Comparing the retinal structures and functions in two species of gulls (*Larus delawarensis* and *Larus modestus*) with significant nocturnal behaviours

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Abstract

Ring-billed gulls (*Larus delawarensis*) and gray gulls (*Larus modestus*) are two species active both by day and night. We have investigated the retinal adaptations that allow the diurnal and nocturnal behaviours of these two species. Electroretinograms and histological analyses show that both species have a duplex retina in which cones outnumber rods, but the number of rods appears sufficient to provide vision at night. Their retinas respond over the same scotopic dynamic range of 3.4 log cd m^{-2}, which encompasses all of the light levels occurring at night in their photic environment. The amplitudes of the scotopic saturated a- and b-wave responses as well as the photopic saturated b-wave response and the photopic sensitivity parameter S are however higher in ring-billed gulls than in gray gulls. Moreover, the process of dark adaptation is about 30 min faster in gray gulls than in ring-billed gulls. Our results suggest that both species have acquired in the course of their evolution functional adaptations that can be related to their specific photic environment.
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1. Introduction

Animals active both by day and by night face complex visual problems. Unlike strictly diurnal or nocturnal species, which are exposed to a restricted range of light intensities during their daily activities, they may cope with great variations of light levels, from the full intensity of the zenith sun to the darkness of night. Thus, in open environment, the ambient light intensity may vary over a range of nearly 9.5 log units during a 24-h cycle (Martin, 1990). Moreover, in some latitudes, light level can change by as much as one log unit every 10 min at dawn and dusk (Lythgoe, 1979).

Most bird species are diurnal, however some gulls *Laridae* are in different aspects of their life cycle partly active at night (McNeil, Drapeau, & Pierotti, 1993). For instance, ring-billed gulls (*Larus delawarensis*) have been reported to forage and fly frequently at night (Burger & Staine, 1993; Hébert & McNeil, 1999), chick feeding and aggressive interactions have sometimes been observed during nighttime in this species (Fetterolf, 1979). Gray gulls (*Larus modestus*) also have nocturnal activities. These birds breed in the desert of Atacama about 30–100 km from the coast of northern Chile and most of their reproductive behaviours take place at night. Thus, beginning in July, adults congregate every day at dusk in large flocks along the coast and initiate spiralling flights until complete darkness, after

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which they leave to their nesting territories in the desert where nest building begins (Guerra, 1987). A few weeks later, courtship and copulation take place exclusively at night. During incubation and brooding, the parents alternate in foraging flights, departing from the coast to the desert at around 21:30 h, they feed their chicks during darkness, and leave the nesting sites before sunrise at around 04:00 h to return back to the coast (Guerra, 1987; Howell, Araya, & Millie, 1974). Outside the breeding season, foraging occasionally takes place at night (Blokpoel, Boersma, Hughes, & Tessier, 1992).

Since light intensity is considered to be the most important selective pressure responsible for specialised adaptations in the eye (Lythgoe, 1979), we hypothesised that ring-billed gulls and gray gulls present visual adaptations that enable them to function in the nocturnal as well as in the diurnal luminance range. Our results support our claim that the retina of each species has acquired in the course of their evolution specific structural and functional adaptations, which can be related to their photic environment.

2. Methods

2.1. Animals

Experiments were performed on 29 ring-billed gulls (350–475 g) and 25 gray gulls (325–400 g) following the guidelines of the Canadian Council on Animal Care (1993). Ring-billed gulls were captured near Montréal (45°50′N, 73°58′W), Canada, between June and September, and gray gulls in Antofagasta (23°65′S, 70°40′W), Chile, during the austral fall (April). All the experiments were done during the day between 08:00 h and 16:00 h in Montreal and Antofagasta. Since the electroretinographic procedure used in this study was not invasive, the birds not kept for histology were allowed to recover from anesthesia and returned to their natural habitat.

2.2. ERG procedures

Birds were anesthetised with a solution of ketamine hydrochloride (50 mg/kg, i.m.; Ketaset, Ayerst) and xylazine hydrochloride (5 mg/kg, i.m.; Rompun, Bayer), and placed on a custom-made recording holder inside a Ganzfeld dome of 41 cm in diameter (LKC Ganzfeld-2503B) so that only the left eye was stimulated. Eyelids and nictitating membrane were retracted with a speculum, the cornea was anesthetised with 0.5% proparacaine hydrochloride and the pupil fully dilated with 1% Tropicamide. A DTL electrode (X-staticsilver coated conductive nylon yarn, Sauquoit Industries, Scranton, PA, USA) was placed on the cornea to act as the active electrode. Reference and ground electrodes (Grass subdermal electrodes, Astro-med Inc., Warwick, RI, USA) were inserted into the scalp and under the skin of the chest, respectively. Responses were evoked to flashes of white light of 0.52 log cd s m^{-2} delivered with a photostimulator (Grass PS-22) through the Ganzfeld and the stimulus luminance was attenuated with Kodak Wratten neutral density (ND) filters (Kodak Ltd., Rochester, NY, USA). Responses were recorded within a bandwidth of 0.3–500 Hz, amplified 10,000×, averaged and stored on hard disc using an EPIC-2000 computer-controlled electrodiagnostic system (LKC Technologies, Inc., Gaithersburg, MD).

Birds were dark adapted for 4 h after which they were prepared as described above under dim red light illumination. Scotopic responses were first obtained using increasing flash luminances from −4.68 to 0.52 log cd s m^{-2} in steps of 0.4 log unit. For each luminance, the responses to six successive flashes presented at an inter-stimulus interval of 10.1 s were averaged. Birds were then light adapted to a white light background of 1.55 log cd m^{-2} for 15 min and photopic responses were obtained using increasing flash luminances of −1.48 to 0.52 log cd s m^{-2} in steps of 0.4 log unit. For each luminance, the responses to 10 successive flashes presented at an inter-stimulus interval of 4.2 s were averaged. Fewer stimuli were used in scotopic (6) than in photopic (10) condition and they were separated by longer intervals to ensure that the retina retained its dark-adapted state.

The kinetic aspect of dark adaptation was studied in an additional group of 14 ring-billed gulls and 10 gray gulls. Birds were light adapted to the above background light for 15 min, the light was then turned off and the ERGs, evoked to a single flash of white light of −1.48 log cd s m^{-2} presented at 10 min intervals, were recorded over a period of 120 min.

The percentage of visual pigment bleached by the background light was evaluated according to Breton, Schueller, Lamb, and Pugh (1994). For a rod outer segment of 32.08–33.24 μm in length and 4.79 μm in diameter, the total pigment content was estimated to be 1.05×10^9 molecules and the effective collecting area 11.92 μm^2. The retinal irradiance was established to be 2.37×10^7 photons μm^{-1} per troland in ring-billed gulls and 3.28×10^7 in gray gulls. Hence, the background light delivered 2.83×10^7 and 3.93×10^7 units of photoisomerisation per rod in ring-billed gulls and gray gulls, respectively, and therefore bleached about 3–4% of the rhodopsin in both species. For this calculation, the pre-retinal media transmissivity and the rod outer segment optical density of rock doves (Columba livia; Bowmaker, 1977) were used since these parameters are not available for ring-billed gulls and gray gulls or any other species of wild birds.

2.3. Histology

Following the photopic ERG recordings (light adaptation), five individuals of each species were given a lethal dose of sodium pentobarbital. Their eyes were removed under this photopic illumination, and the axial length (AL) and equatorial diameter (ED) were measured (Martin, 1986). The left eye was injected with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2) and bathed 30 min in this fixative. The anterior part of the eye was removed at the equator and the retina, still attached to the choroid, was cut into nine sectors using the pecten as a landmark (Fig. 1), according to the procedure of Rojas de Azuaje, Tai, and McNeil (1993). Each sector was further subdivided into smaller portions of approximately 2 mm^2 and kept in the fixative for 3 h. Tissues were then prepared as previously described (Rojas, Ramirez, McNeil, Mitchell, & Martin, 2004).

Transverse semithin (0.7 μm) sections were cut with an ultramicrotome, mounted onto glass slides, stained with toluidin blue and examined with the light microscope. Rods and cones were counted in a field of 310 μm from 15 sections randomly selected for each sector to calculate their average densities. The length and diameter of the outer and inner segments of five rods and five cones randomly selected for each of the 15 sections, as well as the thickness of each retinal layer were measured using a calibrated grating.

To check the presence of a tapetum in the eye of gray gulls, the choroids, as well as the retinal epithelial cells were examined since it has been reported that the tapetum lies in either structure (Arnott,
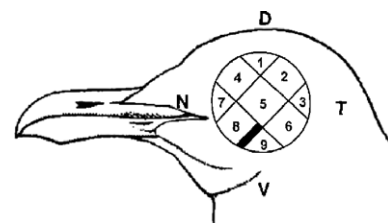

Fig. 1. Schematic representation of the retina showing the nine sectors. D, N, T, and V correspond to dorsal, nasal, temporal, and ventral sectors, respectively. The thick line between sectors 8 and 9 represents the pecten.

Nichol, & Querfeld, 1970; Braekevelt, 1981, 1982, 1983, 1984, 1986; Nicol & Arnott, 1974). The choroid was observed on fresh preparations. The right eye was hemisected along the equator; the vitreous and the retina were removed, thus exhibiting the inner surface of the choroid. The retinal epithelium cells were observed from the pieces of epon-embedded tissues, which were cut into thin (50–70 nm) sections, collected on copper grids coated with formvar, stained with uranyl acetate and lead citrate, and examined with a Jeol JEM-100 transmission electron microscope.

2.4. Data analysis

Luminance–response curves were constructed for the scotopic and photopic a- and b-waves of the ERG by plotting the amplitude of these waves as a function of the stimulus luminance. The amplitude of the a-wave was measured from baseline to trough, that of the b-wave from the trough of the a-wave to the peak of the b-wave or, when no a-wave was present, from baseline to peak.

The thresholds of the scotopic and photopic a- and b-waves were obtained by extrapolating from the luminance–response curves the luminance that produced a criterion response of 10 μV, which was the response amplitude just above the noise level in all the animals tested.

The scotopic and photopic a-wave luminance–response curves were fitted by a least-squares algorithm (Matlab, MathWorks, Natick, MA) to the equation

$$P_3(I, t) \cong \{1 - \exp[-I \cdot S \cdot (t - t_d)^2]\} \cdot R_{m(P3)} \quad \text{for } t > t_d \tag{1}$$

which describes the transduction mechanisms of photoreceptors in vertebrates according to the Lamb and Pugh (1992) model, and where P_3 represents the sum of the individual photoreceptor responses as a function of flash energy I and time t after the occurrence of a short flash, S is a sensitivity parameter that scales the intensity of the flash required to generate a response equal to $1/2 \, R_m$ (semisaturation constant), R_m the maximum response amplitude, and t_d a brief delay (Hood & Birch, 1990, 1993, 1997). Similarly, the scotopic and photopic b-wave luminance–response curves were fitted by a least-squares algorithm to the equation (SAS Information Delivery System for UNIX, Version 6.07.02; SAS Institutes Inc., Cary, NC)

$$V/V_{max} = I^n/(I^n + \sigma^n) \tag{2}$$

which models the activity of the inner retina in vertebrates (Naka & Rushton, 1966, 1967) and where V represents the response to stimulus luminance I, V_{max} the maximum response, σ a parameter of sensitivity which scales the luminance required to generate a response equal to $1/2 \, V_{max}$ (semisaturation constant), and n the slope of the function.

Dark adaptation curves were obtained by plotting the amplitudes of the a- and b-waves as a function of time during recovery from the bleach. Using a least-squares algorithm, the dark adaptation curves were then fitted by a general logistic growth equation estimated to produce the best fit to the a- and b-wave responses in both species (SAS Information Delivery System for UNIX, Version 6.07.02; SAS Institutes Inc., Cary, NC)

$$V = V_{max}/\{1 + T[\exp(-n \cdot t)]\}, \tag{3}$$

where V represents the response to time t, V_{max} the maximum response, T the time at which a response is equal to $1/2 \, V_{max}$, and n the slope of the function.

Statistical analyses were performed using analysis of variance (ANOVA) to evaluate differences between species in their ERG responses and in their retinal cytoarchitecture parameters. Post hoc Holm–Sidak tests were applied for evaluation of significant differences between the groups. Data fitting a non-parametric distribution were tested for significance using the Kuskal–Wallis ANOVA by ranks test with Dunn's post hoc comparison when comparing groups (Statistica for Windows version 5.0; StatSoft Inc., Tulsa, OK). Data are presented as means ± SD and in all cases the $p < 0.05$ level was used to determine statistical significance.

3. Results

3.1. Eye and pupil measurements

The eyes of ring-billed gulls and gray gulls have approximately the same size and present a typical flat shape. The colour of their iris however differs remarkably, the iris of ring-billed gulls being yellow and that of gray gulls dark brown. In ring-billed gulls, the average equatorial diameter (ED) and axial length (AL) measure 17.8 ± 0.9 mm and 16.7 ± 1.7 mm, respectively, compared to 17.0 ± 0.8 mm and 16.0 ± 1.1 mm in gray gulls, which gives a AL:ED ratio of 0.94 for both species. The pupil of ring-billed gulls reaches a maximum diameter of 5.9 ± 0.4 mm, and that of gray gulls 5.3 ± 0.36 mm.

3.2. Retinal function

Fig. 2 shows representative ERG responses obtained from a ring-billed gull and a gray gull to a range of flash stimuli under scotopic (A) and photopic (B) conditions.
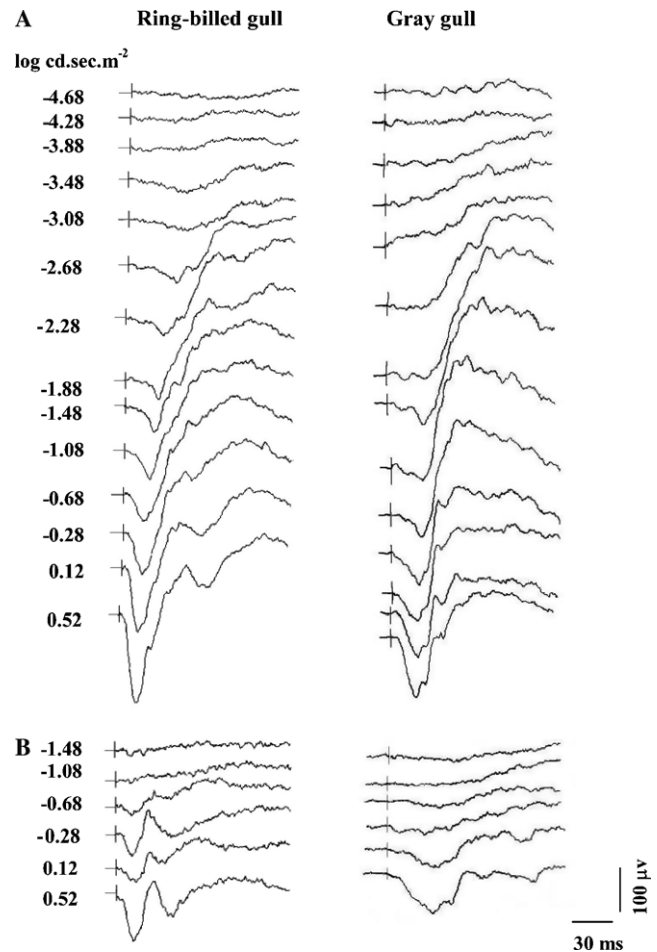

Fig. 2. Representative ERG waveforms obtained for ring-billed gull and gray gull to a range of flash stimuli presented under scotopic (A) and photopic (B) conditions. Abbreviations: *a*, peak of the a-wave; *b*, peak of the b-wave.

It shows that under scotopic condition the general form of the ERGs is very similar in both species. At low stimulus luminances, a large positive potential (b-wave) predominates the waveforms and as luminance increases a negative potential (a-wave) with a shorter latency begins to dip below the baseline. The amplitude of both waves also increases as a function of the stimulus luminance. However, the amplitude of both the a- and b-waves tends to be higher in ring-billed gulls than in gray gulls. Under photopic condition, the morphology of the ERGs of both species appears quite different. Thus, in ring-billed gulls, at the highest luminance (0.52 log cd s m^{-2}), both the a- and b-waves are well defined, whereas in gray gulls the a-wave presents a round shape and the b-wave is barely profiled. In photopic condition, as under scotopic condition, the amplitudes of both waves increase as a function of the stimulus luminance in both species and are higher in ring-billed gulls than in gray gulls.

Fig. 3 provides the mean luminance–response curves derived from the ERGs. The lines represent the least-squares fit of Eqs. (1) and (2) to the amplitude of the a- and b-waves, respectively. The mean values of the parameters of the luminance–response function are given in Table 1. It can be seen that the retina in both species functions over the same scotopic dynamic range, 3.4 log cd s m^{-2}. Thus,

the $10\,\mu V$ criterion threshold for the scotopic a- and b-waves as well as the luminances at which these waves saturate are almost identical in both species. Within this range, however, the retina of both species behaves quite differently. ANOVA tests reveal that under scotopic condition, the amplitudes of saturated responses of the scotopic a-wave (R_m) (Holm–Sidak method, $t = 4.04$; $p < 0.05$) and b-wave (V_{max}) (Dunn's method, $Q = 3.91$; $p < 0.05$) are significantly higher in ring-billed gulls than in gray gulls. Under photopic condition, the parameters S (Holm–Sidak method, $t = 2.48$; $p < 0.05$) and V_{max} (Dunn's method, $Q = 3.80$; $p < 0.05$) are also higher in ring-billed gulls than in gray gulls.

3.3. Kinetic of dark adaptation

The dark adaptation curves obtained from ring-billed gulls and gray gulls after a 15-min period of light adaptation, which bleached about 3–4% of their visual pigment, are shown in Fig. 4. The dots represent the mean amplitude of the a- and b-waves, and the solid lines the least-squares fit of Eq. (3) to the amplitude of both waves. It can be seen that in ring-billed gulls the a-wave reaches saturation more rapidly than the b-wave (Holm–Sidak method, $t = 3.16$; $p < 0.05$). Thus, the amplitude of the a-wave increases
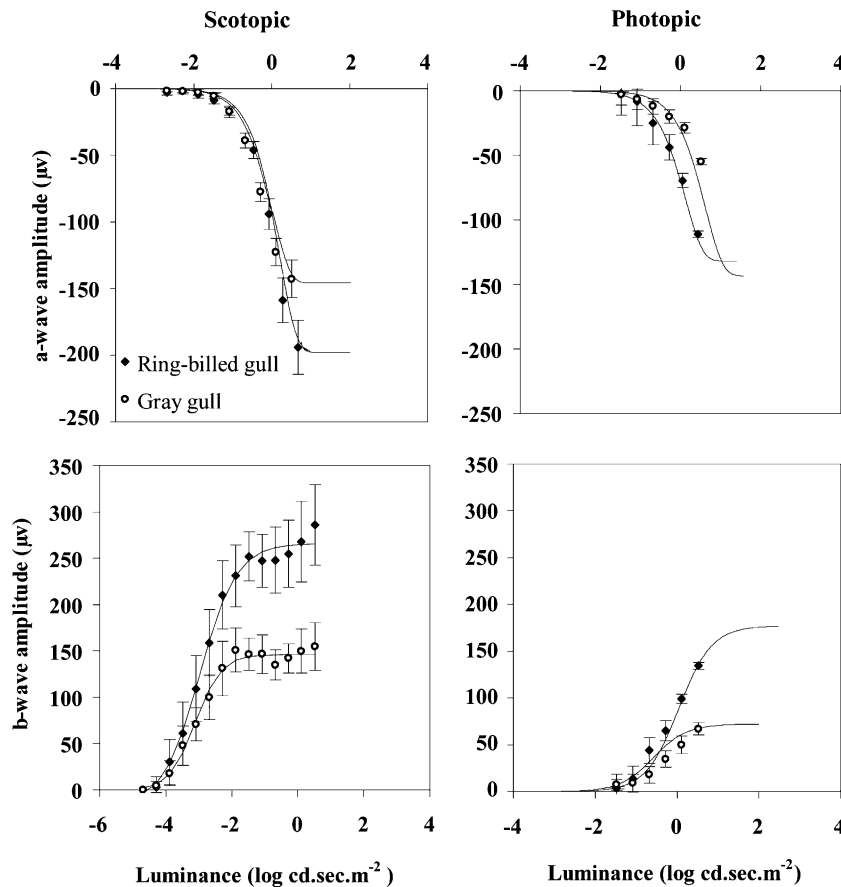

Fig. 3. Luminance–response curves of the a- and b-waves derived from the ERGs. The lines represent the least-squares fit of Eqs. (1) and (2) to the mean amplitudes of the a- and b-waves, respectively.

Table 1
Mean values (±SD) of the parameters of the luminance–response function obtained from ring-billed gulls and gray gulls

Waves	Parameters[a]	Ring-billed gull[b]	Gray gull[b]
Scotopic			
a-wave	10 μv threshold	−1.32 ± 0.10	−1.24 ± 0.31
	R_m	−198.97 ± 34.21[*]	−146.29 ± 37.15
	LR_m	0.53 ± 0.06	0.48 ± 0.08
	S	−0.23 ± 0.11	−0.34 ± 0.23
b-wave	10 μv threshold	−4.21 ± 0.22	−4.25 ± 0.27
	V_{max}	261.78 ± 80.31[*]	149.84 ± 20.78
	LV_{max}	−0.81 ± 0.31	−0.81 ± 0.65
	σ	−2.75 ± 0.40	−3.00 ± 0.43
Photopic			
a-wave	10 μv threshold	−0.88 ± 0.31	−0.54 ± 0.20
	R_m	−131.03 ± 70.28	−143.31 ± 74.52
	LR_m	0.69 ± 0.33	1.19 ± 0.67
	S	−0.04 ± 0.44[*]	0.33 ± 0.38
b-wave	10 μv threshold	−1.22 ± 0.17	−1.36 ± 0.56
	V_{max}	177.47 ± 54.70[*]	72.05 ± 43.43
	LV_{max}	1.55 ± 0.32	2.00 ± 0.58
	σ	0.03 ± 0.39	−0.16 ± 0.26

[a] The parameters represent: threshold (log cd s m^{-2}) which evokes a criterion response of 10 μv; R_m and V_{max}, the maximum responses (μv) estimated from Eqs. (1) and (2), respectively; LR_m and LV_{max}, the luminances (log cd s m^{-2}) at which R_m and V_{max} are obtained, respectively; S and σ, the luminances (log cd s m^{-2}) required to generate half the maximum responses (semisaturation constant) estimated from the Eqs. (1) and (2).

[b] Measurements obtained from 15 specimens.

[*] $p < 0.05$.

slightly for the first 40 min and saturates approximately after 50 min, whereas the b-wave increases more markedly in a nearly linear fashion for the first 40 min, then continues to increase slightly between 40–70 min to finally reach saturation after more than 70–80 min. In gray gulls both the a- and b-waves reach saturation at approximately the same time, the amplitude of both waves increases linearly for approximately the first 40 min and attains saturation after about 50 min. The mean values of the parameters of the dark adaptation function obtained from both species are given in Table 2. It can be seen that in ring-billed gulls, the b-wave reaches saturation about 19.36 min after the a-wave. Furthermore, the time at which the b-wave reaches saturation (T_{vmax}) and half-saturation (T) is approximately twice longer in ring-billed gulls than in gray gulls (Holm–Sidak method, T_{vmax}: $t = 4.81$; $p < 0.05$; T: $t = 3.26$; $p < 0.05$). No difference was observed between species for the a-wave parameters.

3.4. Retinal structures

Light photomicrographs from the central retina (sector 5) of ring-billed gull and gray gull are presented in Fig. 5. In both species, three morphologically distinct photoreceptors were found: single cones, double cones (comprising a principal element closely linked to an accessory one), and rods. Cones and rods were easily differentiated by the
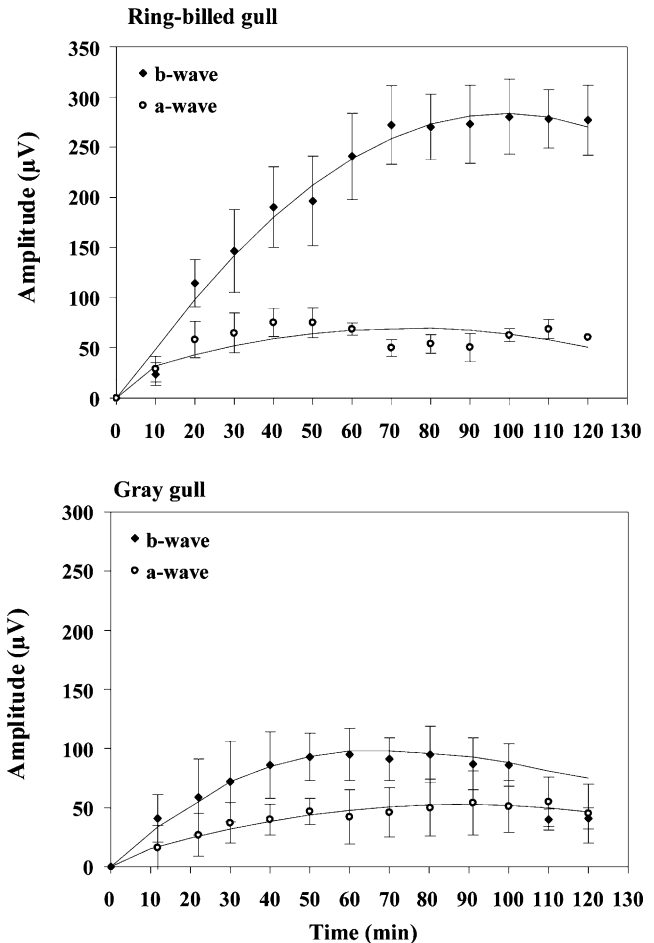

Fig. 4. Dark adaptation curves derived from the ERGs of ring-billed gulls and gray gulls. The lines represent the least-squares fit of Eq. (3) to the amplitude of the a- and b-waves.

Table 2
Mean value (±SD) of the parameters of the dark adaptation function obtained from ring-billed gulls and gray gulls

Parameters[a]	Ring-billed gull[b]	Gray gull[b]
a-wave		
R_{max}	84.35 ± 24.12	57.97 ± 19.12
T_{Rmax}	52.24 ± 16.13	49.09 ± 13.11
T	13.38 ± 11.03	13.03 ± 12.83
b-wave		
V_{max}	246.44 ± 74.46[*]	87.64 ± 42.40
T_{vmax}	71.603 ± 16.32[*]	41.59 ± 13.04
T	31.38 ± 10.10[*]	16.58 ± 12.11

[a] The parameters represent: R_{max} and V_{max}, the maximum response (μv) for the a- and b-wave, respectively; T_{Rmax} and T_{vmax} the time (min) at saturation for the a- and b-wave, respectively; T, the time (min) at half-saturation.

[b] Measurements obtained from 14 ring-billed gulls and 10 gray gulls.

[*] $p < 0.05$.

length and diameter of their outer segments, which are smaller in the cones, and by the presence of an oil droplet at the apical portion of the cone inner segment. In the single cones as well as in the principal element of the
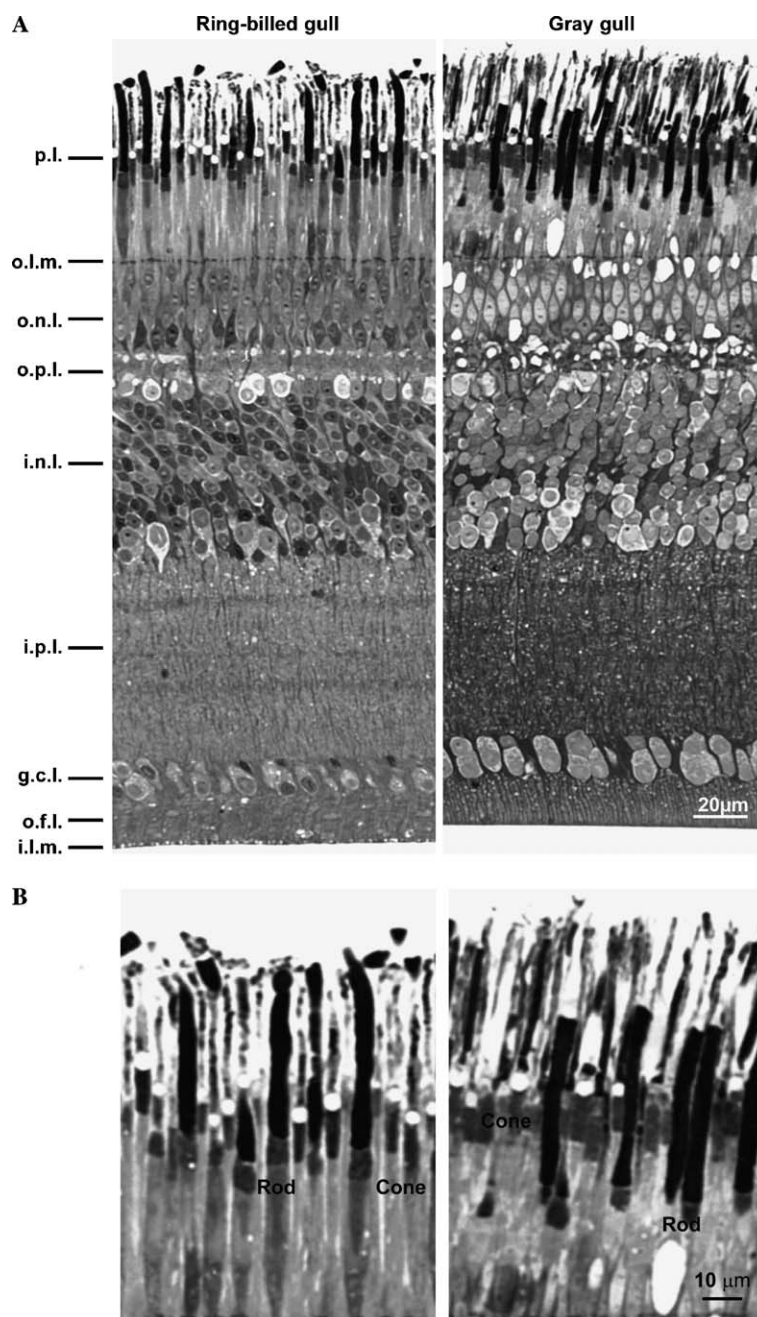
Fig. 5. Light photomicrographs of the central retina (sector 5) of ring-billed gull and gray gull. (A) Toluidine blue-stained transverse sections showing the organisation of the retinal layers. Abbreviations: p.l., photoreceptor layer; o.l.m., outer limiting membrane; o.n.l., outer nuclear layer; o.p.l., outer plexiform layer; i.n.l., inner nuclear layer; i.p.l., inner plexiform layer; g.c.l., ganglion cell layer; o.f.l., optic fiber layer; i.l.m., inner limiting membrane. (B) Magnified view of the photoreceptor layer.

double cones, the oil droplet is large and coloured, whereas in the accessory element of the latter it is smaller and colourless. Table 3 presents the rod and cone morphometric measurements obtained in both species. Since, for either species, no clear difference was observed between retinal sectors, only the overall measurements are given in Table 3. Except for the rod inner segment, which is significantly longer (Holm–Sidak method, $t = 2.87$; $p < 0.05$) in gray gulls than in ring-billed gulls, the other photoreceptor measurements are almost identical in both species.

Because the difference between single cone and principal element of double cones was difficult to assess with certainty at the light microscope, each double cone was counted as two cones for the calculation of photoreceptor density. In both species, cones outnumber rods and comprise about 70% of the total photoreceptors (Fig. 6). The overall relative cone density tends to be slightly higher in gray gulls (55.7/310 µm field ± 7.7) than in ring-billed gulls (52.4/ 310 µm field ± 10.5), while the overall relative rod density is identical in both species (23.2/310 µm field ± 1.0 vs.

Table 3
Overall cone and rod measurements (±SD) of the ring-billed gulls and the gray gulls

	Ring-billed gull[a]		Gray gull[a]	
	Length (μm)	Diameter (μm)	Length (μm)	Diameter (μm)
Single and principal cones				
Outer segment	18.25 ± 4.20	1.76 ± 0.23	17.91 ± 2.75	1.55 ± 0.25
Inner segment	38.74 ± 2.11	1.87 ± 0.52	38.66 ± 2.99	1.45 ± 0.43
Accessory cones				
Outer segment	21.35 ± 3.02	1.32 + 0.20	19.42 ± 2.54	1.75 ± 1.02
Inner segment	33.65 ± 4.00	4.89 ± 0.95	36.85 ± 3.20	4.75 ± 1.20
Rods				
Outer segment	32.08 ± 5.32	4.79 ± 0.62	33.24 ± 3.12	4.79 ± 0.66
Inner segment	31.66 ± 3.28[*]	4.99 ± 0.99	37.41 ± 3.05	4.47 ± 0.59

[a] Measurements obtained from five specimens.

[*] $p < 0.05$.
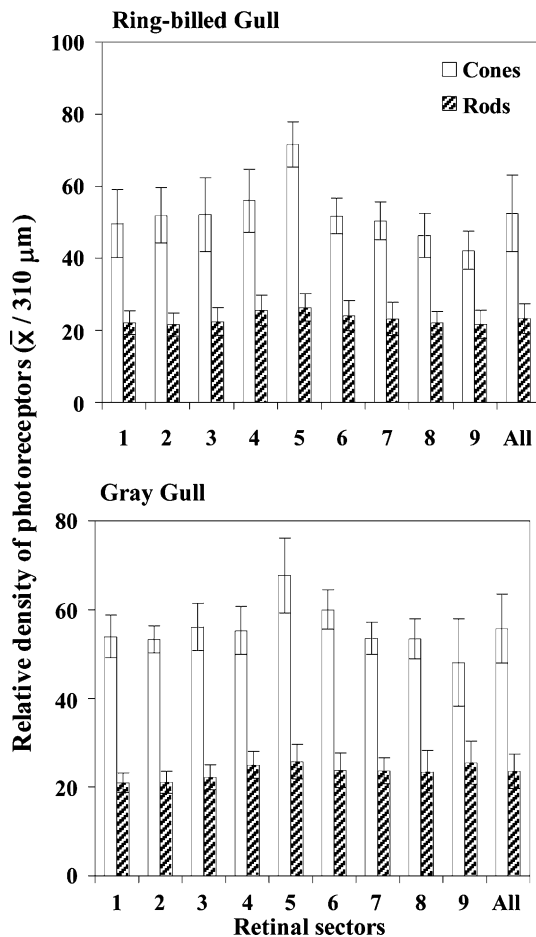

Fig. 6. Mean cone and rod densities (±SD) calculated in five ring-billed gulls and five gray gulls in each of the nine retinal sectors.

23.4/310 μm field ± 3.87). The topographical distribution of cones varies across the retina in approximately the same pattern in both species. Thus, the relative density of cones reaches a peak in the central retina (sector 5) and declines towards the ventral retina where the lowest density is attained in sector 9 (Fig. 6). In contrast, rods are evenly distributed across the entire retina. The rods and cones are therefore not complementary in their distribution, i.e., the increase in cones in the central retina is not compensated by a decrease in rod number. In both species the cone: rod ratio is 3:1 in the central retina while it is 2:1 in all other sectors.

The thickness of the retinal layers was identical in both species and the same sectorial trends were observed (Fig. 7). Thus, the outer and inner nuclear layers, the inner plexiform layer and the ganglion cell layer tend to be thicker in central sector 5, while the optic nerve fiber layer tends to be thicker in ventral sector 9.

With regard to the presence of a tapetum in the eyes of gray gulls, when the retina was removed, the outer surface of the attached pigment epithelium as well as of the choroid appeared dark brown and no reflexive layer was seen. The pigment epithelial cells observed under transmission electron microscope (Fig. 8) presented numerous inclusions such as melanosomes, but no inclusion suggestive of a tapetum.

4. Discussion

Our electrophysiological and histological data show that ring-billed gulls and gray gulls have a duplex retina that allows them to be visually efficient in the nocturnal as well as in the diurnal luminance range. Both species possess a retina clearly dominated by cones, but the number of rods appears to be sufficient to provide vision at night in their natural environment.

Fig. 9 presents the range of luminances that may occur in open habitat during a 24-h cycle. A comparison of Fig. 9 and Table 1 reveals that most of the luminances occurring at night are above the scotopic b-wave thresholds of both species (−4.21 and −4.25 log cd s m^{-2}, respectively), and that the luminance at which their scotopic b-wave saturates (V_{max}) corresponds approximately to the light level occurring just before the end of the civil twilight period under clear sky. Therefore, the retina of both species
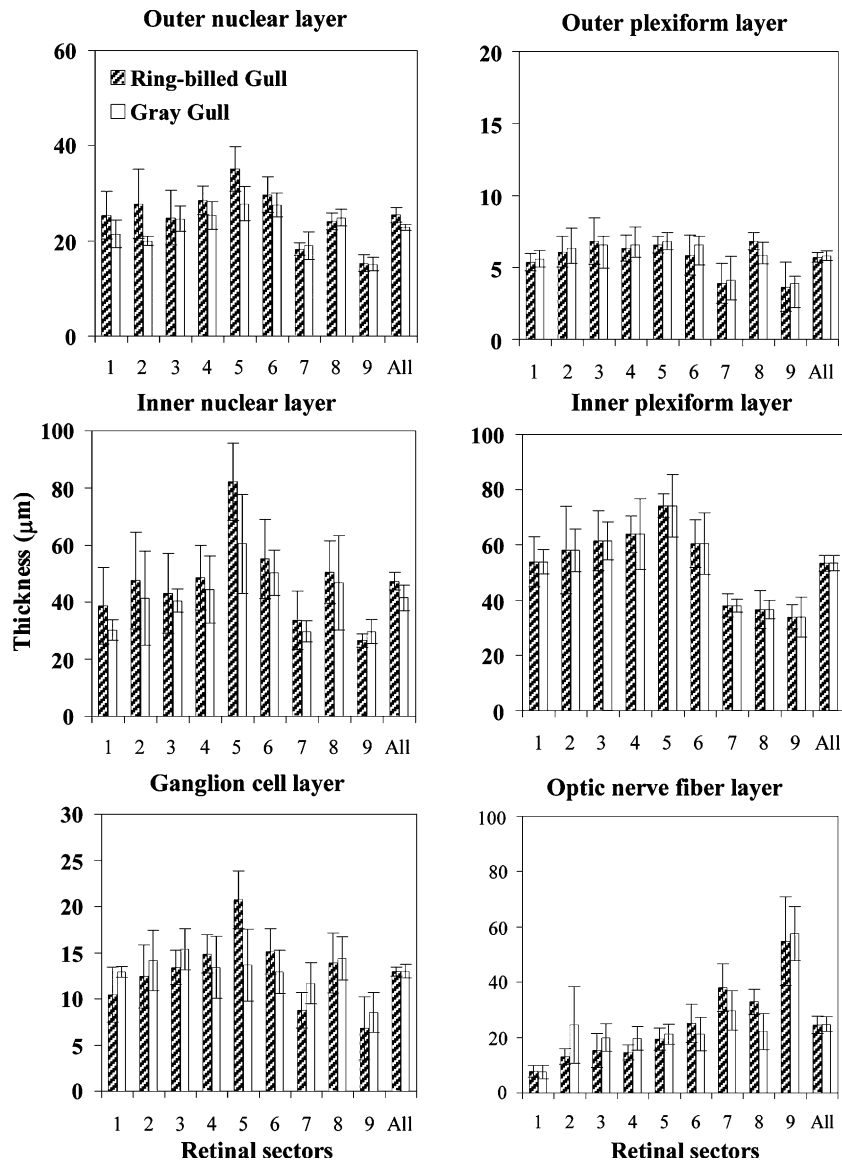
Fig. 7. Mean thickness (μm) of retinal layers (±SD) measured in five ring-billed gulls and five gray gulls in each of the nine retinal sectors.

responds over a scotopic dynamic range of about 3.4 log cd m^{-2}, which encompasses all of the light levels that may occur during the night in their natural photic environment. Whether these levels of luminance are sufficient to allow adequate visual acuity and depth perception for nocturnal flight and/or foraging is difficult to assess. However, our results give information about limits at which these nocturnal behaviours can be guided by vision, and make clear that both species possess a retina which can detect luminances in the nocturnal range.

Although both species have the same scotopic and photopic dynamic ranges, the analysis of the luminance–response reveals that within these ranges their retinas respond differently to light, such that the amplitudes of the scotopic and photopic saturated a- and b-waves (R_m and V_{max}) are significantly higher in ring-billed gulls than in gray gulls. R_m can be related to the number of photoreceptors, and V_{max} to the number of cells within the inner

nuclear layer (INL; bipolar and amacrine cells) (Hood & Birch, 1993). This would suggest that the retina of ring-billed gulls contains a greater absolute number of photoreceptors and cells in the INL than gray gulls, however our histological data do not support this interpretation. Indeed no difference was found between species in the relative rod density or INL thickness, and both species have approximately the same eye size. Therefore, other factors may explain the above physiological difference between species. The ocular pigmentation of ring-billed gulls and gray gulls differs remarkably, the iris of ring-billed gulls being yellow and that of gray gulls dark brown. Some studies have shown that the amplitude of the ERG decreases with a decrease in the transparency and the light-scattering characteristics of the eye through accumulation of eye pigment (Aufdembrinke, 1982; Johansson & Sandström, 2003; Lachenmayr et al., 1994). In humans the amplitude of the ERG responses is higher in individuals with blue eyes
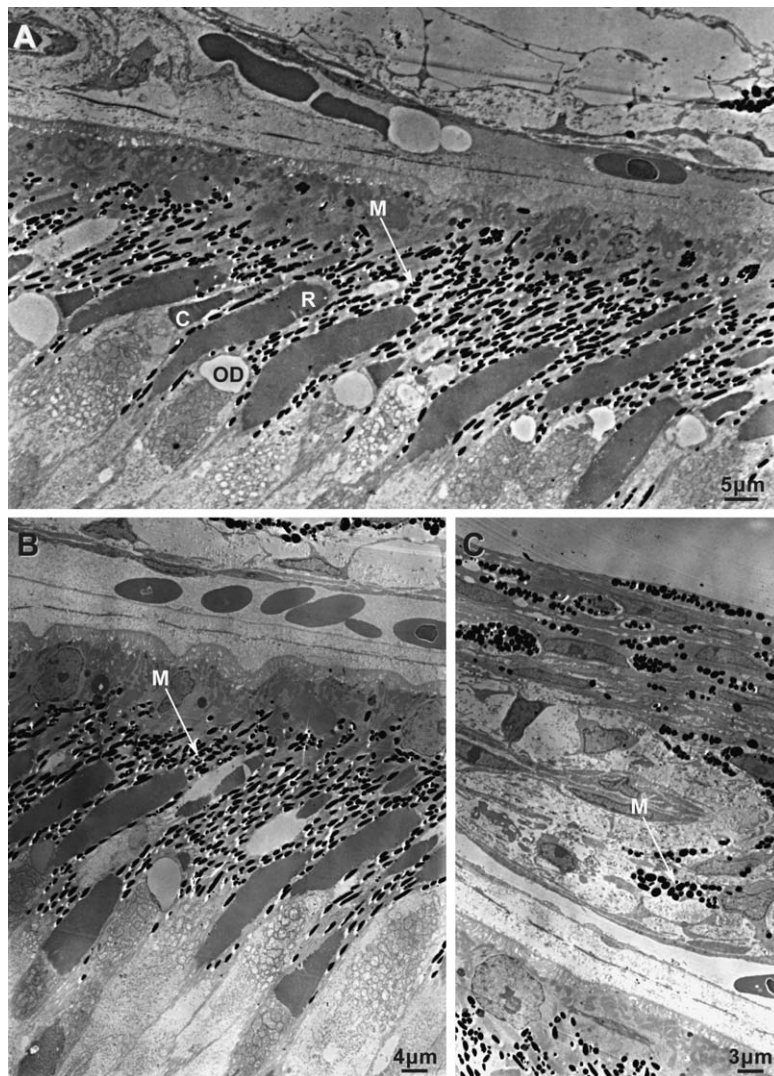
Fig. 8. Electron micrographs through the retina (A), the pigmented retinal epithelium (B), and the choroid (C) of the gray gull. Abbreviations: C, cone; OD, oil droplet; M, melanosome, included within melanocytes.

than with dark brown eyes (Smith & Misiak, 1973). Consequently, the difference we observed in the colour of the iris of ring-billed gulls and gray gulls might explain the ERG discrepancies between the two species.

The phenomena of photostasis, which is a structural and functional adaptive process related to daily variations in luminous environment and whose purpose is to regulate the total amount of photons caught per day, could also contribute to the physiological discrepancies observed. It is well known that rhodopsin concentration and length of the rod outer segment change in response to modifications brought to the luminous environment of animals (Daly, DiLeonardo, Balkema, & Balkema, 2004; Williams, Squitieri, Henderson, & Webbers, 1999). For instance, previous studies have shown that rods of rats raised in a dim light environment contain more rhodopsin, and therefore are more sensitive to light, than those of rats raised in a bright environment (Penn & Williams, 1986; Williams et al., 1999). Since ring-billed gulls live in a habitat (temperate latitude) where the ambient light is less intense than that

of gray gulls (desert at the equator latitude), this would suggest that the rods of ring-billed gulls contain more rhodopsin and therefore are more sensitive to light than those of gray gulls.

The results obtained for the dark adaptation experiment were unexpected and surprising. First, the process of recovery after the bleach was extremely slow in both species. It took more than 70 min in ring-billed gulls and 40 min in gray gulls to reach the saturation level for a bleach of 4–5%. Second, our results give evidence that the two species exhibit different patterns of dark adaptation. In ring-billed gulls, the data show that the sensitivity of the retina is governed by photoreceptors but also by adaptive mechanisms within the inner retina, which are not linked to the sensitivity changes of the photoreceptors during the process of dark adaptation. The discrepancy observed between the a-wave and b-wave demonstrates that, in this species, a non-receptoral adaptive mechanism exists and governs visual sensitivity after 40 min of dark adaptation. In gray gulls, the kinetic of dark adaptation followed by the b-wave
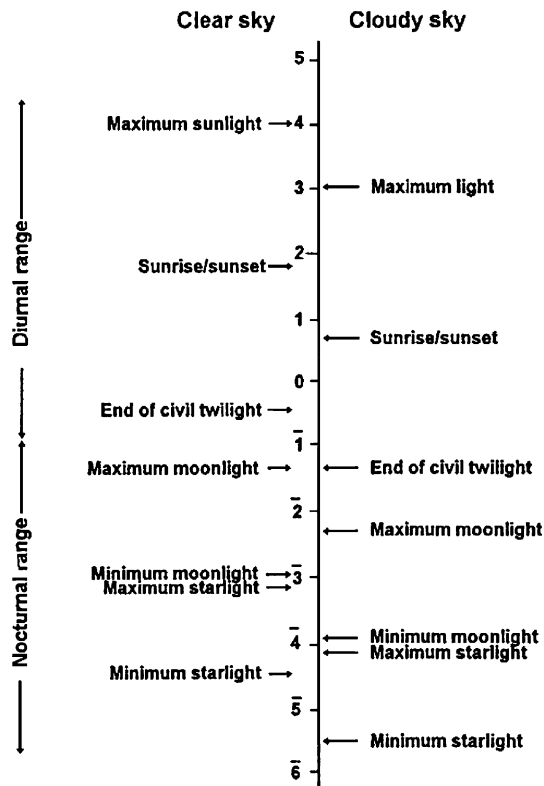
Fig. 9. The range of natural luminance (log cd m^{-2}) of natural surface in open habitats under clear and cloudy skies. Adapted from Fig. 2.5 of Martin (1990).

was exactly the same as the a-wave, suggesting that the sensitivity of the retina of this species is governed mainly by photoreceptors.

Changes in ERG b-wave amplitude during dark adaptation have been related to regeneration of the visual pigment in the dark after exposure to light; in fact, the time course of dark adaptation follows roughly the time course of pigment regeneration (Dowling, 1960). However, some studies have indicated that other factors are involved in this process, such as adjustments within the neural circuitry of the retina (Dowling, 1963; Fain, Matthews, Cornwall, & Koutalos, 2001; Rushton & Cohen, 1954). Other factors may also explain the more gradual attainment of maximum sensitivity. Douglas and Wagner (1982) have shown evidence for a direct relationship between the increase in sensitivity during the course of dark adaptation and retinomotor movements in the rainbow trout (*Salmo gairdneri*). Indeed, when the fishes are shifted from light to darkness, their rods shorten in such a way that the outer segments are removed from the shielding effect of the pigment epithelium, a process that increases the number of rods being exposed to light and, consequently, enhances sensitivity. Such retinomotor movements are also present in birds (Menger, Koke, & Cahill, 2005; Meyer, 1977). In our study, the effect of regeneration of the visual pigment was probably negligible since less than 5% of the rhodopsin was bleached. Therefore, the increase in the amplitude of the b-wave may be attributed to retinomotor movements

and the difference between the two species could be linked to a more rapid retinomotor mechanism in gray gulls than in ring-billed gulls. The difference in ocular pigmentation observed between the two species may also explain the fact that the process of dark adaptation was about 30 min faster in gray gulls than in ring-billed gulls. Previous studies have shown that albino rats have a slower dark-adaptation process than pigmented rats (Behn et al., 2003). This has been related to the fact that calcium, which is involved in the phototransduction cascade, binds strongly to the pigmented tissue due to the presence of melanin (Drager, 1985).

The fact that saturation is attained more rapidly during the process of dark adaptation in gray gulls than in ring-billed gulls may have behavioural and ecological significance. The transition from day to night is more rapid close to the equator than at higher latitudes and days are less bright and nights are less dark at higher latitudes. A more rapid dark adaptation process in condition of rapid transition from day to night could be advantageous to gray gulls which, during the breeding season, fly at sunset from coastal feeding areas to nesting territories in the desert, where they perform most of their activities at night.

The eyes of ring-billed gulls and gray gulls have approximately the same size and their axial lengths (16.7 and 16.0 mm, respectively) and maximum pupil diameters (5.9 and 5.3 mm, respectively) are smaller than what is found in tawny owls (*Strix aluco*) (axial length 28.5 mm and maximum pupil diameter 13.3 mm), a strictly nocturnal species (Martin, 1982). However, compared to diurnal species such as rock doves (*Columba livia*) and european starlings (*Sturnus vulgaris*) (axial length 11.0 and 7.9 mm, respectively, and maximum pupil diameter 4.0 and 2.5 mm, respectively) the values of their axial lengths and maximum pupil diameters are considerably higher. Thus, the eyes of ring-billed gulls and gray gulls seem to be intermediate between the strictly nocturnal and strictly diurnal eyes.

Both gull species possess a duplex retina in which 70% of the photoreceptors are cones and 30% are rods, a proportion similar to what was found in cattle egrets (*Bubulcus ibis*), tricoloured egrets (*Egretta tricolour*) and American white ibises (*Eudocimus ruber*), three strictly diurnal species (Rojas, McNeil, Cabana, & Lachapelle, 1999a). The topographical distribution of the photoreceptors is quite identical in both gull species, with similar higher cone density in central sector 5 resulting in a distinct thickening of this area (Fig. 8). Sectors of higher cone density represent areas of highest visual acuity (Meyer, 1977; Walls, 1967). Furthermore, in both species the rods are evenly distributed across the retina, which may maximise visual sensitivity in all parts of the visual field. Many gull species locate their preys from the water surface as they fly, which, according to Ashmole (1971), requires good vision in all parts of the visual field. The uniform distribution of their rods would therefore have some behavioural and ecological significance since both gulls forage during the night. In most nocturnal shorebird and wading bird species studied so far and that do not forage while flying, rods tend to be more numerous

in sector 5 than in other sectors (Rojas et al., 1999a, Rojas, McNeil, Cabana, & Lachapelle, 1999b). The rod distribution of ring-billed gulls and gray gulls differs from what is generally found in strictly diurnal species, such as american robins (*Turdus migratorius*) and mourning doves (*Zenaida macroura*) in which rods tend to be less numerous in the central retina (McNeil, McSween, & Lachapelle, 2005). Finally, contrary to what has been suggested by Howell et al. (1974), the eye of gray gulls does not contain a tapetum. Under intense flashes, eye-shine can be detected in eyes without a tapetum, due to a small proportion of the light being reflected at the retinal surface before it reaches the photoreceptors. For instance in ostriches (*Struthio camelus*), the light reflection has been attributed to the *lamina vitrea* between the pigment epithelium and choroid (Walls, 1967).

In conclusion, the present study shows that the dynamic range of the retina of ring-billed gulls and gray gulls encompasses all of the light levels that may occur in their natural photic environment. Both gull species live in open habitats, forage and fly between feeding sites or from roosting sites to feeding grounds under nocturnal light levels (McNeil et al., 1993). However, we do not know if vision is sufficient to account for all the nocturnal behaviours that have been observed in both species, notably nocturnal foraging. Gray gulls have been reported to feed at night like sandpipers (*Emerita analoga*) by probing the wet sand behind the retreating waves for mole crabs (Blokpoel et al., 1992). In ring-billed gulls, there is also indication of tactile nocturnal feeding while standing on water bodies (Hébert & McNeil, 1999). This may suggest that both species, like some shorebird species do, e.g., *Catoptrophorus* and *Tringa* species (McNeil & Rompré, 1995; Rompré & McNeil, 1996), could switch from a visual day-time foraging strategy to a tactile strategy at night. However, more behavioural studies are needed in ring-billed gulls and gray gulls to test this hypothesis.

Acknowledgments

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