UNIVERSIDAD NACIONAL DE COLOMBIA
SEDE BOGOTÁ
FACULTAD DE CIENCIAS
DEPARTAMENTO DE BIOLOGÍA

ACTA BIOLÓGICA COLOMBIANA
ARTÍCULO DE INVESTIGACIÓN

REPRODUCTION AND CONSERVATION OF THE MAGDALENA RIVER TURTLE (*Podocnemis lewyana*) IN THE CLARO COCORNÁ SUR RIVER, COLOMBIA

Reproducción y conservación de la tortuga del Río Magdalena (*Podocnemis lewyana*) en el Río Claro Cocorná Sur, Colombia

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Received 28th December 2013, first decision 6th May 2014, accepted 10th May 2014.

Citation / Citar este artículo como: CEBALLOS CP, ROMERO I, GÓMEZ-SALDARRIAGA C, MIRANDA K. Reproduction and conservation of the Magdalena River turtle (*Podocnemis lewyana*) in the Claro Cocorná Sur River, Colombia. Acta biol. Colomb. 2014;19(3):393-400.

ABSTRACT

The Magdalena River Turtle, *Podocnemis lewyana,* is an endangered and endemic turtle from Colombia. Among the most important information needed to conserve endangered species is to identify, monitor, and protect the sites used by the species to reproduce and grow. In this study we report, for the first time, the reproductive output and the nesting beaches of *P. lewyana* in the Claro Cocorná Sur River, a tributary of the Magdalena River drainage. We systematically examined a river transect of 8 km with 14 sandy beaches during two nesting seasons in one year. We recorded a yearly production of 47 clutches, 957 eggs, and two preferred nesting beaches: Alto Bonito with 51 %, and Bélgica with 28.3 % of this reproductive output. AAFUVER, a community-based organization, has led a headstarting program since 2010 to decrease *in-situ* egg mortality due to predation on nesting beaches. AAFUVER collects and incubates the eggs *ex-situ*, raises the hatchlings for one to five months and then releases them into the same river. To understand potential effects of such egg manipulation, we monitored and compared *in-situ* and *ex-situ* incubation temperatures. We found *ex-situ* temperatures below the pivotal temperature known for *P. lewyana* and below the temperatures in nesting beaches. Finally, we monitored hatchlings growth under AAFUVER captive conditions, and found that hatchlings duplicated their body mass during the first three months of age. Egg weight was strongly associated to body weight at hatching, however this association is lost by the third month of age. We strongly encourage supporting this community-based conservation program, and the protection of the Claro Cocorná Sur River as an important nesting and growth habitat for the conservation of *P. lewyana*.

Keywords: growth, incubation temperatures, nesting.

RESUMEN

La tortuga del Río Magdalena, *Podocnemis lewyana,* es una tortuga categorizada como amenazada y además endémica colombiana. Dentro de la información más necesaria para entender el estado de conservación de estas especies esta el identificar, monitorear y proteger sus sitios de reproducción y crecimiento. En este estudio se reporta, por primera vez, el potencial reproductivo y las principales playas de anidación de *P. lewyana* en el río Claro Cocorná Sur, un tributario del río Magdalena. Un trayecto de 8 km del río que albergaba 14 playas arenosas fueron monitoreados sistemáticamente durante

dos estaciones de anidación en un año. Durante este tiempo se registraron 47 nidos, 957 huevos y dos playas de mayor anidación: Alto Bonito con el 51 %, y Bélgica con el 28,3 % de dicha producción anual. AAFUVER, una organización de la comunidad local, ha liderado un programa de reforzamiento poblacional para disminuir la mortalidad de los huevos incubados *in-situ*. AAFUVER colecta e incuba los huevos *ex-situ*, cría los neonatos durante uno a cinco meses, y los libera en el río de origen. Para entender los efectos potenciales de esta manipulación de los huevos las temperaturas de incubación *in-situ* y *ex-situ* fueron monitoreadas y comparadas. Se encontró que las temperaturas *ex-situ* son inferiores a la temperatura pivotal de *P. lewyana* y a las temperaturas en las playas de anidación. Finalmente, se reporta el crecimiento corporal de los tortuguillos dadas las condiciones de cautiverio de AAFUVER. Se encontró una fuerte asociación entre el peso del huevo y el peso de los neonatos a los siete días de edad, sin embargo esta asociación se perdió a los tres meses de edad. Nosotros enfatizamos en la importancia de apoyar este programa de conservación liderado por la comunidad local, y la protección del Río Claro Cocorná Sur como un hábitat importante de anidación y crecimiento de *P. lewyana*.

Palabras clave: anidación, crecimiento, temperaturas de incubación.

INTRODUCTION

The Magdalena River Turtle (*Podocnemis lewyana*) is an endemic turtle of the Magdalena, Sinú and San Jorge rivers and their tributaries in Colombia. In addition to its restricted distribution, this species faces high mortality of eggs and adults due to animal and human predation and habitat alteration associated with artificial manipulations of rivers by hydroelectric dams, river beach mining and forest destruction to grow pasture for cattle (Vargas-Ramírez *et al*., 2007; Gallego-García and Castaño-Mora, 2008; Páez *et al*., 2012). These threats have driven this species to be categorized as Endangered at the national and international level (Tortoise and Freshwater Turtle Specialist Group, 1996; Castaño-Mora, 2002). Other authors however consider its conservation status is even worse, and have suggested listing it as Critically Endangered. The species *P. lewyana* has been reviewed recently (Páez *et al*., 2009b, Páez *et al*., 2012) and of particular interest for conservation actions are those studies providing information on the status of natural populations throughout its distributional range for conservation actions. During the last decade several researchers have studied the reproductive ecology and conservation status of populations of *P. lewyana* in different rivers across its range. For example, a population of *P. lewyana* inhabiting the Prado River in Tolima, a tributary of the Magdalena River was monitored as part of an initiative to create a community-based conservation program (Vargas-Ramírez *et al*., 2007). As part of this program nesting beaches were monitored for two months in 2007, but in spite of their search efforts the authors were able to locate only two nests, perhaps due to the lack of native guides trained to find nests (Vargas-Ramírez *et al*., 2007). The following year the Sinú River was monitored in search of nesting beaches and individuals to estimate the species home range and population structure (Gallego-García and Castaño-Mora, 2008). Five nesting beaches were reported in the mid and low basin of the Sinú River. Though nesting reports were based on anecdotal information, and in spite of the high egg predation by fishermen, authors were able to locate two clutches and report their size and egg morphometry. At the same time, the distribution and abundance of *P. lewyana* in the Magdalena River drainage was also studied (Restrepo *et al*., 2008). Out of the 18 different sites that were visited by the authors, the Chicagua River was found to be the most important for the species, and was classified as exhibiting a high abundance of turtles. Four more sites were reported exhibiting an intermediate abundance, and 13 other sites a low abundance. Among these sites, the Cocorná River was also visited and the authors confirmed the turtle's presence by means of carcasses of depredated turtles, but no live animals were observed. Using anecdotal information from locals the turtle abundance was classified as low (Restrepo *et al*., 2008, Páez *et al*., 2009b).

Currently there is a community-based program for the conservation of *P. lewyana* in the Claro Cocorná Sur River, a program led by the non-governmental organization *Asociación Ambientalista Futuro Verde* (AAFUVER). This program started in 2010 and works in the lower reaches of the Claro Cocorná Sur River, specifically in the river segment between the municipalities of Puerto Nare and Puerto Triunfo, Department of Antioquia (Romero, 2011). Sponsored by the local authorities, this program has included training of the AAFUVER members on fieldwork techniques, and the implementation of a conservation center in the town of Estación Cocorná. The project has now moved on to monitor the nesting activity of *P. lewyana* of neighboring river beaches. Because egg mortality in the river beaches is high due to human and animal predation and river flooding, AAFUVER built a small incubation room to incubate eggs *ex-situ*. After hatching the neonates are maintained in an outdoor pool for one to five months until they are released in wetlands of the same river (Romero, 2011). This headstarting program is expected to increase egg survival and in this way to contribute to the conservation of the local population at this site as has been shown in the congeneric *Podocnemis expansa* (Hernández and Espin, 2006). Data from this monitoring during the last two years have been collected in spite of economic and logistic difficulties; they have not been analyzed previously and thus are included in this study. The present study had three objectives. First, we quantified the reproductive output of *P. lewyana* at this site by carrying out a systematic monitoring of nesting activity during

one year. This data is complemented with unpublished data previously collected by AAFUVER. We specifically recorded the number of nests per beach, clutch size, and egg morphometry. To understand how reproduction varies across space and time we compared our findings across nesting beaches and seasons. Second, given the effects of incubation temperature on sex, size, and growth of neonates (Páez *et al*., 2009a) we compared the incubation temperatures *in-situ* in river beaches with the *ex-situ* temperatures at the AAFUVER incubation room. Finally, we estimated hatchlings body growth given maternal effects and the captive conditions during the first three months at the AAFUVER station before being released into the river.

MATERIALS AND METHODS

Study area

This study was carried out in the lowland of the Claro Cocorná Sur River that runs by the town of Estación Cocorná (6°2'8"N, 74°38'20"W) in the municipality of Puerto Triunfo, Department of Antioquia, Colombia. We monitored 14 sand beaches during two nesting seasons: from 10 December 2012 to 12 January 2013 (herein called the "December season"), and from 13 June to 19 July 2013 ("June season"). These beaches were chosen because AAFUVER had reported them as the main nesting sites of *P. lewyana* in this transect of the river (Romero, 2011).

Egg collection

Boat trips were made every morning starting at approximately 5:00 h. All beaches were inspected for fresh turtle tracks left on the sand the night before with the guidance of AAFUVER members native to this region. Once a nest was found, eggs were recovered with special care to avoid egg rotation and transported to the incubation room within 1-6 hours of collection. Eggs were marked with a pencil to individually identify them, weighed (g), and length and width measured (mm). Most of the clutches from the December season, plus all clutches from the June season were incubated following the AAFUVER protocol as part of their headstarting program. Ten clutches from the December season were incubated as part of another study published elsewhere.

Nest location and incubation temperatures

All natural nests on river beaches were characterized in terms of their depth and relative location with respect to the water and vegetation. This is particularly important because this river is highly dynamic and nesting beaches can be flooded after a strong rain. Likewise, to compare the incubation temperatures *in-situ* (in the natural nests in the river nesting beaches) and *ex-situ* (in the incubation room at AAFUVER center) we recorded temperatures in both locations. *In-situ* incubation temperatures were recorded by inserting packs of three HOBO dataloggers within a zip-lock bag. To facilitate its location, the bag was tied with a rope to the trunk of a nearby plant, or to a wooden stick buried in the sand. Dataloggers were set to record the temperature every one hour and were left buried for 60 days. We buried dataloggers in16 emptied nests at four different beaches: Alto Bonito (n = 8), Bélgica (n = 4), Normandía (n = 3), and Costa Rica (n = 1). *Ex-situ* incubation temperatures were recorded by inserting one datalogger in ten different incubation containers, each with a different clutch. These clutches came from the same beaches: Alto Bonito (n = 6), Bélgica (n = 3) and Normandía (n = 1). The incubation protocol was the same used regularly by AAFUVER as described below.

AAFUVER headstarting program

Podocnemis lewyana nests two times during the year in this region: near the middle and at the end of the year. AAFUVER has been slowly improving their protocol since they started in 2010. Specifically, they have monitored the nesting activity during these seasons: December 2010, June 2011, December 2011, and June 2012. Monitoring results from December 2012 and June 2013 were a joint effort with AAFUVER. Because foxes and humans heavily depredate turtle eggs when they are left *in-situ* in the nesting river beaches, all eggs were transported and incubated at the AAFUVER station.

The incubation room (2 x 3 m) is equipped with shelves, where eggs are incubated in plastic containers fully buried in river sand collected from where the nest was collected. The room temperature varies with natural day and night cycles and additional heat is provided by a light bulb for the entire room. The containers are covered with plastic tied with rubber to maintain humidity. After hatching, neonates are left within the incubation containers a few more days until they completely emerge from the eggshell and the remaining yolk is internalized. Afterwards, hatchlings are moved to an outdoor pool for one to five months. The pool is made of cement, 3 m long x 2 m wide x 1 m deep, with 40 cm of water column, and is provided with a continuous river water (dechlorinated) flow-through system. To avoid bird predation, the pool is covered with a polyethylene mesh. Live "buchón de agua" (*Eichornia crassipens*) are provided to serve as perches and hiding places for hatchlings. In addition, turtles are fed daily with "dinder" or "árbol de mora"(*Chlorophora tinctoria*), "lenteja de agua" (*Lemma minor*), and "plátano topocho" (*Musa paradisiaca*). To monitor the effect of these captivity conditions, we recorded the body growth of hatchlings before being released. The neonates hatched between 3 February and 7 March 2013, and they were weighed at 7 days and 3 to 3.5 months of age. To monitor individual growth, hatchlings were tagged by attaching colored beads to the marginal scutes of their carapace (Galbraith and Brooks, 1984), a technique that has been useful in monitoring individual growth in captive young turtles for several months (Ceballos y Valenzuela, 2011).

RESULTS

Nesting beaches

A total of 47 clutches of *Podocnemis lewyana* were recorded along the Claro Cocorná Sur River in one year. Of this total, 21 clutches were laid during the December 2012 season and 26 nests during the June 2013 season. We missed egg collection from nine nests at the beginning of the June season, and thus we were able to record clutch size in only 17 nests. Including clutches with size data in both seasons (n = 38), we estimated a yearly production of 957 eggs at this site (Table 1). Out of the 14 river beaches that we monitored in a transect of 8 km of this river, we observed that turtles nested on only seven beaches, and of these, turtles heavily used two beaches. The main nesting site was Alto Bonito beach, with a yearly production of 24 clutches containing 488 eggs (51 %). The second main nesting site was Bélgica beach with 13 clutches containing 271 eggs (28.3 %) (Fig. 1). Linear length of nesting beaches varied from approximately 170 to 1.312 m. Nesting beaches were highly dynamic, with a new beach, Buenos Aires, that appeared towards the end of the December season.

The distance of clutches respect to the water and vegetation was measured and we found that clutches (n = 17) were laid closer to the vegetation than to the river water during the June season. Clutches were 25.4 m on average away from the vegetation (range 6.8 to 60 m) and 54.2 m on average from the river (range 15 to 96 m). Nest depth was also recorded during the June season. On average, nests were 16.8 cm deep with a wide variability that ranged from 13 to 27 cm deep. Clutch size was also highly variable during the June season, ranging from 15 to 30 eggs, however we found no relationship between nest depth and clutch size (p = 0.4272, n = 17). We suspect that nest width may have correlated with clutch size; however we did not measure nest width and cannot confirm this idea.

Number of clutches and clutch size variability by season

Counting both nesting seasons, and excluding the nine clutches that we missed in June, we found average clutch size was 21.6 eggs (n = 38), egg weight was 26.3 g, and egg size was 42.4 x 32.5 mm (Table 2).

To better understand the reproductive cycle of *P. lewyana*, we tested for a difference between the two nesting seasons in terms of clutch size and egg size. In absolute numbers, the number of clutches and the number of eggs per clutch were higher in the middle of the year, the June season; nevertheless we found no statistical difference between the number of eggs laid each season (p = 0.411). Interestingly however, eggs laid at the end of the year were heavier (p< 0.0001) and wider (p< 0.0001) than eggs laid in the middle of the year (Table 1). Egg morphometry has not been recorded in previous seasons, thus we do not know if these differences are consistent through time.

Table 1. Number of nests of *Podocnemis lewyana* recorded during two nesting seasons in the Claro Cocorná Sur River, Antioquia, Colombia.

Nesting beach	Beach length (m)	Dec. 2012 season		June 2013 season		Yearly production	
		Clutches	Eggs	Clutches	Eggs	Clutches	Eggs
Alto Bonito	453	11	227	13	261	24	488
Bélgica	821	5	108	8	163	13	271
Buenos Aires	100*	1	19	0	0	1	19
Costa Rica	1312	1	24	1	27	2	51
Florencia I	303	0	0	0	0	0	0
Florencia II	378	0	0	0	0	0	0
Florencia III	665	1	25	0	0	1	25
Florencia IV	324	0	0	0	0	0	0
Florencia V	279	0	0	0	0	0	0
La Culebra	N/A	0	0	0	0	0	0
Normandía I	237	1	21	0	0	1	21
Normandía II	385	1	26	4	56	4	82
Porvenir	177	0	0	0	0	0	0
San Fernando	170	0	0	0	0	0	0
Total		21	450	26	507	47	957

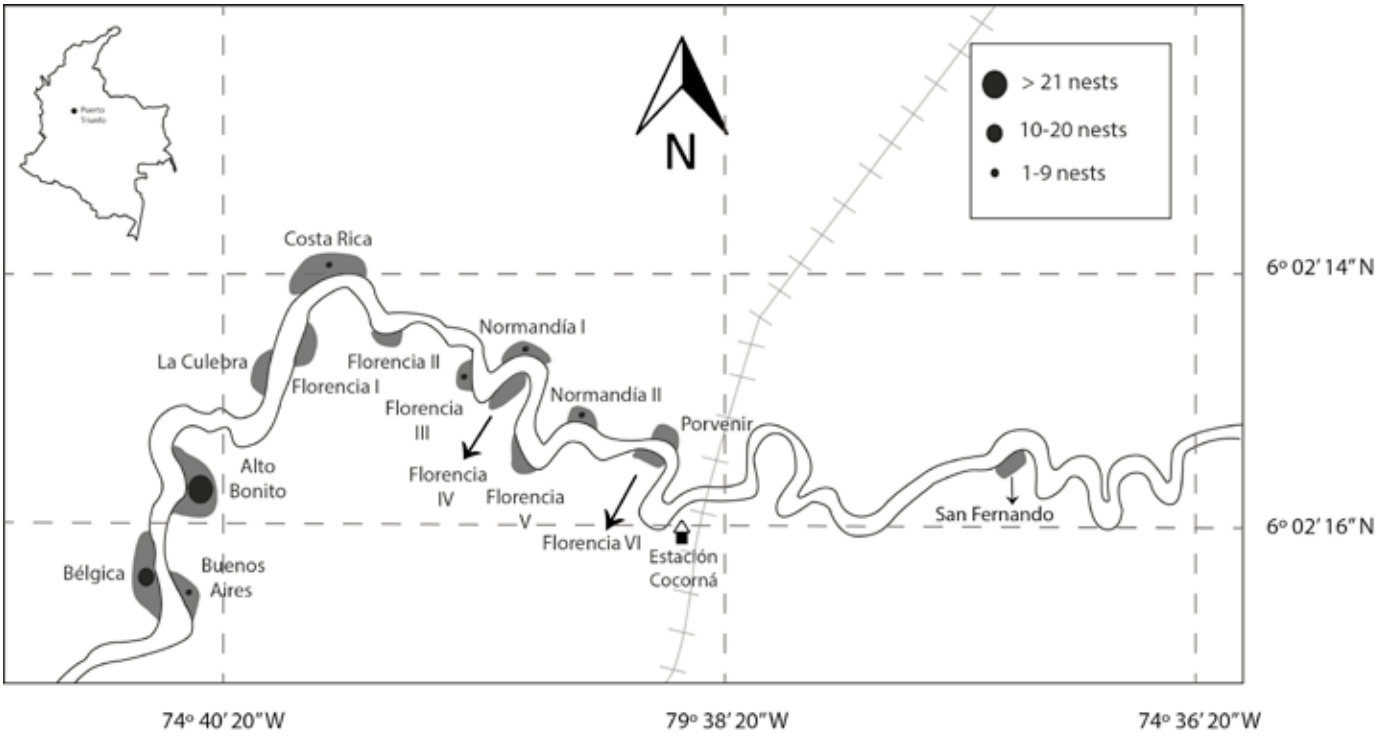

Figure 1. Map of the Claro Cocorná Sur River, Antioquia, showing the nesting activity of *Podocnemis lewyana* on the 14 river beaches monitored during one year in this study.

Table 2. Clutch size and egg morphometry from clutches of *Podocnemis lewyana* monitored during two nesting seasons in the Río Claro Cocorná Sur, Antioquia, Colombia. Bold letters indicate the nesting season with heavier and wider eggs.

	December 2012–January 2013	June–July 2013	Both seasons	Are nesting seasons different?
Clutch size				
n	21	17*	38	
Average	21.2	22.0	21.6	No (p = 0.7453)
Min	11	15	11	
Max	27	30	30	
Egg weight				
n	445	373	818	
Average	27.0	25.5	26.3	Yes (p < 0.0001)
Min	20.0	18.0	18.0	
Max	38.0	31.3	38.0	
Egg length				
N	445	374	819	
Average	42.5	42.2	42.4	No (p = 0.0865)
Min	33.5	32.62	32.6	
Max	49.6	47.9	49.6	
Egg width				
n	445	374	819	
Average	32.9	32.1	32.5	Yes (p < 0.0001)
Min	29.4	21.1	21.1	
Max	38.5	36.6	38.5	

* The total number of clutches in the June season was 26 however we missed egg morphometry data at the beginning of the June season for logistic reasons and thus herein we report data from only 17 nests.

Incubation temperatures

Out of the 16 packs of dataloggers buried in four nesting beaches for two months, we recovered the dataloggers in only seven sites (empty nests): three in Normandia, three in Bélgica and one in Costa Rica. Unfortunately we lost all dataloggers buried in Alto Bonito beach, and because the ropes used to fix them to nearby trees were found sharply cut, we suspect they were stolen. Average sand temperatures were as follows: Normandia: 34.1°C (st. dev. = 4.2°C), Bélgica: 32.8°C (st. dev. = 2.5°C), Costa Rica: 33.7°C (st. dev. = 2.0°C). On the other hand, the temperatures maintained by AAFUVER in their incubation room were lower (average = 31.81°C) and less variable (st. dev. = 1.83°C) than those of natural beaches.

Hatchlings growth

We followed individual body growth of a sample of hatchlings. Their egg weight was 27.2 g (st. dev. = 3.5, n = 175). Body weight at seven days of age was lower, averaging 20.1 g (st. dev. = 2.6, n = 175), but at three months of age their body weight doubled, averaging 40.6 g (st. dev. = 7.8, n = 159). We observed a positive relationship between egg weight and hatchling body weight (p<0.0001, R^2 = 0.78). Nevertheless, three months later, this relationship was diluted as its variance increased considerably (R^2 = 0.22) making this association weak (Fig. 2).

DISCUSSION

Reproductive output

We monitored two nesting seasons of *P. lewyana* at the Rio Claro Cocorná Sur River and were able to document a production of 957 eggs from 47 nests, with an average clutch of 21.6 eggs at this site. The hatching success given the incubation conditions of AAFUVER in recent years has been high: 93 % (n = 65) in 2010, 97.9 % (n = 285) in 2010-2011 (Romero, 2011), and 84 % (n = 432) in December 2012. Hatching success data from June 2013 was not recorded. Thus, assuming an average hatching success from all these years of 91.6 %, applied to the two seasons that were systematically monitored (n = 957 eggs), we estimate that at least 877 *P. lewyana* neonates were produced at this site during that year. This number however may be even higher if we missed nests at the monitored or nearby river beaches. This river then provides an important nesting habitat, and we urge its preservation for the conservation of this endangered species.

In addition to nesting beaches, it is important to mention that this river also provides refuge for juveniles in its associated swamps. AAFUVER and the regional environmental authorities, CORNARE and CORANTIOQUIA, recently started a capture-recapture program aimed at estimating population size at this site. The first monitoring was carried out between 7 July and 4 August 2011 with 72 turtles

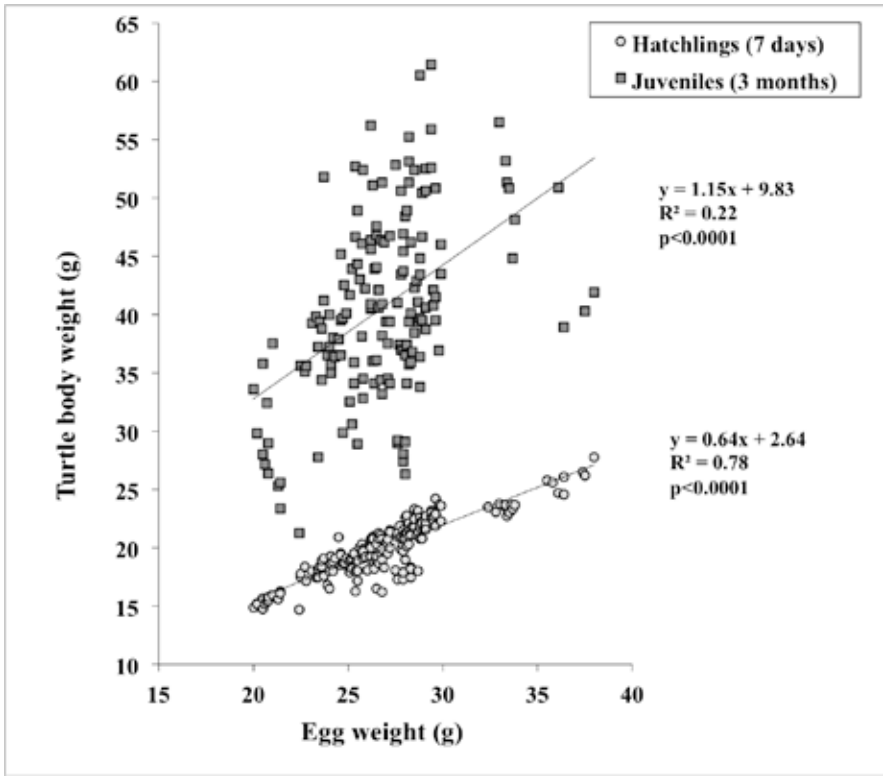

Figure 2. Regression of egg weight on turtle body weight of Podocnemis lewyana at two different ages monitored in this study.

captured (42 males, 19 females and 11 juveniles) in four swamps: Paticos, La Culebra, La Posada, and Buenos Aires (García-Hernández, 2011). The second monitoring was carried out between Dec 30 2012 and 23 Feb 2013, with 109 turtles captured (44 males, 29 females and 36 juveniles) in the same four swamps (García-Hernández, 2013). Most (80.9 %) of these individuals in the second monitoring were under 20 cm of linear carapace length. Considering the total area of these four swamps is 17.68 ha (Londoño *et al*., 2009), the population density counted so far varies between 4.1 and 6.3 juvenile turtles/ha. The fact that these swamps are shallow areas between 1 and 2.7 m deep (Londoño *et al*., 2009), and that larger turtles are mainly found in deeper areas above 3 m (Gallego-García and Castaño-Mora, 2008), explains the high percentage of small turtles found by this capture-recapture studies. One recommendation to improve the AAFUVER conservation program is to encourage capture using different techniques (e.g., traps, nets, diving) in deeper areas of the river in order to include larger turtles in their capture-recapture program.

Nesting seasons and nesting beaches

As in the Sinú, Magdalena and Prado Rivers (Vargas-Ramírez *et al*., 2007; Gallego-García and Castaño-Mora, 2008; Restrepo *et al*., 2008), *P. lewyana* has two nesting seasons in the Rio Claro Cocorná Sur River (this study), December to January and June to July approximately. We found a higher number of nests during the June season; however, egg weight was significantly greater in the December season, and egg weight has an important association on hatchling growth. Thus it is important to keep monitoring and protecting both nesting seasons at this site.

In addition, we found that during the last three years nesting has been concentrated on two river beaches: Alto Bonito and Bélgica beaches (Table 3). These two beaches were not the largest, and cattle were often seen resting or walking on them; however, these livestock do not seem to deter turtles from nesting there. We did not monitored nesting in more distant beaches but it seems important to highlight that these two beaches were the farthest sites from the town of Estación Cocorná, and perhaps turtles prefer these sites because they are less disturbed by human activities.

Incubation temperatures

The average incubation temperature from all nesting beaches monitored in the Claro Cocorná Sur was calculated as 33.5 °C. This temperature is very close to 33.4°C the pivotal temperature reported for *P. lewyana* (Páez *et al*., 2009a), above 32.8 °C reported for nesting beaches in the Magdalena River (Páez *et al*., 2012), and a little above the 33.2 °C reported for nesting beaches in the Sinú River (Gallego-García and Castaño-Mora, 2008). The effect of natural temperatures on sex ratios is hard to predict because it is not only the average temperature during the middle third of the incubation period that is important, but also the temperature variability that defines the accumulated heat experienced by the embryos (Valenzuela, 2001). In addition, the information available on the effect of temperature on sex determination of *P. lewyana* was obtained using a population in the Mompox Depression of the Magdalena River (Páez *et al*., 2009a) and pivotal temperatures may vary with genetics, the environment, and even geographic trends (Ewert *et al*., 2004). However, the incubation temperature achieved with a light bulb at the AAFUVER incubation room was much lower (31.8 °C) than in natural beaches of the Claro Cocorná Sur River. Another recommendation to improve the AAFUVER headstarting program is to increase their *ex-situ* incubation temperature by 1.5 to 2 °C to match temperatures under natural conditions.

Table 3. Number and percentages of nests of *Podocnemis lewyana* in the Claro CocornáSur River by nesting beach. Bold letters indicate the main nesting beaches through time.

Nesting beaches	2010		2011		2012		2013
	June (n = 3)	Dec (n = 14)	June (n = 25)	Dec (n = 7)	June (n =13)	Dec (n = 21)	June (n = 26)
Alto Bonito		21 %	28 %	14 %	54 %	52 %	50 %
Bélgica		57 %	32 %	57 %	23 %	24 %	31 %
Buenos Aires						5 %	
Costa Rica		7 %	8 %			5 %	4 %
Florencia	67 %	14 %	8 %		8 %	5 %	
La Culebra			12 %				
Normandia			8 %	29 %	15 %	10 %	15 %
Porvenir			4 %				
San Fernando	33 %						
Total	100 %	100 %	100 %	100 %	100 %	100 %	100 %

HATCHLINGS GROWTH

The positive relationship between egg weight and initial hatchling size and one month later in *P. lewyana* had been documented already (Páez *et al*., 2009a). Here we find this association is lost by the third month of age; it is likely that other environmental factors such as available food resources (Ceballos *et al*., 2014) are also influencing body weight. Further studies on the effect of different types and amount of food offered in captivity are encouraged to foster hatchlings growth.

CONCLUSIONS

We highlight the Claro Cocorná Sur River as a very important site for the conservation of *P. lewyana* because it provides nesting and growing habitat for this population. We emphasize the importance of community-based programs to conserve native endangered species, as it is the local communities that are live with them every day. The institutional support from the environmental authorities has been vital to empower the community towards a truly long-term conservation goal.

ACKNOWLEDGMENTS

We thank Alvaro Díaz-Pineda, Aurelio Delgado and Alvaro Díaz-Romero for their logistic help and sharing their expertise to locate nests in the field, A. Gómez for designing the map in Fig. 1. Funding was provided by the Comité para el Desarrollo de la Investigación, CODI, and the 2013-2014 Sustainability Program of the University of Antioquia. This work was carried out under protocol approved by the Animal Experimentation Ethics Committee of the University of Antioquia in Act 76, and research permit N°134-0067 issued by the local government authority CORNARE.

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