

Assessing the efficacy of direct conservation interventions: clutch protection of the leatherback marine turtle in the Dominican Republic

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Abstract The beaches of Jaragua National Park in the Dominican Republic are the country's last known major nesting site for the leatherback marine turtle *Dermochelys coriacea*. This nesting aggregation is threatened by widespread illegal egg take, and clutch relocation and artificial incubation have been carried out as protection measures since 1974. We assess the efficacy of such efforts and investigate how artificial incubation may be influencing the success and sex ratios of clutches. We compare hatching success, incubation duration and embryo mortality in in-situ clutches ($n = 43$) with those incubated artificially at sites in the east and west of the Park ($n = 35$ and $n = 31$, respectively). Our results show that in the west, artificial incubation significantly decreases hatching success in clutches. In the east the duration of incubation is increased, which we predict would result in an increase in the number of males from these clutches. Clutch relocation is currently the only viable conservation option for clutches on eastern beaches because of illegal egg take but action is needed to ensure that the natural sex ratio is not distorted. However, on the western beaches in situ clutch incubation seems possible through beach protection. Further community engagement and enforcement are required to improve conservation measures at eastern beaches if long-term, less sustainable intervention is to be avoided.

Keywords Artificial incubation, conservation, *Dermochelys coriacea*, Dominican Republic, hatching success

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Introduction

In critical conservation situations involving threatened species urgent action may be necessary to preserve species and ecosystems (Andrews, 2000). However, in many situations conservation actions have been passed on from individual to individual, with no assessment of whether or not management actions fulfil the conservation objectives (Pullin & Knight, 2003). Evaluation is necessary to demonstrate that conservation actions actually achieve their objectives (Pullin & Knight, 2009; Sutherland et al., 2009).

Following centuries of exploitation marine turtles are recognized as species of conservation concern internationally (Hamman et al., 2010). Illegal egg take by coastal people is still a significant threat to the survival of some populations (Santidrián Tomillo et al., 2008) and has led to a variety of conservation measures, including protection of nesting beaches (Frazier, 2002; Santidrián Tomillo et al., 2009), and relocation of eggs from threatened sites to other beach locations or to enclosed hatcheries (Tuttle & Rostal, 2010; Liles et al., 2011).

On nesting beaches where clutches are handled, management techniques may affect offspring production (Pintus et al., 2009). The handling and transportation of eggs may result in embryo mortality as rotation or vibration can rupture the embryonic membrane (Phillott & Parmenter, 2007), resulting in reduced hatching success of relocated clutches compared to those incubated in situ (Eckert & Eckert, 1990; Özdemir & Türkozan, 2006). Temperature, humidity and oxygen levels in relocated clutches are likely to be different from those chosen by the nesting female, and therefore hatching success, sex ratio and phenotype may be affected (Foley et al., 2000). The temperature experienced by marine turtle embryos during the middle third of development determines the sex of the hatchlings (Yntema & Mrosovsky, 1982) and although a female-biased sex ratio appears to be the norm among marine turtle hatchlings, there have been examples of artificial incubation carried out under cooler conditions resulting in male-biased sex ratios (Morreale et al., 1982; Mrosovsky, 1982; Dutton et al., 1985). Temperature may also affect embryonic development, with very high or very low incubation temperatures increasing embryo mortality rates (Ackerman, 1997; Broderick et al., 2001).

The leatherback marine turtle *Dermochelys coriacea* is categorized as Vulnerable on the IUCN Red List (IUCN, 2012) but some subpopulations are categorized as Critically Endangered. Nesting populations in the Indo-Pacific have declined in recent decades (Spotila et al., 2000; Sarti Martínez et al., 2007), whereas many nesting populations in the Atlantic are stable or increasing (Dutton et al., 2005; McGowan et al., 2008; Witt et al., 2011). The Wider Caribbean Region holds some globally important nesting sites, such as in Trinidad and Tobago (Dow & Eckert, 2011) and on the Caribbean coast of Colombia and Panama (Patiño-Martínez et al., 2008). However, there are many small, widely dispersed sites that may play an important role in species recovery that lack intensive population monitoring effort (Dow et al., 2007).

In the Dominican Republic the leatherback turtle nests sporadically around the coast but the beaches of Jaragua National Park in the south-west have been identified as the last important stable nesting site for the species in the country (mean $126.4 \pm \text{SD } 74.1$ clutches per year, range 17–210 over 5 seasons during 2006–2010; Revuelta et al., 2012). Analyses using genetic markers show that leatherback turtles in the Dominican Republic appear to be connected with other Caribbean populations (Carreras et al., 2013). Although the harvesting of turtles and their products has been banned since 1966 the enforcement of environmental law is weak and the leatherback nesting stock of Jaragua National Park is threatened by widespread illegal egg take (Revuelta et al., 2012). However, there is marked spatial heterogeneity in human predation; almost 100% of eggs are taken illegally on the eastern beaches, whereas egg take is much lower on western beaches (75.9% of clutches could be incubated in situ during 2006–2010; Revuelta et al., 2012). In 1974 a local assistant was trained to carry out relocation and artificial incubation of eggs in styrofoam boxes (Ottenwalder, 1981), placing them in a facility located 10 km from the nesting beach. However, since then this activity has been carried out sporadically, with no strict protocol or scientific monitoring. In 2006 we initiated a project to assess this conservation programme, investigating how artificial incubation may be influencing hatching success and sex ratios of clutches.

Study area

Jaragua National Park is a protected area (1,374 km², of which 905 km² is a marine reserve) situated in the south-west of the Dominican Republic (Fig. 1). There are five main turtle nesting beaches grouped in two areas c. 50 km apart: the western beaches of Bahía de las Águilas (4.4 km) and La Cueva (2.5 km) and the eastern beaches of Mosquera (3.3 km), San Luis (11 km) and Inglesa (1.2 km). The western beaches border calm shallow waters and have fine-grained, white coralline sand (width 4–20 m) backed by low scrub

vegetation. There is a small village (c. 20 inhabitants) between the two beaches, which are visited by several thousand tourists annually.

The eastern beaches lie beyond the Oviedo Lagoon and are generally wider (7–40 m) and steeper than the western ones and have coarser, yellow sand. They are exposed to the prevailing north-easterly winds and currents, which deposit plastic and other debris. Although not frequented by tourists these eastern beaches are patrolled by beachcombers, some of whom partake in illegal egg take for consumption and sale (Revuelta et al., 2012).

Methods

Surveys

During 2006–2007 we carried out preliminary weekly beach surveys on the western and eastern beaches of Jaragua National Park to obtain baseline data on marine turtle nesting activity. Based on the observed differences in egg take between western and eastern beaches, and the availability of human resources, we applied different survey and conservation strategies in each area in subsequent years. Although some artificial incubation was carried out during these two seasons, it was not until 2008 that we were able to test robustly for effects of artificial incubation interventions.

In 2008 we carried out weekly daytime surveys on the eastern beaches during the leatherback nesting season (March–August; Revuelta et al., 2012), with the exception of the remote Inglesa beach, which was monitored twice per month. In 2009, in addition to our weekly surveys, governmental rangers performed nightly beach surveys on Mosquera and San Luis beaches and weekly surveys on Inglesa beach. Because of the high level of egg take at these beaches the strategy chosen was the relocation and artificial incubation of all clutches that were not predated at the time of recording.

On the western beaches researchers and governmental rangers carried out daily surveys during March–August in 2008 and 2009. Night patrols were also carried out 2 or 3 nights per week during the busiest part of the laying season (April–May; Revuelta et al., 2012), to witness laying events. These beaches are rarely visited by individuals involved in illegal activities and access is controlled by rangers. For this reason we left 50% ($n = 22$) and 76.9% ($n = 40$) of clutches incubating in situ in 2008 and 2009, respectively. In these years we limited relocation to clutches that were considered unlikely to hatch without intervention (i.e. clutches laid in a sand road near the beach or those threatened by tidal inundation). From 2010 we left 100% of clutches incubating in situ.

From 2008 we relocated clutches to the park rangers' barracks, for artificial incubation. The facilities in both areas

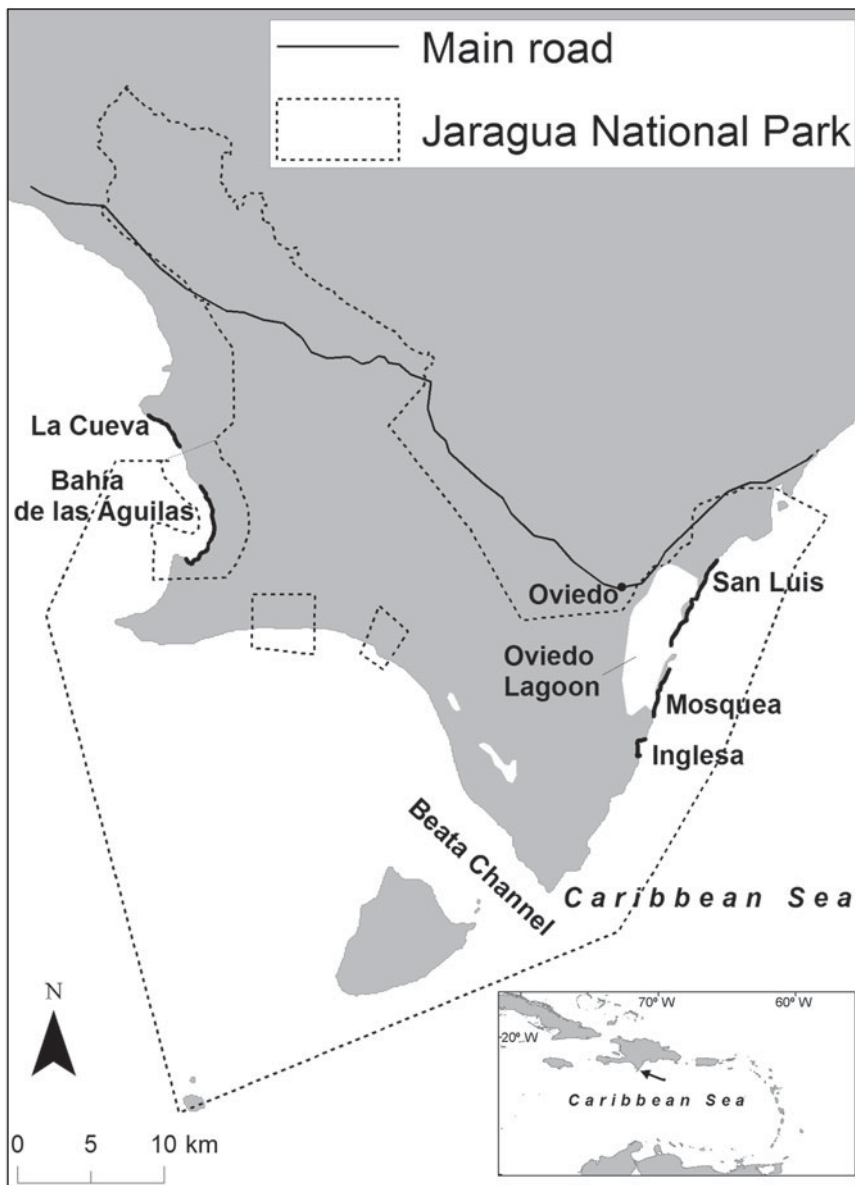


FIG. 1 Locations of the five main nesting beaches for the leatherback marine turtle *Dermochelys coriacea* in the Dominican Republic. The arrow on the inset shows the location of the main map in the Dominican Republic.

are similar in size and have the same characteristics: wooden barracks with concrete floor and corrugated-metal roof. The barracks for the western beaches is on a hilltop c. 2 km from the beaches and is only accessible via a dirt track. This facility is located in an area of arid scrub and is exposed to the sun all day and to dry winds. On the eastern beaches the barracks lies directly behind Mosquea beach, within a forest and near the Oviedo lagoon (Fig. 1), and this area receives more frequent precipitation.

Egg relocation and incubation

During daytime surveys clutches were located by careful probing of the sand with a stick at nesting sites discovered from tracks (Schroeder & Murphy, 1999). During night surveys eggs were collected after the female had returned to the sea.

For artificial incubation we excavated the egg chamber by hand and transferred the yolked eggs into polyethylene exterior boxes with polyurethane foam filling (30 × 50 × 32 cm) for transport and subsequent incubation. Artificial incubation on site before this project did not include yolkless eggs and, because it has not been proved that yolkless eggs have a function in optimizing the clutch environment (Wallace et al., 2006, 2007), we decided to maintain the same practice, excluding the yolkless eggs from the boxes. We tried to mimic the natural clutch arrangement as much as possible, placing yolked eggs in the box with sand from the original nesting beach. We placed 2–3 cm of sand at the bottom and sides of the box to prevent contact between the eggs and the walls of the box. We also placed sand on top of the eggs in the box.

We used Tinytag Plus 2 data loggers (Gemini Data Loggers, Chichester, UK) to record hourly temperatures in

artificially incubated clutches at eastern and western beaches ($n = 12$ and $n = 33$, respectively). In addition, two loggers were placed as controls at nest depth in Mosquea and Bahía de las Águilas beaches during 2008–2009 but these were lost to beach erosion.

When clutches were left in situ we recorded geographical coordinates, using a global positioning system, and camouflaged the tracks of females. We recorded the distance from the nest to the high tide line, and the beach zone where the clutch was located (open beach, at the vegetation border or within the dune vegetation). Once the clutches were camouflaged we estimated the date of hatching based on the estimated mean incubation period for leatherback marine turtles (c. 60 days), and from 10 days before this date we checked the nest area for hatchling tracks.

Study of clutches

During artificial incubation each box was checked daily for signs of hatched turtles. When clutches hatched we excavated them no earlier than 48 hours after the last sign of hatchling emergence. We excavated in situ clutches by hand 1–3 days after hatchling emergence was detected. In both cases data were collected on the numbers of hatched shells and live and dead hatchlings within the clutch. Clutch size was defined as the total number of eggs (hatched and unhatched). Hatching success (expressed as a percentage) was calculated by dividing the number of eggs hatched by the clutch size (Miller, 1999). We opened and examined the contents of all unhatched eggs. Eggs that had partial calcification of the shell or evidence of an early-stage embryo were categorized as early-stage embryonic death (Bell et al., 2003). Eggs that contained dead hatchlings at a late stage of development were categorized as late-stage embryonic death.

For each clutch we calculated incubation duration (defined as the number of days between egg laying and first hatchling emergence). Incubation was considered completed when hatchlings emerged on the top of the sand. A regression test was carried out to describe the relationship between incubation duration and mean temperature during the mid-third of incubation, which is thought to contain the thermosensitive period (the time span outside of which temperature manipulations do not exert any influence on sexual phenotype; Mrosovsky & Pieau, 1991). We estimated hatchling sex ratios, using the conversion curve relating incubation duration to hatchling sex ratio derived from in situ incubated eggs originating from Suriname (Godfrey, 1997). To apply this curve (Godfrey, 1997) for artificially incubated clutches we added 4.1 days to our incubation duration data (Godfrey & Mrosovsky, 1997). Examples of the use of this method and its validation are given by Zbinden et al. (2007) and Katselidis et al. (2012).

Statistical analysis

To understand the effects of incubation methods and nest metrics we carried out data analysis using generalized linear mixed models, which allow both fixed and random factors as well as covariates to be fitted, with random factors controlling for the use of repeated measurements (Schall, 1991). Our data were subject to temporal pseudoreplication caused by repeated measurements of the same nesting females over the season. We therefore included clutch and year as random effects to account for the pseudoreplication among females and potential variation in the response variable across years. The statistical modelling was performed using *lme4* (Bates et al., 2008) in *R v. 2.14.0* (R Development Core Team, 2012) and the graphical output was produced with *sciplot* (Morales, 2012).

We examined the effect of incubation method (artificial incubation at eastern beaches, artificial incubation at western beaches and in situ incubation) on hatching success, using generalized linear mixed model analyses with a binomial error (the most appropriate error distribution for percentage data), with a logit link function. Random effects considered were year and clutch and the covariates included were incubation method, incubation duration (days), clutch size (number of yolked eggs) and laying date.

We used generalized linear mixed models with Poisson error, the most appropriate distribution for count data, and a log link function to examine the effect of incubation method on the number of early- and late-stage dead embryos occurring in clutches, as in Pintus et al. (2009). Maximal models included clutch size (yolked eggs), incubation duration (days) and laying date as covariates, as well as incubation method.

To analyse the effect of incubation method on incubation duration we used a generalized linear mixed model with a Poisson error and a log link function, with the maximal model also including incubation method, laying date and clutch size as covariates.

For each analysis we started with a maximal model with all fixed effects and interactions. The significance of fixed effects was assessed using likelihood-ratio tests (comparing against χ^2 distribution) following deletion from the model until only those terms that explained significant deviance remained, starting with interactions (Weber et al., 2011).

Data exploration techniques were applied as described in Zuur et al. (2010), including multipanel scatterplots to look at the presence of collinearity (correlation between explanatory variables). All two-way interactions were tested but results are only presented if found to explain significant variation. Homogeneity and heteroscedasticity were assessed based on a graphical inspection of the residuals (Zuur et al., 2009).

TABLE 1 Mean number of yolked eggs per clutch, hatching success, incubation duration and % females per clutch of leatherback turtles incubated under artificial (eastern and western beaches) and natural (in situ) conditions in Jaragua National Park, Dominican Republic (Fig. 1), during 2008 and 2009.

	2008			2009		
	Eastern beaches	Western beaches	In situ	Eastern beaches	Western beaches	In situ
Mean no. yolked eggs per clutch ± SD (range; sample size)	65.1 ± SD 18.4 (43–104; 12)	76.3 ± SD 15.1 (47–101; 22)	71.1 ± SD 14.8 (47–110; 22)	70.1 ± SD 16.3 (24–107; 42)	73.2 ± SD 11.9 (54–92; 12)	83.6 ± SD 16.5 (56–120; 40)
Mean hatching success ± SD, % (range; sample size)	53.1 ± SD 16.4 (34.3–79.1; 12)	42.9 ± SD 23.9 (4.8–85.5; 22)	85.3 ± SD 19.9 (5.0–98.2; 22)	57.5 ± SD 26.6 (0.0–93.6; 42)	33.7 ± SD 17.6 (6.0–55.8; 12)	73.6 ± SD 21.8 (12.3–97.9; 40)
Mean incubation duration ± SD, days (range; sample size)	72.6 ± SD 6.1 (66–79; 5)	62.7 ± SD 2.1 (60–67; 20)	64.9 ± SD 1.8 (61–68; 14)	65.6 ± SD 3.8 (59–74; 30)	60.6 ± SD 3.1 (55–64; 11)	62.7 ± SD 2.9 (57–70; 29)
Mean % females (range; sample size)	2.5 ± SD 3.8 (0–8; 5)	41.7 ± SD 23.5 (8–75; 20)	53.6 ± SD 28.5 (0–94; 14)	23.4 ± SD 28.3 (0–85; 30)	57.7 ± SD 26.6 (21–99; 11)	72.9 ± SD 30.7 (0–100; 29)

Results

We studied a total of 109 clutches laid during 2008–2009: 35 incubated artificially at eastern beaches, 31 incubated artificially at western beaches and 43 incubated in situ at western beaches. We could not find 19 of the 62 clutches left in situ, probably because of erosion, rainfall and floods. Table 1 presents descriptive statistics for the number of yolked eggs, hatching success, incubation duration, and sex ratios of clutches. We used data from 2008 and 2009 only to assess the effects of artificial incubation, as no clutches from the western beaches were incubated artificially in 2010.

Effects of incubation method on hatching success

Incubation method had a significant effect on hatching success ($\chi^2 = 76.7, P < 0.001$; Fig. 2a). In situ clutches had greater hatching success than those incubated artificially in both study areas (Table 1; Fig. 2a). We found clutch size ($\chi^2 = 12.7, P < 0.05$), laying date ($\chi^2 = 18, P < 0.01$) and incubation duration ($\chi^2 = 20.1, P < 0.01$) exerted statistically significant effects on hatching success.

Incubation method had an effect on the number of early-stage embryo deaths ($\chi^2 = 16.1, P < 0.05$; Fig. 2b). Clutches from western beaches that were incubated artificially had more early-stage dead embryos than in situ clutches and artificially incubated clutches from the eastern beaches (Fig. 2b). The number of late-stage dead embryos in a clutch was also significantly affected by incubation method ($\chi^2 = 67.4, P < 0.001$; Fig. 2c). Artificially incubated clutches in both study areas had more late-stage dead embryos than in situ clutches (Fig. 2c).

Effects of incubation method on incubation duration

Laying occurred during 9 March–7 June 2008 and 25 March–19 June 2009. There was a significant negative correlation between laying date and incubation duration in 2008, with longer incubation durations recorded at the beginning of the season; however, no such correlation was found in 2009. Incubation method significantly influenced incubation duration ($\chi^2 = 16.0, P < 0.01$). Artificially incubated clutches from the eastern beaches had longer incubation duration than in situ clutches and artificially incubated clutches at the western beaches (Fig. 2d). Mean incubation temperatures were significantly lower in artificially incubated clutches at the eastern beaches compared to artificially incubated clutches at the western beaches (Welch’s *t*-test, $t_{22,9} = -5.66, P < 0.001$; Fig. 3a). Artificially incubated clutches at the western beaches underwent large cycles of diel temperature variation and in some cases exceeded the thermal tolerance range for embryo

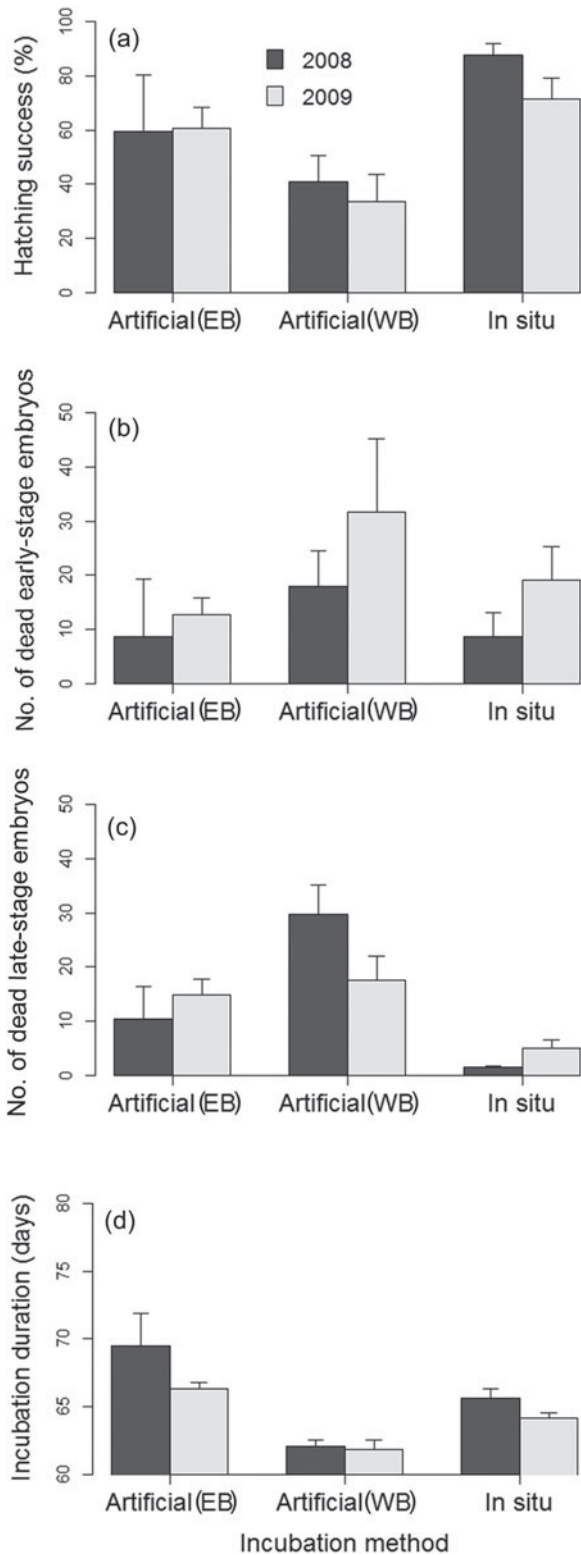


FIG. 2 (a) Hatching success, (b) numbers of dead early-stage embryos, (c) numbers of dead late-stage embryos, and (d) incubation duration for clutches of the leatherback marine turtle incubated artificially on eastern (EB) and western (WB) beaches of Jaragua National Park, Dominican Republic (Fig. 1) and those incubated naturally in situ on western beaches during 2008–2009.

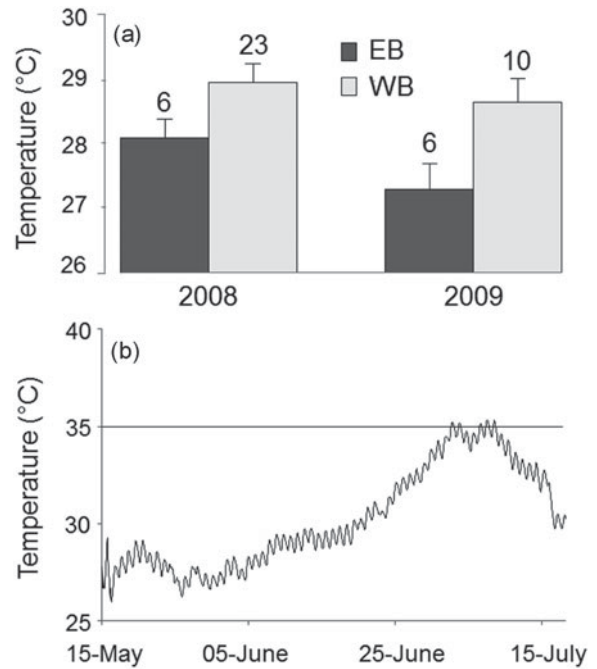


FIG. 3 (a) Mean temperatures for the whole incubation period recorded in artificially incubated clutches at the eastern beaches (EB) and western beaches (WB) of Jaragua National Park, Dominican Republic (Fig. 1) in 2008 and 2009. The numbers over the bars are the standard errors. (b) Hourly recorded incubation temperature profile of one clutch laid on 15 May 2008 and artificially incubated in WB that reached a maximum temperature of 35.3 °C and had 4.8% hatching success. The straight line indicates 35 °C, the upper limit of tolerance for marine turtle embryo development in situ (Ackerman, 1997).

development under laboratory conditions (35 °C; Ackerman, 1997; Fig. 3b). Incubation duration was correlated with mean temperature during the thermosensitive period ($F_{1,42} = 15.9, P < 0.001$; Fig. 4) and thus it can be used to estimate sex ratio (Broderick et al., 2000).

Discussion

Effects of artificial incubation on hatching success

Our results indicate that artificial incubation carried out to protect clutches of leatherback turtles in Jaragua National Park caused changes in both hatching success and sex ratio, when compared to in situ clutches. Artificially incubated clutches, especially those incubated in boxes at western beaches, exhibited lower hatching success than clutches incubated in situ. It is possible that hatching success in some artificially incubated clutches was affected by physical handling of eggs during relocation and transportation (Chan & Liew, 1996), particularly in clutches from the western beaches, which were transported further. However, we believe that the reduced hatching success in artificially incubated clutches is probably a result of incubation

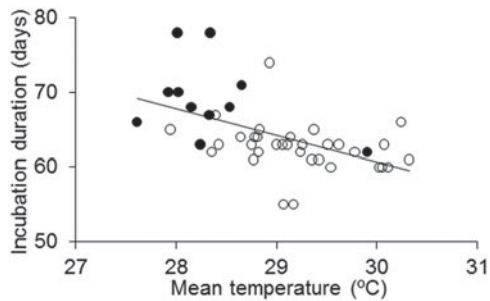


FIG. 4 Relationship between incubation duration and mean temperature during the middle third of the incubation duration for 11 artificially incubated clutches at the eastern beaches (solid circles) and 33 artificially incubated clutches at the western beaches (open circles) of Jaragua National Park, Dominican Republic (Fig. 1). Equation: $y = -3.06x + 152$, $r^2 = 0.28$.

conditions. Restricted exchange of heat, O_2 and CO_2 in boxes increases embryonic mortality, and observed differences in incubation results may be attributable to these differences in gas exchange (Ackerman, 1980; Garret et al., 2010). Most eggs that failed to hatch in situ contained early-stage embryos, mirroring trends seen in other leatherback populations (Eckert & Eckert, 1990; Bell et al., 2004), whereas in artificially incubated clutches most unhatched eggs contained late-stage embryos and many of them appeared to have died just prior to hatching, especially at western beaches. This high late-embryo mortality at western beaches may have been caused by metabolic heat generated by embryo activity (Wallace et al., 2004), because several boxes reached high incubation temperatures ($> 35^\circ C$), which can reduce hatching success (Santidrián Tomillo et al., 2009).

Although the function of yolkless eggs remains unclear (Eckert et al., 2012), studies have indicated that they could be so-called 'production over-runs' of oviducts producing copious albumen for egg clutches and do not have a function to facilitate gas exchange or temperature control within the clutch environment (Wallace et al., 2006, 2007). However, Caut et al. (2006) showed that yolkless eggs had a positive effect on the clutch, protecting yolked eggs from insect predation, and Patiño-Martínez et al. (2010) proposed that space created by the dehydration of yolkless eggs may favour the emergence of neonates. Artificial incubation in boxes protects eggs from predation and we kept hatchlings on the sand surface inside the box until they entered into frenzy before being released. For these reasons, and because other translocation programmes have not found a significant difference in hatching success between clutches incubated with and without yolkless eggs (Dutton & McDonald, 1995; Dal Pont Morisso & Krause, 2004), we believe that not including yolkless eggs in artificially incubated clutches did not affect hatching success.

Effects of artificial incubation on incubation duration

As well as affecting hatching success the artificial incubation treatments also influenced incubation temperature, which determines incubation duration and hatchling sex (Morreale et al., 1982). The longer incubation durations recorded in clutches on eastern beaches could be attributable to environmental conditions because the rangers' barracks where the clutches were incubated was located in a place with higher humidity and probably lower temperatures than the beach. These longer incubation durations resulted in a lower percentage of female hatchlings on eastern beaches. However, as no clutches were incubated in situ on eastern beaches we could not determine if there were significant differences between the sex ratios in artificially incubated and in situ clutches. Given that sand colour and quality can have an effect on thermal conditions on beaches (Naro-Maciel et al., 1999; Weber et al., 2011) it is possible that there are differences in natural sex ratio between beaches. The incubation site at the western beaches is away from the beach in an area of arid scrub, and clutches here reached high temperatures, resulting in similar incubation durations and sex ratios to in situ clutches.

The eastern beaches are subject to illegal take of c. 100% of clutches not relocated (Revuelta et al., 2012) and shortage of human and economic resources prevents in situ clutch protection here. Therefore, clutch relocation is currently the only viable option for increasing recruitment, which has been recommended when illegal take of clutches approaches 100% (Mortimer, 1999). We improved the previous programme by incubating clutches at the barracks of each beach and thereby reducing transportation, replacing styrofoam boxes with polyurethane ones and, since 2011, incubating 50% of clutches on eastern beaches in a hatchery on the beach to mimic natural sex ratios as closely as possible.

For the artificially incubated clutches on western beaches sex ratios were similar to in situ clutches but hatching success was lower. Western beaches are less frequented by walkers, and rangers' facilities were installed there during the project, allowing us to reinforce protection and gradually reduce the number of clutches incubated artificially during the study period. In 2010, considering the low predation rate of in situ clutches, we left all clutches incubating on the beach.

On the eastern beaches of the Jaragua National Park the overall production of hatchlings incubated in boxes during the study period was much greater than would have been expected without protection efforts but this method probably altered natural sex ratios. Further research is needed to improve conservation measures at these beaches, including enforcement of beach protection and the development of an effective hatchery. Our results also indicate that artificial incubation on the western beaches

was an ineffective conservation strategy given the low levels of hatching success recorded, and therefore current conservation is focused on in situ clutch protection. However, the western beaches are subject to unregulated tourism activity and development and are threatened by the expansion of bauxite and limestone mining (Wielgus et al., 2010). Thus, this conservation strategy will only be possible with institutional control over the influx of tourism and coastal development.

It is possible that in the future relocation and artificial incubation will be an option to avoid the extirpation of marine turtle nesting populations threatened by habitat loss to coastal development, or the predicted rise in sea level. Our findings highlight the importance of monitoring and assessing mortality and sex ratio in conservation programmes using these methods.

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