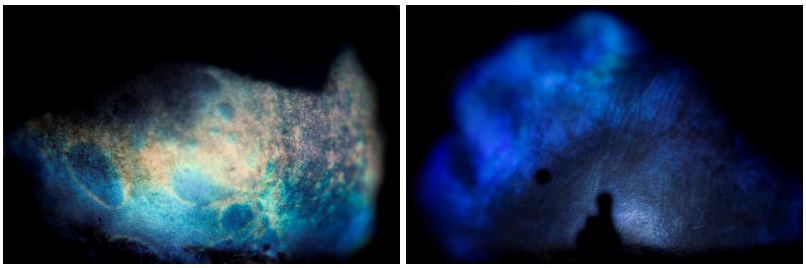
Master Thesis in Biology (60 ECTS)

Seasonal changes in the *Tapetum lucidum* as an adaptation to winter darkness in reindeer

(Rangifer tarandus tarandus)



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Summary

Reindeer (*Rangifer tarandus tarandus*) live at high latitudes and are therefore exposed to extreme changes in environmental illumination. In winter they spend months in relative darkness, in summer they are exposed to months of continuous brightness. Previous investigations have revealed a shift in the wavelength of the light being reflected by the tapetum lucidum (TL) between winter and summer. Since no seasonal changes in the TL have ever been documented in any species before, this finding raised several questions.

In the present study the following issues have been dealt with:

Is this chromatic shift triggered by changing light conditions between summer and winter or regulated by an endogenous clock?

Are there other parts of the visual system that undergo seasonal changes?

What are the physical mechanisms behind this shift?

To find out what is regulating the change in reflected light eyes from animals killed in summer and from animals killed in winter were compared to each other and to eyes from animals kept in altered light conditions.

For this five animals were kept indoors in constant darkness between January and June 2008. The animals were killed and the left eye fixed in formaldehyde. The same was done with a control group that had been kept outside in natural light conditions. Between August and December 2008 four animals were kept indoors in constant light. After this period they underwent the same treatment mentioned above. In December 2008 eyes were obtained from an abattoir in Northern Norway and fixed in formaldehyde.

The eyes of all groups were opened, the tapeta photographed and hue and saturation were analysed using MATLAB.

The statistical analysis showed that eyes from animals in natural light conditions collected in summer are significantly different in hue and saturation from eyes from animals in natural light conditions in winter. It also showed that there is no significant difference between animals experiencing natural light conditions and animals kept in altered light environment during the same season. This strongly indicates that the seasonal changes of the TL results from endogenous mechanisms, such as e.g. a

biological clock and that the prevailing light conditions have no, or only a negligible, impact.

It is known that in some animals, alterations in light conditions lead to changes in distribution of melanin granules of the retinal pigment epithelium (RPE). It has also been shown that the length of the outer segments of the photoreceptors can be influenced by shifts in illumination. Because of this both aspects were examined in the present study.

Histology showed that non-tapetal (peripheral) regions posses a proper RPE, rich in granules which are pigmented by melanin. They are less frequent towards the centre and the RPE of tapetal (central) regions only possess very few or no pigmented granules. These results are in accordance with the function of the tapetum and with findings in other species. There was no evidence to be found for seasonal differences in the pigment distribution within the cells. In both seasons the granules were located adjacent to the apical membrane and not distributed evenly within the cells.

The length of the outer segments of the photoreceptors was measured and the results in winter and summer eyes compared to each other. This showed that the outer segments are significantly shorter in winter than in summer. An unusual shape of the outer segments indicated however that formaldehyde might not have been the suitable fixative medium for this kind of histology work which questions the reliability of the results for the length of the outer segments.

In a final experiment an attempt was made to understand the physical mechanism responsible for the chromatic shift. Earlier investigations of the TL in reindeer revealed that the fibres in this structure are more densely spaced in winter than in summer. A possible explanation for this might be a seasonal change in intraocular pressure. Therefore, intraocular pressure was measured in June and December 2009 on the same group of animals. The results showed a significant seasonal difference with the pressure being higher in winter than summer. To see if change in pressure has an impact on the tapetals structural colour, an external pressure was applied to a piece of summer tapetum of a reindeer. The same was undertaken for a piece of a summer tapetum of a Scottish red deer (*Cervus elaphus scoticus*) and a cow

(*Bos primigenius*). In all three species this led to a change of the reflected light from golden-yellow to blue in reindeer and red deer and pale blue to blue in cow. This suggests that seasonal fluctuations in intraocular pressure could be the mechanism behind the changes in the TL, but also that other ungulate species might be capable of doing the same.

The findings of this thesis strongly suggest that an endogenous circannual clock-like mechanism is causing the chromatic shift in the light reflected by the TL and that seasonal changes in intraocular pressure may be a candidate for the physical mechanism behind the change.

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1. Introduction

To initiate the process of seeing in vertebrates light has to impinge photopigments in the outer segments of the photoreceptors in the retina in the form of photons. This leads to phototransduction which means that the light is converted into an electrical signal. The two types of photoreceptor found in vertebrates are rods and cones. Only cones convey chromatic information and provide high spatial resolution. Rods on the other hand are more sensitive and operate even at low luminance levels. There are a number of mechanisms that can be used to enhance vision at low luminance levels. The most marked of these is the presence of the tapetum lucidum (TL), which means “shining carpet” in Latin. This is a specialization in the back of the eye forming a reflecting structure. It has evolved in parallel in many different species, both invertebrates and vertebrates as a convergent evolution of function (Schwab et al, 2002). Depending on the species, it varies in location, composition and in physical and chemical properties. Its purpose is the same nevertheless: to enhance retinal light sensitivity in scotopic conditions (Greek: skotos = darkness; -opia = condition of sight), that is when levels of luminance are so low that only rods can function.

Where the TL is present it increases the chances of photon captures by reflecting photons back through the photoreceptor layer (Figure 1) (Ollivier et al., 2004; Schwab et al., 2002).

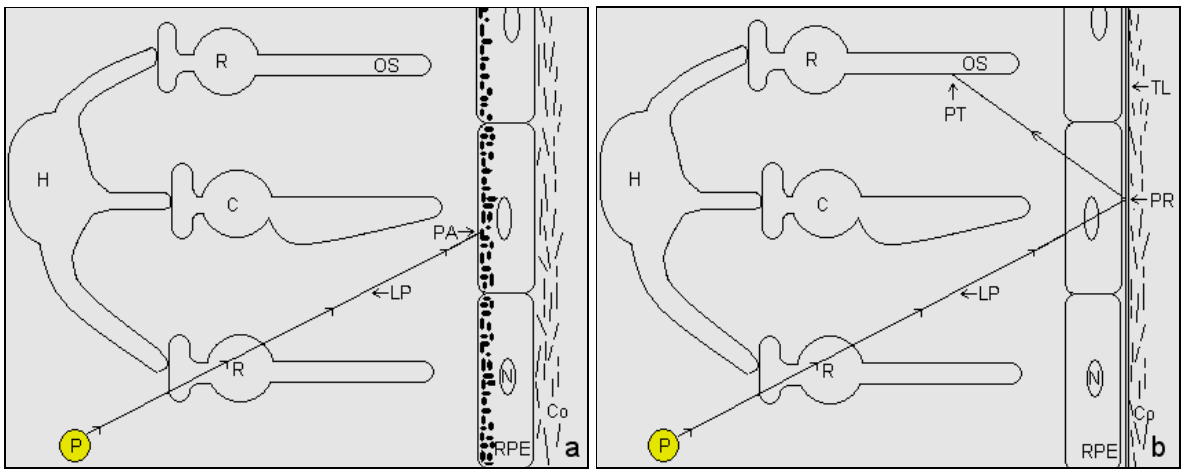

Figure 1 Light travelling in the form of a photon (P) through the retina without hitting a photoreceptor:
a) Pigmented granules (GP) containing melanin found in the RPE absorb the photon (PA = photon absorption); b) Photon travels through the unpigmented RPE, hits the tapetum lucidum (TL) and is reflected (PR = photon reflection); the photon passes through the retina a second time on the way out of the eye. The retina contains the photoreceptors, rods (R) and cones (C). The photon hits the outer segment (OS) of a rod (R) and is absorbed by a photopigment which leads to photo transduction (PT). Horizontal cells (H) collect the signals from several photoreceptors.

In animals that lack a TL only a minority of the photons that enter the eye will hit a photoreceptor and thus induce phototransduction the rest are absorbed by pigments of the retinal pigment epithelium (RPE), the outermost layer of the retina.
Some animals do not have a TL. These are usually diurnal animals, e.g. a lot of birds and some mammals. Evolution of the latter suggests that mammals might have developed a tapetum but lost it secondarily. There is strong evidence that when mammals became distinct from reptilian ancestors they adopted a nocturnal lifestyle. This happened about 200 to 220 million years ago (Crompton et al., 1978). Later a lot of these early mammals changed back to a diurnal lifestyle and/or developed a strong dependency on accurate vision. This led to a loss of the tapetum which was not needed anymore, or even presented a disadvantage by degrading spatial resolution (Ollivier et al, 2004).

In every animal which possesses a TL the same basic principal of how the TL functions is valid, but is generated in different ways.

There are three basic tapetal morphotypes found in vertebrates (Ollivier et al., 2004), which are described in the next paragraph.

1.1. Tapetal morphotypes

1.1.1. Retinal tapetum lucidum

Here the reflective structure is located within the cytoplasm of the retinal epithelium. This type is mainly found in fish but also in reptiles and very rarely in mammals (Ollivier et al, 2004). The retinal tapetum in fish has been divided in two subtypes: the diffuse reflectors, which contain particles of reflecting materials (e.g. uric acid, purines etc.) in small cubes or spheres; and the specular reflectors, which contain layered crystals (Schwab et al., 2002). The first type is mainly found in teleosts and might use Mie scattering (light scattered by spherical particles), the second type is found in abyssal fish and its function resembles that of a mirror. The tapeta in the Virginia opossum (*Didelphis virginian*) and in reptiles are diffuse reflectors. In some reptile species like the American alligator (*Alligator mississippiensis*) in which the tapetum consists of layers of guanine crystals arranged in parallel platelets, the tapetum is occlusible. This animal is potentially active during both during day and night; occluding the tapetum during the day will give it a more accurate picture. In the Virginia opossum the reflective structure is made up of lipoidal spheres which are scattered throughout the retinal epithelium (Schwab et al., 2002).

1.1.2. Choroidal tapetum lucidum

In this type the reflective structure is found in the choroid. It is located external to the retina and directly adjacent to the choriocapillaris, a capillary network on the surface of the TL formed by small blood vessels coming from the choroidal stroma and penetrating the tapetum (Ollivier et al., 2004). To let the light easily pass through to the TL, the RPE, which is the outermost layer of the retina and thus the epithelium directly adjacent to the TL, does not contain any pigment in the tapetal regions. The RPE becomes more pigmented in the outer periphery where there is no TL (Shinozaki et al., 2009). The choroidal TL type can be further categorized into three types:

The guanine type, which is found in elasmobranches; the cellulosum type, for example found in cats and the fibrosum type, present in the group of ungulates. The TL cellulosum is a layer of rectangular-shaped cells packed with organized and highly reflective rodlets and the TL fibrosum is an array of extracellular fibres (Ollivier et al., 2004).

1.2. Aim of the study

Tapeta lucida are found in many animals, still no seasonal change connected to this structure had been documented until recently, when experiments showed that the TL in reindeer reflects yellow light in summer and blue light in winter (Dukes et al., 2003). The aim of this study was to investigate how this seasonal change is controlled, which physical mechanisms lead to it and if there are any other changes connected to it. For this the tapeta lucida of four groups of reindeer receiving different treatments, were compared with each other. One group was held under natural light conditions and killed in mid summer, one group also experienced natural light conditions, but the animals were killed in mid winter. The other groups were held indoors were it was possible to manipulate the light conditions, with one group held in constant darkness and killed in mid summer and the last group held in constant light and killed in mid winter. The experiments with the animals held in altered light conditions were conducted to investigate whether an endogenous clock or the prevailing light conditions regulate the seasonal change. The colour of the tapeta from each group was examined and the results of all the treatments compared with each other.

To further understand the physical mechanism behind the colour shift, intraocular pressure was measured, both in winter and summer, since a seasonal difference in intraocular pressure could lead to a previous found seasonal change in spacing between the collagen fibres of the TL (Dukes et al., 2003). To valuate if it is realistic that a pressure change can lead to the reflecting light shifting wavelengths, external pressure was applied to a piece of TL of a reindeer killed in summer. This experiment was also carried out with summer TL of the Scottish red deer and the domesticated

cow in order to find out if the TL of other ungulates might be capable to a chromatic shift in the light reflected.

Histology work was used to investigate if a seasonal change in photoreceptor outer segment length existed.

A methodology to receive information about the quality and characteristics of colour is to measure its hue, saturation and intensity of it. This are the three attributes a human being can identify in the process of colour perception. Hue defines the dominant wavelength of the colour stimulus whereas saturation indicates the colour purity relative to its brightness. This means that saturation tells how much grey is present within the colour. Of only levels of grey are present the saturation will be zero. The intensity represents the lightness or luminance. Pure white shows the highest intensity and pure black the lowest (Oritz et al., 2001). For this thesis the intensity was not considered of importance and therefore not included in the study.

1.3. Colouration of tapetum lucidum

To understand the change from a yellow summer TL to a blue winter TL, it is important to know why objects are coloured. The colour of an object has the same colour as the light which is leaving it. There are two principles of how the colouration of an object can be produced: Absorption by pigments and interaction with submicron structures. In both cases, a change in wavelength of the incoming light may lead to a wavelength change in the leaving light and thus in the colour of the object.

Pigmentation

When pigments are hit by light they absorb certain wavelengths while reflecting others. The wavelength of the reflected light, i.e. the light leaving gives the object its colour (Yoshioka et al., 2002)

Structural colour

Structural colours are produced when the incoming light interacts with submicron structures of the object. Interactions include diffraction, interference and scattering of light. Diffraction can for example occur in colloid systems of solid, liquid or gaseous nature. Interference can be found on thin films of organized layers of organic or inorganic materials. The TL represents such a thin film and in the case of the TL

fibrosum, the organic material responsible for the interference is collagen which is arranged in layers of ordered collagen fibres. This protein can be found throughout the body including other structures of the eye apart the TL, for example in the sclera. In this case the collagen fibres vary in diameter and are not arranged in a certain order. This leads to a reflection of the whole spectrum of the incoming light and gives the sclera its whiteness, which means the sclera lacks colour (Prum et al., 1994).

1.4. Advantages and disadvantages of the tapetum lucidum

While the TL provides a clear advantage in dim light environments and life styles, including among others densely foliated forests, caves, deep sea and nocturnality, it degrades the acuity of vision by causing scattering of the light. The incoming light is not reflected on the same path by which it entered the eye, but the photons are deflected by the TL at a certain angle depending on the angle of entrance. If such scattered photons hit outer segments they induce phototransduction. This will happen in photoreceptors located in other retina parts than in the area the photons were “supposed” to hit. This leads to a less accurate spatial resolution. On the other hand D’ Angelo et al. (2008) reasoned that the colouration of some tapeta, as in the fibrosum type, is a means of preserving the visual acuity by only reflecting the wavelength the photopigments are most sensitive to and thus reducing the total amount of light reflected. Therefore the degradation of spatial resolution might be reduced.

The disadvantage of the TL in the form of a diminution in acuity might be the reason why animals that live in a bright light environment and are depending mainly on their sight to find food or catch prey either do not posses this structure (e.g. birds of prey) or have pigments which are located in the microvilli of the RPE cells in such a manner that they form sheaths around the outer segments which reach in between the microvilli. Thus outersegments are isolated from each other (e.g. big cats) (Schwab, 2005). However, not all animals which live in scotopic conditions posses a TL. Rats and mice for example are nocturnal, but lack a TL. This might sound contradictory, but those species are not very visual and use their well developed sense of smell for foraging and orientation.

1.5. The tapetum lucidum in *Rangifer tarandus tarandus*

Reindeer in Northern Norway is a semi domesticated animal and developed under a high predation pressure, particularly from grey wolves (*Canus lupus*). This pressure was reduced after the 1960s when the grey wolf was on the verge of becoming extinct in Norway. However in the last two decades the wolf population has been recovering (Skonhoft, 2005). Like in other herbivores, which are a group that is highly predated on, their eyes are located laterally. This limits their stereo optical vision, but gives them a visual field of about 360° and thus the possibility to spot an attacker easily, which will approach most likely from the side or the back (Reimers, 2006). The TL leads to a less accurate picture, but since reindeer do not rely on their eyes to find food, which during winter is buried underneath snow, this is unlikely to present a disadvantage. On the other hand the TL enables them to detect predators by shape and movements even in the dim light conditions of the night and the polar night (time during which the sun does not rise above the horizon).

Ungulates including reindeer possess a TL fibrosum, which is acellular and consists of stacks of closely packed collagen fibrils (Schwab, 2005). The tapeta in this group show colours ranging from gold, green to turquoise. This is also true for the reindeer, but only in summer (Figure 2). In winter it shows a blue TL (Figure 2). This seasonal chromatic shift of the light reflected by this structure has not been documented in any other animal so far (unpublished observations).

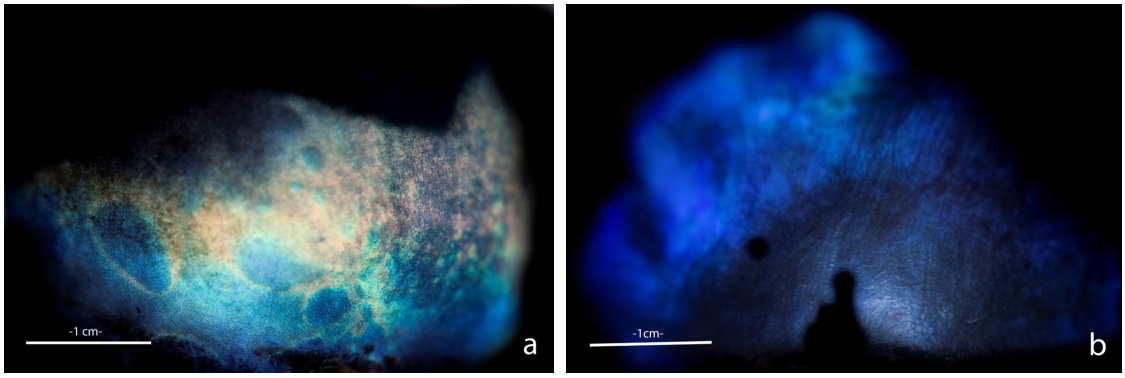

Figure 2 Example of a TL of: a) summer eye, showing the typical golden turquoise structural colour; b) winter eye, showing the typical dark blue structural colour

The TL in reindeer is restricted to the superior fundus and concentrated into more central regions. This means that it is located where ungulates are thought to have their visual streak (D' Angelo et al., 2008), an area of high rod density and thus responsible for accurate vision. In humans the fovea fulfils the same function as the visual streak does in ungulates and other animals. The TL in reindeer is slightly triangular and shifted to one side. The shape is very irregular and there is a significant variance between individuals. Figure 3 shows an example of a fixed reindeer eye from an animal killed in summer. The cornea and the anterior chamber have been removed; the vitreous humor and the retina remained in the eye. The TL is recognized by its yellow colour, which can be seen through the hypo-pigmented RPE covering it. The almost complete absence of pigmented granules allows the photons to hit the TL instead of being absorbed by the pigments. In the black areas the RPE is pigmented properly.

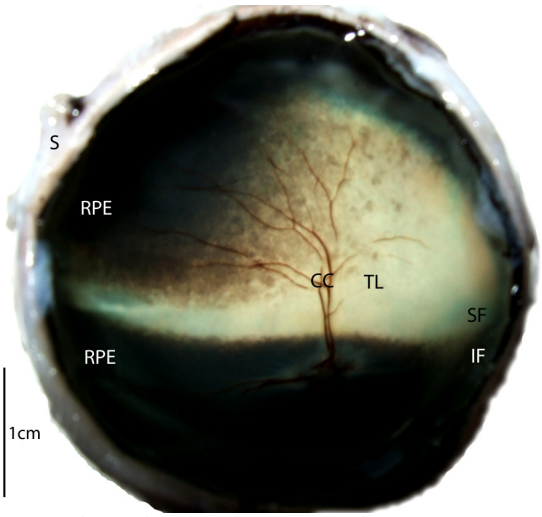

Figure 3 Summer eye; cornea and anterior chamber removed
CC =choriocapillaris; IF = inferior fundus; RPE = retinal pigment epithelium with proper pigmentation; S = sclera; SF = superior fundus; TL = tapetum ludicum, yellow

In preliminary experiments on the TL of the reindeer the wavelength of the reflected light and the total reflectance were measured. This was done on fixed eyes where the lens, anterior chamber and retina had been removed. Both the wavelength and the total reflectance differed in eyes of animals killed in summer from eyes of animals killed in winter (Dukes et al, 2003).

In animals killed in the summer the peak wavelength of the reflected light was 520nm. In animals killed in winter the reflectance was deep blue and peaked at a wavelength of 490nm.

Since no pigments are found in the TL, the colour must be a structural one. The structures responsible for the colouration in the TL fibrosum of the reindeer are the collagen fibres arranged in ordered layers on which the incoming light is scattered. The outgoing light thus receives, depending on the spacing between the collagen fibres, a certain wavelength and gives the TL its structural colour.

The seasonal change in the wavelength of the reflected light from the TL in reindeer eyes was suggested to be the result of a difference in the spacing of the collagen fibres in the TL between summer and winter. Measurements showed that in winter the spacing is narrower than in summer, which according to Bragg's law should result in a spectral shift of reflected light in the direction that was measured; i.e. towards blue (Dukes et al, 2003).

Concomitant with this shift in the wavelength of reflected light, there was an approximately 50% reduction in the total reflectance from the TL in winter. When illuminated with a fibre optic probe the light in the fixed specimens of winter eyes was being scattered through the back of the eye rather than being reflected back directly along a path approximately parallel to the light entering (Dukes et al, 2003).

The shift in the wavelength of the reflected light towards 490nm in winter brings it considerably closer to the peak absorption for mammalian rhodopsin, the rod photopigment, which is approximately 495nm than it is in summer when it peaks at 520nm. This, and the fact that the light scattering during winter through the back of the eye will increase the chance of a photon hitting an outer segment of a photoreceptor, would make the eye more light sensitive during a period when illumination levels are low. Therefore a likely hypothesis is that the changes in the TL function as a means to enhance the sight of the animal in a season when there is almost no light.

Figure 4 shows the distribution of sunshine (white), civil twilight (grey) and darkness (black) in hours for the Tromsø area throughout the year. Sunshine means that the sun is above the horizon, civil twilight is the illumination where the sun is between 0°

and 6° below the horizon and darkness means that the sun is more than 6° below the horizon. The figure illustrates the natural light conditions experienced by the animals kept outdoors and how extreme the seasonal changes in light conditions are in this environment.

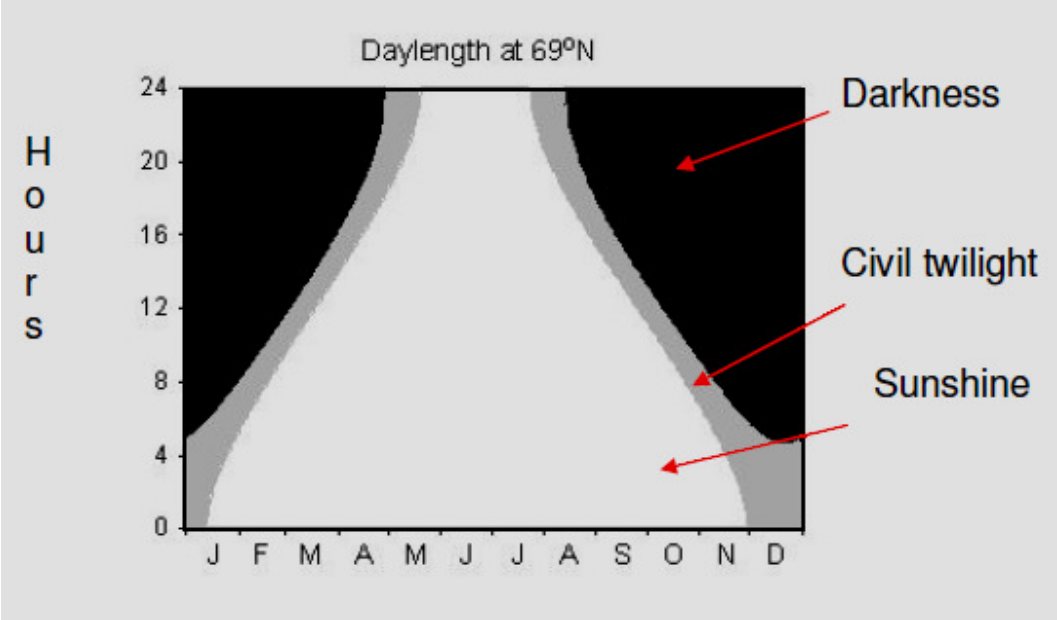

Figure 4 Distribution of sunshine (= white), civil twilight (= grey) and darkness (= black) in the Tromsø area throughout the year in hours

In the environment of the reindeer, not only the amount of light varies throughout the year. There is also a shift in the most dominant wavelength; with the light in winter appearing blue. Thus the chromatic shift of the reflected light towards blue in winter is in accordance with the finding that the light reflected by the TL is of the same wavelength most dominant in the ecological niche of the species' (Schwab et al., 2002).

2. Materials and methods

2.1. Regulating mechanism for seasonal change in the tapetum lucidum

To find out whether an endogenous clock or the changing light conditions are the regulating mechanism behind the seasonal chromatic change, reindeer were kept indoors with altered light conditions opposing the natural conditions outdoors to compare those to animals kept outdoors during the same season. If changes in light conditions were the regulating mechanism the seasonal change in the TL might be influenced by the light pollution which changes illumination levels during winter. Light pollution is created by any artificial lighting and enhanced when the sky is covered in clouds as these reflect the light back to the ground. Animals living close to settlements will experience more light pollution than animals living isolated from human civilization. Therefore eyes from animals kept in Tromsø outdoors during winter and eyes from animals ranging freely in Finnmark County during winter were collected and compared with each other.

2.1.1. Experimental setup

The animals were obtained from herds of semi-domesticated reindeer herded by Sámi pastoralists in northern Norway and brought to the Arctic Biology Department at the University of Tromsø (69°40′33″N 18°55′10″E) where there are extensive facilities for maintaining these animals, both in the open and indoors. Local ethical permission had been obtained for the proposed experimental plan, from the Norwegian Animal Research Authority (NARA).

Six animals kept outdoors in natural light conditions during summer (s.n.l.c.):

Six animals were kept in pens outdoors in normal light conditions from January to June 2008. They were fed *ad libidum* with water and reindeer pellets and also received one serving of reindeer lichens per day. In June 2008 the reindeer were stunned with a bolt gun shot against the forehead and their throats were slit with a knife. As soon as the animal was declared dead the head was cut off. The left eye was removed and fixed in 4% formaldehyde.

Five animals kept indoors in constant darkness during summer (s.24h.d.):
From January to June 2008 five reindeer were kept indoors in darkness. All previous light sources in the room were removed and replaced by two incandescent red bulbs of 25 W were installed, giving approximately 0.1 lux at 'head-level'. This gave enough light to care for the animals properly, but did not have an impact on the experiment, since the rods were not sensitive to the light produced by the red bulbs. These were left on for twenty-four hours a day. Every opening through which light could have entered the room was obstructed and all precautions made to prevent any incidents that would have exposed animals to light. They underwent the same procedure as the five animals kept indoors in darkness.

Four animals kept indoors in constant light during winter (w.24h.l.):
Between August and December 2008 four animals were kept in the same room indoors as the first group. The light was provided by eight pairs of 36 W fluorescent tubes, giving approximately 900 lux at 'head-level' turned on for twenty-four hours a day. The experiment started with five animals of which one died a few days after the animals were moved into the room. It is likely that this animal had been in a bad health condition already prior to arriving at the department and the stress of capturing, transport and the unusual environment might have caused its death. These animals were fed with water and reindeer pellets *ad libidum* and one portion of lichens like the other groups, but also received freshly cut aspen tree (*Populus sp.*), once a day as a response to the death of the fifth animal. In December 2008 these animals underwent ERG procedures on their left eye, were killed in the same way as mentioned above and their eyes processed accordingly.

Animals ranging freely in Finnmark County during winter (w.n.l.c.):
In mid December 2008 fifty-four eyes were collected in an abattoir in Kautokeino (69°14′16″N 23°29′22″E), Norway. The animals had been brought to slaughterhouse the same day of the killing. They originated from herds of semi-domesticated reindeer herded by Sámi pastoralists in the Kautokeino area of Finnmark County. There herds range freely, which means that the animals were exposed to natural light conditions,

far away from any bigger city, town or settlement, which could contribute to “light-pollution”. The eyes were removed right after the animals were killed and fixed in 4% formaldehyde.

All eyes meant for research on the TL were taken to the Institute of Ophthalmology, University College London.
There the eyes were opened by removing the cornea, lens and anterior chamber. The retina was removed and placed into a separate container which also contained 4% Formaldehyde.

2.2. Quantifying hue and saturation of the tapetum lucidum

To quantify the hue and saturation of the tapeta raw photographic images were taken and examined in MATLAB (MathWorks Ltd.). The eyecups were placed into a mould to put them in a stable position and the tapeta were photographed with a Canon EOS 350 D mounted on a tripod. This was undertaken in a completely dark room with the flash being the only light source and a remote control was used to operate the camera.
Raw images were opened in Adobe Photoshop CS4 and everything but the TL was blacked out with the brush tool. The images were saved in jpeg – format of the following dimensions and resolution: Width = 3486 pixels; height = 2304 pixels;
Resolution = 240 pixels/inch. They were grouped according to the treatment the animals received shown in Table 1.

Treatment	Identifier	Number of animals
Kept outdoors in natural light conditions during summer	s.n.l.c.	6
Kept outdoors in natural light conditions during winter	w.n.l.c.	14
Kept indoors in 24 hours of darkness during summer	s.24h.d.	5
Kept indoors in 24 hours of light during winter	w.24h.l.	4

Table 1 Groups included into the hue and saturation measurements. The groups are defined by the treatment the animals received and the season they were killed. Identifier and amount of eyes are shown in the table.

Images of all groups were read into MATLAB and scaled down to 25% by linear interpolation. The RGB colour space (red, green, blue) was converted into the HSV colour space (hue, saturation, value) using the MATLAB command ">>rgb2hsv". Value, which represents the brightness (Chai et al., 1999), was normalized to a range of 0-1, this was not done for hue and saturation since those were already in that range. Masks were created for every image to select the pixels for further processing. To create the mask a two-dimension Gaussian filter with the standard deviation of 16 pixels was applied to the value component of the image and a threshold was set which kept all pixels bigger than 0.5 x the maximum value. This mask was then applied to all three layers of the image (hue, saturation and value). MATLAB calculated mean hue and mean saturation of the images using the hues and saturations of every pixel within the masks. Mean hue and mean saturation were then plotted with saturation as the abscissa and hue as the ordinate in a scatter plot. A bar representing the colour range of hue was added to the images. The mean hue of each image was weighted by the saturation and inserted into the images as a coloured line underneath the bar in the corresponding position related to the bar.

The programme GraphPad Prism 5 was used for the statistical analysis and to create the graphs. The groups were compared with each other in a two-tailed Mann-Whitney-U test.

2.3. Seasonal changes in other parts of the visual system

2.3.1. Preparation of the tissue

Histology work was undertaken to address two issues; the distribution of the pigments in the retinal pigment epithelium and the photoreceptor outer segment length with a possible seasonal difference in both.

Four of the eyes obtained from the abattoir in Kautokeino and three eyes from animals kept in natural light conditions outside and which were killed in summer 2000 at the Arctic Biology Department in Tromsø were processed for this. The eyes were opened as described previously and the retina, the TL and the choroid were carefully removed as one layer. The upper half of this layer was cut into four sections, two from peripheral regions and two from central regions. The sections were each placed between two pieces of filter paper which had previously been wetted with 4% formaldehyde, the fixative medium in which the eyes were kept. The filter paper pieces with the tissue were laid onto a glass slide in a petri dish. To the left and the right side a strip of filter paper which had been folded twice and soaked in formaldehyde was placed and a second glass slide put on top. The strips of filter paper that were placed to either side of the tissue prevented excessive pressure being applied to the TL. Two pennies were put on top to hold the slide in place. To dehydrate the tissue it was exposed to an ascending alcohol series, the concentration steps of ethanol being: 20%, 40%, 60%, 95%, absolute ethanol and absolute ethanol. The sections were kept in each concentration step for thirty minutes after which the ethanol was removed with a plastic pipette and replaced immediately by the next concentration step. After the last step the tissue was placed onto dry filter paper to remove as much of the ethanol as possible. A small petri dish was filled with the preparation solution of Technovit®7100 by Kulzer Gmbh (baseliquid and hardener I). The tissue was removed carefully from the filter paper and put into the preparation solution with the retinal side facing downwards. The sections were kept like this overnight with the petri dish covered. The next day the tissue was cut in smaller pieces, the number varying between nine and thirteen strips, approximately twelve mm long and three mm wide. The strips were transferred into plastic embedding moulds which were then filled with cold-curing resin (Technovit®7100 by

Kulzer Gmbh; preparation solution mixed with hardener II). After the sections were aligned a specimen holder was placed on top of each mould. The specimens were allowed to dry for two days and then removed from the embedding moulds. A motorized rotary microtome (Leica 2050 Supercut) with a glass knife was used to cut the blocks. The thickness of the sections varied from 2.5 µm to 5.0 µm according to the quality of the block.

Sections were straightened on a water surface, placed on a glass slide and left on a hot plate over night at 60°C. The number of sections on a glass slide varied between three and five. The next day they were stained with osmium tetroxide (OsO_4) to distinguish the different structure in the sections especially the outer segments of the photoreceptors. Each section was covered by a small amount of the staining agent for fifteen minutes. The osmium tetroxide was then removed using a glass pipette, which was also used for applying it, and the slide was washed in distilled water. After the glass slides were dried on a hot plate set to 60°C they were put into toliudine blue (tolonium chloride) for 30 seconds washed in distilled water then in 95% ethanol and a second time in distilled water. After the washing they were again left on the hot plate set to 60°C until the water had completely evaporated. A cover slip was mounted on each slide using Depex (by Electron Microscopy Science), a colourless mounting medium.

The slides were examined with an Olympus Bx51 microscope under the magnification of 100 times using immersion oil and pictures were taken using a Nikon digital camera Dxm1200.

2.3.2. Distribution of pigmented granules in the RPE

To address the question of pigment distribution, sections of the nontapetal periphery were compared with central sections in the area of the TL. The absence or presence of pigmented granules in the RPE and their intracellular location was noted. The results from both seasons were compared.

2.3.3. Measurement of photoreceptor outer segments

To measure the outer segments of the photoreceptors, the pictures were opened in Adobe Photoshop CS4. The 'ruler tool' was used to measure the ten longest outer segments in the picture. The programme GraphPad Prism 5 was used for the statistical analysis and to create graphs. In a two-tailed Mann-Whitney-U test the two groups were compared.

2.4. Seasonal changes in intraocular pressure

2.4.1. Intraocular pressure measurements

It has been found that the spacing of collagen fibres in the TL is denser in winter than in summer (Dukes et al., 2003), which could be caused by a higher pressure in the winter eyes than in summer eyes. This led to the investigation of a possible seasonal intraocular pressure (IOP) change. For this the IOP was measured in a group of reindeer in June 2009 and December 2009 with a Tono-Pen (Reichert, Inc, Depew, NY). The tip of the Tono-Pen is pressed against the sclera of the anaesthetised animal and the IOP is given in mmHg. Three readings were undertaken per animal per season. The results from the seasons were compared in a two-tailed Mann-Whitney-U test.

2.4.2. Chromatic shift due to pressure change

To find out if pressure change will result in a shift in reflected light by the TL a piece of a summer TL of a reindeer was exposed to different pressures. The same was done to a summer TL of a Scottish red deer (*Cervus elaphus scoticus*) and a domesticated cow (*Bos primigenius*) to find out if other ungulate species might have the potential for a chromatic change. The TL and the choroid were removed from the eyecups as one layer and a piece of approximately 49 mm² was cut out of each TL. The pieces were placed between two glass slides and a glass vessel put on top. This was performed with to different weights: 15 g, and 45 g, which presented for the surface area of each piece a pressure of 22,517 (15 g) and 67,551 mmHg (45 g).
To minimize the capillary forces between the glass slides the pieces were transferred onto a piece of plastic of the same dimensions and about 0.7 mm thickness before

placing them between the slides. Desiccation of the tissue was prevented by placing the pieces of TL between the slides into a plastic box which was than filled to the brim of the top glass slide with 4% formaldehyde and covered with a lid to minimize evaporation. The tissue was exposed to the pressure for a period of two days. Pictures were taken before and after the experiment with a Canon EOS 350 D in a completely dark room using flash as the only light source.

3. Results

3.1. Hue and Saturation of the tapetum lucidum

Even without further statistical analysis it was possible to distinguish between two distinctive groups by looking at the images of the tapeta. One group consisted of dark blue tapeta from animals killed in winter and the other of rather patchy tapeta showing colours from golden over green to turquoise of animals killed in summer. An example of a summer TL and a winter TL are shown in Figure 5.

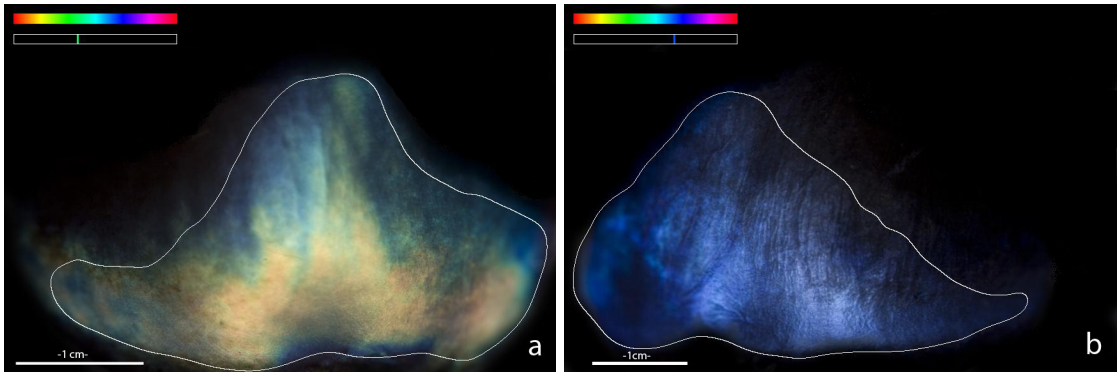

Figure 5 Two examples of TL images processed in MATLAB: a) the dark blue TL of a winter eye: b) the golden-turquoise TL of a summer eye. The white line around the TL represents the border of the mask generated in MATLAB. The bar above the TL is shows the colours of hue; the coloured line underneath the bar represents the mean hue measured in MATLAB within the and weighted by saturation.

This subjective observation is supported by the graphical and statistical analyses.

The scatter plot In Figure 6 shows mean hue and mean saturation of summer tapeta from Tromsø (s.n.l.c. = red; Nr. 24 -29) and winter tapeta from Finnmark (w.n.l.c. = blue; Nr. 1-13), where these two groups can clearly be distinguished from each other. Saturation is plotted on the abscissa and hue on the ordinate. The horizontal whiskers represent the standard deviation for saturation the vertical whiskers the standard deviation for hue.

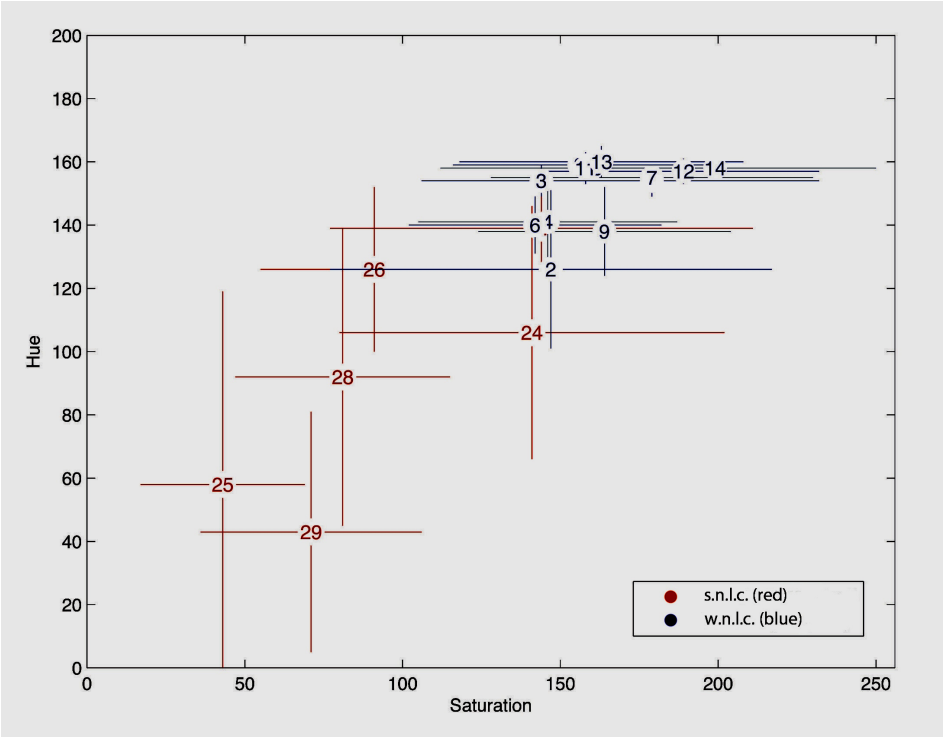

Figure 6 Mean hue and saturation of tapeta lucida from different seasons measured in MATLAB: Animals kept outdoors in natural light conditions in summer (s.n.l.c. = red; Nr. 24-29) and in winter (w.n.l.c. = blue; Nr. 1-13). The horizontal whiskers represent the standard deviation of saturation, the vertical whiskers the standard deviation of hue.

In Figure 7 the two experimental groups were added, i.e. the five animals standing indoors in constant darkness (s.24h.d. = green; Nr. 15-19) and killed in summer and the four animals kept indoors in constant light (w.24h.l. = magenta; Nr. 20-23) and killed in winter. While the eyes of the animals kept in the dark form one group clearly together with the summer eyes, the eyes of the animals kept in light are clearly grouped with the winter eyes. This shows that all tapeta of animals killed in the same season have similar mean hue independent of the treatment the animals received before killing.

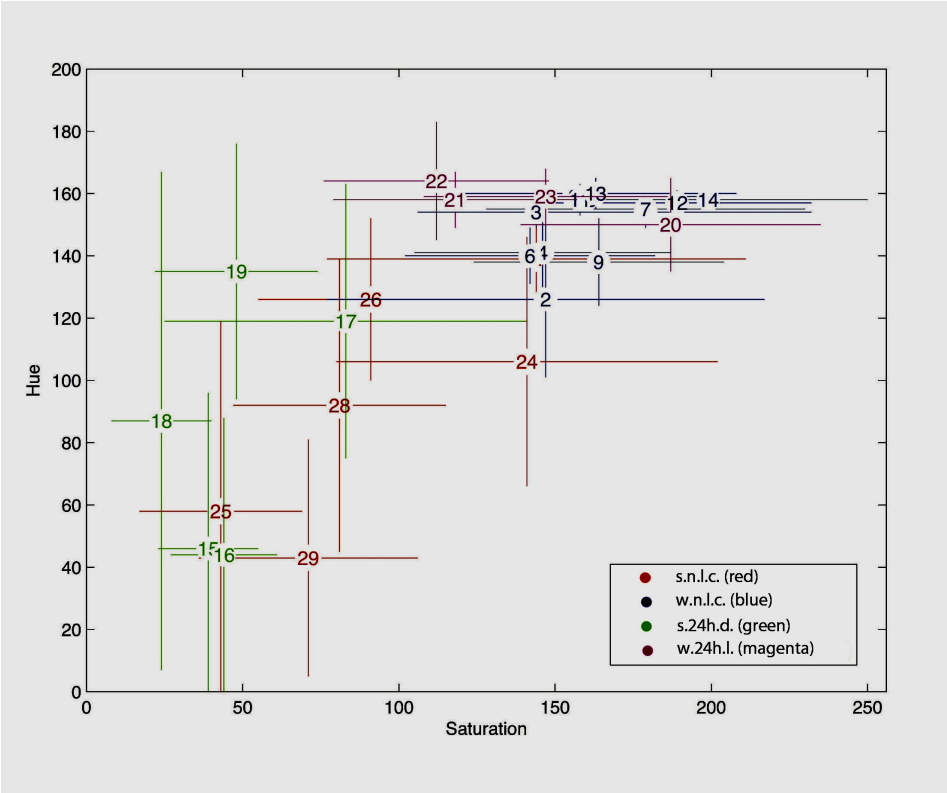

Figure 7 Mean hue and saturation of tapeta lucida from different seasons and under altered light conditions measured in MATLAB:
Animals kept outdoors in natural light conditions in summer (s.n.l.c. = red; Nr. 24-29) and in winter (w.n.l.c. = blue; Nr. 1-13). Animals kept indoors in constant darkness in summer (s.24h.d. = green; Nr. 15-19) and in constant light in winter (w.24h.l. = magenta; Nr. 20-23). The horizontal whiskers represent the standard deviation of saturation, the vertical whiskers the standard deviation of hue.

The statistical analyses show that there is a significant difference (p = 0.0013; Mann-Whitney U-test) in hue between the winter (w.n.l.c. = winter in natural light condition i.e. kept outside in Finnmark) and summer group (s.n.l.c. = summer in natural light condition i.e. kept outside). However there is no significant difference between winter and the light room group (w.24h.l. = winter inside in 24 hours of light) (p = 0.2391) and between summer and the dark room group (s.24h.d. = summer inside in 24 hours of darkness) (p = 0.7922) (Figure 8).

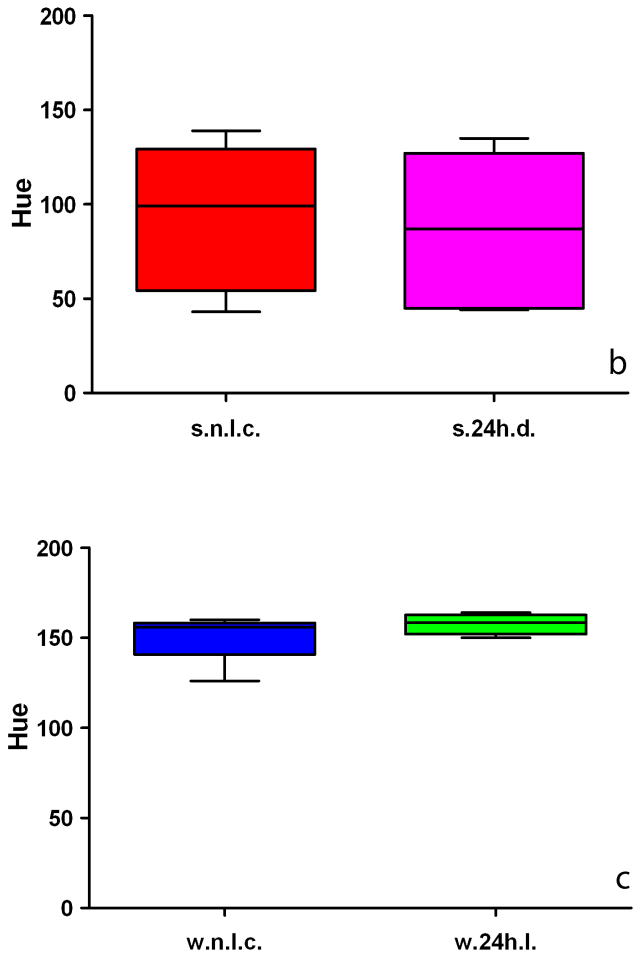

Figure 8 Mean hue of tapeta lucida from different seasons and under altered light conditions measured in MATLAB: a) summer under natural light conditions (s.n.l.c = red) compared to winter under natural light conditions (w.n.l.c. = blue); b) summer under natural light conditions (s.n.l.c) compared to altered light conditions in dark room during summer (s.24h.l. = green); c) winter under natural light conditions (w.n.l.c) compared to altered light conditions in light room during winter (w.24h.d. = magenta). The range of hue is covered by the boxes with the black horizontal line found within the box representing the mean hue. The whiskers are denoting the standard deviation.

The graphs clearly show a difference in mean hue between summer and winter, but also that winter tapeta have a far smaller range in hue than summer tapeta. This means that tapeta in summer have a broader colour range than in winter, which is in accordance with the observation that the tapeta in summer look irregularly coloured from golden to green to turquoise while they are uniformly dark blue in winter.

The statistical analyses for the saturation show the same pattern: A significant difference between winter and summer ($p = 0.0010$; Mann-Whitney U-test) (Figure 9)

no significant difference between winter and light room (p = 0.2021) (Figure 10) and summer and dark room (p = 0.5368) (Figure 9).

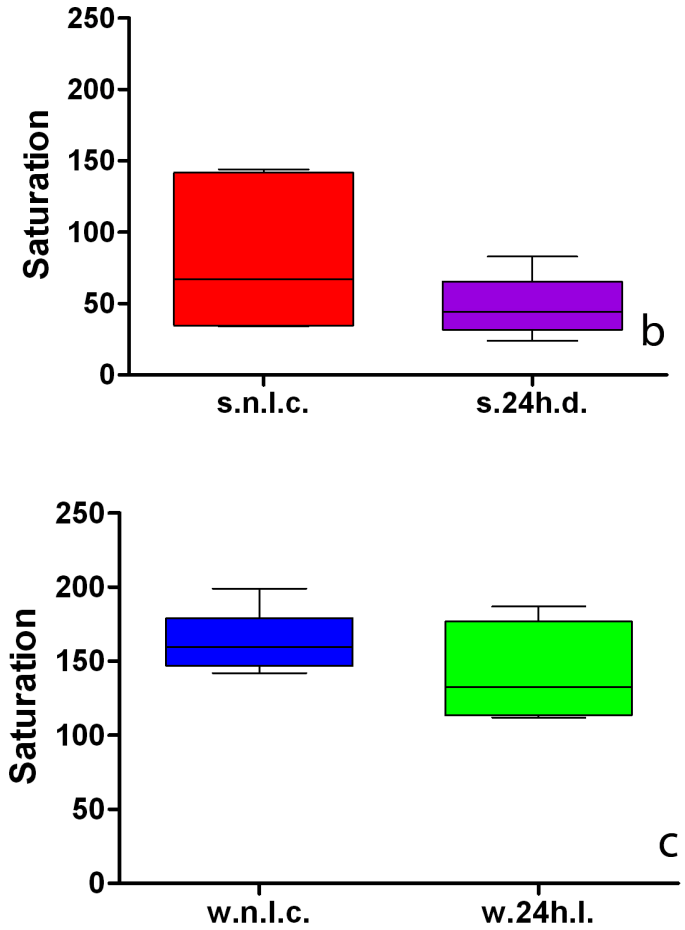

Figure 9 Mean saturation of tapeta lucida from different seasons and under altered light conditions measured in MATLAB: a) summer under natural light conditions (s.n.l.c = red) compared to winter under natural light conditions (w.n.l.c. = blue); b) summer under natural light conditions (s.n.l.c) compared to altered light conditions in dark room during summer (s.24h.l. = green); c) winter under natural light conditions (w.n.l.c) compared to altered light conditions in light room during winter (w.24h.d. = magenta). The range of saturation is covered by the boxes with the black horizontal line found within the box representing the mean saturation. The whiskers are denoting the standard deviation.

Even if there is no statistically significant difference between winter and light room and summer and dark room, looking at the images leaves the impression that those groups are slightly different from each other:

The tapeta of animals kept in constant darkness and killed in June seem to be less patchy and more uniformly golden compared to the ones from the control group, which also show green and turquoise (Figure 10).

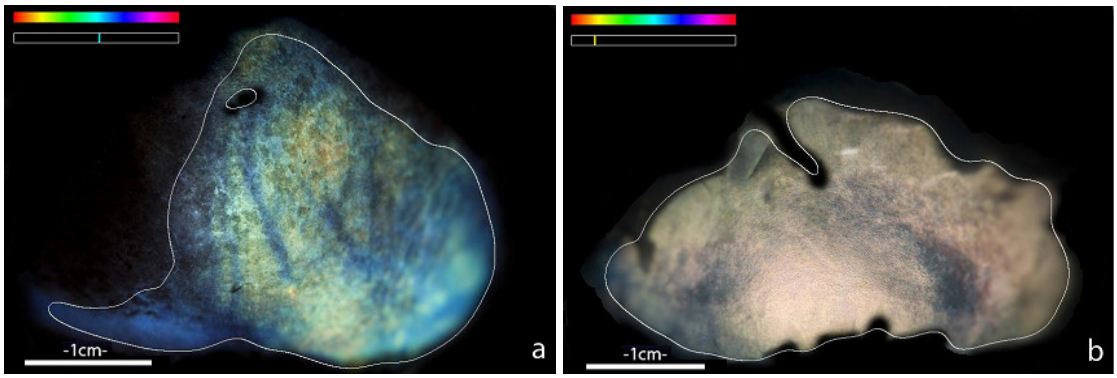

Figure 10 Tapeta from animals killed in mid June: a) outdoors in natural light conditions; b) indoors in constant darkness

The animals kept in constant light indoors and killed in December seem to have a slightly greener TL than the animals which experienced natural light conditions outside (Figure 11).

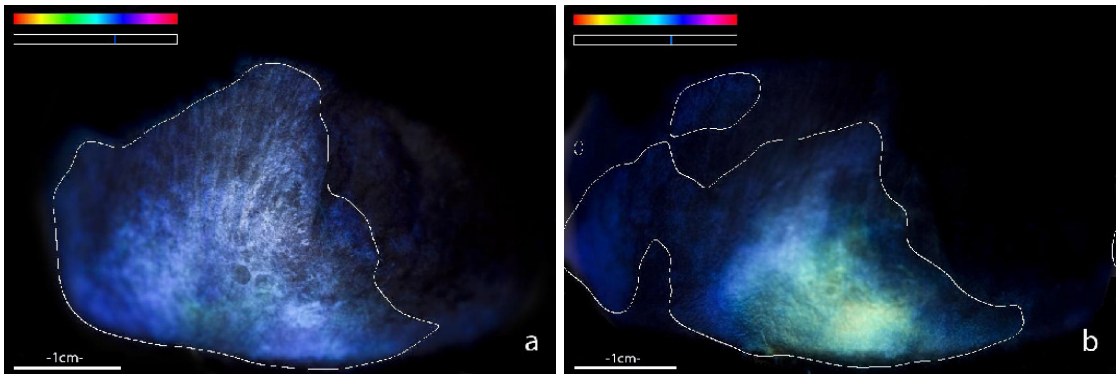

Figure 11 Tapeta from animals killed in mid December: a) outdoors in natural light conditions; b) indoors in constant light

3.2. Histology

3.2.1. Distribution of pigments in the RPE

Two aspects were addressed: The distribution of pigmented granules over the whole area of the RPE and the intracellular distribution of this tissue.

Distribution across the RPE

The histology showed that in the peripheral or non-tapetal regions, the RPE is strongly pigmented while the central regions did not show any or just very few pigmented granules. This is true for both winter and summer eyes (Figure 12).

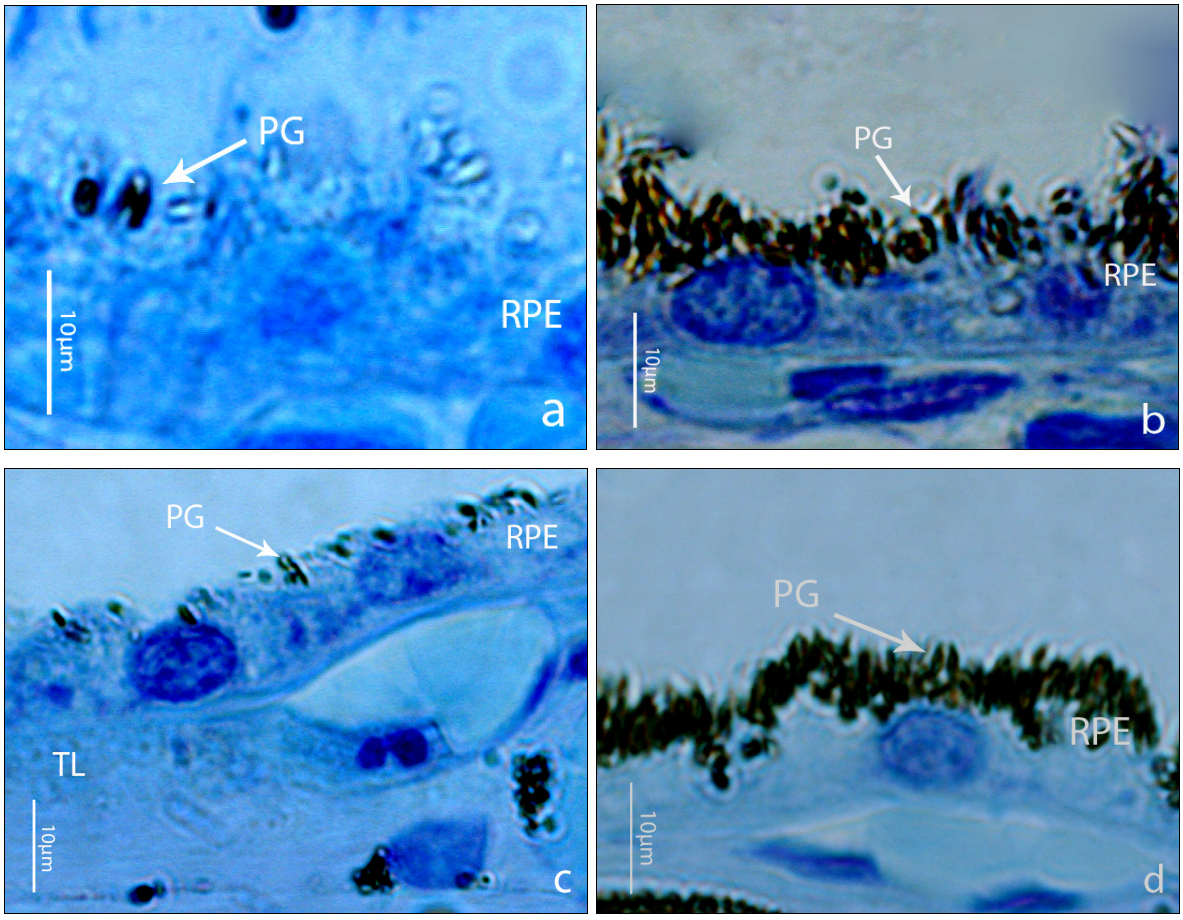

Figure 12 Distribution of pigmented granules in the RPE of reindeer in different seasons: a) the hypopigmented periphery of RPE in an animal killed in summer; b) richly pigmented central RPE in an animal killed in summer; c) the hypopigmented periphery of RPE in an animal killed in summer; b) richly pigmented central RPE in an animal killed in summer,
PG = pigmented granule; RPE = retinal pigment epithelium; TL = tapetum lucidum

Distribution within the RPE cells
The pigmented granules in RPE were located adjacent to the apical membrane of the cells and not distributed anywhere else within the rest of the cell. This was consistent in all areas of the RPE in both summer and winter eyes (Fig. 12 b) and c)).

3.2.2. Measurement of photoreceptor outer segments

While measuring the length of the outer segments of the photoreceptors it was noticed that these were of an unusual shape with the tips looking 'bloated'. The deformation was found in both winter and summer eyes and throughout the entire retina (Figure 13). The consistency of the finding suggests that this might be an artefact caused by an unsuitable fixative medium.

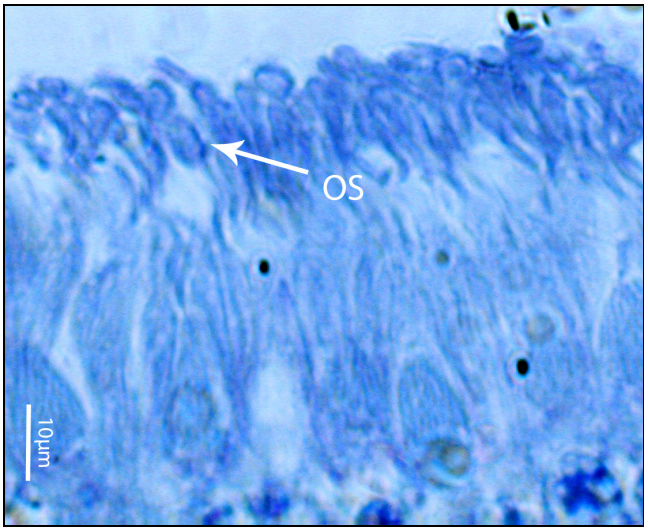

Figure 13 Deformation of the outer segments of photoreceptors in the retina of a winter eye; OS = outer segment

The measurement was nevertheless undertaken, but the results may not be trustworthy. It showed that the outer segments in winter eyes were significantly shorter than in summer eyes ($p = 0.0095$; Mann-Whitney U-test) (Figure 14).

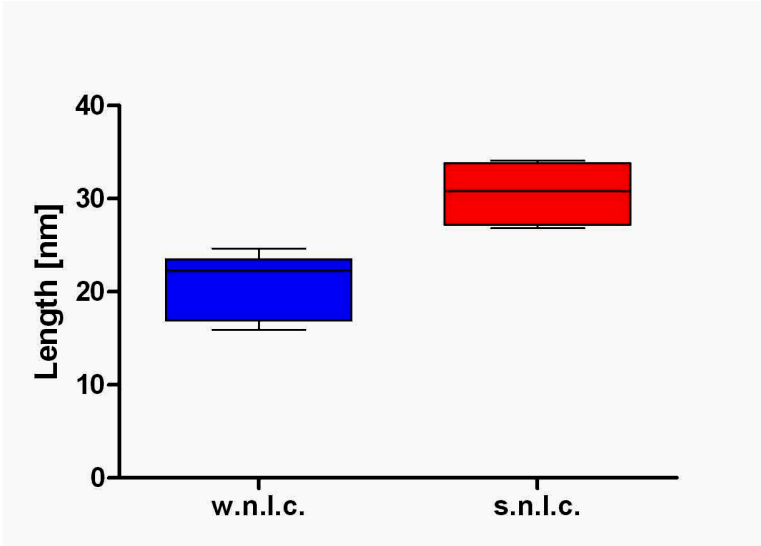

Figure 14 Outer segment lengths of photoreceptors in nm; winter eyes (w.n.l.c.) compared to summer eyes (s.n.l.c.). The range of outer segment length is covered by the boxes with the black horizontal line found within the box representing the mean length. The whiskers are denoting the standard deviation.

3.3. Seasonal changes in intraocular pressure

3.3.1. Intraocular pressure measurements

The measurements of intraocular pressure showed a significant difference between summer and winter with the mean pressure being 5 mmHg higher in winter ($p < 0{,}001$; Mann-Whitney U-test). Figure 15 shows the intraocular pressure in mmHg winter and in summer.

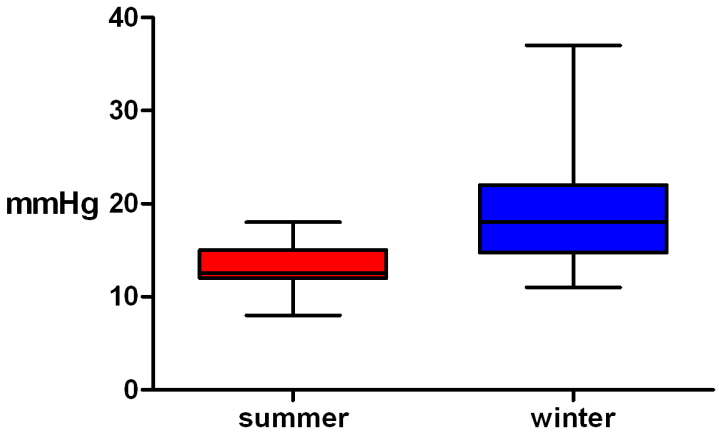

Figure 15 Intraocular pressure in mmHg in summer (red) and winter (blue). The range of intraocular pressure is covered by the boxes with the black horizontal line found within the box representing the mean intraocular pressure. The whiskers are denoting the standard deviation.

3.3.2. Chromatic shift due to pressure change

When the spacing between the fibres of the TL was experimentally reduced by applying pressure in the form of weight onto the tissue, it showed a shift from yellow to blue. This could be partly reversed after the pressure was removed and a few drops of fixative medium added. This was true for reindeer and Scottish red deer and cow.

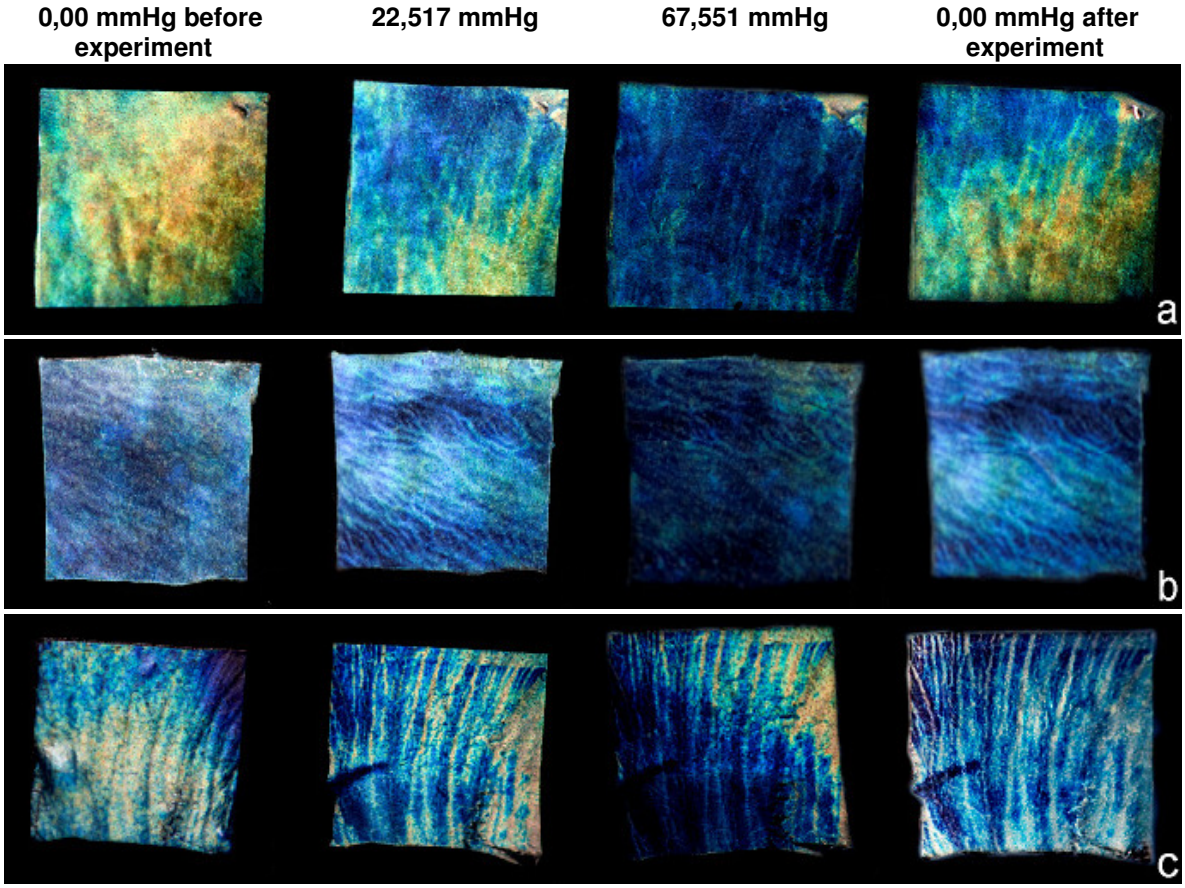

Figure 16 Pieces of summer tapeta of a) reindeer, b) cow and c) red deer showing chromatic shift in the light reflected due to being exposed to different pressure:
before experiment without pressure; 22,517 mmHg; 67,551 mmHg; after experiment without pressure

4. Conclusion

The TL of reindeer kept indoors in light conditions opposing the actual natural light conditions outside (in constant darkness during summer; in constant light during winter) were significant different from the TL of reindeer which experienced those natural light conditions. This strongly indicates that an endogenous circannual clock is regulating the seasonal chromatic shift in the reflected light from golden-green in summer to blue in winter. Altering the natural light conditions seems to have a very marginal impact.

The histology showed that the distribution of pigmented granules in the RPE in reindeer does not differ from other ungulates (Shinozaki et al., 2009), where non-tapetal regions are richly pigmented and tapetal regions show an almost complete lack of pigmented granules. The results also suggest that there is no seasonal change in pigment distribution.

Since the results of the measuring of the outer segment lengths are not reliable no real conclusions can be drawn from them.

The measurements of intraocular pressure in the same animals in summer and winter showed a significant seasonal difference with the pressure being higher in winter. This, in combination with the finding that a piece of yellow – green summer TL will turn blue when exposed to pressure, supports the hypothesis that intraocular pressure change may be a physical mechanism leading to a change in spacing of collagen fibres of the TL fibrosum and thus to a shift in the light being reflected by this structure. That a chromatic shift could also be provoked in the tapeta of two more ungulates living further in the south i.e. Scottish red deer and cow, by applying pressure to those indicates that other species which have a TL fibrosum might be capable of this change.

5. Discussion

5.1. Hue and saturation of the tapetum lucidum

The fact that there was a significant difference shown in hue and saturation between winter and summer tapeta, but not between tapeta from animals kept in altered light conditions and animals kept outside in natural light conditions during the same season, strongly indicates that an endogenous circannual clock is responsible for the chromatic shift of the light reflected by the TL.

Inner clocks, which are found in every living organism, make sure that the appropriate physiological processes or behaviour happen at the right time of the day or the year. The advantage of an endogenous clock regulating metabolism and behaviour in an organism over regulation by an outside stimulus might be that the change is already completed when the environment or lifestyle demands it (Morgan, 2004). If the changing light regime was the only regulating mechanism behind the seasonal shift in TL, the change might be initiated too late and would lag behind the season. The animal could thus not use the advantage of a blue TL to its full extent. It has been shown that reindeer have a rather weak circadian (Latin for “circa diem”, which translates into “about a day”) inner clock (Van Oort et al., 2007), this might appear to contradict the finding of a circannual (Latin for “circa annus”, which translates into “about a year”) inner clock in the present study. It has to be born in mind though that these animals live in an environment where there are long periods when there is no, or almost no, change between night and day. In Tromsø for example the polar day, the period in which the sun never sets bellow the horizon lasts between mid May to the end of July (Figure 5). In winter on the other hand there are seven weeks (between end of November to mid January) in which the light intensity never exceeds that of twilight (Van Oort et al., 2007). This extreme difference in illumination between summer and winter becomes more and more overt the higher the latitude; with an extreme on the North and South Pole where there are six months in which the sun never sets and six months in which the sun stays below the horizon. This means that adaptation to the illumination change from night to day for a reindeer living in the Arctic and Subarctic might not be as important as adaptation to the change from summer to winter. This is not only true for reindeer of

course, but also for most organisms living in the vicinity of either Pole. The other extreme is found on the equator, where there is no difference in seasons, here circadian rather than circannual clocks are important.

The subjective observation of a slight colour difference between the animals kept in natural light conditions outdoors, and the animals kept indoors in altered light conditions during the same season, might be understandable when one looks closer at how endogenous clocks work. Inner clocks are not completely independent from outer signals needing these to be entrained regularly. This means that the endogenous clocks are set in phase with the changes in their environment. These signals are called zeitgebers. Different stimuli can serve as zeitgebers, of which light is the most important one. This can be explained by the fact that the cycling of dark and light is the most dominant recurring stimulus on earth. If an organism is not exposed to its appropriate zeitgebers at a certain time, the endogenous clock can not be entrained and it runs freely. This is true for both circadian and circannual clocks, with circadian clocks oscillating with a rhythm around twenty-four hours and a circannual clock with a rhythm at about a year. Endogenous clocks which are not entrained and run freely are out of phase with the environment and the changes or processes in the body happen too early or too late (Moore, 1997). Keeping the reindeer inside with no exposure to the natural light conditions, zeitgebers might not have been able to stimulate them during a phase at which the animals are sensitive to them. This could have led to the circannual clock running free which could have resulted in the chromatic shift occurring earlier or later than it usually would have done during natural light conditions. This could explain the subjective observation of the difference between the TL of animals kept outdoors in natural light conditions and animals kept indoors in the same season in altered light conditions.

5.2. Distribution of pigments in the RPE

Distribution across the RPE

Almost no pigmented granules were found in the RPE of central or tapetal regions while peripheral or non-tapetal regions were richly pigmented. This confers with the findings in other animals (Shinozaki et al., 2009) and the function of the TL as a

means of enhancing light sensitivity in scoptic conditions. A pigmented RPE in tapetal regions would lead to absorption instead of a reflection of the incoming photons by the TL. This would prevent the photons from passing through the retina a second time and thus from hitting an outer segment of a photoreceptor for phototransduction (Shinozaki et al., 2009).

Melanolysosomes have been found in bovine RPE of non-tapetal regions. They are believed to be responsible for the destruction of melanin through autophagy (Bermann et al., 1974). The same mechanism may cause the lack of pigment granules in tapetal regions in reindeer.

Distribution within the RPE cells

This aspect of the visual system was being examined because pigment migration induced by bright light has been observed in some animals, for example fish (Burnside et al., 1983). Another reason for examining distribution of pigments in the RPE was the finding that some outer segments of the photoreceptors stuck to the RPE in a summer eye (Figure 17).

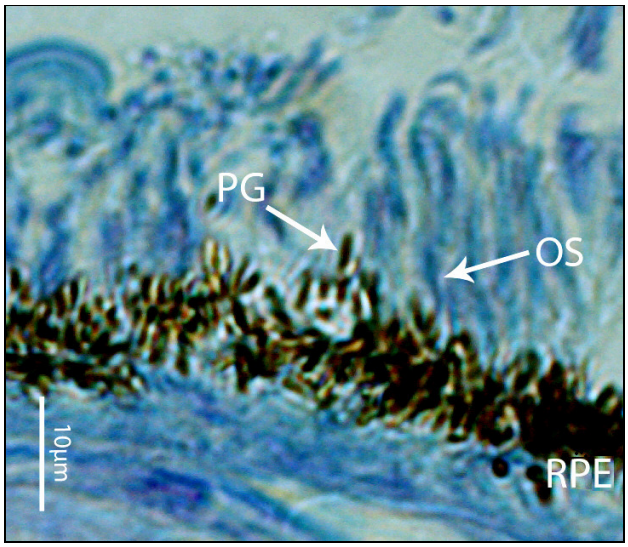

Figure 17 summer eye; peripheral region; outer segments stuck to RPE
OS = outer segments; PG = pigmented granule; RPE = retinal pigment epithelium

This led to the assumptions that in summer the pigment granules in the RPE could migrate towards the retina and into the apical microvilli of the RPE between the outer segments, thus making these “stick” to the RPE. The advantage for the animal would

be the isolation of the outer segments from adjacent outer segments by means of a pigment sheath. This is found in some predators and would lead to a higher acuity (Schwab, 2005).

The histology however showed that there was no difference in the distribution of the pigments in the RPE between summer and winter, in both seasons the granules were only found adjacent to the apical membrane. This lack of seasonal change is not surprising considering what was mentioned above: Unlike in predators, for the survival of a reindeer it is of a higher importance to recognize shape and movement rather than to see an accurate picture, this being true for summer and winter.

5.3. Measurement of photoreceptors outer segments

These aspects of the visual system were being examined because albino rats showed a change in outer segments length when kept in constant darkness (Schremser et al., 1994).The measuring of the outer segments turned out to be difficult and the results not reliable since the outer segments were distorted. It is possible, that formaldehyde was not the right fixative medium for preserving the retina for this kind of histology work.

Because of the unreliability of the measuring results of the outer segments, the issue of whether those are longer in one season than in the other could not be resolved here. Experiments in albino rats (Schremser et al., 1994) showed that when kept in constant darkness, these have longer outer segments than albino rats kept in cycling dark-light conditions or constant light conditions. This is due to a higher production rate of outer segments while the shedding rate stays the same. The longer outer segments might function to enhance the chance of photon capture by the photoreceptors and thus improve vision. In similar experiments done on pigmented mice, this difference in outer segment length could not be found (Beshares et al., 1979). However, albino rats, like any albino, are due to a genetic mutation. Such animals are not suitable to be used as a standard. This does not mean that there is no seasonal change in the outer segment length in reindeer, but it does not support the theory either.

There is an important difference between the rodents (rats, mice) and the reindeer in regards of the shape to the pupil. The pupils of rats and mice are circular while reindeer possess pupils that are horizontal slits which gives them a wide visual field. They pupils in reindeer can dilate to a stage where almost no iris can be seen, thus more light can enter the eye. This might mean that the reindeer does not have to produce outer segments, an energy demanding process, at a higher rate in constant darkness than it does under normal conditions or in constant light.

5.4. Colour shift of the reflected light caused by external pressure applied to the tapetum lucidum

The seasonal fluctuation in IOP seems a possible candidate for the physical mechanism behind the changes in the TL. The IOP is generated by the aqueous humor, a fluid produced by the ciliary bodies in the posterior chamber of the eye. Through the pupil the aqueous humor travels into the anterior chamber where it is drained out of the eye via the Schlemm's canal. The IOP is regulated by the rate of production and/or drainage of the aqueous humor (Boron et al., 2004).

The pressures needed to lead to the changes shown in Figure 16, 22.517 and 67.551 mmHg, are very high compared to the mean intraocular pressure, 13.3 mmHg in summer and 18.3 mmHg in winter, and its mean seasonal change, 5 mmHg. This could lead to the assumption that the seasonal pressure change can not possibly be the cause for the seasonal chromatic shift found in the light reflected by the TL. It is important to note, that the pieces of TL used for the experiment had been fixed in 4% formaldehyde. Fresh, untreated tissue will very likely react to a much lower pressure values than used for this experiment. In the *in vivo* situation factors like the shape of the eye, the location of the TL in the eye and its relationship to other ocular structures could be influencing how pressure is perceived by the TL. Another factor to be considered is the time scale. A small pressure change over a long time period e.g. several weeks or months may be as effective as a bigger pressure change over a short time period, like in the experiment. The experiment could not mimic any of these factors, but since its cause was only to investigate whether pressure change

experienced by the TL was capable at all to result into a chromatic shift in the reflected light, it was not required to.

The fact that the TL of other ungulates turn blue when put under pressure might not be as surprising as it seemed at first. All ungulates have a TL fibrosum, the structure not differing much between the species (Ollivier et al., 2004). Treating them in the same way in an experiment one can expect similar results.

What exactly leads to the different spacing between seasons is still to be determined. No seasonal chromatic shift in cow (*Bos primigenius*) or in Scottish red deer (*Cervus elaphus scoticus*) has ever been reported. In this context it is important to keep in mind that even if the cow, due to domestication, is now found in rather high latitudes, its ancestor, the aurochs (*Bos primigenius primigenius*) (Edwards et al., 2007) and the Scottish red deer (Gyllensten et al., 1983) both evolved in an environment with far less dramatic seasonal differences in light regimes than the ones that are found in the environment the reindeer evolved in. Thus there is no need for a seasonal chromatic shift of the light reflected by the TL in either of those species.

5.5. Concluding comments – Future studies

It would be of interest to examine the eyes of calves before the first winter to see if the annual endogenous clock has to be entrained by experiencing a first period of arctic night. An examination of the TL of other polar animals, such as the muskoxen (*Ovibos moschatus*), the Arctic fox (*Alopex lagopus)* and of course the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) could provide information about the evolution of the seasonal change in TL and its value for animals living in Polar Regions. Since the eyes of the first two species are quite hard to obtain, it will only be practical to sample eyes from the Svalbard reindeer, but this being the closest relative to the Eurasian mountain reindeer of those three species, it is also of the biggest immediate interest. For this, animals were shot on Svalbard in January 2010.

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