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Photoperiodic regime influences onset of lens opacities in a non-human primate

Marko Dubicanac [Corresp., 1], **Julia Strueve** [2], **Nadine Mestre-Frances** [3], **Jean-Michel Verdier** [3], **Elke Zimmermann** [1], **Marine Joly** [4]

[1] Institute of Zoology, Tierärztliche Hochschule Hannover, Hanover, Lower Saxony, Germany

[2] Clinic for Small Animals, Tierärztliche Hochschule Hannover, Hanover, Lower Saxony, Germany

[3] Department of Health and Biology, INSERM, Montpellier, France

[4] Centre for Comparative and Evolutionary Psychology, University of Portsmouth, Portsmouth, United Kingdom

Corresponding Author: Marko Dubicanac
Email address: marko.dubicanac@tiho-hannover.de

Background. Opacities of the lens are typical age related phenomena which have high influence on photoreception and consequently circadian rhythm. In mouse lemurs, a small bodied non-human primate, a high incidence (more than 50% when > 7 years) of cataract has been previously described during aging. Previous studies showed that photoperiodical induced accelerated annual rhythms alter some of mouse lemurs' life history traits. Whether a modification of photoperiod also affects the onset of age dependent lens opacities has not been investigated so far. The aim of this study was therefore to characterize the type of opacity and the mouse lemurs' age at its onset in two colonies with different photoperiodic regimen. **Methods.** Two of the largest mouse lemur colonies in Europe have been investigated; Colony 1 with a natural annual photoperiodic regime and Colony 2 with an induced accelerated annual cycle. A Slit-lamp was used to determine opacities in the lens and a subset of all animals which showed no opacities in the lens nucleus in the first examination but developed first changes in the following examination were further used to estimate the age at onset of opacities. In total 387 animals were examined and 57 represent the subset for age at onset estimation. **Results.** The first and most common observable opacity in the lens was nuclear sclerosis. Mouse lemurs from Colony 1 showed a delayed onset of nuclear sclerosis compared to mouse lemurs of Colony 2 (4.35 ± 1.50 years *vs.* 2.75 ± 0.99 years). For colony 1, the chronological age was equivalent to the number of seasonal cycles experienced by the mouse lemurs. For colony 2, in which seasonal cycles are accelerated by factor 1.5, mouse lemurs had experienced 4.13 ± 1.50 seasonal cycles in 2.75 ± 0.99 chronological years. **Discussion.** Our study showed clear differences in the age at the onset of nuclear sclerosis formation between lemurs kept under different photoperiodic regimes. Instead of measuring the chronological age, the number of seasonal cycles (N = 4) experienced by a mouse lemur

can be used as an estimation for risk of beginning NS formation. Ophthalmological investigations should be taken into account when animals older than 5 - 6 seasonal cycles are used for experiments in which unrestricted visual ability has to be ensured. This study is the first to assess and demonstrate the influence of annual photoperiod regime on the incidence of lens opacities in a non-human primate.

Photoperiodic regime influences onset of lens opacities in a non-human primate

Marko Dubicanac[1], Julia Strüve[2], Nadine Mestre-Francés[3], Jean-Michel Verdier[3], Elke Zimmermann[1], Marine Joly[1,4]

julia.strueve@tiho-hannover.de; jean-michel.verdier@univ-montp2.fr; nadine.frances-mestre@univ-montp2.fr; elke.zimmermann@tiho-hannover.de; marine.joly@port.ac.uk

[1]Institute of Zoology, University of Veterinary Medicine Hannover, Bünteweg 17, 30559 Hannover, Germany

[2]Small Animal Clinic, University of Veterinary Medicine Hannover, Bünteweg 4, 30559 Hannover, Germany

[3]Molecular Mechanisms in Neurodegenerative Diseases (MMDN) Univ. Montpellier, Montpellier, F-34095 France; Inserm, U1198, Montpellier, F-34095 France; EPHE, Paris, F-75014 France; PSL Research University, Paris, F-75005 France

[4]Present address: Centre for Comparative and Evolutionary Psychology, King Henry Building, King Henry 1st Street, Portsmouth, PO1 2DY, United Kingdom

Corresponding author:

Marko Dubicanac[1]

Email: marko.dubicanac@tiho-hannover.de

Abstract

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described during aging. Previous studies showed that photoperiodical induced accelerated annual rhythms alter some of mouse lemurs' life history traits. Whether a modification of photoperiod also affects the onset of age dependent lens opacities has not been investigated so far. The aim of this study was therefore to characterize the type of opacity and the mouse lemurs' age at its onset in two colonies with different photoperiodic regimen.

Methods. Two of the largest mouse lemur colonies in Europe have been investigated; Colony 1 with a natural annual photoperiodic regime and Colony 2 with an induced accelerated annual cycle. A Slit-lamp was used to determine opacities in the lens and a subset of all animals which showed no opacities in the lens nucleus in the first examination but developed first changes in the following examination were further used to estimate the age at onset of opacities. In total 387 animals were examined and 57 represent the subset for age at onset estimation.

Results. The first and most common observable opacity in the lens was nuclear sclerosis. Mouse lemurs from Colony 1 showed a delayed onset of nuclear sclerosis compared to mouse lemurs of Colony 2 (4.35 ± 1.50 years *vs.* 2.75 ± 0.99 years). For colony 1, the chronological age was equivalent to the number of seasonal cycles experienced by the mouse lemurs. For colony 2, in which seasonal cycles are accelerated by factor 1.5, mouse lemurs had experienced 4.13 ± 1.50 seasonal cycles in 2.75 ± 0.99 chronological years.

Discussion. Our study showed clear differences in the age at the onset of nuclear sclerosis formation between lemurs kept under different photoperiodic regimes. Instead of measuring the chronological age, the number of seasonal cycles (N = 4) experienced by a mouse lemur can be used as an estimation for risk of beginning NS formation. Ophthalmological investigations should be taken into account when animals older than 5 - 6 seasonal cycles are used for experiments in which unrestricted visual ability has to be ensured. This study is the first to assess and demonstrate the influence of annual photoperiod regime on the incidence of lens opacities in a non-human primate.

Introduction

Opacities of the lens are typical age-dependent pathologies with high impact on circadian rhythm in humans. Cataract even represents the most common cause for visual restriction in elderly humans (Pascolini, Mariotti. 2012). It is a phenomenology causing opacities in the lens which start as small dots or thin strings. The opacity eventually can occupy the whole lens and highly impacts eyesight which can result in complete blindness. Cataract and its different forms can be caused by a huge variety of reasons like e.g. age (Truscott. 2005; Vinson. 2006; Yoon, Kim, Shin. 2015; Žorić, Miric, Kisic. 2015), radiation e.g. UV-light, see for instance (Cruickshanks, Klein, Klein. 1992; Delcourt et al. 2000; Tang et al. 2015), malnutrition (Heseker. 1995; Ohta et al. 1997; Meyer, Sekundo. 2005; Nourmohammadi et al. 2008; Yonova-Doing et al. 2016) or metabolic diseases (Miglior et al. 1994; Klein et al. 1995; McCarty et al. 1999).

A typical manifestation in the aging lens is age related nuclear cataract (ARN cataract) which starts forming in the centre until it finally fills out the whole lens. Oxidation seems to play a central role in the pathogenesis of this special form (Truscott. 2005; Vinson. 2006; Yoon, Kim, Shin. 2015; Žorić, Miric, Kisic. 2015). Antioxidants, especially reduced glutathione (GSH) are important defence-mechanisms which protect the lens proteins from oxidation. Lens nuclei affected by cataract show significantly lowered concentrations of GSH. This may be due to the aging lens which starts to form a barrier around the nucleus which prevents GSH and other antioxidants to enter the core (Sweeney, Truscott. 1998; Moffat et al. 1999). As a result oxidised proteins in the core start to accumulate and form light scattering structures which prevent light from reaching the retina and finally occupying the whole lens which cause total blindness.

An important differential diagnosis to ARN cataract is nuclear sclerosis (NS). NS is caused by an increased density of nuclei in the lens core and represents a physiological progress of the aging lens. It affects farsightedness more than near vision, is not discussed to cause complete blindness but reduced vision and plays part in the formation of presbyopia. Since the lens capsule cannot expend, the remaining nuclei of apoptotic cells as well as chromophores and insoluble crystalline aggregates are more and more compressed in the center of the lens which finally leads to visual opacities restricted to the lens core (Gwin, Gelatt. 1981; Glover, Constantinescu. 1997; Keil, Davidson. 2001). The genetic background is still not clear but studies suggest a polygenetic and environmental impact on familial aggregation instead of one major gene in humans (Klein et al. 2005). In contrast to ARN cataract even advanced stages of NS do allow an investigation of the retina by using indirect ophthalmoscopy and therefore can optically be differentiated from each other.

Cataract is not only found in the human lens, it is also commonly described in various animals like e. g. dogs (Rubin. 1974; Narfstrom. 1981; Gelatt et al. 2003), horses (Matthews. 2000) as well as in non-human primates like macaques (Sasaki et al. 2011) and also frequently in the gray mouse lemur (Beltran et al. 2007).

The gray mouse lemur belongs to the smallest primates worldwide (Mittermeier et al. 2010). Mouse lemurs are nocturnal and therefore have developed relatively large eye sizes (9.4 mm in diameter) compared to their skull (Kirk. 2004; Ross, Kirk. 2007). The combination of a relatively large eye size and higher life expectancy in captivity makes mouse lemurs highly prone to eye diseases. A whole variance of diseases have already been determined (Beltran et al. 2007), as e.g. corneal degeneration and dystrophy, pupil seclusion and most frequently cataract. In animals older than 7 years, cataract was diagnosed in more than 50% of all investigated animals by Beltran et al. Most frequently anterior and posterior subcapsular cataract was described

92 concerning incipient cataract forms and all stages of progression were observed (incipient,
93 immature, mature and hypermature). The lack of adequate animal models in cataract research
94 and the high incidence of cataract in mouse lemurs make these animals a highly interesting model
95 (Truscott. 2011).
96 Nowadays the gray mouse lemur (*Microcebus murinus*) is suggested to represent a promising
97 non-human primate model in aging (Perret. 1997; Cayetanot et al. 2005; Gomez et al. 2012;
98 Languille et al. 2012; Zimmermann, Radespiel. 2014; Zimmermann et al. 2016) and Alzheimer's
99 research (Austad, Fischer. 2011; Verdier et al. 2015). With a life expectancy of about 8 years in
100 the wild (Zimmermann et al. 2016) and up to 18.5 years (Weigl, Jones. 2005) in captivity, mouse
101 lemurs live much shorter than other non-human primates. Most notably are deficiencies in
102 behaviour and cognition (Nemoz-Bertholet, Aujard. 2003; Joly, Deputte, Verdier. 2006; Trouche
103 et al. 2010; Joly et al. 2014), aggregation of abnormal phosphorylated tau protein (Bons et al.
104 1995) and ß-amyloid plaques (Mestre-Frances et al. 2000) as well as cerebral atrophy (Dhenain et
105 al. 2000; Kraska et al. 2011). Mouse lemurs are also in the centre of interest for evolutionary
106 research since they show highly flexible adaptations to their natural habitats and a high cryptic
107 diversity between species (Zimmermann, Radespiel. 2014). The Broad Institute has also recently
108 sequenced the genome of mouse lemurs (GenBank accession number ABDC00000000).
109 The photoperiod has major impact on the annual rhythm of mouse lemurs regarding physiological
110 constitutions like body weight, locomotion, lifespan and sexual function (Perret. 1997; Cayetanot
111 et al. 2005) or life history patterns such as female body mass at first reproduction, female age at
112 first reproduction as well as longevity (Zimmermann et al. 2016). As long-day breeders mouse
113 lemurs breed when day-length overstep 12 hours of sunlight (rainy season/summertime in
114 Madagascar) which applies to six months out of twelve months per year under natural conditions
115 (Perret. 1997). Under artificially accelerated light conditions the non-breeding as well as the
116 breeding season can be shortened to a total amount of 8 months which equally accelerates the

reproductive capability. This physiological characteristic revealed an interesting peculiarity in the aging mechanism in mouse lemurs. Animals held under accelerated photoperiodic conditions age faster and show typical age related symptoms earlier e.g. grey fur around the eyes and flatting of the snout as well as age dependent pathologies like cataract (Perret. 1997; Dubicanac et al. 2014), have lower body weight, show locomotion activity patterns which resemble those of aged mouse lemurs, have a shortened lifespan equivalent to the shortened photoperiodic year and males show earlier sexual activity (Perret. 1997; Cayetanot et al. 2005). This dependency is due to the alternance of periods of dry and wet season which strictly dictates breeding seasons in these parts of Madagascar. The age of these animals seems therefore to be based on the numbers of seasonal cycles instead of chronological age (Perret. 1997).

The main aim of this study was to characterize the type of the beginning opacity and to compare the age at onset in two colonies with different photoperiodic regimes. Since it was described that mouse lemurs age faster when kept under accelerated photoperiodic cycles, we hypothesize that animals kept under accelerated photoperiodic cycles should develop age-related cataract and/or nuclear sclerosis earlier than animals kept under a normal photoperiodic regime.

Material & Methods

Animals and maintenance

We examined mouse lemurs (*Microcebus murinus*) housed in two licensed breeding colonies kept under different photoperiodic regimes, at the Institute of Zoology at the University of Veterinary Medicine Hannover, Germany (for details in housing conditions see (Wrogemann, Radespiel, Zimmermann. 2001); Hannover breeding license number 42500/1H) and at the University of Montpellier 2, France (Agreement No. ≠ C-34-172-23). The animals are kept in cages with up to

four individuals at constant temperature and humidity, have unrestricted access to water and receive fresh food each day. In both facilities all animals were born in captivity and kept under artificial light conditions with a reversed light cycle.

The photoperiodic regime in Hannover (Colony 1) is based on annual photoperiodic cycles in Madagascar. The photoperiodic year lasts 12 months (8 months long-day period and 4 months short-day period). In Montpellier (Colony 2) the photoperiodic regime is accelerated. Therefore the photoperiodically triggered reproductive "year" lasts 8 months (5 month long-day period and 3 months short-day period) instead of 12 months. Studies show that aging processes in gray mouse lemurs can be accelerated by the factor 1.5 when kept under these conditions (Perret. 1997; Languille et al. 2012; Dubicanac et al. 2014; Zimmermann et al. 2016).

Colony 1 was investigated three times, between March and April in 2012, 2013 and 2014. Colony 2 has been investigated twice in May 2012 and 1 year later, in May 2013. In total 387 animals have been investigated, 100 animals in colony 1 (49 males, 51 females) and 287 animals in colony 2 (130 males, 157 females) ranging from 3 months to 13.6 years. To determine potential eye diseases each animal underwent an ophthalmological investigation.

Ophthalmologic investigation

The examinations in this purely observational study were all licensed by the respective authorities (Hannover licence number, 33.9-42502-05-11A200, LAVES to Elke Zimmermann; Montpellier license number, B-34-8 to Nadine Mestre-Frances) and comply with animal care regulations, the applicable national law and adhere to the legal requirements of both countries.

All animals are habituated to weekly handling procedures for health checks minimizing stress during the ophthalmological examination. The sleeping-boxes (equipped with a lockable door) were used for transportation from the animal cage to the examination room. All examinations were conducted at the end of the sleeping period/beginning of the activity period.

In the beginning of the examination each animal's pupillary reflex was tested using the Slit-lamp. The investigation room was dimmed so the pupil can naturally widen, followed by a light impulse of the Slit-lamp to induce the reflex. A reaction under 2 seconds was considered healthy. The intraocular pressure was determined using indirect tonometry (TonoVet®; ICare, Finland Oy). For the valuation of the measured values we used references obtained in a previous study (Dubicanac et al. 2016). To determine potential eye pathologies, both eyes of a lemur were investigated with a slit-lamp bio-microscope (SL-14; Kowa, Eickemeyer, Germany) and indirect ophthalmoscope (Omega 100; Heine, Ettenheim, Germany). Mydriatic eye-drops (Mydrum®, Chauvin ankerpharm GmbH, Berlin, Germany) were used to widen the pupil and make the examination of the lens and retina possible. All ocular findings were noted down on a self-prepared testing sheet similar to those used in clinical ophthalmologic investigations.

Special attention within the ophthalmologic investigation was given to the slit-lamp examination. Each eye was scanned carefully to determine any kind of opacity within the lens. While any kind of opacity was noted and estimated in size, special attention was given to opacities in the centre of the lens. Therefore all animals showing no opacities during the first investigation were reinvestigated with special patience in the following year.

UV-light

UV-light emission has been measured using a UV-light meter (UV LIGHT METER, YK-35UV, Lutron Electronic Enterprise Co., LTD., Taiwan). The measurable wavelength reached from 290-390 nm and therefore includes the UV-A/-B range. The measurement was carried out when the white light was turned on "day-time" as well as when the white light was turned off and red light turned on "night-time". In both facilities no UV-light could be detected. The measured value at "day-time" as well as at "night-time" was zero for all rooms ($W/m^2 = 0$).

Data Analysis

For each colony we determined the number of animals showing any kind of opacity and the age at its onset by selecting animals which showed no opacity in the first investigation (see Figure.1), but were positively tested in the second investigation one year later (see Figure.2). This subset consists of 27 animals from colony 1 and 30 animals from colony 2 which all showed nuclear sclerosis as the first observable opacity. The age groups of both facilities were separately analysed for mean, range, standard deviation and median. Animals showing other pathologies than cataract or nuclear sclerosis were excluded from the analysis. Findings for both colonies were compared using the Mann-Whitney-U test and either chronological age and or the number of seasonal cycles.

Software for statistical analysis

All statistical analysis was performed using SPSS 23.0 for Windows. Significance level was set at $P = 0.05$.

Results

Overall diagnosed eye pathologies in both colonies

51 out of 100 (51 %) in colony 1 and 192 out of 287 (66.9 %) in colony 2 showed certain stages of cataract and/or NS. (see Table 1.) Out of these animals which showed opacities, NS was the most frequent opacity (45 out of 51 (88.2 %) cases in colony 1; 184 out of 192 (95.8 %) cases in colony 2) followed by incipient anterior cortical cataract (28 out of 51 (54.9 %) cases in colony 1; 86 out of 192 (44.8 %) cases in colony 2). Other less frequent findings were incipient posterior cortical cataract (6 cases in colony 1; 15 cases in colony 2), incipient anterior & posterior

207 epithelial cataract (3 cases in colony 1; 6 cases in colony 2), immature nuclear cataract (1 case in
208 colony 2) and mature cataract (5 cases in colony 1).
209 Other pathologies like glaucoma, synechia, corneal degeneration, hyphema, posterior lens
210 luxation and phthisis bulbi were diagnosed sporadically.

211 **Cataract and NS findings during aging**

212 The first visual opacity the investigated mouse lemurs developed was nuclear sclerosis, which
213 usually becomes denser with age. Indirect ophthalmological investigations of the retina through
214 this type of opacity are still possible (see Figure.2). Some older animals (n = 4; ≥ 10 years) were
215 additionally affected by nuclear cataract. This type of opacity does not allow a complete free
216 investigation of the retina (see Figure.3). Animals frequently showed cortical cataract in addition
217 to NS and/or nuclear cataract (n = 107; ≥ 1.7 years) (see Figure.3). In several animals mature
218 cataract could be observed (see Figure.4).

219 **Onset of lens opacity in the colonies**

220 In all animals nuclear sclerosis was the first observable opacity. In colony 1 the mean age of
221 animals with first signs of nuclear sclerosis was 4.35 ± 1.50 years (the median was 3.9 years; the
222 range was 1.8 – 7.9 years). In colony 2, the mean age was 2.75 ± 0.99 years (the median was 2.5
223 years; the range was 1.5 – 5.3 years). For colony 1, the chronological age was equivalent to the
224 number of seasonal cycles experienced by the mouse lemurs. For colony 2, mouse lemurs had
225 experienced 4.13 ± SD 1.50 cycles (median = 3.73 years; range = 2.2 – 8.0 years) before showing
226 first signs of lens opacity.
227 Therefore when taking into account their chronological age, mouse lemurs showing nuclear
228 sclerosis for the first time were older in colony 1 than in colony 2 (Mann-Whitney-test, N_{total} =
229 57, $N_{colony1}$= 27, $N_{colony2}$ = 30, U = -4.030, P < 0.001, see Figure.5).

This difference between colonies did not hold if the number of seasonal cycles experienced by the mouse lemurs was considered (Mann-Whitney-test, $N_{total} = 57$, $N_{colony1} = 27$, $N_{colony2} = 30$, $U = -0.424$, $P = 0.671$, see Figure.6).

Discussion

What was first, cataract or nuclear sclerosis?

In our study we identified nuclear sclerosis being the first developing age-dependent lens opacity. Indirect ophthalmological investigations of the retina through the opacity were possible in even more advanced stages of NS. The reason for complete blindness in old age on the other hand seems consequently be caused by forms of cataract like e.g. ARN cataract, which do not allow an unrestricted view of the retina and has only been found in old animals.

NS starts to form in the nucleus of the lens which still allows an ophthalmologic examination of the retina. In older humans and e.g. dogs it usually affects farsightedness more than near vision and plays a role in the development of presbyopia, while in rhesus monkeys the loss of ciliary muscle motility alone seems to influence the accommodation ability (Glover, Constantinescu. 1997; Strenk, Strenk, Koretz. 2005). In humans NS is caused by an increased density of nuclear lens cells. Since the lens capsule cannot expend, the remaining nuclei of apoptotic cells are more and more compressed what finally leads to visual opacities (Glover, Constantinescu. 1997; Keil, Davidson. 2001). However, mouse lemurs do show high incidence of progressive cataract formation in old age leading to complete blindness and therefore unlikely to be affected by nuclear sclerosis alone. An explanation of both, the possible investigation of the retina in the beginning and progressive loss of eyesight with old age, could be a combination of a physiological pathway (NS formation) and a pathogenic pathway (nuclear cataract). ARN cataract e.g. is commonly described to accompany nuclear sclerosis in veterinary medicine

(Peiffer. 1991; Glover, Constantinescu. 1997) and could explain the later loss of eyesight. For this type of cataract oxidation seems to play a central role in its pathogenesis (Truscott. 2005; Vinson. 2006; Yoon, Kim, Shin. 2015; Žorić, Miric, Kisic. 2015). Antioxidants, especially reduced glutathione (GSH) are important defence-mechanisms which protect the lens proteins from oxidation. Lens nuclei affected by cataract show significantly lowered concentrations of GSH. This may be due to the aging lens which starts to form a barrier around the nucleus which prevents GSH and other antioxidants to enter the core (Sweeney, Truscott. 1998; Moffat et al. 1999). As a result oxidised proteins in the core start to accumulate and form light scattering structures which finally can affect the whole lens and cause total blindness. In contrast to NS, ARN cataract does have an impact on the ophthalmological investigation since a disruption of the spatial arrangement of the lens fibres occurs which obscures a detailed view on the retina.

Previous examinations in these animals performed in other colonies did not observe nuclear sclerosis as the initial impairment, which usually would be expected in aging eyes in other species (Beltran et al. 2007). Although we partly confirm the high incidence of anterior and/or posterior cataract forms, in our study NS was the most common opacity followed by anterior cortical cataract. A difference in the genetic backgrounds would be the more obvious explanation since the animals in both colonies originate from different wild animal populations.

Nuclear sclerosis onset and its dependency on the photoperiod

In our study, mouse lemurs showing first signs of NS in colony 1 were significantly older than those from colony 2 (when chronological age is considered). The number of seasonal cycles experienced by mouse lemurs seems therefore to determine the onset of nuclear sclerosis. These results match well with our presumption that NS may be the cause for the initial opacity in the lens of mouse lemurs and that its onset depends more on photoperiodic cycles than on chronological age. As a physiological process NS onset is in line with other physiological aging

effects, like gray fur around the eyes and flatting of the snout, which also show progression depending on photoperiodic cycles (Perret. 1997; Cayetanot et al. 2005; Languille et al. 2012). In humans it is assumed that both environmental and polygenetic effects play a role in the etiology of NS (Klein et al. 2005). Our results in gray mouse lemurs point towards a photoperiod-dependent onset of NS. As other physiological aging processes in the gray mouse lemur the onset of NS is accelerated when photoperiodic cycles are shortened.

The effect of opacities in the lens on circadian photoreception and rhythm represents an important field in human research. In humans crystalline lens opacities progressively increase with age causing a continual loss of circadian photoreception. 10-years old humans therefore have a 10 time higher circadian photoreception than a 95-years old person (Turner, Mainster. 2008). The loss of circadian photoreception highly affects the physiological and mental state and a diversity of cardiovascular, respiratory, endocrine, rheumatological and neurological diseases has been linked to variations in circadian rhythms (Klerman. 2005).

This general loss of responsiveness to light has also been shown in aging mouse lemurs (Gomez et al. 2012). Though in our study defined minor lens opacities (like e.g. incipient lens opacity and beginning NS) are likely to have no effect on photoreception, it remains unclear which impact very dens opacities have on the mouse lemurs' photoreception. The general age-dependent loss of photoreception and the impairment of photoreception caused by lens opacities could increase each other's effects leading to severe pathophysiological changes. Further investigations could reveal changes in the physiological state similar to those in humans, making this animal model interesting for a diversity of new medical research fields.

Causes of cataract which have to be considered and excluded

Although nuclear sclerosis was the predominant opacity that we could observe, several forms of cataract were found as well (incipient anterior cortical cataract, incipient posterior cortical

cataract, incipient posterior/anterior epithelial cataract, immature nuclear cataract and mature cataract). Usually the observed forms showed slow or no progression but still can have serious impact on vision in older individuals which makes it necessary to exclude possible reasons.

UV-light seems to be predominantly associated to cortical cataract formation (Cruickshanks, Klein, Klein. 1992; Delcourt et al. 2000; Tang et al. 2015) but may potentially occur in any lens layer. Since no UV-light was detectable within the facilities we can exclude this kind of radiation as an inducing factor.

Another important factor for cataract formation is diabetes mellitus (diabetes type 2). This kind of cataract usually shows fast progressive expansion up to few months (Basher, Roberts. 1995; Beam, Correa, Davidson. 1999; Li, Wan, Zhao. 2014) and is mainly associated with cortical cataract (Miglior et al. 1994; Klein et al. 1995; McCarty et al. 1999; Li, Wan, Zhao. 2014). Although we could observe cortical cataract frequently, it showed slow or no progressive spreading.

It is unclear whether insufficient supply of antioxidative substances like vitamin E, C, B as well as essential amino acids as tryptophan, phenylalanine, histidine and carotenoids may induce or accelerate cataract development (Heseker. 1995; Ohta et al. 1997; Meyer, Sekundo. 2005; Nourmohammadi et al. 2008). At least Vitamin C is described of being protective against both nuclear cataract formation and progression in humans (Yonova-Doing et al. 2016). In both facilities regular additions of vitamins and minerals are offered in mashed fruit mixtures to ensure sufficient supply. Based on this nutrition management we preclude cataract formation caused by malnutrition in the first place.

Conclusion

In our study, nuclear sclerosis represented the earliest stage and the most common opacity in the mouse lemurs' lens. Here we showed clear differences in the onset of NS formation between two

colonies kept under different photoperiodic regimes when measured in chronological age. The number of seasonal cycles experienced by the mouse lemurs was the main determinant for the onset of the lens opacities.

The number of seasonal cycles (N = 4) experienced by a mouse lemur can be used as an estimation for risk of beginning NS formation (around 4 years in colony 1 and around 3 years in colony 2) and further studies which necessitate visual fitness in mouse lemurs. Ophthalmological investigations should be taken into account when animals older than 5 - 6 seasonal cycles are used for experiments in which unrestricted visual ability (e.g. unimpaired accommodation) has to be ensured. Due to the exceptional high incidence of opacities in the lens of mouse lemurs, this is of utmost importance for further potential aging research studies on mouse lemurs.

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List of Abbreviations

ARN cataract: age related nuclear cataract; NS: nuclear sclerosis

Authors' contributions

MD, MJ, EZ, JS have conceived, coordinated and designed the study. Data from the screening of both colonies was acquired by MD and MJ. Statistical analysis was conducted by MD. All authors contributed in drafting, reading and approving the final manuscript.

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Table 1(on next page)

Overview of cataract/NS incidences in both colonies.

This table shows the total amount of animals positively tested for cataract and/or NS at any stage.

1

	Colony 1		Colony 2	
	Number of investigated animals	Number of animals with cataract and/or NS	Number of investigated animals	Number of animals with cataract and/or NS
Age (in years)	**100**	**51**	**287**	**192**
0-1	**11**	**0**	**42**	**0**
1-2	**19**	**1**	**47**	**22**
2-3	**9**	**1**	**70**	**47**
3-4	**21**	**13**	**31**	**26**
4-5	**8**	**6**	**28**	**28**
5-6	**5**	**4**	**22**	**22**
6-7	**6**	**6**	**30**	**30**
7-8	**3**	**3**	**11**	**11**
8-9	**9**	**9**	**3**	**3**
9-10	**2**	**1**	**1**	**1**
10-11	**2**	**2**	**2**	**2**
11-12	**4**	**4**	**-**	**-**
12-13	**0**	**0**	**-**	**-**
13-14	**1**	**1**	**-**	**-**

2

Figure 1

Eye of a two year old mouse lemur.

This lens shows no opacities.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

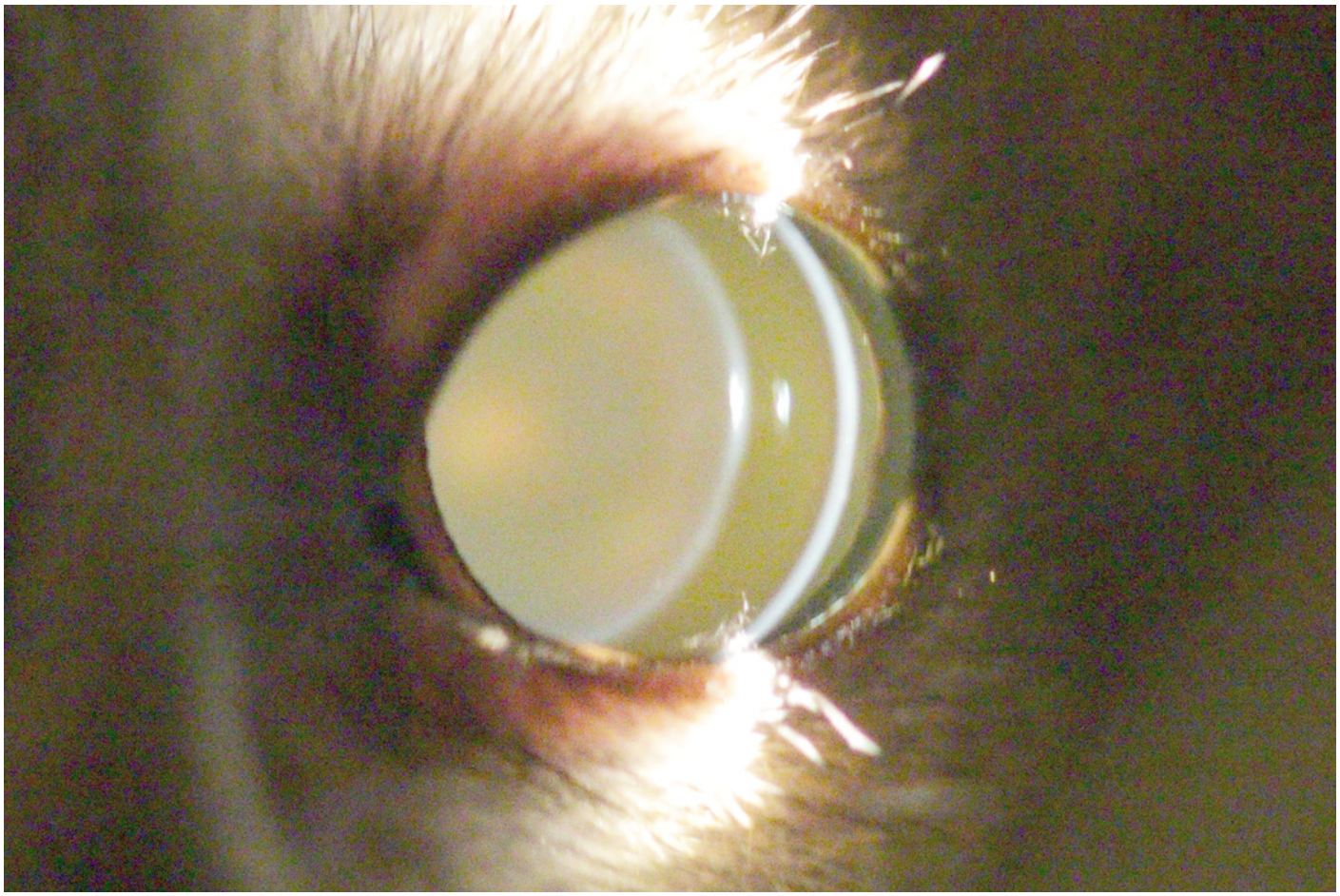

Figure 2

Eye of a four year old mouse lemur.

The lens shows first signs of nuclear sclerosis in the center of the lens.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

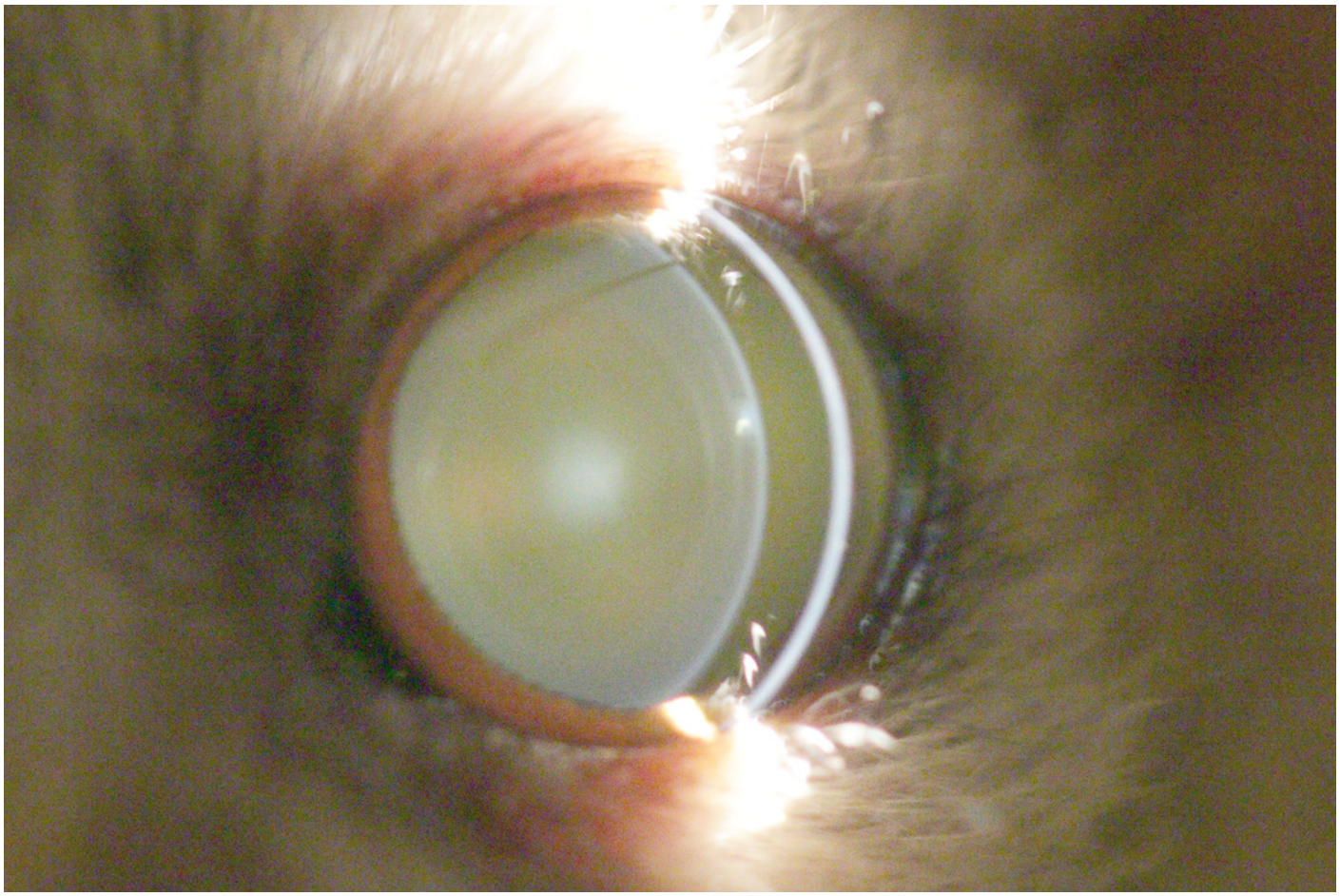

Figure 3

Eye of an eight year old mouse lemur.

The lens shows immature, nuclear cataract with additional incipient anterior cortical cataract.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

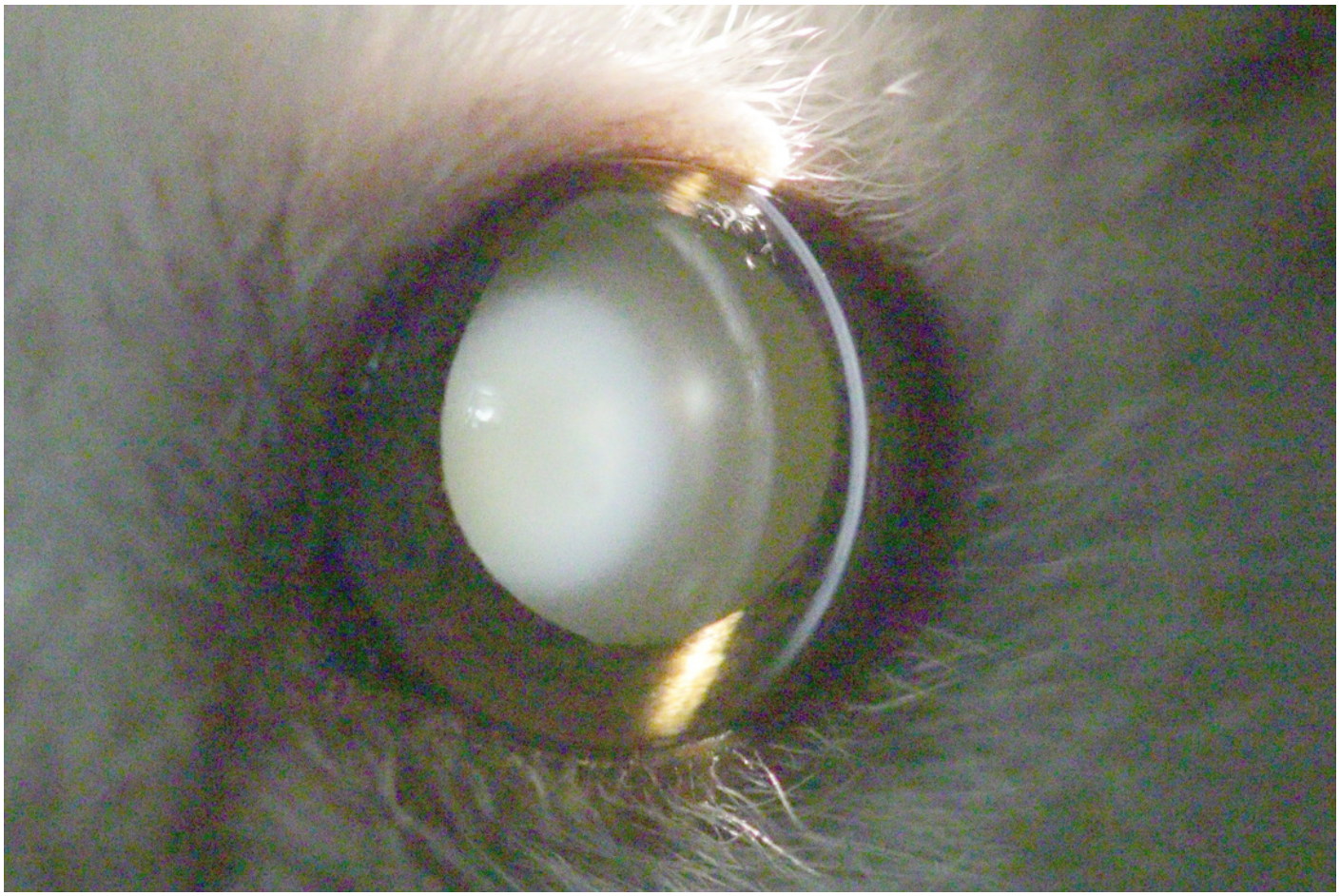

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Figure 4

Eye of an 11 year old mouse lemur.

The lens is affected by mature cataract.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

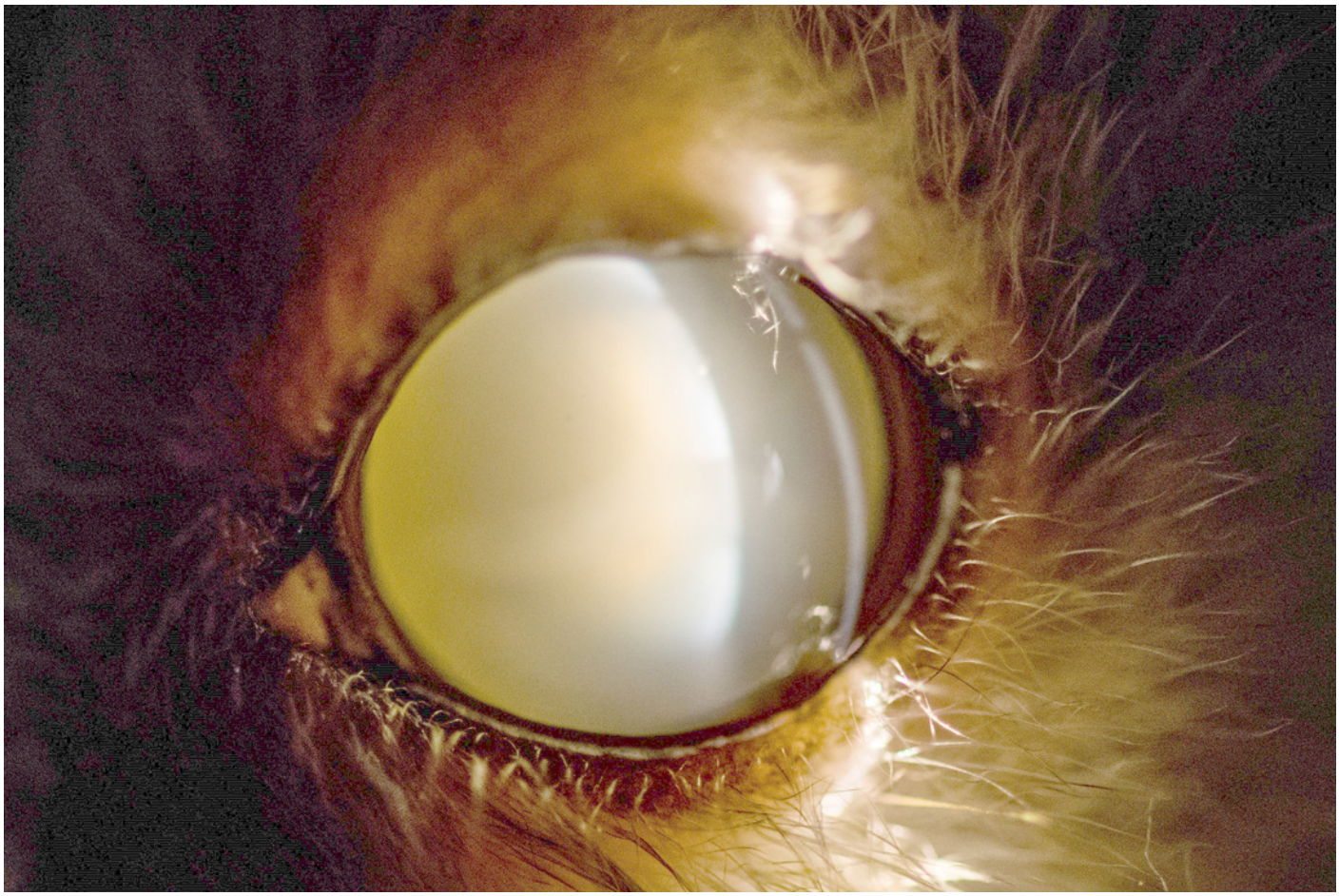

Figure 5

Age at the onset of nuclear sclerosis in mouse lemurs of two colonies with different photoperiodic cycles, measured in chronological years.

Chronological age of mouse lemurs showing first signs of nuclear sclerosis in both investigated colonies. Nuclear sclerosis is present at a significant younger age in colony 2 than colony 1 ($N1 = 27$; $N2 = 30$; $P < 0.001$).

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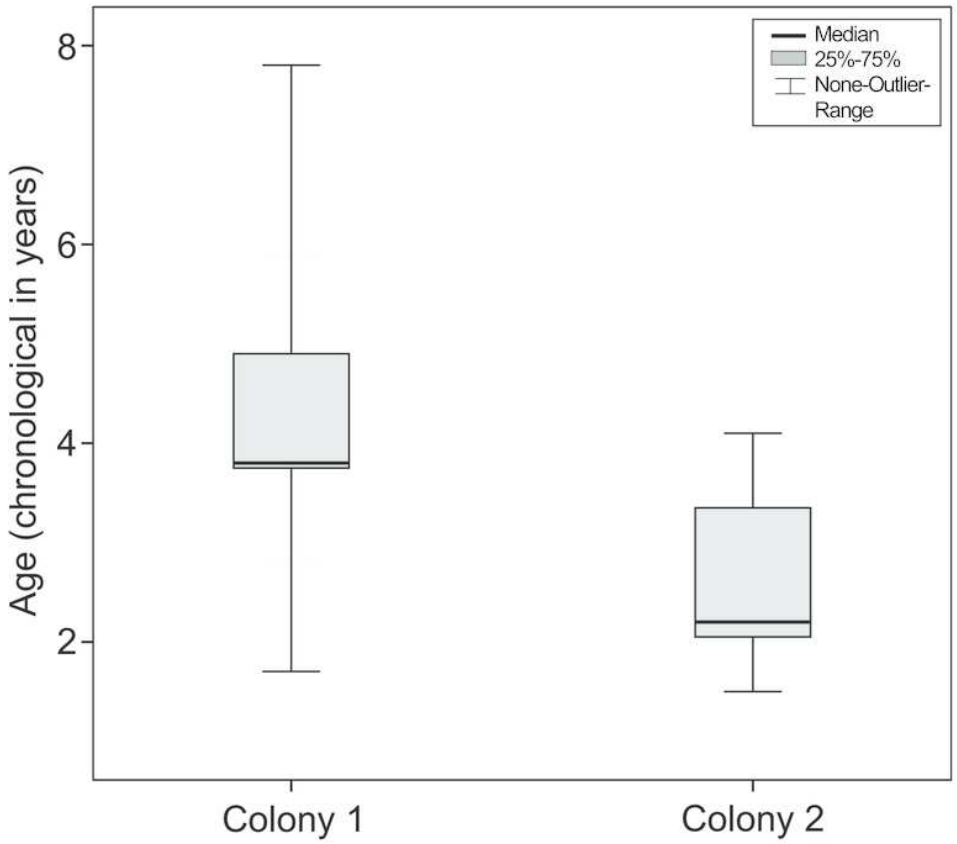

Figure 6

Age at the onset of nuclear sclerosis in mouse lemurs of two colonies with different photoperiodic cycles, measured in number of seasonal cycles.

Number of seasonal cycles experienced by mouse lemurs showing first signs of nuclear sclerosis in both investigated colonies. The onset of NS does not significantly differ between both colonies (N1 = 27; N2 = 30; P = 0.671).