradual decrease with increased retention time. Despite previous studies having found a relation between body condition and swimming performance (Burgess *et al.* 2006; Ischer *et al.* 2009), in the present study the correlation between swimming performance and size index was not strong enough to explain the decrease in power stroke rate. It can therefore be assumed that the decrease in swimming performance was mainly due to retention time.

According to the data, after 12 hr there was a 10% drop in the median power stroke rate, from 140-126 strokes min⁻¹, and after 48 hr this drop increased up to 16%, from 140-117 strokes min⁻¹. These findings seem to be in line with previous studies, where hatchlings gradually decrease their power stroke rate as they move through the frenzy period, and when dog paddling and resting become more

frequent (Wyneken & Salmon 1992; Burgess *et al.* 2006). However, the decrease found over hours of retention in this study was not as marked as that found by previous studies. For example, the first significant decrease happened after 12 hr, while Pereira *et al.* (2011) reported a rapid decrease of power stroke rate of green turtles during the first 2 hr of swimming, followed by a slower decrease after 8-12 hr. Pilcher & Enderby (2001) found that from 4-6 hr of retention the hatchlings used at the end of the trials exhibited a more erratic power stroke instead of a continuous one. In their experiment they quantified swimming speed and found a significant reduction after 3 hr of retention, and after 6 hr, a drop by over 12%. Although the results of the present study were not quite as pronounced, in terms of recommendations for hatchery management they do support previous findings. Moreover, because hatchlings need to be released after sunset, if they are not released immediately after emergence, which usually occurs soon after sunset, 24 hr would need to pass until the next release when power stroke rate would have significantly declined. Having an energetic and rapid swimming performance can be important for survival, as hatchlings do not display any other predation avoidance mechanism (Gyuris 1994). Consequently, hatchlings should be released right after emergence to avoid this reduction in swimming performance.

Populations of the endangered green turtle in Asia are believed to have declined over the last decades, including Sri Lankan populations (Shanker & Pilcher 2003). The highest mortality rate in sea turtles occurs during the first stages of their lives, between incubation, crawling to the sea and swimming away from shore (Crouse *et al.* 1987). An experiment by Pilcher *et al.* (2000) found hatchlings suffer 40-60% mortality within the first two hours in the sea, but once they reach deeper waters this predation rate decreased by two thirds. With such high levels of mortality, it is important that hatchery management practice seeks to maximize the chances of survival of the hatchlings by minimizing the depletion of the energy they need for the frenzy swim. The present study further reinforces the idea that time of retention has a negative impact on hatchlings, by reducing crawling and swimming performance. The reduction of swimming performance in this case can be considered the most potentially significant outcome in terms of survival, as hatchlings cannot be protected while they swim to deep waters.

In addition, this retention may affect the natural migration of hatchlings. Okuyama *et al.* (2009) suggested that retention of hatchlings decreases their probabilities of experiencing the natural migration of wild hatchlings. Releasing hatchlings offshore to reduce their mortality rate and minimize the effects of retention on their migratory route is a practice carried out by some hatchery operators (Hewavisenthi 1993). In the past hatcheries have been discouraged from using this strategy as it may disturb the imprinting mechanism of hatchlings, which may affect females in their return to the natal beaches for nesting (Pritchard 1980). However, according to Lohmann & Lohmann (1996), sea turtles may be able to use the earth's magnetic field to return to their natal nesting beaches using a bicoordinate magnetic map. Hence, offshore release may be a good solution;

nonetheless, further study is required. Another strategy followed by hatcheries is to feed the hatchlings prior to release (Rajakaruna *et al.* 2013). The effects of feeding on hatchling condition needs to be investigated, but this is complicated by the likelihood that not all hatchlings will consume the same amount of food in these early stages. Therefore, it still seems that the best practice would be to release hatchlings just after emergence.

The effectiveness of sea turtle hatcheries relies on improving current practices (Tisdell & Wilson 2005). This study provides experimental evidence supporting the importance of releasing hatchlings immediately after emergence. Hatcheries may be somewhat resistant to the idea of adjusting their ecotourism policies to maximize hatchling survival upon release, as this would mean that the collection and release of hatchlings should take place in the dark, which may be less appealing for tourists. However, it is possible to combine tourism and conservation, following best practice guidelines (IUCN 2005), which recommend releasing at least 90% of the hatchlings from each nest immediately after emergence, and holding the remaining hatchlings as a tourist attraction. Tourists could release these remaining hatchlings, despite being fewer in number, the next day at the sunset. In this way, Sri Lanka, and turtle hatcheries worldwide, would be able to improve their contribution to sea turtle conservation whilst maintaining much needed tourist revenue.

Acknowledgements. We thank Kosgoda Sea Turtle Conservation Project (KSTCP), and especially D. Perera, (the director of KSTCP) for allowing us to spend time at the hatchery and providing their assistance for everything that we needed. We also thank the Durrell Institute of Conservation and Ecology for the financial support.

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