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First observations on courtship and nesting behavior of *Kinosternon vogti* (Testudines: Kinosternidae)

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ABSTRACT

Kinosternon vogti is one of the most narrowly distributed kinosternid turtles in Mexico; it is apparently restricted to small streams and ponds in the urban area of Puerto Vallarta, Jalisco and Bahía de Banderas, Nayarit. There is little information on the biology of this recently described and endangered species, particularly regarding reproductive biology. We report reproductive behaviors on two mating pairs of *K. vogti* in captivity. Males exhibited combat for copulating with female. During courtship, the male blocks the female movements, the female bites male´s rostrum scute, the male displays titillation posture, and finally the male moves to position himself above the female. The reproductive season begins with courtship in August-September and ends with egg-laying in November-December. Six and two eggs per clutch (non-viable eggs; average size, n = 5: 28.83 ± 1.19 x 15.95 ± 0.55 mm). Minimal reproductive size of females and males were CL (carapace length) = 88.37 mm and CL = 78.7 mm, respectively.

Key Words: Clutch Size; Laying Eggs Period; Mating; Mature Size; Vallarta Mud Turtle.

Kinosternon is one of the most diverse genera of turtles in Mexico (Legler and Vogt, 2013), and two new species were recently discovered. The Vallarta Mud Turtle (*Kinosternon vogti* López-Luna *et al.,* 2018) was described from the city of Puerto Vallarta, Jalisco, and the Cora Mud Turtle (*K. cora* Loc-Barragán *et al*., 2020) was described from the coastal plains in the state of Nayarit, both exhibit a restricted distribution (López-Luna *et al*., 2018; Loc-Barragán *et al*., 2020). The distribution of the Vallarta Mud Turtle is limited to the urban area of Puerto Vallarta and Bahía de Banderas, Nayarit, which are located on the western-central coast of Mexico, where its known to exist in small streams and ponds at low densities (López-Luna *et al*., 2018; Rosales-Martínez *et al*., 2021).

Kinosternon vogti was recently cataloged as Endangered by Mexican law (Secretaría de Medio Ambiente y Recursos Naturales, 2019) based on low population size, as only 30 individuals are known to science. Moreover, the 30 individuals that have been reported were observed within urban areas and human-made habitat (F.G.C-M. pers. obs.).

To date, the life history of this species is limited to anecdotal observations of diet (Ramírez-Ramírez *et al*., 2019), morphology (López-Luna *et al*., 2018) and reproduction (Montaño-Ruvalcaba *et al*., 2020). For example, Montaño-Ruvalcaba *et al.* (2020) provided the only information on reproduction of this species by dissecting an adult female in early November and observed four oviductal eggs (one crush egg, and the size of the oviductal eggs was

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26 x 15 mm, 28 x 15 mm, and 28 x 16 mm), and approximately 18 enlarged follicles.

Herein, we provide additional data on the reproductive biology of *K. vogti* based on a video of 97 s, recorded on 23 October 2017 at the Laboratory of Biodiversity and Ecosystem Services in the Centro Universitario de la Costa of the Universidad de Guadalajara. From this video, four photos were extracted that exhibit the courtship and mating behaviors (Fig. 1). During these recording turtles were being held in a 40 l glass aquarium (water depth < 11 cm) that measured 26 cm wide x 50 cm long x 35 cm tall. The individuals in the video were the paratypes of the original description of *K. vogti* (López-Luna *et al.*, 2018): female #1 Carapace length (CL) = 88.37 mm, male #1 CL = 80.0, and male #2 CL = 83.5 mm.

In addition, we captured three individuals on a dirt road within Puerto Vallarta City; a female in October 2019 (female #2: CL = 95.18 mm), and one female (female #3: CL = 90.71 mm) and one male #3:

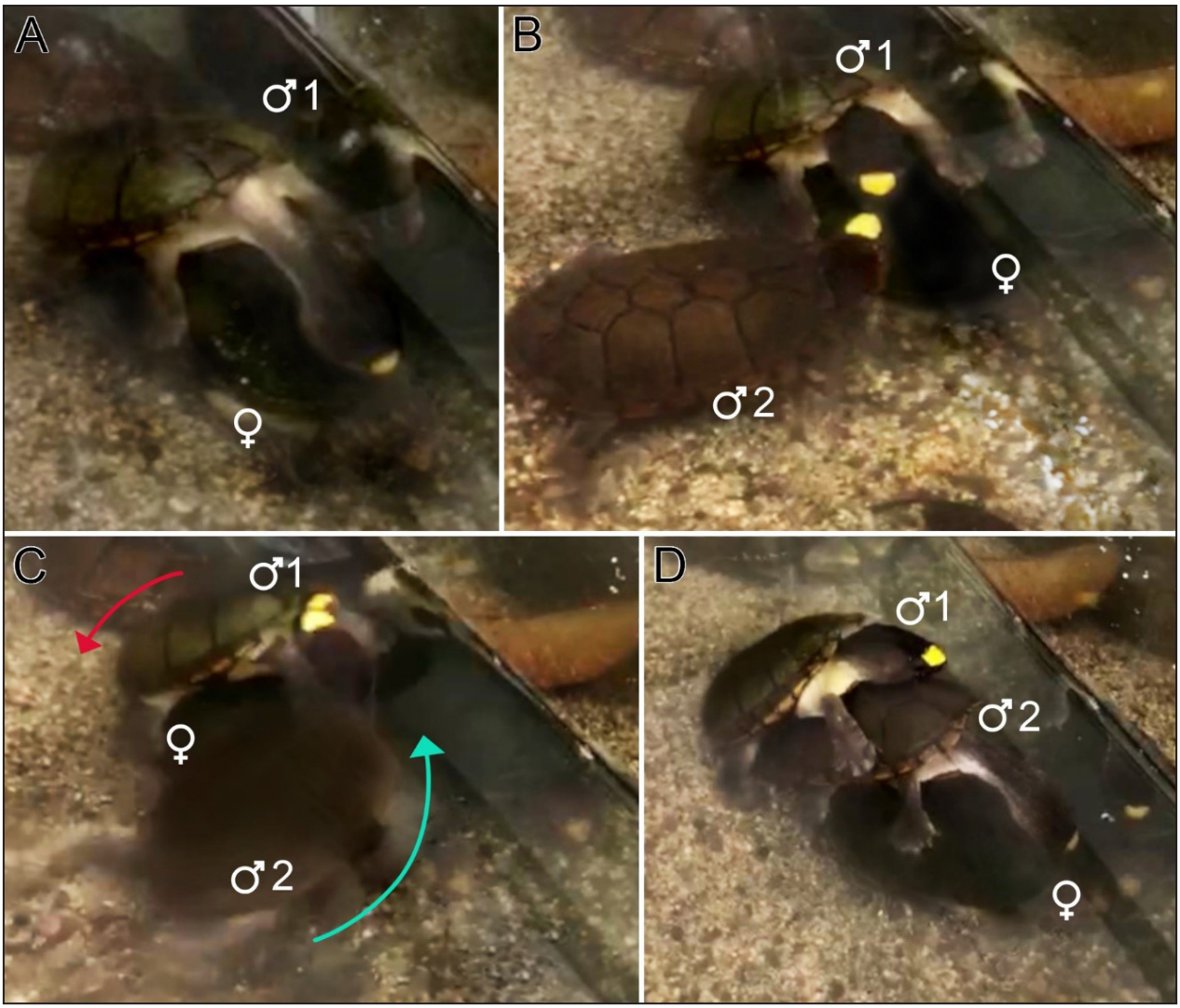

Figure 1. Reproductive combat behavior sequence between two captive *Kinosternon vogti* males. A) Male #1 in mount of female #1. B) Confrontation between males. C) Slam of male #2 (the direction is indicated by the green arrow) on male #1 to knock him off the mount of female #1 (the red arrow indicates the direction of his movement). D) Male #2 mounts female #1 and this in turn is mounted by male #1.

(CL = 78.7) mm in August 2020. These individuals were placed in a terrarium that consisted of a dry area (43 cm wide x 30 cm long x10 cm tall), and a freshwater area (75.5 cm wide x 97.5 cm long x 10 cm tall). On 18 August 2020 courtship and mating behavior was observed in these two individuals and captured in a 96 s recording (https://www.youtube.com/watch?v=3CvuPf8pAzE; cellular phone camera iPhone 7). In both videos the observations were made underwater.

In the images of the first video, male #1 is seen mounting female #1 (Fig. 1A), and at the same time male #2 approaches male #1 and faces him head-to-head (Fig. 1 B). Male #2 then propels himself with his head and body, knocking male #1 out of the mount (Fig. 1C). Male #2 then assumed the mount of female # 1, then male #1 attempted to mount male # 2 (Fig. 1D).

Competition between males (male-male competition) to mate with females like we observed in *K. vogti* is an uncommon behavior in other kinosternid species (previously observed in *K. hirtipes*, a sister species of *K. vogti*), as they are primarily known to mate via forced insemination (Berry and Shine, 1980). Moreover, mating behavior between males, like we observed when male #1 mounted male #2, has been previously documented and it is speculated that this occurs due to a low capacity for sexual recognition (mainly by smell) or by intrasexual behavior with unknown function (Mahmoud, 1967).

In the second video, male #3 was observed mating with female #2 underwater, which is a common behavior in other kinosternid species (Legler and Vogt, 2013). In this video, male #3 extended his neck and placed himself in front of the female #2, impeding her ability to advance by making lateral movements left and right (Fig. 2A). Female #2 responded by biting the yellow rostral scute male # 3 a total of eleven times (Fig. 2B).

When facing female # 2, male # 3 walked forward on the bottom of the terrarium. Also, during this process, six movements that involved one or both front legs were observed with variable duration. Our first impression of these movements was that the male was making swimming type propulsion and changes of direction. However, on two occasions the male executed a pair of horizontal movements with his front legs in front of female # 2 (Fig. 2C). We interpret that this movement pattern of the forelegs was used to attract the attention of the female and facilitate mounting, as has also been suggested by Liu *et al.* (2013) in other freshwater turtles.

The swimming behavior observed in male #3, where the male executes a series of stereotyped movements with his forelimbs has been described as "titillation" and has been observed in other species of freshwater turtles (Liu *et al.*, 2013). This behavior is a type of visual signal, in which the male places their front legs parallel to the female's head in order to vibrate and drum the claws against the female's eyes and interocular region (Kramer and Burghadt, 2010; Liu *et al.*, 2013). In the case of *K. vogti*, male # 3 performed the "titillation" several centimeters away from female # 2 (Fig. 2C), which could be a variant of titillation or a different courtship behavior that has not been described.

The mating behavior occurred after a final stroke of male # 3 and a bite of female # 2 on the male's rostral yellow patch. Subsequently, female # 2 turned, positioning the posterior end of her carapace towards male # 2, and then the male approached the right posterior side of the female and proceeded to mount her (Fig. 2D). Once mounted, with his plastron in contact with the female's carapace, he tried to grasp her posterior margin of the carapace by grabbing her with his hind legs, and in order not to fall from the mount he placed both his front and hind legs on the lateral scutes, as well as the head and neck extended in contact on the middle and anterior part of the carapace (Fig. 2E). This postural pattern of mounting falls within one of the two postures used by freshwater turtles during mounting and couple (Liu *et al.*, 2013). The couple was not consummated.

The courtship observed in *K. vogti* in the two videos from this study is consistent with behavior that has been observed in other *Kinosternon* species (Legler and Vogt, 2013). At least three behaviors have been described in *Kinosternon*: tactile (male approaching another turtle from behind to smell it and apparently determine its sex; which was not observed in this work), mounting and intromission, and biting and rubbing (Mahmoud, 1967). We did not observe the couple, but after 13 weeks, on 7 November 2020, female #2 laid six eggs, and on 3 December 2020 female #3 laid three eggs (for this last female, the courtship occurs on 16 September 2020, but not recorded in video). We don't have evidence to define the exact date of fertilization of both females # 2 and # 3, therefore it was not possible to establish egg retention period. In addition, no courtship or other spawning was observed in months before or after those documented in this note.

Egg laying by females took place in dug holes in the groundwood pulp substrate of the terrarium. No eggs exhibited band formation in the shell that would have indicated normal embryo development (Ewert, 1985), thus the incubation period was unknown. We did not control the incubation temperature and humidity, which could have affected normal embryo development (Packard *et al.*, 1987). From female #2 and female #3, two and three eggs (the remaining

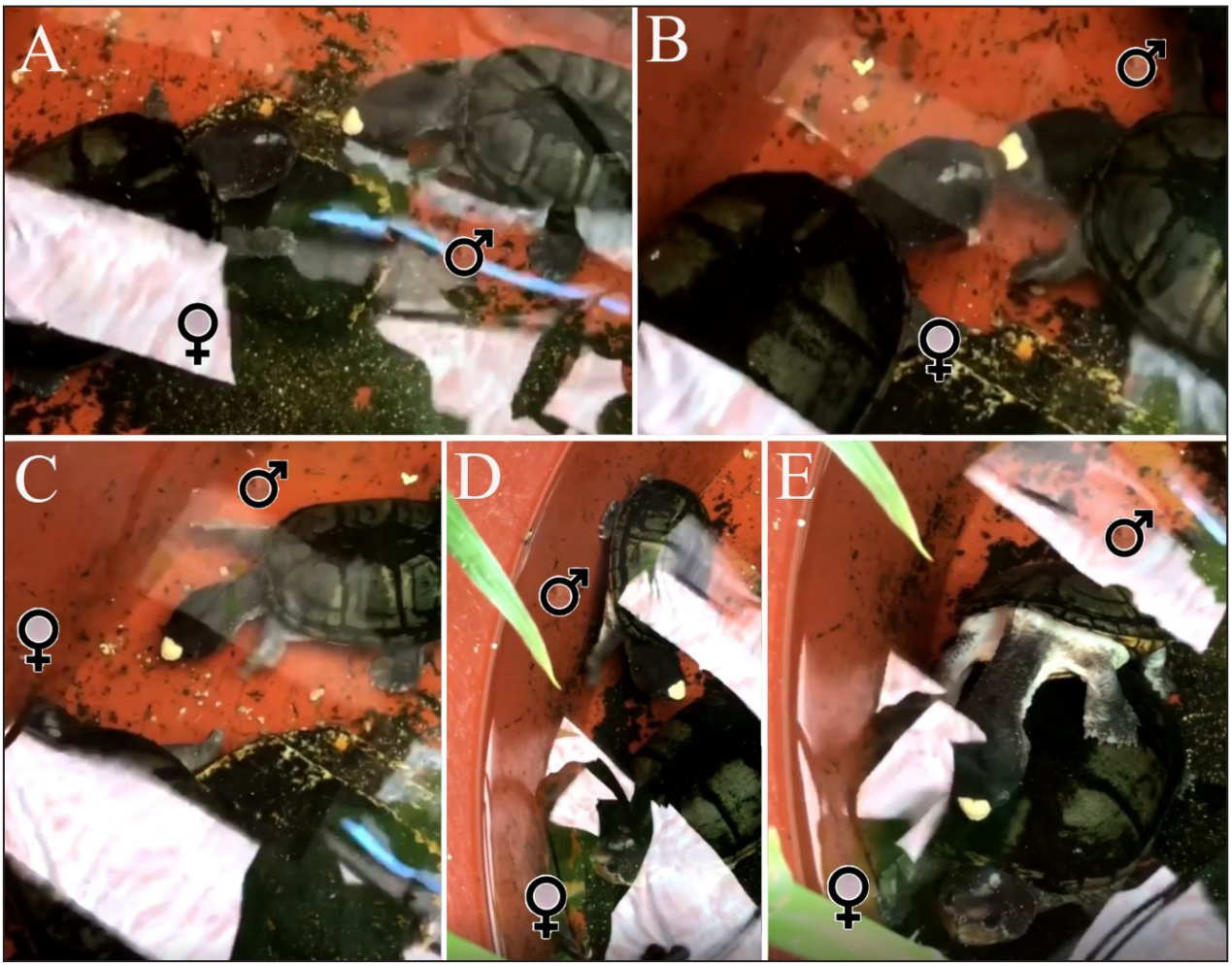

Figure 2. *Kinosternon vogti* female #2 and male #3, courtship and mount. A) Male block the advancement of the female. B) Female bites the male's rostral scale. C) Male in titillation posture. D) Approximation of the male to the back of the female. E) Male in mount on the female. Images from video available at https://www.youtube.com/watch?v=3CvuPf8pAzE. ♀ = female #2; ♂ = male #3.

four eggs were broken) were measured respectively. On their major and minor axes eggs measured: 26.88 x 15.17 mm, and 27.23 x 15.30 mm (Fig. 3); 28.49 x 16.14 mm, 29.38 x 16.71 mm, and 29.70 x15.88 mm. The average size for the five eggs was 28.83 ± 1.19 x 15.95 ± 0.55 mm.

Based on the observations presented in this work, female #1 (CL = 88.37 mm) and male #3 (CL = 78.7 mm) represent the lowest record of sexual maturity size for each sex in this species. The reproductive body-size, clutch size, and the egg traits are within the ranges observed for other species of the genus *Kinosternon* (Legler and Vogt, 2013) and even for this same species (similar clutch size and egg straits observed in Montaño-Ruvalcaba *et al.*, 2020). *Kinosternon acutum* is similar to *K. vogti* in that it has a small body size (CL = 116 mm), and the minimum CL of sexual maturity females is between 77 mm and 97 mm, and as small as 74 mm in males (Legler and Vogt, 2013). Clutch size (one to three) and egg size (33 x 17 mm) in *K. acutum* are also similar to *K. vogti*, but *K. acutum* are known to lay two to four clutches per year (Legler and Vog,t 2013).

Observations on the reproductive biology of *K. vogti* in captivity is an important first step to identify the reproductive season for this species. Based on our observations and the anecdotal observations published previously, the reproductive season in *K. vogti* seems to be from mid-August to early December (end of summer and beginning of winter). Thus, with seasonal reproductive activity, *K. vogti* can make use of trophic and spatial resources for courtship provided by the streams and temporary ponds it inhabits, formed during the summer rains (García-Oliva *et al.*, 2002), as do other species of the genus in Mexico (Iverson, 1996).

Figure 3. *Kinosternon vogti* female #2 with two of its unviable eggs laid in captivity.

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