Journal of Herpetological Medicine and Surgery Intraocular Pressure in the Sonoran Desert Tortoise (Gopherus morafkai) Using Rebound Tonometry --Manuscript Draft--

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Corresponding Author:	Julio Alfonso A Mercado Rodriguez, MVZ, MPVM Universidad Autonoma de Baja California Mexicali, Baja MEXICO					
First Author:	Julio Alfonso A Mercado Rodriguez, MVZ, MPVM					
Order of Authors:	Julio Alfonso A Mercado Rodriguez, MVZ, MPVM					
	Itza Denisse Vizcarra Alcantar					
	Cesar Augusto Flores Dueñas, PhD					
	Alberto Barreras Serrano, PhD					
Abstract:	A complete ophthalmic examination includes the measurement of intraocular pressure (IOP). IOP has been recorded in several species of reptiles, and differences between species have been recognized. This study measured IOP in Sonoran Desert tortoises (Gopherus morafkai) presented to a veterinary hospital. The IOP observed in this study (right eye 15.8 mm Hg ± 2.66 and left eye 16.8 mm Hg ± 2.75) was higher than those reported in aquatic chelonians. This study demonstrates the reliability of rebound tonometry (i Care® Tonovet Plus) for measuring IOP in Sonoran Desert tortoises, providing a robust method for future research. It is not recommended that IOP values be extrapolated between species and collection methods.					

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4	Julio Alfonso Mercado Rodríguez ^{1*} , Itza Denisse Vizcarra Alcantar ¹ , Cesar Augusto Flores						
5	Dueñas ² , Alberto Barreras Serrano ³						
6							
7	¹ Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja						
8	California, Fracc. Campestre s/n, Mexicali, B.C., México						
9	² Hospital Veterinario de Pequeñas Especies. Instituto de Investigaciones en Ciencias						
10	Veterinarias, Universidad Autónoma de Baja California, C. Río Mocorito Sn, Ex-Ejido						
11	Coahuila, 21360 Mexicali, B.C., México						
12	³ Estudios de Posgrado. Instituto de Investigaciones en Ciencias Veterinarias, Universidad						
13	Autónoma de Baja California, Fracc. Campestre s/n, Mexicali, B.C., México						
14	*Corresponding author: juliomr@uabc.edu.mx						
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30 Introduction

Mexico is a significant habitat for three species of desert tortoises of the genus 31 Gopherus: G. berlandieri (Texas tortoise), G. flavomarginatus (Mapimi tortoise), and G. 32 morafkai (Sonoran Desert tortoise). The Sonoran Desert tortoise was previously included in 33 the species G. agassizii, which is now the Mojave Desert tortoise. There is also a debate 34 over the existence of a fourth species within the genus and with a natural range within 35 Mexico, G. evgoodei (Southern Sonora-Northern Sinaloa) (Edwards et al., 2015, 2016). 36 Mexican tortoises are protected. The Mapimi tortoise is the only species classified 37 as "endangered" in Mexico (NORMA Oficial Mexicana NOM-059-SEMARNAT-2010). 38 Previously, the Mojave Desert tortoise was considered under "special protection," but since 39 40 the Sonoran Desert tortoise was recognized as a separate species, the protection classification for this species within Mexico has not yet been reconsidered by the 41 authorities (NORMA Oficial Mexicana NOM-059-SEMARNAT-2010); however, 42 ownership of this species is illegal within Mexico. The Sonoran Desert tortoise population 43 (including Arizona's) is listed as vulnerable by the IUCN (Averill-Murray et al., 2023). 44 45 Tortoises have greatly suffered from the impacts of human activities on their habitat, including habitat destruction, e.g., through livestock farming and road construction. 46 Additionally, droughts, vehicular trauma, and capture for pet ownership or zoological 47 collection have negatively affected the population. Tortoises are regularly kept in human 48

49 care in the cities of Baja California despite being a non-native species in this state (Edwards
50 *et al.*, 2015).

51	Ocular diseases are common and widely reported in reptiles (Williams, 2012). These
52	diseases can manifest as systemic or ophthalmic, and it is essential to differentiate and
53	diagnose them accurately (Allgoewer et al., 2002; Reyes-Olivares et al., 2016; Mayer et al.,
54	2010; Agostinho et al., 2023; Fumero-Hernandez et al., 2023). Examination of intraocular
55	pressure (IOP) is essential as it provides crucial information for identifying conditions such
56	as glaucoma and uveitis (Williams, 2012). IOP has already been reported in various species
57	of reptiles such as the green iguana (Iguana iguana), Andros Island iguana (Cyclura
58	cychlura cychlura), central bearded dragon (Pogona vitticeps), red-eared slider (Trachemys
59	scripta elegans), loggerhead sea turtle (Caretta caretta), Kemp's ridley sea turtle
60	(Lepidochelys kempii), box turtle (Terrapene spp.), Hermann's tortoise (Testudo hermanni),
61	red-footed tortoise (Chelonoidis carbonarius), yellow-foot tortoise (Chelonoidis
62	denticulatus), San Cristóbal giant tortoise (Chelonoidis niger chathamensis), European
63	pond turtle (Emys orbicularis) and Texas tortoise (Gopherus berlandieri) (Selmi et al.,
64	2002; Selmi et al., 2003; Selleri et al., 2012; Wojick et al., 2013; Delgado et al., 2014;
65	Espinheira et al., 2015; Schuster et al., 2015; Rajaei et al., 2015; Gornik et al., 2016;
66	Cordeiro de Araujo et al., 2017; Petritz et al., 2019; Martin de Bustamante et al., 2020;
67	Rowatt et al., 2020; Lamagna et al., 2021; Masterson et al., 2022).
68	Differences in IOP have been documented between species and between collection
69	methods, whether applanation tonometry or rebound tonometry. Rebound tonometry is
70	more efficient in small eyes and allows data to be collected quickly in shy species, such as
71	chelonians. This work reports IOP values obtained in Sonoran Desert tortoises in human
72	care in Mexicali, Baja California, Mexico.

73

74 Methods

75 This study was carried out through a voluntary request to owners who wanted to 76 know the health status of their Sonoran Desert tortoises in Mexicali, Baja California. This 77 species is protected, and ownership as pets is forbidden (Averill-Murray et al., 2023). 78 However, they are commonly seen as patients in private practice. Twenty tortoises born in captivity were evaluated. Thirteen females and seven males were included in the study. The 79 animals had a mean weight of 2.604 kg (SD = 1.9) for the females and 2.443 kg (SD = 1.6) 80 for the males. The exact ages of the tortoises were difficult to obtain since some people had 81 not owned the tortoise since its birth, and age was considered subjectively based on the 82 time of ownership that could be documented, varying from 3 to 24 years. Other variables, 83 such as carapace length and body condition (subjective), were obtained but were not 84 85 considered as part of the analysis. At the time of the evaluation, no changes to the eyes or adjacent structures were reported or observed directly using an ophthalmoscope. The 86 tortoises were evaluated in June 2023. According to the medical history, all tortoises were 87 healthy during the examination. Tortoises were placed on top of a circular container above 88 the surface of the examination table to prevent contact of their limbs with the table surface 89 and therefore movement. This restraint method allowed the tortoises to relax and stick their 90 heads out, out of curiosity. 91 IOP was measured using a rebound tonometer (i Care® Tonovet Plus, Icare Finland 92

OP was measured using a rebound tonometer (1 Care Finland Oy, Vantaa, Finland) placing it perpendicular to the cornea of the tortoise, approximately 3-5 mm from the cornea. Six IOP readings (mm/Hg) were taken from each eye (right and left) using the "dog" configuration, as this has been used previously in other *Gopherus* species (Rowatt *et al.*, 2020). IOP data were analyzed using a linear model, including the effects of 97 eye (right and left), sex (male and female), eye x sex interaction, and residual error. 98 Assumptions of normality and common variance for residual error were evaluated using the 99 Shapiro-Wilk and Levene's tests, respectively. The means were separated using the 100 honestly significant difference test (Tukey's procedure). Effects were considered significant 101 when the value of $P \le 0.05$. The analysis was performed using the SAS/STAT[®] GLM 102 procedure (SAS/STAT[®], 2023).

103

104 **Results**

Manifestations of pain or discomfort were not observed, but there was a response of 105 surprise when seeing or feeling the blunt-tipped needle of the tonometer in the cornea; the 106 107 tortoises closed their eyes or hid their heads momentarily but allowed us to continue obtaining the measurements. IOPs were obtained without excessive delay and with 108 109 consistency. It is important to note that local anesthetics are not required for this type of tonometry. No tortoises had to struggle or be forced during IOP collection. No eye 110 problems were reported after IOP collection in any tortoise sampled. The i-Care Tonovet 111 112 Plus offers a measurement range of 10 - 60 mmHg, with a precision of ± 2.5 mmHg (10-30 mmHg) and $\pm 10\%$ (> 30-60 mmHg), and with a repeatability (coefficient of variation) of < 113 8%. The effects included in the linear model on IOP values are shown in Table 1. 114 No significant differences (P > 0.05) were observed for interaction and main effects 115 of eye (right and left) and sex (male and female). Table 2 reports the IOP results obtained in 116 this study and those of other chelonian species, including the type of instrument used for 117 greater comparative ease for the clinician. 118

119

120 Discussion

This work establishes the average IOP values in Sonoran Desert tortoises obtained in healthy animals with the i Care® Tonovet Plus. IOP differences are recognized between reptile species. Slight differences have been found depending on the between species, restraint method (physical or chemical), and instrument used. Differences in IOPs as measured by applanation have been documented between red- and yellow-footed tortoises, both in the genus *Chelonoidis* (Selmi *et al.*, 2002; Selmi *et al.*, 2003).

It would seem likely that the IOP would be higher in aquatic-diving species to 127 128 counteract the effects of water pressure; however, in aquatic species such as the European pond turtle and the red-eared slider, the IOP is lower than that found in our study. The same 129 is true for the marine species such Kemp's ridley sea turtles and loggerhead sea turtles for 130 which lowest values documented in chelonians have been obtained (Delgado et al., 2014; 131 Rajaei et al., 2015; Petritz et al., 2019; Fumero-Hernandez et al., 2023). IOP measured in 132 133 box turtles (*Terrapene* spp.) has also been found to be lower than in other terrestrial chelonians and slightly higher than or equal to aquatic chelonians (Espinheira et al., 2015). 134 135 The IOP measured in the Sonoran Desert tortoises was slightly higher than those of 136 other terrestrial species, such as the San Cristóbal giant tortoise and the Texas tortoise, where rebound tonometry was also used (Rowatt et al., 2020; Masterson et al., 2022). 137 The body size of chelonian species seems unrelated to IOP, based on the IOP 138 reported in species of different sizes. This has also been observed in other vertebrate 139 species with no significant correlation between body size and IOP (Zouache et al., 2016). 140 The most crucial difference observed is between terrestrial and aquatic animal species. 141 Anatomical differences between terrestrial and aquatic reptile species could explain such 142 differences, e.g., the presence or total or partial absence of the *conus papilaris* (homologous 143 to the pecten oculi of birds) could be a key structure for these differences. This structure is 144

145	found in the retina and participates in retinal nutritional processes and regulation of
146	intraocular fluid (Ringvold, 2022). However, further study of ocular anatomy of various
147	species is needed to understand factors influencing physiological IOP.
148	Rebound tonometry is a reliable method for obtaining IOP measurements in
149	Sonoran Desert tortoises. Extrapolating IOP results between species or methods is not
150	recommended, and the clinician should consider such differences when interpreting results
151	from species where IOP has not been reported yet.
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237

239Table 1. Mean intraocular pressure (IOP) values in Sonoran Desert tortoises (Gopherus

240 *morafkai*). SD values are shown in parentheses. OD = right eye, OS = left eye, M = male, F 241 = female.

	Μ	Male		Female		Sex		Eye	
	OD	OS	OD	OS	M	F	OD	OS	
IOP	15.42 (2.63)	16.28 (2.75)	16 (2.76)	17.07 (2.95)	15.85 (2.62)	16.53 (2.85)	15.8 (2.66)	16.8 (2.75)	

Table 2. Average intraocular pressure (IOP) reported in different species of chelonians, including the type of restraint, measurement method, and equipment used.

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Species	n	IOP (mm Hg)	Restraint	Type of Tonometry	Equipment	Reference
Marine						
Lepidochelys kempii	25/20	6.5±1.0(h setting) 3.8±1.1(no definida)	None	rebound	Tonovet	Gornik, et al. 2016
Caretta caretta	11	5 (range 4 to 9)	Manual	rebound	Tonovet	Petritz, et al. 2019
Terrapine				rebound		
Trachemys scripta elegans	17	11.32±1.57	None	rebound	Tonovet	Delgado, et al. 2014
Trachemys scripta elegans	17	10.2±0.66	None	rebound	Tonolab	Delgado, et al. 2014
Trachemys scripta elegans	17	6.31±5.59	Manual At neck	rebound	Tonolab	Delgado, et al. 2014
Trachemys scripta elegans	17	6.75±6.01	Manual at head (rostral)	rebound	Tonolab	Delgado, et al. 2014
Trachemys scripta elegans	11	-1.79±1.17	Chemical	rebound	Tonolab	Delgado, et al. 2014
Trachemys scripta elegans	13	-1±0.76	Chemical	rebound	Tonolab	Delgado, et al. 2014
Emys orbicularis	22	5.42±0.96	None	rebound	Tonovet	Rajaei, et al. 2015
Semi-aquatic/terrestrial				rebound		
Terrapene spp.	103	-	Manual	rebound	Tonovet	Espinehira, et al, 2015
Terrapene carolina major	69	6.7±1.4	Manual	rebound	Tonovet	Espinehira, et al, 2015
Terrapene carolina triunguis	24	8.3±1.5	Manual	rebound	Tonovet	Espinehira, et al, 2015
Terrestrial						
Geochelone carbonaria	25	15.3±8.81	Manual	applanation	Tonopen	Selmi, et al. 2002
Geochelone denticulata	15	14.2±1.2	Manual	applanation	Tonopen	Selmi, et al. 2003
Testudo hermanni	26	15.74±0.20	Manual	rebound	Tonovet	Selleri, et al. 2012
Chelonoidis chathamensis	39	13.38±3.81	Manual	rebound	Tonovet	Masterson, et al. 2022
Gopherus berlandieri	61	13.8±2.4	Manual At head (rostral)	rebound	Tonovet	Rowatt, et al. 2020
Gopherus morafkai	20	16.3±2.7	None	rebound	Tonovet	This study