

The Day 53 Experiment:

**Using late-term, artificial incubation to increase hatchling production in
leatherback sea-turtle (*Dermochelys coriacea*) nests**

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Abstract

In the 2006 nesting season of leatherback sea turtles (*Dermochelys coriacea*) on Sandy Point National Wildlife Refuge, St. Croix, U.S. Virgin Islands, late-term, artificial incubation was used to increase hatch success. Around day 50 of development, all live eggs in a clutch were extracted from their beach location, placed in Styrofoam boxes and incubated in the lab until pipping. Approximately 24 hours after initial signs of hatching, boxes were excavated according to traditional management protocols. Hatchlings were released on Sandy Point the evening following excavation. Full term hatch success was determined as a percentage of eggs that produced viable, live hatchlings divided by the total full term eggs in the box. Comparisons of day 53 hatch success data and naturally incubated nest data were made. Late-term artificial incubation was found to significantly increase hatch success of the leatherback. Hatchling production was also significantly increased. Labor, time and cost were not determined to adversely hamper a large-scale, day 53 installation. With further study, this technique may prove a significant advance in permanently increasing hatch success of leatherbacks and provide an opportunity to determine possible factors responsible for late-term mortality.

1. Introduction

Leatherback sea turtles exhibit significantly lower hatch success (50-55% on average) when compared to other sea turtle species (Whitmore and Dutton 1985). Sandy Point National Wildlife Refuge (SPNWR) typically produces a mean annual hatch success range of 40-60%, with a trend of decreasing hatch success since 1992 (Fig. 1) (Garner *et al.* 2006). The 2005 season exhibited the lowest hatch success in the past 25 years of the leatherback conservation project's history, at only 40.28% (Garner *et al.* 2005). Significant portions of this embryonic mortality occur in late developmental stages (Garner *et al.* 2005; Eckert and Eckert 1990). Late-term mortality remained a factor in 2006 with an average of 18 late term mortalities (12 full-term pipped and 6 full-term unpipped dead) occurring per nest (Garner *et al.* 2006).

High hatchling production remains a vital source in preventing a decline in adult recruitment (Dutton *et al.* 2005). The continuing decline in hatch success due to late-term embryonic mortality (Garner *et al.* 2005) may ultimately threaten the adult population of leatherbacks at Sandy Point. The "day 53" experiment was originally conceived in the 1990's to determine if artificial incubation of live eggs after day 53 had the potential to minimize late-term mortality, thus increasing the hatch success of the leatherback turtle (Dutton, *pers.comm.*). If successful, late term artificial incubation could serve as a conservation strategy for maximizing hatchling production at Sandy Point. In an attempt to bolster the declining hatch success observed since 1992 (Garner *et al.* 2005), the day 53 experiment was re-implemented in the 2006 nesting season. Nests selected for the experiment were excavated around day 50 of natural incubation and all live eggs placed in Styrofoam boxes to complete their development artificially. The day of extraction was altered from the previous standard of 53 days to ensure that the live eggs had only

recently transitioned from stage 28 (mid-embryonic development) to stage 29 (late-embryonic development) (Miller 1985; Whitmore and Dutton 1985). Despite this change, the original name of the experiment was maintained.

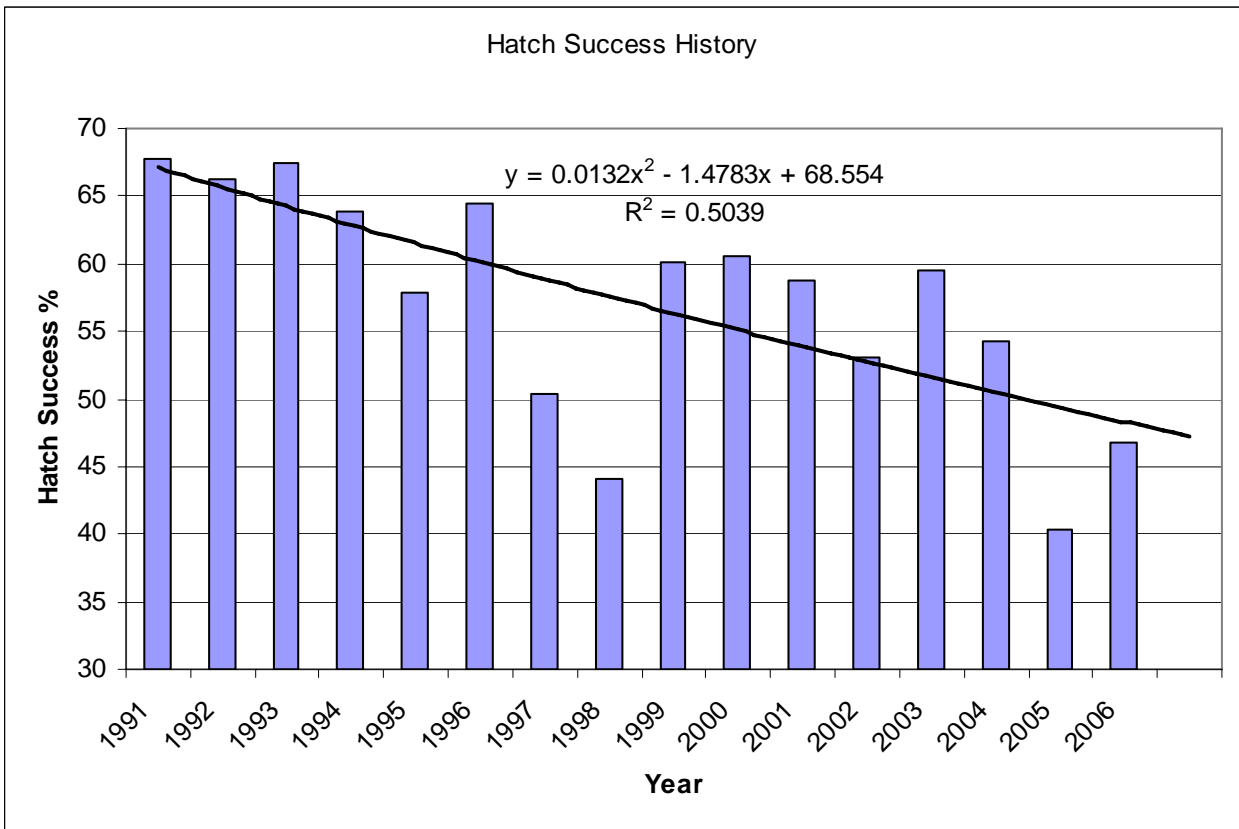


Figure 1. Hatch success history from the 1991 to 2006 nesting seasons (from Garner *et al.* 2006).

Since its conception in the 1990's, the day 53 experiment has been performed once, although with limited numbers of nests and unpublished results. In the first day 53 experiment conducted under Donna Dutton, the hatch success of artificially incubated eggs reportedly increased to between 90% and 100% of full-term eggs (Dutton, unpublished data).. Despite potentially favorable results, the experiment demanded considerable time and labor combined with physically intense conditions. These factors contributed to a lack of interest in installing a large-scale day 53 experiment. However, acquiring conclusive data remained imperative for (1) determining whether

this project has the potential to increase overall hatch success; and (2) assessing the feasibility of implementing a day 53 program as part of established management protocols.

Three main objectives were evaluated during the course of this study. The first objective evaluated the potential for maximizing late term hatch and emergence success. Late-term, artificial incubation was hypothesized to produce an average hatch success between 90-100% and an emergence success close to 100%. As a result, hatchling production was expected to increase significantly when compared to naturally incubated nests. Secondly, large-scale, artificial late-term incubation was evaluated in terms of labor time and effort required to produce the desired results. Evaluation of these factors will determine the feasibility of implementing day 53 as a future strategy for resource management and leatherback conservation policies. Lastly, the viability of using day 53 excavations as an emergency management strategy in cases of extreme beach weather conditions and erosion was also investigated.

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2. Methods

2.1. Study Site

All nests used for this experiment were located between stakes 169-195, at Sandy Point National Wildlife Refuge on St. Croix, U.S. Virgin Islands. Extraction of day 53 nests began in late July of the 2006 nesting season.

2.2. Nest Selection

A strict criterion was developed to select nests in order to maximize effectiveness of the experiment. All data used for these criteria was found in the St. Croix Sea Turtle Database (WIMARCS[®]) and included the following: (1) Nests extracted were between days 48-53 of incubation. (2) The number of eggs in the nest was greater than 70. In the case of *in situ* nests where an egg count was unavailable, the mother's egg count history was evaluated. Historically "low egg" mothers were not chosen. (3) Hatch success of previous nests from the 2006 season were evaluated for each potential candidate. Only mothers with a history of low undeveloped and unfertilized egg counts (<15%) were chosen. (4) Location on the beach was initially assessed to ensure a balanced geographic distribution of nests; however, this was not possible because the majority of nests that met the criteria were located between stakes 169 and 175. Relocated nests were preferred over *in situ* nests since they tend to have significantly lower hatch success (Garner *et al.* 2005).

2.3. Extraction Procedure

Before beginning the nest extraction, a candling station was set up within arm's distance of the nest site. The candling station consisted of an inverted Styrofoam box, coated with non-toxic black paint that prevented light from penetrating the box. This box will be referred to as the "black-box". Two small eyeholes were carved out of the "top" of the black-box, while the side contained a hole large enough to place one hand (with flashlight) through. Wet sand was placed underneath the box in order to avoid contact of the extracted egg with hot surface sand. This

candling station was only required if nest extraction occurred before nightfall. A black box is not required for night-time candling.

Clean Styrofoam boxes were used as artificial incubators. These boxes were prepared by first punching holes throughout the length and width of the box's underside. An inch deep layer of damp sand taken from the overburden of the nest was packed evenly in the bottom of the box. The nest was then hand dug (in lieu of using a small plastic "scoop" shovel typically used for excavations) so as not to inadvertently damage any eggs. When the top layer of eggs was reached, a 24-gauge Cu/Cn wire thermocouple was inserted into the natural nest and initial nest temperature measured using a BAT-12 (Physiotemp) thermocouple meter. Each egg was removed individually from the nest and kept in the same orientation to minimize any possible negative effects of handling (Chan, 1985). Extracted eggs were passed from the nest into the candling station within 10 seconds of removal. Inside the candling station, each egg was candled using a high-intensity Maglite® beam to determine the viability of the embryo. The presence of clearly defined blood vessels inside the egg indicated a live embryo. Eggs that glowed yellow when candled were considered dead and not put in the incubation box. When the viability of an egg could not be determined by candling, other methods were used. The presence of calcium mobilization (Bilinski *et al.* 2001) and a rough texture on the outside of the eggshell indicated a full term embryo. On occasion, movement of the live embryo through the shell aided in determining viability. When the viability of the egg could not be determined conclusively, it was included with the live eggs. Dead eggs were opened and staged to determine level of embryonic development if any (Whitmore and Dutton, 1985).

Live eggs were closely packed into the prepared Styrofoam incubator box without denting nearby eggs. Each layer within the box contained between 9 and 12 eggs. When three layers of

eggs were complete, the thermocouple probe was placed in the center. When a total of 45 eggs were in the box, 2-3 paper towel sheets were thoroughly wetted with freshwater and placed evenly over the top egg layer. Another inch of damp sand was then placed over the paper towel layer without allowing any sand to be in contact with the egg surface. The thermocouple housing remained on top of the damp sand for easy reading access. A Styrofoam lid was placed on top of box. The box was then labeled with the mother's original tag ID, date laid, date extracted and number of eggs in the box. If additional viable eggs remained in the original nest, the above procedure was repeated for a second box containing the remaining eggs.

2.4. Emergency strategies

Occasionally, some viable eggs (<1%) were inadvertently broken during extraction. In this case, the broken egg was removed from the nest carefully and quickly to avoid blood vessel breakage and fluid loss. The egg was completely wrapped in 2 layers of wet paper towels and kept in the cool darkness of the black box. After the nest box was completely extracted, any broken eggs were placed on the top layer of damp sand and their paper towels kept moist.

Upon excavation, some (< 7 %) hatchlings had visible maggots crawling in their yolk sac and other orifices. The affected orifice of the hatchling was rinsed with freshwater running perpendicular to the orifice for about 5 minutes to draw the maggots out. Betadine[®] (Povidone Iodine solution) was then liberally applied to the affected area. After treatment, these hatchlings were kept separate from unaffected hatchlings until release.

2.5. Nest Maintenance

An enclosed room with ventilation to the outdoors was used as the egg lab. Wooden shelves were built into the walls with a 1 x 1 cm² wood block running along the back length of the shelves. Before nest extractions began, the suitability of this room's temperature flux was monitored for approximately two weeks using a temperature data logger. The egg room proved to be a suitable incubation environment as it did not exhibit extreme temperatures (<25°C or >32°C) that may be detrimental to developing eggs and/or hatchlings (Garner 2006). Incubation boxes were transported via truck from the beach to the egg room. The boxes were placed on the shelves so that the back of the box was elevated at least 10° above the shelf plane. Styrofoam drainage trays were placed underneath the boxes to collect eluting fluids. Boxes were misted daily with enough fresh water to keep the top layer of sand damp. Temperature readings using the BAT-12 gauge were also taken daily at the same time. Four nests were monitored for temperature to ensure that the temperature range of day 53 nests fell within the acceptable range of 25°C to 32°C for nest boxes (Garner 2006).

2.6. Nest Excavation

When fluid was observed draining from the boxes, it was assumed that pipping had begun, and date of initial hatching was recorded. Usually within two days, several hatchlings began to emerge and the boxes were promptly excavated. Hatchlings and eggs were categorized and separated into four groups. Hatchlings with little or no yolk-sac were placed in a separate box filled with an inch of fresh sand and covered with wet paper towels until release on Sandy Point. Release occurred at dusk of the same day. Hatchlings with un-absorbed yolk-sacs were kept in a second container filled with a layer of fresh, damp sand, and covered with a moist towel until their yolk sacs were fully absorbed and they were ready for release. Pipping eggs were kept in a third,

separate container layered with fresh sand. These eggs often had to be turned around and placed with the hatchling head facing upwards in the box. Wet paper towels also covered these eggs to avoid desiccation. A clean Styrofoam box with holes punched out of the bottom and a fresh layer of damp sand was prepared for any unhatched, live eggs. These eggs were carefully moved to their new box and a damp paper towel placed over them. Any dead eggs in the box were opened and staged per Whitmore and Dutton, 1985.

2.7. Data Analysis

Total hatch success and full term hatch success of each day 53 nest was calculated using the following equations:

$$(1) \quad \% \text{ Total Hatch Success} = \frac{H}{T}$$

$$(2) \quad \% \text{ Full Term Hatch Success} = \frac{H}{FTP + FTU + H}$$

Where T stands for total number of eggs laid at oviposition and H equals hatched shells. FTP stands for full-term eggs pipped (dead), and FTU stands for full-term unpipped (dead).

Total emergence success was calculated using similar equations and variables:

$$(3) \quad \% \text{ Total Emergence Success} = \frac{H - D - L}{T}$$

$$(4) \quad \% \text{ Full Term Emergence Success} = \frac{H - D - L}{FTP + FTU + H}$$

Where D stands for dead hatchlings found in the nest, and L stands for live hatchlings in the bottom of the nest.

Identical equations were used to determine total hatch and emergence successes for all leatherbacks with data available in the 2006 season (Garner *et al.* 2006).

Significance of data acquired was determined using Student's t-tests. A significance level of $P < 0.05$ was deemed to be significant.

3. Results

A total of 24 nests were selected for use in the day 53 experiment. Upon excavation, four nests contained zero viable full term eggs and two nests were not found. Additionally, 5 nests contained less than 25 live full term eggs and were therefore not included in the statistics. These nests were, however, included in hatchling production totals. The remaining 13 nests met the appropriate criteria needed for effective statistical analysis. Summary data regarding hatch success and hatchling production of day 53 nests is represented in Tables 1 and 2.

A total of eighteen day 53 nests successfully released 783 hatchlings. These hatchlings accounted for approximately 6.73% of total hatchling output of Sandy Point in 2006, despite the 18 nests accounting for only 4.83% of total nests this season¹. Nests incubated on the beach in 2006 produced an average of 34.87 ± 16.44 hatchlings per nest, whereas day 53 nests produced an average of 43.55 ± 23.57 hatchlings per nest. The average number of hatchlings produced per day 53 nest was not significantly greater when compared to all nests incubated naturally, or *in situ* nests only ($P = 0.072$ and $P = 0.24$ respectively). However, the average number of hatchlings

¹ Based on calculations of 783 day 53 hatchlings / 11, 638 total hatchlings produced in 2006; and 18 day 53 nests / 373 total nests

produced per day 53 nest *was* significantly greater when compared to relocated nests (43.55 ± 23.57 versus 30.19 ± 15.00 hatchlings per nest, $P = 0.016$).

Total hatch success of the day 53 nests ($62.78\% \pm 20.1\%$) was significantly higher ($P = 0.0127$) than total hatch success for all nests incubated at Sandy Point ($46.83\% \pm 24.57\%$), as was total emergence success of nest boxes compared to all Sandy Point nests ($61.73\% \pm 20.32\%$, $P = 0.0048$). Full term hatch success for the Day 53 nest boxes ($88.59\% \pm 12.2\%$) was also significantly higher than the full term hatch success of Sandy Point nests ($68.01\% \pm 26.73\%$, $P < 0.001$). Full term emergence success of nest-boxes ($87.32\% \pm 14.30\%$) also proved significantly higher than nests hatched naturally ($66.11\% \pm 26.73\%$, $P < 0.001$).

No significant difference between the use of one box and two boxes for incubation was observed ($P = 0.0516$). The difference between emergence success of nests with greater than 70 eggs and those with less than 70 eggs within the total nesting population was not found to be significant ($P = 0.409$). There was, however, a significant difference between emergence success of nests incubated naturally (with greater than 70 eggs), and emergence success of day 53 nests (that contained greater than 70 eggs) but were excavated late term and incubated artificially ($P < 0.001$).

Temperatures monitored in a sub-sample of boxes were consistent with those obtained by Garner (2006), and fell within the acceptable range of 25°C to 32°C for incubating eggs. Temperatures fluctuated approximately $1\text{-}1.5^{\circ}\text{C}$ per day.

Table 1. Summary statistics of hatch and emergence success used in statistical analysis

Original Tag #	Date Laid	Date Emerged	Day Extracted	Day hatched	(1) % Total Hatch Success	(2) % Full Term Hatch Success	(3) % Total Emergence Success	(4) % Full Term Emergence Success
XXZ053	6/5/06	8/02/06	53	58	85.00%	97.14%	85.00%	97.1%
AAC275	6/8/06	8/02/06	50	55	62.22%	68.29%	57.78%	63.4%
MK82	6/9/06	8/01/06	51	53	51.76%	63.77%	47.06%	58.0%
MK31	6/10/06	8/04/06	50	55	50.93%	75.34%	48.15%	71.2%
AAR914	6/11/06	8/04/06	49	55	83.53%	98.61%	83.53%	98.6%
AAR577a	6/12/06	8/07/06	48	56	50.96%	98.15%	50.96%	98.1%
AAV651	6/13/06	8/06/06	49	54	66.67%	85.29%	66.67%	85.3%
AAG996	6/15/06	8/07/06	50	53	89.16%	98.67%	89.16%	98.7%
MJ82	6/17/06	8/11/06	49	55	88.76%	97.53%	88.76%	97.5%
PPQ148	6/20/06	8/15/06	49	56	56.34%	93.02%	56.34%	93.0%
AAR577b	6/21/06	8/18/06	49	58	24.32%	90.00%	24.32%	90.00%
AAG906b	6/24/06	8/23/06	49	61	65.82%	91.23%	64.56%	89.5%
MK56	7/01/06	8/26/06	50	56	40.70%	94.59%	40.23%	94.60%
		Mean	49.69	55.77	62.78%	88.59%	61.73%	87.32%
		Standard Deviation	1.25	2.20	20.1	12.2	20.32	14.30
		Min	48	53	22.5%	63.8%	24.32%	58.0%
		Max	53	61	89.2%	98.7%	89.16%	98.7%
		Median	49	55	62.2%	94.6%	59.03%	91.51%

Table 2. Summary of hatchling production for day 53 nests used in statistical analysis.

Original Tag #	Date Laid	Date Emerged	Full Term Eggs	Dead Hatchlings	Hatched Shells	Full Term Pipped (Dead)	Full Term Unpipped (Dead)
XXZ053	6/5/2006	8/02/06	70	0	68	2	0
AAC275	6/8/2006	8/02/06	82	4	56	25	1
MK82	6/9/2006	8/01/06	69	4	44	21	4
MK31	6/10/2006	8/04/06	73	3	55	4	14
AAR914	6/11/2006	8/04/06	72	0	71	0	1
AAR577a	6/12/2006	8/07/06	54	0	53	1	0
AAV651	6/13/2006	8/06/06	68	0	58	7	3
AAG996	6/15/2006	8/07/06	75	0	74	0	1
MJ82	6/17/2006	8/11/06	81	0	79	1	1
PPQ148	6/20/2006	8/15/06	43	0	40	2	1
AAR577b	6/21/2006	8/18/06	30	0	27	3	0
AAG906b	6/24/2006	8/23/06	57	1	52	4	1
MK56	7/1/2006	8/26/06	37	0	35	0	2
		Total	811	12	712	70	29

4. Discussion

4.1. Eggs and Egg Handling

The possibility of stress on eggs during extraction and transport is a concern. Handling of sea turtle eggs late in development may not be as sensitive a factor as in early development (Limpus *et al.* 1979; Chan *et al.* 1985) but care was taken not to alter egg orientation. However, eggs late in development may be sensitive to additional effects of nest pressure, atmospheric pressure and egg air cell pressure, as seen in other shelled embryos such as ostriches (Meir and Ar 1990). During extraction of all nests, a variety of egg sizes and shapes were observed. Some eggs were misshapen with a bloated appearance. On occasion, some of these bloated eggs spontaneously split open when touched. Others split open simply when they were uncovered. If substantial blood vessel breakage was avoided when the split occurred and the egg promptly wrapped in wet paper towel sheets, these eggs often survived to hatching. They were, however, more susceptible to fly and maggot invasion in this state. Egg-splitting incidences did provide some insight into the developmental orientation of late term embryos. In most cases, the split occurred dorsally along the eggshell. The hatchling embryo head was always facing downward and carapace oriented upwards. A similar state was observed in drowned, full-term pipped embryos. Embryo orientation, or failure to adjust position after pipping, could therefore be another factor in late-term mortality. Another interesting factor regarding this particular type of egg is that they showed very little calcium mobilization from the eggshell and were difficult to candle effectively. Often, undeveloped or early-term dead eggs looked very similar to these bloated eggs. These eggs tended to be found in the top layer of eggs in the natural nest.

The “traditional” live egg was rounder with significant calcium attrition observed on the eggshell. These eggs were often indented in points of contact with surrounding eggs. When

extracted and placed in the box, they tended to “undent” themselves within 15 minutes. Attempts were made to mark these different egg types and correlate them with hatch success, but time and labor constraints of the project prevented a full investigation.

Transport along the extremely rough Sandy Point road could not be avoided. It would be difficult to assess whether this transportation has effects on hatch success, although the high hatch success observed in day 53 eggs suggests that late term eggs are less susceptible to the impacts of movement. Early term eggs, which are more susceptible to handling, such as those used in the 2006 maternal impact study may be more significantly impacted by the jarring transport through Sandy Point (Limpus *et al.* 1979; Chan *et al.* 1985; Garner 2006). Conclusive data, however, is not available to determine the degree of impact that transport may have in either of these circumstances. Significant care in terms of slow driving must be maintained when transporting eggs in order to minimize possible negative effects.

Maggots and flies were encountered in a small percentage of hatchlings/nest boxes. Maggots tended to infest broken eggs and hatchlings with yolk sacs. Consistent removal of egg fluids and washing fluid trays, as well as prompt excavation of nests is required. Additionally, wiping the inside of a nest box once the presence of flies was observed helps remove any fly eggs and minimize the potential for future problems. Many eggs that were affected occurred in nest boxes with poorer hatch success (i.e. a higher number of dead eggs). Any affected hatchlings were successfully treated and disinfected as previously mentioned. Ensuring that all full term eggs and hatchlings with yolks sacs are placed in a fresh box with fresh sand after excavation minimizes this issue. Fungal problems were not observed and therefore were not an issue in affecting hatch success as in past incubation studies (Phillott 2002). Most eggs going into the box were free of a visible fungal presence. Hatched shells and dead eggs developed fungus covering similar to that

found in natural nests, though less prevalently than in natural nests (*pers. observ.*). Although it may be impossible to completely eliminate flies and/or fungus, strict disinfectant techniques for maintaining the egg lab such as described in Phillott (2002) should be adhered to in future studies.

4.2 Hatchlings and Hatch Success

Previous efforts to artificially incubate sea turtle eggs as a conservation strategy have been largely abandoned to due to the potential to disrupt a nesting rookery's natural sex-ratio (Schwartz *et al.* 1980; Ruiz *et al.* 1981; Mrosovsky 1982; Dutton *et al.* 1985). However, day 53 incubation occurs after the pivotal sex-determining period (Bull 1980; Standora and Spotila 1985; Dutton *per. comm.*) thus preserving the natural sex-ratio of hatchlings produced by day 53 nest boxes. Without the complication of sex-determination, the day 53 experiment's value as a management protocol can be evaluated solely in terms of maximizing the hatch success of leatherback nests, hatchling production and time/labor benefits.

The day 53 experiment fulfilled its goal of raising hatch success of leatherback nests. Though full-term hatch and emergence success did not rise to the expected range of 90-100%, the rise to $88.59\% \pm 11.99\%$ and $87.32\% \pm 14.05\%$ respectively, made a significant difference in hatchling output. Because this experiment was largely conducted without formally established protocols, small adaptations in methods were required throughout the experiment. Two examples of this occurred in the first two nest boxes completed (AAC275 and MK82). Drainage holes in the incubation boxes were initially punched out in only one end of the box. This provided insufficient drainage for the large volume of liquid that needed to drain. Upon excavation, many of the full-term pipped hatchlings were found dead with their heads facing downwards in egg fluid, presumably drowned. This contributed to the large numbers of full term pipped and unpipped

reported in these two nests (Table 2). In response to this issue, all subsequent boxes had holes punched out throughout the bottom of the box. The large numbers of full term pipped seen in the initial nests were avoided in subsequent samples.

Some dead full-term pipped hatchlings were also found with crushed yolk sacs. A small amount of emergence space in packed boxes of more than 70 eggs (Table 2) may have contributed to this problem as hatchlings pushed their way to the surface. Therefore, boxes were excavated within one day of initial hatchling emergence. Avoiding the socially-facilitated “rush” when the majority of the hatchlings emerge (Witherington *et al.* 1990) might have aided in providing a better hatch and emergence success observed in the first nest XXZ053, excavated one day after hatching. Delaying excavation an extra day or two, allowing for nests to “naturally emerge”, resulted in decreased hatch and emergence success. This was evidenced in nests such as AAC275 and MK82, which were excavated approximately 2-3 days after hatching and experienced decreased hatch success.

In six of the 13 nests used, eggs were portioned among two boxes with a maximum of 45 eggs per box. This method of splitting nests was employed in order to decrease the potential for full term mortality in nest boxes due to hatchling crowding. No significant difference in hatch success between the one-box and two-box method was observed. However, the associated P value of 0.0519 suggests that a larger sample size might indeed indicate a better hatch success in boxes of 45 eggs or less. This requires further investigation. Future applications of the day 53 experiment should seek to further standardize the methodology employed in order to maximize hatch and emergence success.

The increased hatchling production observed in the 18 nests that contained full term eggs is encouraging. A total of 783 hatchlings were produced from only 18 nests. This is a significant

number of hatchlings when taking into consideration only 11,638 hatchlings were estimated to emerge from a total of 373 nests in 2006 (Garner *et al.* 2006). This, along with the significantly increased hatch and emergence success observed in day 53 nests, also demonstrates the potential of this experiment to minimize the decreased hatch success observed in relocated leatherback nests (Eckert and Eckert, 1995; Alexander *et al.* 2004; Garner *et al.* 2005). Relocation remains a vital management tool in saving 40-60 % of nests laid annually on Sandy Point (Garner *et al.* 2005). When combined with day 53 incubation, relocation hatch success and ultimately hatchling production, could be maximized. This could potentially reverse the downward trend in hatch success observed over past decade (Figure 1.). Maximizing hatchling production would also serve to maximize adult recruitment into the Sandy Point population and assist the USFWS and WIMARCS in meeting their goals for species recovery.

4.3 Management Implications

The possibility of implementing day 53 protocol as a permanent management tool was investigated during the course of this project. A typical nest extraction performed by two people required approximately 1 hour to complete. This time was calculated from initial triangulation of the nest site and continued until the completed nest box was placed in the truck. Depending on both the distance of the nest site from beach exits and the nest size, time ranged from 35 minutes to about 1.5 hours per nest. This does not include travel time, time required to monitor the nest boxes daily, or time required for excavation and release of hatchlings. Daily maintenance of one incubating nest required a maximum of 10 minutes of time per day. However, excavations were often spread out over the course of four or five days, requiring an average of approximately 30 minutes per day, per nest. In large-scale incubations, several boxes requiring excavation on the

same day would require significant amounts of time for personnel. All nest boxes were extracted during the day. Night extractions are possible but if combined with other extensive adult and nest management fieldwork, they might prove too exhaustive in terms of time requirement and physical exertion. A continuous period of at least 1.5 hours is required per nest using one person. This would not fit in with night-time patrol schedules that occur every 45 minutes. Carrying the boxes is physically difficult since each full box may weigh up to 30 pounds and must be carried from the bottom. The awkward rectangular shape of the box also made this a challenging task. If a dedicated day 53 project was implemented, sufficient time and labor must be provided either at night or during the day for the extractions and excavations. At least two, trained individuals would be required to conduct the extractions and assist with nest box/equipment transport. One person is needed to monitor the nest boxes daily and conduct excavations. Sufficient space requirements in the egg lab would also be necessary for large-scale implementation. Assuming no other nest box experiments are ongoing, the WIMARCS facility should be capable of housing such an experiment. In addition, there were no problems keeping nest boxes in the acceptable range of incubation temperature (Garner 2006). Therefore, if dedicated time, space and qualified labor are provided, no other logistical factors should prove problematic. Additional funding for equipment and labor would also be required.

Although considerable time and labor investment are required for this program, it is not an unreasonable task. The effort and effectiveness of this project can be maximized by focusing on relocated nests. I believe the value of this experiment in increasing relocated hatch success outweighs potential logistical issues. Of the 373 nests laid in 2006, 180 were relocated (Garner *et al.* 2006). If the day 53 experiment had been performed in only 50 of these nests, around 700 additional hatchlings could have been produced. With a decrease observed in the female

population nesting at Sandy Point in the past 5 years, as well as declining nest numbers (Garner *et al.* 2005, 2006), significant efforts must be put forth to maximize hatchling output. In years such as 2006, with low numbers of nesting females and nests, hatchling production is extremely important to maintain. Therefore, I would recommend that efforts which serve to produce additional hatchlings, such as the day 53 experiment, be applied as often as possible, especially in years with limited nesting.

Using the Day 53 experiment as an emergency technique in case of severe weather and abnormal beach conditions is another possibility. However, evaluation of the experiment's potential as an emergency strategy could not be proved conclusively in 2006. During the time period extractions were carried out, no major storms or severe rainfall passed over Sandy Point. Timing of severe weather cannot be anticipated so the number of nests that could be saved by emergency day 53 nests would be limited: only nests within the latter third of incubation could be taken within the day 53 protocol. Eggs could potentially be removed from the beach before the latter third of incubation, yet this strategy runs the risk of increased sensitivity to handling, skewing of the sex-ratio and is beyond the scope of this paper. In the case of an extreme hurricane with the potential to wipe out all nests, the benefits of saving as many nests as possible regardless of developmental stage, might be beneficial. In such a case, evacuation priority should be given to eggs in the later stages of development. These would have the best chances of benefiting from artificial incubation.

4.4. Future Research

This experiment assumes that removing full-term eggs from natural environmental conditions increases hatch success. Since artificial conditions of the incubation boxes produced a higher hatch success, one or more conditions present in nest boxes and not on the beach may facilitate hatching. These factors may include gas exchange (Prange and Ackerman 1974; Ackerman 1980, 1981; Wallace *et al.* 2004; Ralph *et al.* 2005), temperature (Bustard and Greenham 1968; Maloney *et al.* 1990) incubation time (Cagle *et al.* 1993; Booth and Astill 2001) moisture (Cagle *et al.* 1993) and sand particle size (Stancyk and Ross 1978; Mortimer 1990). Few studies have been performed to determine the unique environmental constrictions Sandy Point places on its incubating nests. Therefore, high late term mortality observed on the beach might be attributed to one or more of these factors. The day 53 experiment makes a case for furthering our study of late term mortality. In addition to its clear conservation benefits, the day 53 technique can be used as a comparison tool for understanding conditions that create low late term mortality in the boxes and conditions that create high mortality on the beach. Further investigation into nest parameters such as temperature, gas exchange, and moisture in artificially incubating nest boxes, versus naturally incubating nests should be examined.

Additional effects of the experiment must also be determined. The shorter incubation period as well as unnatural emergence of hatchlings must be evaluated in terms of hatchling fitness and short term survival. Day 53 nests were observed to produce “better looking” hatchlings than those observed at Sandy Point. Hatchlings appeared well-hydrated and extremely active. Hatchling carapaces were thick and round, and showed none of the indentation and/or abnormal shape normally found in natural nests (*pers. observ.*). These hatchlings were also spared an energetically expensive emergence through 60 cm of sand and long beach crawl to the ocean.

Some areas on Sandy Point required hatchlings to emerge from 90 cm of sand, while other areas required hatchlings to crawl over 50 meters from nest to waterline (Garner *et al.* 2006). Energy expenditure during may impact overall energy budgeting for swimming, predator avoidance and finding food. This may ultimately have survival consequences for the first few days at sea (Booth and Astill 2001; Reece *et al.* 2002). Fitness parameters for sea turtle hatchlings are vague and questions regarding the effect of size and energy expenditure on hatchling fitness remain unanswered (Reece *et al.* 2002). It is believed, however, that an increase in moisture results in an increase in hatchling mass and ultimately increased survivability (Tracy *et al.* 1978). It is predicted that hatchling survivability is maximized in day 53 nests due to decreased energy expenditure and optimal environmental conditions during late term incubation. Further studies on hatchling size, energy expenditure and resulting fitness are recommended to determine any beneficial or possibly negative consequences artificial incubation might have on hatchlings.

5. Conclusion

Maximizing the hatchling output of Sandy Point is extremely important in increasing the adult recruitment of Sandy Point (Dutton *et al.* 2005). The day 53 experiment achieved a hatch success high enough to significantly increase the hatchling production of Sandy Point. With adequate personnel, time and physical space for the nest boxes, the day 53 experiment can be implemented as a management protocol to increase hatch success in relocated nests. A strict methodology must be followed in order to achieve these results. For future studies, it is recommended that possible biotic and abiotic factors affecting late-term mortality on the beach be investigated in relation to day 53 nests.

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