Report on the 2015 incubation efforts of diamondback terrapin *(Malaclemys terrapin)* eggs at the Bermuda Government's Department of Conservation Services

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Table of Contents

Executive summary.. 2
Introduction... 3
Methods... 3
Nest surveys and egg collection.. 3
Care of incubated eggs 3
Care of hatchling terrapins... 4
Examination of failed egg material... 4
Results 4
Control group... 4
Incubator group 5
Discussion.. 6
Literature Cited 8
Appendix 1 (Figures) 9
Fig. 1. Aerial photographs of the sand bunkers on the Mid Ocean Golf Course.…..….9
Fig. 2. Graph showing the incubation temperatures of the control group…............….10
Fig. 3. Graph showing the incubation temperatures of the incubator group……........11
Fig. 4. Road fatality of a mature female diamondback terrapin……………………...12
Fig. 5. Road fatality of a hatchling diamondback terrapin…………………………..….13
Fig. 6. Example of a diamondback terrapin crossing sign...13
Appendix 2 (Tables)……………………………………………………...…….……14
Table 1. Summary of the egg data for the control group………….………..……….14
Table 2. Summary of the egg data for the incubator group…………….……………14

Executive summary

The investigation into the hatching success of wild diamondback terrapin eggs versus incubated terrapin eggs continued in Bermuda during 2015. Previous studies have shown that there is a very low hatching success rate for this species in Bermuda (Outerbridge, 2012, 2013, 2014a, 2014b) 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 not a significant difference in the hatching success rates between the clutches of eggs that were left in the wild (14.5%) versus those that were placed within an incubator (19%) at the Bermuda Government's Department of Conservation Services. A total of 19 hatchling terrapins (11 from the incubator group and eight from the control group) were produced from the 21 monitored nests in 2015. Fourteen were released within the mangroves surrounding Mangrove Lake at the conclusion of this study and five were released in the same habitat surrounding Trott's Pond. The road fatality of three large diamondback

terrapins was also recorded in June 2015, in addition to the continued documentation of hatchling death from this source of mortality.

Introduction

The artificial incubation of diamondback terrapin eggs collected from the wild was first attempted at the Bermuda Aquarium Museum and Zoo in 1994 and then resumed annually beginning in 2012 at the Department of Conservation Services (DCS) in an effort to increase the annual hatching success observed in Bermuda's native 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 (Outerbridge, 2012; 2013; 2014b).

Methods

Nest surveys and egg collection

Nesting surveys were performed twice daily (between 09:00 and 10:00 and then again between 16:00 and 17:00) between Monday and Friday in the sand bunkers on the 5th, 6th and 7th holes of the Mid Ocean golf course from June 17th to July 8th, 2015. Encountered nests were haphazardly divided into two groups; eleven control clutches and ten 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 Appendix 1).

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 two 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 5th hole. One data logger was also installed inside the incubator to log the daily temperatures of the incubator group. Both were deployed on June 18th and collected on September 14th (incubator) and on September 25th (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 eleven nests that comprised the control group were all located between June 17th and June 29th. Seven clutches were monitored in the sand bunkers on the 5th hole, one clutch was from a bunker on the 6th hole and three clutches were from bunkers on the 7th hole. Excavation of all 11 control nests (i.e. 6A1, 5D1, 5D2, 7A1, 7A2, 5C1, 5D4, 5D5, 5D6, 7C1, 5D7) occurred on September 25th 2015, between 88 days and 100 days after egg deposition. A total of 62 eggs were found, of which only nine (14.5%) successfully produced hatchlings – one of which was found dead at the time of excavation (scutes and skeletal remains tangled within a grass root ball). Forty eggs (64.5%) showed no visible evidence of embryonic development, 13 eggs contained dead embryos in various stages of development (five appeared to be in stages 9-17 and eight appeared to be in stages 18-25 following Yntema's classification (1968)). Only two clutches in the control group produced hatchlings, and all were found within their respective nest chambers at the time of excavation. See Table 1 in Appendix 2 for a full summary of the egg data.

Eight hatchlings were released onto the mangrove leaf litter between the prop roots at the water line; three hatchlings at Mangrove Lake and five at Trott's Pond. Only one (12.5%) had carapace scute abnormalities; an extra vertebral scute and an extra costal scute.

The incubation temperatures for the control group ranged from 24.4-38.4°C (mean 30°C; SD 2.6°C) between June 18th and September 25th 2015 (Figure 2 in Appendix 1). The incubation period was not monitored.

Incubator group

A total of 58 eggs were found within the ten nests that comprised the incubator group (i.e. 5A1, 5D3, 7A3, 7A4, 6B1, 7A5, 6B2, 7A6, 7C2, 7A7). Two clutches came from the sand bunkers on the 5th hole, two clutches came from a bunker on the 6th hole and six clutches came from bunkers on the 7th hole. All were discovered between June 18th and July 8th. Of those 58 eggs, 11 (19%) developed into hatchlings. Forty-two eggs (72.4%) showed no visible evidence of embryonic development and five (8.6%) contained dead embryos in various stages of development. Of the five dead embryos, one appeared to be in stages 1-8 and four appeared to be in stages 18-25. Five clutches (50%) in the incubator group produced hatchlings. The overall hatching success rate for this group was 19%. See Table 2 in Appendix 2 for a full summary of the egg data.

The 11 hatchlings were taken to Mangrove Lake and released onto the mangrove leaf litter between the prop roots at the water line. Three of these terrapins (27.3%) had abnormal carapace scute patterns, which included extra marginal scutes (n = 1), extra vertebral scutes (n = 2) and extra costal scutes (n = 1). Two of the three individuals came from the same clutch.

The temperatures for the incubator group ranged from 26.3-28.8°C (mean 27.3°C; SD 0.4) between June 18th and September 14th 2015 (Figure 3 in Appendix 1). The incubation period ranged from 62 – 67 days (mean 64.3 days, n = 5 clutches). This is consistent with previous studies (Outerbridge, 2014a; 2014b).

Discussion

The results of the 2015 incubation study did not show 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 is consistent with the results of the 2013 incubation study (Outerbridge, 2013) but differs from those undertaken in 2012 and 2014 (Outerbridge, 2012; 2014b).

The daily temperatures recorded in the nests of the control group often reached, and exceeded, the 35°C+ lethal threshold for developing turtle embryos (see Cunningham, 1939). While such incidences of heat shock may be partly responsible for the observed low hatching success of the eggs in the control group, lethal incubation temperatures 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).

Environmental contamination is also thought to contribute to the low hatching success of this species on Bermuda. Total petroleum hydrocarbons, polycyclic aromatic hydrocarbons and a variety of heavy metal residues (arsenic, cadmium, chromium, copper, iron, lead, nickel, zinc and mercury) were found in fresh whole diamondback terrapin eggs, aquatic gastropods (which are a primary food source for the terrapins) and benthic sediment from the pond environments inhabited by the terrapins. Some of these contaminants are known to have mutagenic and teratogenic effects at the observed concentrations. Bermuda's diamondback terrapins evidently live and feed in wetland habitats characterized by chronic, multifactorial contamination that renders their main food source potentially dangerous to consume (see Outerbridge, 2014a).

The penetration of plant roots into diamondback terrapin eggs and the trapping effect it has upon hatchlings has been previously observed in Bermuda (see Outerbridge, 2014b) and is a recognized source of (limited) predation within some terrapin populations in the U.S.A.

In September 2015, the LookBermuda Media Company filmed the release of the hatchlings into the mangrove swamp. This company has also expressed the desire to film a diamondback terrapin hatching from an egg in 2016.

Between 2012 and 2015, 68 hatchlings from the incubator were released within the mangrove leaf litter surrounding Mangrove Lake (*n* = 29, 2012; *n* = 12, 2013; and *n* = 16, 2014; *n* = 11, 2015). The mean incubation temperatures recorded during each year was 27.5°C (2012), 28.8°C (2013), 27.1°C (2014) and 27.3°C (2015). Given that incubation temperatures of 24-27°C reportedly produce male hatchlings (Jeyasuria et al., 1994; Roosenburg and Kelly, 1996) and the temperature range 28.5-29.5°C produces mixed sexes for this species (Jeyasuria et al., 1994; Roosenburg and Place, 1994), the hatchlings from 2012, 2014 and 2015 were considered to be male while the hatchlings from 2013 were considered to be a mixture of both sexes. The decision to incubate the majority of the eggs at male producing temperatures was made in an effort to boost the number of male diamondback terrapins in Bermuda's wild population, which was recorded to be only 22 (Outerbridge, 2014a).

On June 16th 2015 a mature terrapin was encountered as roadkill on the cart path adjacent to the NE portion of Mangrove Lake (Figure 4 in Appendix 1). This terrapin had sustained massive injuries to the carapace and plastron and was found, upon closer inspection, to be carrying eggs - all of which had been broken as a result of the injury. Road fatalities have been recorded in the past from Bermuda for this species; crushed hatchlings (Figure 5 in Appendix 1) were reported in 2009, 2010, 2012 and 2015 (G. Hutchings, pers. comm.; M.O. pers. obs.) and in June 2015 two crushed adult terrapins were found, on separate occasions, on Harrington Sound Road close to the junction of Paynter's Road (T. Neijmeijer, pers. comm.) While these numbers are low, it is worrying that valuable adults are being removed from a population which is already characterized by a very low number of individuals (Outerbridge, 2014a). To address this issue, the Mid Ocean Club board of directors has been asked to consider the idea of installing cautionary signs at selected locations advising motorists to drive slowly because of terrapins in the area (Figure 6 in Appendix 1). Furthermore, they are considering the option of installing speed bumps on selected cart paths.

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Appendix 1.

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.

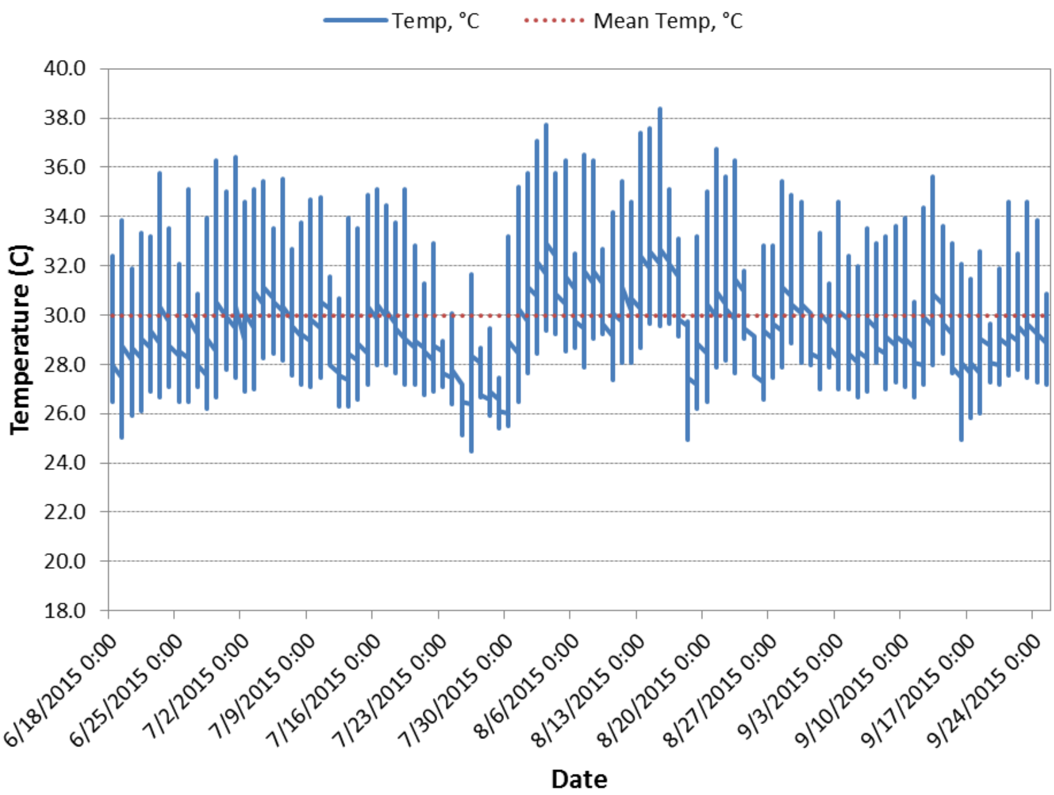

Figure 2. Graph showing the daily incubation temperatures of the control group.

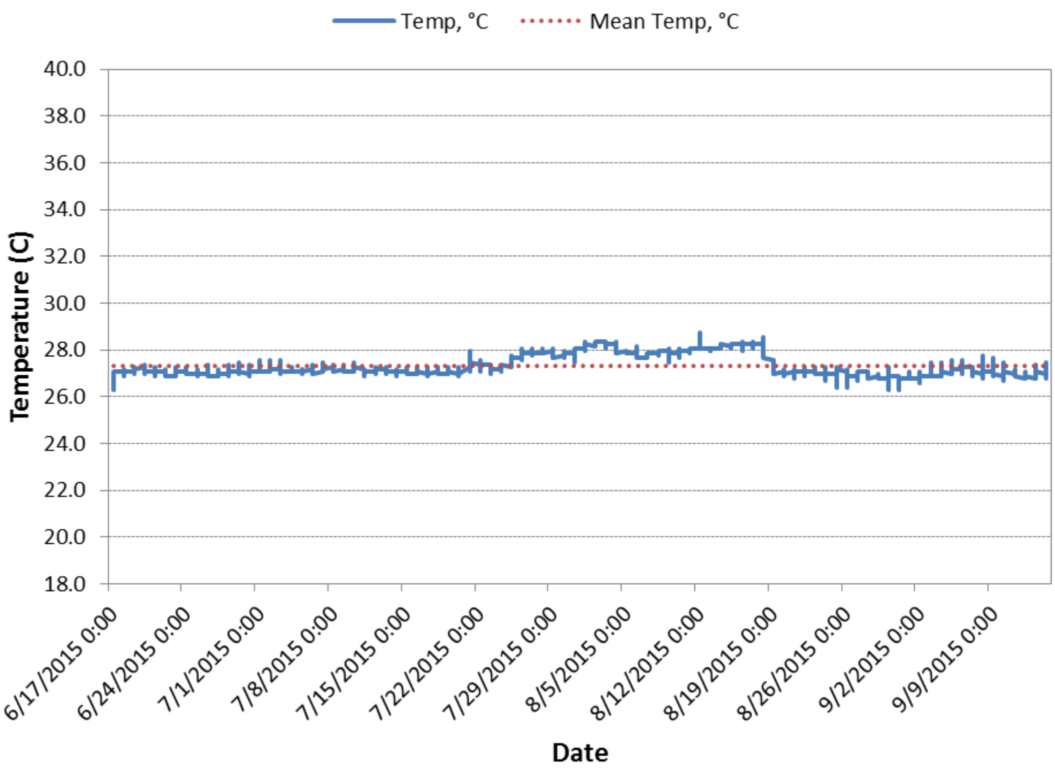

Figure 3. Graph showing the daily incubation temperatures of the incubator group.

Figure. 4. Road fatality of a mature female diamondback terrapin on a cart path adjacent to Mangrove Lake at the Mid Ocean golf course.

Figure. 5. Road fatality of a hatchling diamondback terrapin on a cart path adjacent to South Pond on the Mid Ocean golf course.

Figure. 6. Example of a diamondback terrapin crossing sign that is being considered for installation at selected locations on the Mid Ocean golf course.

Appendix 2.

Table 1. Summary of egg data for the control group (n=11 clutches).

Nest ID	Lay Date	# eggs	# emerged hatchlings	# dead in nest or torn shells	# un-hatched embryos	# eggs with no visible embryo	hatching success
6A1	unknown	8	5	0	2	1	62.5%
5D1	unknown	8	3	1	3	1	37.5%
5D2	unknown	6	0	0	0	6	0%
7A1	June 22	5	0	0	0	5	0%
7A2	June 22	7	0	0	0	7	0%
5C1	June 22	7	0	0	7	0	0%
5D4	June 23	5	0	0	1	4	0%
5D5	June 25	2	0	0	0	2	0%
5D6	June 26	4	0	0	0	4	0%
7C1	June 29	7	0	0	0	7	0%
5D7	June 29	3	0	0	0	3	0%
	TOTALS	*62*	*8*	*1*	*13*	*40*	***14.5%***

Table 2. Summary of egg data for the incubator group (n=10 clutches).

Nest ID	Lay Date	# eggs	# emerged hatchlings	# dead in nest or torn shells	# un-hatched embryos	# eggs with no visible embryo	hatching success
5A1	June 18	5	0	0	0	5	0%
5D3	June 18	6	2	0	4	0	33.3%
7A3	June 22	9	4	0	1	4	44.4%
7A4	June 23	5	1	0	0	4	20%
6B1	June 24	3	2	0	0	1	66.7%
7A5	June 24	6	0	0	0	6	0%
6B2	June 30	5	0	0	0	5	0%
7A6	June 30	4	0	0	0	4	0%
7C2	July 3	8	0	0	0	8	0%
7A7	July 8	7	2	0	0	5	28.6%
	TOTALS	*58*	*11*	*0*	*5*	*42*	***19%***