Spatial Vision in Birds: Anatomical investigation of spatial resolving power

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Anatomical investigation of spatial resolving power

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Abstract

Avian eyes are big both in relative and absolute terms, thus the importance of vision to birds is obvious. Even though the general eye plan is rather conservative throughout the group, there is a great variation in visual capabilities. In this thesis I present four studies on different aspects of spatial vision in parrots, procellariiform seabirds and birds of prey. In Paper I we studied retinal ganglion cell topography and anatomical spatial resolution in two Australian parrots, budgerigar and Bourke's parrot, inhabiting open terrain. Differently than expected, we did not find a horizontal visual streak, an elongated area of increased ganglion cell density, which would be predicted by the "terrain theory". In addition, we found that anatomical spatial resolution based on ganglion cell density is lower than behaviourally determined visual acuity. In Paper II, we compared spatial resolution and optical sensitivity in two procellariiform seabirds with contrasting nesting and foraging strategies. As predicted, the Leach's storm-petrel had lower visual acuity than the Northern fulmar, however similar optical sensitivity at the level of single rod photoreceptor. Additionally, both species had a well-pronounced horizontal visual streak that supports the "terrain theory". In Paper III, we studied the development of the visual and olfactory system in the Leach's storm-petrel juveniles. Our results indicated, that fine-tuning of retinal ganglion cell topography does not happen early in development, and that the ganglion cell layer continues to mature throughout the nest period. In addition, we found that two-weeks old juveniles lack basic phototactic and optokinetic reflexes. In Paper IV we used transmission electron microscopy and immunohistochemistry to investigate the presence of double cones in the foveae of the red kite, common buzzard, Eurasian sparrowhawk and peregrine falcon. The double cone-free zones in the central fovea differed in size between species. We also found double cone-free zone in the temporal fovea of the common buzzard and peregrine falcon, but not in the Eurasian sparrowhawk. In three species of raptors, in which we studied opsin expression, we found violet and green sensitive cones in the central fovea, and thus assume that raptors have all four types of single cones in the central fovea. These findings indicate, that single cones, not double cones, mediate the high spatial resolution in the raptor fovea.

Key words: Vision, Visual ecology, Visual spatial resolution, Retinal ganglion cells, Development, Double cones, Birds, Parrots, Seabirds, Raptors

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"The outcome of any serious research can only be
to make two questions grow,
where only one grew before"
Thorstein Veblen

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Original papers and manuscripts

Paper I **Mitkus, M**., Lind, O., Chaib, S. & Kelber, A. (2014) Retinal ganglion cell topography and spatial resolution of two parrot species: budgerigar (*Melopsittacus undulatus*) and Bourke's parrot (*Neopsephotus bourkii*). *J. Comp. Physiol. A* 200, 371 – 384

Paper II **Mitkus, M**., Nevitt, G., Danielsen, J. & Kelber, A. Spatial resolution and optical sensitivity of a DMS-responder and a non-responder: Leach's storm-petrel and Northern fulmar. Manuscript, to be submitted to *Functional Ecology*

Paper III **Mitkus, M**., Nevitt, G. & Kelber, A. Development of the visual and olfactory system in Leach's storm-petrel nestlings. Manuscript

Paper IV **Mitkus, M.,** Olsson, P., Toomey, M., Corbo, J. & Kelber, A. The central fovea of raptors lacks double cones. Manuscript, to be submitted to *Journal of Comparative Neurology*

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Author contributions

Paper I MM performed 90% of the lab work, developed methods, analysed most of the data and wrote the manuscript with feedback from OL and AK.

Paper II MM planned and performed the experiments with some contributions from AK and JD, analysed all data and wrote the manuscript with feedback from AK, JD and GN.

Paper III MM planned and performed the experiments with some contributions from AK, analysed all data and wrote the manuscript with feedback from AK and GN.

Paper IV MM had the idea for the project, planned it in collaboration with AK, MT, PO and JC, did the sampling and TEM work with contributions from PO, analysed data and contributed to writing the manuscript together with the other authors.

Papers, which are not part of the thesis:

Lind, O., Sunesson, T., **Mitkus, M.** & Kelber, A. (2012) Luminance dependence of spatial vision in budgerigars (*Melopsittacus undulatus*) and Bourke's parrots (*Neopsephotus Bourkii*). *J. Comp. Physiol. A* 198, 69-77

Lind, O., **Mitkus, M.,** Olsson, P. & Kelber, A. (2013) Ultraviolet sensitivity and colour vision in raptor foraging. *J. Exp. Biol.* 216, 1819-1826

Lind, O., **Mitkus, M.,** Olsson, P. & Kelber, A. (2014) Ultraviolet vision in birds: the importance of transparent eye media. *Proc. R. Soc. B* 2014 281, 20132209, 1-9

Populärvetenskaplig sammanfattning (Summary in Swedish)

Fåglar har fascinerat människor i århundraden. Färgen på deras fjädrar, deras flyghöjd och deras förmåga att fånga små byten har skapat den allmänna uppfattningen att fåglar har en fantastisk syn. Föreställningen att fåglar har skarpare syn än människor är väletablerad hos jägare och fiskare och finns att hitta i litteraturen. Det finns emellertid tusentals fågelarter och deras synförmåga skiljer sig åt. Idag känner man endast till några få rovfåglar som kan se finare detaljer – spatial upplösning – än människor. I den här avhandlingens kappa ger jag en översikt av de teoretiska begränsningarna för spatial upplösning och hur denna egenskap kan mätas, och jag ger en bild av mångfalden av fågelögon och deras utveckling. Sammanlagt lägger jag fram fyra originalstudier om fåglars näthinna och spatiala upplösning.

I den första studien undersökte vi två papegojor, undulater och bourkesparakiter. Dessa papegojor lever i ett öppet landskap i centrala Australien och vi förväntade oss att de skulle ha ett brett lateralt område av skarp syn i näthinnan för att i detalj kunna övervaka händelser utmed horisonten. Det hade de emellertid inte. Vi upptäckte även att de har relativt låg anatomisk synskärpa som inte stämmer överens med uppskattningar av synskärpan från beteendestudier.

I den andra studien undersökte vi två petrellfåglar: klykstjärtad stormsvala och stormfågel. Den klykstjärtade stormsvalan är en mindre, kryptiskt färgad fågel som bygger bo i hålor och förlitar sig på luktsinnet för att hitta mat. Stormfågeln är en större och färgglad fågel som bygger bo i öppna landskap och är mindre beroende av luktsinnet för att hitta mat. Våra resultat visar att stormfågeln har skarpare syn som den kan använda för att hitta andra fåglar ute till havs. Dessutom hade båda arter näthinnor med breda lateral områden för skarp syn, vilket man kunde förvänta sig av fåglar som behöver upptäcka föremål utefter horisonten på ett öppet hav.

I den tredje artikeln studerade vi hur syn- och luktsystemen utvecklas i den klykstjärtade stormsvalans ungfåglar. Vi upptäckte att synen inte är färdigutvecklad hos två veckor gamla ungar och näthinnan fortsätter att utvecklas under hela tiden i boet. Vi visar även att ögonstorleken ökar lika mycket som luktbulberna från två veckors ålder till mogen ålder.

I den sista studien letade vi efter dubbeltappar i fovean hos fyra rovfåglar. Dubbeltappar antas vara de synceller som ger skarp syn och för denna funktion bör dubbeltappar vara rikligt representerade. Vi hittade emellertid inga dubbeltappar i den centrala regionen av foveorna. Däremot hittade vi lila- och grönkänsliga enkeltappar och antar att alla enkeltappar finns representerade i fovean hos rovfåglar. Således förmedlar enkelltappar, men inte dubbeltappar, hög spatial upplösning hos rovfåglar.

Introduction

Animals have evolved various senses to extract information from the environment. Sensory information is present in many forms including light, sound, vibration and others, but only light delivers instantaneous information about the position of objects and enables immediate long-distance interactions. Not surprisingly vision is used to guide so many behaviours including locomotion, orientation, navigation, prey detection, predator avoidance, communication and mate choice. For some of these behaviours coarse visual information is sufficient, but for others high precision in detecting the direction of light is needed. Depending on the lifestyle, some animals cope well with coarse images, but others require high spatial resolution.

Birds have fascinated humans for centuries. A long lasting notion that birds have sharper vision than humans is common not only in the idle talks of hunters and fishermen, but can be also found in the text books. However, even though the basic morphological plan of the avian eye is as uniform throughout the group as it is in other vertebrates (Walls 1942), the great deal of variation exists in its function, and especially so in its spatial resolving power. To date only some raptorial birds are known to possess higher spatial resolution than humans do (Fischer 1969, Reymond 1985, 1987), whereas some others have more than ten times lower visual acuity (Harmening et al. 2009).

This thesis comprises four chapters. Chapter 1 introduces theoretical principles, which set the limit to the spatial resolution. Chapter 2 briefly overviews the diversity and morphology of avian eyes and Chapter 3 presents some knowledge on avian eye development. In Chapter 4 several techniques used to measure spatial resolution in birds are presented with the main emphasis on the anatomical methods, which were used in Papers I, II, III and IV.

In Paper I we studied retinal ganglion cell topography and anatomical spatial resolution in two parrot species, the budgerigar (*Melopsittacus undulatus*) and the Bourke's parrot (*Neopsephotus bourkii*). We discussed methodological issues in detail and compared the results with the behaviourally determined visual acuity values obtained from the literature. In Paper II we investigated retinal ganglion cell topography, anatomical spatial resolution and optical sensitivity in two procellariiform seabirds with contrasting foraging strategies, the Leach's storm-petrel (*Oceanodroma leucorhoa*) and the Northern fulmar (*Fulmarus glacialis*), and discussed the results from the perspective of foraging ecology. In Paper III we

compared the development of the visual and olfactory systems in the Leach's storm-petrel juveniles. Paper IV presents the result of the search of the double cones in the central and temporal foveae of four raptor species, the common buzzard (*Buteo buteo*), the Eurasian sparrowhawk (*Accipiter nisus*), the red kite (*Milvus milvus*) and the peregrine falcon (*Falco peregrinus*).

Chapter 1 Principles of Spatial Resolution

Both, evolution and technology have to follow the same rules of physics. Having had a camera in your hands helps to understand the basic working principles and structure of an eye. Resolution and sensitivity are two main properties of any type of eye or camera. Interestingly, the wave nature of light sets the limit to the resolution and the quantum nature of light limits the sensitivity. The trade-off between these two sides of the light is what determines the structure of an eye.

Diffraction limit and the optical cut-off frequency

The main point in understanding spatial resolution is that the image of a point is not a point. Due to the wave nature of light, the image of a distant point light source, such as a star, falls on a retina as a pattern of a specific light intensity distribution. This optical phenomenon known as diffraction sets the ultimate limit to spatial resolution.

The parallel rays of light coming from a distant object may be more accurately considered as the propagation of a flat wavefront. When passing through the lens, the peripheral parts of the wavefront have to cross less optical material than the central part, which therefore gets delayed. This results in refraction - the wavefront becomes curved (Fig. 1.1a). Refracted light continues to the focal point, where different parts of the same wavefront meet. Because some light was delayed in the lens, different components of the wavefront arrive at the focal point in different phases. Those parts, which are in phase, interact constructively and reinforce each other, while other parts, which are out of phase, interact destructively and cancel each other out. This phenomenon of interference results in a diffraction pattern, which, if light passed through a circular aperture, has a central bright spot surrounded by a series of alternating dark and bright rings (Fig. 1.1b). Thus, the point of light is no longer a point.

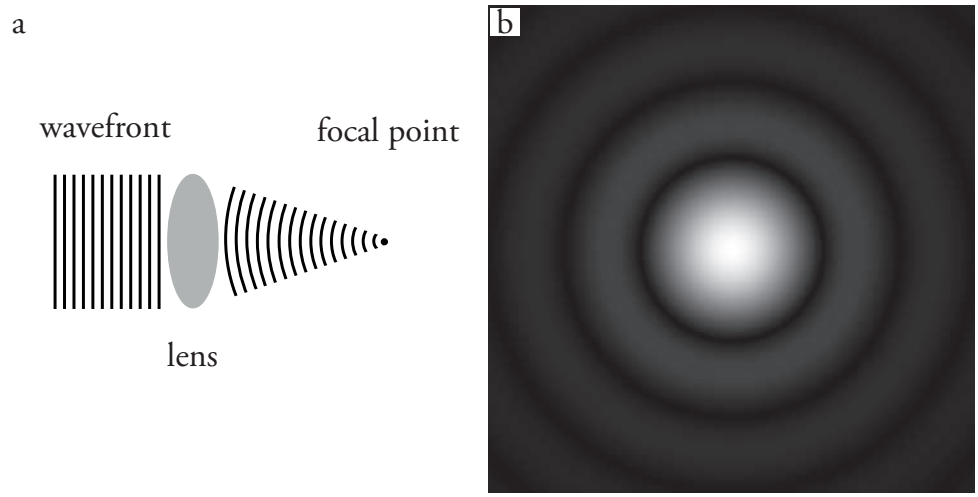

Fig. 1.1. The light from a distant point source propagates as a flat wavefront. a - When passing through a lens the light is refracted and focused to the focal point. b - The image of the point source is not a point, but, if passed through a circular aperture, a diffraction pattern known as the Airy disc. (b courtesy of Wikimedia Commons)

The central bright spot of the diffraction pattern is known as Airy disc. The width (ω) of the bright spot at its half maximum intensity is used as a measure of the Airy disc (Land and Nilsson 2012). Its angular size (expressed here in radians) depends on the wavelength of light (λ) and the diameter of the aperture (D):

$$\omega = \lambda/D. \qquad [1]$$

The larger this spot, the more blurred is the image of the distant point. If the image of any other object is smaller than approximately the half width of the Airy disc, it is blurred out (Land and Nilsson 2012). Thus the finest spatial frequency (expressed in cycles/radian) that the optics can pass is the reciprocal of the half width of the Airy disc:

$$\nu_{co} = 1/\omega = D/\lambda. \qquad [2]$$

This is also represented in the modulation (contrast) transfer function (MTF) (Fig. 1.2), which illustrates how the contrast in the image is attenuated when passing through the optics. In an ideal optical system the highest spatial frequency, which is passed, is indeed limited by diffraction. This equation indicates, that the larger the pupil (and the lens) of the eye, the higher spatial resolution it can have. However, larger lenses introduce other optical imperfections, which compromise the spatial resolution of an eye.

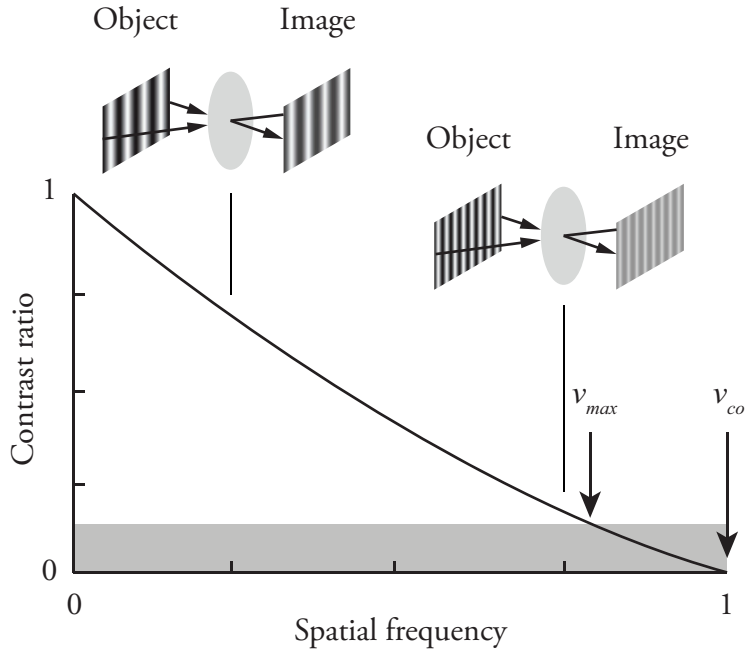

Fig. 1.2. The contrast transfer function. Because of diffraction, even the theoretically ideal lenses convert a high contrast object into a lower contrast image. The contrast ratio between image and object decreases as the detail in the object gets finer. At the optical cut-off frequency (v_{co}) there is no contrast left in the image. The shaded area at the bottom of the figure illustrates how low photon numbers limit the maximum spatial frequency (v_{max}) to the fraction of the cut-off frequency (v_{co}) at low light levels. The contrast between black and white stripes of the grating pattern is defined as the difference of their intensities divided by their sum: Contrast = $(I_{max}-I_{min})/(I_{max}+I_{min})$. (Modified from Land and Nilsson 2012)

Optical defects

Apart from the main optical limitation intrinsic to the nature of light, there are other optical problems, which can degrade the image on its way to the retina. The most common and important are defocus, spherical and chromatic aberrations.

Imperfect focus

Objects in a 3D world are in different distances from the eye. Because nearby objects are brought to a focus further from the lens than distant objects, many eyes have to have a mechanism of accommodation. In fish, which have non-flexible lenses, accommodation is achieved by moving the lens further away or closer to

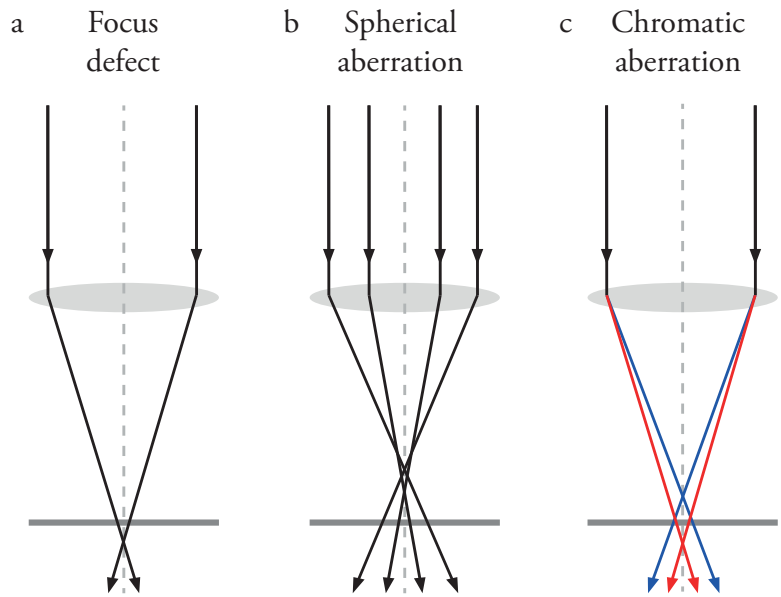

Fig. 1.3. Optical imperfections. a - Image is focused behind the focal plane. b - Spherical aberration – the light, which passes further away from the optical axis, is focused closer to the focal plane than the light passing near the axis. c - Chromatic aberration – even if entering the lens at a same position, light of short wavelengths (blue) is refracted more strongly than light of long wavelengths (red).

the retina. Other vertebrates can change the refractive power of the lens or cornea, or both, by changing the curvature of the refracting surfaces. If the image is refracted too much or too little it will be focused in front or behind the retina, and thus will be blurred (Fig. 1.3a). Generally, small eyes with small apertures suffer less from defocus, because small apertures provide larger depth of focus (De Valois and De Valois 1990).

Aberrations

In lenses with a short focal length, peripheral light has to be bent much more than light entering closer to the optical axis. In lenses with a spherical surface this is hard to achieve, and peripheral rays are refracted too much resulting in spherical aberration (Fig. 1.3b). One way to reduce this problem is to have a non-spherical lens. Indeed the front surface of the human lens is hyperbolic, while the rear surface is parabolic (Martin 1983). Another solution to the problem is to have a lens with a refractive index gradient, which is the case in many vertebrate species (Land and Nilsson 2012).

Another type of optical imperfection is longitudinal chromatic aberration, which occurs because light of different wavelengths is refracted differently even if it enters a homogenous lens at the same point. Light of short wavelengths is refracted more strongly than light of long wavelengths, which means that blue light is focused closer to the lens than red light (Fig. 1.3c). To overcome this problem fish and some other vertebrates have evolved multifocal lenses (Kröger et al. 1999).

Both types of aberrations become more severe when the aperture is large relative to the focal length. Thus while a large aperture in a large eye can help to reduce diffraction, the large eye size itself cannot help to reduce aberrations. In a given eye with a fixed focal length, the best image quality can be achieved when diffraction blurring is about equal to the aberration blurring. Indeed, in humans, pupil diameter can be changed from 2 to 8 mm (Land and Nilsson 2012), but the best image quality in bright light is produced when the pupil is 2.4 mm in diameter (Miller 1979).

Retinal sampling frequency

According to the sampling theorem, a sampling system with N sampling units can fully resolve all spatial frequencies below N/2 (De Valois and De Valois 1990). In other words, one sampling unit is needed for each node and antinode of a sinusoidal grating, thus the number of sampling units needed is twice the spatial frequency of the grating. To put it simply, a spatial frequency of for example 30 cycles/degree can be fully sampled by 60 sampling units per degree of visual angle. Expressed in the opposite way, 60 sampling units in one degree of visual angle can sample all spatial frequencies below 30 cycles/degree (De Valois and De Valois 1990). How small do these sampling units, which can be simply called photoreceptors, have to be to sample the spatial frequency at the optical cut-off limit?

Each eye has a point, called a nodal point, that light passes through without being bent (Fig. 1.4). The distance from the nodal point to the focal plane in the retina is called posterior nodal distance (*PND*, colloquially often simply called the focal length). An object of a size *O* at a distance *U*, and the image of the object on the retina of a size *I*, then have the following relationship:

$$O/U = I/PND. \qquad [3]$$

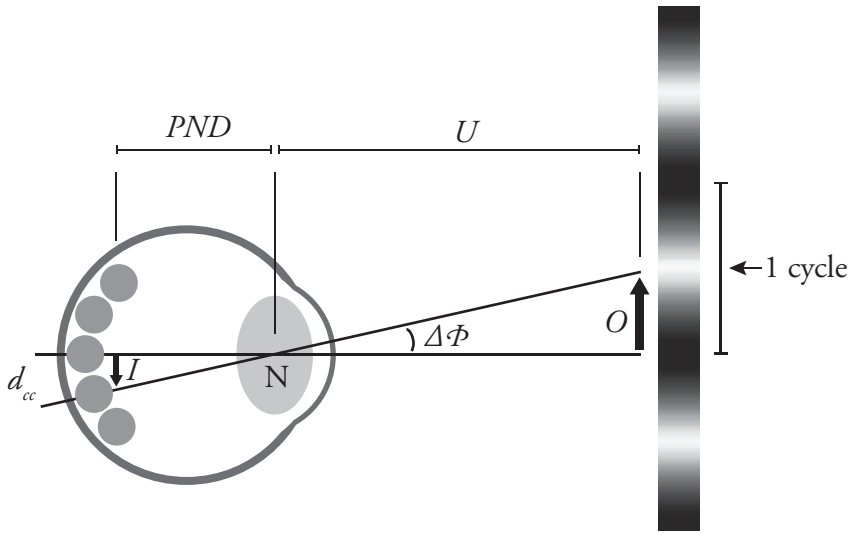

Fig. 1.4. Each eye has a nodal point N, that light passes through without being bent. The distance from the nodal point to the image plane is the Posterior Nodal Distance *PND*. The object *O*, at a distance *U* from the nodal point, and its image *I* on the retina subtend the same angle $\Delta\phi = O/U = I/PND$. The angular separation between two adjacent receptors, packed in a square array, and sampling node and antinode of the sinusoidal grating is also $\Delta\phi = d_{cc}/PND$, where d_{cc} is the receptor centre-to-centre distance. The angular width of one period (cycle) then is $2\,\Delta\phi$, therefore the sampling frequency of the eye is $\nu_R = 1/(2\Delta\phi) = PND/2d_{cc}$. (Modified from Land and Nilsson 2012)

It is easy to see from this relationship that the angular separation ($\Delta\phi$; in radians) between two adjacent receptors sampling node and antinode of the sinusoidal grating (Fig. 1.4) is:

$$\Delta\phi = d_{cc}/PND, \qquad [4]$$

where d_{cc} is the receptor centre-to-centre distance. Then, for the spatial frequency at the diffraction limit, the angular distance (in radians) between neighbouring receptors, when they are packed in a square array, is:

$$\Delta\phi_R = 1/2\nu_{co} = \lambda/2D. \qquad [5]$$

However, in the highest density regions, photoreceptors are usually packed in a hexagonal instead of a square array (Snyder and Miller 1977); then the angular distance (in radians) between the receptors is:

$$\Delta\phi_H = 1/\sqrt{3}\nu_{co} = \lambda/D\sqrt{3}. \qquad [6]$$

Therefore, in order to sample the image provided by the diffraction-limited optics, the receptor centre-to-centre distance has to be:

$$d_{cc} = \Delta\phi_H \, PND = \lambda PND/D\sqrt{3}. \qquad [7]$$

Do photoreceptors of such size exist? In humans, photopic vision is maximally sensitive to light of 555 nm wavelength, and the best image quality is achieved when the pupil diameter is 2.4 mm (Miller 1979). With a *PND* of 16.7 mm the d_{cc} is 2.2 μm. This indeed agrees with histological measurements, which report distances between adjacent cones in the human fovea of 2.0-2.3 μm (Snyder and Miller 1977).

Can photoreceptors be even narrower? Yes, but not much. The narrowest photoreceptors in both vertebrates and invertebrates are about 1 μm wide (Land and Nilsson 2012). Again, due to the wave nature of light, when photoreceptor diameter approaches the wavelength of light, total internal reflection no longer holds. Photoreceptors do not act as a light-guiding fibre anymore. Light starts to interfere and creates waveguide modes with uneven energy distribution. Some part of this energy even travels outside the photoreceptor and can be captured by neighbouring photoreceptors causing deterioration in resolution (Snyder 1979). Thus, when the smallest photoreceptor diameter is achieved, the only way to increase spatial resolution further is to increase *PND* and therefore eye size:

$$\nu_R = PND/(d_{cc})(\sqrt{3})(57.3), \qquad [8]$$

where factor 57.3 converts spatial frequency unit from cycles/radian to cycles/degree, because 1radian = 180°/π, or 57.3°.

However, independently of the eye size, the brightest naturally occurring light levels are needed (close to 10^5 cd/m^2) to resolve spatial frequencies of about 96% of the optical cut-off limit. Humans and some raptorial birds can indeed reach these limits. If the light level is 100 times lower, only frequencies of about 80% of the cut-off limit can be resolved (Snyder and Miller 1977), as is schematically indicated in the MTF (Fig. 1.2). Indeed as light level drops, spatial resolution deteriorates drastically (Fig. 4.1).

Sensitivity

There are 10^{10} times more photons available under bright sunlight than under an overcast starlit sky (Martin 1990). Every photographer knows three main ways how to take better pictures when it gets darker. Reducing the *F-number (*focal length/aperture diameter - *f/D)* of the lens increases image brightness *(1/F-number)*^{2}. Increasing the ISO value of the camera chip (the gain) increases pixel sensitivity. Prolonged exposure time increases the amount of photons collected.

The first two mechanisms can be easily seen in the equation describing photoreceptor sensitivity S (measured in square micrometres times steradians (μm^2 sr; Warrant and Nilsson 1998):

$$S = 0.62\, D^2\, (d/f)^2\, P_{abs}, \qquad [9]$$

where a larger pupil diameter D and shorter focal length f are analogous to a lower *F-number* in a camera lens. The absorption rate P_{abs}, which is comparable to the ISO value of the matrix, can be approximated by:

$$P_{abs} = kL/(2.3+kL) \qquad [10]$$

for white light. Parameter k is the absorption coefficient describing the proportion of light absorbed per micrometre of the receptive structure length (L), for example the outer segment of vertebrate rod or cone. It depends on the density of photopigment and the way it is packed. It is specific to the photoreceptor type and, for example, is 0.028 for a human rod (Warrant and Nilsson 1998). Longer photoreceptor outer segments can pack more photopigment, thus larger L means higher P_{abs}.

As the photoreceptor diameter d needs to be small for better spatial resolution (to the limits described above) and the absorption coefficient k is limited by the maximum packing density of photopigment, the only way to increase sensitivity further is to increase photoreceptor outer segment length or lower the *F-number* of the eye. (However, long photoreceptors with highly packed photopigment suffer from high levels of thermal noise, thus a compromise with optimal signal to noise ratio has to be found). Once the limits in these parameters are reached, narrow receptors (small d) can be grouped into bigger units by summing their signals neuronally (computationally larger d; Warrant 1999). This mechanism, called spatial summation, allows using the same system for better resolution at high light levels and for lower resolution, but higher sensitivity in dim light. Yet another way to increase sensitivity even more, though with a substantial cost of motion blur, is to integrate photon catch over a longer period of time (Warrant 1999). This so called temporal summation has exactly the same effect as choosing a longer exposure time in photography.

The number of photons caught per photoreceptor determines the contrast that can be detected at each light level (Land and Nilsson 2012). Due to the random nature of photon availability, Poisson statistics applies and the relation between photon numbers (N) and detectable contrast (C) is as simple as:

$$N > 1/C^2, \text{ or } C > 1/\sqrt{N}. \qquad [11]$$

For instance, in order to detect 10% contrast, at least 100 photons are needed. If we look back at the contrast transfer function (Fig. 1.2), we see that low photon numbers, which limit the minimum detectable contrast, also set the lower value for the optical cut-off frequency.

Thus, as we see, resolution and sensitivity - two features, which describe eye performance – get intertwined together. The wave nature of light sets the limit to spatial resolution as it determines the minimum size of photoreceptors due to effects of waveguide optics, and the optimum size of the pupil due to deleterious effects of diffraction and aberrations. The quantum nature of light sets the limit to sensitivity, which limits the capability of the eye to make full use of its potential spatial resolving power.

Chapter 2 The Avian Eye

As now we know something about the theoretical constrains on optical systems, we will briefly look into the structure and diversity of avian eyes. These eyes, which are no more spectacular than the eyes of any other vertebrate (Walls 1942), however, fascinated humans for centuries, probably for no better reason than that they look at us from above.

General structure

The eye is an expensive organ and energy investment in it has to pay back. As avian eyes are big both in relative and absolute terms (Walls 1942) the importance of vision to birds is obvious. It is the ostrich (*Struthio camelus*), not the elephant, which has the largest terrestrial eye, reaching 50 mm in axial length (Martin 1985) and weighing more than the brain (Burton, 2008). Other "heavy-eyed" birds are the wedge-tailed eagle (*Aquila audax*), Kori bustard (*Ardeotis cori*) and European nightjar (*Caprimulgus europaeus*) (Burton 2008). Indeed, the eyes of some birds are so large that the interorbital bony septum is as thin as paper or even has a hole, and the eyes meet at the median sagittal plane (Fig. 2.1b). Because there is so little room in the orbit most birds have low amplitudes of eye movements (7-20° in various passerines, Fernández-Juricic et al. 2010; but up to 39° in tufted titmouse (*Baeolophus bicolor*), Moore et al. 2013), with nocturnal birds being extreme in having only minute eye movements (ca 1° in great horned owl (*Bubo virginianus*); Steinbach and Money 1973). This shows that in some species, the eyes are as big as the skull can accommodate, and some birds (like the Eurasian golden plover (*Pluvialis apricaria*)) even needed to build an extra structure on the skull to accommodate the huge nocturnal eyes (Fig. 2.1a; Martin and Piersma 2009). On the other extreme are kiwi birds, which have the relatively smallest eyes of all birds (Walls 1942, Martin et al. 2007), and one can find the reason in the behaviour and ecology of this nocturnal non-flying stranger of the bird kingdom.

Apart from their variation in size, avian eyes vary in shape too. However, they share one common property that the nearly hemispheric posterior segment of the eye globe is much larger than the anterior part (Jones et al. 2007). Thus, bird eyes are never spherical as they are in e.g. humans, seals or mice (Land and Nilsson 2012). There is also an asymmetry along the horizontal plane with the temporal

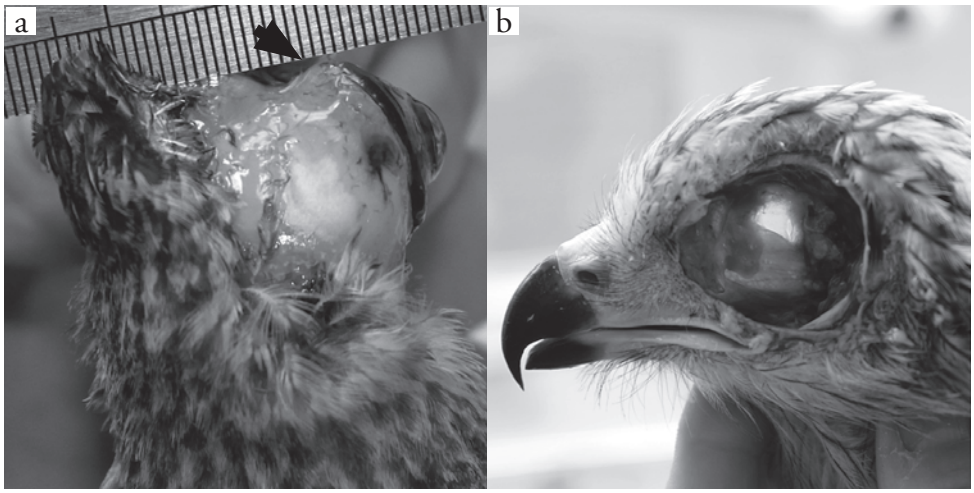

Fig. 2.1. The eye size is maximized in some avian species. a - The head of the Eurasian golden plover (*Pluvialis apricaria*) as viewed from the back. Feathers and skin are partially removed to expose large supraorbital protrusions - the supraorbital aliform bones (indicated with an arrowhead). These bones increase orbital volume and allow accommodation of relatively large eyes in a small skull. b - Freshly enucleated head of the red kite (*Milvus milvus*). The hole in the interorbital septum, which is almost 1 cm wide, indicates that eyes of a large relative and absolute size are touching each other in the centre of the skull of this species.

side of the eye being more extensive than the nasal side (Jones et al. 2007), which is easily visible in some owls and waders (pers. obs.). But along the vertical plane avian eyes are either flat (like in most diurnal birds with low resolution), globose (as in many diurnal birds with high resolution), or tubular (typical in owls, but also in some raptors). A ring of ossical bones connects the anterior and posterior parts of the eye and creates the sclero-corneal sulcus, to support the optical system of the eye against intraocular pressure, which rises slightly during accommodation (Walls 1942).

Optical system of the eye

Morphologically avian eye is of the camera-type (Land and Nilsson 2012) and its optical system consists of the cornea, aqueous humour, lens and vitreous humour (Fig. 2.2a). In terms of function, it can be considered as a linear magnification system (Martin 1983) and can be described, in a simplified manner, by the focal length and minimum *F-number* of an equivalent theoretical single lens.

Since in a vertebrate eye, the image of the object is in a different refractive medium than the object itself, it is the anterior focal length, which is of interest here. The anterior focal length is equal to the posterior nodal distance (*PND*) and

can be calculated by using Gullstrand's simplified (No. 2) schematic eye model (Land and Nilsson 2012). This model uses the radii of curvature of the cornea and the lens, their positions and refractive indices (Fig. 2.2b), which can be considered as the main parameters to describe the optical system of the camera-type eye. Variation in these parameters, or in other words variation in optical eye designs, is reflected in ecological needs of the animals.

One of the challenges posed by an amphibious lifestyle is a need to see relatively well both in air and under water. Diving birds need to cope with the loss of corneal refractive power while submerged, but still need to have not over-focused image while on land. One solution to this problem is a relatively flat cornea, which does not contribute much to the total refractive power of the eye (Kröger and Katzir 2008). Indeed, penguins have the flattest corneae of all birds (Martin 1999). Other diving species, with more rounded corneae, thus more equal refractive power distribution between the cornea and the lens, have to use strong accommodative mechanisms when under water (Meyer 1977).

Avian accommodation is always for near vision and usually has a range of around 20 diopters (Meyer 1977). Unlike fish or mammals, birds have lenticular as well as corneal accommodation (Land and Nilsson 2012). Crampton's muscles pull the cornea and reduce its radius of curvature to increase refractive power. Brücke's muscles contract the lens and sometimes so much that it is squeezed through the pupil supported by muscular iris and ossical bones.

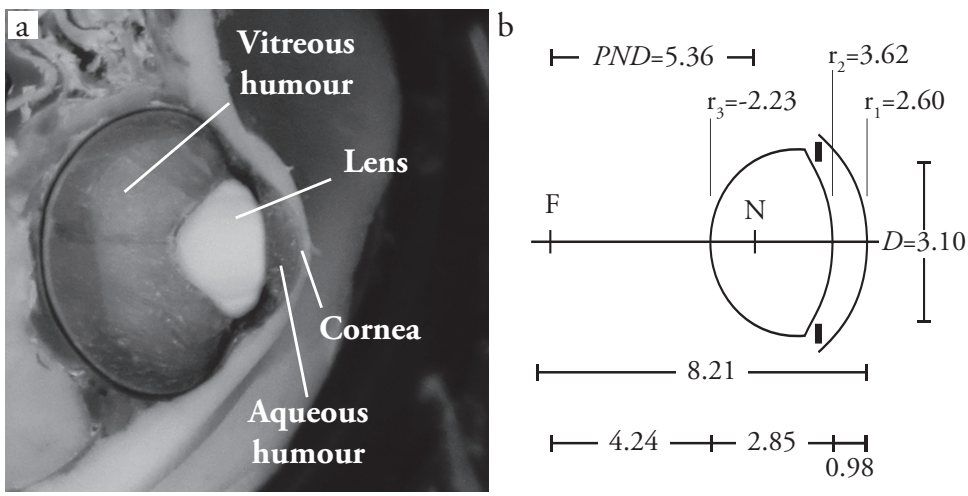

Fig. 2.2. Optical system of the avian eye. a - Hemisected frozen eye of the Leach's storm-petrel (*Oceanodroma leucorhoa*) showing the main optical components. b – Schematic representation of the simplified schematic eye model (*PND* – Posterior Nodal Distance; F – focal plane; N – nodal point; *D* – pupil diameter; r_1, r_2, r_3 – radii of the cornea front, lens front and lens rear surfaces respectively).

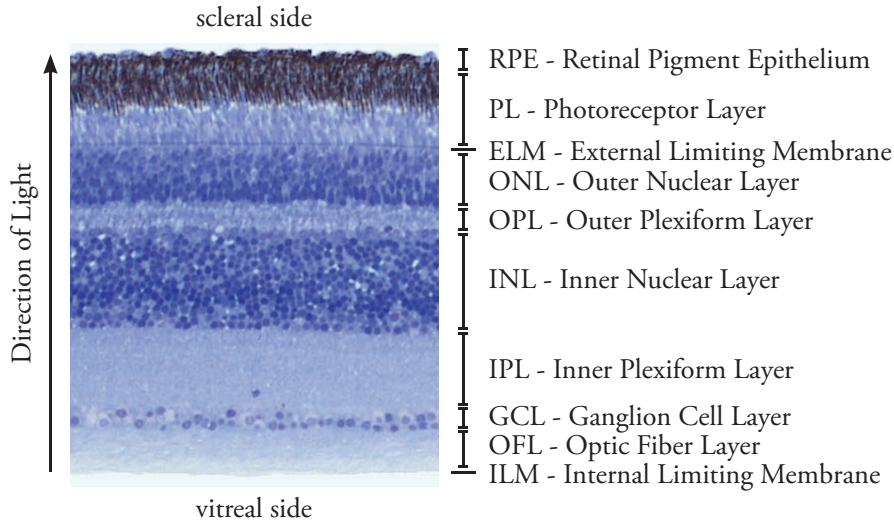

Fig. 2.3. Cross-section through the Leach's storm-petrel (*Oceanodroma leucorhoa*) central retina showing the retinal layers. The RPE contains cells, which protrude their apical processes filled with melanin granules into the PL, where inner and outer segments of the rods, single and double cones are situated. The nuclei of photoreceptors are positioned in the ONL. The OPL contains synapses connecting the photoreceptors to the bipolar and horizontal cells with nuclei in the INL, which also contains amacrine cells. Accordingly, the IPL is built up of synapses connecting bipolar and amacrine cells to the retinal ganglion cells (RGCs) in the GCL. Some displaced RGCs are found in the IPL and some displaced amacrine cells are present in the GCL. The axons of the RGCs heading to the optic nerve form the OFL.

Again unlike in fish or mammals, the avian lens is soft and flexible, and especially so in diving birds. By squeezing the lens through the pupil American dipper (*Cinclus mexicanus*) can create 50 diopters (Meyer 1977), hooded merganser (*Mergus cucullatus*) 67 diopters, and common goldeneye (*Bucephala clangula*) 78 diopters of extra power (Sivak et al. 1985) to compensate for the loss of corneal refractive power upon submersion.

Apart from accommodation and image formation, the optical system also controls the spectrum of light, which reaches the retina. Part of the light is scattered and part is absorbed mainly by the cornea and lens, and mostly in the short-wavelength region between 300 and 400 nm. Thus the optical system also works as a long-pass filter for the incident light travelling to the retina (Lind et al. 2013, 2014).

Retina

The avian retina is of the typical vertebrate type with an inverted design (Land and Nilsson 2012). It has several distinct neuronal layers, which light has to pass before reaching photoreceptors that are located outermost (see Fig. 2.3 for naming and abbreviations). Compared to the retinae of most other vertebrates, the avian retina is considerably thicker (Walls 1942). This increased thickness is attributable to the high density of cells in the inner nuclear layer and the high complexity of neurite arborisation in the inner plexiform layer (Martin 1985). These layers are especially thick in the regions with high photoreceptor density, which can be as much as double of that in the human fovea (Miller 1979, Coimbra et al. 2015).

Photoreceptor layer

Birds have three types of morphologically distinct photoreceptors: rods, double cones and single cones (Martin and Osorio 2008). Rods are active in dim light (scotopic conditions) and mediate achromatic vision. Cones are active in bright light (photopic conditions) and mediate both chromatic and achromatic vision. At intermediate light levels (mesopic conditions) all photoreceptors are functioning. Not surprisingly, rods dominate the retina of nocturnal birds, while cones are the main photoreceptors in diurnal species (Nalbach et al. 1993).

Birds have four types of single cones. Ultraviolet or violet sensitive cones (UVS/VS, containing sws1 type of light sensitive pigment), short-wavelength sensitive cones (SWS, sws2-pigment), medium-wavelength sensitive cones (MWS, rh2-pigment) and long-wavelength sensitive cones (LWS, m/lws-pigment) are responsible for colour vision (Hart 2001). Double cones consist of principle and accessory member (both containing m/lws-pigment) and are suggested to mediate motion perception and high resolution achromatic vision in bright light (Martin and Osorio 2008, Lind and Kelber 2011).

Photoreceptor size and shape varies not only between species, but also between different regions of the retina. However, the general structure and position of organelles within the photoreceptive cell is conservative (Fig. 2.4). Each cone type, but not the rod, at the end of the inner segment, contains a specific pigmented oil droplet, which, similarly to the ocular media, acts as a high-pass filter and further attenuates light reaching the light-sensitive pigments in the disks of the outer segment. Apart from spectral filtering, oil droplets also have an optical function, and together with the ellipsoid have a substantial effect on the light guiding through the inner structures of the cones to their outer segments (Wilby et al. 2015). Once the light is converted into electrical signals, they are sent to the brain via the complex network of neurons in the remaining layers of the retina.

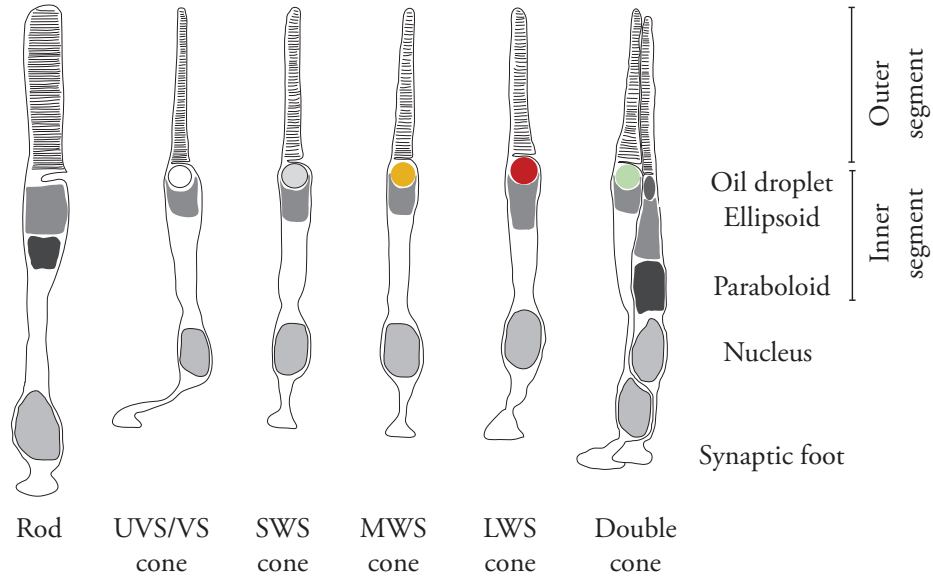

Fig. 2.4. Schematic drawing of the domestic chicken (*Gallus gallus domesticus*) rod, single cones and double cone representing six types of avian photoreceptors. UVS/VS – ultraviolet or violet sensitive cone, SWS – short wavelength sensitive cone, MWS – medium wavelength sensitive cone and LWS – long wavelength sensitive cone. The ellipsoid is the region, where mitochondria are located. Paraboloid (called hyperboloid in rods) is the region, where glycogen is stored. (courtesy of Peter Olsson)

Other retinal layers

The basic avian retinal design and its division in layers essentially resemble those of other vertebrates (Nalbach et al. 1993). In general, the signal from photoreceptors is sent to the retinal ganglion cells via dedicated bipolar cells, with horizontal and amacrine cells providing intraretinal interactions (Land and Nilsson 2012). On top of this basic plan, there is a lot of variation (Güntürkün 2000). However, our knowledge on components and structure of the avian inner nuclear layer and plexiform layers is very limited. Still today the classical Cajal's work from 1893 is often referred to as the main source of knowledge on avian retinal complexity. The reason for that is "a general belief from past research…that, for many cell types, the response characteristics and functions are similar across all vertebrate groups" (Martin 1985 p. 344). Therefore most of our knowledge on retinal organization and function comes from the research on mammalian model species (Masland 2012). Nevertheless, it is known that a lot of neural processing, which happens outside the retina in invertebrates, takes place already in the retina of vertebrates (Cronin et al. 2014). A complex neural network performs first computations and extracts relevant features for acuity, contrast, sensitivity and colour determination (Rodieck 1998).

Accordingly, in the high acuity areas of the retina high photoreceptor densities result in large numbers of bipolar and ganglion cells as compared to the peripheral regions (Nalbach et al. 1993). At these specialized locations, the convergence ratio from photoreceptors to ganglion cells is low and in some birds the ratio is 1:1 or close to this (Oehme 1964, Fite and Rosenfield-Wessels 1975, Coimbra et al. 2015). Finally, all the signals from the retinal ganglion cells are sent to the brain via the optic nerve, thus ganglion cells, not the photoreceptors, are the bottleneck for the information capacity of the eyes (Land and Nilsson 2012).

Retinal specialisations

Apart from neural convergence and pre-computation within the retina, another way to reduce the amount of visual information sent to the brain, and also the thickness of the optic nerve, is to "concentrate visual effort" on the areas of "interest". For species living in open habitats all approaching objects are imaged in a narrow horizontal band on the retina (Hughes 1977, Land and Nilsson 2012). This lead Hughes (1977) to propose the "terrain theory" to explain why species from open habitats have a narrow elongated band of increased RGC density. Indeed, prey or predatory mammals from open savannah (Hughes 1977), waterfowl (Lisney et al. 2013), or shallow water fish (Collin and Pettigrew 1988) all have a zone of higher acuity stretching horizontally across the retina, the so called visual streak. In Paper II we investigated RGC topography in two procellariiform seabirds, the Leach's storm-petrel and the Northern fulmar (Fig. 2.5), and found well-pronounced visual streaks in both species corroborating the theory. Other animals from more cluttered three-dimensional environments, like forest dwellers (Hughes 1977), ground-feeding birds (Dolan and Fernández-Juricic 2010), or coral reef fish (Collin and Pettigrew 1988), have a region in the retina, where RGCs are concentrated in a circular area instead of an elongated streak. However, as more and more species were studied, some examples contradicting the theory emerged (Hayes and Brooke 1990, Lisney et al. 2012, Tyrrell et al. 2013). Additional factors, like prey capture and handling technique, were suggested to have an effect too.

In Paper I we looked at RGC distribution in two parrot species, budgerigars and Bourke's parrots. Even though both species inhabit open terrain of the central Australia, all but one specimen lacked the visual streak that would be predicted by the "terrain theory". In budgerigars, apart from the area centralis we also found a presumptive area nasalis, which has never been reported in birds before. Higher visual acuity in the nasal retina serving temporal visual field would have an advantage for budgerigars while climbing, when birds use the beak as a support, and head mobility is therefore restricted.

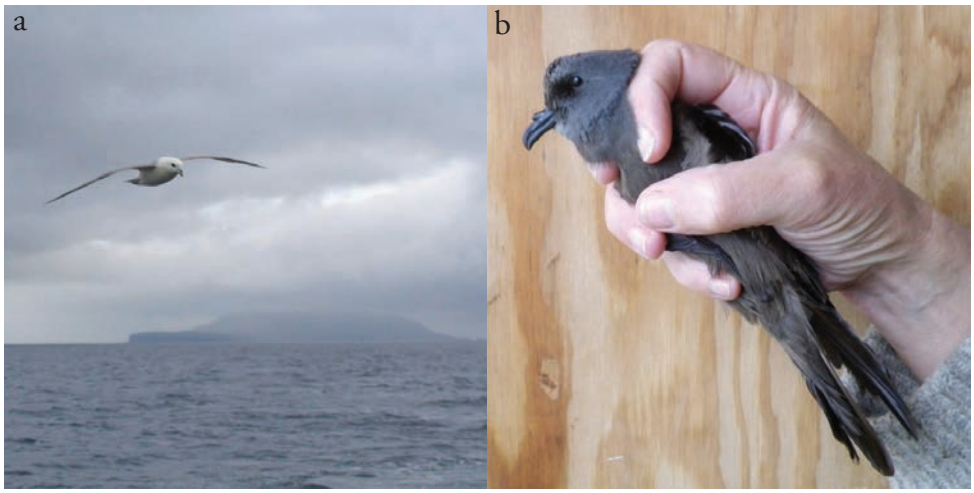

Fig. 2.5. Northern fulmar (*Fulmarus glacialis*) in its most natural environment (a). Leach's storm-petrel (*Oceanodroma leucorhoa*) in its most unnatural environment (b). (b courtesy of Almut Kelber)

However, neither Bourke's parrots nor other parrot species studied recently (Coimbra et al. 2014) had similar retinal specialisation. Several other explanations for the presence of the presumptive area nasalis were discussed in Paper I. They include domestication, age, and general intraspecific variability, which were also observed in pigeons (*Columbia livia domestica*) (Querubin et al. 2009) and Japanese quails (*Coturnix japonica*) (Budnik et al. 1984, Ikushima et al. 1986, Lisney et al. 2012). The effect of domestication on the avian visual system is an interesting question, which deserves more research in the future.

Apart from areae and visual streaks, many bird species have a third type of retinal specialisation called fovea. It is a structural depression in the retina caused by a partial or full radial displacement of ganglion and inner nuclear layers to the sides. Some foveae are shallow, but some are deep with steep walls (Meyer 1977). Snyder and Miller (1978) suggested that a fovea acts as a negative lens of a telephoto system increasing spatial resolution at that particular area of the retina. High variation of foveal depth, size and shape within single species (Oehme 1964, Paper II) suggests that the function of foveal depression might be different from the widely spread hypothesis of the magnifying telephoto lens. Reymond (1985, 1987) has shown that spatial resolution in raptors with deep foveae can be explained without this assumption. Indeed, optical, anatomical and behavioural spatial resolution match quite closely in the wedge-tailed eagle – the animal with the highest visual acuity discovered so far.

Chapter 3 Development of the Eye

Most of the knowledge on avian eye development undoubtedly comes from the research on domestic chickens (*Gallus gallus domesticus*). From regulatory genes that control early neural development to experimental manipulations on visual environment during maturation, studies on chickens build the basis of our understanding on the development of visual function in birds (Mey and Thanos 2000). However, in this chapter, we will briefly look only into the development of the retina and the growth of the main optical compartments of the eye.

Development of the retina

The vertebrate retina develops from the neural epithelium. Early in development the anterior portion of the neural tube divides into the forebrain, midbrain and hindbrain. The posterior part of the forebrain invaginates and forms two bilaminated eyecups. The outer layer of the eyecup forms into retinal pigment epithelium, and the inner layer gives a rise to the neural retina (Mey and Thanos 2000).

In chickens, during the initial stages of retinal development, progenitor cells proliferate uniformly across the retina. Around the fifth embryonic day (abbreviated as E5), proliferation slows down in the centro-temporal retina and by E8 progenitor cell division is found only in the retinal periphery. Accordingly, the oldest cells in the developing chicken retina are found in the centre, and subsequent neuron differentiation and maturation proceeds in an approximate centro-peripheral direction (Mey and Thanos 2000). However, a temporal-nasal gradient also exist as the nasal retina continues to grow for a longer period than the temporal retina (Rager et al. 1993).

The first retinal neurons, which start to differentiate, are retinal ganglion cells (RGCs), followed by the photoreceptors, amacrine cells, horizontal cells, and finally bipolar cells. However, the period of each cell type generation overlaps with one another, and the photoreceptors are the last cells, which stop to proliferate. In chickens, RGCs are morphologically developed at E16, but before that a wave of apoptosis sweeps through the retina and more than one third of all initially built RGCs die (Hughes and McLoon 1979, Straznicky and Chehade 1987). Photoreceptor cells mature much later. Even though opsin expression starts

seven days before hatching (E14, Bruhn and Cepko 1996), retinal oil droplets attain their mature colour and size only 15 days post-hatch in chickens (López et al. 2005). While the retina grows in size, it also grows in thickness at the same time.

Retinal stratification, the formation of retinal layers (depicted in Fig. 2.3), is happening as a result of vertical cell migration mainly between E6 and E16. The RGCs and displaced amacrine cells move to form the ganglion cell layer; amacrine, horizontal, bipolar and displaced ganglion cells form the inner nuclear layer, and photoreceptors stay on the scleral (ventricular) side to form the outer nuclear layer. Growing neurites and dendritic branching result in plexiform layers, which separate the nuclear layers. The first synapses appear in the inner plexiform layer at E13, however in the outer plexiform layer synapses start to form only on E17. Three days before chickens hatch (E18) only 3% of axons in the optic nerve are covered in myelin, suggesting that most of the myelination takes place during early post-hatching development (Mey and Thanos 2000).

Knowledge on retinal development in birds other than chicken is scarce. In chickens, which are relatively mature and independent after hatching (precocial birds), photoreceptor inner segments start growing 10 or 11 days before hatching (E9-E10; Wai and Yew 2002, Mey and Thanos 2000). In pigeons, which hatch relatively immature and require parental care (altricial birds), budding photoreceptor inner segments are found only on day 2 post-hatching (Bagnoli et al. 1985). By day 9 post-hatching most of the receptor outer segments of pigeons are present, but they continue to grow and reach adult length only by day 20 post-hatching (Bagnoli et al. 1985). In chickens, receptor outer segments appear 3 days before hatching (E18) and get their adult form 1 day before hatching (E20) (Olson 1979), even though photoreceptors still continue to proliferate until some time after hatching (Mey and Thanos 2000). In pigeons, synapses in the outer plexiform layer do not mature before day 9 after hatching (Bagnoli et al. 1985), while in chicken the first mature synapses are found 3 days before hatching (E18) (Mey and Thanos 2000). Apparently, the pigeon eye is much less developed at the time of hatching than the chicken eye. These data illustrate that timing of the retinal development in birds highly depends on the level of precocity.

Even though at the time of hatching the chicken visual system is basically functioning (Over and Moore 1981, Mey and Thanos 2000), other retinal developmental processes take place as the eye continues to grow. In the area centralis of the post-natal mammalian retina cell density increases due to cell migration, but in the periphery it decreases because of general eye growth and retinal stretch (Hendrickson and Provis 2006). In birds the formation of the area centralis was investigated only in chickens (Straznicky and Chehade 1987, Prada et al. 1991, Rager et al. 1993, Chen et al. 2004). Differently from mammals, chicken RGC density continues to drop after hatching and reaches the adult level at the age of four weeks (Ehrlich 1981, Straznicky and Chehade 1987).

Preferential cell death and differential retinal expansion increase the centro-peripheral gradient in RGC density and contributes to the well-pronounced area centralis (Straznicky and Chehade 1987, Rager et al. 1993), the region with the highest visual acuity.

In Paper III, we investigated the development of the RGC topography in the Leach's storm-petrel juveniles. Even though Leach's storm-petrels are classified as semi-precocial birds (Ricklefs et al. 1980), the chicks spend their first 61-67 days before fledging in a long and dark underground burrow. We had a chance to investigate juveniles at the age of 2, 4 and 6 weeks post-hatching. RGC topography varied not only between, but also within different age classes, however at least one specimen in each age class had a well-pronounced visual streak (Figs. 2, 3, 4 in Paper III), similar to that found in the adult birds (Fig. 1 in Paper II). These findings indicate that fine-tuning of RGC topography does not happen early in development, and RGC layer continues to mature throughout the nest period. Differently from chickens, the peak RGC density of Leach's storm-petrels did not decrease after week 2 post-hatching. It was similar in all investigated age groups, and was comparable to that of adult birds (Paper II). Unfortunately, we had no chance to study younger birds or to do more elaborated retinal investigations to reveal more about the retinal development in this species.

Eye growth

While the retina develops from the neural epithelium, the lens starts to form from the epidermal epithelium as soon as the retinal cup approaches it (around 1.5 days after start of incubation; Mey and Thanos 2000). As the task of the optical system is to bring light rays into focus on the retina, the growth of the optical components, and their product – the focal length, has to match the growth of the eyeball.

Different techniques are used to obtain optical parameters for the schematic eye model. It can be measured in a live animal, freshly fixed eyes or frozen eyes. The measures from different techniques agree quite well (Lind and Kelber 2009, Avila and McFadden 2010), especially when the natural level of variation and the number of assumptions used in constructing schematic eyes are considered (Martin 1983). To date, optical parameters of growing eyes exist for four species: domestic chicken (e.g. Schaeffel and Howland 1988, Avila and McFadden 2010), American kestrel (*Falco sparverius*) (Andison et al. 1992), common barn owl (*Tyto alba*) (Schaeffel and Wagner 1996) and Leach's storm-petrel (Paper III). Data summarised in figure 3.1 show how optical structures change during the first two months post-hatching in these four species.

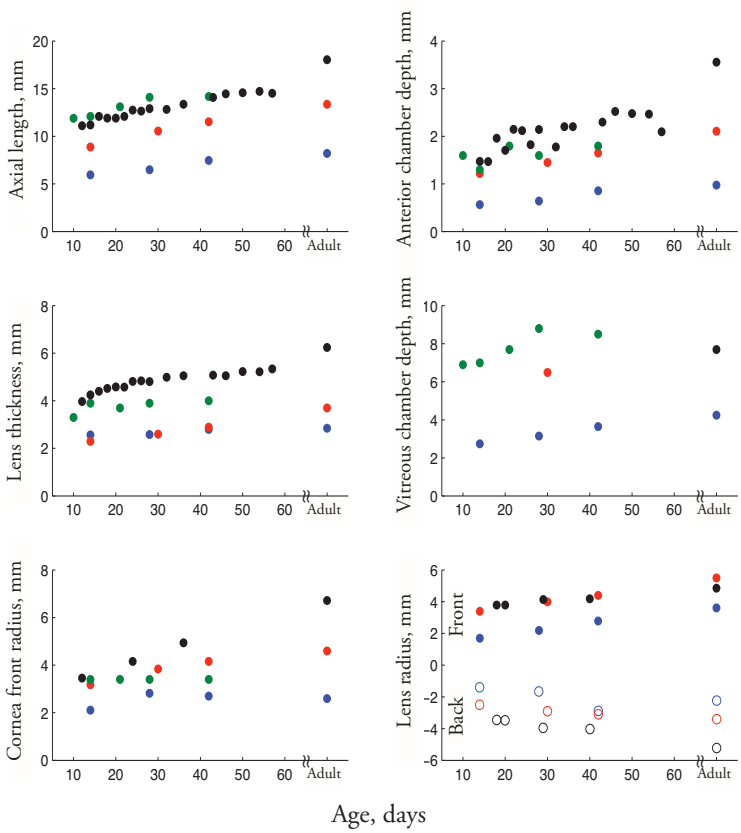

Fig. 3.1. Schematic eye parameters of the growing eyes. Blue symbols – Leach's storm-petrel (*Oceanodroma leucorhoa*) (Paper III), red symbols – domestic chicken (*Gallus gallus domesticus*) (Schaeffel and Howland 1988), green symbols – American kestrel (*Falco sparverius*) (Andison et al. 1992), black symbols – common barn owl (*Tyto alba*) (Schaeffel and Wagner 1996). Open symbols in the bottom right panel represent the radius of the lens back surface.

Chicken, American kestrel and common barn owl show considerable differences in ametropia soon after they open their eyes. Chickens are hyperopic soon after hatching (+3 to +6.5 diopters), American kestrels are highly myopic (-20 to -25 diopters) while common barn owl chicks show a high variation in the refractive power of the eye when the eyelids open (-4 to +4 diopters). However, chickens achieve near emmetropia in just 7 days, while it takes 10 days for American kestrels, and 2 weeks for the common barn owl juveniles. In the study presented in Paper III we did not perform measurements on emmetropization as this was difficult to achieve under field conditions. However, we did preliminary negative phototaxis and optokinetic nystagmus experiments, which indicated that spatial vision is still not functioning at 2 weeks post-hatching in Leach's storm-petrel juveniles.

Chapter 4 Measuring Spatial Resolution

We saw that the wave nature of light sets the limit to spatial resolution, and the quantum nature of light limits the optical sensitivity. We looked at the avian eyes, which have to compromise between both in order to provide enough information to their bearer to serve its ecological needs. In this chapter we will discuss how we can determine the spatial resolution in birds.

Behavioural measurements

The most common behavioural method to estimate visual acuity is a classical two-alternative forced choice discrimination task, where an animal has to choose between two simultaneously presented stimuli (Donner 1951, Martin and Gordon 1974, Dąbrowska 1975, Fox et al. 1976, Lind et al. 2012). The negative stimulus contains sinusoidal gratings of dark and lights bars, while the positive stimulus is a homogeneous field of the same mean luminance as the negative one. Once the animal is trained to choose the homogeneous stimulus field, which is rewarded with food, trials are repeated with randomised stimulus position and level of 'difficulty'. The percentage of correct choices for each spatial frequency is plotted in a psychometric function, and the highest spatial frequency at which animal does no more correct choices than could be predicted by chance is considered to be the spatial resolution threshold. The lowest behavioural visual acuities among birds are reported for the common barn owl (2.3-4.5 cyc/deg, Harmening et al. 2009, Orlowski et al. 2012) and the highest for the wedge-tailed eagle (138 cyc/deg; Reymond and Wolfe 1981, Reymond 1985) (Table 4.1). Raptors are the only group of all animals that have higher spatial resolution than humans.

Table 4.1. An overview of spatial resolution thresholds in birds.

Species name	Spatial resolution (cyc/deg)	Stimulus luminance (cd/m^2)	Reference
Common barn owl (*Tyto alba*)	2.6–4.0/4.1	2.7/30	Harmening et al. 2009
House finch (*Carpodacus mexicanus*)	4.7g	-	Dolan and Fernández-Juricic 2010
House sparrow (*Passer domesticus*)	4.8g	-	Dolan and Fernández-Juricic 2010
Carolina chickade (*Poecile carolinensis*)	5.0g	-	Moore et al. 2013
European robin (*Erithacus rubecula*)	6/6	1.53/19.1	Donner 1951
Tufted titmouse (*Baeolophus bicolor*)	6.6g	-	Moore et al. 2013
Leach's storm-petrel (*Oceanodroma leucorhoa*)	7.1g	-	Paper II
Great horned owl (*Bubo virginianus*)	6–7.5	80	Fite 1973
American mourning dove (*Zenaida macroura*)	7.6g	-	Dolan and Fernández-Juricic 2010
Reed bunting (*Emberiza schoeniclus*)	2.17/7.8	1.53/19.1	Donner 1951
Bourke's parrot (*Neopsephotus bourkii*)	9.2g/9.4	-/62	Paper I/Lind et al. 2012
Canada goose (*Branta canadensis*)	9.6g	-	Fernández-Juricic et al. 2011
Japanese quail (*Coturnix japonica*)	9.7g	-	Lisney et al. 2012
Yellowhammer (*Emberiza citrinella*)	2.17/9.7	1.53/19.1	Donner 1951
Grey partridge (*Perdix perdix*)	10.2g	-	Lisney et al. 2012
Tawny owl (*Strix aluco*)	8.8/12/8–11.1	5/50.5/160	Martin and Gordon 1974
Budgerigar (*Melopsittacus undulatus*)	6.9g/11.7	-/50	Paper I/Lind et al. 2012
Mallard (*Anas plathyrynchos*)	11.9g	-	Lisney et al. 2013
Domestic chicken (*Gallus gallus domesticus*)	4.2-6.4/7/8.3-12.8g	3.5/16/-	De Mello et al. 1992/Jarvis et al. 2009/Ehrlich 1981, P. Olsson (pers. com)
Ring-necked pheasant (*Phasianus colchicus*)	12.9g	-	Lisney et al. 2012
Pigeon (*Columba livia domestica*)	3/6/12/18	0.85/3.2/32/317	Hodos et al. 1976

Table 4.1 cont.

Species name	Spatial resolution (cyc/deg)	Stimulus luminance (cd/m^2)	Reference
Silvereye (*Zosterops lateralis*)	18.5c	-	Coimbra et al. 2015
Blue Jay (*Cyanocitta cristata*)	15–19g	-	Fite and Rosenfield-Wessels 1975
Ostrich (*Struthio camelus*)	19.8g	-	Boire et al. 2001
Brown honeyeater (*Lichmera indistincta*)	20.2c	-	Coimbra et al. 2015
Indian peafowl (*Pavo cristatus*)	20.6g	-	Hart 2002
Common blackbird (*Turdus merula*)	22.5/22.5	1.53/19.1	Donner 1951
Common chaffinch (*Fringilla coelebs*)	22.5/22.5	1.53/19.1	Donner 1951
Yellow-rumped thornbill (*Acanthiza chrysorrhoa*)	25.6c	-	Coimbra et al. 2015
Sacred kingfisher (*Todiramphus sanctus*)	26g	-	Moroney and Pettigrew 1987
Rook (*Corvus frugilegus*)	29.5/29.5	9.5/60	Dąbrowska 1975
Common magpie (*Pica pica*)	23.7–29.5/29.5–33.3	9.5/60	Dąbrowska 1975
Red wattlebird (*Anthochaera carunculata*)	40.8c	-	Coimbra et al. 2015
Laughing kookaburra (*Dacelo novaeguineae*)	41g	-	Moroney and Pettigrew 1987
Northern fulmar (*Fulmarus glacialis*)	44.7c	-	Paper II
American kestrel (*Falco sparverius*)	46c/39.7–71.4e	–/93e	Dvorak et al. 1983/Gaffney and Hodos 2003
Griffon vulture (*Gyps fulvus*)	104	980	Fischer 1969
Egyptian vulture (*Neophron percnopterus*)	135	1150	Fischer 1969
Indian vulture (*Gyps indicus*)	135	995	Fischer 1969
Brown falcon (*Falco berigora*)	52/57/73/76c	2/20/2000/-	Reymond 1987
Wedge-tailed eagle (*Aquila audax*)	58/115/138/142c	2/20/2000/-	Reymond 1985

Note: if not indicated, values are obtained from behavioural experiments, otherwise by: c - photoreceptor centre-to-centre distance or photoreceptor density counts, g – retinal ganglion cell density counts, e -electroretinogram.

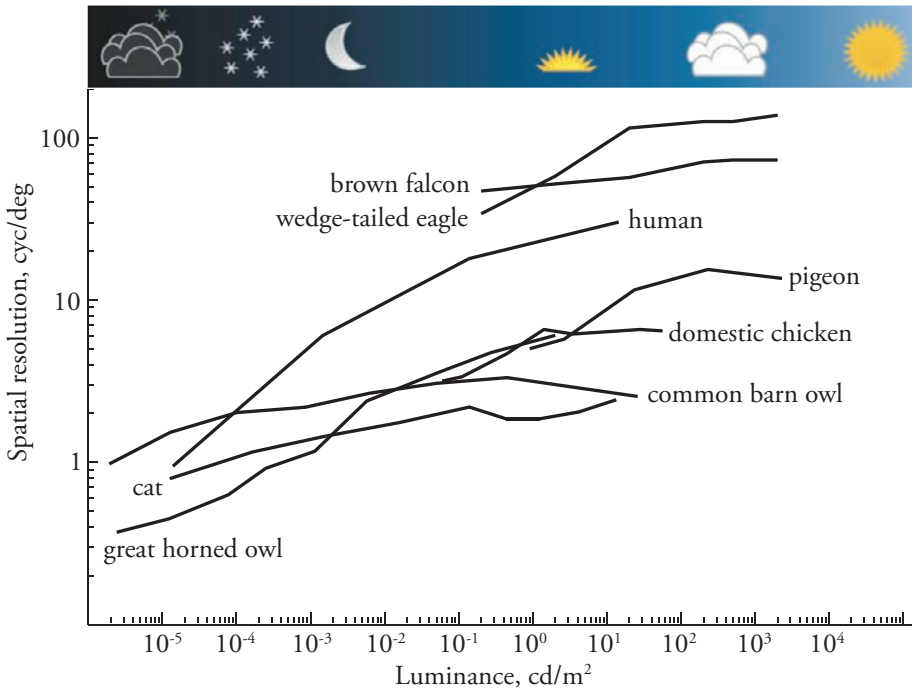

Fig. 4.1. The spatial resolution - luminance function of six species of birds, cat and human. Data assembled from: wedge-tailed eagle (*Aquila audax*) - Reymond 1985, brown falcon (*Falco berigora*) - Reymond 1987, pigeon (*Columba livia domestica*) - Hodos et al. 1976, domestic chicken (*Gallus gallus domesticus*) - Gover et al. 2009, great horned owl (*Bubo virginianus*) - Fite 1973, common barn owl (*Tyto alba*) - Orlowski et al. 2012, human and cat - Pasternak and Merigan 1981. The symbols on top depict relative sky conditions representing the luminance level.

The age (Hodos et al. 1991, Porciatti et al. 1991, Ghim and Hodos 2006) or the race of tested domesticated animals (Over and Moore 1981, Jarvis et al. 2009) can have an effect on the spatial resolution threshold. However, one really important parameter is the luminance of the stimulus. Spatial resolution deteriorates as the light level drops, but many behavioural studies have investigated spatial resolution only at one or two luminance levels (Table 4.1), and only few species have been tested at several light levels (Fig. 4.1).

The same two-alternative forced choice procedure can be repeated with stimuli of different contrast. Collected data allow to establish a contrast sensitivity function (CSF), which describes the ability of the visual system to discriminate intensity contrast over a range of spatial frequencies (Fig. 4.2). The typical achromatic CSF has an inverted U-shape with maximum contrast sensitivity somewhere in the lower half of spatial frequencies, and a roll-off at both low and high spatial frequencies. Decline of sensitivity at high frequencies is explained by the imperfections of optics and decline at the low end is suggested to be due to lateral inhibition in the receptive fields (De Valois and De Valois 1990).

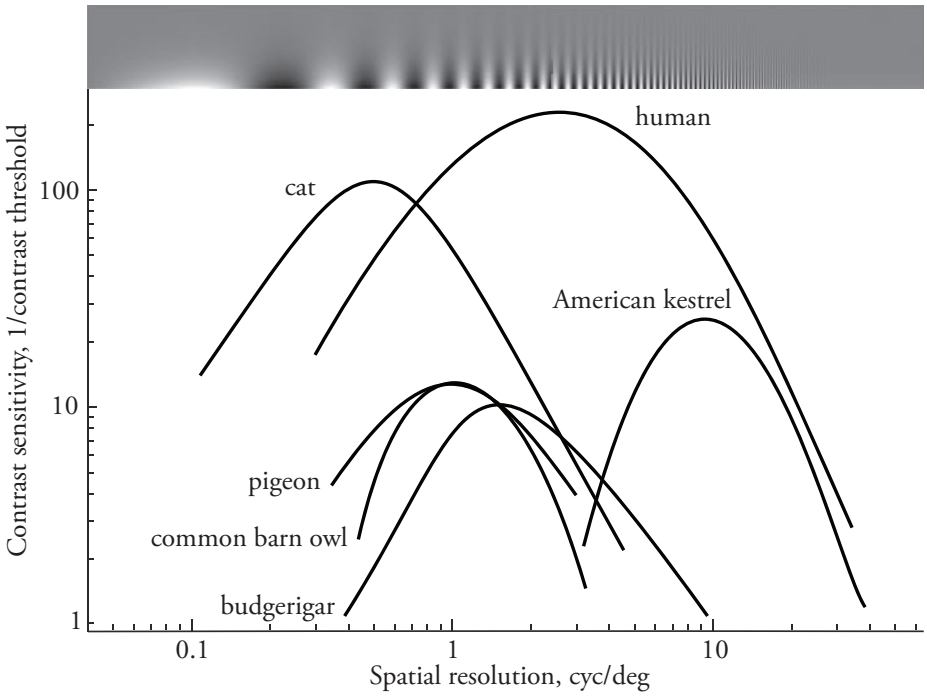

Fig. 4.2. Contrast sensitivity function of four species of birds, cat and human. Data assembled from: pigeon (*Columba livia domestica*) - Hodos et al. 2002, common barn owl (*Tyto alba*) - Harmening et al. 2009, budgerigar (*Melopsittacus undulatus*) - Lind et al. 2012, American kestrel (*Falco sparverius)* - Hirsch 1982, cat - Bisti and Maffei 1974, human - Berkley 1976.

While the shape of the CSF is similar for most animals, the peak contrast sensitivity and its position, as well as the frequency range vary from species to species (Ulrich 1981) (Fig. 4.2, Table 4.2). In general, birds have lower contrast sensitivity and narrower band-width as compared to mammals. Which mechanisms are responsible for this phenomenon is still not fully resolved, however several possible hypotheses exist (summarized in Ghim and Hodos 2006). The highest contrast sensitivity among birds was recorded for the American kestrel (31; Hirsch 1982), and the lowest for the common barn owl (6; Ghim and Hodos 2006) (Table 4.2). An effect of age on contrast sensitivity is indicated for pigeons, American kestrels (Ghim and Hodos 2006) and Japanese quails (Lee et al. 1997).

Behavioural tests determine the ultimate spatial resolution of animals, but are hard to perform as they involve keeping birds in captivity and tedious training procedures (Martin and Gordon 1974, Reymond 1985, 1987, Lind et al. 2012). The alternative methods are described below.

Table 4.2. An overview of spatial contrast sensitivity thresholds in birds.

Species name	Peak spatial contrast sensitivity	Position of peak sensitivity (cyc/deg)	Stimulus luminance (cd/m^2)	References
American kestrel (*Falco sparverius*)	30e/31	2.1e/10	94e/40	Ghim and Hodos 2006/Hirsch 1982
Wedge-tailed eagle (*Aquila audax*)	14	10	20	Reymond and Wolfe 1981
Common barn owl (*Tyto alba*)	6e/13	1a/1	94e/2.74	Ghim and Hodos 2006/Harmening et al. 2009
Domestic chicken (*Gallus gallus domesticus*)	12	1	16	Jarvis et al. 2009
Budgerigar (*Melopsittacus undulatus*)	10	1.4	50	Lind et al. 2012
Japanese quail (*Coturnix japonica*)	9.9	1	94	Ghim and Hodos 2006
Pigeon (*Columba livia domestica*)	4.6e/8e/9.9	0.7e/1.3e/0.8	16e/94e/100	Hodos et al. 2002/Ghim and Hodos 2006/ Hodos et al. 2002
Bourke's parrot (*Neopsephotus bourkii*)	7	2.4	62	Lind et al. 2012
Red-bellied woodpecker (*Melanerpes carolinus*)	6.7	0.8	94	Ghim and Hodos 2006
Common starling (*Sturnus vulgaris*)	6.2	1.1	94	Ghim and Hodos 2006

Note: if not indicated, values are obtained from behavioural experiments, otherwise by: e – electroretinogram.

Electrophysiological measurements

Spatial resolution, as well as contrast sensitivity, can be also measured using electroretinograms (ERGs). During ERG measurements the bird is anesthetized and immobilized. Cycloplegia (paralysis of the ciliary muscle to hinder accommodation) and mydriasis (dilation of the pupil) are usually induced by vecuronium bromide. The recording electrode is inserted through the eyelid to contact the sclera or the cornea. The reference electrode is inserted in the other eye in a similar manner. The ground electrode is inserted subcutaneously in the scalp. The bird eye is positioned in front of the stimulus monitor where gratings of varying spatial frequency and contrast are presented. The gratings are phase reversed usually at 7.5-7.7 Hz. The CSF using ERG was measured for the

Japanese quail (Lee et al. 1997), pigeon (Hodos et al. 2002), American kestrel, common barn owl, common starling (*Sturnus vulgaris*) and red-bellied woodpecker (*Melanerpes carolinus*) (Ghim and Hodos 2006). The spatial resolution and peak contrast sensitivity measured with this method are somewhat lower as compared to the behavioural estimations in pigeon and American kestrel (Table 4.2).

Anatomical measurements

Most studies on avian visual acuity are based on retinal whole-mount technique (Stone 1981, Ullmann et al. 2012) and RGC density counts. The peak cell density, instead of centre-to-centre distance is most often used, and the Nyquist limit of spatial resolution (F_n) for an assumed two-dimensional hexagonal array of RGCs is calculated as:

$$F_n = 0.5 \times RMF \times (2G/\sqrt{3})^{1/2}, \quad [12]$$

where G is ganglion cell density in cells/mm^2 and RMF is the retinal magnification factor, which represents the distance on the retina subtended by 1° of visual space, and is calculated as:

$$RMF = 2\pi PND/360. \quad [13]$$

Neither does RGC density approximate a hexagonal array, nor are they packed in two dimensions, especially in the area of the highest cell density. Depending on species and retinal eccentricity, RGCs can be positioned in several lamina, the highest, up to seven or eight layers, reported in the perifoveal region of the sacred kingfisher (*Todiramphus sanctus*) (Moroney and Pettigrew 1987). As RGC nuclei are much wider than photoreceptor inner segments, this type of packing is a result of high RGC-photoreceptor ratio and extremely high photoreceptor density, which indeed approximates a two-dimensional hexagonal array very well (Snyder and Miller 1977, Kram et al. 2010).

In Paper I we present and discuss several difficulties and other assumptions associated with this method. Briefly, first, it is important to measure the actual PND of the species studied. Often the PND is approximated by using a fixed PND:axial length ratio, which was established based only on a very limited number of species (Pettigrew et al. 1988). As this ratio can vary highly even between closely related species with similar activity patterns (0.55 for the Manx shearwater (*Puffinus puffinus*) (Martin and Brooke 1991) and 0.67 for the northern fulmar (Paper II)) the spatial resolution estimate can be affected. Second, disregard of the retinal shrinkage during the histological tissue processing, inclusion of non-ganglion cells in the RGC counts, or underestimation of the RGC density due to multiple cell layers can affect precise estimation of spatial resolution. However,

the variability in anatomical spatial resolution due to the errors in the RGC density or PND evaluation may still be in an acceptable range as the inter-individual variation in visual acuity observed in behavioural studies is usually very high (summarised in Paper I).

Other studies, especially those on raptorial birds (Snyder and Miller 1978, Miller 1979, Reymond 1985, 1987) use photoreceptors instead of RGCs as retinal sampling units. Coimbra et al. (2015) suggested that the peak density of retinal neurons with the lowest population in the retinal pathway determines spatial resolution. Indeed, in a primate fovea, where two to four midget RGCs connect to each photoreceptor (Perry and Cowey 1988, Curcio and Allen 1990, Wässle et al. 1990), spatial resolution based on RGC density would be overestimated. Whether a midget-like system exists in any bird species is currently unknown, however, Oehme (1964) traced RGC to photoreceptor connections in the central and temporal foveae of a common buzzard and common kestrel (*Falco tinnunculus*), and found one RGC for each foveal cone. Depending on how far and how asymmetrically the RGCs are displaced in the fovea, regular RGC counts in whole-mounts can provide highly unreliable density estimations. Therefore, Coimbra et al. (2015) recommended to use RGC density in afoveate, and photoreceptor density in foveate bird species. Indeed, as mentioned above, Reymond (1985, 1987) has shown that the theoretical spatial resolution based on the peak cone density in the central fovea of brown falcon (*Falco berigora*) and wedge-tailed eagle closely match behavioural visual acuity.

In Paper II, where we studied RGC topography and spatial resolution in two procellariiform seabird species, we found a fovea in the centre of visual streak in the Northern fulmar, but not in the Leach's storm-petrel. In addition to the retinal whole-mounts we did cross-sections through these areas and found that Leach's storm-petrel had thicker neuronal layers, but no retinal indentation (Fig. 3 in Paper II). The Northern fulmar, however, had a fovea, and we estimated photoreceptor density in the foveal pit based on the oil droplet diameter. Therefore, following the presented reasoning, we calculated spatial resolution from the peak RGC density in Leach's storm-petrel (7.1 cyc/deg), but from the photoreceptor density in the fovea of the Northern fulmar (44.7 cyc/deg).

We did not find a fovea in the two parrot species studied in Paper I. Still, we calculated spatial resolution based on both peak RGC density and oil droplet diameter in order to compare these values to the visual acuity thresholds measured in behavioural studies. Surprisingly, spatial resolution measured behaviourally (Lind et al. 2012) was higher than resolution based on RGC density, but lower than resolution based on oil droplet diameter in both species. As different individuals were used for behavioural and anatomical studies with budgerigars, the mismatch could be possibly explained by inter-individual variation. However, the same Bourke's parrots were investigated in both studies, and the mismatch between these results remains unclear.

Yet another underlying assumption in the estimation of anatomical spatial resolution is that there are no rods in the high-acuity region, and that all types of cones in this region contribute equally to the high acuity tasks. Rod-free areas were indeed found in the area centralis of the chicken (Bruhn and Cepko 1996) and in the fovea of the pigeon (Querubin et al. 2009), some passerines (Coimbra et al. 2015) and the Northern fulmar (Paper II). However it is generally assumed that only double cones mediate achromatic vision in birds (Campenhausen and Kirschfeld 1998, Osorio and Vorobyev 2005, Lind and Kelber 2011). This assumption is rarely addressed in studies on avian resolution.

As mentioned before, raptorial birds are the only animals with spatial resolution higher than humans. They are also known to possess two foveae, a deep central fovea, projecting to the lateral visual field and a deep or shallow temporal fovea projecting to the frontal visual field (Meyer 1977). Therefore, if double cones are assumed to mediate achromatic vision in birds, the raptor fovea should be densely packed with double cones. This reasoning led us to Paper IV, where we used transmission electron microscopy (TEM) and immunohistochemistry to investigate the presence of the double cones in the central and temporal foveae of four raptor species.

Based on TEM micrographs, we found that the central fovea of the red kite and common buzzard had a double cone-free zone of approximately 200 μm in diameter, but it was only around 30 μm in the peregrine falcon. We could not determine the size of double-cone free area in the central fovea of the Eurasian sparrowhawk, but results from the immunohistochemistry show that it also lacks double cones. The temporal fovea of the common buzzard and peregrine falcon had small (25-30 μm) double-cone free zones, however the Eurasian sparrowhawk temporal fovea contained double cones. In addition to this, we found VS and MWS cones in the central fovea of the common buzzard, peregrine falcon and Eurasian sparrowhawk. These results indicate, that most likely not only double cones, but also single cones contribute to achromatic high-resolution vision in birds, in general.

Concluding Remarks

Although this thesis has an extremely general title *Spatial Vision in Birds*, it is clear that only some aspects of spatial vision, and actually only some aspects of spatial resolution, could be covered in this study. However, even though work on only eight species is presented in this thesis, I have to admit, that I have touched more bird eyes than any other regular teenager. Around 0.5% of all extant bird species have donated their eyes to us during these years, though many results are still waiting to see the daylight.

However, in the studies presented here we see RGC topography maps in four new bird species, high intra-species variation in the peak RGC density, a mismatch between RGC based anatomical resolution and behavioural visual acuity, a putative area nasalis never before reported in birds, first estimations of spatial resolving power in procellariiform seabirds, RGC topography development in only the second bird species, foveal depth profiles for two new raptor and one seabird species, the absence of double cones and presence of V and MVS cones in the raptor fovea, indicating the contribution of single cones to high resolution vision.

Still, work presented in this thesis adds just a little bit to the knowledge on bird spatial vision. While the theoretical constraints on spatial resolution are very well understood, retinal development, retinal function and bird visual ecology have enormous amounts of unanswered questions. Some of them stem directly from each of the studies presented in this thesis.

The effects of domestication have been investigated in chickens, quails and pigeons, but has selective breeding already affected the pet birds? How does anatomical and behavioural spatial resolution match in other bird species (Paper I)? Can seabirds really forego vision for olfaction? What can they really see on the ocean (Paper II)? Retinal development has been studied in detail in chickens, but how does the retina develop in other birds (Paper III)? How does the avian fovea form (Paper II and III)? What is the real function of the double cones? How is their distribution in the retina controlled? Are double cones missing from the shallow central foveae (Paper IV)?

"The outcome of any serious research can only be to make two questions grow, where only one grew before" Thorstein Veblen

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