# Intraocular pressure using rebound tonometry in the Galápagos marine iguana (*Amblyrhynchus cristatus*)

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## Abstract
The Galápagos marine iguana (*Amblyrhynchus cristatus*) is the world's only marine lizard and is capable of swimming and diving to depths of several meters to forage for food. Thus, the physiology of this species must accommodate both terrestrial and underwater environments. Until now, no studies have evaluated ocular health parameters in this species. Free-ranging Galápagos marine iguanas (n=26) were captured in the field. Intraocular pressure (IOP) was measured using rebound tonometry at the beginning and end of the handling period. Tear production was measured using Schirmer tear test (STT) and endodontic absorbent paper point test (EPPT). Baseline physiologic parameters including heart rate and body temperature, as well as morphometric parameters (body weight, total length, snout vent length, hemipene sulcus length), and baseline blood parameters (packed cell volume, total protein, lactate) were evaluated. The mean IOP was 9.4 mm Hg (SD±1.4) and is comparable to other terrestrial iguanid species. Mean STT was 4.1 mm/minute and EPPT was 11.1 mm/minute. The IOP did not vary with snout vent length, total weight, hemipene sulcus length, or between the right and left eye. The IOP was higher at the beginning of handling compared to the end of handling, likely due to immediate stress associated with capture. The IOP did not vary with packed cell volume (PCV), total protein (TP), or lactate. These results represent a first step toward establishing a baseline for ocular health parameters in marine iguanas.

**Keywords:** Intraocular pressure, Galápagos marine iguana, *Amblyrhynchus cristatus,* ophthalmology, rebound tonometry, tear production

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## Introduction

The Galápagos marine iguana (*Amblyrhynchus cristatus*) is an endemic reptile species in the Galápagos archipelago and is considered the world's only marine lizard. Galápagos marine iguanas forage primarily on marine algae off rocky shores, diving to depths of several meters and holding their breath for an extended period of time (Wikelski and Trillmich, 1994; Wikelski and Wrege, 2002). The Galápagos marine iguana is currently listed as vulnerable and the San Cristóbal subspecies (*Amblyrhynchus cristatus martensi)* is listed as endangered under the IUCN Red List (MacLeod *et al*., 2020). Major threats include El Niño events (Steinfartz *et al*., 2007) and anthropogenic factors such as introduced predators and ocean pollutants (French *et al*., 2001; Wikelski *et al*., 2001; Wikelski *et al*., 2002). The bulk of the literature on marine iguanas has focused on natural history and behavioral ecology, but few studies have focused on the health of wild populations.

Measurement of intraocular pressure (IOP) is an important baseline parameter for the determination of ocular health. Intraocular pressure represents a balance of fluid production and outflow within the eye (Cervino, 2006; Leiva *et al*., 2006; Telle *et al*., 2019). Studies have been performed in a variety of reptile species including European pond turtles (*Emys orbicularis*), yacare caiman (*Caiman yacare)*, red-eared sliders (*Trachemys scripta*), loggerhead sea turtles (*Caretta caretta*), eastern box turtles (*Terrapene carolina*), bearded dragons (*Pogona vitticeps*) and the San Cristobal giant tortoise (*Chelonoidis chathamensis*) (Chittick and Harms, 2001; Delgado *et al*., 2014; Rajaei *et al*., 2014, 2015; Ruiz *et al*., 2015; Schuster *et al*., 2015; Espinheira Gomes *et al*., 2016; Masterson *et al*., 2022). These studies have improved our understanding of reptile ocular physiology, as well as the importance of species-specific reference intervals for intraocular pressure, since reptilian eye anatomy is quite variable and linked to environmental and behavioral differences (Rival *et al*., 2015).

Field studies on wild populations of reptiles are limited. The purpose of this study was to evaluate ocular parameters in healthy adult free-ranging Galápagos marine iguanas including IOP and tear production. Additional goals were to evaluate the effect of handling time on IOP in this species, and to compare IOP measurements with morphometric and hematologic parameters, including packed cell volume, total protein, and lactate.

## Materials and methods

The study was conducted during a two-day sampling period on San Cristóbal Island in the Galápagos archipelago. The study site was on La Loberia beach (0° 55′ 40″ S, 89° 36′ 43″ W). Sampling was performed in July 2018 as part of a marine iguana population health assessment authorized by the Galápagos National Park Service (permit No. PC-

59-18 to G.A. Lewbart). All handling and sampling procedures were consistent with standard vertebrate protocols and veterinary practices and approved by the Universidad San Francisco de Quito.

Animals (n=26) were hand-captured within 100 m of the shoreline. The iguanas were restrained with leather restraint gloves and held in ventral recumbency without sedation during the ophthalmic examinations. Care was taken not to place pressure around the neck during restraint. Blood collection, sexing, and body measurements were taken with the animals in dorsal recumbency. After handling the iguanas were released back onto the beach at their initial capture site.

Immediately after capture, IOP measurements were taken of the right and left eye using a rebound tonometer (TonoVet®, iCare, Tiolat, Helsinki, Finland). A complete physical examination, including ocular examination by use of handheld ophthalmoscope, was performed by a single evaluator (JC). Total body weight, snout to vent length (SVL), and total length (TL) were measured. Sex was determined through external characteristics, as well as with the use of a hemipene sulcus probe using methods previously described by Dellinger *et al*. (1990). The IOP measurements were repeated at the conclusion of the examination by the same investigator (JC).

Intraocular pressures were measured using the Tonovet® rebound tonometer with the setting on undefined patient (p). Disposable probes were used and changed between every five individuals. The tonometer was held in position perpendicular to the iguana and approximately five mm from the corneal surface (Fig. 1).

**Figure 1: Obtaining Tonovet® intraocular pressures using manual restraint of a marine iguana (*Amblyrhynchus cristatus*) in ventral recumbency.**

Three measurements were taken in both the right and left eye. Each measurement consisted of six applications of the probe onto the cornea in accordance with the operating protocol of the Tonovet®. If the machine displayed an error message, additional measurements were taken until the machine displayed results in accordance with the machine's acceptable standard deviation. The sequence of eyes tested (left or right first) varied among individuals depending upon how the animal was restrained against the handler's body.

Tear production was measured in a subset of iguanas using an endodontic absorbent paper point test (EPPT) and the Schirmer tear test (STT; n=5). The periocular skin was determined to be dry in all animals prior to taking measurements. Size 30 endodontic paper points (Parallax® Veterinary Absorbent Paper Points, Shipps Dental, Marana, Arizona, 85658 USA) were used. The tapered end of the clean points was placed into the fornix in both the right and left eye simultaneously (Fig. 2). The point was removed after 60 seconds and the length of moisture that was wicked up the side of the point was measured in millimeters. Schirmer tear tests were performed with standard TearFlo® sterile tear measurement strips (HUB pharmaceuticals LLC, Plymouth, Michigan, 48170 USA) using the same procedure.

**Figure 2: Application of the endodontic absorbent paper points (EPP) in the fornix of an adult marine iguana (*Amblyrhynchus cristatus*).**

Heart rate was measured on each iguana using an ultrasonic doppler flow detector (Model 811B, Parks Medical Electronics Inc, Aloha, Oregon, 97078 USA) with the probe placed in the left axillary region. Heart beats were counted by audible doppler sounds and counted for 30 seconds and doubled to get the heart rate per minute. Body temperature was taken using an EBRO® Compact Thermocouple thermometer (model EW-91219-40, Cole-Parmer, Vernon Hills, Illinois 60061 USA) placed 2-3 cm into the cloaca.

Venipuncture was performed in 15 iguanas as part of an ongoing health monitoring project. Samples were evaluated for packed cell volume (PCV), total protein (TP) and lactate. Blood was collected in a heparinized 3.0 mL syringe with a 22-gauge needle from the ventral coccygeal vein within 5 minutes of capture. A minimum sample of 1.0 ml was evaluated. A portable microhematocrit centrifuge revealed the PCV and total protein was evaluated with a refractometer. Lactate was measured with a Lactate Plus™ analyzer (Nova Biomedical, Waltham, Massachusetts 02454 USA).

Descriptive statistics (mean±SD; median, first and third quartiles) were calculated and reported for IOP, STT with EPPT (Lange *et al*., 2014), body weight, SVL, TL, heart rate, body temperature, probe length, lactate, hematocrit, and total protein. Comparisons between IOP values in left and right eyes, both at the start of the physical exam and at the end, were made using the non-parametric Wilcoxon signed-rank tests. Correlations between IOP and body weight, body length and hematologic parameters (PCV, TP and lactate), were investigated using the Pearson correlation coefficient ($r$). Values of $p \leq 0.05$ were considered statistically significant.

## Results

Ocular examinations were considered unremarkable in all animals. All iguanas were determined to be adults based on a total snout vent length greater than 224 mm, which is the cut off for external differentiation of sex by Dellinger and Van Hegel (1990). Based on external sexual characteristics and cloacal probe depth, seven females, 16 males, and three individuals of undetermined sex were sampled. The mean handling time was 18±0.03 minutes. The mean time between the IOP measurements at the beginning and end of handling was 12±0.02 minutes. The mean intraocular pressure for both the right and left eye was 9.4±1.4 mm Hg. Descriptive statistics for ocular tests, morphometric characteristics, and blood parameters are summarized in Table 1.

The mean tear production measured by STT for all eyes was 4.1±1.1 mm/min. The mean STT for the right eye was 4.0±1.58 mm/ min, while the mean for the left eye was 4.2±0.44 mm/min. Tear production measured by endodontic absorbent paper point test (EPPT) had a mean of 11.2±2.2 mm. The mean EPPT for the right eye was

11.1±2.6 mm and the mean for the left eye was 11.2±1.4 mm. No adverse effects were noted from the application of either method of tear production.

**Table 1: Descriptive statistics of intraocular pressure and other parameters in 26 marine iguanas (*Amblyrhynchus cristatus*) on San Cristóbal, Galápagos: July, 2018.**

| Parameter | Mean ± SD | Median (1st Quartile, 3rd Quartile) |
|---|---|---|
| IOP mm Hg left (n = 26) | 8.9 ± 1.4 | 9.0 (8.0, 9.0) |
| IOP mm Hg right (n = 26) | 8.9 ± 1.4 | 9.0 (8.0, 10.0) |
| IOP mm Hg start n = 23) | 9.4 ± 1.4 | 10.0 (8.0, 10.0) |
| IOP mm Hg end (n = 22) | 8.4 ± 1.3 | 8.5 (7.0, 9.0) |
| EPPT mm (n = 10) | 11.2 ± 2.2 | 11.5 (10.25, 12.0) |
| STT mm (n = 5) | 4.1 ± 1.0 | 4.0 (4.0, 4.75) |
| | | |
| Body weight kg (n = 26) | 4.7 ± 1.9 | 5.1 (2.9, 6.4) |
| Total length cm (n = 26) | 99.7 ± 14.0 | 105.7 (87.0, 110.6) |
| Snout-to-vent length cm (n = 26) | 39.2 ± 5.8 | 40.0 (34.8, 43.7) |
| Heart rate bpm (n = 25) | 17.1 ± 3.3 | 16.0 (15.0, 19.5) |
| Body temperature C (n = 23) | 30.6 ± 4.3 | 31.5 (26.4, 34.4) |
| Cloacal Probe Depth (n = 26) | 4.8 ± 1.4 | 5.0 (3.8, 5.9) |
| Lactate mmol/L (n = 23) | 5.1 ± 3.2 | 3.9 (2.7, 7.2) |
| Hematocrit % (n = 15) | 32.6 ± 4.8 | 33.0 (28.0, 36.0) |
| Total Protein g/dL (n = 15) | 6.4 ± 0.4 | 6.4 (6.0, 6.7) |

Intraocular pressure was significantly (*p*≤0.05) lower on the left eye (median=8.0 mm Hg) and right eye (median=9.0 mm Hg) at the end of handling, compared to values at the start of handling (median for left eye=10.0 mm Hg; median for right eye=10.0 mm Hg) (Table 2). In addition, IOP measurements were not correlated with body weight (r=0.26; p=0.18), total length (r=0.20; p=0.30) or SLV (0.24; p=0.20). Finally, IOP measurements were not correlated with examined blood parameters, which included PCV (r=0.10; p=0.69), TP (r=0.06; p=0.82), and lactate (r=0.31; p=0.12).

**Table 2: Paired comparisons of intraocular pressure between left and right eyes at start and end of a physical examination of 26 marine iguanas (*Amblyrhynchus cristatus*)***

| | Left eye | Right eye | p |
|---|---|---|---|
| At start (n = 26) | 10.0 (9.0, 10.0) | 10.0 (8.7, 10.0) | 0.59 |
| At end (n = 21) | 8.0 (7.0, 9.0) | 9.0 (8.0, 9.0) | 0.45 |
| | **At start** | **At end** | **p** |
| Left eye (n = 23) | 10.0 (9.0, 10.0) | 8.0 (7.0, 9.0) | < 0.01 |
| Right eye (n = 22) | 10.0 (8.7, 10.0) | 9.0 (8.0, 9.0) | 0.05 |

*Data are reported as median (1st, 3rd quartiles).

## Discussion

This study provides the first measurements of intraocular pressure in Galápagos marine iguanas. Rebound tonometry allowed for quick and easy measurement of intraocular pressure in the field as the animals did not require sedation or application of topical anesthetic agents. The animals showed no signs of discomfort during or after the measurements. The Tonovet® worked well for measuring intraocular pressure within the small working area that is accessible in the marine iguana ocular surface.

Relative to other iguanid species, the mean IOP of marine iguanas (9.4±1.4 mm Hg) was lower than that of green iguanas (*Iguana iguana*; 18.0±1.7 mm Hg) (Araujo *et al*., 2017) but higher than that of the Andros Island iguana (*Cyclura cuchlura cuchlura*; 4.8±1.88 mm Hg) (Wojik *et al*., 2013). This may be due to variations in settings on the tonometer itself or represent physiologic differences between species. Limited information exists about intraocular pressures in other iguanids. In comparison to other aquatic reptiles, the mean IOP of rehabilitated juvenile Kemp's ridley sea turtles (*Lepidochelys kempii*) was 3.8±1.1 mm Hg (Gornik *et al*., 2016); for red-eared sliders (*Trachemys scripta elegans)* the mean was 5.4±1.7 mm Hg (Delgado *et al*., 2014). These values are lower than the IOP of animals evaluated in this study. The variation observed among reptilian species highlights the importance of species-specific studies when using rebound tonometry for evaluating ocular health.

In general, intraocular pressure is higher for aquatic animals, which is likely due to increased external pressures below the water surface (Colitz *et al*., 2012). This consideration makes the marine iguana a particularly interesting subject for study, since these lizards spend considerable time both on land and under water. This study did not show intraocular pressures as high as those seen in deeper diving species such as cetaceans (Colitz *et al*., 2012) and penguins (Sheldon *et al*., 2017). A possible explanation is that marine iguanas remain in shallow water throughout their lives and thus do not need to accommodate the high-water pressures encountered by deep-diving animals that forage in the open sea. Another possible contributor to intraocular pressure that has not been extensively evaluated in marine animals is the relationship between corneal thickness and globe size. Additional research on the relationship between diving and ocular pressure are needed to further explore this finding.

The measurements in this study were taken in the morning. A study of intraocular pressure in bearded dragons (*Pogona vitticeps*) revealed that the lizards had higher intraocular pressure in the morning compared to the evening, suggesting possible variation with diurnal rhythms (Schuster *et al*., 2015). Whether daily variation occurs in marine iguanas is unknown. Given the constraints of fieldwork and the opportunistic sampling of iguanas during the study period, controlling for time of day was not possible. Further studies are needed to evaluate the effect of diurnal rhythms on ocular pressure in this species.

The IOP at the time of capture was significantly higher than the IOP at the end of the handling period (approximately 18 minutes later). This is suspected to be due to the initial period of stress at the time of capture, thereby raising systemic blood pressure secondary to catecholamine release and subsequently increasing intraocular pressure. Studies in humans have shown an increase in intraocular pressure with exercise, likely due to an increase in systemic blood pressure (Karabatakis *et al*., 2004), and this phenomenon may be occurring in the iguanas sampled. Future work could focus on obtaining blood pressures in the field. The difference in mean IOP at the beginning and end of the handling period, however, would not be considered clinically significant.

Measurement of tear production was considered subjectively challenging in this species. Due to the muscular anatomy of the eyelids of the marine iguana, manipulating the Schirmer tear test (STT) strips to contact the inferior palpebrae was difficult compared to other reptile species. By contrast, the endodontic paper points were more easily applied into the fornix near the medial canthus of the eye. Prior studies in green iguanas (*Iguana iguana*) were found to have mean tear production with STT and EPPT of 1.0±0.5 mm/min and 8.5±2.4 mm/min, respectively, based on a study performed with 20 individuals (Araujo *et al*., 2017). This value is lower than the tear production in the green iguana study was lower than that of our study, which revealed a mean STT of 4.1 mm/minute and mean EPPT of 11.1 mm/minute. This may be due to differences in methodology, as the study in green iguanas utilized a different brand of endodontic paper points, although the size of strips was the same. Another interesting possibility is that differences in tear production might reflect difference in physiology between the two species. The marine iguana has unique physiologic adaptations to handle the elevated salt load in their marine environment (Wikelski and Trillmich, 1994). Previous plasma chemistry studies on marine iguanas have shown elevated sodium and chloride relative to other reptiles, indicating a systemic effect (Lewbart *et al*., 2015). Local excretion of salt occurs at the paired nasal salt glands. While these glands were considered too deep for sampling by the tear measuring methodologies, it is possible that the increased salt, and thus homeostatic water absorption, led to higher overall tear production compared to terrestrial iguanid species. Caution is required in interpreting these results, however, and further studies are needed to explore tear production in this species.

Evaluation of hematologic parameters was not found to have any correlation with IOP in Galápagos marine iguanas. The mean lactate identified in this study was 5.1±3.2 mmol/L and is considered relatively high in comparison to canine and feline reference intervals. Elevated lactate can indicate anaerobic cellular metabolism, and can increase from exercise or physiologic stress, and may predispose to acid-base disturbances. Lactate values in this study are similar in comparison to a previous study evaluating the health of free-ranging Galápagos marine iguanas which evaluated blood gas, biochemistry, and hematology parameters in this species (Gleeson, 1980; Lewbart *et al*., 2015).

This study is the first to measure IOP and other ocular health parameters in Galápagos marine iguanas and helps expand our knowledge for the use of conservation and management practices. The findings provide a foundation for future research of ocular health for this species.

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