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REVIEW

Captivity breeding model and aspects on management of the *Kinosternon scorpioides*

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SUMMARY

Captivity projects for wild animals require scientific technical bases to subsidize them. In this sense we present a simple and efficient model to use in the captivity breeding of *K. scorpioides* as well as for another chelonians. This species is widely consumed by Amazonian populations, having social, cultural and economic importance. In this work, we compiled important information for the management of these animals. The data presents a technical and scientific guide for the implementation of new breeding, especially the maintenance and reproduction of the species.

ADDITIONAL KEYWORDS

Mud Turtle.
Chelonians.
Wild Fauna.

Modelo de criação em cativeiro e aspectos sobre o manejo de *Kinosternon scorpioides*

RESUMO

Projetos de cativeiro para espécies silvestres necessitam de bases técnico-científicas para subsidiá-los. Nesse sentido, apresentamos um modelo simples e eficiente que pode ser aplicado na criação em cativeiro de *K. scorpioides*, ou mesmo para outros quelônios. Esta espécie é amplamente consumida pelas populações Amazônicas, tendo sua importância econômica, social e cultural. Neste trabalho compilamos informações importantes do manejo desses animais. Os dados apresentados servirão de base técnico-científica para a implantação de novos cativeiros, especialmente quanto à manutenção e reprodução da espécie.

PALAVRAS-CHAVE ADICIONAIS

Tartaruga do Lodo.
Quelônios.
Fauna Silvestre.

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INTRODUÇÃO

The turtles are among the main sources of protein feed of families in the Amazon region, where their meat and eggs are used and enjoyed for many generations (Redford and Robinson, 1991; Rebelo and Pezzuti, 2000; Conceição, 2013). The growth of human populations and the use of more effective catching techniques, however, have led populations of these animals to declining in various regions, compromising the sustainability of this resource (Kemenes and Pezzuti, 2007; Waldez *et al*., 2013).

In the Brazilian Amazon, *K. scorpioides* is popularly known as *jurará* or *muçuã*, and has an important role in the human communities of the region. Especially in the eastern region of the Brazilian Amazon the species is used as a food resource and source of income for some families. It is sold in adult and juvenile forms, especially between May and August, when the females have eggs, an important period of the reproductive cycle. As a result, this is one of the most exploited species in the region (Cabrera and Colantonio, 1997; Castro, 2006; Pereira *et al*., 2007a; Barreto *et al*., 2011).

Law No. 5197 for the protection of Brazilian Fauna (Brazil, 1967) forbids the hunting of wild species, excluding the hunting for the subsistence (to survival). *K. scorpiodes* and other turtles, instead, continues to be exploited including commercial purposes by communities (Castro, 2006) and its activity become them criminals. This problem could be remedied by encouraging the creation of the species in captivity, as the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA), an institution that regulates the exploitation of wild animals, allows the breeding of *K. scorpioides* for sale and consumption (IBAMA in 169/2008). However, such activity has not yet been developed due to the lack of information surrounding the captive management of these animals.

For effective decisions to protection and management of the natural environment, as well as the planning of projects that encourage captive breeding, a guide for the captivity management and a comprehensive review of available knowledge about the species is needed. Our data collection aims to describe the captivity simple model for *K. scorpiodes* developed during seven years of experience in Universidade Estadual do Maranhão – UEMA (Brazil) as well as to compile information to support new captivity breeding of this important Amazon resource.

The scientific breeding of *K. scorpioides* in UEMA (KSSB) was authorized in 2008 by the Brazilian Institute for the Environment and Renewable Natural Resources of the state of Maranhão (IBAMA-MA) (1899339/2008). The project is located at the Veterinary Medical School of the Maranhão State University (Campus Paulo VI, São Luís, Maranhão, Brazil) where research into the biology of the species under controlled conditions is carried out.

Below we describe the characteristics of the captivity and management of animals, discussing important information for the implementation of the breeding.

Recruitment of animals to captivity

Success full recruitment needs to consider some aspects of natural history of the animal, like the distribution of the species, the behavior and the genetic variability.

To KSSB animals from the wild area were recruited since 2008 from the same locality of the Maranhão State (São Bento city) in different months of the year, been possible to find animals during all the year. We recruited a total of 48 animals, all adults (average size 14,6 cm), in the proportion of 38 females and 10 males. The animals were collected manually by fishermen. This easy collection is determined by characteristics of their natural history and makes the turtle an interesting species for captive breeding.

K. scorpioides live in groups and are small in size, reaching up to 15 cm in carapace length. They usually walk slowly and, being semi-aquatic fresh water turtles, share different environments, both aquatic and terrestrial, simultaneously (Rocha and Molina, 1987; Delduque, 2000). *K. scorpioides* release an odoriferous substance through their body surface when handled or molested (Rocha and Molina, 1990).

In terms of the biological features of the species, during the dry season (summer), when weather and food conditions are unfavorable due to high temperatures and low humidity, they can be found buried under the ground in a state of low metabolism and energy supply. This mechanism is known as estivation and is entirely linked to the oscillations of environmental temperatures, resulting in the species regulating its body temperature in order to avoid thermal stress (Pereira *et al*., 2007b).

K. scorpioides is extensively distributed around the world, being found in Argentina, Bolívia, Colômbia, Costa Rica, Equador, El Salvador, Guiana Francesa, Guatemala, Honduras, México, Nicarágua, Panamá, Paraguai, Peru, Suriname, Venezuela and Brasil (Berry and Iverson, 2011). In Brazil, *K. scorpioides* is found in the states of Mato Grosso, Rondônia, Amazônia and Pará (Costa *et al*., 2010), as well as on São Luís Island (Maranhão), in the Tapajós (Pará) (Pritchard and Trebbau, 1984), Manicoré, and Madeira Rivers (Amazonas) (Iverson, 1992; Cabrera and Colantonio, 1997; Berry and Iverson, 2001); the Serra Sul, Serra dos Carajás (Pará) (Carvalho-Jr *et al*., 2008); Cachoeira do Nazaré, the Machado River (Rondônia) (Cabrera and Colantonio, 1997; Berry and Iverson 2001), Aripuanã and the Aripuanã River (Mato Grosso) (Costa *et al*., 2010); the Tapirapé River (Mato Grosso) (Pritchard and Trebbau, 1984; Iverson, 1992; Cabrera and Colantonio, 1997) and in the town of Porteirinha (Minas Gerais) (Silveira *et al*., 2011) **(figure 1)**.

The collection in only one site for KSSB is supported by the studies of molecular markers that indicate the existence of considerable genetic variability in the species, whem RAPD loci (*Random Amplified Polymorphic DNA*) of a *K. scorpioides* population from the Animal Germplasm Bank of the East Amazon (animals from Salvaterra city, Marajó Island, state of Pará, Brazil) was studied. The authors of this study reinforced the importance of the conservation of the species, both in captivity and in the natural environment (Silva *et al*., 2011).

Information about more efficient molecular markers for the species is still limited. Iverson *et al*. (2013) and Spinks *et al*. (2014) included *K. scorpioides* in a phylogenetic study, generating sequences for mitochondrial (cytochrome b, 16S and 12S ribosomal gene) and nuclear DNA (RAG one and two and C-*mos* genes); and for 14 nuclear loci (10.305 bp) respectively. These studies reinforce the need to analyze more specimens of *K. scorpioides* to review proposed subspecies, indicating possible species included in the same taxon.

Physical structure of the captivity

The KSSB space has a total area of 159.92 m², consisting of five bays with screened walls made from galvanized iron. Each bay has an area of 13.94 m², forming part of an overall building area of approximately 91.0 m². There is also a free space of approximately 68 m². Each bay has a brick tank covered in ceramic, an access ramp for the animals, available water and a drainage runoff. The dimensions of the tank are: 2, 10 m in length, 1.30 m of width and 0.30 cm of depth. The bays contain trees, gravel and sand **(figure 2)**. During the year, the medium temperature of the environment of the bays is 28^0 C. There is no covered area to protect the animals of the rain, but there is shadow to protect of the sun. The breeding area has considerable natural ventilation and an open area.

These characteristics provide to the space a similar scenario of the natural area, since the animals in wild inhabit areas that flood in the rainy season or are permanently flooded. Barreto *et al*. (2009) in a survey conducted on Curupu Island, in the state of Maranhão, Brazil, identified the presence of *K. scorpioides*, showing that the species can also be found in beach areas, characterized by dunes, grasses and salt water.

In KSSB the animals are handled daily during refilling of the water tank and monitored in order to evaluate their health conditions. During the cleaning procedure, all the animals are removed from inside the bays and cleaning products are not used.

Feeding

The dietary nutritional requirement for turtles and protein concentrations in the diet is indicated between 20 and 40% according to Andrade (2008). The animals are fed once a day, preferably in the morning. They are fed on alternate days with commercial fish food (Nestle Purina® Pet Care Company, Brazil - 32% protein) and

Figure 1. Distribution of *Kinosternon scorpioides* in South America. The following regions are highlighted: , Argentina, Belize, Bolivia, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Trinidad, Venezuela and Brazil, especially the states of Acre, Alagoas, Amapá, Amazonas, Bahia, Ceará, Goiás, Maranhão, Mato Grosso, Minas Gerais, Pará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Rondônia, Sergipe and Tocantins (Pritchard and Trebbau, 1984; Iverson, 1992; Cabrera and Colantonio, 1997; Berry and Iverson, 2001; Carvalho-Jr *et al.,* 2008; Costa *et al.*, 2010; Silveira *et al.*; 2011; Acosta *et al.*, 2013). Adapted from Dijk *et al.* (2014) (Ocorrência de *Kinosternon scorpioides* na América do Sul. São destacadas as seguintes regiões: Argentina, Belize, Bolívia, Colômbia, Costa Rica, Equador, El Salvador, Guiana Francesa, Guatemala, Guiana, Honduras, México, Nicarágua, Panamá, Paraguai, Peru, Suriname, Trinidad, Venezuela e Brasil, especialmente os estados do Acre, Alagoas, Amapá, Amazonas, Bahia, Ceará, Goiás, Maranhão, Mato Grosso, Minas Gerais, Pará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Rondônia, Sergipe e Tocantins (Pritchard e Trebbau, 1984; Iverson, 1992, Cabrera e Colantonio, 1997; Berry e Iverson, 2001; Carvalho-Jr *et al.*, 2008; Costa et al., 2010; Silveira *et al.*, 2011; Acosta *et al.*, 2013). Adaptado de Dijk *et al.* (2014).

the feed is administered in water, in the proportion of 10 g per animal.

Muçuã has the ability to metabolize different types of food, being classified as omnivores, as they feed on fish, fruits, seeds, algae, plant remains, amphibians, shrimp and crabs (Vanzolini *et al.*, 1980 Acuña-Mesén *et al.*, 1983; Delduque, 2000 Vogt, 2008). According to Berry and Iverson (2001), this species can be considered predominantly carnivorous in the wild and can display cannibalistic behavior when underfed or playing the female with another male of the species (Pritchard and Trebbau, 1984).

Sexual dimorphism

Currently, KSSB breeding includes a herd of 112 animals, of which 31 are males and 81 are females, distributed according to size between young and adults. Adult animals and young are distributed separately in bays according to their age. Juveniles are kept in plastic boxes containing sand inside in order to make the most natural environment.

The morphological and biometric patterns of *K. scorpioides* are identified through major characteristics that are associated with their habits and behavior in the environment. The turtles have a protective bony shell, formed by the joining of the carapace and plastron by a bony bridge. This feature provides a protective mechanism for the limbs, head and neck. According to Castro (2006) and Pereira *et al*. (2007a) some morphological characters differ considerably between the genders. In general, the average size of the carapace and plastron of females is between 15.26 and 13.35 cm whereas in males, these values range from 14.79 and 12.30 cm. Delduque (2000) mentions that the size of the carapace varies between 15.0 cm and 27.0 cm, with plastrons of different colors and movable plates. Weight can also vary between genders. Females which have an average weight of 430.08g are heavier than males, which have an average weight of 315.05 g (Castro, 2006; Pereira *et al*., 2007a). Studies have shown that female shell size and morphology can differ in environments with distinct ecological characteristics (Rocha and Molina, 1987; Delduque, 2000).

Studies by Bramble *et al*. (1984) demonstrated that the relative development of *Kinosternon* plastron can be strongly influenced by local environmental conditions, especially the aquatic habitat. Species from wetlands, perennial and shallow environments have flatter shells, in order to hide from predators, while the hooves of species from deeper environments are larger and taller (Acunã-Mesen, 1994). The species name comes from the tail of the animal, which has a format similar to the sting of the scorpion, hence the name *scorpioides*. In males, the tail is longer, facilitating the introduction of the penis into the cloaca of the female, serving as a fixing support until ejaculation. The plastron has a concave depression, which facilitates mounting and copulation. In the female, the tail is shorter and the plastron is straight. Males have a corneal fingernail, used to hold the female during copulation (Castro, 2006; Pereira *et al*., 2007a).

Internal anatomy

Figure 2. Scientific breeding of UEMA for breeding *K. scorpioides* (a), especially the tank (b) (Instalações do criadouro científico para *K. scorpioides* da UEMA (a), especialmente o tanque (b)).

The knowledge of some aspects of the internal anatomy of K. *scorpiodes* has been important to veterinary care of the animals in captivity. The morphology of *K scorpioides* liver was studied by Machado-Júnior *et al*., (2005), who observed robust and delimited structures with a large brown-purple colored organ cranio-medially located in relation to the heart and caudo-medially located in relation to the small intestine. It has five lobes, two on the left and three on the right of the median plane of the body, with the gall bladder positioned between the right lobes; differing from evolutionarily closely related species such as salamanders, snakes and other vertebrates.

Histological and immune histochemical studies of the esophagus of *K. scorpioides* was carried out by Pereira *et al*. (2005). The authors characterized and reported the presence of important structures of the esophageal regions, which consist of the mucosal, submucosal, muscular and adventitious or serous layers. Caliciform cells interposed between the ciliated epithelial cells were observed in the mucosa tunica. The muscularis mucosa is prominently formed by two layers of smooth muscle fibers, while the submucosa consists of connective tissue with collagen fibers infiltrated by lymphocytes rich in vessels and nerve plexus. The muscular layer consists of smooth muscle fibers and an inner circular and an outer longitudinal muscle layer. The tunica adventitia consists of loose connective tissue, fat cells, blood vessels and nerve terminals.

In terms of the organs of the circulatory system, the aorta is twofold in origin at the base of the heart (left and right artery). These organs extend from the heart through the ventral region and continue in this direction to the caudal portion of the body of the animal, sending out visceral branches from the left aorta (Oliveira *et al*., 2009).

According to Machado-Júnior *et al*. (2006) and Carvalho *et al*. (2010) the sexual organs of these reptiles are similar to those of other classes of turtle: the female genital system consists of a pair of ovaries and a pair of oviducts. Males have a pair of yellow ovoid-shape testicles, the color of which ranges from a light to a golden yellow, according to the reproductive period. They are asymmetrically positioned within the celoma and fixed by the mesorquio and the mesocolon, followed by a convoluted epididymis that continues to the deferent ducts, opening inside the cloaca. The penis has the format of a ventral groove and consists of a base, body and glans. The body has two penile cavernous bodies, separated by a groove through which the semen flows, and the glans is blackish. The penile urethra is absent.

Reproductive features

In KSSB, females during laying times are monitored. Some eggs can be placed in artificial incubators, but others remain in their own environment until birth. The eggs are measured and weighed and placed in an artificial incubator, followed daily until hatching. Preliminary studies showed that the eggs show a 83.3% hatching rate, with an average of 195 days incubation period (Anunciação *et al*., 2011).

The pen is monitored daily and the animals are separated into nurseries after birth. Seasonality influences the reproductive process of *K. scorpioides* and the cycle can be synchronized by climatic, dietary and hormonal factors. Another important environmental factor for *Kinosternon*, as well as for other reptiles, is temperature, since the process of egg incubation and sex determination is controlled by this factor (Ferreira-Júnior, 2009; Viana *et al*., 2013).

It was noted that the mating behavior of *K. scorpioides* created in captivity occurred during the rainy season and was not observed during the dry season. Copulation always occurred in the water, with the male courting the female, followed by pursuit, dominance and mounting, resting all four legs on the carapace and making ventral movements with the tail in search of the cloacal opening, until the introduction of the penis (Chaves, 2011).

The seasonal copula contrasts with a finding that the female's ovaries are sexually mature throughout the year (Chaves *et al*., 2012). On the other hand, seasonal variation in the morphology of the epididymis (its organization into *rede testis*, efferent duct and epididymal duct) and variation of sperm production have been reported among males. Differences in the morphology of the tubular and luminal diameters were found, with the highest average size found in the

Table I. Laying period of *Kinosternon scorpioides* in captivity found in different bibliographical references (Períodos de postura de ovos de Kinosternon scorpioides em cativeiro encontrados em diferentes referências bibliográficas).

Oviposition period	Spawning habitat	Reference
April to January	Captivity	Araujo (2007)
May to October	Captivity	Silva *et al*. (2006)
April to August	Zoobotanic Park of Emilio Goeldi Museum (PA)	Castro (2006)
February to December	São Paulo Zoo (SP)	Basho and Molina (2000)
March to August	São Paulo Zoo (SP)	Rocha and Molina (1990)

Adapted from Araújo (2009).

rainy season, the reproductive period for *K. scorpioides*. Sperm production was observed throughout the year, but was higher during the rainy season. Reports on sperm production in *K. scorpioides* showed that Sertoli cells play a key role in the production, as well as the efficiency and lifetime of sperm. In males, the body weight, gonad size, testicle size and morphology and hormonal concentrations are related to environmental conditions, reflected in the seasonal reproductivity of the species (Viana *et al*., 2013; 2014a; 2014b; 2014c and Sousa *et al*., 2014).

Costa *et al*., (2009) followed the ovarian cycle of *K. scorpioides* and verified the development of pre-ovulatory follicles between the months of October 2005 to March 2006. In the months of April to August 2006, the deposition of calcium was observed in a period corresponding to the end of the rainy season and the beginning of the dry season.

In terms of the oviposition of captive-bred animals, Chaves (2011) reported that females can lay eggs all year. Silva (2011) however, states that the laying of females kept in captivity occurs primarily during the dry season, confirming the reproductive seasonality of the species. Several studies found differentiated laying periods for *K. scorpioides* eggs. The egg laying months in different geographic regions and environments are shown in **table I**.

Castro (2006) found that the female can lay eight to nine eggs in each laying period in clayey soil and that these hatch in about 150 days. Araújo *et al*. (2012) reports that the size of the females can be related to the rate of egg laying, with larger females possessing higher laying frequency in relation to smaller females. Temperature influences the incubation period through the exchange of heat between bodies. The embryo development rate is generally 50% above the average temperature that defines males and females. In this regard, embryonic development is positively related to temperature, as the higher the temperature, the greater the rate of embryo development (Ackerman, 1997 and Ferreira-Júnior, 2009).

Sex determination in turtles can be synchronized by the influence of external factors, such as incubation temperature, in early stages of embryonic development, corresponding to the first trimester. During this stage, the process is still reversible (Bull and Vogt, 1981; Morosovsky and Pieau, 1991). The sex determining temperature in *K. scorpioides* was reported by Ferreira-Júnior (2009), who indicated that males develop at temperatures of around 26°C and females at around 30°C.

CONCLUSIONS

1. Knowledge of the natural history of a species is the first step to the in captivity management, that can provide efficient conservation programs. *K. scorpioides*, due to its economic and cultural importance in Amazonian communities, as well as its extensive exploration, needs programs that meet such goals.

2. More information regarding the reproductive aspects, biology, morphology, sexual development and geographic distribution of the genetic variability of this species is still required to complete the basis for its management.

3. The data presented here support the implementation of K. *scorpioides* breeding. Its easy collection, captive maintenance and reproduction indicate that the production of these animals can be an economically viable alternative to Amazonian populations.

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