Research Article

Success of Delayed Translocation of Loggerhead Turtle Nests

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ABSTRACT Sea turtle embryo mortality in natural nests due to environmental and anthropogenic factors can be very high. To increase hatching success of these endangered species, nest translocation to hatcheries immediately after egg-laying is a common management tool. To test the viability of delayed translocation, we moved 50 loggerhead sea turtle (*Caretta caretta*) nests to a beach hatchery after various times (0–96 hr) after egg-laying at Boavista Island (Republic of Cabo Verde, western Africa). We transported eggs in a rigid plastic container, being careful to maintain their original vertical orientation. Delayed translocation times of 0 hours, 12 hours, 24 hours, 84 hours, or 96 hours after egg-laying did not have any effect on hatching success, incubation period, or hatchling size and mass. Delayed translocation slightly increased the duration of the translocation process because of extra precautions taken (e.g., maintaining axial orientation, protecting eggs from mechanical shocks). We conclude that delayed nest translocation can be done in a safe and effective way, thereby increasing the efficiency of the whole monitoring program. Finally, delayed translocation, accompanied by an evaluation of fertility, would seem to permit the removal of undeveloped eggs and to facilitate their subsequent exploitation by local communities without affecting turtle nesting success. (JOURNAL OF WILDLIFE MANAGEMENT 71(7):2290–2296; 2007)

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KEY WORDS Conservation, hatchery management practices, loggerhead, nests translocation, sea turtle.

In many sea turtle nesting beaches, factors such as human poaching, predation by domestic or wild carnivores, flooding, or beach erosion cause extreme and systematic embryo mortality. In these beaches, nest translocation is a common management tool (Wyneken et al. 1988). However, the use of this technique is controversial because of its possible detrimental effect on hatching success (Eckert and Eckert 1990), hatchling fitness (Booth and Astill 2001*a*, *b*; Reece et al. 2002), or hatchling sex ratio (Mrosovsky and Yntema 1980, Morreale et al. 1982). During the extraction, transport, and relocation of clutches, eggs are exposed to rotational or vibrational movements caused by human manipulation, and such movements could negatively affect embryonic development.

Traditional protocols for translocation or relocation of sea turtle nests advise moving eggs within 2–12 hours after egg-laying to protect developing embryos (e.g., Miller 1999, Mortimer 1999, Shanker et al. 2003). However, this recommendation generally obliges the manager to move eggs at night, decreasing visibility, reducing the manager's ability to detect nests, interfering with risk assessment and decision-making, and potentially compromising the convenience of translocation to the new location. Most of these problems can be solved by a diurnal delayed translocation. Moreover, nests located in risky areas that are not found immediately after egg-laying have been deemed untranslocatable. However, the advantages of a delayed translocation may be attenuated by reducing embryo survival due to egg manipulation during translocation.

In this article, we experimentally tested the viability of delayed translocation in the loggerhead turtle (*Caretta caretta*), moving nests from the beach to a hatchery at different times after oviposition (0–96 hr, including nighttime and daytime translocations). If delayed traslocation is viable, we predict no differences in embryo mortality, incubation duration, and hatchling sex ratio, size, and mass compared with these measurements from nests traslocated immediately after egg-laying.

STUDY AREA

We conducted field studies on an important loggerhead nesting area on Boavista Island, Republic of Cabo Verde, western Africa (16.18°N, 22.92°W; Fig. 1; López-Jurado et al. 2000*a*). There was a long history of loggerhead turtle hunting and meat consumption by the population of Cabo Verde. Since 1998, continuous conservation efforts (e.g., reinforcement of the local sea turtle law, monitoring and protection of nesting beaches, implementation of environmental education programs) have been conducted in Boavista to protect sea turtles, as proposed in López-Jurado et al. (2000*b*).

METHODS

To test the influence of delayed translocation on embryonic development, we translocated 50 doomed nests (located in flooding or silty areas) to a hatchery at different times after egg-laying, being careful to maintain the original vertical orientation of the eggs during the translocation procedures. We compared the success of these nests with that of nests naturally incubated in the beach and with that of 134 doomed nests that were translocated to the same hatchery

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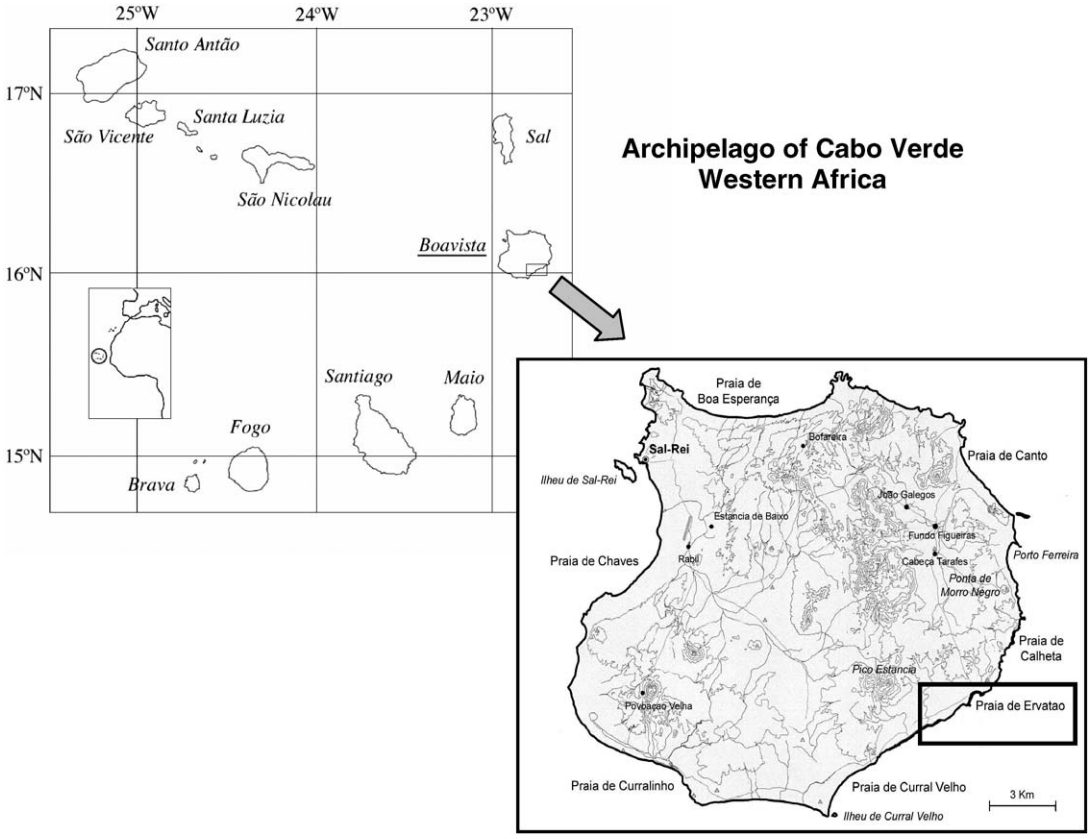

Figure 1. Map of Cabo Verde Archipelago, Boavista Island (Republic of Cabo Verde, western Africa), and the study site (framed area).

using a standard protocol that transports eggs within the first 2 hours after egg-laying, without concern for egg vibration or orientation during the movement process. We collected all nests from 2 southeastern Boavista beaches (Ervatão and Ponta Cosme) that have a high density of turtle nests (Fig. 1). The artificial hatchery (50×15 m) was established on a Benguinho beach located between Ervatão and Ponta Cosme. We arranged nests in parcels of 1 m^2 for every nest, distributing nests of different treatments in a block design.

We conducted translocations from 6 August to 15 October, and all translocated nests were laid within the first 7 days of this period. Immediately after laying, we marked and randomly assigned the 50 nests to 1 of the 5 experimental treatments (10 nests/treatment), which consisted of translocations at 0 hours, 12 hours, 24 hours, 84 hours, and 96 hours after egg-laying. We translocated nests for 0-hour, 24-hour, and 96-hour treatments at night, whereas we moved nests translocated 12 hours and 84 hours after laying during the day. We considered 4 days (96 hr) as the practical time interval that a translocation could be feasible. After the fourth day, it is very difficult to identify turtle tracks and nest location. We included diurnal translocations to assess the effect of day temperature on egg mortality rate.

We translocated nests from treatment 0 hours to the hatchery immediately after egg-laying as control nests (following the traditional standard protocol of nest translocation). The rest of the treatment nests remained on the beach without any experimental manipulation until we translocated them. The translocation procedure was identical for all nests. We conducted nest excavation and egg extraction by hand while wearing latex gloves. During egg extraction, we maintained the original position of eggs in the nest. After egg extraction, we marked the top of all eggs with a graphite pencil. We carefully placed eggs (arranged in columns and rows) into a rigid plastic container ($24 \times 35 \times 19$ cm) with moist beach sand, maintaining oviposition order and the axial orientation. We transported eggs on foot, avoiding jerking or rotational movements. We reburied clutches in the hatchery in standardized hand-dug cavities that resembled natural nests in shape, size, and sand characteristics (max. nest depth of 50 cm). We used an umbrella or a dry towel to protect clutches that had diurnal translocation treatment from the sun. Two people performed the translocation operations. All experimental translocations lasted 30–70 minutes, depending on the distance from the original nest location to the hatchery. During translocation, eggs remained $\leq$10 minutes in the air.

We measured fertility of nests where possible during delayed translocations. We assessed fertility of eggs by the presences of a "white fertility circle" described by Blanck and Sawyer (1981:167). After the first 24 hours of incubation, a white spot (Blanck and Sawyer 1981, Miller 1985) is perceptible on the top of fertile eggs of loggerheads. Those eggs that had creamy aspect or that did not present a

Table 1. Description of visual subcategories classification of embryonic death stages described from Miller (1985) used in nest exhumations during 2005 loggerhead nesting season, Boavista Island (Republic of Cabo Verde, Western Africa).

Visual subcategories	Miller embryonic stages	Coarse brief morphological stage descriptions to differentiation
1	<19	Signs of initial development (e.g., embryonic disk, blood remnants,)
2	19–20	Eyes present
3	21–22	Eyes and anterior foreflippers present
4	23	Digital ridges are visible on the 4 digital plates
5	24–25	Digital ridges are well developed and the scutes are defined
6	26	All scales are present on the flippers and some superficial pigmentation occurs
7	27	Vol of the yolk is greater than the vol of the embryo; embryo not well pigmented yet
8	28	Vol of the yolk is equal to that of the embryo; pigmentation has intensified but is lighter than that of a hatchling
9	29	Ratio of embryo vol to yolk ranges 4:1; basic hatchling pigmentation and morphology

white spot after 24 hours (Blanck and Sawyer 1981, Miller 1985) were examined closely with a flashlight to identify any sign of development. If no sign was seen, we classified the eggs as infertile.

Alternatively, we translocated 134 nests to the hatchery using standard protocols (Eckert et al. 2000). We removed eggs during or immediately after oviposition (within the first 0.5 hr), when vibration or rotational movements do not affect embryonic development (Limpus et al. 1979, Parmenter 1980). We transported eggs to the hatchery in plastic bags without concern for the original vertical orientation, and we reburied the eggs into hand-dug cavities equal in shape and size to experimental nests. All translocations were finished within 30–90 minutes of egg-laying, depending primarily on distance from the nest location to the hatchery. Finally, we marked and monitored 75 natural (nontranslocated) nests on Ervatão ($n = 34$) and Ponta Cosme ($n = 41$) beaches. We counted the clutch size in these nests during egg-laying. We left nests in their original location until hatching.

We placed a round plastic net (45 cm in diam, 50 cm in ht) over all nests 45 days after egg-laying to retain hatchlings to count and measure them after emergence. After day 45, we checked all nests daily during the night and at daybreak to record hatchling emergence. Within 6 hours of emergence, we weighed 10 hatchlings to the nearest 0.1 g, and we measured straight carapace length (SCL) and straight carapace width to the nearest 0.1 mm. We then released hatchlings in the nest surroundings and permitted them to reach the water. We excavated each nest 5 days after the last hatchling emergence. We classified exhumed material into shells of hatched eggs, dead hatchlings, dead pipped hatchlings, live hatchlings, live pipped hatchlings, undeveloped eggs, and 9 eyesight subcategories of visual embryonic stages defined from Miller (1985; Table 1).

Eggs classified as undeveloped contained no visible sign of an embryo. Because on a few occasions we detected that some hatchlings had escaped from the nest enclosure, we took reported emergence success to be either the number of empty complete eggshells during exhumation or the number of emerging hatchlings, depending upon which was greater. We calculated early embryonic mortality considering the number of dead eggs with no detectable sign of embryonic development plus those eggs that did not develop past stage 1 (Table 1) divided by initial number of eggs ([no. undeveloped + no. ≤stage 1]/initial no. eggs).

We defined the incubation period as the number of days between egg-laying and the most massive hatchling emergence, which in 96% of cases was the first emergence recorded. To estimate hatchling sex ratio, we used curves from Marcovaldi et al. (1997) and Mrosovsky et al. (1999) that relate loggerhead sex ratio and incubation duration. We used the mean of incubation duration (d) from nests hatched from 20 September to 6 November, the period within experiment nests hatched. To apply Marcovaldi et al. (1997) curve, we subtracted 4.1 days of our data as hatch emergence interval accepted for loggerheads (Godfrey and Mrosovsky 1997) and corroborated in Cabo Verde (E. Abella, Estación Biológica de Doñana-Consejo Superior de Investigaciones Cientificas, personal observations).

We used a one-way multivariate analysis of variance (MANOVA) to evaluate significant differences in mortality rate, incubation time, and hatchling length, width, and mass among experimental treatments. We conducted a similar MANOVA to compare incubation success from nests used in the experiment with that of nest incubated in the hatchery and 2 beaches. We conducted univariate analysis of variance (ANOVAs) to analyze the influence of experimental treatments or nest locations on each dependent variable. We used post hoc Tukey's test for pairwise comparisons. To compare early embryo mortality among experimental treatments, we used a Kruskal–Wallis nonparametric test because of heterogeneity of variances. To evaluate the relationship between hatchling SCL and mass, we used an analysis of covariance (ANCOVA) where hatchling mass was the dependent variable, hatchling SCL was the covariate, and the location was the effect.

RESULTS

The translocation date had no overall effect on embryonic development (MANOVA: Wilks $\lambda = 0.643$, $F_{4,16} = 1.19$, $P = 0.282$). Univariate subsequent ANOVAs indicated that translocation at 0 hours, 12 hours, 24 hours, 84 hours, or 96 hours after egg-laying had no effect on hatching success ($F_{4,45} = 0.099$, $P = 0.982$), incubation period ($F_{4,43} = 0.425$, $P = 0.790$), hatchling size (length: $F_{4,43} = 0.41$, $P = 0.802$;

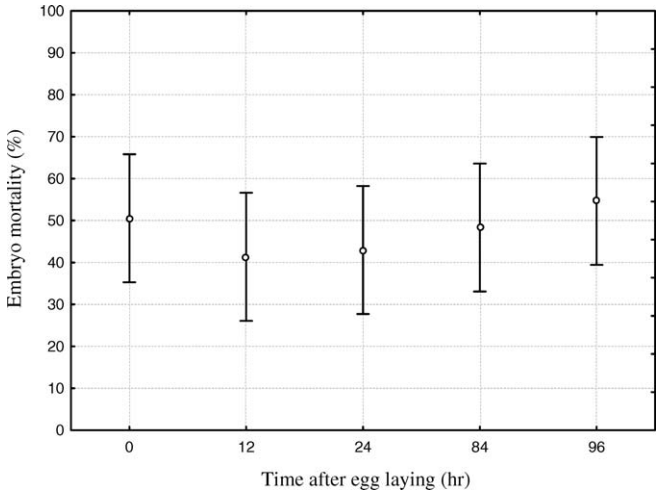

Figure 2. Effect of translocation at 0 hours, 12 hours, 24 hours, 84 hours, and 96 hours after egg-laying on mortality rates of eggs from 50 loggerhead sea turtle nests at Boavista (Republic of Cabo Verde, western Africa), 2005.

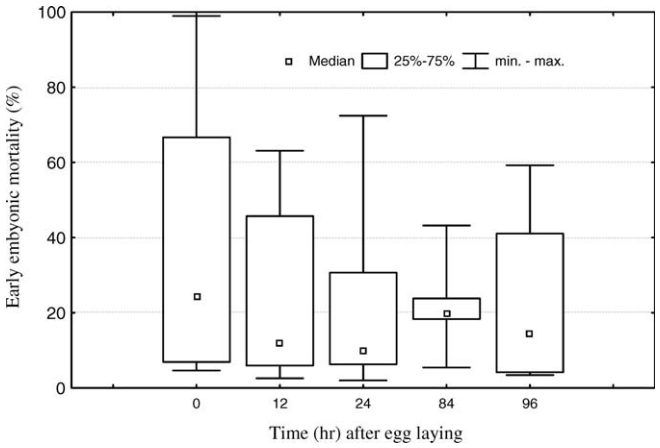

Figure 3. Effect of translocation at 0 hours, 12 hours, 24 hours, 84 hours, and 96 hours after egg-laying over early embryo mortality rates in 50 loggerhead sea turtle nests on Boavista (Republic of Cabo Verde, western Africa) during 2005 nesting season.

width: $F_{4,43} = 0.02$, $P = 0.999$), or hatchling mass ($F_{4,43} = 0.672$, $P = 0.615$). Embryonic mortality was similar in all treatments (Fig. 2). Hatchling length, width, and mass (Table 2) were similar among nests translocated at different times ($F_{3,12} = 0.91$, $P = 0.545$). There was no effect of translocation treatment on early embryonic mortality (Kruskal–Wallis: $\chi^2 = 6.40$, df = 4, $P = 0.171$; Fig. 3).

When comparing nests used in this experiment ($n = 50$) with protected nests incubated in the hatchery ($n = 134$) and in 2 close nesting beaches called Ervatão ($n = 34$) and Ponta Cosme ($n = 41$), we found overall differences on embryonic development (MANOVA: Wilks $\lambda = 0.726$, $F_{5,15} = 4.23$, $P < 0.001$). Univariate ANOVAs indicated differences among nest locations in most of the recorded variables: mortality rate ($F_{3,262} = 24.25$, $P < 0.001$; Fig. 4), incubation duration ($F_{3,213} = 4.91$, $P < 0.001$), hatchling width ($F_{3,205} = 3.55$, $P = 0.015$), and hatchling length ($F_{3,209} = 3.67$, $P = 0.013$; Table 3). Hatchling mass ($F_{3,209} = 1.068$, $P = 0.364$) did not vary among groups (Table 3).

Experimental nests had similar hatching success to that of Ervatão beach and hatchery, whereas nests from Ponta Cosme beach had lower success (Tukey's test: $P < 0.001$). Hatchery mortality was lower than that from beaches (Tukey's test: $P < 0.01$). Natural beach egg mortality would have been even higher if we had not translocated a number of doomed nests to the hatchery. In length, hatchlings from experiment were larger than hatchlings from Ervatão ($P < 0.05$), whereas hatchling width did not show differences among locations ($P > 0.05$).

In the 4 nest locations, sex ratio was skewed toward females. However, we only found a significant difference in female production between the experimental nests and Ervatão ($P < 0.01$; Fig. 5). We found that hatchery had longer incubation periods and thus lower female production (approx. 15%) than natural beach nests ($P < 0.02$; Fig. 5). Conversely, Ervatão presented shorter incubation periods and higher female production ($\bar{x} = 90.64\%$) than experiment ($\bar{x} = 73.63\%$) and hatchery ($\bar{x} = 68.76\%$; $P < 0.01$).

The number of nests from where it was possible to measure hatchlings differed from the number of initial nests sampled because several nests had no hatchling emergence or because in a few nests hatchlings escaped before we could measure them.

Hatchling mass was correlated to hatchling SCL ($r = 0.801$, $F = 378.72$, $P < 0.001$). This relationship was not affected by the nest location (ANCOVA: $F_{3,208} = 2.184$, $P = 0.091$; Fig. 6).

Table 2. Hatchling length, width and mass found in translocation at 0 hours, 12 hours, 24 hours, 84 hours, and 96 hours after egg-laying of 50 nests of loggerhead sea turtles from Boavista (Republic of Cabo Verde, western Africa) in 2005.

	SCL (mm)[a]				SCW (mm)[b]				Mass (g)				
Treatment (hr)	$\bar{x}$	SCL	Max.	Min.	$\bar{x}$	SD	Max.	Min.	$\bar{x}$	SD	Max.	Min.	*n*
0	42.5	0.62	41.2	43.7	33.2	0.47	32.3	34.1	15.8	0.62	14.6	17.1	8
12	42.8	0.56	41.7	43.9	33.3	0.43	32.4	34.1	16.1	0.55	15.0	17.2	10
24	43.1	0.56	42.0	44.3	33.1	0.43	32.3	34.0	16.6	0.55	15.5	17.7	10
84	42.6	0.568	41.4	43.7	33.1	0.43	32.3	34.0	16.5	0.55	15.4	17.7	10
96	43.3	0.56	42.2	44.5	33.2	0.43	32.3	34.1	17.0	0.55	15.9	18.2	10

[a] SCL = straight carapace length.
[b] SCW = straight carapace width.

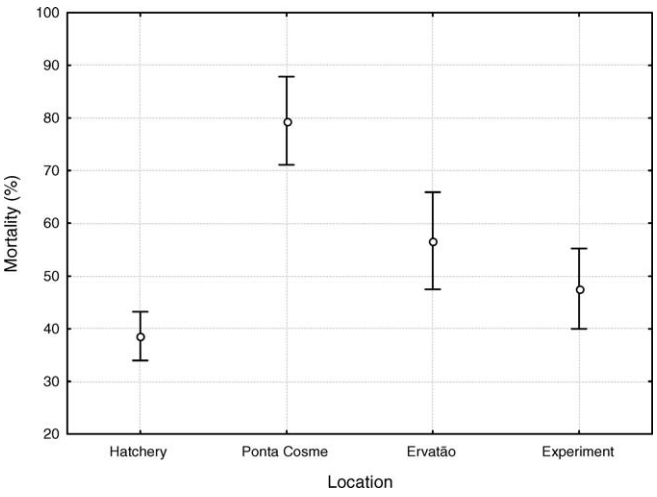

Figure 4. Egg mortality in loggerhead sea turtle nests incubated in the hatchery ($n = 134$), the delayed translocation experiment ($n = 50$), and the natural Ponta Cosme ($n = 41$) and Ervatão ($n = 34$) beaches, from Boavista (Republic of Cabo Verde, western Africa) during 2005 nesting season.

At 0 hours and 12 hours, it was not possible to identify fertile eggs reliably using the white spot technique. However, after 24 hours, the estimation of the number of developing embryos did not vary among treatments, and all mean values were >90% (min. = 75%, max. = 100%) after this time (Table 4).

DISCUSSION

The results of our field translocations (nocturnal or diurnal) to a beach hatchery confirm that a delayed nest translocation can be done in a safe and effective way if appropriate care is taken, thereby increasing the efficiency of the whole monitoring program and saving a greater number of nests. We found no significant differences in mortality rates between immediate and delayed translocations. In addition, newborns emerging from delayed translocation nests showed no differences in size and mass from those emerging from immediately translocated nests. We also compared data from the field experiment with those from hatchery and natural beaches, and we found that emergence rates of the experiment were higher than those from some beaches. If we had not moved translocated nests to a safer location, their hatching success would be close to zero. Thus, the percentage of emergence success resulting of a translocation (delayed or not) would be usually higher than that expected for a doomed nest. Only 2 studies have reported data on delayed translocations (Raj 1976, Harry and Limpus 1989). Results from these studies are difficult to compare because techniques and methodologies were very different, not only because they finished nest incubations under laboratory conditions but also because translocation times and transport methods were different. However, both studies reported high emergence rates from delayed translocations.

The incubation period was slightly larger in the hatchery compared with natural nests. We think that such difference could be due to variation of chamber depth between translocated nests and natural nests. All hatchery and experimental nests had the same depth (50 cm), whereas we have to expect that in situ nest depths presented variations according to different nesting females. We also suggest that incubation substrate could have affected the incubation period, because both Ponta Cosme and Ervatão beaches presented high soil variability. Heat accumulation (Wibbels 2003) and gas exchange (Packard and Packard 1988) change with substrate and directly impact incubation period. Temperature affects embryo development, incubation duration, and sex ratio; long incubation times imply low temperatures and lower female production, and short incubation times imply high temperatures and high percentage of females (Wibbels 2003). We estimated that the percentage of females produced on Ponta Cosme and Ervatão beaches during the period selected to detect changes in sex ratios due to translocations was between 83.3% and 90% and in the hatchery and the translocation experiment that the percentage was between 70% and 75%. Despite that translocations implied a decrease in female production due to changes in temperature, the sex ratios were within natural parameters that had been found in other loggerhead populations (Wibbels 2003).

Identification of embryonic development is not possible within the first 12 hours after oviposition because no signs of the white fertility circle (Blanck and Sawyer 1981, Chan 1989) are perceptible until after ≥24 hours have passed. At 24 hours, recognition of embryonic development required an accurate observation with a flashlight because the white spot sometimes still looked diffuse. After 24 hours, the recognition of viable eggs is very easy. At 84 hours and 96

Table 3. Incubation duration (ID; d), hatchling length (SCL[a]; mm), width (SWL[b]; mm), and mass (g) in 4 groups of nests: the delayed translocation experiment, the hatchery, Ervatão Beach, and Ponta Cosme Beach during 2005 loggerhead sea turtle nesting season, Boavista (Republic of Cabo Verde, western Africa).

	Exp. ($n = 48$)				Hatchery ($n = 127$)				Ervatão ($n = 24$)				Ponta Cosme ($n = 10$)			
	SCL	SCW	Mass	ID	SCL	SCW	Mass	ID	SCL	SCW	Mass	ID	SCL	SCW	Mass	ID
$\bar{x}$	42.9	33.2	16.5	56.4	42.4	32.7	16.2	56.8	41.8	32.3	16.1	54.5	41.6	31.9	15.6	54.2
SD	0.21	0.21	0.33	0.53	0.13	0.13	0.2	0.33	0.30	0.30	0.35	0.68	0.47	0.46	0.54	0.91
Min.	42.5	32.8	16.0	55.4	42.1	32.4	15.9	56.1	41.2	31.7	15.4	53.1	40.7	31.0	14.5	52.4
Max.	43.3	33.6	16.9	57.4	42.6	32.9	16.5	57.4	42.4	32.8	16.7	55.8	42.6	32.8	16.7	56.0

[a] SCL = straight carapace length.
[b] SCW = straight carapace width.

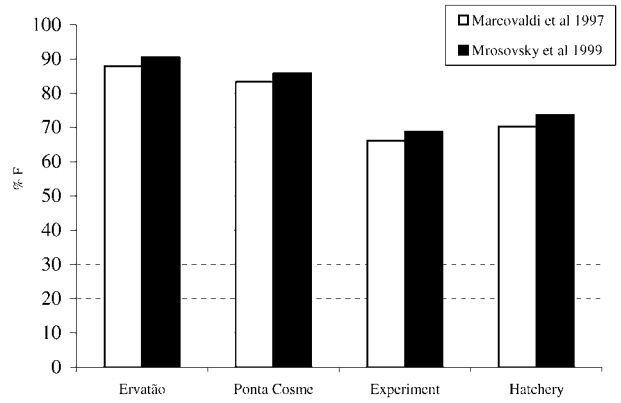

Figure 5. Estimated percentage of females for Ervatão, Ponta Cosme, hatchery, and experiment on Boavista (Republic of Cabo Verde, western Africa) loggerhead sea turtle nesting population in 2005. We calculated the sex-ratios by applying Marcovaldi et al. (1997) and Mrosovsky et al. (1999) curves that relate sex ratio and incubation duration in the same species.

hours, the fertile white spot covered approximately 33% of the shell, in contrast to 5–10% found in eggs observed at 24 hours. Delayed translocation, accompanied by a skilled evaluation of fertility (Abella et al. 2006), would seem to permit the removal of undeveloped eggs. Doing that, we can increase hatching success of translocated nests by eliminating dead eggs that can negatively affect development of contiguous live eggs. The unfertile, removed eggs from delayed translocated nests could be used by local communities, in places where egg collecting is a significant cause of live egg loss. This application could be a valuable and sustainable conservation measure to reduce or prevent nest poaching.

Finally, delayed translocation slightly increased the duration of the translocation process (approx. 15–20 min), because of the additional precautions (e.g., maintaining axial orientation, protecting eggs from mechanical shocks; Limpus et al. 1979, Parmenter 1980).

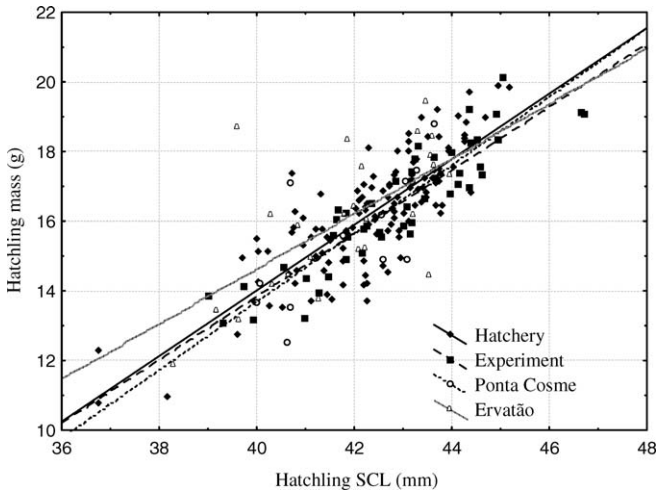

Figure 6. Relationship between loggerhead sea turtle mean hatchling length (straight carapace length [SCL]) and mass for all groups (location effect) during 2005 nesting season, Boavista (Republic of Cabo Verde, western Africa). Each data point represents one nest.

Table 4. Percentage of loggerhead eggs where embryonic development can be recognized as a function of time elapsed after egg-laying found during 2005 nesting season in Boavista Island (Republic of Cabo Verde, western Africa).

Time after egg laying (hr)	$\bar{x}$	SD	Lower 95% CL	Upper 95% CL	*n*
0	0				10
12	0				10
24	96.52	1.915	90.66	98.38	9
84	91.55	1.816	87.89	95.21	10
96	95.28	1.816	91.62	98.96	10

MANAGEMENT IMPLICATIONS

A careful delayed translocation avoiding egg rotation can significantly improve the translocation efficiency on sea turtle conservation programs because many doomed nests found later than the first 12 hours after laying, following turtle tracks, could be moved to a hatchery or transplanted to a safe part of the beach. During a diurnal translocation, it would be easier to evaluate whether a nest is located in a risky area, improving decision-making and the translocation process. Finally, diurnal delayed translocation would not interfere with turtle nesting and the tagging of other nesting females.

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