Population structure, size and morphometry of the white-lipped mud turtle *Kinosternon leucostomum postinguinale* in pond systems in Quindío, Central Andes of Colombia

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ABSTRACT - We analyzed the population structure, size and morphometry of *Kinosternon leucostomum postinguinale* present in ponds of three localities of La Vieja River basin in the Department of Quindío: National Park of Coffee (NPC, Montenegro municipality), La Palmita village (La Tebaida municipality) and Roble River low area (Quimbaya municipality). Twenty-three days of sampling were conducted from May to October 2017. To locate turtles, we implemented an active search method by walking. Each manually captured individual was measured, weighed and sexed in the field. In total, 61 turtles were captured, most of them in the National Park of Coffee (n = 44). The populations in all three locations were dominated by adults with a 1:1 ratio of females to males. Juveniles (three) were only found in the National Park of Coffee, suggesting that this locality is a refuge for the species. Turtle linear dimensions to body mass relationship was allometric; juveniles grew faster than adults. Preanal and postanal length differed between males and females, allowing the efficient determination in mature turtles. Sexual maturity in both males and females is estimated to be reached at a straight maximum carapace length of 9.5 cm.

INTRODUCTION

Colombia has the seventh highest number of turtles and ranks second after Brazil in the Neotropics (Páez, 2012a; Turtle Taxonomy Working Group, 2017; Forero-Medina et al., 2016) with 34 species (six marine and 28 freshwater). However, little is known about the biology, natural history and distribution of many species. In addition, turtles are considered one of the most threatened vertebrate groups (Gibbons et al., 2000; Páez, 2012a; Turtle Taxonomy Working Group, 2017; Vargas-Ramírez et al., 2017), with more than half of the species in a threat category (Páez, 2012a).

It has been recognised that environmental stressors can alter the structure of animal populations and reduce their size, leading to local extinction (Dodd, 1990; Gibbons et al., 2000; Rueda-Almonacid et al., 2007; Morales-Betancourt et al., 2012). In Colombia, the risks and threats to turtle populations are diverse. These include degradation and loss of their ecosystems, such as ponds and wetlands; the introduction of foreign and exotic species that compete directly and indirectly for resources; indiscriminate hunting and illegal trade including the pet trade and as magic-religious objects (Rueda-Almonacid et al., 2007; Morales-Betancourt et al, 2012; Páez, 2012a). These factors in addition to late sexual maturity and low juvenile survival, means that recovery of turtle populations in their natural environment even under optimum conditions, will be very slow (Rueda-Almonacid et al., 2007; Páez, 2012a). One of the species is *Kinosternon leucostomum*, but most information on population and reproductive aspects are derived from the subspecies *K. l. leucostomum*, which is distributed from the south-eastern Mexico, to Honduras and Nicaragua (Turtle Taxonomy Working Group, 2017), Although the subspecies *K. l. postinguinale* has a wide distribution from southern Nicaragua to Peru, few studies have been carried out on its ecology (Medem, 1962; Berry & Iverson, 2001; Rueda-Almonacid et al., 2007; Giraldo et al., 2012b; Rodríguez-Murcia et al., 2014). Populations of *K. l. postinguinale* in Colombia are affected by: (1) illegal trafficking in the south-west, due to the pet trade (Giraldo et al., 2012b; Rodríguez-Murcia et al., 2014); (2) transformation of the forest, as in the inter-Andean valleys (Valle del Cauca River and Magdalena River Valley) and the Sinú River (Córdoba), where their habitat has been transformed for pastures, agricultural crops and sugarcane plantations; and (3) the drying of wetlands and water pollution, where freshwater reservoirs have high accumulation of industrial and agricultural pollutants, which could drastically affect the viability of populations (Castaño-Mora et al., 2005; Rodríguez-Murcia et al., 2014). Recently *K. l. postinguinale* has been recorded in the Department of Quindío (Arango-Lozano et al., 2018). However, there are no data on the species´ population ecology in this Andean Department, which makes research critical for assessing population status and developing management strategies. The objectives of this study were to evaluate the structure of population, size and shell morphometry of *K. l. postinguinale* in ponds at three locations in the Department of Quindío.

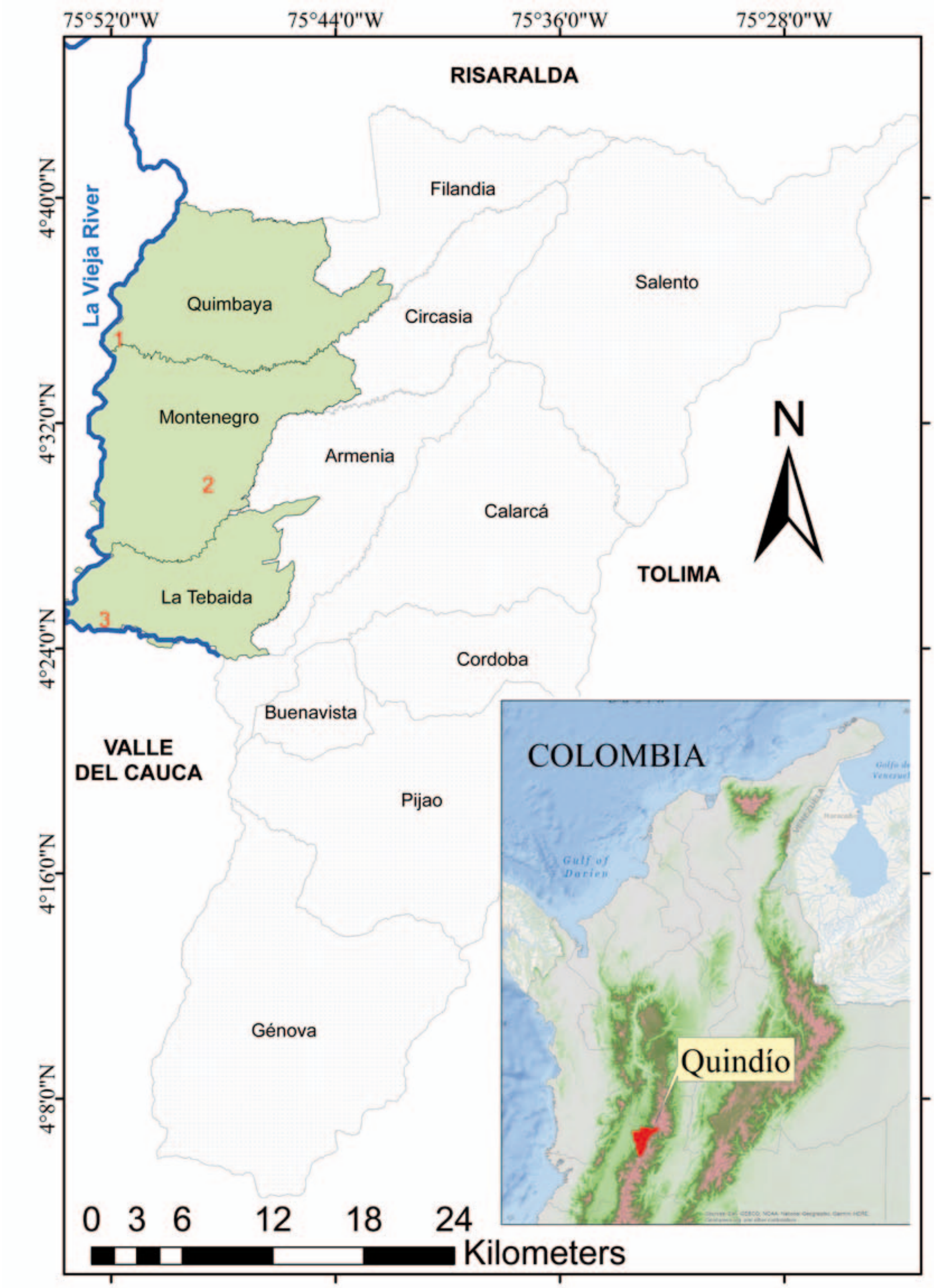

Figure 1. Location of the study area and each of the locations in the Department of Quindío, Colombia; 1 = Roble River low area; 2 = National Park of Coffee; 3 = La Palmita, Pisamal

MATERIALS AND METHODS

The study area included three locations in the La Vieja River basin, which covers the whole of Quindío and part of the Departments of Risaralda and Valle del Cauca, in western central Colombia. The landscape of the basin is dominated by an agriculture (livestock and crops) with numerous tributaries, and small areas of native vegetation, mainly *Guadua angustifolia* forest (Botero-Botero et al.,, 2016). The region has two seasons of low rainfall (June-September and January-March) and two of high rainfall (October-December and April-May, > 2000 mm per year) (Román-Valencia, 1993; Ramírez et al., 2014). Our three sites were selected where individuals of *K. l. postinguinale* had previously been observed: (1) Roble River low area (4° 34'34.38" N, 75° 52'5.94" W, 975-1100 m, Roble River sub-basin; (2) National Park of Coffee (NPC, 4°32´22.08" N, 75˚ 46´6.28" W, 970-1250 m, Espejo River sub-basin), and (3) La Palmita village, Valle de Pisamal (4° 24'53.05" N, 75° 52'34.43" W, 1000-1200 m, Quindío River sub-basin; Fig. 1).

Sampling was carried out monthly from May to October 2017 at each of the study locations. To locate turtles, we used the method of free-search in the field, using visual and manual inspection, actively looking for turtles in wetlands, swamps and temporary ponds with abundant vegetation (Rueda-Almonacid et al., 2007; Giraldo et al., 2012b; Rodríguez-Murcia et al., 2014). Two people, one on each side of a pond or wetland, inspected the perimeter by touch, feeling for similar shapes to the shell of the turtle (Arango-Lozano et al., 2017). Once detected, each individual was hand-captured and uniquely marked with notches on the marginal shields following Cagle (1939).

For each individual, we recorded the straight maximum carapace length (SCL), straight maximum plastron length (SPL), preanal length (from last edge of plastron to cloaca, PRL), postanal length (from cloaca to edge of tail, POSL), carapace maximum height (CH), straight maximum carapace width (SCW) and body mass (MASS). After measuring, each turtle was released where it was captured in the shortest time possible (to minimise the stress of its handling). Sex was determined based on the preanal and postanal length of the tail, which are greater in males. Adults were sexed if SCL ≥ 10 cm, since at this size they can be considered mature sexually (Rueda-Almonacid et al., 2007; Rodríguez-Murcia et al., 2014), although we found smaller turtles, which were thought to be juveniles, and after results, we determined them as adult males.

The size and sexual structure of the populations in each of the sampled localities was established, and differences in the presence of adults (males and females) and juveniles among localities were evaluated using an independence test, and the proportions were compared using a goodness-of-fit test (Rodríguez-Murcia et al., 2014; Arango-Lozano et al., 2017). The SCL-body mass relationships for the combined populations were determined by a least squares linear regression after transformation to logarithms. Geometric similarity (retention of shape) was indicated when the regression coefficient was in agreement with 3.0 or when mass was the dependent variable 3.0 (e.g. Meek, 1982; Meek & Avery, 1988).

Based on the adults, the morphometric measurements of males and females between locations were compared using a Kruskal-Wallis multiple comparison test and a subsequent Dunn test (Giraldo et al., 2012a; Arango-Lozano et al., 2017). In addition, the mean morphometric measurements were calculated, and a student's *t*-test was used to determine if there were differences between sexes (Giraldo et al., 2012a; Arango-Lozano et al., 2017). Finally, the relationships of SPL to PRL and PRL to POSL by sex were evaluated with an analysis of covariance (Arango-Lozano et al., 2017). The statistical analyses were carried out in the free use software R version 3.4.2 (R Development Core Team, 2017).

RESULTS

During 504 man-hours of sampling effort (168 man-hours per site), 61 individuals were captured, 44 in NPC, seven in La Palmita and ten in the Roble River low area (Table 1). There were no significant differences in the sex ratio among study locations (χ^2 independence test = 0.88; df = 2; P = 0.64). However, significant differences were found in the adult to juvenile ratio among localities. Each population of *Kinosternon leucostomum postinguinale* was dominated by adults; with juveniles only found in NPC (Table 1).

Table 1. Sex ratios of adults *K. l. postinguinale* and adult to juvenile ratios at the different study locations in Quindio; M = male, F = female, A = adult, J = juvenile. The empty cells indicate samples sizes that are too small for analysis.

Locality	M	F	J	M:F	χ^2	P	A:J	χ^2	P
NPC	18	23	3	1:1.27	0.609	0.43	13.6:1	32.80	0.01
La Palmita	3	4	-	1:1.30	0.14	0.70	-	-	-
Roble River	6	4	-	1.5:1.0	0.40	0.52	-	-	-
Total	27	31	3	1:1.15	0.27	0.59	19.3:1	49.59	< 0.01

The straight maximum carapace length to body mass was allometric in *K. l. postinguinale* ($M = 0.1143L^{3.06}$; $r^2 = 0.967$; P < 0.01; Fig. 2). Also, when performing the multiple comparison analysis between localities, significant differences were found in PRL sizes for females of Roble River low area and NPC (*Q* = 1.94; P = 0.02), for CH in both females of La Palmita and Roble River (*Q* = -2.3; P = 0.01), and of Roble River and NPC (*Q* = 2.03; P = 0.02). In each of the measures, the sizes were greater for females of Roble River low area. On the other hand, for males no significant differences were found for any of the morphometric variables among the locations (Tables 2 and 4).

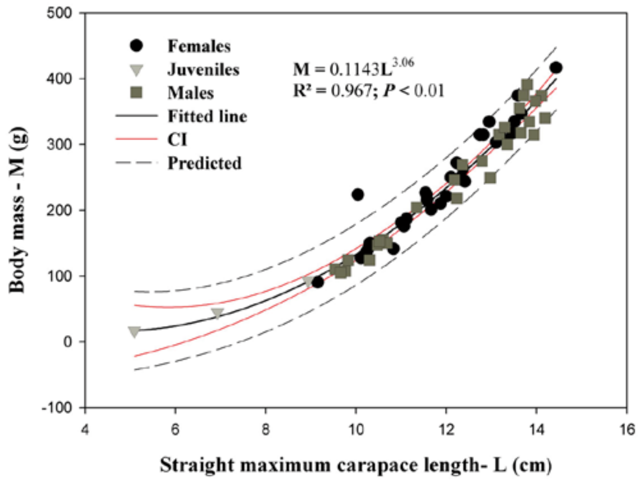

Figure 2. Relationship of the straight maximum carapace length (L) and body mass (M) variables in 61 individuals of *K. l. postinguinale*

From the analysis of comparison of morphometric variables only preanal length (*t* = 14.20; P < 0.01) and postanal length (*t* = 14.6; P < 0.01), differed by sex, being larger in males (Tables 3 and 4). The preanal region was greater in males than in females of similar sizes. The relationship

Table 2. Multiple comparison of size of the morphometric variables for males and females of *K. l. postinguinale* among the study localities. Q = Dunn test. SCL = straight maximum carapace length; SPL = straight maximum plastron length; PRL = preanal length; POSL = postanal length; CH = carapace maximum height; SCW = straight maximum carapace width; MASS = body mass. Values in bold type indicate a difference in greater size (e.g PRL) between two compared populations (e.g Roble River – NPC).

		Males		Females	
Variable	**Comparison**	**Q**	**P**	**Q**	**P**
SCL (cm)	La Palmita – Roble River	0.56	0.28	-1.04	0.14
	La Palmita - NPC	1.30	0.09	0.03	0.48
	Roble River - NPC	0.87	0.19	1.40	0.08
SPL (cm)	La Palmita - Roble River	0.03	0.48	-0.70	0.24
	La Palmita - NPC	0.49	0.31	0.43	0.33
	Roble River - NPC	0.60	0.27	1.34	0.08
PRL (cm)	La Palmita - Roble River	-0.23	0.40	-1.36	0.08
	La Palmita - NPC	-0.21	0.41	0.17	0.40
	Roble River - NPC	0.07	0.47	1.94	**0.02**
POSL (cm)	La Palmita - Roble River	0.16	0.43	-0.56	0.28
	La Palmita - NPC	-0.51	0.30	-0.80	0.20
	Roble River - NPC	-0.92	0.17	-0.09	0.46
CH (cm)	La Palmita - Roble River	1.00	0.15	-2.30	**0.01**
	La Palmita - NPC	1.33	0.09	-1.03	0.14
	Roble River - NPC	0.25	0.40	2.03	**0.02**
SCW (cm)	La Palmita - Roble River	0.80	0.21	-0.66	0.25
	La Palmita - NPC	1.10	0.13	0.42	0.33
	Roble River - NPC	0.26	0.39	1.28	0.09
MASS (g)	La Palmita - Roble River	0.47	0.31	-0.38	0.34
	La Palmita - NPC	1.13	0.12	0.87	0.19
	Roble River - NPC	0.78	0.21	1.38	0.08

Table 3. Comparison of size of morphometric variables between sexes of *K. l. postinguinale*; n = Number of samples; "χ" = Mean; *t* = value of the Student's *t*-test. SCL = straight maximum carapace length; SPL = straight maximum plastron length; PRL = preanal length; POSL = postanal length; CH = carapace maximum height; SCW = straight maximum carapace width; MASS = body mass. Values in bold type indicate significant differences of size (e.g SCL) between sexes.

Variable	**Males**		**Females**		***t***	**P**
	n		**n**			
SCL (cm)	27	12.2	31	11.95	0.78	0.43
SPL (cm)	27	10.61	31	10.73	0.34	0.73
PRL (cm)	27	1.68	31	0.80	14.20	**< 0.01**
POSL (cm)	27	1.97	31	0.99	14.60	**< 0.01**
CH (cm)	27	4.43	31	4.18	1.21	0.22
SCW (cm)	27	7.85	31	7.77	0.30	0.76
MASS (g)	27	249.9	31	241.80	0.34	0.73

between SPL and LPR in adults, differed significantly by sex (Ancova; $F = 273.3$; $P < 0.001$, Fig. 3). Both the influence of the covariate SPL ($F = 5.7$; $P = 0.02$) and the interaction of sex and the covariate SPL were significant ($F = 10.7$; $P = 0.0018$). Thus, the length of the preanal region in males is not correlated with SPL (Pl = 0.003Spl + 1.65; $r^2 = 0.0002$; $P = 0.93$; n = 27), but it is in females (Pl = 0.14Spl – 0.73; $r^2 = 0.79$; $P < 0.001$; n = 31; Fig. 3).

The relationship of PRL and POSL also differ significantly by sex (Ancova; $F = 375.8$; $P < 0.001$; Fig. 4). Both the influence of the covariate PRL ($F = 16.06$; $P < 0.001$) and the interaction of sex and the covariate PRL were significant ($F = 18.3$; $P < 0.001$). Length of the postanal region in males is correlated with PRL (Posl = 0.94Prl + 0.37; $r^2 = 0.53$; $P < 0.001$; n = 27), but not in females (Posl = -0,03Prl + 1.02; $r^2 = 0.003$; $P = 0.75$; n = 31; Fig. 4).

DISCUSSION

Most of turtles were captured in the NPC, possibly due to the protection offered by the National Park of Coffee Foundation; where fishing and hunting are prohibited and there is 24 hour surveillance, which favours both *Kinosternon leucostomum postinguinale* and *Chelydra acutirostris* as reported by Arango-Lozano et al. (2017). In addition, according to Rodríguez-Murcia et al. (2014), a factor that affects the population size of *K. l. postinguinale* is hunting for human consumption and by the pet trade. Therefore, it is possible that the low number of encounters in La Palmita and Roble River low area is due to free access by fishermen and hunters. The greater incidence of adults with respect to juveniles captured in this study may reflect to the search methodology, which might be biased against smaller individuals, as indicated by Rodríguez-Murcía et al. (2014). Also, the presence of juveniles only in NPC may be due to its refuge status but possibly the white-lipped mud turtle has some different habitat features not found elsewhere.

All adults in the study populations of *K. l. postinguinale* in Quindío had a sex ratio close to parity. Similar information to that reported in populations of white-lipped mud turtles in the Department of Caldas by Rodríguez-Murcia et al. (2014) where although the proportion in some ponds was skewed to males, the general population was 1:1; all the populations studied in Valle del Cauca were 1:1 Vallejo (2013). Since *K. leucostomum* exhibits a pattern of temperature-dependent sex determination (Yntema, 1979), with clutches incubated at constant temperatures above 28 °C producing only females (Ewert & Nelson, 1991; Vogt & Flores-Villela, 1992), it is possible that temperature in the clutches of the Department of Quindío follow a pivotal parameter, where it is stable enough not to bias the sex of neonates and to a certain extent, be reflected in the adult population (Valenzuela & Ceballos, 2012, Rodríguez-Murcia et al., 2014). Also, skewed sex ratios in adults may simply be a product of mortality rates in juveniles (Páez, 2012a, b; Ewert & Nelson, 1991; Arango-Lozano et al., 2017).

Relationship of linear dimension size to body

Table 4. Mean, minimum (Min) and maximum (Max) values of morphometric variables of adults and juveniles of *K. l. postinguinale*, captured in the study localities. SCL = straight maximum carapace length; SPL = straight maximum plastron length; PRL = preanal length; POSL = postanal length; CH = carapace maximum height; SCW = straight maximum carapace width; MASS = body mass

NPC	Variable	N	Mean	Min	Max
Males	SCL (cm)	18	11.94	9.53	14.19
	SPL (cm)	18	10.44	8.66	13.30
	PRL (cm)	18	1.71	1.34	2.79
	POSL (cm)	18	2.02	1.37	2.70
	CH (cm)	18	4.40	3.25	6.65
	SCW (cm)	18	7.76	6.36	9.69
	MASS (g)	18	233.11	107.72	391.20
Females	SCL (cm)	23	11.76	9.15	13.66
	SPL (cm)	23	10.58	8.19	12.56
	PRL (cm)	23	0.77	0.45	1.04
	POSL (cm)	23	1.00	0.67	1.17
	CH (cm)	23	4.12	2.56	5.06
	SCW (cm)	23	7.67	5.56	9.34
	MASS (g)	23	228.71	90.70	374.20
Juveniles	SCL (cm)	3	6.99	5.09	8.94
	SPL (cm)	3	5.96	4.30	7.81
	PRL (cm)	3	0.45	0.40	0.50
	POSL (cm)	3	0.47	0.40	0.50
	CH (cm)	3	2.65	1.71	3.68
	SCW (cm)	3	4.91	3.69	6.17
	MASS (g)	3	51.93	17.00	93.50
La Palmita	**Variable**	**N**	**Mean**	**Min**	**Max**
Males	SCL (cm)	3	13.27	12.35	14.11
	SPL (cm)	3	11.25	10.76	11.51
	PRL (cm)	3	1.61	1.38	1.83
	POSL (cm)	3	2.01	1.84	2.11
	CH (cm)	3	4.78	4.42	5.30
	SCW (cm)	3	8.36	7.59	8.82
	MASS (g)	3	314.33	269.00	374.00
Females	SCL (cm)	4	11.82	10.04	12.82
	SPL (cm)	4	10.75	10.45	11.04
	PRL (cm)	4	0.77	0.75	0.79
	POSL (cm)	4	0.98	0.91	1.12
	CH (cm)	4	3.76	3.40	4.42
	SCW (cm)	4	7.81	7.69	7.99
	MASS (g)	4	259.63	223.90	314.60
Roble River (low area)	**Variable**	**N**	**Mean**	**Min**	**Max**
Males	SCL (cm)	6	12.51	9.66	13.94
	SPL (cm)	6	10.83	8.54	12.03
	PRL (cm)	6	1.68	1.46	1.83
	POSL (cm)	6	1.81	1.19	2.15
	CH (cm)	6	10.83	8.54	12.03
	SCW (cm)	6	7.85	6.36	8.73
	MASS (g)	6	268.18	104.80	374.20
Females	SCL (cm)	4	12.89	10.83	14.43
	SPL (cm)	4	11.62	9.01	13.23
	PRL (cm)	4	1.03	0.71	1.21
	POSL (cm)	4	0.99	0.85	1.18
	CH (cm)	4	5.01	4.00	5.47
	SCW (cm)	4	8.30	6.87	9.32
	MASS (g)	4	299.75	141.70	416.70

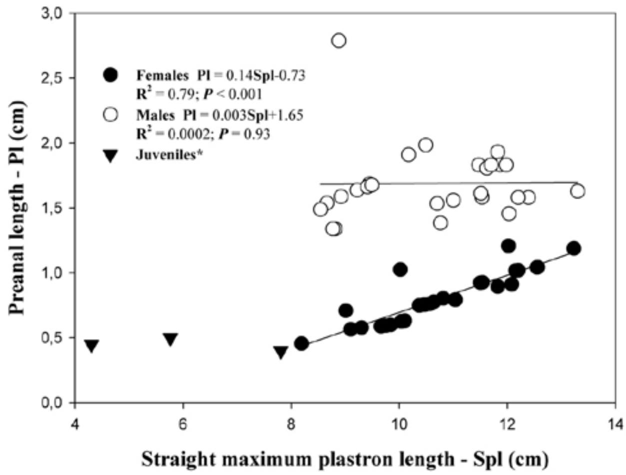

Figure 3. Relationship of straight maximum plastron length and preanal length in 27 males and 31 adult females of *K. l. postinguinale*. *Juveniles, were not include in the statistical analysis.

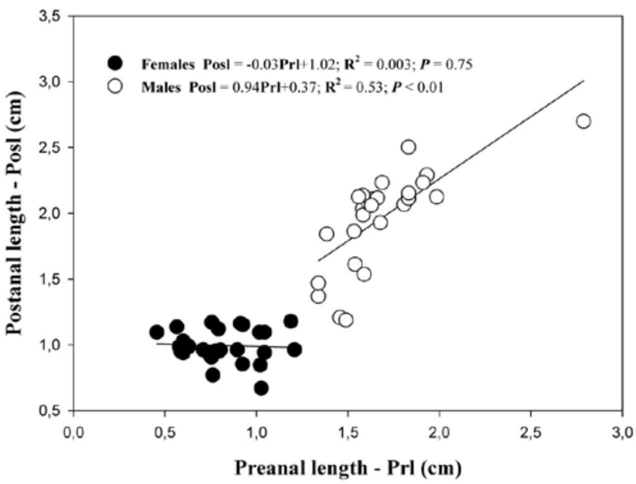

Figure 4. Relationship of the preanal length and postanal length in 27 males and 31 adult females of *K. l. postinguinale*

mass, showed that growth of the 61 individuals of *K. l. postinguinale* was allometric but between linear shell measurements was isometric. When reaching approximately SCL = 10 cm growth slows. Thus, while juveniles invest more energy in growth, adults invest more in reproduction (Congdon & Gibbons, 1990; Páez, 2012b; Arango-Lozano et al., 2017). On the other hand, multiple comparison of morphometric variables between the study localities did not record differences in the size of males, whereas, in females, only The Río Roble low area had larger measures of PRL and CH than those in La Palmita and NPC. However, the small sample size (n = 4) for females from La Palmita and Roble River limited our ability to detect differences between localities.

PRL and POSL were strongly sexually dimorphic in *K. l. postinguinale*, with males having larger preanal and postanal lengths than females. Rodríguez-Murcia et al. (2014), reported the same pattern in the Department of Caldas. The longer tail in the adult male allows it to grasp the female during copulation by means of a pronounced corneal nail at the end of the tail (Giraldo et al., 2012b). The larger preanal region in males reflects the location of the penis (Miller & Dinkelacker, 2007).

Between sexes, there is a significant difference in the growth of preanal length, which is greater in males, presumably since they reach the maturity size (8 cm SPL), PRL remains at a relatively constant value as they grow, while in females, it grows scaled to body size. However, when the PRL is very small (Juveniles, Fig. 3), there is no evidence of its growth to scale in the males, which could be explained by both; very few data of juveniles in this study, and the difficult measurement of small live animals in the field, since the tail of the turtle can be stretched inwards or outwards, which makes accuracy difficult.

Rueda-Almonacid et al. (2007) reported the size at maturity is 10 cm SCL and 8 cm SPL for females and 12 cm SCL and 10 cm SPL for males. However, based on our data for PRL and POSL (Figs. 3, 4), we suggest that sexual maturity may be reached by 9.5 cm SCL and 8.5 cm SPL, at least in males in Quindío, although this should be confirmed by anatomical studies. Similarly, further research on population density, habitat use, and reproductive ecology in *K. l. postinguinale* are needed in order to generate management strategies for this species at least at the local level.

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