Determination of Reference Values for Tear Production and Intraocular Pressure in Pygoscelid Penguins of the Antarctic Peninsula

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Research Article

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Abstract

According to literature review, this is the first study investigating tear production and intraocular pressure in pygoscelid penguins living in their natural habitat. This study also provides reference values for penguins kept under professional care at zoos and rehabilitation centres. This study aimed to establish normal values for standard ocular tests, including the Schirmer tear test (STT-1) and intraocular pressure test (IOP), for penguins belonging to the genus Pygoscelis (P), namely, the Adélie (*Pygoscelis adeliae*), gentoo (*Pygoscelis papua*) and chinstrap (*Pygoscelis antarctica*) penguins. Ophthalmic measurements were made from the Antarctic. In the left eye of each penguin, the amount of tear production (TP) was determined with the STT-1 and the IOP was measured using a Tonovet® rebound tonometer. No macroscopic findings affecting the eyelids, third eyelid, cornea or anterior eye camera were detected in the eyes of the examined penguins. The mean STT-1 and mean IOP values of 129 and 120 adult penguins were determined as 10.163 ± 4.054 mm/min and 38.852 ± 13.188 mmHg, respectively. A statistical difference at the level of $p < 0.001$ was determined between the islands for the mean IOP values. While no statistically significant difference was detected between the penguin species for the mean IOP values, between the locations was found to be significant ($p < 0.001$).

Statistical differences at a level of $p < 0.05$ were determined for the mean TP values between the all locations and for the mean IOP values between the all locations ($p < 0.001$) and only Lions Rump - Ardley I ($p = 0.023$). A statistical difference of $p < 0.05$ was detected between the chinstrap and gentoo for the mean STT-1 values. This study, which is aimed at reporting the first literature data in this field, has shown the need for further more detailed studies to elucidate the impact of different locations, daily time intervals and seasons on the STT and IOP values of penguins.

Highlights

- This study is the first study investigating tear production and intraocular pressure in Pygoscelid penguins
- Tear production (TP) amount was obtained by Schirmer Tear Test (STT-1) and intraocular pressure (IOP) The mean IOP (±SD mmHg) was obtained by Tonovet® rebound tonometer
- No macroscopic findings affecting the eyelids, third eyelid, cornea or anterior eye camera were found in the eyes of the examined penguins.
- STT and IOP in Pygoscelis penguins were decleared of 10.163±4.054 mm / min and 38.852±13.188 mmHg.
- No statistically significant difference was detected between the species for the mean IOP values ($p=0.854$), yet the statistical difference between the locations was found to be very significant ($p<0.000$).

1 Introduction

Ocular disorders bear significance for survival and self-sustainment. Thus, ocular examination, which not only enables the collection of basic medical data and the protection of the health of animals under professional care, but also constitutes an integral part of the monitoring of wild animals, is of major importance (Prashar et al. 2007; Harris et al. 2008; Mercado et al. 2010; Reuter et al. 2010, 2011; Ghaffari et al. 2012; Labelle et al. 2012; Kuhn et al. 2013; Molter et al. 2014; Bliss et al. 2015; Gonzalez-Alonso-Alegre et al. 2015; Meekins et al. 2015; Wills et al. 2016; Woodhouse et al. 2016a; Kinney et al. 2017; Sheldon et al. 2017). *Pygoscelis* penguins are found mostly in the higher latitudes of the sub-Antarctic and the Antarctic. All three *Pygoscelis* species have an IUCN threat status of Least Concern, though regionally, Adélie and chinstrap penguins are known to be in decline (Dunn et al., 2016), while gentoo penguins (*Pygoscelis papua*) are increasing. Eye sight is of critical importance for penguins as it aids in migration, orientation, and foraging (Nesterova et al. 2010). Thus, vision disorders adversely affect the capability of these animals to adapt to their physical and social environment. In penguins, interpretation of ocular findings and diagnostic tests are very difficult (Bliss et al., 2015) Ocular disorders are relatively common, especially among free-living birds (Moore et al. 2017). While numerous bacteria, viruses, fungi, and parasites have been isolated from diseases of the avian ocular surface (Griggs 2019), only a few studies are available on the ophthalmic parameters of several penguin species, including ocular bacterial flora, intraocular pressure, and tear production (Suburo et al. 1988; Martin 1999; Swinger et al. 2009;

Nesterova et al. 2010; Bliss et al. 2015; Sila et al. In a study conducted by Swinger et al. (2009), the ocular bacterial flora and ophthalmic parameters of 28 captive penguins kept at a zoo were investigated. However, there is no study of three species belonging to the pygoscelid genus. However, to our knowledge, there is no published study on the ocular infections of penguins. the first documented report of the unilateral pyogranulomatous ocular lesion in a Gentoo penguin chick, living in its natural habitat in Antarctica (Çakır Bayram et al. 2021).

The Schirmer tear test (STT) and intraocular pressure (IOP) measurements serve as the main diagnostic tools for multiple ocular diseases. In view of differences existing between avian species, there is a need for establishing species-specific reference values for IOP and the STT (Prashar et al. 2007; Harris et al. 2008; Mercado et al. 2010; Reuter et al. 2010, 2011; Ghaffari et al. 2012; Labelle et al. 2012; Işler et al. 2013; Kuhn et al. 2013; Molter et al. 2014; Bliss et al. 2015; Gonzalez-Alonso-Alegre et al. 2015; Meekins et al. 2015; Ansari Mood et al. 2016, 2017; Wills et al. 2016; Woodhouse et al. 2016a; Kinney et al. 2017; Sheldon et al. 2017)(. The amount of tear produced serves as an important parameter for the assessment of the pathological condition of the ocular surface. The STT has long been the gold standard for determining the amount of tear production (Hamor et al. 2000; Swinger et al. 2009; Işler et al. 2013; Komnenou et al. 2013; Smith et al. 2015; Ansari Mood et al. 2017). Furthermore, the STT is the most commonly used test for diagnosing ocular diseases in veterinary medicine (Lewin et al. 2020).

IOP can be described as the balance between the production and secretion of the aqueous humor. Abnormally high or low IOP is considered as an indicator of ocular diseases, such as uveitis and glaucoma (Swinger et al. 2009; Ansari Mood et al. 2017). While contact tonometry is repeatable and yields almost precise results, in the last decade, the use of the TonoVet® (Icare Finland, Oy) (McLennan), ICare® Tonovet (TV01; Icare Finland Oy, Helsinki, Finland) (Gloe Shawna) and TonoVet Plus® (Icare) (Mustikka) rebound tonometers for the measurement of IOP in domestic, laboratory, exotic and wild animals has gained popularity.

As rebound tonometry requires only an instant contact between the probe and corneal surface (Chui et al. 2008; Rodrigues et al. 2020). Being a minimally invasive technique, rebound tonometry can be safely performed for corneal diameters as small as 1.4 mm (Reuter et al. 2011; Molter et al. 2014; Woodhouse et al. 2016b; Sheldon et al. 2017). Rebound tonometry, known not to require topical anaesthesia, measures the deceleration of the probe which is rapidly and repeatedly bounced against the cornea (Prashar et al. 2007; Harris et al. 2008; Mercado et al. 2010; Reuter et al. 2011; Labelle et al. 2012; Molter et al. 2014; Bliss et al. 2015; Gonzalez-Alonso-Alegre et al. 2015; Ansari Mood et al. 2016, 2017; Wills et al. 2016; Woodhouse et al. 2016b; Kinney et al. 2017; Sheldon et al. 2017). The Tonovet veterinary device has been specifically designed for use in animals, and generates calibration curves for IOP measurement in small animals using different settings (D for dogs, H for horses, P for other species) (Ollivier 2003).

Normal STT-1 values (Harris et al. 2008; Storey et al. 2009; Ghaffari et al. 2012; Barsotti et al. 2013; Kuhn et al. 2013; Beckwith-Cohen et al. 2015; Meekins et al. 2015; Ansari Mood et al. 2016) and IOP values measured with the Tonovet® rebound tonometer have been reported for various domestic and wild animals (Jeong et al. 2007; Prashar et al. 2007; Harris et al. 2008; Reuter et al. 2011; Molter et al. 2014; Meekins et al. 2015; Ansari Mood et al. 2016, 2017; Wills et al. 2016; Kinney et al. 2017; Church et al. 2018; Ady Y. Gancz et al. 2020). However, very limited information is available on these ocular parameters in penguins living in their natural Antarctic habitat. Normal IOP and TP values have been determined with diagnostic ophthalmic tests in species of the order Sphenisciformes, including the macaroni penguin (*Eudyptes chrysolophus*) (Bliss et al. 2015; Woodhouse et al. 2016a), southern rockhopper penguin (*Eudyptes chrysocome*) (Bliss et al. 2015; Woodhouse et al. 2016a; Church et al. 2018), black-footed penguin (*Spheniscus demersus*) (Mercado et al. 2010; Gonzalez-Alonso-Alegre et al. 2015), Humboldt penguin (*Spheniscus humboldti*) (Swinger et al. 2009; Sheldon et al. 2017), gentoo penguin (*Pygoscelis papua*), king penguin (*Aptenodytes patagonicus*), and chinstrap penguin (*Pygoscelis antarctica*).

Statistically significant differences have been reported to exist between IOP values measured with the Tonovet® rebound tonometer in relation to the species, age and ocular pathologies of animals (Reuter et al. 2011; Komnenou et al. 2013; Bliss et al. 2015; Sheldon et al. 2017). Some studies have reported the absence of statistically significant sex-related differences between STT-1 values and Tonovet® rebound tonometer-produced IOP values in young and old birds (Trbolova and Ghaffari

2012). Some other studies have suggested that, in animals, STT and IOP values do not significantly differ for age, sex or the left/right eye (Swinger et al. 2009; Mercado et al. 2010; Reuter et al. 2011; Işler et al. 2013; Bliss et al. 2015; Gonzalez-Alonso-Alegre et al. 2015; Ansari Mood et al. 2016, 2017; Woodhouse et al. 2016a). Using the Schirmer tear test and Tonovet® rebound tonometer, Sheldon et al. (Sheldon et al. 2017) established reference ranges for tear production and IOP in wild Humboldt penguins.

Reports have been published, which provide normal IOP and STT values for the Humboldt penguin (Swinger et al. 2009; Sheldon et al. 2017), macaroni penguin (Bliss et al. 2015; Woodhouse et al. 2016b), southern rockhopper penguin (Bliss et al. 2015), black-footed penguin (*Spheniscus demersus*), gentoo penguin (*Pygoscelis papua*), king penguin (*Aptenodytes patagonicus*), and chinstrap penguin (*Pygoscelis antarctica*) (Mercado et al. 2010; Gonzalez-Alonso-Alegre et al. 2015; Church et al. 2018). Despite the availability of literature reports on tear production and intraocular pressure in various penguin species, the majority of the populations investigated in these reports cover captive penguins kept under professional care at zoological institutes or wildlife rehabilitation centres (Swinger et al. 2009; Mercado et al. 2010; Church et al. 2013; Bliss et al. 2015; Gonzalez-Alonso-Alegre et al. 2015; Woodhouse et al. 2016b).

On the other hand, there is no published literature data on normal STT-1 and Tonovet® rebound tonometer-produced (Icare® Oy, Finland (TV01)) IOP values in pygoscelid penguins. Thus, the present study was aimed at establishing reference ranges of clinically normal STT-1 and IOP values for three penguin species belonging to the genus Pygoscelis, namely, the Adélie penguin (*Pygoscelis adéliae*), chinstrap penguin (*Pygoscelis antarctica*), and gentoo penguin (*Pygoscelis papua*).

Based on literature review, this is the first study on STT-1 and Tonovet® rebound tonometer-aided (Icare® Oy, Finland (TV01) IOP measurements in pygoscelid penguins, performed in open air in their natural habitat.

2 Materials And Methods

2.1 Locations and times of study

A bilateral cooperation project titled "The cytological, microbiological and ophthalmic evaluation of ocular surface samples from Antarctic penguins", conducted with the Chilean Antarctic Institute (INACH) within the scope of the 55th Antarctic Scientific Expedition (ECA55), was implemented in the 2018–2019 period, under the Third National Antarctic Scientific Expedition (TAE III) organised by the Polar Research Centre (PolRec) of Istanbul Technical University. The project was approved under the Turkish Antarctic Programme (Antarctic Specially Protected Area, ASPA, No: 150, Permit Ṅ- 21- 2019,' Permit Ṅ- 07-2019). The locations visited (between 62° 06' S – 058° 09' W and 64° 52' S – 063° 32' W) in the study included Harmony Point/Nelson Island (Antarctic Specially Protected Area No. 133) (ASPA 133), Doumer Island/Yelchoo Base, Cabo Legoupil/General Bernardo O'Higgins Base, Ardley Island (Antarctic Specially Protected Area No. 150) (ASPA 150) and Lions Rump, King George Island (Antarctic Specially Protected Area No. 151) (ASPA 151). (Table 1)

The islands were visited 3 times for sampling. A zodiac was used for transport from the ship to the locations. A different entry point was used during each visit. According to the penguin species to be sampled, either the Louis entry point (Refuge Balive, Brazilian Refuge "Astronomo Cruls" -R1ANF/P, Ardley I), Braillard entry point (Julio Ripomonti Refuge, Ardley II), or Faro entry point (Ardley III) were used (Fig. 1).

2.2 Method of capture and handling

The capture and handling of the penguins were performed as described by González-Acuña et al. (González-Acuña et al. 2013)and in accordance with the standard methods laid down for the Ecosystem Monitoring Programme by the Commission for the Conservation of the Antarctic Marine Life Resources (CCAMLR 2004). During the sampling procedure, the penguins were restrained in an upright vertical position by applying gentle pressure to the wings, base of the skull, and beak. During ophthalmic examination, the penguins were restrained manually in a facedown position. According to standard procedure, the captor restrained each penguin in such a way that the ventrum of the animal lay on his legs. While holding the wings of the animal with one hand, the captor fixed the legs in an extended position with his other hand. In the meantime, the body of the penguin leaned onto the abdomen of the captor. To avoid any measurement failure or technical error, all diagnostic tests were performed by a same researcher (Bliss et al. 2015). For the purposes of data collection, only one ocular sample was taken from each physically restrained animal in the shortest time possible. Penguins away from their nesting sites, going to feeding or returning from the ocean, were selected for sampling. Penguins were caught with a tool with a long handle and a wide net (such as a fish or butterfly net).the wings were determined with an arm, by holding them by their feet without causing much irritation. then they were prepared for sampling by holding the feet and wings in horizontal shape. Each penguin was captured manually and restrained for approximately 6 min. After collecting samples and taking measurements, birds were immediately released (Gonzalez-Acuna et al.,2016).

2.3 Ophthalmic tests

Owing to the specific geographical structure of the Antarctic Peninsula and the sudden changes that occur in its weather conditions, the penguins were not able to be physically restrained and subjected to ophthalmic measurements in a closed, protected and silent environment. The penguins were macroscopically examined for signs of possible ocular infection and sight impairment. For this purpose, the menace reflex test was conducted bilaterally by waving a hand in front of both eyes. To avoid any air flow-related false positive result, the hand was waved at a distance of at least 30 mm to the tested eye. The menace reflex was considered to be present, when the penguin responded to the visual threat by continuous head movement, blinking of the eye or opening its mouth in a threatening manner. When the penguin moved its head or blinked its eye once and did not repeat this response to continuous hand waving, then the reflex was considered to be inconsistent (Kuhn et al. 2013). In all of the penguin species examined, the third eyelid was transparent and displayed its normal structure (Figs. 2A-2C).

The animals were exposed to minimal stress under physical restraint, yet a major difficulty encountered in applying the standard procedure was not being able to allow the time required for the penguins to calm down.

This also negatively affected the collection of accurate data on their blinking frequency. Thus, the ocular tests were applied to the left eye only. The major difficulty of performing ophthalmic measurements in both the left and right eyes was the need for two persons to capture and restrain a penguin and the requirement to ensure that all measurements were performed by a single person. In view of these requirements and in order to minimise the handling period, tear production measurements with the Schirmer tear test (STT-1) (129 penguins) and IOP measurements (120 penguins) with the TonoVet® tonometer were performed in only the left eye of each penguin. Thereby, the handling period allowed for in the permission document was abided by (Supplementary video).

2.3. Method of Schirmer tear test type (STT)

The Schirmer 1 test (STT-1) was performed by a single person, according to the manufacturer’s instructions and by using strips of sterile standardized filter paper, which were 35 mm long and 5 mm wide. The standardized strips (Schirmer-Tränentest®; Vet Eickemeyer, Tuttlingen, Germany) were placed in the lower conjunctival fornix for one minute. The strips were bent at the dented part, and by means of a dry forceps the bent part was placed in the exterior one-third of the lateral canthus of the left lower eyelid (Figs. 2D-2F). The amount of tear absorbed by the strip was measured in millimetre/minute with the aid of the millimetric scale on the strip. Care was taken to handle the strips only by the sides to avoid contact with any object or moisture before sampling (Bliss et al. 2015; Sheldon et al. 2017). (Supplementary video. Minute range: 00.25–00.55).

2.4 Method of Intraocular pressure

During macroscopic examination and the ophthalmic measurements and tests, the penguins were manually restrained in a facedown position by the captor. Intraocular pressure readings were performed with a rebound tonometer (TonoVet®; Icare, Helsinki, Oy, Espoo, Finland), using a P calibration setting installed in the tonometer by the manufacturer. Measurements were performed according to the manufacturer’s instructions, maintaining the tonometer in a horizontal position and holding the probe at a distance of 4–8 mm to the cornea. With one hand, the beak of the penguin was held and gentle pressure was applied to the occipital base of the skull. Measurements were made from the cornea center of the left eye. To avoid readings of the third eyelid and the generation of false results, while collecting data, care was taken to ensure that the probe came in contact with the cornea only, when the third eyelid (nictitating membrane) (Fig. 2A-2C) had been contracted. The tonometer digitally displayed the IOP value on its screen each time the cornea was touched (Sheldon et al. 2017). After the fifth touch, an average of the previous 5 readings was generated automatically. The average measurement, as calculated by the tonometer (highest and lowest values excluded), was recorded (Sheldon et al. 2017). Readings were not able to be performed in some animals, either due to their excessive movement under restraint or as a result of stormy, rainy or snowy weather. Therefore, only data pertaining to the animals, in which the 5 readings were able to be successfully completed, were analysed (Fig. 2G-2I). Supplementary video. Minute range: 02.35–02.47).

2.5. Statistical analyses

The study data were collected from 04 January to 06 February in 2019. Analyses were conducted using the TURCOSA cloud (Turcosa Analytics Ltd. Co., Turkey) statistical software (https://turcosa.com.tr/). The normal distribution of numerical variables was analysed with Shapiro-Wilk test of normality and Q-Q graphics. One-way analysis of variance (ANOVA) (Tukey's test was used as a multiple comparison test.) was used for the comparison of more than two groups. Student's t test was used to compare two independent groups. Tukey's test was used as a multiple comparison test. For any comparison, if the *p*-value obtained was lower than the significance level used ($\alpha < 0.05$), it was concluded with 95% confidence that there were statistically significant differences between the values compared.

3 Results

None of the examined penguins presented with any macroscopic ocular finding affecting the eyelids, nictitating membrane, cornea or anterior ocular chamber. The mean STT-1 value, calculated for measurements performed in six different regions in 129 adult penguins, was 10.163 ± 4.054 mm/min with a range of 3–25 mm/min. The highest maximum value was measured at Harmony Point as 25 mm/min. In other locations, this value ranged between 18 mm/min. For mean STT-1 values by location, insignificance was set as p = 0.088 in the ANOVA test. However, the specific geographical structure of the Antarctic Peninsula and the sudden and major changes in its weather conditions led to different numbers of samplings having been performed in the different locations. For this reason, despite the balanced distribution of standard deviations, a difference of 1/4 of the maximum value of 12 was observed between the minimum and maximum mean. (Table 2).

The overall range and mean tear production amounts calculated for the three pygoscelid species investigated in this study, namely, the Gentoo, Adélie and chinstrap penguin, were 3–25 mm/min and 9.789 ± 3.908 mm/min, 11.143 ± 2.340 mm/min and 11.944 ±4.964 mm/min, respectively.

For mean STT-1 values by penguin species, insignificance was set as p = 0.091 in the ANOVA test. Excluding the few numbers of Adélie penguins sampled, the two independent samples t-test demonstrated that the gentoo and chinstrap species significantly differed for the STT-1 values (p = 0.037) (Table 3).

Descriptive values of IOP by locations and penguin species are presented in Table 4. The mean IOP value of 120 penguins was 38.852 ± 13.188 mm Hg. The highest maximum value was measured as 69 mm Hg in Ardley III. In the other locations, this value ranged between 50–63 mm Hg. Due to adverse weather conditions, only one value could be measured in the chinstrap penguin. Due to the same reason, IOP values could not be measured at Harmony Point. Statistically significant differences (Tukey's HSD) were detected between Ardley III-Bernardo O'Higgins Base (p < 0.001), Ardley III-Ardley I (p < 0.001), Lions Rump-Ardley I (p = 0.023), Ardley III-Ardley II (p < 0.001), and Lions Rump-Ardley III (p < 0.001).

The overall range and mean IOP values calculated for the Pygoscelid species investigated in this study, namely, the gentoo, Adélie and chinstrap were 15–69 mmHg, 39.27 mmHg ± 11.5, 38.43 mmHg ± 14.8 and 29 mmHg±0.0 respectively. There was no statistically significant difference between the species for mean IOP values (p = 0.854) (Table 5).

4 Discussion

This is the first study reporting IOP and TP in free ranging pygoscelid penguins The present study is the first on the use of the Tonovet® rebound tonometerin penguins living in their natural habitat. In the present study, it was ascertained that the mean STT and IOP values of 129 and 120 adult penguins were 10.16 ± 4.05 mm/min and 38.85 ± 13.19 mmHg, respectively. A statistical difference at the level of $p < 0.05$ was determined between the islands for the mean IOP values. While no statistically significant difference was detected between the species for the mean IOP values ($p = 0.854$), the difference observed between the locations was found to be statistically very significant ($p < 0.001$).

Based on literature review, there is no previous study on ophthalmic findings of penguins living in their natural habitat, and thus, diagnostic ophthalmic reference values have not been established for these animals. As all of the studies conducted to date have been performed under professional care in zoological institutions or wildlife rehabilitation settings in controlled environments, there is need for further research. On the contrary to the expectation of observing differences among species, differences having been observed between the study locations was attributed to the very harsh Antarctic weather conditions characterized by sudden daily changes and to an equal number of samplings not being able to be performed in the investigated penguin species. The multiple comparisons of the study locations for the mean IOP values having demonstrated the presence of differences is in support of this attribution. The mean STT-1 and IOP values determined for each pygoscelid penguin species in this first study on tear production and IOP in wild penguins will serve as reference values not only for future studies to be conducted in the Antarctic Peninsula, but also for captive penguins kept under professional care. Yet there is still need for further research in different locations, time periods, and species, excluding the gentoo penguin.

It has been reported that it may not be possible to develop a standardized tear test for birds, due to anatomical and physiological differences observed between species for tear drainage and lacrimal ducts (Smith et al. 2015). As only very few ophthalmologic studies have been previously conducted in penguins, limited information is available on ocular examination, data interpretation and the difficulty of applying diagnostic tests in these birds during ophthalmologic examination(Martin and Young 1984; Suburo and Scolaro 1990; Pigatto et al. 2005). The establishment of reference ranges for each species is highly important in avoiding erroneous diagnostic interpretations during ophthalmic examination (Stiles et al. 1994; Korbel, L; P. 1998; Harris et al. 2008; Reuter et al. 2011; Labelle et al. 2012).

To date, studies aimed at determining IOP and STT values in penguin species belonging to the order Spheniscus have been conducted in animals kept under professional care in artificial marine and freshwater environments at either wildlife rehabilitation centres or zoos (Table 6) (Swinger et al. 2009; Mercado et al. 2010; Bliss et al. 2015; Gonzalez-Alonso-Alegre et al. 2015; Woodhouse et al. 2016a; Church et al. 2018). In their study on macaroni penguins (*Eudyptes chrysolophus*) and southern rockhopper penguins (*Eudyptes chrysocome*) kept at zoos and aquariums in North America, Woodhouse et al. (Woodhouse et al. 2016a) assessed the impact of multiple factors, including husbandry conditions, the presence/absence of cataract and concurrent ocular pathologies, as well as the body position during physical restraint, on IOP values in penguins. Based on literature review, this is the first study on tear production and intraocular pressure in pygoscelid penguins, namely, the Adélie chinstrap and gentoo living in their natural habitat on the southern Antarctic islands. Different from previous controlled studies conducted in a closed environment, this study was carried out in open air, in the natural marine habitat of penguins. Measurements were performed in the animals during their daily routine, such that they were exposed to dust, ocean water spray, strong winds, snow and abrupt weather changes at an average environmental temperature of -4 ^{0}C. The objective of the present study was to determine IOP and STT-1 values in clinically normal pygoscelid penguins that would serve as a reference for future research in this field.

When assessing the impact of species-specific anatomical and physiological differences, stress and geographical conditions on parameters such as tear production in raptorial birds, it should be noted that data comparison is able to be made only under optimal conditions (Jeong et al. 2007). In view of differences observed between species for IOP values (Table 7) and in agreement with previous studies (Sheldon et al. 2017), higher IOP values having been detected in pygoscelid penguins was attributed to these species diving up to 30 m beneath the ocean surface, exposing their cornea to high levels of external pressure, and thus, was considered to be an adaptive function related to underwater foraging. The only study previously conducted on these ocular parameters in populations living in their natural habitat was carried out in the Punta San Juan Conservation Area in Peru (Sheldon et al. 2017). This study presents, for the first time, IOP values detected in pygoscelid penguins living in their natural habitat in the Antarctic Peninsula. It was observed that IOP values varied with the age of the penguin, as well as with the year and location. In previous studies conducted by Swinger and Mercado (Swinger et al. 2009;

Mercado et al. 2010), IOP values were determined in healthy penguins, but the factors influential on these values were not investigated. Swinger et al. (Swinger et al. 2009) reported to have determined higher IOP values that fell within a larger range, compared to values previously detected in zoo animals. Suggesting that the higher IOP values they had detected were an adaptation of the animals to the higher atmospheric pressure they were exposed to during underwater dives, these researchers also indicated the necessity for further research to confirm their hypothesis (Mercado et al. 2010). In the present study, while no statistically significant difference was determined between the penguin species for the mean IOP values ($p = 0.854$) (Table 5), the study locations significantly differed for both the STT-1 and IOP values ($p < 0.05$, $p < 0.001$) (Table 2, Table 4).

The scarcity on ophthalmic findings in penguins makes it difficult to interpret ocular examination findings and diagnostic test results in these animals. Thus, it is of great importance to establish reference values for routinely used ocular parameters such as IOP and STT in penguins. Several studies have been carried out to determine STT and IOP values in the Humboldt penguin (*Spheniscus humboldti*) (Swinger et al. 2009; Sheldon et al. 2017), macaroni penguin (*Eudyptes chrysolophus*) (Bliss et al. 2015; Woodhouse et al. 2016a), southern rockhopper penguin (*Eudyptes chrysocome*) (Bliss et al. 2015), black-footed penguin (*Spheniscus demersus*), gentoo penguin (*Pygoscelis papua*), king penguin (*Aptenodytes patagonicus*), and chinstrap penguin (*Pygoscelis antarctica*) (Mercado et al. 2010; Gonzalez-Alonso-Alegre et al. 2015; Church et al. 2018) (Table 6). To date, only Sheldon et al. (Sheldon et al. 2017) have attempted to establish reference values for tear production with the Schirmer tear test and for IOP values with rebound tonometry in wild Humboldt penguins living in their natural habitat. Compared to values previously detected by (Sheldon et al. 2017) in Humboldt penguins living in their natural habitat (Table 6), the present study demonstrated higher values (38.85±13.19 mmHg) falling within a larger range (16–69 mmHg), which were attributed to the harsh Antarctic weather conditions characterized by sudden changes. l.

IOP measurements by rebound tonometry have been previously performed in the Humboldt penguin (*Spheniscus humboldti*) (Sheldon et al. 2017), macaroni penguin (*Eudyptes chrysolophus*) (Bliss et al. 2015), southern rockhopper penguin (*Eudyptes chrysocome*) (Bliss et al. 2015; Woodhouse et al. 2016b), black-footed penguin (*Spheniscus demersus*) (Mercado et al. 2010; Gonzalez-Alonso-Alegre et al. 2015), gentoo penguin (*Pygoscelis papua*), king penguin (*Aptenodytes patagonicus*) and chinstrap penguin (*Pygoscelis antarctica*) (Church et al. 2018). The mean IOP values of healthy macaroni and southern rockhopper penguins were ascertained as 42.0±9.7 mmHg and 32.9 ± 6.2 mmHg, respectively. No statistically significant difference was detected in these two penguin species for sex or the left/right eye. In previous research on the use of tonometry in penguins, the mean IOP value calculated for a healthy eye was determined to be above 28 mmHg, and thus, was significantly higher than values previously reported in several other avian species (Table 4, Table 5) (Mercado et al. 2010; Bliss et al. 2015; Gonzalez-Alonso-Alegre et al. 2015). A relatively lower mean IOP value of 20.4±4.1 mmHg was reported for the Humboldt penguin (*Spheniscus humboldti*), but it should be noted that this value was obtained using the applanation tonometry technique, which is known to yield significantly lower IOP values in penguins and other birds, in comparison to rebound tonometry (Swinger et al. 2009). Therefore, it is required to make a comparison of penguin IOP values obtained with the same tonometry technique (Reuter et al. 2010). Although scarce, IOP values obtained with rebound tonometry have been reported for some penguin species (Table 7). The results of the present study are in agreement with those reported in previous studies on the use of TonoVet in penguins. When compared to the IOP ranges previously reported for other avian species, the mean IOP values determined in pygoscelid penguins in the present study were found to be higher (Table 4, Table 5).

Studies available on the use of STTs in penguins are limited to the macaroni penguin (*Eudyptes chrysolophus*) and rockhopper penguin (*Eudyptes chrysocome*) (Swinger et al. 2009; Bliss et al. 2015; Woodhouse et al. 2016b; Sheldon et al. 2017), and of these studies, only 2(Swinger et al. 2009; Sheldon et al. 2017) have reported STT-1 values.(Swinger et al. 2009) reported a STT range of 1–12 mm/min and a mean STT value of 6.45 ± 2.9 mm/min for the Humboldt penguin. Different results have been reported for animals rehabilitated in freshwater and marine environments. Accordingly, researchers have reported mean STT values of 4.8 mm/min, and 8.5 mm/min for penguins kept in experimental marine and freshwater environments, respectively, and thus, have demonstrated a significant difference between the two habitats. The mean STT value of freshwater penguins was two-fold of that of marine penguins, and this was attributed to differences between the supraorbital glands of these species (Swinger et al. 2009). In avian species, the Harderian gland, situated in proximity to the base of the nictitating membrane, is the main source of tear fluid (Chieffi et al. 1996; A Bayón et al. 2007). Harris et al.(Harris et al. 2008) suggested that owls and penguins produced a smaller volume of aqueous tear owing to the smaller size or absence of lacrimal glands, when compared to other birds (Korbel et al. 1994). Similarly, Meekins et al.(Meekins et al. 2015) reported that tear production varied greatly among birds of different size and phylogenetic classification. STT-2 values previously reported for macaroni penguins (*Eudyptes chrysolophus*) and rockhopper penguins (*Eudyptes chrysocome*) kept at zoos were found to be similar to the STT-1 values detected in pygoscelid penguins in the present study (Table 6).

In their research aimed at establishing STT and IOP ranges for some raptors, Barsotti et al. (Barsotti et al. 2013) determined the presence of significant inter-species differences. When compared to values previously reported for other avian species, the mean IOP and STT-1 values determined in the present study have been observed to be similar to some values, and higher or lower than some other values (Table 7). The differences observed could be related to a marine adaptation serving as an advantage to penguins during underwater diving and foraging.

The number of ophthalmic measurements performed in the different locations vary due to the sudden changes that occurred in the Antarctic weather. While the number of penguins sampled in the Ardley SPA (Ardley III) for STT-1 measurements was 24, IOP values were measured in 33 penguins. Strong winds that blew during the visits, which made it very difficult to place the filter papers in the conjunctival fornix without causing any harm to the penguins, prevented the completion of the measurements in some animals. To avoid any stress to the animals, the restraint of the penguins was not prolonged and the measurements were not repeated. Heavy rain encountered during the visits to the Harmony Point caused the tonometer to display values outside the normal range. As the repetition of tonometer measurements would require the prolonged restraint of the animals, causing increased stress that would prevent the achievement of accurate results, IOP values were not measured. Ophthalmic measurements were not able to be made at Doumer Island/Yelchoo Base due to adverse weather conditions.

In the present study, it took time for the animals to calm down after being captured and physically restrained for clinical tests and observations. In view of the data being collected from the animals under physical restraint and with an aim to prevent any error, data was collected from only one eye in each animal. Apart from two persons being needed to restrain the animals and perform tests on them, another major difficulty encountered was placing the STT test strips in the conjunctival fornix. For the correct placement of the strips, it was required to open the eyelid and at the same time apply the test. Furthermore, given the small size of the eyes of penguins, it should be noted that placing the strips in the conjunctival fornix without touching the cornea is almost impossible, and eventually irritates the eye and causes artifactitious tear production. We consider these aspects to be important for researchers and practitioners, when diagnosing subtle pathological changes in tear production. Moreover, there is a need for further veterinary research on tear production and ocular surface measurements in penguin species other than those investigated in the present study.

In conclusion, this study presents both IOP values measured with a Tonovet® rebound tonometer and STT-1 values detected in clinically healthy pygoscelid penguins, and shows the need for further research to establish reference values for wild animals living in their natural habitat. This study will constitute a reference for future studies to be conducted in different locations and time periods.

Declarations

Ethıcs Approval

This study was performed in line with the principles of the Antarctic Environmental Protection Protocol. Also, Van Yüzüncü Yıl University Animal Experiments Local Ethics Committeehas confirmed that no ethical approval is required(dated 31 May 2018 and numbered 32).

They also stated that in these studies, no living thing belonging to the continent was harmed and that they were approved by national and international documents, including permissions, before the expedition.

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Contributions

Latife Çakir Bayram designed the research and made measurements and tests, and from the field wrote the main manuscript text , Cafer Tayer İŞLER edited and wrote the ophthalmological part of the article and Görkem EKEBAŞ prepared tables and figures.

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Tables

Table 1. Geographical position (Latitude and longitude of study locations) with ophthalmic examination types and the number of penguins made ophthalmic measurement.

Study Locations	Number of penguins†	Latitude S DD°MM' SS"	Longitude W DD°MM' SS"
1*	[ab]17	63° 19' 20"	57° 54' 04"
2	[ab]25	62° 07' 57"	58° 08' 09"
3	[b] 17	62° 18' 31"	59° 12' 34"
4	[ab]29	62° 18 '34”	58° 55' 34”
5	[ab]17	62° 12' 35”	55° 55' 43”
6	[a]33,[b]24	62° 12' 34”	58° 56' 01”

* : Study Locations Numbers:1. Cabo Legoupil/ General Bernardo O'higgins Base. 2. Lion Rump area, King George Island ASPA (151). 3. Harmony point /Nelson Island (ASPA 133).4-6. Ardley island (ASPA 150). 4.Ardley I. 5. Ardley II.6. Ardley III.

DD MM SS : Degree Minute Second

† : Type of Ophthalmic examination: [a]Intraocular pressure measurement with Icare® rebound tonometer

[b]Measurement of tear secretion by Schirmer Tear Test -1

Table 2. Comparison of tear production amounts (mm/min) by locations.

Locations	n	STT-1		95% CI		p
		Mean	SD	Lower	Upper	
O'Higgins	17	9.412	3.906	7.404	11.420	0.088
Harmony	17	12.177	5.015	9.598	14.755	
Ardley I	29	9.379	3.479	8.056	10.703	
Ardley II	17	11.177	3.844	9.200	13.153	
Ardley III	24	9.542	3.563	8.037	11.046	
Lions Rump	25	10.120	4.409	8.300	11.940	
Total	129	10.163	4.054	9.457	10.869	

SD : Standard deviation of the mean

95% CI : 95% confidence interval for mean STT-1 value

p : >0.05

Table 3. Comparison of tear production amounts (mm/min) by species.

Species	n	STT-1		95% CI		p
		Mean	SD	Lower	Upper	
Adélie	7	11.143	2.340	8.979	13.307	0.091
Chinstrap	18	11.944	4.964	9.476	14.413	
Gentoo	104	9.789	3.908	9.028	10.549	
Total	129	10.163	4.054	9.457	10.869	

SD : Standard deviation of the mean

95% CI : 95% confidence interval for mean STT-1 value

p : >0.05

Table 4. Comparison of IOP amounts (mmHg) by locations.

Locations	n	IOP		95% CI for Mean		p
		Mean	SD	Lower	Upper	
O'Higgins	17	35.412^{b}	10.272	30.131	40.693	<0.001
Ardley I	29	37.276^{b}	7.964	34.247	40.305	
Ardley II	17	36.412^{b}	9.559	31.497	41.327	
Ardley III	33	51.091^{c}	8.225	48.175	54.007	
Lions Rump	25	29.920^{a}	9.046	26.186	33.654	
Total	120	38.852	13.188	37.039	41.242	

$^{a, b, c}$: Means with a different superscript are significantly different at an alpha level of 0.05 according to Tukey's HSD test.

SD : Standard deviation of the mean

95% CI : 95% confidence interval for mean IOP value

p : <0.001

Table 5. Comparison of IOP amounts (mmHg)by species.

Species	n	IOP		95% CI for Mean		p
		Mean	SD	Lower	Upper	
Adélie	7	38.429	14.831	24.712	52.145	0.854
Gentoo	113	39.274	11.545	37.122	41.426	
Total	120	38.852	13.188	37.039	41.242	

SD : Standard deviation of the mean

95% CI : 95% confidence interval for mean IOP value

p : >0.05

Table 6. Reference values of intraocular pressure and tear production from the healthy eyes of 8 breeds of of captivity and nonAntarctic penguins as determined by Schirmer tear test-1 strips and Icare® rebound tonometer .

	Tear production (mm)/min				IOP technique, mm Hg				References
Species	**Technique**	**mean**	**±**	**SD, range**	**Tonometer**	**mean**	**±**	**SD,range**	
Humboldt penguin *(Spheniscus humboldti)*	(STT-I)	9	±	4, (2-20)	(TP-D)	28	±	9, (3-49)	Sheldon, et al (2016)
	(STT-I)	6.45	±	2.9, (1-12)	(TV-XL)	20.4	±	4.1	Swinger RL, 2009
Macaroni penguin *(Eudyptes chrysolophus)*	(STT-II)	12.1	±	5.43	(TV-D)	29.1	±	7.1	Bliss CD
			±		(TV-D)	42.0	±	9,7	Woodhouse SJ. 2016
Rockhopper penguin *(Eudyptes chrysocome)*	(STT-II)	11.0	±	3.96	(TV-D)	24.1	±	5.09	Bliss CD,2015
					(TV-D)	32.9	±	6.2	Woodhouse SJ. 2016
Black-footed penguin *(Spheniscus demersus)*					TV-D; Icare®)	31.8	±	3.3	Gonzalez-Alonso-Alegre EM 2015
					TV-D	30.4	±	4.3 OD	Mercado, J. A.2010
					TV-D	28.1	±	6.8 OS	
					TV-H	25.06	±	4.35 OD	
					TV-H	25.05	±	5.56 OS	
• Southern Rockhopper *(Eudypteschrysocome)* • Gentoo penguin *(Pygoscelis papua)* • King penguin *(Aptenodytespatagonicus)* • Chinstrap penguins *(Pygoscelis antarctica)*					Tono- Pen XL®;	6	±	4-13	Church,M.2018
					TonoVet®-	16	±	4-22	

OD: Rigt Eye ; OS:Left Eye

Table 7. Summary of intraocular pressure (IOP) and tear production data from studies Icare®rebound tonometry and the Schirmer tear test in Avian species

Species	Tear production (mm/min) STT-1				IOP (mmHg)				References
	mean	±	SD	range	mean	±	SD	Range (technique)	
American flamingo (Phoenicopterus ruber)					11.1	±	2.3	8–21 (56) OS (TonoVet®-P)	Molter et al.2014
					10.9	±	1.8	7–15 (28) OD(TonoVet®-P)	Molter et al.2014
	12.3	±	4.5	4–20 (18)	9.5	±	1.7	7–13 (16) (TonoVet®-P)	Meekins et al.2015
					16.1	±	4.2	(Tonopen XL)	Meekins et al.2015
Barn owl17 (Tyto alba)					10.8	±	3.8	5–16 (6) (TonoVet®-D)	Reuter et al.2011
Common kestrel (Falco tinnunculus)					9.8	±	2.5	4–15 (141) (TonoVet®-D)	Reuter et al.2011
Eurasian sparrowhawk17 *(Accipiter nisus)*					15.5	±	2.5	10–23 (47) (TonoVet®-D)	Reuter et al.2011
Long-eared owl17 *(Asio otus)*					7.8	±	3.2	4-13 (21) (TonoVet®- D)	Reuter et al.2011
Northern goshawk17 *(Accipiter gentilis)*					18.3	±	3.8	12–29 (58) (TonoVet® -D)	Reuter et al.2011
Peregrine falcon17 *(Falco peregrinus)*					12. 75	±	8.00	5–21 (7)	Reuter et al.2011
Red kite17 *(Milvus milvus)*					13.0	±	5.5	4–19 (8) TonoVet® -D)	Reuter et al.2011
White-tailed sea eagle17 *(Haliaeetus albicilla)*					26.9	±	5.8	17–41 (29)	Reuter et al.2011
Tawny owl *(Strix aluco)*					9.4	±	4.1	3.0-17 (27)	Reuter et al.2011
Long-eared owl *(Asio otus)*					7.8	±	3.2	4.0-13.0 (21) TonoVet®-D	Reuter et al.2011
Eurasian Sparrowhawk *(Accipiter nisus)*					15.5	±	2.5	10.0- 23.0 (47)	Reuter et al.2011
Common Buzzard *(Buteo buteo)*					26.9	±	7.0	14.0- 44.0 (86)	Reuter et al.2011
Eurasian eagle owl *(Bubo bubo)*					10.5	±	1.6	7–14 (20) TonoVet ®-P	Jeong et al.2007
					9.35	±	1.81	TonoPen®XL ®	Jeong et al.2007
Bald eagle12 *(Haliaeetus leucocephalus)*	14	±	2	8–19 (32)	21,5	±	1.7	Tonopen® XL	Kuhn et al.2013

Amazon parrots *(Amazona ventralis)*	7.9	±	2.6	0-13 (48)					Storey et al.2009
Common buzzard *(Buteo buteo)*	12.5	±	2.7					(20) Tonopen® XL	Barsotti at al.2013
Eurasian tawny owl *(Strix aluco)*	3.12	±	1.92					(20)	Barsotti at al.2013
Little owl	3.5	±	1.96					(20) Tonopen® XL	Barsotti at al.2013
European kestrel	6.20	±	3.67					(20)	Barsotti at al.2013
Ostrich *(Struthio camelus)*	16.3	±	5 (40)					13.0–22.5 (40) Tono-Pen Vet®	Gaffari et al.2012
Duck	6.2	±	2.2 (96)		10.2	±	2.2		Ansari Mood et al. 2017
Geese	5.5	±	2.6 (104)		9.1	±	2.0	TonoVet ®-P, icare	Ansari Mood et al. 2017
Pigeon					6.0	±	0.9	OD 3-9 (100)	Ansari Mood et al.2016
					6.1	±	1.0	6.1±0.9 OS TonoVet ®-P (100)	Ansari Mood et al.2016
Cooper's Hawk				9.0-12.0	10.7	±	1.4	9.0-12.0 (TonoVet®-Icare-P) (6)	Labella et al.2012
Turkey vulture				10.0-12.0	11.7	±	1.0	10.0-12.0 (6)	Labella et al.2012
Red -Taileed Hawk				14.0-34.0	19.8	±	4.9	14.0-34.0 (44)	Labella et al.2012
American Kestrel				8.0-9.0	6.8	±	1.7	5.0-9.0 (8)	Labella et al.2012
Eastern Screech owl					6.3	±	1.3	5.0-8.0 (4)	Labella et al.2012
Great -Horned Owl					9.9	±	2.2	6.0-14.0 (15)	Labella et al.2012
Barn owl *(Tyto alba)*	3.6	±	2.2 (29)					Tonopen® XL	Beckwith et al.2015
Scops owl *(Otus scops)*	1.0	±	0.5 (23)						Beckwith et al.2015
Long -eared owl *(Asio Otus)*	1.25	±	1.00 (4)						Beckwith et al.2015
Little owl *(Athena noctua)*	2.5	±	0.7 (4)						Beckwith et al.2015
Eurasian eagle-owl *(Bubo bubo interpositus)*	12.0	±	7.0 (4)						Beckwith et al.2015

Pharaoh eagle owl *(Bubo bubo ascalaphus)*	15	±	0 (2)						Beckwith et al.2015
Black kite *(Milvus migrans)*	7.4	±	5.7 (10)						Beckwith et al.2015
European Honey buzzard *(Pernis apivorus)*	7.5	±	2.2 (18)						Beckwith et al.2015
Western march harrier *(Circus aeruginosus)*	12.0	±	5.6 (4)						Beckwith et al.2015
Short-toed Snake-eagle *(Circaetus gallicus)*	7.5	±	3.5 (4)						Beckwith et al.2015
Montagus Harrier *(Circus pygargus)*	8.0	±	2.8 (4)						Beckwith et al.2015
Common buzzard *(Buteo buteo)*	13.7	±	4.4 (20)						Beckwith et al.2015
Steppe buzzard *(Buteo buteo vulpinus)*	3.0	±	0 (2)						Beckwith et al.2015
Long-legged Buzzard *(Buteo rufinus)*	12.5	±	10.0 (4)						Beckwith et al.2015
Common kestrel *(Falco tinnunculus)*	5.8	±	4.0 (2)						Beckwith et al.2015
Barbary falcon *(Falco pelegrinoides)*	3.0	±	0.0 (2)						Beckwith et al.2015
Lesser kestrel *(Falco naumanni)*	2.0	±	0.0 (2)						Beckwith et al.2015
Eastern Screech owl	2	<	median	2-6	9.0	±	1.8	(6-14) (22) TonoVet®-P	Harris et al. 2008
					14.0	±	2.4	(9-20) (22) TonoVet®-D	Harris et al. 2008
American white pelicans (Pelecanus erythrorhynchos)					9.0	±	1.41	TonoVet®-P	Kinney et al. 2017
Great Rhea				21 OD 20 OS				Tonopen® XL	Church et al. 2013
Chicken					17.51	±	0.13	(210) TonoVet®-Icare	Prashar et al. 2007
Great grey owls (Strix nebulosa)	9.8	±	2.8	5.0-16.0 (23)	9.6	±	2.6	4.0- 14.0 TonoVet®-P, Icare	Wills et al. 2016

Snowy owls (Bubo scandiacus)	9.8	±	2.4	6.0-15.0 (19)	9.1	±	1.9	4.0- 12.0 TonoVet®-P, Icare	Wills et al. 2016

Figures

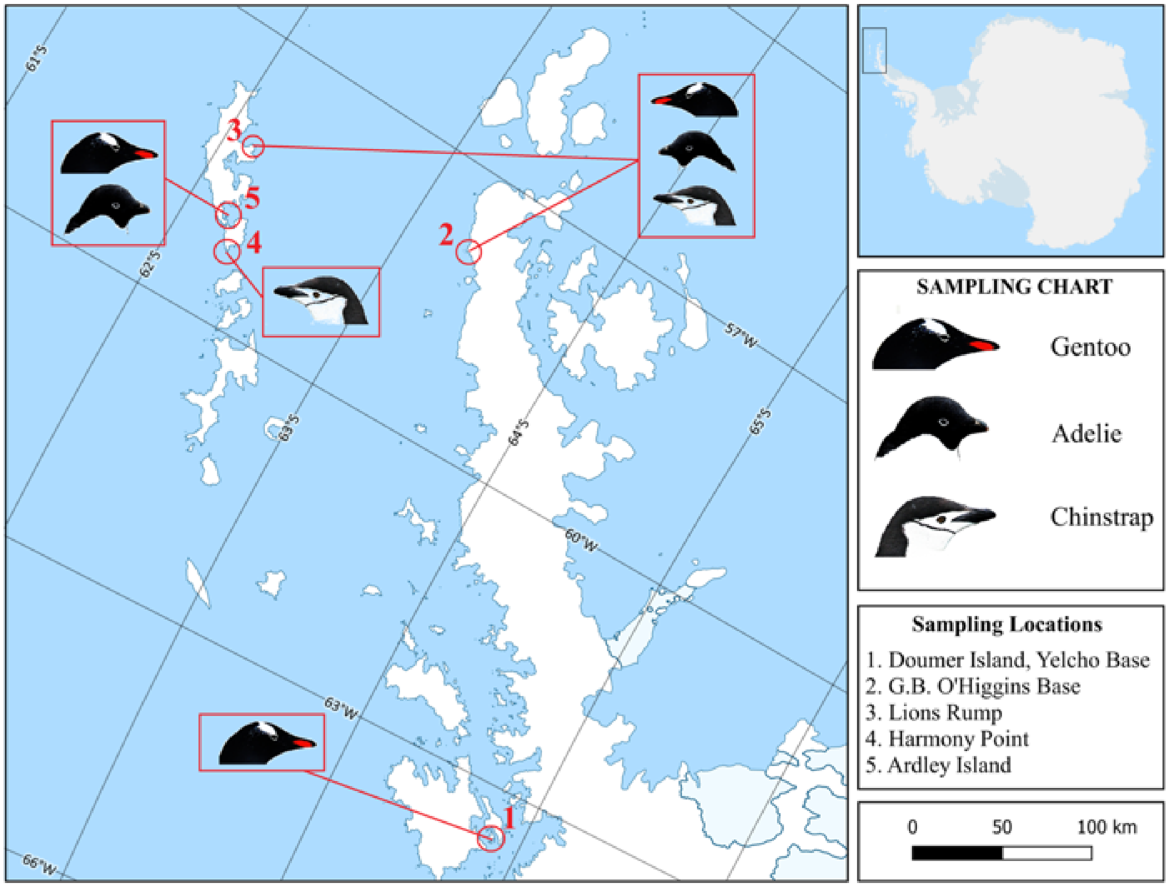

Figure 1

Location of the study area. Red dots indicate the location of the colonies of Pygoscelid penguins

Figure 2

Normal eye of a three species of pygoscelid penguins (Pygoscelis). Nictitating membranes can be seen slightly covering the eye of A) Gentoo penguin, *Pygoscelis papua* (yellow arrow), B) Chinstrap penguin, *Pygoscelis antarctica* (yellow arrow), C) Adélie penguin, *Pygoscelis adeliae (red arrow).* The transparent nictitating membrane is apparent ventromedially (arrows). D-I) Ophthalmic evaluation of pygoscelid penguins (Pygoscelis) that was subjected to STT-1 (D-F), with the TonoVetâ (Icareâ, Finland ,Oy) rebound tonometer (G-I). D) Schirmer tear test being performed on an, Adélie penguin, *Pygoscelis adeliae .* The strip had been inserted into the inferior lateral conjunctival fornix. Strips were best situated in the temporal third of the ventral conjuntival fornix to avoid displacement by the nictitans. E) The image depicts the placement of a Schirmer tear strip in the lower conjunctival fornix on Gentoo penguin F) The strip was then removed, and the tear production was measured in millimeters H-I) Manual restraint of penguins during exam. Measurement of intraocular pressure using a veterinary rebound tonometer on the P setting in an pygoscelid penguins. The Icareâ rebound tonometer measuring intraocular pressure in the left eye of an gentoo penguin. The probe is positioned approximately 5 mm from the corneal surface before deployment.

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