Report on the 2014 incubation efforts of diamondback terrapin *(Malaclemys terrapin)* eggs at the Bermuda Government's Department of Conservation Services

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Executive summary

The investigation into the hatching success of wild diamondback terrapin eggs versus incubated terrapin eggs continued in Bermuda during 2014. Previous studies have shown that there is a very low hatching success rate for this species in Bermuda (Outerbridge, 2012, 2013a, 2014) as opposed to some regions in the U.S.A. (Cook, 1989; Roosenburg et al., 2003). The results of the present study showed that there was a significant difference in the hatching success rates between the clutches of eggs that were left in the wild (3.8%) versus those that were placed within an incubator (27.6%) at the Bermuda Government's Department of Conservation Services. A total of 18 hatchling terrapins (16 from the incubator group and two from the control group) were produced from the 20 monitored nests in 2014. Two hatchlings emerged unobserved from a sand bunker on the 7th hole on the Mid Ocean golf course while 16 were released within the mangroves surrounding Mangrove Lake at the conclusion of this study.

Introduction

The artificial incubation of terrapin eggs collected from the wild was first attempted at the Bermuda Aquarium Museum and Zoo in 1994 and then resumed in 2012 and 2013 at the Department of Conservation Services (DCS) in an effort to increase the annual hatching success observed in Bermuda's terrapin population. The results of those incubation attempts showed that there was a variable difference in the hatching success rates between the clutches of eggs that were left in the wild (control group) versus those that were placed within an incubator at DCS.

Methods

Nest surveys and egg collection

Daily nesting surveys were performed in the sand bunkers on the 5th, 6th and 7th holes of the Mid Ocean golf course from May 10th to June 12th, 2014. Encountered nests were haphazardly divided into two groups; 10 control clutches and 10 incubator clutches. The control clutches were left *in-situ* and their locations were marked with metal stakes and surveyors tape. The incubator clutches were gently excavated (taking care not to rotate the eggs) and transported back to DCS where they were artificially incubated in a tabletop Precision Scientific mechanical convection incubator (Model 6M). Alphanumeric notations were assigned to each of the above sand bunkers to aid in the identification of individual nests (Figure 1 in the Appendix).

Care of incubated eggs

Each clutch of eggs was placed on a 3 cm deep bed of autoclaved, coarse vermiculite which had been moistened with sterile water in 1:1 proportions by weight inside of a 2-quart plastic container measuring 12 cm x 12 cm x 5 cm. The containers were placed in the incubator and covered throughout the incubation period to retain moisture. Each plastic container had eight small holes (*ca.* 1 mm diameter) drilled into the sides for ventilation. The incubation temperature was set at 27°C for the duration of the incubation period.

The eggs were checked weekly for the first 50 days to look for evidence of fungal contamination upon the surface of the shells, then twice daily (once at 09:00 and then again at 17:00) there-after to look for evidence of hatching. Eggs that became infected with fungi were removed from their respective containers to prevent contaminating the remaining eggs in the clutch.

The daily temperatures for the entire incubation period of the control group were recorded hourly using one digital thermometer (HOBO pendant data logger) deployed in the largest sand bunker on the 5^{th} hole. One data logger was also installed inside the incubator to log the daily temperatures of the incubator group. Both were deployed on May 14^{th} and collected on August 22^{nd} (incubator) and on September 12^{th} (control).

Care of hatchling terrapins and examination of failed egg material

The protocols for the care of hatchlings and the examination of failed egg material are described in Outerbridge (2012).

Results

Control group

The 10 nests that comprised the incubator group were all located between May 10^{th} and June 12^{th}. Four clutches were monitored in the sand bunkers on the 5^{th} hole, two clutches from bunkers on the 6^{th} hole, three clutches from bunkers on the 7^{th} hole and one was monitored at the terminal end of the canal at Mangrove Lake. Excavation of six control nests (7A1, 5D1, 6B1, 7B1, 5D2, 6B3) occurred on August 6^{th} 2014; the remaining four clutches (5D4, 5D5, 7A6, ML canal) were excavated on September 12^{th} 2014. A total of 53 eggs were found, of which only two (3.8%) had developed into hatchlings. Forty-nine eggs (92.5%) showed no visible evidence of embryonic development, one egg contained a dead embryo (stage of development was undetermined due to advanced decomposition) and one egg contained the scutes and skeletal remains of a hatchling in addition to a grass root ball (Figure 2 in the Appendix). Only one clutch in the control group produced hatchlings (n = 2), both of which had emerged prior to nest excavation. The overall hatching success rate in the control group was 3.8%. See Table 1 in the Appendix for full summary of the egg data.

The incubation temperatures for the control group ranged from 19.6-39.2°C (mean 28.6°C; SD 3.7) between May 14^{th} and September 12^{th} 2014 (Figure 3 in the Appendix). The incubation period was not monitored.

Incubator group

A total of 58 eggs were found within the 10 nests that comprised the incubator group. Three clutches came from the sand bunkers on the 5^{th} hole, one clutch came from a bunker on the 6^{th} hole and 6 clutches came from bunkers on the 7^{th} hole. All were discovered between May 14^{th} and June 12^{th}. Of those 58 eggs, 16 (27.6%) developed into hatchlings. Thirty-five eggs (60.3%) showed no visible evidence of embryonic development and seven (12.1%) contained dead embryos in various stages of development (note that 15 eggs were removed from their respective containers during the course of the incubation study because of fungal infection). Of the seven dead embryos, three appeared to be in stages 1-8 following Yntema's classification (1968) and four appeared to be in stages 18-25. Four (40%) clutches in the incubator group produced hatchlings. The overall hatching success rate for this group was 27.6%. See Table 2 in the Appendix for full summary of the egg data.

The 16 terrapins that hatched in the incubator group were taken to Mangrove Lake and released onto the mangrove leaf litter between the prop roots at the water line. Four of these terrapins (25%) had abnormal carapace scute patterns, which included extra marginal scutes (n = 4) and extra vertebral scutes (n = 1).

The temperatures for the incubator group ranged from 23.0-27.7°C (mean 27.1°C; SD 0.2) between May 14^{th} and August 22^{nd} 2014 (Figure 4 in the Appendix). The incubation period ranged from 64 – 67 days (mean 65.3 days, n = 4 clutches).

Discussion

The results of the 2014 incubation study showed significant difference between the hatching success of the eggs left *in-situ* within the sand bunkers versus the eggs that were collected and artificially incubated *ex-situ* at DCS. This discrepancy is similar to that found in the 2012 incubation study (3.4% *in-situ* vs. 44.6% *ex-situ* hatching success), but contrasts with the results of the incubation study undertaken in 2013 (32.3% *in-situ* vs. 24.6% *ex-situ* hatching success). In contrast, Cook (1989) and Roosenburg et al., (2003) reported hatching success rates of over 90% for wild terrapins in some regions of the U.S.A. Herlands et al., (2004) reported 32-50% hatching success rates for artificially incubated terrapin eggs collected from road-killed females, however they suggested that their (comparatively) lower hatching success may have resulted from fungal infection in the vermiculite-filled containers, improper functioning of their incubator, egg dehydration, rotation of the recovered eggs once they were placed on the moistened vermiculite and the time of day the terrapins eggs were retrieved. Most of these factors were not applicable to the present study; the Bermuda terrapin eggs were collected after natural deposition (rather than from road-killed females), the eggs did not experience any rotation during collection or transport, the incubator at DCS did not experience any malfunction during operation and the incubated eggs were kept moist throughout the incubation period. However, some eggs were removed from their respective containers during the course of the incubation study because of fungal infection.

Lazell and Auger (1981) and Stegmann et al. (1988) reported negative incidences of plant-animal interaction between dunegrass *Ammophila breviligulata* roots and diamondback terrapin eggs and hatchlings in the U.S.A. (e.g. penetration and entanglement). The latter reported mortality rates of 25% for terrapin hatchlings that developed in grassy areas of their study site, in which affected terrapins died during development or when they tried to emerge through a confining mass of roots. The penetration of bluegrass *Poa annua* roots into diamondback terrapin eggs has been observed in Bermuda (personal observations) and hatchling entanglement has now been observed (this investigation). This represents another threat to Bermuda's terrapin population in addition to those described in the diamondback terrapin recovery plan (Outerbridge, 2013b).

The daily temperatures recorded in the nests of the 2014 control group often reached, and exceeded, the 35°C+ lethal threshold for developing turtle embryos (see Cunningham, 1939). Such incidences of heat shock may be partly responsible for the observed low hatching success. Lethal incubation temperatures, however, do not appear to be a factor affecting the relatively low hatching success of the artificially incubated eggs. Temperatures recorded in the incubator during the present study were well within the tolerated thermal regime for this species (see Jeyasuria et al., 1994; Roosenburg and Kelly, 1996; Wood and Herlands, 1997). Furthermore, those temperatures were within the thermal range known to produce all male hatchlings (Jeyasuria et al., 1994). This sex bias was intentional and done in an effort to boost the number of male diamondback terrapins in Bermuda's wild population, which is known to be low (Outerbridge, 2014).

The continued monitoring of hatching success to determine long term trends, combined with the transfer of hatchlings to the mangrove swamp developmental habitat, is planned for 2015.

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Appendix

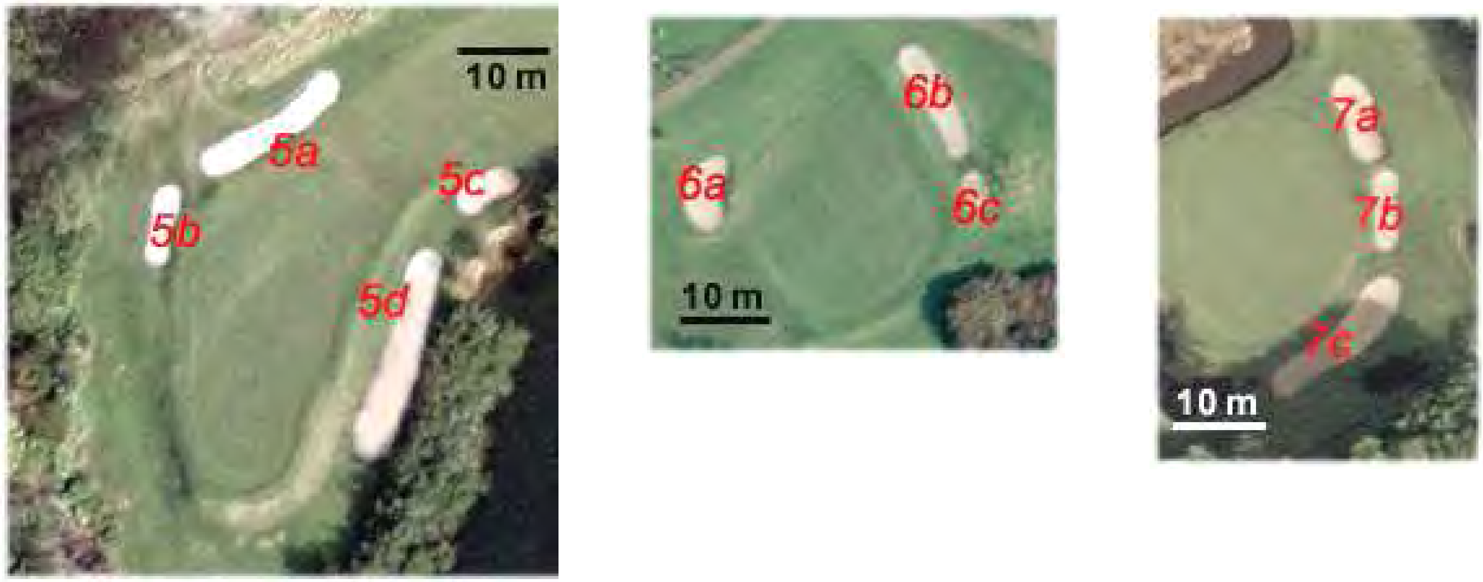

Figure 1. Aerial photographs of the sand bunkers on the 5th, 6th and 7th holes of the Mid Ocean golf course showing the alphanumeric notations assigned to each bunker.

Figure 2. Contents of a diamondback terrapin egg showing hatchling remains (bones and scutes) and grass roots.

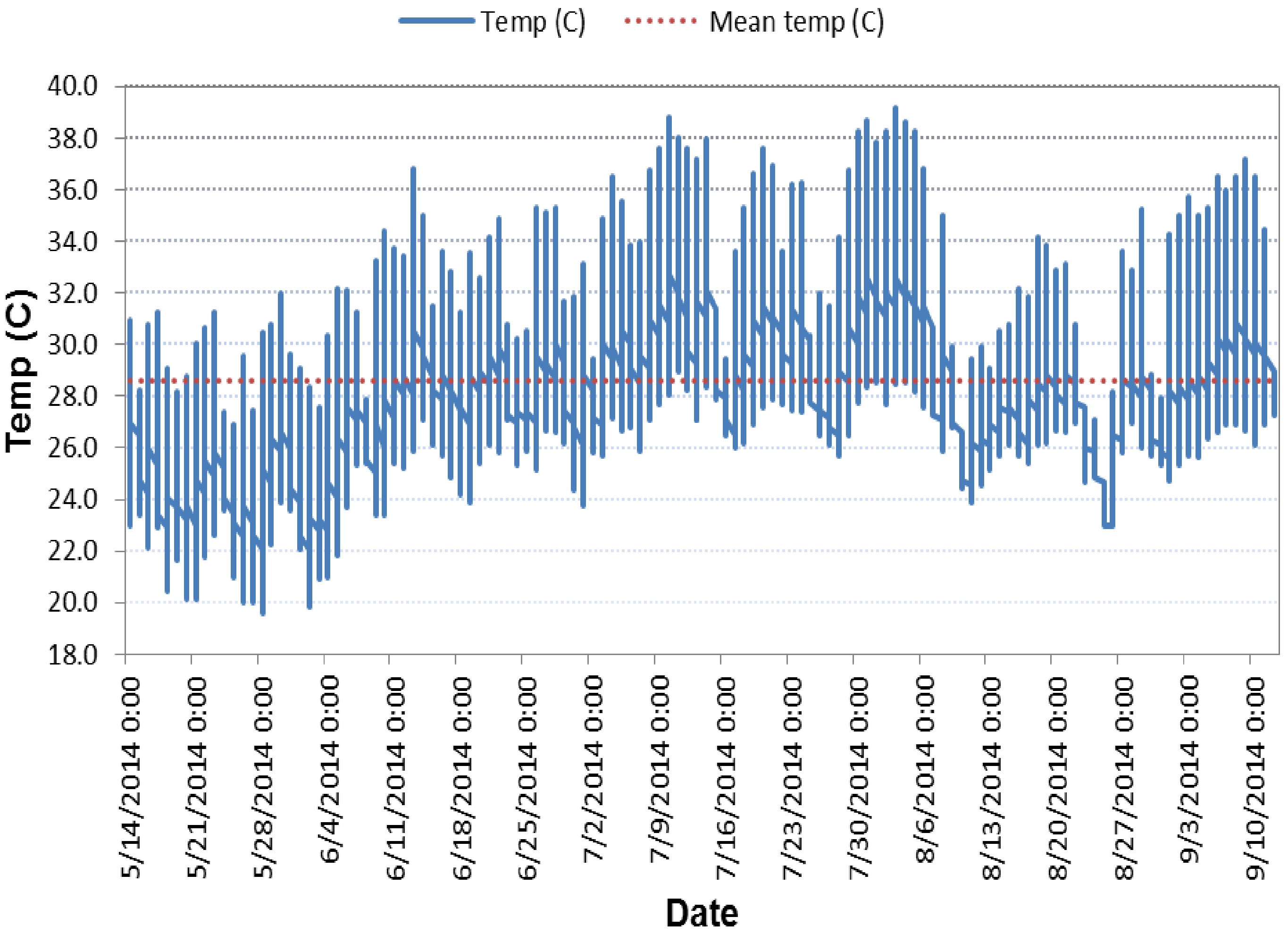

Figure 2. Graph showing the daily incubation temperatures of the control group.

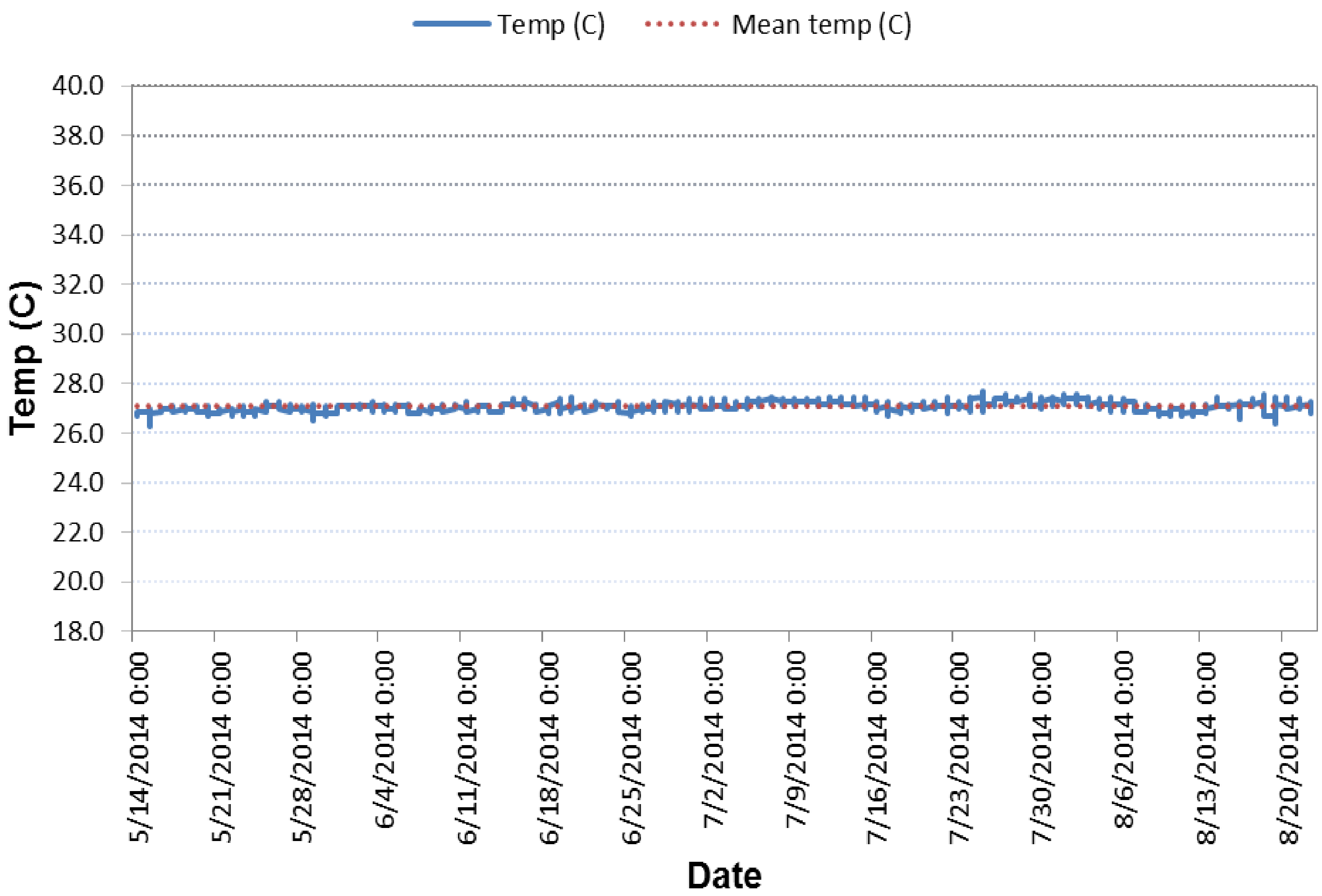

Figure 3. Graph showing the daily incubation temperatures of the incubator group.

Table 1. Summary of egg data for the control group.

Nest ID	Lay Date	# eggs	# emerged hatchlings	# dead in nest or torn shells	# un-hatched embryos	# eggs with no visible embryo	hatching success
7A1	May 10 2014	8	0	0	0	8	0%
5D1	May 13 2014	9	0	0	0	9	0%
6B1	May 13 2014	2	0	0	0	2	0%
7B1	May 15 2014	2	0	0	0	2	0%
5D2	May 19 2014	5	0	0	0	5	0%
6B3	May 23 2014	10	0	0	0	10	0%
5D4	May 28 2014	5	0	0	0	5	0%
5D5	June 2 2014	3	0	0	0	3	0%
7A6	June 4 2014	4	2	1	1	0	50%
ML canal	June 12 2014	5	0	0	0	5	0%
	TOTALS	*53*	*2*	*1*	*1*	*49*	

Table 2. Summary of egg data for the incubator group.

Nest ID	Lay Date	# eggs	# emerged hatchlings	# dead in nest or torn shells	# un-hatched embryos	# eggs with no visible embryo	hatching success
7C1	May 14 2014	3	3	0	0	0	100%
6B2	May 16 2014	5	0	0	2	3	0%
5D3	May 21 2014	7	3	0	3	1	43%
7A2	May 28 2014	6	0	0	0	6	0%
7A3	May 29 2014	1	0	0	0	1	0%
7A4	May 29 2014	7	0	0	0	7	0%
7A5	May 29 2014	7	0	0	0	7	0%
7C2	June 2 2014	8	0	0	2	6	0%
5D6	June 3 2014	6	6	0	0	0	100%
5D7	June 12 2014	8	4	0	0	4	50%
	TOTALS	*58*	*16*	*0*	*7*	*35*	