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RESEARCH ARTICLE

Effects of Intranasal Butorphanol-Diazepam Combination on Ultrasonographic Ocular Biometry, Tear Production, and Intraocular Pressure in Yellow-Legged Gulls (*Larus michahellis*)

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Abstract

The aim of this study was to evaluate the effects of intranasal butorphanol-diazepam sedation on tear production, intraocular pressure (IOP), and ocular biometry in Yellow-legged Gulls (*Larus michahellis*). Eighteen gulls (nine juvenile, nine adult) undergoing rehabilitation were included. Measurements from the left (n=18) and right (n=18) eyes were analyzed separately for Schirmer Tear Test I (STT-I), IOP, and ultrasonographic parameters. Sedation was achieved with intranasal diazepam (8mg/kg) and butorphanol (1mg/kg). The body weights of the gulls were 670.55±35.84 g for juveniles and 782.22±61.61 g for adults. The sedation protocol used in this study produced statistically significant decreases (P<0.05) in juvenile animals in STT-I, anterior chamber depth (ACD), vitreous chamber depth (VCD), axial globe length (AGL), equatorial globe width (EGW) and pecten oculi length (POL). Statistically significant increases (P<0.05) were detected in lens axial height (LAH) and axial globe height (AGH). In adults, statistically significant decreases (P<0.05) were observed in central corneal thickness (CCT), ACD, and VCD while LAH, EGW and AGH showed statistically significant increases (P<0.05). Although decreases in IOP and increases in lens thickness (LT) were noted in both groups, no statistically significant differences were detected. These results indicate that intranasal butorphanol-diazepam provides reliable sedation for ophthalmic assessment in Yellow-legged Gulls without adverse effects; however, its influence on ocular parameters should be considered when interpreting measurements under sedation.

Keywords: Gulls, Intraocular pressure, Sedation, Schirmer test, Ultrasound

INTRODUCTION

The Yellow-legged Gull (*Larus michahellis*; family Laridae) is a common coastal seabird along the shores of Samsun, Türkiye. It is omnivorous, feeding on fish, crustaceans, worms, and the eggs and chicks of other birds [1]. Adults are characterised by long wings, a broad hooked bill with a prominent red spot extending onto the upper mandible, and a darker dorsum with a bluish hue. Although populations have increased markedly in recent decades and the species is considered invasive, information on its visual physiology and ocular anatomy remains limited [1].

Handling and restraint of wild birds often provoke pronounced stress responses. Sympathetic stimulation and catecholamine release may lead to tachycardia, tachypnoea, hyperthermia, and hypertension, with sudden death possible in compromised individuals [2,3]. Sedation reduces responsiveness to external stimuli, suppresses struggling and vocalisation, and minimises the risk of injury to handlers and stress-related complications in patients. This is particularly valuable in aggressive species such as gulls and raptors, facilitating examination, diagnostic imaging, sample collection, and minor procedures [2]. Intranasal administration offers a practical, non-invasive route for sedation, especially in field settings where intravenous access may be challenging; however, its effects on ocular parameters in this species have not been documented.

Species-specific ophthalmic reference values are essential for diagnosis and clinical decision-making. Tear production, which reflects corneal epithelial health, is commonly assessed using the Schirmer tear test I (STT-I), although modifications exist [4,5]. Measurement of intraocular pressure (IOP) is critical for identifying ocular hypertension and the risk of glaucoma, as sustained elevation can cause irreversible retinal and optic nerve damage [4,6]. Ocular ultrasonography is a routine, non-invasive modality for assessing ocular structures based on echogenic differences [7].

The objective of this study was to defines baseline values for tear production, IOP, and ocular ultrasonography in Yellow-legged Gulls, and to determine whether intranasal butorphanol-diazepam sedation influences these parameters. Given the significant interspecies variation in avian ocular anatomy, morphometric values obtained from one species cannot be reliably applied to another without risking diagnostic misinterpretation. Furthermore, recognising potential differences between sedated and non-sedated measurements is crucial for accurate clinical assessment. Accordingly, species-specific and sedation-dependent reference values provide a necessary foundation for distinguishing physiological variation from pathological change and thereby support precise clinical diagnosis and appropriate management in avian ophthalmology [4,8].

Material and Methods

Ethical Statement

All procedures were approved by the Ondokuz Mayıs University Animal Experiments and Local Ethics Committee (Approval No: 2025-11). This study was conducted with the permission of the Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (Ref. No: E-72784983-288.04-18471322, Date: 17.03.2025).

Animals

The study population comprised Yellow-legged Gulls *(Larus michahellis)* that had completed treatment and rehabilitation at the Ondokuz Mayıs University. The gulls were housed in a 1 m^3 (1 x 1 x 1 m) stainless steel cage in a room with a 22-24°C temperature and 40%-60% humidity, with a 12 h light and 12 h dark cycle. All patients were provided with ad libitum water and 100 g of fresh fish twice daily. All birds were capable of oral food intake, demonstrated flight ability, and were scheduled for imminent release into the wild. Sex could not be determined, and none presented with ocular pathology. A total of 18 gulls (nine juveniles and nine adults; 36 eyes) were examined. Age determination of the specimens in this study was performed based on plumage characteristics, bill and iris coloration, following the standard criteria previously established for the Yellow-legged Gull. Adult individuals (≥4 years old) were defined as those exhibiting a fully white head, pale grey mantle, characteristically patterned black-and-white wing tips, yellow bill with a red gonys spot, pale iris, and fully developed plumage. Juvenile individuals were characterised by pink legs; a dark bill; a brown iris; a grey-streaked head; grey-brown feathers with pale edges on the upperparts and wing coverts, underparts mottled grey-brown; primaries and secondaries dark, with similar pattern; primary coverts dark brown; white tail with broad dark terminal band. This classification ensured clear and reliable comparative analyses between age groups [9,10].

Experimental Design and Procedure

Ophthalmic examinations were performed between 09:00 and 16:00 h. Initial examinations were performed without sedation. Assessments were conducted in the following order: Schirmer tear test I (STT-I), intraocular pressure (IOP) measurement, and ocular ultrasonography. The left eye was examined first, followed by the right. Ultrasonography was performed last, as acoustic gel and probe application could potentially influence tear production and IOP. Sedated examinations were repeated 24 h later using a neuroleptanalgesic protocol. After a 4-hour fasting period, each gull underwent the sedation protocol.

Drugs and Intranasal Administration

Diazepam 8 mg/kg (Diapam 10 mg/2 mL I.M./I.V, Osel Pharmaceuticals®, Türkiye) and butorphanol 1 mg/kg (Nalgosed 10 mg/mL, Bioveta Pharmaceuticals®, Türkiye) were combined in a single syringe and administered intranasally via a 22 G intravenous catheter with the needle removed. The catheter was inserted approximately 5-6 mm into each nostril, and the solution delivered slowly and evenly between both nares *(Fig. 1)*. Deep sedation was defined as the absence or marked reduction of reflexes such as wing extension/withdrawal and pedal withdrawal. The same examination protocol was then repeated.

Fig 1. Intranasal administration of butorphanol-diazepam combination

Ophthalmic Examination

Tear production was measured using STT-I strips placed in the conjunctival fornix for 1 min *(Fig. 2)*. IOP was assessed using a rebound tonometer (TonoVet®, Icare Finland Oy, Vantaa, Finland) applied perpendicularly to the corneal surface *(Fig. 3)*. Each IOP measurement was repeated three times, and mean values recorded. Ocular ultrasonography was performed with a Vetus 9 ultrasound system (Mindray Animal Care, Shenzhen, China) equipped with a C11-3s convex array transducer *(Fig. 4)*. With the probe oriented horizontally, the following parameters were measured: central corneal thickness (CCT), anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), axial globe length (AGL), equatorial globe width (EGW), and pecten oculi length (POL). With the probe held vertically, lens axial height (LAH) and axial globe height (AGH) were recorded *(Fig. 5)*.

Statistical Analysis

Data normality was assessed using the Kolmogorov-Smirnov test. Descriptive statistics were presented as mean ± standard deviation (SD). To ensure data homogeneity and address potential age-related physiological variations, the study population was stratified into two groups: Juveniles and Adults. Comparisons between non-sedated (baseline) and sedated measurements for ocular parameters (IOP, STT-I, and ultrasonographic indices) were performed within each age group using the Paired Samples t-test. Comparisons of baseline values between the juvenile and adult groups were conducted using the Independent Samples t-test. A P-value of <0.05 was considered statistically significant. All analyses were

Fig 2. Measurement of tear production using STT-I

Fig 3. Measurement of intraocular pressure using a rebound tonometer

Fig 4. Ocular ultrasonography

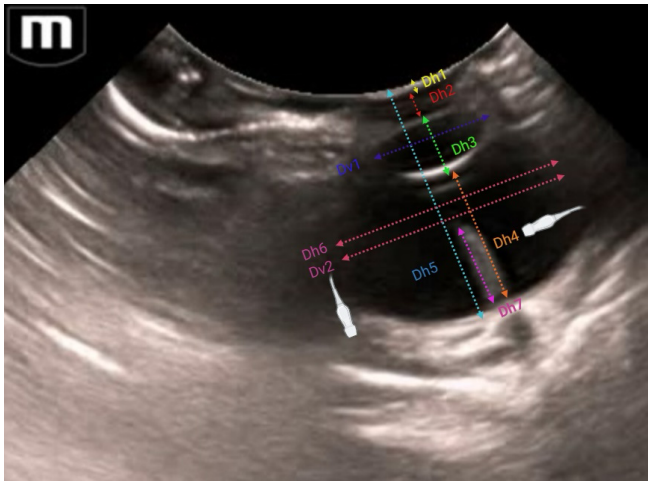

Fig 5. With the probe held horizontally, central corneal thickness (Dh1), anterior chamber depth (Dh2), lens thickness (Dh3), vitreous body depth (Dh4), axial globe length (Dh5), eyeball centre width (Dh6) and pecten oculi length (Dh7) were measured. When the probe was held vertically, lens height (Dv1) and eyeball centre height (Dv2) were measured

conducted using SPSS software (version 21.0; IBM Corp., Armonk, NY, USA).

Results

The body weights of the Yellow-legged Gulls were recorded as 670.55±35.84 g for juveniles and 782.22±61.61 g for adults. During examinations without sedation, gulls exhibited continuous wing flapping, resistance, and pecking behaviour. In particular, during ocular ultrasonography and the Schirmer I test, birds continued to struggle despite gentle restraint of the beak by assisting personnel, making examinations difficult to perform. In contrast, all examinations in sedated gulls were completed smoothly and without complication. The protocol provided an adequate depth of sedation for ocular ultrasonography and other ophthalmic assessments in all individuals. The mean onset time of deep sedation was 11.7±3.4 min.

No statistically significant differences were detected between left and right eyes (P>0.05); however, measurements from each eye were analyzed separately for STT-I, IOP, and ultrasonographic parameters. When the same parameters under sedated and non-sedated conditions were compared between juvenile and adult groups, no statistically significant differences were detected (P>0.05) *(Table 1)*. In the analysis, measurements were compared within each age group (juvenile and adult) for sedated and non-sedated parameters. This approach ensured the homogeneity of comparisons within each group, providing a fair and balanced evaluation. Accordingly, *Table 2* presents the parameters for juvenile and adult gulls, with sedated and non-sedated values shown separately within each age group.

The sedation protocol produced statistically significant decreases (P<0.05) in juveniles regarding STT-I, ACD,

Table 1. *Comparison of non-sedated and sedated ocular measurements between juvenile and adult gulls*

Ocular Parameters	Group	n (Eye)	Mean ± SD	P-value
IOP (mm/Hg)	J	18	12.278±1.127	0.920
	A	18	12.222±2.045	
IOPSED (mm/Hg)	J	18	9.388±1.5771	0.075
	A	18	10.888±3.085	
STT-I (mm)	J	18	12.055±3.171	0.104
	A	18	13.667±2.589	
STT-ISED (mm)	J	18	10.166±3.776	0.801
	A	18	9.889±2.698	
CCT (cm)	J	18	0.059±0.007	0.833
	A	18	0.060±0.008	
CCTSED (cm)	J	18	0.060±0.006	0.380
	A	18	0.058±0.008	
ACD (cm)	J	18	0.151±0.023	0.475
	A	18	0.157±0.025	
ACDSED (cm)	J	18	0.144±0.029	0.629
	A	18	0.149±0.025	
LT (cm)	J	18	0.452±0.021	0.180
	A	18	0.462±0.022	
LTSED (cm)	J	18	0.466±0.033	0.319
	A	18	0.475±0.021	
LAH (cm)	J	18	0.875±0.045	0.101
	A	18	0.899±0.040	
LAHSED (cm)	J	18	0.893±0.045	0.291
	A	18	0.911±0.057	
VCD (cm)	J	18	0.999±0.057	0.540
	A	18	1.008±0.026	
VCDSED (cm)	J	18	0.990±0.047	0.401
	A	18	1.003±0.047	
AGL (cm)	J	18	1.672±0.076	0.237
	A	18	1.704±0.082	
AGLSED (cm)	J	18	1.656±0.058	0.135
	A	18	1.688±0.066	
EGW (cm)	J	18	1.753±0.051	0.605
	A	18	1.742±0.075	
EGWSED (cm)	J	18	1.746±0.077	0.838
	A	18	1.751±0.085	
AGH (cm)	J	18	1.779±0.075	0.454
	A	18	1.761±0.061	
AGHSED (cm)	J	18	1.788±0.080	0.283
	A	18	1.760±0.076	

Table 1. Continue

Ocular Parameters	Group	n (Eye)	Mean ± SD	P-value
POL (cm)	J	18	0.680±0.022	0.054
	A	18	0.663±0.028	
POLSED (cm)	J	18	0.671±0.025	0.541
	A	18	0.677±0.034	

n: *total samples,* ***SED:*** *sedated,* ***A:*** *adult,* ***J:*** *juvenile,* ***CCT:*** *central corneal thickness,* ***ACD:*** *anterior chamber depth,* ***LT:*** *lens thickness,* ***VCD:*** *vitreous chamber depth,* ***AGL:*** *axial globe length,* ***EGW:*** *equatorial globe width,* ***POL:*** *pecten oculi length,* ***LAH:*** *lens axial height,* ***AGH:*** *axial globe height*

Table 2. *Non-sedated and sedated ocular measurements in juvenile and adult gulls*

Ocular Parameters	Juvenile (n=18 eye)		Adult (n=18 eye)	
	Mean ± SD	*P-value*	Mean ± SD	*P-value*
IOP (mmHg)	12.278±1.127	0.507	12.222±2.045	0.208
IOPSED (mmHg)	9.388±1.577		10.888±3.085	
STT-I (mm)	12.055±3.171	0.007*	13.667±2.589	0.127
STT-ISED (mm)	10.166±3.776		9.889±2.698	
CCT (cm)	0.059±0.007	0.345	0.060±0.008	0.001*
CCT SED (cm)	0.060±0.006		0.058±0.008	
ACD (cm)	0.151±0.023	0.005*	0.157±0.025	0.001*
ACDSED (cm)	0.144±0.029		0.149±0.025	
LT (cm)	0.452±0.021	0.764	0.462±0.022	0.082
LTSED (cm)	0.466±0.033		0.475±0.021	
LAH (cm)	0.875±0.045	0.001*	0.899±0.040	0.001*
LAHSED (cm)	0.893±0.045		0.911±0.057	
VCD (cm)	0.999±0.057	0.001*	1.008±0.026	0.001*
VCDSED (cm)	0.990±0.047		1.003±0.047	
AGL (cm)	1.672±0.076	0.001*	1.704±0.082	0.192
AGLSED (cm)	1.656±0.058		1.688±0.066	
EGW (cm)	1.753±0.051	0.006*	1.742±0.075	0.001*
EGWSED (cm)	1.746±0.077		1.751±0.085	
AGH (cm)	1.779±0.075	0.001*	1.761±0.061	0.006*
AGHSED (cm)	1.788±0.080		1.760±0.076	
POL (cm)	0.680±0.022	0.002*	0.663±0.028	0.340
POLSED (cm)	0.671±0.025		0.677±0.034	

** P<0.05, parameters showing statistically significant differences*
n: *total samples,* ***SED:*** *sedated,* ***CCT:*** *central corneal thickness,* ***ACD:*** *anterior chamber depth,* ***LT:*** *lens thickness,* ***VCD:*** *vitreous chamber depth,* ***AGL:*** *axial globe length,* ***EGW:*** *equatorial globe width,* ***POL:*** *pecten oculi length,* ***LAH:*** *lens axial height,* ***AGH:*** *axial globe height*

VCD, AGL, EGW, and POL, while statistically significant increases (P<0.05) were detected in LAH and AGH. In adults, statistically significant decreases (P<0.05) were observed in CCT, ACD, VCD, and AGH, whereas LAH and EGW showed significant increases (P<0.05). Although decreases in IOP and increases in LT were noted in both groups, no statistically significant differences were detected.

On ultrasonography, the cornea appeared hyperechoic with a slightly convex contour in all gulls. The anterior chamber was anechoic, consistent with its aqueous humour content. The lens was clearly delineated, with both anterior and posterior capsules visible as hyperechoic lines. The vitreous body was anechoic, and the pecten oculi was visualised at the 5 o'clock position as a tubular echogenic structure extending from the retina. The retina appeared as a hyperechoic line, separated from the orbital wall by a thin anechoic interface. The scleral and choroidal layers could not be differentiated, and equatorial and axial globe dimensions could therefore not be measured with precise margins. In these regions, measurements were taken approximately by aligning the probe parallel to the lens and relative to the ciliary region *(Fig. 5)*. The shape of the globe was conical in all birds.

Discussion

Establishing normal anatomical and physiological values for the eye in healthy wild birds is essential for diagnosing ocular pathologies that may arise in clinical or rehabilitation settings. Ophthalmological studies in wild and exotic avian species have demonstrated that ocular parameters can vary markedly between species, and in some cases, even between breeds within a species [4,11,12]. Several investigations have reported normal ocular values for a variety of bird species [13]. By focusing on a single species, our study presents STT-I, IOP, and ultrasonographic ocular biometry values in clinically healthy, rehabilitated *L. michahellis* suitable for release into the wild.

Several methods have been employed to evaluate tear production in birds, including Schirmer I, modified Schirmer, and phenol red thread tests [14]. The choice of test may vary according to the size and anatomical configuration of the conjunctival fornix. As each method reflects different layers of the tear film, they should not be compared directly [14, 15]. Accordingly, when establishing reference values, the specific test used must be explicitly reported. STT-I values are also thought to vary with orbital size and the relative dimensions of the lacrimal glands [14]. In our study, although STT-I values decreased with sedation in both juvenile and adult animals, this reduction was not statistically significant. When the groups were compared within themselves, allowing for a more homogeneous evaluation, the decrease in STT-I values remained notable, and this reduction was statistically significant in juveniles (P=0.007). This finding is consistent with the well-recognised transient xerophthalmic effect of sedatives and opioids [16]. Similar reductions in STT-I values following sedation have been reported in other species with

different sedative and anaesthetic protocols [16-18]. In wild and exotic birds, IOP has been measured using indentation (Schiøtz), applanation (Tono-Pen), and rebound (TonoVet) tonometers [19,20]. IOP values have been shown to vary across parrot and raptor species, as well as depending on the device used for measurement [19-21]. In our study, TonoVet rebound tonometry revealed no significant differences in IOP between juvenile and adult groups. Similarly, when age groups were evaluated within themselves, sedation caused a noticeable decrease in IOP values; however, this reduction was not statistically significant (P>0.05). Anaesthetic agents and clinical procedures are known to exert variable effects on IOP. Ketamine, for instance, increases extraocular muscle tone and may raise IOP, whereas most other sedatives and anaesthetics generally reduce IOP depending on the depth of anaesthesia [22,23]. Nevertheless, some studies have reported minimal changes in IOP with ketamine anaesthesia [22]. IOP is also influenced by systemic blood pressure, head and neck positioning, and body posture [24]. Therefore, it is important to characterise species-specific effects of sedatives and anaesthetics on IOP.

When juveniles and adults were compared with each other -both for non-sedated and sedated measurements- no significant differences were detected for any ultrasonographic parameter (P>0.05). However, when each age group was evaluated within itself, certain changes became apparent. In both juveniles and adults, a decrease in IOP (P=0.507) and an increase in LT (P=0.208) were observed, but these changes were not statistically significant. For all remaining parameters, significant changes were detected either within the juvenile group, the adult group, or within both age groups. While Lens Thickness (LT), measured in the horizontal plane, showed a non-significant increase, Lens Axial Height (LAH), measured in the vertical plane, significantly increased in both age groups. The correlation between reduced IOP and increased vertical lens dimensions (LAH) is consistent with previous reports. Reduced pressure within the anterior chamber and vitreous body has been associated with lens expansion and elongation [25,26]. Numerous studies in both human and veterinary medicine have documented IOP reductions following anaesthetic administration [25,27,28]. The decrease in IOP and increases in LT are thought to result from reduced aqueous and vitreous humour production or increased elimination of these fluids, thereby lowering intraocular volume. Such changes can lead to measurable structural alterations in corneal thickness, anterior chamber depth, and lens configuration [25,29]. Considering these findings, the fact that the sedation protocol used in our study caused changes in the anatomical structures of the eye is consistent with the data reported in the literature.

When sedated and non-sedated parameters were compared between juvenile and adult subgroups, no statistically significant differences were detected. However, when sedated and non-sedated values were compared within each age group, statistically significant differences emerged in several parameters. These within-group comparisons provided a more homogeneous age-based distribution, but studies with a larger sample size would more clearly reveal how the sedation protocol affects ocular parameters in Yellow-legged Gulls. Nonetheless, age-related physiological variability and inter-individual differences may have increased variance within the combined dataset, thereby influencing the statistical outcomes. In addition, minor variability associated with measurement techniques and individual stress responses may have contributed to the observed limitations. *Larus michahellis* is a sexually monomorphic species, and reliable morphological sexing is currently not possible. Molecular sexing methods were not applied in this study due to technical limitations and practical constraints. Some studies have reported morphometric measurements such as head, bill, wing, and tarsus lengths as potential indicators of sex [30], but geographic variation among populations and overlapping values between sexes make these predictions unreliable. To avoid inconsistent or speculative results, all data were analyzed without sex differentiation. This represents a limitation of the present study. Further studies with larger sample sizes and more homogeneous age groupings are warranted to clarify age-related effects and corroborate the present findings. The aim of this study was to evaluate the effects of intranasal butorphanol-diazepam sedation on tear production, intraocular pressure (IOP), and ocular biometry in Yellow-legged Gulls *(Larus michahellis)*. In our study, group classifications were performed to achieve as precise and homogeneous distributions as possible. We believe that each chemical agent may exert different effects in different species, and therefore, species-specific studies are one of the most important considerations. Consequently, it may provide preliminary data to support future reference studies with larger sample sizes, contributing to species-specific evaluation of ocular parameters.

Declarations

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author (C.N.) on reasonable request.

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Authors' Contributions: CN: contributed to the study design, fieldwork organization, sedation protocol implementation, data collection, data management, and manuscript preparation. BDE: contributed to data collection, supplementary material documentation, and manuscript preparation. KSI: performed statistical analyses, contributed to the interpretation of results, and assisted in manuscript preparation. HON: assisted in the literature review, image selection, manuscript formatting and supervisor. All authors critically reviewed and approved the final version of the manuscript.

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