

The sex-determination pattern in crocodylians: A systematic review of three decades of research

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Abstract

1. Sex in crocodylians is not determined by chromosomes, but by egg incubation temperature, where different temperatures produce different clutch sex ratios. Two patterns have been proposed to describe these changes in sex ratios: a 100% female proportion at low and high temperatures with male predominance at intermediate ones (FMF) or a simpler pattern with a single female-to-male transition (FM). Over the last three decades, researchers have provided empirical information to support either of these two patterns in different species; however, no consensus has been reached partly because data have not been analysed as a whole.
2. Here, we aimed at gathering the existing data on these patterns to provide models of temperature-dependent sex determination in those crocodylians studied so far.
3. Potentially relevant publications were searched on Web of Knowledge, Scopus, Scielo and Science Direct. Studies that reported results on the sexual identity of crocodylian hatchlings obtained from constant temperature incubation treatments were considered. Using statistical models varying in their underlying assumptions, we evaluated which sex-determination pattern was best supported for the studied crocodylians and constructed species-specific and latitude-specific models.
4. Based on the 8,458 sexed hatchlings studied throughout 31 studies, we show that the evidence supports a shared FMF pattern in all the crocodylian species for which enough data are available. We find that such pattern changes between species and at different latitudes.
5. These results suggest a lability of the FMF crocodylian sex-determination pattern, a key feature under the present climate change scenario.

KEYWORDS

alligator, caiman, crocodile, Crocodylia, incubation temperature, sex ratio, systematic review, temperature-dependent sex determination

1 | INTRODUCTION

The study of sex determination in reptiles, and in particular in crocodylians, has been a matter of study for several decades (Deeming, 2004; Deeming & Ferguson, 1989; Janzen & Paukstis, 1991; Janzen

& Phillips, 2006; Lang & Andrews, 1994; Valenzuela, 2004). After the discovery of the absence of sex chromosomes in crocodylians (Ohno, 1967), researchers aimed at identifying the moment at which sex was established and the factors responsible for triggering sexual differentiation. More than three decades ago, Ferguson and Joanen

(1982) established experimentally that in *Alligator mississippiensis* sex was determined within a specific time frame during egg incubation and that the temperature experienced by the embryo at this moment was the main factor driving the differentiation of its gonads. This mechanism had already been identified in chelonians (Pieau, 1974; Pieau & Dorizzi, 1981) and termed temperature-dependent sex determination (TSD; Bull & Vogt, 1979). After *A. mississippiensis*, further research has suggested that TSD is displayed by all crocodylians (Deeming, 2004; Lang & Andrews, 1994).

The TSD mechanism translates into temperature threshold patterns in which below and above a particular incubation temperature value the predominant sex of the hatchlings in clutch changes (Ewert, Jackson, & Nelson, 1994). Three TSD threshold patterns have been described so far in crocodylians and in reptiles in general (Ewert et al., 1994; Valenzuela & Lance, 2004). The first pattern, MF (for Male–Female) or TSD Ia, establishes that low incubation temperatures lead to the predominance of males and high temperatures to that of females. In the second pattern, termed FM or TSD Ib, we have the inverse pattern, with a prevalence of females at low temperatures, and males at high ones. Finally, in the FMF or TSD II pattern, low and high temperatures produce a predominance of females, and intermediate temperatures produce mainly males. Associated with these patterns, two parameters are pertinent. First, the transitional range of temperature (TRT) describes the range of values within which both sexes are produced, although at different proportions. Second, every TRT has an associated pivotal (or threshold) temperature (PT) at which both sexes are produced at equal ratios (1:1) (Valenzuela & Lance, 2004). Thus, the FMF pattern presents two TRTs and two PTs, while the FM and MF patterns present only one value for each parameter.

Temperature-dependent sex-determination patterns and their associated parameters have been mainly determined through the incubation of eggs at constant temperatures under laboratory conditions (Janzen & Paukstis, 1991). In such experiments, sets of eggs are incubated separately at different specific, controlled temperatures; once hatched, the sex of hatchlings has to be determined to establish the sex ratio produced under each temperature. Worth of consideration is the fact that hatchling sexing usually involves the sacrifice of the individual for the observation of its gonads, as alternative non-invasive methods are often less accurate, more expensive or require rearing individuals for over a year (Eplanova & Roitberg, 2015; Leslie, 1997; Yntema & Mrosovsky, 1980). The study of TSD in the field is further complicated by the fluctuation of nest temperature throughout incubation due to factors such as weather, sun exposure, rainfall events or thermal gradients within nests, among others (Georges, Doody, Beggs, & Young, 2004). Mean nest temperature has been generally used to predict sex ratio of hatchlings; however, fluctuations make the relationship between mean temperature and sex ratio less clear (Georges et al., 2004).

Since the discovery of TSD in *A. mississippiensis* (Ferguson & Joanen, 1982), several researchers have accumulated information on the sex ratios obtained at various temperatures in the different species of the Crocodylia. The work has involved the study of species both from the Alligatoridae and from the Crocodylidae families,

and the study of the phenomenon both under laboratory and field conditions in distinct locations. The accumulation of these data has translated into original studies and literature reviews of existing results (Deeming, 1989; Lang & Andrews, 1994; Deeming, 2004). However, the limited per-study sample size, associated with the aforementioned deleterious hatchling sexing method, has resulted in limited identification of the patterns in most species; *A. mississippiensis* stands apart as it has clearly been the subject of a most thorough examination of its TSD pattern. Also, a lack of statistical rigour in the analysis of the information produced at the study level has contributed to this uncertainty, as most studies have determined the pattern from a simple visual examination of the data.

Statistics became an element in the analysis of the sex-ratio data after the work of Girondot (1999) and Godfrey, Delmas, and Girondot (2003). In these papers, the development of mathematical models to describe the FMF and FM patterns and their fit to data through maximum likelihood provided rigour to the description of these patterns. An accompanying R package, *embryogrowth* (Girondot, 2018), was also a contribution to widen the use of these models at the level of particular studies. However, to our knowledge, no statistical analysis of the entire available information on the patterns has yet been performed.

Presently, following a systematic review protocol, we identified and gathered the existing information on the TSD pattern in Crocodylia. We analysed the entire body of evidence to determine a model of this pattern in those species for which limited information is available, while accounting for the multiple sources of uncertainty associated with the disparate origin of the data and the evolutionary relationships among the species. We also considered the potential latitudinal variation of the patterns as this has been demonstrated in other reptiles (Bull, Vogt, & McCoy, 1982; Ewert, Lang, & Nelson, 1999). Up to our knowledge, this is the first attempt, after more than three decades of research, to gather the existing data on the TSD in Crocodylia in order to derive unified TSD patterns. A clear identification of these patterns is essential to forecast the composition and viability of populations under scenarios of shifts in the incubation temperatures, potentially associated with current global climate change (Neuwald & Valenzuela, 2011; Valenzuela et al., 2019), and to propose effective management and conservation policies (Girondot, Delmas, & Rivalan, 2004).

2 | MATERIALS AND METHODS

2.1 | Systematic search

To determine a general pattern of sexual determination in crocodylians, we performed a systematic review of the existing literature, complemented with references contained in previous reviews on the subject. The review started with a preliminary search of information on the TSD pattern in (non-avian) reptiles, through a revision of books, book chapters, theses, and review literature. From the 14 sources gathered (Table S1), we reviewed their references, considering as relevant those that addressed the TSD pattern in reptiles, and, together with the sources, assembled a first set of relevant documents.

From the sources, we also gathered a glossary of terms related to the subject. The terms were divided into five groups: temperature, determination, ratio, incubation and reptiles (Table S2). For a paper to be considered as relevant for the systematic review, it would have to contain in its title, abstract or keywords at least one of the following Boolean combinations of terms:

1. sex* AND Temperature AND Determination AND Reptiles,
2. sex* AND Ratio AND Temperature AND Reptiles, and
3. sex* AND Incubation AND Temperature AND Reptiles,

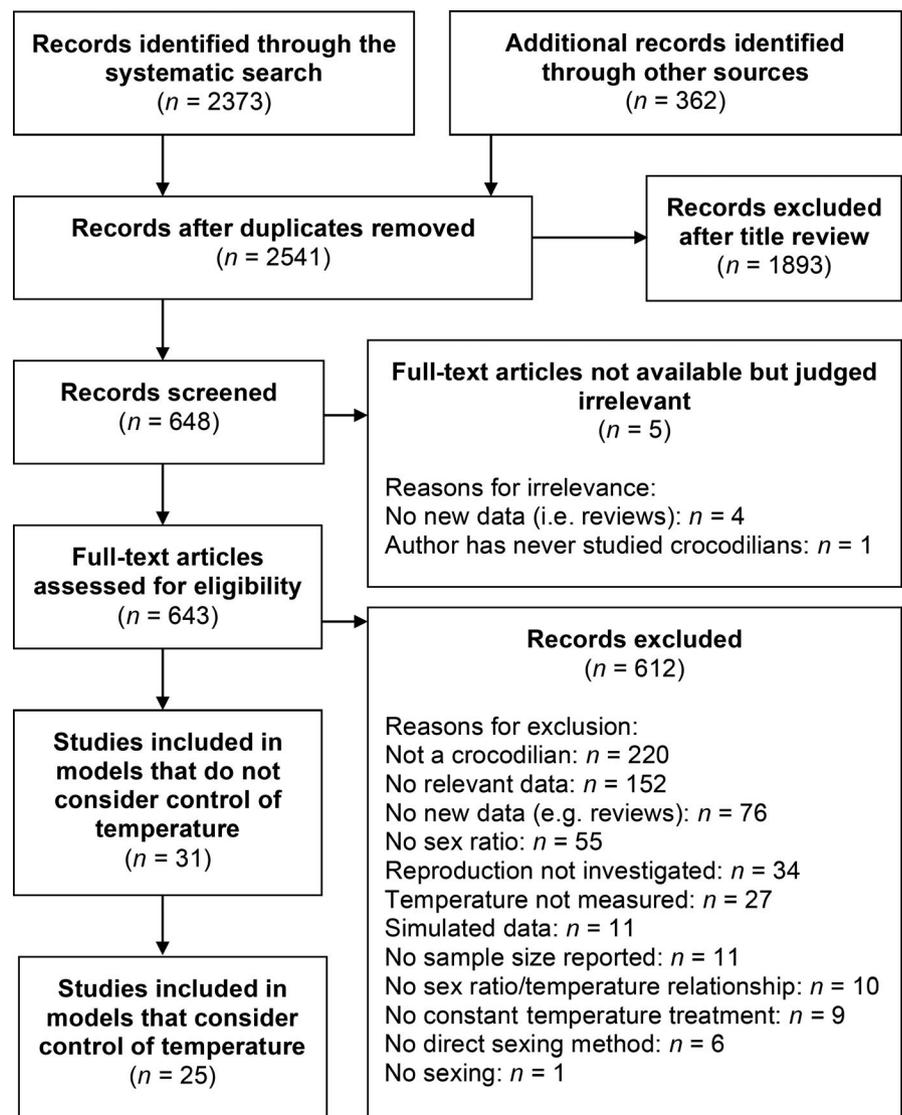
where capital-letter terms refer to any of the terms contained in the groups and sex* to any term with this prefix.

The systematic search was conducted in four search engines on 3 November 2014: Web of Knowledge, Scopus, Scientific Electronic Library Online (SciELO) and Science Direct in both Spanish and English. The titles produced by these engines, together with the preliminary searched documents, were assembled into a single list. From this list, records underwent two screening processes: one that excluded records based on the title, and a subsequent one based

on the full document (Figure 1). For each record, two authors independently evaluated its eligibility. To keep the database up to date, a second title search was performed on 7 June 2018.

From each document, we extracted 21 pieces of information: (a) country, (b) study site, (c) latitude (in decimal degrees), (d) first author, (e) year when the study was performed, (f) reference, (g) journal, (h) contributor (EJG, MM-L, MAM-G, RG-M), (i) family, (j) genus, (k) species, (l) type of experiment (field or laboratory), (m) sexing method, (n) reported evidence for sex-ratio change (none sought; no evidence of TSD found; quantitative evidence of TSD, when a subjective appraisal of results was performed; qualitative evidence, when a statistical analysis was performed and the null hypothesis of no effect was rejected), (o) pattern stated for TSD when evidence was argued to exist, (p) stated pivotal temperature (i.e. those at which a 50:50 sex proportion is obtained) of the FM and MF sections, (q) stated TRT of the FM and MF sections, (r) sample size, (s) mean incubation temperature ($^{\circ}\text{C}$), (t) standard deviation around this mean ($^{\circ}\text{C}$) and (u) number of male and female hatchlings. Species names are reported following The Reptile Database (www.reptile-database-org). When a study applied a treatment on the eggs during

FIGURE 1 Flow diagram of the study selection in the performed systematic search. The 25 studies included in the models that considered control of temperature were as follows: Ferguson and Joanen (1982, 1983), Webb, Manolis, and Buckworth (1983), Hutton (1987), Webb, Beal, Manolis, and Dempsey (1987), Joanen and McNease (1989), Lang, Andrews, and Whitaker (1989), Deeming and Ferguson (1991), Aguilar (1994), Guillette et al. (1994), Lang and Andrews (1994), Rhodes and Lang (1995), Conley et al. (1997), Leslie (1997), Pinheiro, Mourao, Campos, and Coutinho (1997), Mohanty-Hejmadi et al. (1999), Piña et al. (2003), Stoker et al. (2003), Charruau (2010), Marcó et al. (2010), Elsey and Lang (2014), Simoncini et al. (2014), López-Luna et al. (2015), Charruau, Martínez, Cantón, and Méndez de la Cruz (2017) and Marcó et al. (2017). The six studies also included in the models that did not consider control of temperature were as follows: Charruau (2012), Lance and Bogart (1991, 1992), Milnes, Allen, Bryan, Sedacca, and Guillette (2004), Milnes, Bryan, Medina, Gunderson, and Guillette (2005) and Moore et al. (2010)



incubation (e.g. injection of a hormone), only the data from the control treatment were used. To avoid input errors, all extracted data were double-checked. The above information was integrated into a single database (González et al., 2019). The protocol used to perform this systematic review has not been previously published.

2.2 | Statistical analyses

The number of hatchlings of each sex obtained at each incubation temperature was modelled as a binomial process, and its relation with temperature was modelled with a logistic function, that is if $PF(t)$ and $PM(t)$ are, respectively, the probability of obtaining a female and a male hatchling at a given temperature t :

- $PF(t) = 1/(1 + \exp(-f(t)))$, and
- $PM(t) = 1 - PF(t)$,

where $f(t)$ was one of three options:

1. A linear model, with $f(t) = \beta_0 + \beta_1 \cdot t$, corresponding to a single transition pattern (i.e. FM or MF).
2. A quadratic model, with $f(t) = \beta_0 + \beta_1 \cdot t + \beta_2 \cdot t^2$, corresponding to a pattern with two transitions (i.e. FMF or MFM).
3. A flexible model, with $f(t) = \sum_i \beta_i \cdot a_i(t)$ a 2D thin plate regression spline with basis functions $\{a_i(t)\}$ (Wood, 2006).

Note that, by considering a flexible model, we were not assuming that the change over temperature follows a predetermined pattern—as equations 1 and 2 do. We instead allowed the data to inform the shape of the relationship. However, to keep the observed probabilities within the $[0, 1]$ interval, we kept the assumption of a logistic relationship and, to reduce model “wrigginess” (i.e. the amount of its oscillation along the temperature gradient), we kept to a minimum the number of knots to divide the range of observed temperatures into segments of equal length (i.e. $\max\{i\} \leq 4$ in equation 3. In this way, we are considering a partially specified model sensu Wood (2001).

To account for differences in the sex determination patterns due to differences between studies, we included random effects on each study as:

$$f_i(t) = f(t) + u_i,$$

where u_i is a Gaussian-distributed random error associated with the i -th study.

Given the low number of cases for most of the studied species (see Section 3.1), we decided to analyse the whole dataset accounting for the taxonomic hierarchy between the species to borrow strength from those better studied (Gelman & Hill, 2007; Halstead, Wylie, Coates, Valcarcel, & Casazza, 2012). To account for the relationships between these species, we included nested random effects for all model parameters at the species, genus and family levels as:

$$f_{ijkl}(t) = f_i(t) + v_{0j} + v_{1j} \times t + x_{0k} + x_{1k} \times t + y_{0l} + y_{1l} \times t,$$

where v_{0j} and v_{1j} , x_{0k} and x_{1k} , and y_{0l} and y_{1l} are bivariate Gaussian random errors associated with the respective j -th species, k -th genus and l -th family. We recognize that the inclusion of the phylogenetic relations between species in the model would have been a better approach, but no software is yet available that allows this; nonetheless, a hierarchical model, as the one we propose here, is a valid alternative (McMahon & Diez, 2007).

To account for differences in study quality associated with the degree of control of the incubation temperature, either due to environmental fluctuation (under natural conditions) or differential degree of control of temperature by the incubator (under laboratory conditions), we included weights in the log-likelihood function inversely related to the reported standard deviation in incubation temperature:

$$l(F_{\text{obs},ijkl}, M_{\text{obs},ijkl} | t_{\text{obs},ijkl}, s_{\text{obs},ijkl}) = \sum w_{ijkl} \times (F_{\text{obs},ijkl} \times \ln(P_F(t_{\text{obs},ijkl})) + M_{\text{obs},ijkl} \times \ln(P_M(t_{\text{obs},ijkl}))),$$

where $w_{ijkl} = 1/s_{\text{obs},ijkl} \cdot s_{\text{obs},ijkl}$ is the standard deviation around the observed incubation temperature $t_{\text{obs},ijkl}$ and $F_{\text{obs},ijkl}$ and $M_{\text{obs},ijkl}$ are the reported number of female and male hatchlings obtained at that temperature.

Finally, to consider latitudinal differences in the patterns we used latitude (l) as an additional explanatory variable of differences in sex ratios. To include this variable in the flexible model, we used tensor product bases to relate the marginal spline bases associated with each explanatory variable (Wood, 2006). Thus, we had the temperature- and latitude-dependent flexible model:

4. A flexible model, with $f(t, l) = \sum_i \sum_j \alpha_{ij} \cdot a_i(t) b_j(l)$ a 3D thin plate regression spline with bases functions $\{a_i(t)\}_i$ and $\{b_j(l)\}_j$.

To evaluate the impact of excluding those cases for which no standard deviation in temperature was reported (6.95% of all cases), we also developed the above models without weights, using the entire database (Statistical analyses section in Supporting Information S1).

To construct these models, we used the `lme4` (Bates, Maechler, Bolker, & Walker, 2015) and `gam4` (Wood & Scheipl, 2014) packages in R (R Core Team, 2018). The small-sample-corrected Akaike's information criterion (AICc) was used to perform model selection with the `MumIn` package (Barton, 2018). Figures were made with the `ggplot2` package (Wickham, 2016). Pivotal temperatures and transitional ranges were calculated from the model with the smallest AICc through finite differences.

3 | RESULTS

3.1 | Systematic search

A systematic search of the literature exploring the number of female and male hatchlings produced at different incubation temperatures

resulted in 2,373 records. However, successive exclusion criteria reduced the number of relevant documents to 31 (Figure 1). These studies translated into 331 reported sex ratios (cases) obtained at different temperatures for nine Crocodylia species. The number of hatchlings sexed totalled 8,458 distributed over more than 30 years (Figure S1). Most of these hatchlings were from *A. mississippiensis* (4,611), and for the rest of the species, sample size was relatively low (522 ± 304). Also, because of the deleterious methods usually followed to determine sex in hatchlings, sample size has decreased over the last years ($r_s = -0.26$). In terms of temperature control during incubation under laboratory conditions, this has increased over time ($r_s = 0.49$). More recently, studies performed under natural conditions have become increasingly relevant to the study of TSD, and consequently, temperature variation is wide in these studies (1.07°C vs. 0.24°C on average under laboratory conditions; Figure S1).

3.2 | Statistical analyses

After considering three models to describe the TSD pattern as a function of incubation temperature only (Figure S3, the best-supported one assumed no pre-established pattern (AICc values in Table S3). This model supported an FMF pattern over an FM for those species on which information over most of the FMF temperature range exists: *A. mississippiensis*, *Crocodylus acutus*, *Crocodylus johnsoni*, *Crocodylus niloticus* and *Crocodylus porosus* (Figure 2a,d,e,g and i, respectively).

However, differences between species exist and these occur at this taxonomic level rather than at higher ones; species-specific patterns differed in terms of the pivotal temperatures (i.e. those at which a 50:50 sex proportion is obtained) and the temperature ranges at which mixed clutches are obtained (Table 1). On average, female-biased sex ratios are produced at temperatures under $31.7 \pm 0.6^\circ\text{C}$ and above $33.3 \pm 0.3^\circ\text{C}$, with male-biased ratios produced within these values. In general, female-only temperature ranges are wider than those producing strictly males. Furthermore, in some species (with *C. johnsoni* the clearest example, Figure 2e), there was no evidence of incubation temperatures yielding only males. Transitional ranges of temperature (i.e. those producing mixed clutches; for this study, a clutch was considered mixed if it presented, at most, a 1:19 ratio) were asymmetrical, with wider ranges at the MF transition (Table 1).

When latitude was considered as an additional explanatory variable of the variation in crocodylian sex-determination patterns, a better fit of the data was obtained (Table S3). Model 4 shows that, at least for those species inhabiting the northern hemisphere and with sufficient information (i.e. *A. mississippiensis*, *C. johnsoni* and *C. porosus*), the male-biased section of the FMF pattern tends to widen towards higher temperatures (Figure 3). Although caution should be put in interpreting patterns from species poorly represented, it is clear that in *A. mississippiensis* sex-ratio patterns vary with latitude (Figure 3a).

Notably, the models considered thus far accounted for the fact that studies differed in their degree of control of the temperature throughout incubation; when this element was not considered in the modelling procedure, similar general patterns were obtained.

We refer the reader to Supporting Information S1 for the associated species-specific models (Figure S2) and the corresponding latitude models (Figure S4). There, we also point out the differences in the results when the degree of control is excluded from the analyses (Statistical analyses section).

4 | DISCUSSION

Our analysis supports a common FMF pattern in Crocodylia, which agrees with what several authors had previously reported for a variety of its species (Deeming & Ferguson, 1989; Lang & Andrews, 1994; Leslie, 1997; Piña, Larriera, & Cabrera, 2003; Deeming, 2004; Valenzuela, 2004; Marcó, Piña, Simoncini, & Alejandro, 2010; Charruau, 2012). In terms of the agreement of the pivotal temperatures and transitional ranges reported by previous authors and those derived from our temperature-dependent model, this is relatively good (Table S5).

Not reported previously were the asymmetries between the transitional ranges of temperature (i.e. those producing mixed clutches), with wider ranges at the MF transition. Noteworthy is the fact that in the species for which fewer data are available, the extent of this asymmetry is less reliable due to model uncertainty (confidence bands in Figure 2). Nevertheless, for those species for which clear asymmetries exist, these suggest that the species could maintain mixed-sex clutches with relatively high nest temperatures, an aspect pointing towards resilience to high temperatures (Hulin, Delmas, Girondot, Godfrey, & Guillon, 2009). Such resilience, coupled with behavioural responses such as nesting site and depth selection, and breeding time choices (Mitchell & Janzen, 2010; Urban, Richardson, & Freidenfelds, 2014), could allow crocodylians to maintain population viability under future increases in both average environmental temperature and its fluctuation due to climate change.

Notwithstanding, the search for pivotal temperatures and transitional ranges at the species level loses relevance when we acknowledge the fact that factors other than incubation temperature play a role in the TSD patterns expressed under natural conditions. As shown by our temperature- and latitude-dependent model, the pattern is relatively flexible in evolutionary times, as the differentiation of the patterns occurred not only at the species level, but also with respect to latitude, which can be considered a proxy to population. By considering the latitude of the location from where the eggs were retrieved and not that of the laboratory where eggs were incubated, we could separate the effects of incubation temperature and latitude. Therefore, an existing interaction between both explanatory variables suggests that factors prior to incubation modify the patterns (Ah-King & Nylin, 2010). In this interaction, male-biased sex ratios were estimated to occur at cooler temperatures in temperate regions compared with subtropical ones. Ewert et al. (1999) found the same pattern in the American snapping turtle and interpreted it as an evolutionary strategy to guarantee the production of males in more temperature-variable regions. This could also explain the latitudinal differences described by our model.

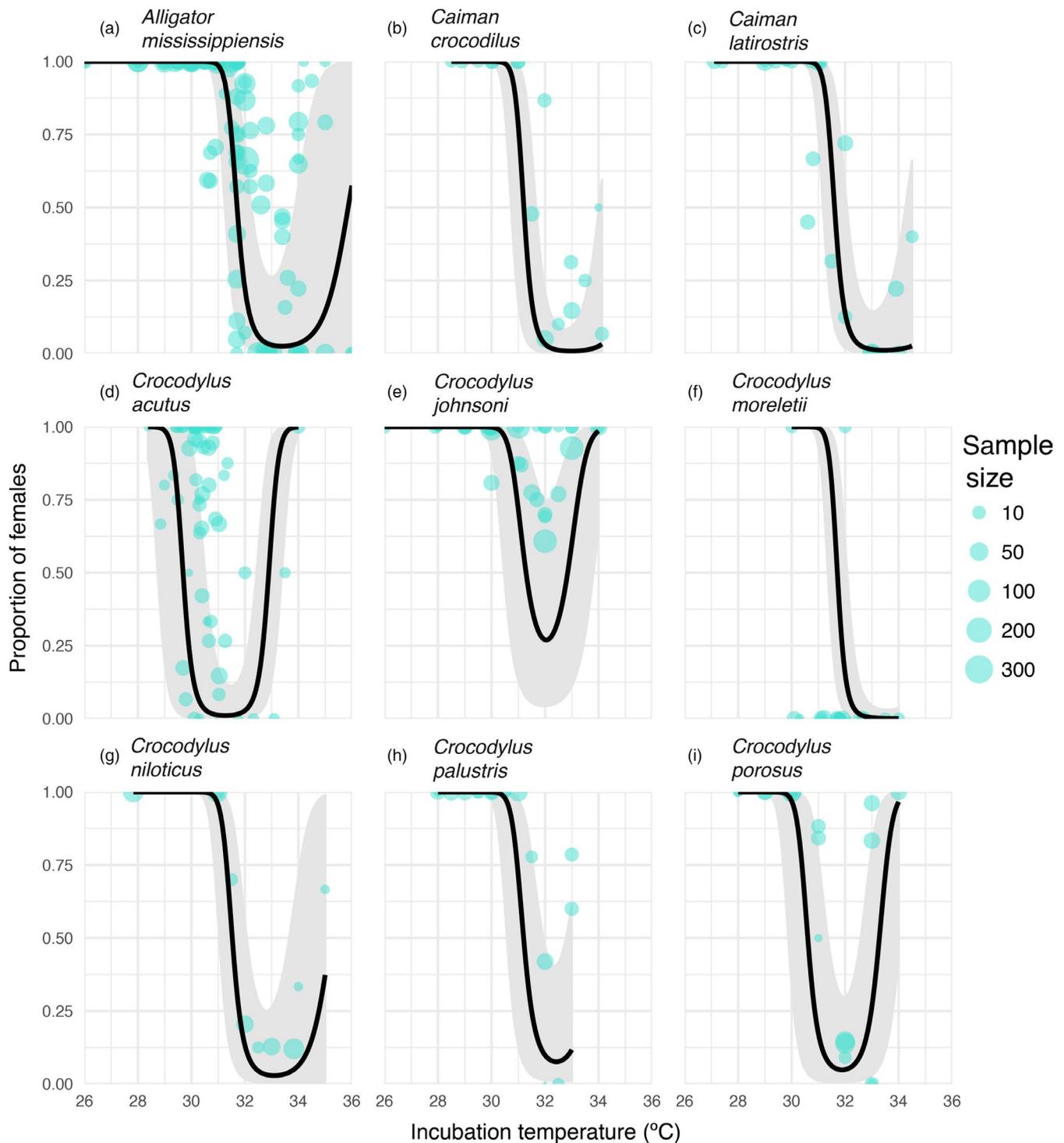


FIGURE 2 Temperature-dependent sex-determination patterns in nine Crocodylia species (a-i). Each circle corresponds to an observed proportion of females in a particular study; sample size corresponds to number of hatchlings sexed. 95% confidence intervals are in grey

Finally, it is worth noting that in most species, being *Crocodylus moreletii* the only exception, no single temperature was identified as producing male-only clutches (Figure 2). Many studies interested in the genetic basis of temperature sex determination rely on the existence of such temperature value to avoid performing a direct sexing of the organisms (Gabriel, Blumberg, Sutton, Place, & Lance, 2001; Janes, Elsey, Langan, Valenzuela, & Edwards, 2013); our results

suggest that this practice should be discouraged in crocodylians. This seems to be true even in *A. mississippiensis*, a species considered to produce 100% male clutches at particular incubation temperatures (Lang & Andrews, 1994).

This is the first study to attempt a systematic review and a rigorous statistical analysis of all the existing literature on the temperature-dependent sex-ratio pattern in Crocodylia. Previous studies had

TABLE 1 Statistics associated with the temperature-dependent sex-determination patterns in crocodilians

Species	Female–male transition		Male–female transition		Most male-biased	
	TRT (width) (°C)	PT (°C)	TRT (width) (°C)	PT (°C)	Prop.	Temp. (°C)
<i>Alligator mississippiensis</i>	31.2–32.6 (1.4)	31.7	34.3–NE	35.9	0.98	33.4
<i>Caiman crocodilus</i>	30.8–31.8 (1.0)	31.2	NE–NE	NE	0.99	33.0
<i>Caiman latirostris</i>	31.1–32.3 (1.2)	31.6	NE–NE	NE	0.99	33.5
<i>Crocodylus acutus</i>	29.2–30.4 (1.2)	29.7	32.2–33.4 (1.2)	32.9	0.99	31.3
<i>Crocodylus johnsoni</i>	30.6–32.0 (1.4)	31.3	32.0–33.7 (1.7)	32.8	0.73	32.0
<i>Crocodylus moreletii</i>	31.3–32.2 (0.9)	31.7	NE–NE	NE	1.00	34.5
<i>Crocodylus niloticus</i>	31.0–32.4 (1.4)	31.5	33.8–NE	NE	0.97	33.1
<i>Crocodylus palustris</i>	30.6–32.4 (1.8)	31.2	32.4–NE	NE	0.92	32.4
<i>Crocodylus porosus</i>	30.1–31.7 (1.6)	30.6	32.0–33.9 (1.9)	33.3	0.95	31.9

Abbreviations: NE, no data available to estimate its value; PT, pivotal temperature; TRT, transitional range of temperature.

focused either on the statistical description at the single-study level (Girondot, 1999; Godfrey et al., 2003) or on the critical appraisal of the available information (Lang & Andrews, 1994). Furthermore, those studies considering a statistical analysis of the patterns pre-imposed particular functions to describe them (e.g. those considered by Godfrey et al., 2003). By regressing flexible functions against the data, we allowed these to inform the shape of the pattern (Wood, 2001), avoiding any predisposition towards a particular mathematical description of the pattern. Another consideration, already recognized by Godfrey et al. (2003), is variation in the control of incubation temperature. By favouring data from well-controlled experiments, we reduced the impact of noisy sex ratios, which distorted some of the species-specific patterns (cf. Figure 3 and Figure S2). However, this decision reduced sample size by 6.95% and therefore reduced the information provided to the model on the underrepresented species, which could introduce a bias on the patterns. Thus, we considered important to provide the unweighted models in Supporting Information S1 to allow the reader to contrast the results obtained under both scenarios.

Despite our best efforts, four elements should be taken into account when considering our results. Firstly, we could not include many crocodilian species. In the case of *Alligator sinensis*, *Gavialis gangeticus*, *Caiman yacare*, *Crocodylus siamensis* and *Paleosuchus trigonatus*, only anecdotal or low quality information (e.g. no sample size was reported) was available. For other species, no single study on this subject has been performed; this is the case of *Melanosuchus niger*, *Paleosuchus palpebrosus*, *Tomistoma schlegelii*, *Osteolaemus tetraspis*, *Crocodylus novaeguineae*, *Crocodylus suchus*, *Crocodylus mindorensis*, *Crocodylus intermedius*, *Crocodylus rhombifer* and *Mecistops cataphractus*. The latter four are of particular importance due to their critically endangered status (IUCN, 2018), and thus, we encourage their study. Secondly, the MF section of the pattern presented small sample size. This reduces the confidence in the statistics associated with this section (pivotal temperatures and transitional ranges of temperature) as well as the generality of the FMF pattern. A special case was *C. moreletii*, for which only two studies were available, but the most extensive one

presented male-only clutches, a result possibly due to the amount of pollution in the area (López-Luna, Hidalgo-Mihart, Aguirre-León, González-Ramón, & Rangel-Mendoza, 2015). Because knowledge of the response crocodilian species have to high incubation temperatures will become more relevant under a scenario of higher climate temperatures (Neuwald & Valenzuela, 2011), it is important that future work focuses on this information gap.

Thirdly, some cases were discarded because the authors did not report any measure of the uncertainty in temperature uniformity over the incubation period. We considered this information as a measure of study quality (Koricheva, Gurevitch, & Mengersen, 2013), as less confidence can be reasonably assigned to those studies not concerned with keeping temperature constant, an element of particular significance for those temperatures within the transitional range. As a consequence, observational studies in natural conditions had a lesser impact on the shape of the patterns than laboratory studies. We consider this an appropriate approach, as variation in natural studies is not only wide, but also differs in the way it is reported (e.g. variation in temperature can be based on a single measure in different nests, or on multiple measurements over time in a single nest). Another potential measure of quality could be the accuracy in the sexing method (Bull, 1987), but this validation was absent in most studies and would have further reduced statistical power. Therefore, it is important that future studies clearly acknowledge quality aspects such as sample size, variation around mean incubation temperature and sexing method accuracy.

Finally, unexplained variation remained in the best model, suggesting that either (a) another temperature statistic, other than mean incubation temperature, could be a better predictor of sex ratio (e.g. proportion of development spent at a particular temperature; Georges, Limpus, & Stoutjesdijk, 1994) or (b) other environmental aspects independent from latitude may play a role in sex determination; thus, we recommend that authors should report additional information, such as duration of exposure relative to entire developmental process, egg size, incubation humidity, incubation duration or clutch origin, which will certainly help improve our understanding of this phenomenon.

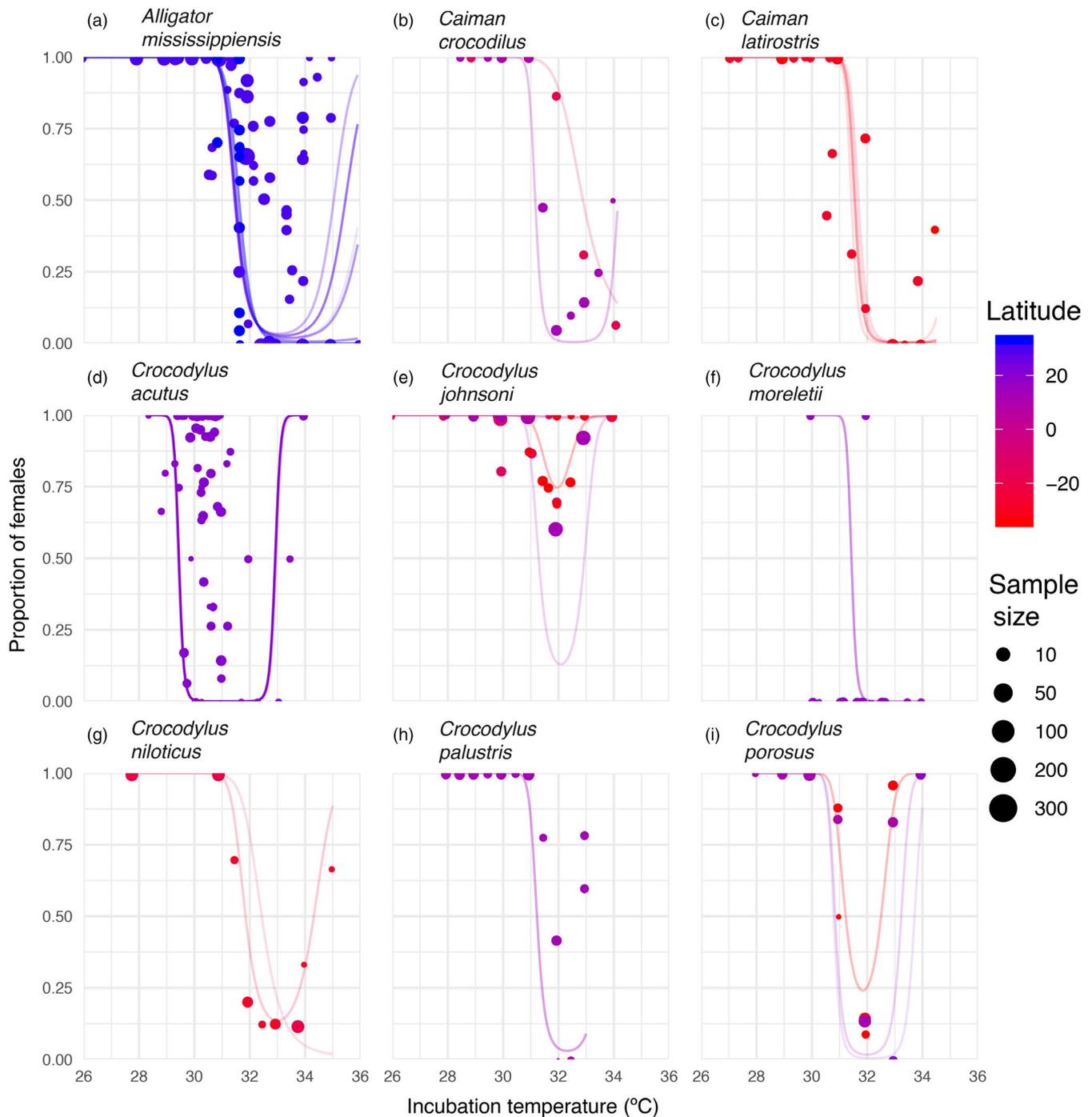


FIGURE 3 Temperature- and latitude-dependent sex-determination patterns in nine Crocodylia species (a-i). Patterns estimated from a flexible model fitted to systematically gathered data. The transparency of the lines relates to the amount of information available at different latitudes. Each circle corresponds to an observed proportion of females in a particular study, with its size reflecting the number of hatchlings sexed

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AUTHORS' CONTRIBUTIONS

E.J.G., M.M.-L., M.A.M.-G., R.G.-M., P.C. and J.A.G.-C. conceived the ideas and designed methodology; E.J.G., M.M.-L., M.A.M.-G. and R.G.-M. performed the systematic search and extracted the data;

E.J.G. analysed the data; E.J.G., P.C. and J.A.G.-C. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.1t2p835> (González et al., 2019).

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