Received: 8 February 2019 | Revised: 26 April 2019 | Accepted: 9 May 2019

DOI: 10.1111/vop.12681

ORIGINAL ARTICLE

WILEY

Effective mydriasis in juvenile loggerhead turtles (*Caretta caretta*) following topical administration of rocuronium bromide and 10% phenylephrine

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Abstract

Objective: To determine the combined mydriatic effects of topical rocuronium bromide and phenylephrine in juvenile loggerhead turtles and identify any adverse effects associated with treatment.

Animals studied: Eleven juvenile loggerhead turtles (*Caretta caretta*).

Procedures: Four 20 μL drops of rocuronium bromide and four 20 μL drops of 10% phenylephrine were placed into the right eye at 2-minute intervals of 5 turtles, while the same volume of saline was administered to six control turtles. A pupilometer recorded pupil measurements at rest and following a light stimulus at 2, 15, 30, 60, 120, 150, 180, 210, 240, 300, and 360 minutes following delivery of the final drop to the ocular surface. Intraocular pressure (IOP) was also measured at similar time points.

Results: The nonilluminated and light-stimulated pupillary diameter of the right eye of treated turtles was significantly greater than baseline starting at 120 and 15 minutes, respectively. Light-stimulated pupillary diameter of treated eyes was greater than that of control eyes from time 15 minutes until the end of the treatment period. No systemic side effects were noted over a 24 hours period following treatment and all turtles showed normal behavior and appetite. No mydriasis was noted in either eye at 24 hours and the anterior segment was normal.

Conclusions: A combination of topical ophthalmic rocuronium bromide and 10% phenylephrine is safe and effective for mydriasis in juvenile loggerhead turtles.

KEYWORDS

chelonian ophthalmic examination, loggerhead turtle, pharmacologic mydriasis, phenylephrine, rocuronium, sea turtle

1 | INTRODUCTION

Loggerhead sea turtles (*Caretta caretta*) are one of the largest of the *Cheloniidae* family, the hard-shelled sea turtles, and one of the most abundant sea turtle species in rehabilitation centers. The geographic range they occupy is worldwide and includes the Mediterranean Sea as well as the Atlantic, Pacific, and Indian Oceans.[1] Similar to all sea turtle species, loggerhead turtle populations have declined significantly in the last century due to human activities including bycatch mortality from the fishing industry, hatchling mortality from light pollution, and habitat loss. Loggerhead turtles are currently considered vulnerable to extinction by the International Union for the Conservation of Nature (IUCN) and are protected under the Convention on International Trade in Endangered Species (CITES).[1]

Sea turtle ocular anatomy is similar to other chelonians, with several adaptations for aquatic life. These include a flattened cornea, a spherical lens, and a thickened, partially ossified sclera.[2] As in other species, mydriasis in sea turtles is integral to being able to perform a complete fundic examination and cataract surgery.[2,3] Originally, it was believed that all reptile irises were composed of striated muscle exclusively. Therefore, neither parasympatholytic nor adrenergic agents would be effective.[4] However, recent studies have shown that neuromuscular blockade alone is insufficient to cause mydriasis in red-eared slider turtles (*Trachemys scripta elegans*)[5] and adrenergic agents are required, in conjunction with neuromuscular blockade to achieve mydriasis in this species.[6-8] Rocuronium bromide is a nondepolarizing neuromuscular blocking agent, which has a more rapid onset of action compared to vecuronium and has been successfully used topically for mydriasis in several avian species, whose irises also contain striated muscle.[9-13] To our knowledge, this drug has never been used for mydriasis in any chelonian species. The objective of this study was to determine the combined mydriatic effects of topical rocuronium bromide and phenylephrine in juvenile loggerhead turtles and identify any adverse effects associated with this therapy.

2 | MATERIALS AND METHODS

2.1 | Animals

Twelve 10-month-old loggerhead sea turtles of unknown sex, having an average weight of 620 g (range, 525-751 g) were initially included in this study. These turtles were obtained from the wild immediately upon hatching for a noninvasive study of electromagnetic navigation. They were housed in a recirculating system of 3800 L, with each turtle occupying its own 128 L tank. Water quality parameters were as follows: temperature 24-26°C, salinity 25-28 ppt, ammonia <0.5 ppm, nitrite < 0.5 ppm, nitrate < 200 ppm, pH 7.2-8.5, and coliforms <1000 MPN/100 mL. The turtles were fed a frozen omnivorous or carnivorous marine fish diet for the first month and switched to a Mazuri® Omnivore Aquatic Gel (Mazuri Exotic Animal Nutrition) supplemented with vitamin C and ReptoCal® (Spectrum Brands Pet, LLC).

The systemic health of these animals was determined based on a complete physical examination, packed cell volume, total plasma proteins, and a biochemical profile. In addition, each turtle received an anterior segment ophthalmic examination including tonometry (SL 17, Kowa Medical and Tonovet, iCare) by a board certified veterinary ophthalmologist (HDW). Indirect ophthalmoscopy was not possible through the undilated pupil. Based on these results, one of the twelve turtles was excluded from the study because it did not have a normal pupillary light reflex in either eye. The remaining eleven animals were deemed healthy with no evidence of ophthalmic disease. This study was approved by the Institutional Animal Care and Use Committee (IACUC) at University of North Carolina at Chapel Hill.

2.2 | Drug treatment protocol

To determine an effective dose, drug protocol, and preliminary safety data, a pilot study (data not shown) was performed on a separate population of juvenile green sea turtles (*Chelonia mydas*) and Kemp's ridley sea turtles (*Lepidochelys kempii*) temporarily housed at a rehabilitation center. Topical application of phenylephrine or rocuronium alone did not result in appreciable pupillary dilation. This earlier work was approved by the Institutional Animal Care and Use Committee (IACUC) of the North Carolina Aquariums. In the current study, turtles assigned to either treatment (n = 5 animals) or control (n = 6 animals) groups by drawing numbered pieces of paper from a bag. Two turtles received rocuronium (Rocuronium bromide 10mg/mL, XGen, Big Flats, NY) first followed by phenylephrine(10% phenylephrine Hydrochloride Ophthalmic Solution, Akorn), while the other three received phenylephrine followed by phenylephrine. Each turtle was manually restrained with the apex of the right cornea positioned upwards, and four consecutive 20 μL drops of each drug (PR-100, Rainin Classic Pipette, Mettler Toledo), each 2 minutes apart, were instilled onto the corneal surface. The turtles in the control group were handled identically but received an equivalent volume of saline in the same dosing manner. Following drug administration and between pupillometry, turtles were individually housed in plastic holding boxes lined with wet towels and kept in a darkened room. The turtles were misted with water as needed throughout the study. All turtles were returned to their tanks after the final measurement on the first day. Respiratory rates, activity level, and overall mentation were monitored throughout the treatment period, specifically during each handling event

for pupil measurement. Approximately 24 hours after the initial drop administration, a physical examination and anterior segment ophthalmic examination were repeated on all turtles. In addition, their appetite, respiratory rate, and activity level were monitored during this time as part of their routine husbandry.

2.3 | Pupillometry and intraocular pressure measurements

For pupil measurement, the turtles were placed on the pupilometer (A2000, Neuroptics) positioning platform which required minimal manual restraint. The cameras were adjusted so both pupils were in focus simultaneously. The pupilometer was then set to record baseline pupillary diameter for 3 seconds before delivering a 3 sec light stimulus of 3.7×10^3 cd/m^2 to both eyes. Pupil diameter was recorded for 9 seconds after the light stimulus stopped. Background illumination at the level of the cornea during all testing was 3×10^{-3} cd/m^2.

Pupillometry was performed immediately prior to drug administration (time 0) and repeated at 2, 15, 30, 60, 120, 150, 180, 210, 240, 300, and 360 minutes following delivery of the final drop to the ocular surface. Intraocular pressure (IOP) was also measured (TonoVet, "d" setting, iCare) by the same operator (MCW) immediately after pupil measurements were obtained at all time points in both treatment and control groups until 300 minutes after final drop delivery.

2.4 | Data analysis

Resting pupillary diameter was tabulated as the pupillary diameter immediately prior to delivery of the light stimulus. Maximal constriction was recorded as the smallest pupillary diameter observed during the 3 seconds of light stimulation. To facilitate data analysis, results were segmented into six groups as outlined in Table 1. A random effect model was used to evaluate differences per group over time, where the subjects were a random effect, time point and group as within-subject random effect factors, respectively, and time point zero was the baseline. Post hoc treatment comparisons at individual times were tested using Tukey's adjustment to preserve a nominal 5% type I error rate. Adjusted *P*-values among groups and time point combinations were reported. An F test comparing the difference between the light-stimulated (groups 1,3, and 5) and non light-stimulated (groups 0, 2, and 4) eyes was also conducted, and *P*-values were reported without adjustment (R v. 3.5.2, The R Foundation, www.r-project.org). Differences in intraocular pressure over time between treatment groups were evaluated using a univariate analysis of variance (SPSS v. 25, IBM Corporation, www.ibm.com). For all tests, $P \leq 0.05$ was considered significant.

TABLE 1 Description of the groups into which the data were segmented for statistical analysis

Group ID	Description
Group 0	Nonilluminated pupil diameter of nontreated turtles (OD and OS)
Group 1	Light-stimulated pupil diameter of nontreated turtles (OD and OS)
Group 2	Nonilluminated pupil diameter of the untreated eye of treated turtles (OS)
Group 3	Light-stimulated pupil diameter of the untreated eye of treated turtles (OS)
Group 4	Nonilluminated pupil diameter of the treated eye of treated turtles (OD)
Group 5	Light-stimulated pupil dimeter of the treated eye of treated turtles (OD)

3 | RESULTS

The pupils of all turtles constricted in response to light prior to treatment at time zero, and there was a significant difference in the light-stimulated pupillary diameters (groups 1,3, and 5) compared with the nonilluminated pupil diameters (groups 0, 2, and 4) (adjusted $P < 0.0001$) at baseline (Figures 1 and 2). Nonilluminated pupil diameter of control turtles did significantly increase over the course of the study (adjusted *P*, 0.05 to <0.0001) compared to their baseline; however, light-stimulated pupil diameter did not change significantly at any time point in control turtles (adjusted $P = 1.0000$). Nonilluminated and light-stimulated pupil diameter of the right eye of the treated turtles (groups 4 and 5, respectively) significantly increased in size over time compared to baseline. This difference was significant (adjusted $P < 0.001$) starting at 120 minutes for nonilluminated eyes, and at 15 minutes for light-stimulated eyes (adjusted $P < 0.0001$), and this persisted to the end of the study period. The non light-stimulated and light-stimulated left eye of the treated turtles (groups 2 and 3, respectively) showed no significant difference in pupillary diameter from baseline at any time point (adjusted *P*, 0.2917 to 1.0000), which is expected as this eye did not receive any treatment.

There was a significant difference between the light-stimulated and nonilluminated pupillary diameter in control eyes at all time points (adjusted *P*, 0.0515 to <0.0001). There was no difference in nonilluminated pupil diameter between the right eye of treated turtles and the control

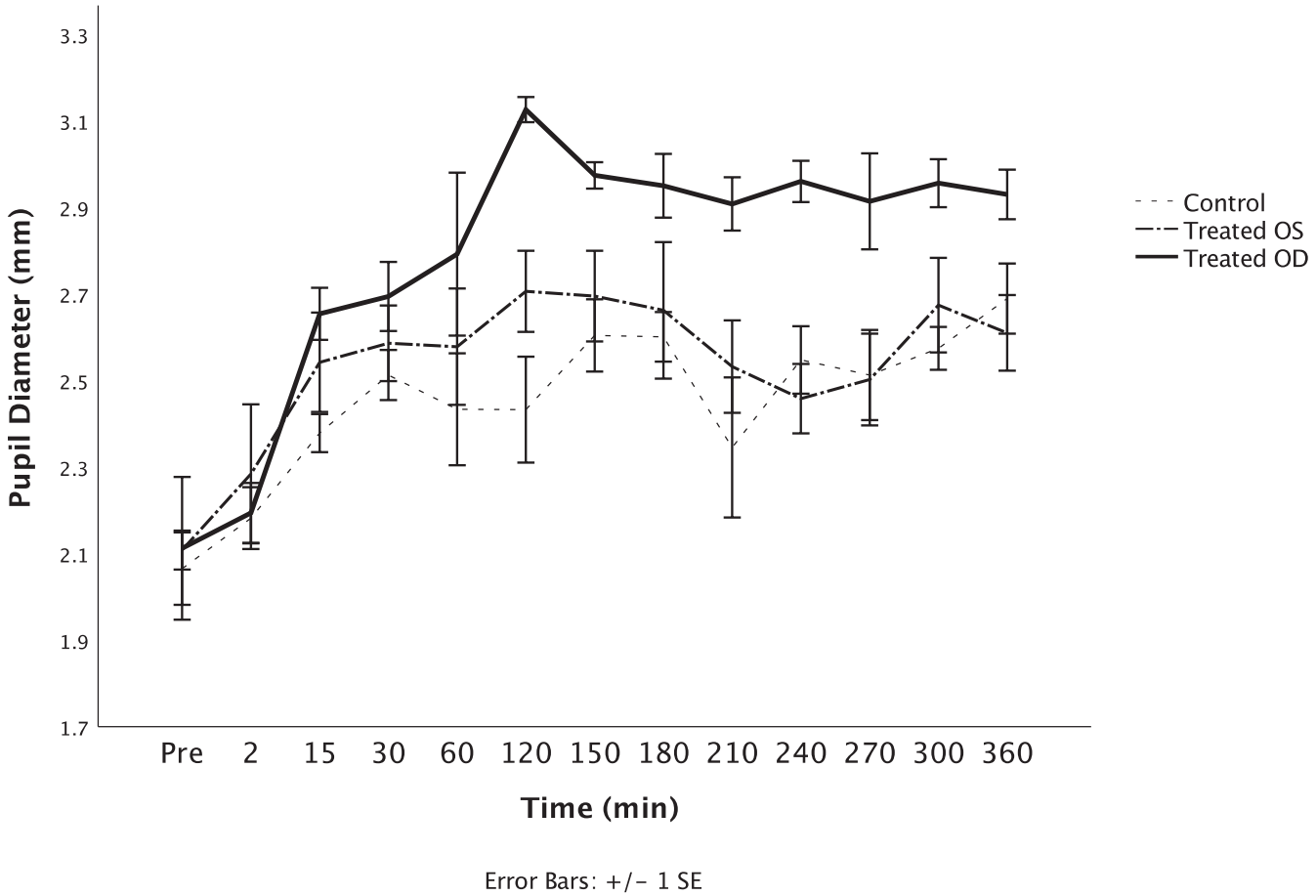

FIGURE 1 Mean ± SE pupil diameter (mm) in juvenile loggerhead sea turtles *(Caretta caretta)* after topical administration of four drops each of rocuronium bromide and 10% phenylephrine to the right eye (treatment OD), saline solution to the right eye (control), or no therapy (treatment OS) without light stimulation

turtle eyes (adjusted *P*, 0.1365-1.0000) nor was there any difference between nonilluminated pupillary diameter of the left eye of the treated turtles and the control turtle eyes (adjusted $P = 1.0000$). With light stimulation, pupillary diameter of the right eye of treated turtles was significantly larger than that of control turtle eyes starting at time 15 (adjusted $P = 0.0001$) and this difference persisted until the end of the treatment period (adjusted $P < 0.0001$). While there was not a significant difference in pupillary diameter between the light-stimulated left eye of treated turtles compared to light-stimulated control turtle eyes at any time point (adjusted *P*, 0.4051-1.0000), a trend toward a difference is visible in the graph (Figure 2). Supporting the presence of this trend is the fact that a difference in pupillary diameter between nonilluminated pupillary diameter and light-stimulated pupillary diameter was only detected at time point 2 (adjusted $P = 0.0008$) and at time 360 (adjusted $P = 0.0019$). This suggests maximal constriction may have been inhibited by treatment delivered to the opposite eye.

There was no difference in IOP over time between treated and control eyes ($P = 0.87$). No adverse systemic effects were noted, and all turtles maintained normal respiratory rates, mentation, and activity levels throughout the treatment period. All turtles were observed to eat their normal diet following treatment, and no apparent visual deficits were noted when they were placed back in their individual tanks. No abnormalities were noted on their anterior segment ophthalmic examination the following day.

4 | DISCUSSION

Mydriasis was successfully achieved following topical ophthalmic application of four drops of rocuronium bromide and four drops of 10% phenylephrine in loggerhead turtles. In the treated eyes of turtles, light-stimulated pupillary diameter increased in size from baseline and was significantly larger than the light-stimulated pupillary diameter of control turtles starting at 15 minutes, and this persisted to the end of the 360-minute study period. Peak pupil diameter (least square mean 3.13 mm) was achieved at 120 minutes in the treated eye. A difference in pupil size from baseline without light stimulation in the treated right pupil was noted starting

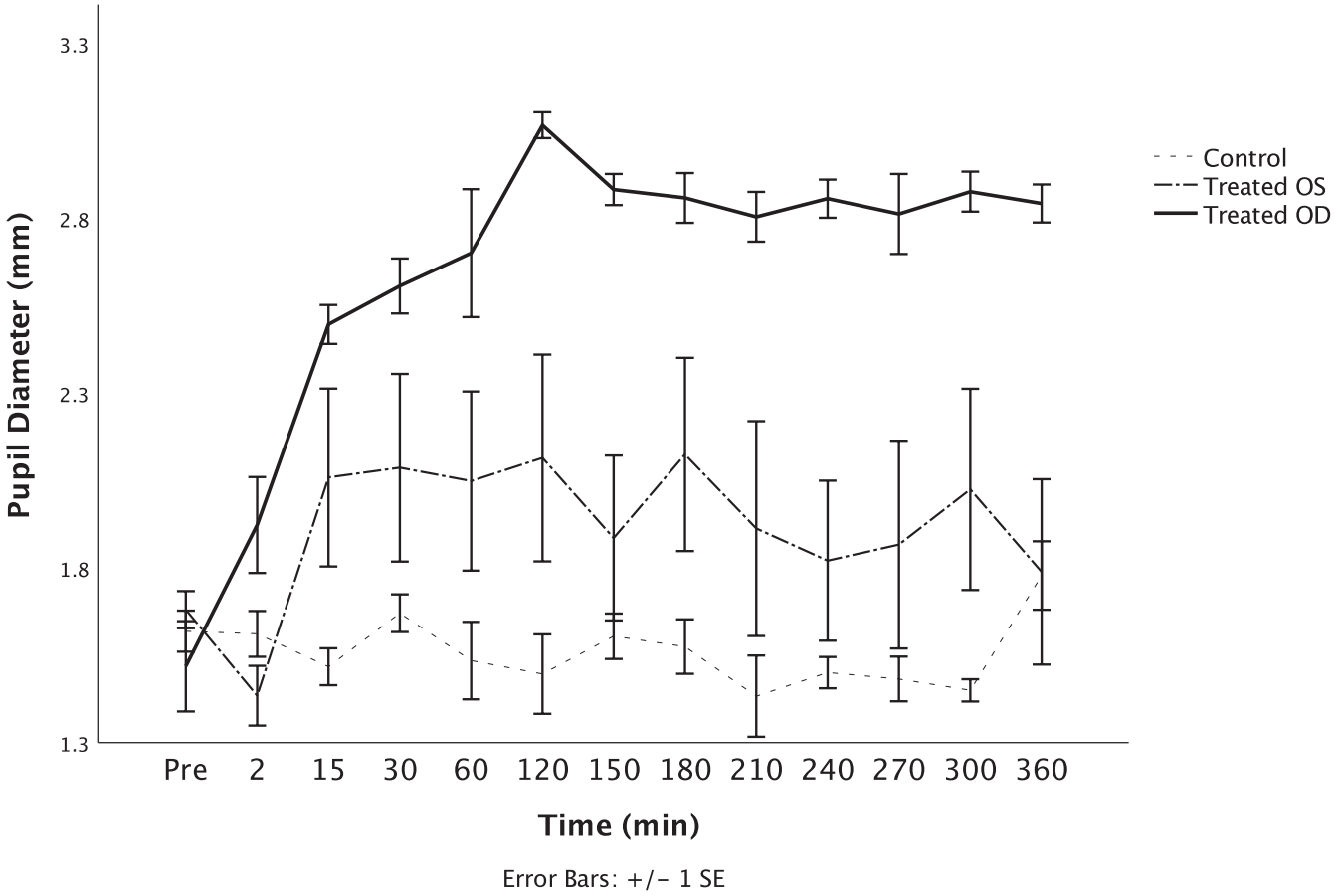

FIGURE 2 Mean ± SE pupil diameter (mm) in juvenile loggerhead sea turtles *(Caretta caretta)* after topical administration of four drops each of rocuronium bromide and 10% phenylephrine to the right eye (treatment OD), saline solution to the right eye (control), or no therapy (treatment OS) following a three-second light stimulation

at 120 minutes; however, there was no statistical difference in the size of the treated right pupil compared to the control pupil in the absence of a light stimulus. The size of the control pupil did significantly increase over time, which may represent normal changes caused by circadian rhythms or dark adaptation. All mydriasis had resolved when the turtles were clinically evaluated 24h after drug application, but no additional pupil measurements were obtained at that time due to logistical constraints. Therefore, it is difficult to accurately predict the total duration of mydriasis achieved from this protocol. Recovery following topical administration of vecuronium bromide and phenylephrine in red-eared sliders started approximately 110-120 minutes after drug application.[6]

There was a worrisome trend in pupillary diameter of the left (nontreated) eye of turtles in the treatment group. The increase in light-stimulated pupillary diameter in the nontreated eye suggests drug absorption in that eye. There are two possible routes for the drugs to have reached the untreated eye. The first is for the applied drops to spill over and out of the palpebral fissure and then trickle down the side of the turtles' head into the opposite eye as the turtle was being restrained with one cornea pointing up. This restraint method was deemed best for topical absorption in a similar study in parrots,[12] which is why it was used in this study. This should not be a concern if this drug protocol is used in adult loggerhead turtles as manual restraint in this manner is impractical with mature turtles given their large size. The second route involves systemic absorption of the drug and hematogenous delivery to the opposite eye, which may be possible as sea turtles have large, well-vascularized salt glands dorsomedial to each eye. We consider this unlikely given the lack of systemic effects, such as changes in respiratory rate and generalized muscle weakness, but acknowledge it cannot be ruled out.

The neural pathways responsible for pupil response have not been evaluated in any species of sea turtle. In red-eared slider turtles, however, the pathways resemble those in mammals and birds.[14] An early study[15] in another reptile, the American alligator (*Alligator mississippiensis*), suggested control of the iridial sphincter was similar to that in mammals, but a subsequent study showed the alligator iridial sphincter contains both smooth and striated muscle.[16] In red-eared sliders, successful mydriasis was only achieved following disruption of both parasympathetic and sympathetic innervations to the musculature of the iris via administration of both a

nicotinic cholinergic antagonist, vecuronium bromide, and an adrenergic agonist, phenylephrine.[6,7] These findings suggest that chelonians may possess a dilator pupillae homologous to mammals. To our knowledge, investigations have not been carried out on the iridial muscles and receptors of any sea turtle. The ocular morphology of the leatherback sea turtle (*Dermochelys coriacea*) has been examined, but the authors did not elucidate any of details of the iridial musculature.[17]

The requirement of two different mydriatic agents to achieve mydriasis in red-eared slider turtles is consistent with the pilot studies performed for this current project, in which varying concentrations of rocuronium bromide and 10% phenylephrine administered individually did not successfully produce any significant mydriasis. By contrast, successful mydriasis in several avian species was achieved with topical administration of a single neuromuscular blocking agent.[9-13] The total dose of rocuronium bromide (1.07-1.52 mg/kg; median, 1.42 mg/kg) and 10% phenylephrine (10.65-15.24 mg/kg; median, 14.23 mg/kg) used in the present study was based on pilot data. These doses are higher than those used in a previous study of three red-eared slider turtles[6] (body weights 0.6-1.2 kg; median not available), which used vecuronium bromide (0.53-1.06 mg/kg; median not available) and phenylephrine (3.33-6.667 mg/kg; median not available). A notable difference between these two species is the presence of the viscous secretions of the salt gland in sea turtles. It is possible this difference in tear film composition result in significantly less drug absorption.[17] Therefore, extrapolation of these doses to other chelonian species should be performed with caution.

The juvenile sea turtles used in the pilot study were significantly larger (2.1-3.5 kg; median 2.9 kg) than in the final study population. Despite this size difference, successful mydriasis was achieved in both groups at this same dose, and no adverse local or systemic effects were noted. The degree of mydriasis was substantial and would likely lead to much easier ocular fundus examination and easier visualization of the lens during intraocular surgery. No local or systemic adverse effects were noted in previous studies which utilized vecuronium bromide and phenylephrine topically in red-eared slider turtles.[6,8] The use of neuromuscular blocking agents should always be done with caution, particularly in awake patients without airway protection. To our knowledge, no adverse effects of these agents have been reported after topical use in any reptile species; however, adverse systemic effects, ranging from mild tachypnea to acute death, have been reported in several avian species after topical administration of pancuronium,[18] alcuronium,[19] and vecuronium.[18] In addition, bradycardia is a reported side effect of topical 10% phenylephrine in clinically normal domestic dogs.[20] Heart rate was not monitored in this study, as manual restraint for Doppler cardiac assessment or auscultation often leads to artifactual tachycardia in this and other wild reptilian species. However, the respiratory rate, activity level and general mentation were monitored in both the pilot and main study, and no abnormalities were noted in any turtle at any time point. Pharmacologic mydriasis has been reported to affect the intraocular pressure in humans,[21] horses,[22] dogs,[23] and cats[24] causing both increased and decreased IOP depending on the species and the mydriatic agents used. No significant changes in the IOP of the treatment turtles were noted at any time points in relationship to the saline-treated control turtles in the current study. In addition, the IOP measurements for both the treatment and control groups was within the reported range for juvenile loggerhead sea turtles held in a dorsoventral position.[25]

In previous mydriatic studies with red-eared slider turtles,[6,7] pupil diameter was measured manually from images obtained during the treatment period. In avian species with large ocular size, pupil diameter has been measured directly with a pupillary gauge.[9-11] Use of a pupilometer offers significant advantages over these methods including accuracy of measurement in patients with small ocular size and evaluation of pupillary response to a controlled, repeatable light stimulus. In veterinary medicine, pupillometry is frequently used in laboratory rodent models,[26,27] but to our knowledge this technique has not been reported previously in reptiles.

In future studies, ocular histology of loggerhead turtles can be used to further evaluate the iridal musculature. Additional studies should also be performed on adult loggerhead turtles prior to clinical use to ensure that dosages used on juvenile turtles in this study are effective in larger turtles. In addition, the effects of general anesthesia, as required for cataract surgery, on the efficacy of this protocol are unknown. For now, we conclude that the topical combination of rocuronium and 10% phenylephrine successfully induces reversible mydriasis in juvenile loggerhead turtles, without local or systemic adverse effects. These findings represent the first published report of successful mydriasis in any sea turtle species.

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How to cite this article: Petritz OA, Westermeyer HD, Whitehead MC, et al. Effective mydriasis in juvenile loggerhead turtles *(Caretta caretta)* following topical administration of rocuronium bromide and 10% phenylephrine. *Vet Ophthalmol*. 2019;00:1–7. https://doi.org/10.1111/vop.12681