

Effects of climatic conditions on sex ratios in nests of broad-snouted caiman

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Keywords

Caiman latirostris; climate change; nesting; sex determination; temperature.

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Editor: Mark-Oliver Rödel

Received 05 December 2013; revised 05 March 2014; accepted 07 March 2014

doi:10.1111/jzo.12140

Abstract

Caiman latirostris has temperature-dependent sex determination and is potentially susceptible to environmental temperature fluctuations and, thus, to the global climate change phenomena. Considering the potential consequences of increasing temperatures for *Ca. latirostris* offspring, we examined the effects of climatic conditions on sex ratios produced by caimans in wild nests and in particular how climate variables affect nest temperature and the percentage of females produced. We also explored the potential consequences of a hypothetical 0.5 and 1.0°C increase in nest temperature on caiman populations. The proportion of females produced from nests in the wild varied among reproductive seasons, as mean nest temperatures varied between 27.1 and 33.9°C. However, after seven seasons the sex ratio biased toward females, and only during extreme events (strong El Niño Southern Oscillation event, La Niña) was there a reduction in the percentage of females produced in the wild. In the hypothetical scenarios of global warming, we predict a decrease of unisexual female nests, with nests containing both sexes or unisexual male nests becoming more frequent. Entire clutches might be lost if nest temperatures rise above 34.5°C for extended periods. However, it is possible that females modify their nesting timing and behavior to select thermally suitable nest environments.

Introduction

Global warming has a potential effect on living organisms (Setyawan, 2009). It may influence health, spatial distribution, growth and reproductive output (Michaels, 2008; Simpson, 2009). Understanding how contemporary climatic variation affects species may help to predict ecological consequences of global climate change (GCC; Weatherhead, 2005). This scenario, in turn, can lead to a reformulation of species communities, reflecting differential changes in species, and to numerous extirpations and possibly extinctions (Monasterio *et al.*, 2013).

Climatic changes affect all organisms but the effect may be more important for ectotherms that have temperature-dependent sex determination (TSD) such as crocodylians, many turtles, some lizards and tuatara (Valenzuela & Lance, 2004). Thus, the increasing temperatures due to climate change (Intergovernmental Panel on Climate Change, 1997) may affect incubation temperature of egg-laying species, their embryonic development, sex ratios and hatching success (Piña *et al.*, 2007a; Charruau, 2012), unless they modify their nesting behavior and phenology (Simoncini, Cruz & Piña, 2013). As an example of the potential effects of temperature

rising, studies on *Chrysemys picta* estimated that an increase of 4°C in environmental temperature may produce exclusively unisexual female cohorts (Janzen, 1994a).

Caiman latirostris (Crocodylia: Alligatoridae) has TSD (Piña, Larriera & Cabrera, 2003); eggs incubated in laboratory conditions between 29 and 31°C produce 100% females, whereas eggs incubated at 33°C produce 100% males. Moreover, when nest temperature exceeded 34.5°C for a long period, embryos experience high mortality (Piña *et al.*, 2003). Therefore, this species may be affected by global warming; however, we have to keep in mind that laboratory conditions are different from natural conditions. For details on nesting habitat and nest description, see Larriera & Imhof (2006).

Previous studies focused on incubation temperatures of wild reptile nests, in aspects such as sex ratio, hatching time, egg survival, hatchling performance and phenotypic traits (Deeming & Ferguson, 1988). In the case of crocodylians, these studies focused on body size, energy reserves and nest temperature (Valenzuela & Lance, 2004). The relationship between incubation temperatures and hatchling sex ratios under natural conditions was studied in 6 out of the 23 recognized species of crocodylians, but only one study extended for 5 years [not including El Niño Southern Oscillation (ENSO)

events] of field nesting seasons for *Alligator mississippiensis* showing a strong female bias of hatchlings (Elsey & Lang, 2014).

Given the potential threat of climate change on *Ca. latirostris*, a TSD species, we studied (1) the effects of climatic conditions during seven reproductive seasons on sex ratios of caimans produced from nests in the wild; (2) how climate variables affect internal nest temperature and the percentage of females produced; (3) the potential consequences of a hypothetical 0.5 and 1.0°C increase of nest temperature on caiman population viability.

Materials and methods

Field work was carried out in eight sites in northern Santa Fe Province, Argentina. Data on proportions of females produced from individual clutches came from seven reproductive seasons (1999–2006 except for 2003), and three different nesting environments used by *Ca. latirostris*: floating vegetation, savanna and forest. Eggs were harvested in the wild using standard techniques by Proyecto Yacaré (Larriera & Imhof, 2006). We made sure to harvest the eggs after the sexual differentiation has occurred, that is, after 45 days of incubation (Piña *et al.*, 2007a), which was confirmed by monitoring the nests in the wild and knowing the nesting date or by the difference between the date of birth and arrival date to the incubator (no more than 30 days). We excluded nests with clear signs of depredation.

Clutches were marked and transferred to the laboratory where they were kept under controlled conditions of temperature (31.0 ± 0.5°C) and humidity (95–98%). In every season, we recorded the number of hatchlings per nest, and then we marked the neonates for later sex identification. Thus, 1284 individuals were kept in captivity for a year until their sexed could be visually determined, based on external secondary sexual characteristics (Piña *et al.*, 2003). At least 15 individuals were sexed per nest.

During egg harvesting period, we compiled a database of meteorological data from January 1 to February 15 of each year in order to include the thermosensitive period (TSP) of every nest of the study. The climatic database contained the following information: average air temperature (recorded by data loggers in each study site), a standard deviation from the mean air temperature, accumulated precipitation (mm) and total number of days with precipitation for this period. We included rainfall because heavy rains may cause nests cooling immediately after (because of the effect of rain temperature itself) or may raise the nest temperature due to decomposition of plant material in the days after the rain occurred and, thereby, affect the sex ratio of offsprings produced. Meteorological data were obtained from the climatological database and corresponded to the site localities where the nests were harvested. These databases came from Facultad Ingeniería y Ciencias Hídricas/Universidad Nacional del Litoral, published on the Web sites <http://www.santa-fe.gov.ar/gbrn/regpluv> and www.smn.gov.ar. Finally, we used these local climatic variables with the aim of identifying the relationship between climate and sex ratios produced in nature.

We acquired the proportion of females produced in the seven seasons from a total of 78 nests (11% of the total nests counted in the study area). In order to test if all seasons and nesting environments produced the same sex ratios, we compared data from each site with a non-parametric analysis of variance (Kruskal–Wallis), because normality or homoscedasticity assumptions were not reached. To detect if sex ratio among years was affected by ENSO events (Niña and Niño) data, we used Wilcoxon test. The presence of different phases of ENSO was identified through the multivariate ENSO index (MEI; Wolter, 2004). Because climatic data came from eight different sites and not all of them produced nests in every year of our 7-year study, we grouped the nests within each site considering the season (26 groups; Table 1). We analyzed the relationship among percentages of females of *Ca. latirostris* produced in every study site and breeding season and climatic variables using principal component analysis (PCA); for this purpose we used SPSS 17.0. (SPSS Inc., Chicago, IL, USA).

In a parallel study, we collected temperature data from the interior of 10 *Ca. latirostris* nests in the wild with data loggers every hour to relate changes in thermal conditions inside the nests and the proportion of females produced. Data loggers were placed inside the nests (among eggs) immediately after oviposition, and remained there until the nests were collected, once TSP was over (after 45 days). Later, eggs were transferred to the laboratory to complete their embryonic development at 31.0 ± 0.5°C and 95–98% of relative humidity. When the caimans of those 10 nests hatched, we recorded the number of hatchlings, marked and kept them in captivity for a whole year, as previously described.

From these 10 nests, we calculated mean temperature (±standard deviation), the coefficient of variation (CV; ratio of standard deviation and mean temperature expressed in percentage) and the number of days that embryos stayed around 31°C (±0.5°C) or 33°C (±0.5°C) during the TSP, temperatures that produce 100 and 0% females, respectively (Piña *et al.*, 2003). We also used the meteorological data for the harvested nests as previously mentioned. This database was analyzed using PCA to determine what environmental variables better explained the internal nest temperature and the percentage of females produced. Later, we checked the associations between environmental and biological variables, with simple regressions; additionally, we compared the CV of internal and external temperature by Student's *t*-test. Finally, with the proportion of females produced and the recorded temperature in those 10 nests, we estimated possible scenarios of climate change using the model that best fit the data and simulating nest temperature increases of 0.5 and 1.0°C.

Results

The percentage of females produced from nests in the wild varied between 49 and 87% among seasons (H_6 $_{77} = 12.97$; $P = 0.038$; Table 2). We did not find differences in the percentage of females produced among the three nesting environment sites where caimans lay their eggs (floating vegetation: 29 nests, savanna: 39 nests, and forest: 10 nests; H_2 $_{77} = 0.60$,

Table 1 Percentage of females ± SD produced in the study sites in each breeding season, and the number of nests and number of offspring sexed is presented between parenthesis

Reproductive season	Pueblo 114	Lucero	Fisco	Espín	Cacique	Cañada Ombú	Alejandra	Gallareta
1999–2000		59.3 ± 31.7 (5; 82)	100 (1; 19)					
2000–2001	66.0 ± 34.4 (3; 47)	18.3 ± 5.6 (2; 34)	76.5 (1; 21)				46.9 ± 41.3 (10; 160)	
2001–2002		91.7 (1; 18)		86.1 ± 15.7 (8; 129)				
2003–2004		69.6 ± 39.6 (4; 63)		81.2 ± 26.6 (6; 93)	54.9 ± 26.7 (5; 79)	62.0 ± 12.2 (2; 33)		77.8 ± 31.4 (2; 33)
2004–2005		93.3 ± 9.4 (2; 39)			100 ± 0 (2; 34)	90.0 ± 14.1 (2; 36)		
2005–2006		100 (1; 18)		60 (1; 17)		79.6 ± 6.4 (3; 48)		
2006–2007		100 (1; 17)		100 (1; 16)		56.8 ± 9.7 (2; 32)	100 ± 0 (2; 34)	

sd, standard deviation.

Table 2 Mean sex ratios produced from the seven reproductive seasons studied

Reproductive season	N of nests		Unisexual total nests	% unisexual females nests
	(N of hatchlings)	% females hatchlings		
1999–2000*	6 (101)	66.2 ± 32.8	1	14.3
2000–2001**	16 (262)	48.7 ± 37.7	3	12.5
2001–2002	9 (147)	86.7 ± 14.8	4	44.4
2003–2004	25 (395)	68.9 ± 28.3	5	20.0
2004–2005	9 (164)	86.9 ± 17.7	4	44.4
2005–2006#	7 (116)	81.5 ± 14.9	2	28.6
2006–2007	6 (99)	85.6 ± 22.7	4	66.7
Total	78 (1284)	71.1 ± 29.9	23	29.3

*ENSO events a drier season (strong Niña event) and # a weak Niña event. Reproductive season 2003–2004 corresponds to a wet season (strong Niño event). The number of individuals sexed each year is presented between parenthesis.

°The only record of unisexual male nest produced in the wild.

$P = 0.737$). Only the strong ENSO events of La Niña (1999/2000 and 2000/2001), when rainfall was almost absent and higher nest temperatures were recorded, reduced the percentage of females produced [$W = 157.5$; degrees of freedom (d.f.) = 1; $P = 0.021$]. Conversely, weak La Niña events produced similar results compared with those years without such phenomena ($W = 201.0$; d.f. = 1; $P = 0.086$). From 78 nests studied, 29.5% produced exclusively females (Table 2) and only one nest (1.3%) produced exclusively males (Niña 2000/2001 season; Fig. 1). Overall results from the seven seasons reveal a female-biased sex ratio (71%).

From the original database with 26 groups (site season; Table 1) of nests and climatic variables, we excluded Lucero 2000/2001 group because of its high CV and standardized residuals. Then, from the correlation matrix of 25 groups and climatic variables, the PCA shows that the first two components contributed to 70% of the variance. We found associations between climate variables, but none of them was linked to the percentage of females (Table 3).

From the nests with data loggers, mean nest temperature was 30.8°C (±1.4, $n = 10$) during the first 68 days out of 75 days of total incubation period, and 30.3°C (±1.7, $n = 10$) during the TSP (c. 22 days, between days 19 and 40 of incubation; Piña *et al.*, 2007a). During incubation, the mean (air) temperature over all study sites was 25.7°C (±2.6). The mean CV of temperature within the nests was 5.6% (range = 2.9–8.8), which is lower than the external environmental temperature CV (9.8%, range = 7.2–13.2; t -test, $t = 3.67$, d.f. = 9, $P = 0.002$), suggesting that the nests acted as a thermal buffer to climatic variations.

Three nests with internal data loggers showed similar mean temperatures during TSP, but different standard deviations (e.g. 30.6 ± 2.7, 30.7 ± 1.2 and 30.8 ± 1.9°C; nests E, F and G; Fig. 1), and produced 45, 100 and 67% of females, respectively. These nests differed in the proportion of days they were exposed to temperatures close to 31 and 33°C (Table 4). We

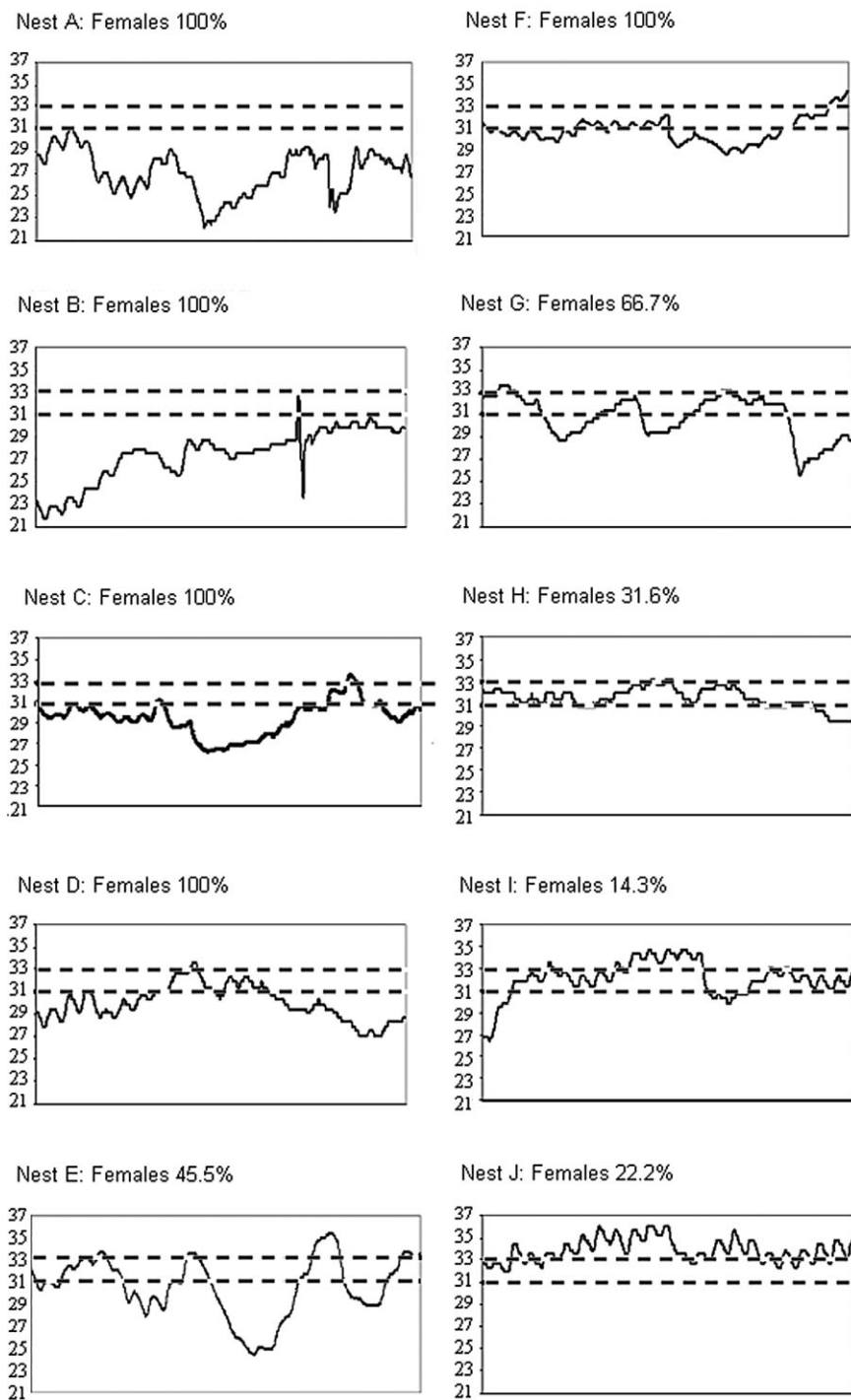


Figure 1 In each plot, the internal temperature of each nest during the thermosensitive period is presented (22 days in continuous line). Temperature was recorded with data loggers (HOBO H01-001-01, Onset Computer Corporation, Cape, Cod, MA, USA). Dashed lines indicate the temperature of 31°C (producing 100% females) and 33°C (producing 0% females).

found that during the TSP (22 days), nests with 10 or more days with temperatures close to or lower than 31°C produced 100% females.

The PCA of temperature within the nests and external climate variables explains 74% of data variation in the first two components. We observed a positive association between

the percentage of females produced and the standard deviation of external mean air temperature during the TSP (eigenvector > 0.77). We also found a negative association between percentage of females produced and mean internal nest temperature during the TSP (eigenvector = -0.91), and between mean external air temperature during the TSP and

the number of days at 33 and 32°C (eigenvector > -0.76) [PC1: unisexual female nests (0.87) and PC2: days with 31°C (0.87); number of rainfall events during the TSP (0.89)].

The percentage of females produced in the nests with data loggers was affected by nest temperature during TSP (Fig. 2). Considering that an incubation temperature lower than 30°C produced 100% females, we only considered nests with temperatures higher than 30°C during the TSP ($n = 7$) for this model. This relationship was best described by a cubic regression (model: % of females = $8038.024 - 365.466 t + 0.112 t^3$; $r^2_{adj} = 0.63$, $P = 0.062$; Fig. 2). During the TSP, we also found a positive relationship between nest and air temperature (model: temperature of nests TSP = $-3.42 + 1.32 \text{ air temp}^2$; $r^2_{adj} = 0.34$, $P = 0.049$), whereas the variation of mean air temperature was inversely related to the mean nest temperature (model: temperature of nests TSP = $36.74 + (-2.46) \text{ variation of mean temp}^2$; $r^2_{adj} = 0.45$, $P = 0.021$).

From the 10 nests with data loggers, five produced both sexes and five produced exclusively females. From this last result and the possible scenarios taking into account the best model fit obtained (cubic regression; Fig. 2), we predict that if the temperature inside the nest increases from 0.5 to 1.0°C (in agreement with a conservative potential scenario of global warming), there will be a decrease in the amount of unisexual female nests and an increase in nests of both sexes. Additionally, an increase in the percentage of nest producing 100% males (based on the data of Piña *et al.*, 2003) is possible.

Table 3 Eigenvectors show the associations of original variables with the first principal component (PC1) and second principal component (PC2)

Original variables	Eigenvectors	
	PC1	PC2
% females	-0.30	-0.22
Mean air temperature TSP	0.60	-0.69
Mean air temperature deviation TSP	0.75	-0.33
Unisexual nests	-0.23	0.44
Number of rainfall events TSP	0.51	0.74
Accumulated rainfall TSP (mm)	0.82	0.40

Those highlighted in bold had an association greater than 0.60. TSP, thermosensitive period.

Discussion

We evaluated the sex ratio of hatchlings of *Ca. latirostris* produced from wild nests corresponding to several consecutive seasons. Our data are useful for the management of crocodilian species because they may help to predict the sex ratios produced in the wild in relation to climatic variables and also for estimating possible consequences due to climatic change.

Global warming is expected to alter incubation temperatures of wild nests of reptiles and may potentially result in hatchlings of only one sex in TSD species, particularly for species where only one sex is produced at higher temperatures. This scenario may generate strongly biased sex ratios in populations and eventually lead to extinction (Huey & Janzen, 2008; Escobedo-Galván *et al.*, 2011; Charruau, 2012). For these reasons, it is important to understand and survey temperature regimes in wild nests of crocodilians and account for the hatchling sex ratios, mostly because several crocodilians are managed and are TSD species.

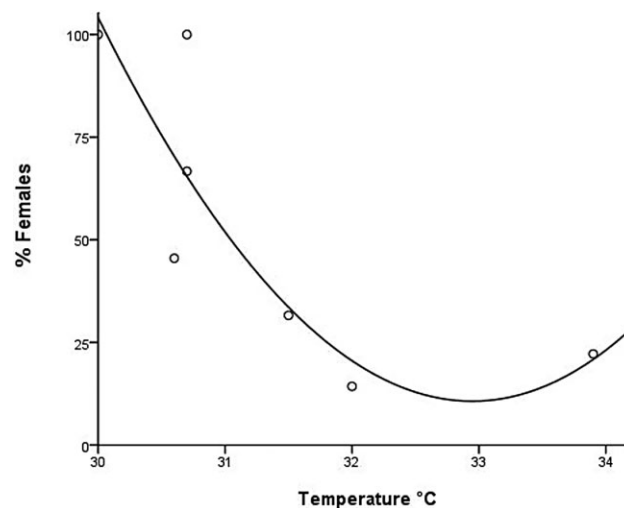


Figure 2 Relationship between the percentage of females produced and the average temperature of the nest during the thermosensitive period, recorded with data loggers (HOBO H01-001-01, Onset Computer Corporation). Line represents the better fitted model (% of females = $8038.024 - 365.466t + 0.112 t^3$).

Table 4 Temperature (°C) recorded in 10 wild nests during the incubation period and the sex ratios of hatchlings produced

Data obtained from the conditions of the nest	Nest A	Nest B	Nest C	Nest D	Nest E	Nest F	Nest G	Nest H	Nest I	Nest J
Mean temperature (°C)	27.1	27.4	29.4	30.0	30.6	30.7	30.8	31.5	32.0	33.9
Standard deviation	1.98	2.33	1.61	1.57	2.7	1.15	1.87	0.91	1.64	1.04
Coefficient of variation	7.3	8.5	5.4	5.2	8.8	3.7	6.1	2.9	5.1	3.1
Days with 31°C	1	0.2	1	5.2	4.0	10	5	11	2	1
Days with 33°C	0	0	0.5	0.5	7.5	3	2.5	1	9	15
Percentage of females (%)	100	100	100	100	45.5	100	66.7	31.6	14.3	22.2

Highlighted in boldfaced are the three nests with similar average temperatures but different standard deviations.

The proportion of females produced in the wild from the three different habitat types did not differ, showing that habitat type plays a minor role on the internal temperature of the nests. In contrast, nest site selection by nesting females has been shown to influence nest temperature and ultimately hatchling sex ratios in freshwater turtles and lizards (Braña & Ji, 2000; Mitchell, Maciel & Janzen, 2013). However, variation in climate characteristics among reproductive seasons influenced on *Ca. latirostris* sex ratios produced, particularly when strong ENSO events occur.

Previous studies reported that rainfall shows a clear relationship with the decrease of internal nest temperatures and leads to a female-biased proportion of hatchlings (Campos, 1993; Rhodes & Lang, 1996; Lance, Elsey & Lang, 2000). However, even knowing the environmental conditions outside the nests (e.g. temperature and precipitation) during the TSP it was not possible to predict the percentage of female *Ca. latirostris* for each breeding season in our study. Apparently, rainfall may reduce nest temperature under certain conditions or elevate it under others; it appears that combinations of microbial respiration of mound material and moisture or shade and moisture of the nest cause different nest temperatures. These different thermal responses of nests to humidity may result from different combinations of materials used to build nests and the exposure to solar radiation (Campos, 1993; Janzen, 1994b; Shine & Harlow, 1996).

The proportion of females produced every year ranged from 49 to 87%. It is important to mention that these minimum and maximum values of females occurred in consecutive years [2000/2001 – Niña event (corroborated in MEI; Wolter, 2004) – and 2001/2002]. The same situation was reported by Rhodes & Lang (1996) for *A. mississippiensis* in two consecutive reproductive seasons, when in an unusual dry nesting season it produced a male-biased sex ratio (38% of females). In the present study, we observed an overall sex ratio biased toward females (71% of hatchlings). Only during a prolonged drought and high temperatures typical of strong Niña events (1999 and 2001) was a low proportion of females (49%) produced. If the frequency of Niña events increases, as a consequence of GCC, a reduced production of females in natural nests may result as a consequence of high temperatures and drought. Although, Lance *et al.* (2000) reported a male-biased sex ratios (range from 54 to 74% males) in a 5-year study of American alligators.

Ewert, Jackson & Nelson (1994) mentioned that in most reptiles with TSD, clutches are unisexual because unisexual nests would be an advantage to minimize inbreeding (Parrot & Logan, 2010). However, Janzen (1994b) found that 66% of nests were unisexual for *Ch. picta* in a 4-year study. Here, we found that only 29.5% of nests were unisexual (22 nests produced 100% females and one nest 100% male hatchlings). Thus, our results do not support the hypothesis of high production of unisexual nests. Besides, the hypothesis of unisexual nests advantage is not consistent for long-lived iteroparous organisms (such as crocodiles and turtles) because descendants of several annual cohorts may interbreed (Shine, 1999); thus, the impact of unisexual nests will not be important.

Although data from incubation in laboratory conditions are important to establish bases and study parameters, they do not represent what is actually happening in the wild (e.g. Shine, Elphick & Harlow, 1997). Natural incubation of *Ca. latirostris* eggs allowed us to observe variation in the percentage of females among nests from different reproductive seasons and even nests within the same reproductive season. However, our field-based data on nest temperature and resultant sex ratios are consistent with our laboratory experiments (Piña *et al.*, 2003).

According to Shine (1999), reptile nest temperatures in nature (including those with TSD) fluctuate around values that may produce both sexes. In our study we found that the mean temperature inside *Ca. latirostris* nests clearly affected sex determination of hatchlings, and that in most nests (70%) the mean internal temperature was lower or close to 31°C (27.1–33.9°C range), a temperature that produces female-biased nests (Piña *et al.*, 2003). Previous studies show a variety of results among the sexes produced in wild nests in present day Crocodylia. For example, wild nests of *Crocodylus niloticus* tend to be female biased (Leslie & Spotila, 2012), whereas wild nests of *Cr. acutus* tend to be male biased (Charruau, 2012).

Nest temperatures of *Ca. latirostris* were higher than environmental temperatures (about $5.0 \pm 1.9^\circ\text{C}$), similar to *A. mississippiensis* (nests 4.4°C ; Joanen & McNease, 1969). Previous studies reported that air temperature and rainfall affect the internal temperature of the nest (Ferguson & Joanen, 1983; Campos, 1993). Thus, external conditions may serve as indicators of the sex ratio produced from developing embryos. Our results show that temperatures inside and outside nest are related, and the low mean temperatures are associated with an increase in production of females. Several factors may act simultaneously as Magnusson *et al.* (1990) proposed for *Paleosuchus trigonatus* nests, where sunlight, metabolic heat of embryos and decomposition rates of plant material may increase internal nest temperature, whereas rainfall events and variation in weather conditions may decrease mean temperature of nests.

The proportion of females produced in the 10 nests with data loggers is similar to the percentage in the 78 wild nests of this study (68.0 ± 36.4 and $71.0 \pm 29.9\%$, respectively). Assuming that climate change may produce an air temperature increase of $0.7\text{--}1.2^\circ\text{C}$, we conservatively estimated an increase of $0.5\text{--}1^\circ\text{C}$ of the internal nest temperature. Consequently, this increase of temperature might decrease the proportion of females produced to 53 and 44% depending on the scenario, according to the best fit model (cubic regression), compared with 71% produced at present (as shows in Table 5). If the mating system of caimans is linked to this female : male ratio (70:30), an increase in global temperature may lead to bias in the mating system and possibly a reduction in the production of nests. Additionally, at higher incubation temperatures the occurrence of nests of both sexes increases, but beyond higher temperatures (34.5°C) hatching success is reduced (Piña *et al.*, 2003; Warner & Shine, 2009). Furthermore, whether this change persists through time or increases at a higher rate may affect population viability (Huey &

Table 5 Hatchlings sex ratio produced in wild nests, and under two different scenarios of global warming (with an increase in internal temperature of the nests of 0.5 and 1°C)

% Sex produced	Results of		
	this study	+0.5°C	+1°C
100% females	5 nests	3 nests	2 nests
100% males	0	0	0 nest
Females and males	5 nests	6 nests	7 nests
No hatching	0	1 nests	1 nest
Mean percentage of females \pm sd	68.0 \pm 36.4	53.0 \pm 36.8	44.0 \pm 36.4

sd, standard deviation.

Janzen, 2008; Escobedo-Galván *et al.*, 2011). However, female ectotherms may potentially buffer their progeny from the effects of climate change through active thermoregulation in viviparous species, and by changing their nesting behavior in oviparous species (Shine & Harlow, 1996), as well as moving their distribution.

Alligator and caiman hatchlings show a strong sex ratio bias to females, reaching a 10:1 female : male ratio (Woodward & Murray, 1993). Over 7 years our hatchling sex ratio (7:3, F : M) is similar to that reported for *A. mississippiensis* (Rhodes & Lang, 1996; Lance *et al.*, 2000), *Cr. johnstoni* (Webb, Buckworth & Manolis, 1983) and *Cr. palustris* (Lang, Andrews & Whitaker, 1989). This ratio may be affected by extreme events of La Niña, leading to a ratio near 50:50. However, Lance *et al.* (2000) in *A. mississippiensis* pointed out that prior to dispersion, juveniles show a sex ratio where males are more abundant than females. This was also observed for *Ca. latirostris* from the state of São Paulo in Brazil (Marques, 2013). Unfortunately, there are no data on *Ca. latirostris* on the sex ratio of juveniles in our study site.

It has been suggested that a greater proportion of hatchling females may be advantageous because of a differential mortality between sexes during the first years of life, where males supposedly have greater survival than females (Lance *et al.*, 2000) in large populations ($N \approx K$). This differential mortality may be associated with a slower growth rate in females (Lang & Andrews, 1994). Then, the surplus of females at hatching would ensure recruitment to breeding stock of both males and females. According to Lance *et al.* (2000), only 60% of *A. mississippiensis* females survive the first year, and females at classes II (50–139 cm) and III (140–179 cm), according to Ross & Godshalk, 2003) are in a 1:3 ratio, which was observed in the small population of *Ca. latirostris* in Brazil (Marques, 2013); however, these numbers may underestimate the real number of females according to Portelinha, Verdade & Piña (2012).

Another possible hypothesis is that a large number of females at hatching could lead to a larger number of nests in case of high survivorship of both sexes. We know that *Ca. latirostris* females have similar survivorship and grow faster than males in captivity (Piña *et al.*, 2007b), where intraspecific competitions are low. So, in a reduced population ($N \ll K$) with almost absent intraspecific competition, we

expect no differential mortality; so we predict that the number of breeding male and female stock is more even in large populations and biased to females in low-density populations.

In this study, we show evidence of the effect of environmental temperature on nest temperature as previously reported in other species (Georges *et al.*, 2004). We also observed that internal nest temperature affects the proportion of females produced in wild *Ca. latirostris* nests. However, we failed to predict the proportion of females in the nests, based on the temperature and rainfall data recorded by weather stations. Proportion of females produced in wild nests might be affected by other factors such as nest location, insolation and nest plant material, making sex ratio of hatchlings produced every year difficult to estimate (Ferguson & Joanen, 1983; Rhodes & Lang, 1996). Major changes in climate may occur in the future; therefore, *Ca. latirostris* (and other TSD species) may be susceptible to incubation temperature-induced changes in sex ratio and embryo mortality. However, changes in the phenology of this species may enable *Ca. latirostris* to maintain nest temperatures at optimal temperatures despite an increase in global temperature (Simoncini *et al.*, 2013).

Acknowledgments

We thank K. Rice, Proyecto Yacaré members, A. Woodward and the 'gauchos' for their field work. This study was supported by Proyecto Yacaré, Yacaré Santafesinos, PICT-ANPCyT2008N220 and N404, CAI+DPI50120110100222 LIPACT47 and PAIS (CSG/SS/IUCN). This is publication number 87 from Proyecto Yacaré.

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