Management and relocation of nests of *Podocnemis expansa* (Schweigger, 1812) (Testudines, Podocnemididae) on the Crixás-Açu River, Brazil

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Abstract

Podocnemis expansa nests in the sandbanks of the Crixás-Açu River in central Brazil during the dry season. With the return of the rainy season, the river banks are once again inundated, flooding nests and eggs and drowning hatchlings. This study evaluated relocation as an alternative to manage nests at risk of flooding. Forty *P. expansa* nests from four beaches of the Crixás-Açu were relocated to nurseries constructed in tanks filled with sand from the same river. Nine nests were covered with palm fronds to simulate shading conditions and to lower the incubation temperature. The base of the egg chamber was at a depth of 60 cm in all the nests. The duration of incubation varied for sunlit (49.1 ± 2.35 days) and shaded nests (51.7 ± 1.75 days), but did not change with nest position (center or edge of nurseries). Hatching success was 47.3 ± 13.6%, and did not vary significantly with sunlight/shade or nest position. The mean incubation temperature during the thermosensitivity period varied from 33 ± 1.56 °C to 34.1 ± 1.40 °C. The lowest mean temperature during the thermosensitivity period (31.9 ± 0.95 °C) and the highest proportion of males (up to 45% of hatchlings) were observed in a shaded nest. Most sunlit nests produced only females. The results suggest that relocation is a viable alternative in managing nests at risk of flooding during the rainy season in the Crixás-Açu. Moreover, artificial shading was shown to be an option to increase the proportion of males.

Keywords: hatching, incubation, nursery, sex ratio, giant Amazon River turtle.

Manejo e transferência de ninhos de *Podocnemis expansa* (Schweigger 1812) (Testudines, Podocnemididae) no Rio Crixás-Açu, Brasil

Resumo

Durante o período de maior vazante do rio Crixás-Açu na região central do Brasil, nos meses de setembro e outubro, *Podocnemis expansa* (tartaruga-da-amazônia) desova nos bancos arenosos que surgem nas suas margens. Com o retorno do período chuvoso em novembro é comum que as praias deste rio sejam inundadas afogando ovos e filhotes. Este trabalho avalia a transferência como alternativa de manejo dos ninhos que seriam naturalmente alagados. Foram transferidos 40 ninhos de *P. expansa* oriundos de quatro praias do rio Crixás-Açu para berçários construídos em tanques de alvenaria que foram preenchidos com areia oriunda do próprio rio. Nove ninhos foram cobertos com folhas de palmeira para simular o sombreamento e diminuir a temperatura da incubação. A base da câmara de ovos de todos os ninhos situou-se a 60 cm de profundidade. A duração de incubação variou entre os ninhos ensolarados (49,1 ± 2,35 dias) e ninhos sombreados (51,7 ± 1,75 dias), mas não foi afetada pela posição do ninho, se nas bordas ou no centro dos berçários. O sucesso da eclosão foi de 47,3 ± 13,6% e não foi afetado pelo sombreamento ou pela posição do ninho nos berçários. A temperatura média de incubação durante o período termossensitivo variou de 33 ± 1,56 °C a 34,1 ± 1,40 °C. Os ninhos ensolarados produziram 100% de fêmeas em sua maioria. Os resultados mostram que a transferência é uma alternativa viável para o manejo dos ninhos que seriam alagados durante as cheias do rio Crixás-Açu e o sombreamento artificial dos ninhos é uma alternativa para aumentar a produção de machos nos berçários.

Palavras-chave: eclosão, incubação, berçário, razão sexual, tartaruga-da-amazônia.

1. Introduction

Sex determination of the giant Amazon river turtle *Podocnemis expansa* (Schweigger, 1812, Podocnemididae) depends on the egg incubation temperature (Alho et al., 1984; Valenzuela et al., 1997; Malvasio et al., 2002a,b. The nesting environment, as well as its physical and climatic characteristics determine the temperature inside nests, which reveals the crucial importance of choosing appropriate oviposition sites to the reproductive success of the species (Ferreira Júnior, 2009). The reproductive cycle of *Podocnemis* and hydrological cycles are synchronously related. During the period of receding water, sandy beaches are formed on the riverbanks. These beaches are utilized as nesting areas by *P. expansa* (Alho and Pádua, 1982), *P. unifilis* Troschel (Ferreira Júnior and Castro, 2010), *P. sextuberculata* Cornalia (Pezzuti and Vogt, 1999), *P. lewyana* Duméril (Correa et al., 2010), and *P. erytrocephala* Spix (Batistella and Vogt, 2008). In the search for the most suitable beaches for nesting, females must choose sites that are topographically high enough to prevent the flooding of nests at the start of the rainy season. Moreover, the temperatures in these environments must be suitable to allow complete embryo development and promote sex ratios that ensure the species' population levels (Valenzuela et al., 1997).

The reproduction of *P. expansa* is affected by the climatic conditions of the west-central region of Brazil. For example, when the rainy season starts earlier than usual, sudden rises in water level, locally called *repiquete,* may be caused by rains in the headwater regions. This phenomenon causes the beaches to flood and results in the drowning of embryos (Alho and Pádua, 1982; Mitchell and Quiñones, 1994). In the Crixás-Açú River basin, this phenomenon is quite common, and is the main cause of losses of *P. expansa* nests (Ferreira Júnior and Castro, 2006b). In order to reduce the potential loss of large numbers of hatchlings, RAN/ICMBio (Reptile and Amphibian Conservation and Management Center, an organization that has formed a partnership with the Brazilian Institute of the Environment and Natural Renewable Resources) recommends the relocation of nests to topographically higher sites. However, simply raising the level of the nests on a beach is not the only aspect to consider. Equally important is the choice of an appropriate site to ensure that the temperature inside relocated nests matches that of the original nests. This must be considered in nest relocations, since the differences in grain size (Ferreira Júnior et al., 2003) and mineral composition of sediments (Milton et al., 1997; Naro-Maciel et al., 1999) may substantially change the nest's heat-transfer regimen (Ferreira Júnior and Castro, 2006b), affecting sex determination of embryos. In nest relocations, the new nesting site should provide the appropriate conditions to produce a sex ratio that is similar to that observed in natural nests (Tuttle and Rostal, 2010).

Knowing the sex ratio of embryos born in a natural environment is an essential element in management and conservation projects for species with temperature-dependent sex determination (Morreale et al., 1982; Girondot et al., 1998). In turn, understanding how a given physical trait influences nest temperature is important to elucidate the factors that influence sex determination of *P. expansa*. This knowledge is useful in the charting of areas expected to be appropriate for the relocation of nests, and in the establishment of conservation areas and environmental management practices. The present study evaluated the role of incubation site features in a pattern of temperature variation in relocated nests of *P. expansa*.

2. Materials and Methods

Eggs of *Podocnemis expansa* were collected in four beaches located near the outpost of RAN/ICMBio on the Crixás-Açú River (13° 03' 10" S and 50° 14' 27" W), located in the municipality of Nova Crixás, state of Goiás, Brazil. During the incubation period of *P. expansa*, flooding of beaches is common in the region, killing the hatchlings and embryos. A more detailed description of the beaches of the Crixás-Açu was given by Ferreira Júnior and Castro (2006b). In order to minimize the effects caused by collection, transport, and handling of eggs (Malvasio et al., 2005), only recent nests (up to two days old) were selected. Eggs were stored in styrofoam boxes filled with sand from the inner nest. This procedure was adopted to maintain the nest temperature and humidity, as well as to prevent the eggs from shaking and rotating during translocation.

Two brick and mortar tanks, originally built to be used in experiments with the breeding of *P. expansa* in captivity, were used as nurseries. The 60 m^3 (W = 3 m; L = 10 m; H = 2 m) tanks were filled with sand from the Crixás-Açu beaches one day before the beginning of the relocations. For this reason, the sediment was not quite compact, which made it necessary to use a PVC tube (25 cm in diameter) to assist in the digging and compaction of the walls of the egg chamber. When eggs were placed in the nest, the tube was removed carefully, avoiding moving and rotating the eggs. The distance between nests was 40 cm, and the base of the egg chamber was 60 cm deep (the same interval and size of nests in the natural environment, according to Ferreira Júnior and Castro, 2003). Rainfall data were obtained from the National Hydrological Information System (Hidroweb) from the Brazilian Water Management Agency (ANA), collected at the gauge station located in São Miguel do Araguaia, nearly 50 km distant from the nursery. Thirty-one nests relocated to the nursery were exposed to sunlight throughout the incubation period. Nine other nests were shaded, covered with fronds of a palm tree (*Cocos nucifera* L.), to observe the influence of shading on the nest temperature and on the hatchling sex ratio. Samples of sand from the inner sediments of 26 nests were collected for grain size analysis. The temperature of seven nests was monitored using digital thermographs (Novus, Termotag), inserted in the middle of the egg chamber, and recorded every 15 minutes during the incubation period. The thermographs were distributed in nests located in the center and along the edge of the nurseries to assess the influence of the concrete walls on heat distribution. If the nest's surface caved in, the nest was dug out. The numbers of living hatchlings, of dead hatchlings (animals that exited the egg but died in the nest), of retained hatchlings (animals that broke the eggshell but died before leaving the egg completely), and number of unhatched eggs were recorded. These data were used to calculate hatching success, here defined as the ratio of the number of living hatchlings to the total number of eggs. The duration of incubation was calculated as the number of days elapsed between oviposition and nest opening.

The sex of the turtles was identified based on the histological gonad analysis of 70 hatchlings from nests where the temperature was measured. Ten hatchlings were taken at random from each nest to determine the sex and calculate the sex ratio. The specimens were euthanized with 25% sodium thiopental injected intracardially. Slides were stained with hematoxylin-eosin and inspected in an optical microscope. Ovaries and testes were differentiated according to the criteria defined by Malvasio et al. (2002a).

Sediment grain size was established using eight fractions, according to the classification of Folk. Granulometric analysis was conducted by sieving, and the percentage of each grain size class was arcsine-transformed to normalize the data. Variation in incubation duration, hatching success, and grain size between shaded and sunlit nests in the center and in the border of nurseries was assessed using the Kruskal-Wallis test at the 5% significance level. The influence of grain size on the duration of incubation and on hatching success was tested using multiple linear regression. Because of the high colinearity of data, some granulometric fractions were excluded from the calculations. Hatching success was arcsine-transformed to normalize data. These tests were conducted using the Statistica 6.0 software. The null hypothesis that the mean temperature during the thermosensitivity period did not affect hatching of males and females was tested using the binary logistic regression (Hosmer and Lemershow, 2000) in MINITAB 13.0.

Table 1. Variation in incubation duration, hatching success, and grain size in terms of the degree of shading and position of 26 nests of *Podocnemis expansa* in nurseries along the Crixás-Açu River.

Parameter	Shading intensity		Nest position	
	H	p	H	p
Incubation duration	9.27	0.004	0.893	0.345
Hatching success	0.16	0.691	1.021	0.281
Grain size				
Gravel	1.252	0.274	1.102	0.304
Granule	1.136	0.286	0.114	0.736
Very coarse sand	0.611	0.434	2.037	0.154
Coarse sand	0.08	0.776	1.488	0.223
Medium sand	0.854	0.356	3.788	0.052
Fine sand	0.505	0.477	3.589	0.058
Very fine sand	1.192	0.286	0.421	0.517

3. Results

Grain size did not vary between shaded and sunlit nests, irrespective of nest position (edge or center of nurseries) (Table 1). All samples analyzed were classified as "gravelly sand" (sensu Folk and Ward, 1957), with a prevalence of coarse sand fractions (Table 2). The mean duration of incubation was longer in the shaded nests (51.7 ± 1.75 days) compared to the sunlit nests (49.1 ± 2.35 days), though it was not affected by the position of the nest in the nurseries (Table 3). The mean hatching success was 47.3 ± 13.6% (ranging between 11.97 and 73.53%), and was not affected by the degree of shading or the position of the nest in the nursery (Table 3). Grain size and clutch size did not influence the incubation duration and hatching success of *P. expansa* (Table 4). The number of retained hatchlings (26.7 ± 17.43%) was higher than the number of hatchlings that died inside the nests (1.2 ± 2.7%). The proportion of retained hatchlings reached 44%, while the highest proportion of dead specimens was 13.7%. A considerable number of specimens had "folded" carapaces and plastrons, although these animals did not show any irregular scute pattern.

The percentage of females varied between 55% (in shaded nests) and 100% (in sunlit nests) (Table 3). The temperature of monitored nests increased in the incubation period, though with two sharp drops that signalled the beginning of the second third of the incubation period (Table 4 and Figure 1). These drops were associated with

Table 2. Grain size distribution of sediments of nests of *Podocnemis expansa* monitored using thermographs (percent values), along the Crixás-Açu River.

Nest	Very coarse sand	Coarse sand	Medium sand	Fine sand
18	9.49	17.82	40.73	21.48
22	20.12	41.61	26.7	3.82
26	19.22	40.95	25.64	3.18
33	20.3	45.34	23.41	3.05
36	6.59	16.49	43.37	26.09
39	20.44	41.92	23.98	3.94
45	21.51	46.31	21.92	2.79

Table 3. Incubation temperature, sex ratio, hatching success and incubation duration in relocated *P. expansa* nests.

Mean temperature (°C)	Mean temperature 2/3 (°C)	Temperature range (°C)	Females (%)	Incubation duration (Days)	Hatching success (%)	Clutch size
34 ± 1.73	33.5 ± 1.37	6.9 (30.1-37)	100	48	51.46	130
33.7 ± 1.63	32.9 ± 1.0	5.7 (31-36.7)	100	51	55.34	103
34.1 ± 1.67	33.2 ± 1.18	5.9 (31.4-37.3)	100	51	43.47	114
33.9 ± 1.37	33.2 ± 1.08	5.1 (31.5-36)	100	49	47.71	109
34.1 ± 1.40	33.5 ± 1.22	5.5 (31.4-36.9)	100	48	49.46	93
33 ± 1.56	31.9 ± 0.95	6.2 (29.6-35.8)	73	49	54.74	95
34.0 ± 1.79	33.2 ± 1.12	7 (30.4-37.4)	90	48	32.81	128

Table 4. Influence of grain size and number of eggs on incubation duration and hatching success of 26 relocated nests of *Podocnemis expansa* from the Crixás-Açu River. Because of high colinearity (hc), some grain size fractions were excluded from correlations. Results are expressed as mean ± standard deviation.

Variable	Mean	Incubation duration		Hatching success	
		t	p	t	p
Incubation duration (Days)	49.1 ± 2.35			–0.287	0.777
Number of hatchlings	104.6 ± 23.05	–1.453	0.162	–1.053	0.305
Grain size					
Gravel	2.02 ± 1.06	–0.86	0.401	0.749	0.463
Granule	5.9 ± 2.52	1.572	0.132	1.084	0.291
Very coarse sand	18.8 ± 4.12	0.384	0.705	hc	hc
Coarse sand	40.5 ± 7.98	hc	hc	hc	hc
Medium sand	26.7 ± 6.45	hc	hc	hc	hc
Fine sand	5.2 ± 5.99	hc	hc	0.373	0.713
Very fine sand	0.76 ± 0.17	0.801	0.433	–0.226	0.824

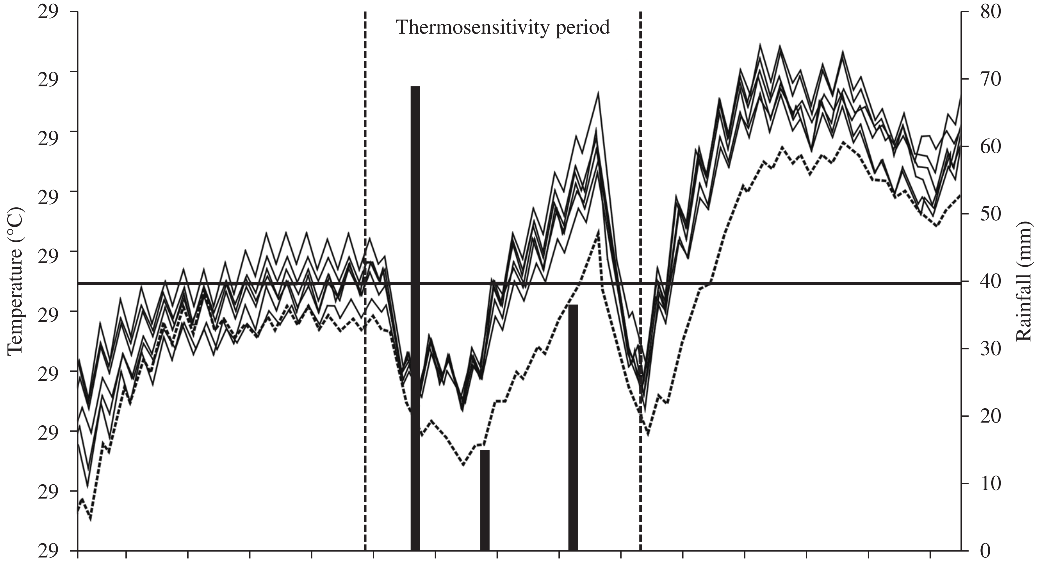

Figure 1. Daily variation of temperature inside nests and of rainfall in the region of the Crixás-Açu River, in October. The temperature dotted line is from a shaded nest. The horizontal line in the center indicates the pivotal temperature of *Podocnemis expansa* for the population inhabiting the Javaés River (Lubiana and Ferreira Júnior, 2009).

the rains during these two periods. The rainfall reached 68.8 mm on a single day in October, when the first drop in temperature was observed. The rainy season started between mid-October and mid-November, with an eventual total rainfall of 284.3 mm.

The mean temperature of nests during incubation varied between 33 ± 1.56 °C and 34.1 ± 1.4 °C. During the thermosensitivity period of sex determination, the second third of the incubation period (Bull and Vogt, 1981), nest temperature varied between 31.9 ± 0.95 °C and 33.5 ± 1.37 °C. These values were above the pivotal temperature, except in shaded nests where temperatures were lower, leading to a higher production of males (Figure 1). The lowest mean temperature in the second third of the incubation period (31.9 °C) was observed in a shaded nest. Incubation temperature was responsible for the sex ratio observed. The risk rate in the binary logistic regression showed that the mean incubation temperature provides a more cogent explanation for the female-biased sex ratio, than the mean temperature during the second third of the incubation period (Table 5). The peaks in the beginning of the second third of the incubation period reduced the mean temperature in the interval, making it lower than the mean temperature measured for the entire incubation period.

4. Discussion

The sand used in this experiment was dug from a single location in a beach of the Cixás-Açú River, which explains why grain size did not vary across the different nests in the nurseries, as occurs in nests that turtles dig along the nesting beaches (Ferreira Júnior and Castro, 2003; 2005). Since nests were transferred one day after the sand was placed in the nursery, there was no time for this sediment to compact. Bjordnal and Bolten (1992) stated that the walls of the egg chamber normally collapse in beaches where the sediment is drier and less compacted, which eventually decreases hatching success of *Chelonia mydas* (Linnaeus). The gradual and progressive compaction of sand during incubation reduces empty spaces in the egg chamber, compressing the eggs and hindering embryo development. This compaction of sand is the reason for the irregular or folded shell of hatchlings, and although it caused neither malformation nor defects in scute patterns (Malvasio et al., 2005; Salera Júnior et al., 2009), it restricted the movement of hatchlings of *P. expansa*. Also, sand compaction was an obstacle to hatchlings retained in the eggs, since the effect decreased the space available inside the egg chamber. Temperatures above 36 °C and below 28 °C reduce the survival rates of *P. expansa* embryos (Valenzuela et al., 1997; Lubiana and Ferreira Júnior, 2009). In our study, temperatures inside the nests exceeded 35.8 °C, reaching 37.4 °C in nest 39, which remained exposed to sunlight throughout the incubation period. Of the seven nests where temperatures were monitored, nest 39 showed the lowest hatching success, indicating that the high temperatures increased the death rate of the embryos and hatchlings.

The incubation temperature of *P. expansa* is high, since the species' distribution is limited to tropical regions (Pritchard and Trebbau, 1984; Rueda-Almonacid et al., 2007). Furthermore, females of this species typically choose sandy, sunlit beaches without vegetation to nest

Table 5. Binary logistic regression between incubation temperature and hatchling sex ratio of *Podocnemis expansa* from the Crixás-Açu River.

Models	Coefficient	Standard error	Z	p	Risk rate and limits (95%)		
					Rate	Low	High
Female							
Constant	–63.1	31.93	–1.98	0.048			
Temp. second third	2.02	0.985	2.05	0.04	7.56	1.09	52.13
G = 4.616; p = 0.032; gl = 1; Hosmer x^2 = 0.963; p = 0.618; df = 2							
Female							
Constant	–87.4	44.96	–1.94	0.052			
Mean temperature	2.69	1.347	2	0.046	14.78	1.05	207.2
G = 4.24; p = 0.039; df = 1; Hosmer x^2 = 2.35; p = 0.503; df = 3							
Male							
Constant	63.1	31.9	1.98	0.048			
Temp. second third	–2.02	0.985	–2.05	0.04	0.13	0.02	0.91
G = 4.61; p = 0.032; gl = 1; Hosmer x^2 = 2.349; p = 0.503; gl = 3							
Male							
Constant	–4.8	0.43	–11.04	0			
Mean temperature	49.68	1.347	–2	0.046	0.07	0	0.95
G = 4.24; p = 0.039; df = 1; Hosmer x^2 = 0.963; p = 0.618; df = 2							

Z) standardized value of the coefficient according to the reduced normal curve; p) level of significance, * significant test; df) degrees of freedom.

(Alho et al., 1984; Ferreria Júnior and Castro, 2003). The environmental characteristics of the areas where *P. expansa* chooses to nest have led the species to develop a higher pivotal temperature, compared to other reptiles (Valenzuela and Lance, 2004). Experiments conducted in the laboratory under constant incubation temperatures indicate that the pivotal temperature is above 32.6 °C for the species living in the Caquetá River, Colombia (Valenzuela, 2001), and 33.5 °C for the turtles inhabiting the Javaés River, state of Tocantins, Brazil (Lubiana and Ferreira Júnior, 2009). The pivotal temperature was estimated as 34.5 °C in nests in the Trombetas River, state of Pará, Brazil (Alho et al., 1984). In a natural scenario, where temperatures oscillate daily, the mean incubation temperature is not a good prediction factor for sex determination (Georges et al., 1994; Valenzuela et al., 1997; Valenzuela, 2001). In addition, the sex ratio is influenced by oscillations in the pivotal temperature, since the rate of embryo development is directly correlated with incubation temperature (Bull and Vogt, 1981; Mrosovsky et al., 1984, 2009; Marcovaldi et al., 1997). Due to the daily oscillations in temperature, eggs may be exposed to temperatures either below or above the pivotal value, and the length of time that temperatures remain at a certain level will define the embryo's sex (Georges et al., 1994). When the incubation temperature is above the pivotal temperature, the embryo develops more quickly, with a higher proportion of females – even when the mean final temperature is near the pivotal temperature (Bull and Vogt, 1981; Pieau and Dorizzi, 1981; Mrosovsky et al., 1984, 2009; Marcovaldi et al., 1997).

In the present study, the mean temperatures of relocated nests were near the pivotal temperature of *P. expansa* as observed in the Javaés River (Lubiana and Ferreira Júnior, 2009). Similar climate conditions can be found in the Crixás-Açu River and Javaés River regions, which suggests a pivotal temperature of around 33.5 °C for the turtle population in the former. Our data demonstrate that although the mean temperatures were around the pivotal temperature, the eggs were exposed to temperatures above 33.5 °C, which led to the evolution of a sex ratio biased towards females. Male hatchlings were observed mainly in shaded nests, which also had milder temperatures in the nursery. Shading of nests caused the incubation temperature to drop and increased the incidence of males, as also reported by Alho et al. (1984) and Valenzuela (2001).

The second fortnight of October marked the beginning of the rainy season, with rainfall reaching 284.3 mm, sufficient to raise the river level and flood part of the beaches, drowning thousands of hatchlings and embryos (Ferreira Júnior and Castro, 2006a). Therefore, the results obtained in the present study demonstrate that the relocation of nests exposed to flooding is a feasible alternative for environmental management of the local population of *P. expansa*. However, the observed high percentage of females may lead to an imbalance in the sex ratio. Therefore, shading the nests is recommended in order to increase the number of males produced. Further studies on the population structure of *P. expansa* of the Crixás-Açu River should be conducted, mainly to evaluate the species' sex ratio. Apart from this, the results obtained in the present study point to the need to develop new alternatives to reduce nest temperature and to increase hatching success in relocated nests. The use of denser, more compact sand may reduce the losses caused by the sliding of egg-chamber walls and minimize the compression of eggs and embryos, thus contributing to higher hatching success rates and decreasing the incidence of folds in hatchlings' shells.

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