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Multifocal lenses in a monochromat: the harbour seal

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SUMMARY

Previous photorefractive results from harbour seals indicated the presence of a multifocal lens. This was surprising because the evolution of multifocal lenses has served to compensate for chromatic aberration in animals with colour vision, which harbour seals as monochromats should not be capable of. To gain insight into the lens optics of these animals, we extended our photorefractive measurements in live seals under water and in air and, additionally, analyzed eight extracted juvenile harbour seal lenses with schlieren photography and a laser scanning technique. The results from all methods applied support the presence of multifocal lenses in harbour seals. However, the functional significance of multiple focal lengths in harbour seal lenses remains speculative. Interestingly, the slit pupils of harbour seals cannot be considered to be an adaptation to the multifocal optical system of the eye.

Key words: harbour seal, *Phoca vitulina*, lens, multifocal.

INTRODUCTION

The optical function of aquatic eyes can largely be understood by studying the optics of the lens because, in most cases, the lens is the dominant refractive element with the eye immersed in water. The cornea is optically almost ineffective in this situation (Mathiessen, 1886) as its two surfaces are nearly parallel and are bordered by aqueous media, water and aqueous humour, of nearly the same refractive index.

Marine mammals are secondarily adapted to the aquatic environment. The spherical state of their crystalline lenses compensates to some extent for the loss of corneal refractive power under water (Jamieson and Fisher, 1972). So far, little is known about lens optics in marine mammals in general and in pinnipeds in particular. In whales, measurements revealed that the harbour porpoise (*Phocoena phocoena*) lens brings parallel light to a focus in front of the retina (Matthiessen, 1886; Matthiessen, 1893; Kröger, 1989; Kröger and Kirschfeld, 1992; Kröger and Kirschfeld, 1993). In these eyes, the cornea acts as a diverging lens under water. To our knowledge, the only study examining lens optics in a pinniped species, the hooded seal (*Cystophora cristata*), was conducted by Sivak et al. (Sivak et al., 1989). The authors reported that hooded seal lenses are spherical in shape and have short focal lengths. According to the results of that study, the lenses are well corrected for spherical aberration.

While performing photorefractive measurements on harbour seal eyes (Hanke et al., 2006), we observed ring-shaped brightness distributions under water, reminiscent of the brightness distributions indicative of multifocal lenses (Kröger et al., 1999; Malkki and Kröger, 2005). The evolution of such lenses served to compensate for the chromatic defocus that occurs because the refractive index of any transparent material increases with decreasing wavelength. Consequently, the focal length of a lens is a function of the wavelength of light (longitudinal chromatic aberration, LCA). This implies that at any time, only a narrow band of wavelengths can be in focus on the retina in the absence of a compensatory mechanism. Light of other wavelengths is defocused (chromatic defocus). Chromatic defocus is especially unwanted in species capable of colour vision and with eyes with a short depth of focus. The evolution of multifocal lenses has solved the problem of chromatic defocus as these lenses create well-focused images at the wavelengths of maximum absorbance (λ_{max}) of the cone photoreceptors (Kröger et al., 1999). First described for a cichlid fish (Kröger et al., 1999), multifocal lenses seem to be widespread among vertebrates (Malkki and Kröger, 2005; Malmström and Kröger, 2006; Karpestam et al., 2007; Gustafsson et al., 2008; Lind et al., 2008).

The ring-shaped brightness distributions in photorefractive images as a first indication of multifocal lenses in harbour seals were unexpected as harbour seals are said to be colour-blind because of the absence of the short-wave-sensitive cone type (Peichl and Moutairou, 1998; Crognale et al., 1998; Peichl et al., 2001; Newman and Robinson, 2005; Levenson et al., 2006); however, this still leaves the possibility of mesopic colour vision, which is currently under investigation in our lab. Furthermore, the ring-shaped brightness distributions in seal pupils were atypical because they could only be observed under water and only along the optical axis. These contradictory findings, together with the fact that no detailed information on pinniped lenses is available, led us to examine lens optics in juvenile harbour seals with modern optical techniques as described by Malkki and Kröger (Malkki and Kröger, 2005). In addition, we repeated and extended our photorefractive measurements under water and in air in two live, adult seals.

MATERIALS AND METHODS

Infrared (IR)-photoretinoscopy

Experimental animals

Photorefractive measurements were performed on two live harbour seals, *Phoca vitulina* (Linneaus 1758), at the Marine Science Centre

(www.msc-mv.de), Cologne, Germany. Both animals ('Enzo', 3 years old; 'Sam', 12 years old) were experimentally experienced and had already taken part in various experiments on vision. All experiments were conducted in accordance with the German animal protection law.

Photorefractive measurements

Photorefractive measurements under water and in air were performed using IR-photoretinoscopy as developed by Schaeffel et al. (Schaeffel et al., 1987) and described in detail by Hanke et al. (Hanke et al., 2006). The IR-photoretinoscope (Fig. 1A, inset) consisted of a metal shield that covered one half of the lens aperture (lens: Cosmicar/Pentax, F=50 mm, *f*/1:1.4, Hamburg, Germany; with two extension rings, resulting in an operating distance of 0.5 m) of an IR-sensitive monochrome CCD camera (The Imaging Source, Bremen, Germany). The light of 13 IR-LEDs (light emitting diodes) on the IR-retinoscope, eccentrically arranged in four horizontal rows, entered the eye, was reflected and produced a brightness distribution in the pupil. Photorefractive measurements under water and in air were performed in darkness in order to dilate the pupils. The camera and retinoscope were placed at a distance of 0.5 m in front of the eye with the knife edge of the retinoscope (edge of the metal shield in the lens aperture) (Fig. 1A, inset) orientated horizontally. For underwater measurements, the animal climbed onto a small platform and immersed its head in a water-filled aquarium made of glass (Fig. 2). After lowering the head, the eyes were close to the aquarium's front window.

Lens measurements

Material

Harbour seal lenses were studied at the Research and Technology Centre West Coast, Büsum, Germany. We were able to analyze the lenses of four harbour seal pups aged from newborn to approximately two weeks (Table 1). In Germany, harbour seals are protected by the Trilateral Wadden Sea Agreement and the Seal Management Program. As part of this program, abundant seal pups are rehabilitated in seal stations. Prior to entering the rehabilitation program, the seal pup's health status is judged, according to guidelines set by the official bodies of the German province of Schleswig Holstein. The seal's constitution has to meet well-defined criteria because only seals with a realistic chance of survival should be rehabilitated. If the criteria are not met, the seal pups are euthanized. We analyzed the lenses of one seal who was euthanized by the veterinary (seal 3) and of two seals that died during the rehabilitation program (seal 2 and seal 4). In addition, during our stay at the German Wadden Sea coast, one harbour seal that was kept permanently at the Friedrichskoog seal station gave birth but the pup was non-viable. We were offered the opportunity to analyze the eyes of this seal pup as well (seal 1). Ages and causes of death are given in Table 1 for all seals.

Preparation of the lenses

To facilitate the removal of the eye out of the orbit, large parts of the eyelids were removed. The eye muscles were detached from the eyeball and the optic nerves were cut. Due to the thickness of the lens and its close adherence to the vitreous (Fig. 3B), the eye was opened anteriorly *via* the corneal periphery. First, the cornea and iris were completely removed. Second, the lens was excised by cutting the zonular fibres almost all around the lens. Some zonular fibres with their associated ciliary body and neighbouring sclera (approximately 5 mm×5 mm) (Fig. 3A) were left attached. This served as a handle, which allowed the lens to be placed in the setups with minimal manipulation. During dissection, the vitreous was found to be strongly adherent to the posterior surface of the lens (Fig. 3B), as is encountered in the young human eye (Sachsenweger, 2003). Although in each case an attempt was made to remove this vitreous carefully from the lens, without damage, it was not always certain that removal was complete, except in seal 4.

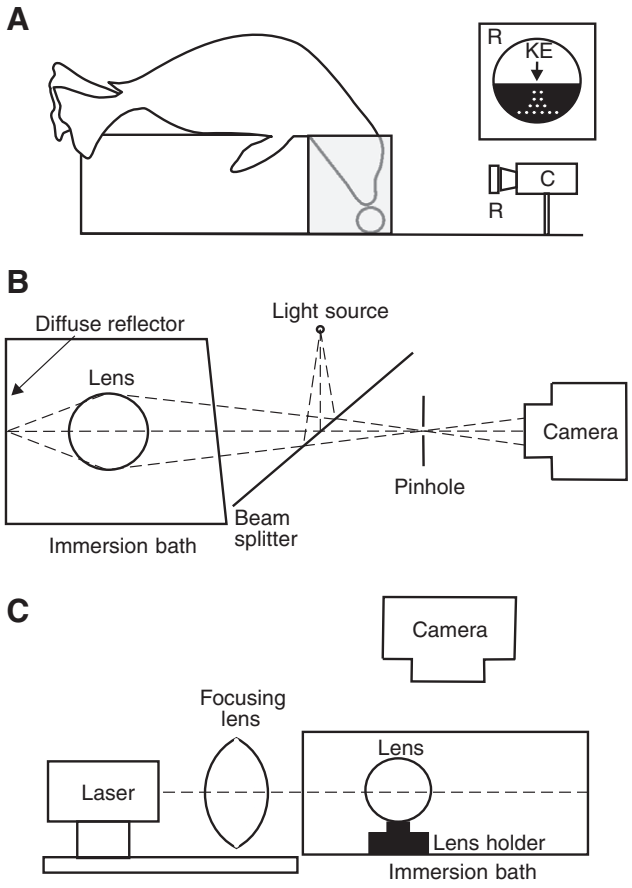

Fig. 1. Schematic representation of the experimental setups used to measure harbour seal lenses. (A) IR-Photoretinoscopy under water. A retinoscope (R) with 13 IR-LEDs arranged eccentrically and a knife edge (edge of metal shield in the lens aperture; KE) arranged horizontally was attached to a video camera (C). IR-light entered the eye, was partly reflected and caused variation in brightness in the pupil. The seals were trained to lower their heads into an aquarium from an elevated platform. (B) Schlieren photography. White light (point source, cold light laboratory lamp, 3200 K) was reflected by a beam splitter into the optical axis. The lens, immersed in PBS (pH=7.4, 290 mosmol, 20°C), focused the light beam on a diffuse reflector. Reflected light was focused by the lens on the pinhole and recorded by a camera. (C) Laser scan. The lens was positioned on a holder in the middle of the immersion bath filled with PBS to which a small amount of microparticles was added. A 5 mW green (537 nm) diode-pumped, solid-state laser was used to scan through a meridional plane of the lens. The upwards scattered light was recorded by a video camera.

The lens of the first eye was extracted between 0.5 and 2.5 h after the death of the animal (Table 1). The second eye was excised approximately one hour later, after the measurements on the first lens had been completed.

Schlieren photography

The portable setup described by Malkki and Kröger (Malkki and Kröger, 2005) was used in this study. In schlieren photography,

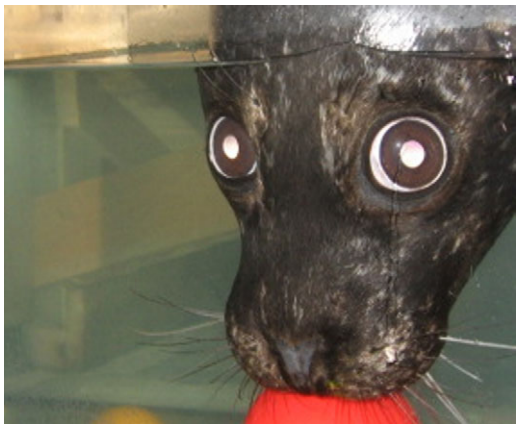

Fig. 2. Harbour seal 'Enzo' during underwater photorefractive measurements. The seal immersed its head into an aquarium placing its snout on a red ball (target). This way, the seal's eyes came close to the aquarium's front window. Pupils are almost completely circular because measurements were performed in darkness.

white light from a point source (standard cold light laboratory lamp running at 3200 K) was reflected by a beam splitter into the optical axis (Fig. 1B). The seal lens was suspended in an immersion bath filled with phosphate buffered saline (PBS, pH=7.4, 290 mosmol, approximately 20°C) and focused the light beam on a diffuse reflector at the rear side of the immersion bath. Reflected light was focused by the lens on the pinhole and was recorded by a digital video camera (Sony DCR-TRV620E PAL, Sony Corporation, Tokyo, Japan). Different from Malkki and Kröger (Malkki and Kröger, 2005), it was not necessary to further magnify the picture.

Each freshly excised lens was inserted directly into the schlieren setup with the posterior pole of the lens facing the diffuse reflector. The posterior pole was identified from the lens's orientation in the eye-cup. Care was taken to keep the lens orientated correctly throughout all experiments.

Laser scanning

Laser scan setup

The video camera used in schlieren photography was transferred to the portable laser scan setup (Malkki and Kröger, 2005), video recording the immersion bath at a distance of 10 cm from above (Fig. 1C). The image's long axis was slightly turned (approximately 10 deg.) relative to the laser beam in order to minimize aliasing effects. The immersion bath contained PBS (pH=7.4, 290 mosmol, approximately 20°C) to which one drop of microparticles had been added to scatter light and, therefore, gain a high visibility of the laser beam. The seal lens was then positioned on a holder in the middle of the immersion bath with the help of the forceps used in schlieren photography. The posterior pole of the lens was facing the rear side of the immersion bath. The position of the lens was further modified by manually rotating the lens and/or the lens holder with respect to the laser beam until the optical axis of the lens was aligned with the laser beam. This could be achieved by adjusting the zonular fibre ring to be perpendicular to the laser beam when viewing the immersion bath from the top and from the side. The remaining piece of sclera was removed immediately after positioning of the lens. The large size of the seal's lens obviated the need for magnification used for measuring fish lenses (Malkki and Kröger, 2005). A 5 mW green (537 nm) diode-pumped, solid-state laser was used to scan the lens. The laser beam was focused with an F=100 mm lens with the meniscus of the focused laser beam placed directly in front of the seal lens that re-collimated the beam. The laser beam was then adjusted in height until it passed through the centre of the seal lens without being deflected. The translation stage on which the laser and its focusing unit were mounted could only be moved to scan two thirds of the large seal lenses. Therefore, the same lens was scanned twice, once starting from the left side and once from the right side, while the camera was video recording the light of the laser beam, which was scattered upwards by the microparticles. Laser scanning experiments were performed with only one wavelength because the obtained results are largely independent of the wavelength, except for a longitudinal shift, within the visible range of the spectrum (Kröger and Campbell, 1996).

Analysis of laser scans

From each scan, 200 frames were exported in TIFF (tagged image file format). We used a custom-written program (in IDL 6.0 developing environment, Research Systems, Boulder, CO, USA), which has been described in detail by Malkki and Kröger (Malkki and Kröger, 2005), for analysis of the back centre distance (BCD, axial distance between the lens centre and the intercept between the exit beam and the optical axis of the lens) as a function of beam entrance position (BEP, lateral distance between the entrance beam and the optical axis). This function describes the longitudinal spherical aberration (LSA) of the lens. The two scans per lens (see above) were analyzed separately. After acquiring laser beam data at each laser position by the program, an image was generated by averaging all exported frames. On this image, the axial and equatorial diameters of the lens were determined manually. As we always had laser scans extending over only approximately two thirds of the equatorial diameter of the lens, we had to estimate the equatorial diameter. However, there were always enough data points from the second half of the lens to adjust the optical axis of the lens in the last step of analysis (Malkki and Kröger, 2005). In Microsoft Excel (The Microsoft Corporation, Redmond, WA, USA), the results from both scans through each lens were averaged because there was very good agreement in the region covered by both scans.

Measurement of refractive index and osmolarity of the aqueous and vitreous humours

Extraction of aqueous and vitreous

Before opening the eye, an injection needle was gently inserted into the anterior chamber of the eye *via* the cornea and approximately 1 ml of aqueous was extracted with a syringe. The syringe and its content were immediately deep-frozen at –80°C until measurements could be performed. Some of the vitreous was cut out of the eye-cup after removing the lens and placed into Eppendorf tubes. These tubes were also stored at –80°C.

Table 1. Age and life history of all animals used for schlieren photography and laser scanning, as well as time between death and start of the experiments in hours

Seal	Age at death	Life history	Time between death and experimental start (h)
1	2 hours	Death after birth	2
2	14 days	Death due to illness	1.5
3	15 days	Euthanized	0.5
4	17 days	Death due to illness	2.5

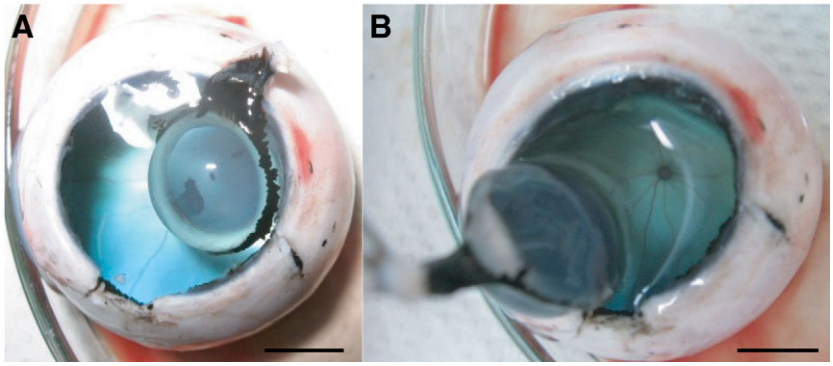

Fig. 3. Dissection of harbour seal lenses. (A) Lens lying on the vitreous humour after removal of the cornea and iris, and after cutting the zonular fibre ring. (B) Posterior half of the lens showing the tight coupling between lens and vitreous. The lens was indirectly handled with a pair of forceps holding a small piece of sclera with some attached zonular fibres and the lens. Scale bar, 5 mm.

Determination of eye and lens dimensions, refractive index and osmolarity

Equatorial diameter of the eye-cup and both diameters of the lenses were measured with sliding callipers after the optical measurements had been performed. The accuracy of the measurements was estimated as 0.1 mm. For the determination of refractive index and osmolarity of the aqueous and vitreous humours, the deep-frozen samples were thawed. Refractive index was determined at 22°C with a digital Abbe refractometer (DR 5000, Krüss Optic, Hamburg, Germany). Osmolarity was measured with an osmometer (The Advanced Micro Osmometer Model 3300, Advanced Instruments, Norwood, MA, USA).

RESULTS

Photorefractive measurements

As described earlier by Hanke et al. (Hanke et al., 2006), ring-shaped brightness distributions are apparent on photorefractive pictures obtained under water (Fig. 4A–C). However, extended measurements revealed that these rings are always visible, even if recordings were off-axis and the eye was slightly ametropic. One very prominent ring appears in the periphery of the lens but only when the pupil is fully dilated (Fig. 4A–C). Two faint rings are present at approximately 0.5 lens radius (R) and 0.75 R (Fig. 4A). All rings are more pronounced in the adult seal (Fig. 4C). In air, somewhat masked by reflections from the corneal surface (Hanke et al., 2006) and by corneal distortions, rings are not as prominent but can still be detected between approximately 0.65 and 0.75 R (Fig. 4D).

Schlieren photography

On schlieren photographs, up to four coloured rings are visible (Fig. 5). A red outermost ring and two bluish rings are clearly present on the pictures of all lenses except for seal 3 where the outer blue ring was thin and incomplete (Fig. 5C). The overall pattern is variable between the lenses of different individuals and the rings are often inhomogenously coloured (e.g. Fig. 5C). Especially in the centre, colours spread out in a fan-shaped manner towards the periphery of each lens. On the schlieren photographs of the lenses of the first two seals (Fig. 5A,B), there are soft transitions between the rings. Pictures of the lens of seal 1 (Fig. 5A) indicate that the lens is slightly larger than the coloured part indicates.

Due to the complete removal of the vitreous humour from the lenses of seal 4 (Fig. 5D), the respective schlieren photographs are most reliable. There are two broad bluish rings, one thin dark-blue ring and a red outer ring. These features are in keeping with the results of photorefractive measurements in the young seal under water (Fig. 4A) with the two bluish rings correlating to the two more central rings and the thin dark-blue ring corresponding to the prominent ring at approximately 0.85 R. The lens sutures are prominent on all schlieren photographs (Fig. 5). They stretch over almost 0.8 R and are of cross-like appearance.

Laser scans

The results are irregular for the central BCDs between 0 and 0.3 R (Fig. 6A–D) where the accuracy of the method is low (Malkki and Kröger, 2005). The obtained LSA curves vary between individuals. However, as a common feature, all LSA curves, except for the scans of the newborn (Fig. 6A), show two peaks in the periphery (Fig. 6B–D, long arrows). Furthermore, a sharp decline in BCD towards the outermost periphery is evident in all analyzed lenses (Fig. 6A–D, short arrows). The mean LSA curve of all, except for the lenses of the neonate, is presented in Fig. 7A. Two peaks of slightly different BCDs can be seen (first peak at BEP 0.67 R, BCD 3.77 R; second peak at BEP 0.87 R, BCD 3.75 R). The mean BCD between 0.3 and 0.6 R is 3.58 R. BCD increases minimally towards the centre by 0.05 R (1.4% of mean value 3.58 R). At 0.9 R, BCD

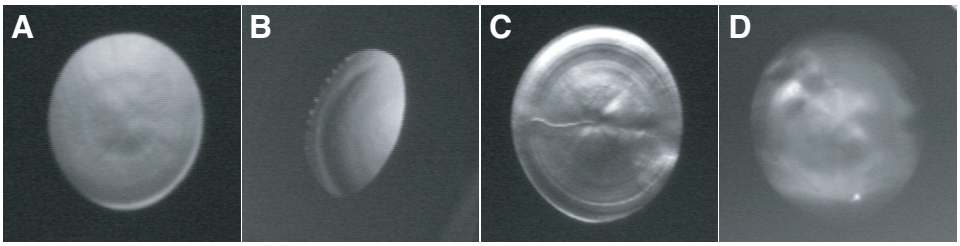

Fig. 4. Photorefractive images of a young (3 years old) harbour seal under water axially refracted with lateral (A) and frontal camera position (B) and an older (12 years old) harbour seal under water (C) and in air (D). On the underwater pictures (A–C), two rings in the centre and one pronounced ring in the periphery can be seen. Corneal cloudiness in the older seal leads to a central stripe in the brightness distribution under water (C), and irregularities in corneal topography to spot-like distortions in air (D) that mask the slightly visible rings.

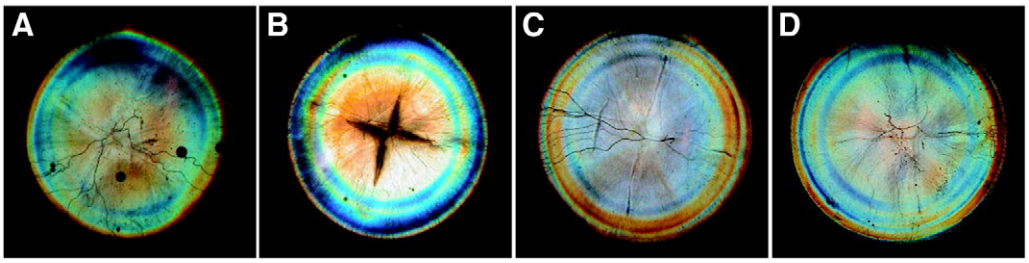

Fig. 5. Results of schlieren photography. For each seal, one typical picture is presented in chronological order of measurement (A, picture of seal 1; B, seal 2; C, seal 3; D, seal 4). Coloured rings and lens sutures are prominent.

drops steeply by approximately 0.5 R (14% of mean value 3.58 R) before increasing again up to values of approximately 5 R. The mean LSA curve (Fig. 7A) mirrors the results of photorefractive measurements in the live seals under water (Fig. 4A–C) and the schlieren photographs of the best dissected lens (Fig. 5D) as again two broad rings and a thin sharp ring can be discerned clearly. Fig. 7B shows the averaged picture of one laser scan presenting all laser beams entering the lens and the way they are deflected by the lens.

Eye and lens dimensions, refractive index and osmolarity of the aqueous and vitreous humours

All eye and lens dimensions as well as refractive index and osmolarity measurements are listed in Table 2. Due to the dissection process and the need for minimizing the time between the animal's death and the start of the experiments, only the equatorial diameter of the eye-cup could be measured with a mean (±s.d.) value of 33.0±0.08 mm (Table 2). The equatorial diameter of the eye-cup was slightly larger in older animals (Table 2).

The lenses were clear and almost spherical in shape. In all lenses, the equatorial diameter is slightly larger than the axial diameter. Mean axial lens diameter (±s.d.) is 10.90±0.19 mm, mean equatorial diameter (±s.d.) 11.69±0.33 mm (Table 2).

The aqueous and vitreous humours both have approximately the same mean refractive index of 1.335 (aqueous, 1.33498±0.00032, N=7; vitreous, 1.33454±0.00010, N=6; Table 2). Mean osmolarity of both media is 343.6 mosmol (aqueous, 344.571±22.397 mosmol, N=7; vitreous, 342.667±12.127 mosmol, N=6; Table 2).

DISCUSSION

Our data suggest that harbour seal lenses are equipped with multifocal lenses of the basic vertebrate design (Malkki and Kröger, 2005; Malmström and Kröger, 2006; Karpestam et al., 2007; Gustafsson et al., 2008; Lind et al., 2008). Although methodological limitations have to be considered (see Methodological limitations, below), we assume the distinctive refractive zones to be of functional significance for vision in harbour seals (see Optical status of harbour seal lenses, below).

Methodological limitations

Photorefractive measurements in live animals

We ascribe the main role in the generation of the rings in photorefractive images to the lens because of the similarity of the rings obtained by all methods applied. Scattering of light inside the lens is an unlikely cause for the occurrence of the rings because scattering would make them appear dark. Corneal shape and irregularities have an effect on the brightness profiles in air (Fig. 4D) (Hanke et al., 2006) but their effect on underwater photorefraction is expected to be negligible.

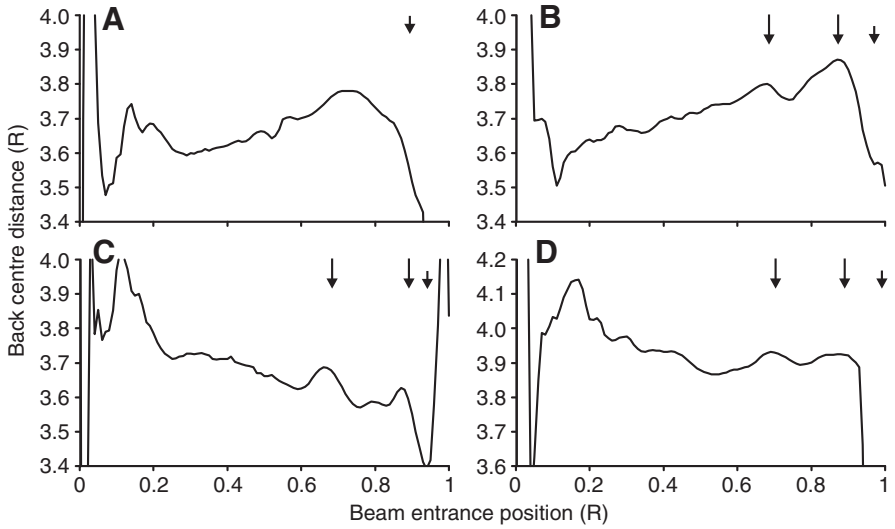

Fig. 6. Results of laser scanning displaying the longitudinal spherical aberration (LSA) curves of harbour seal lenses measured at 537 nm. (A–D) LSA curves of the right eyes of seal 1 (A), seal 2 (B), seal 3 (C) and seal 4 (D). Long arrows mark peaks, short arrows mark steep declines in back centre distance.

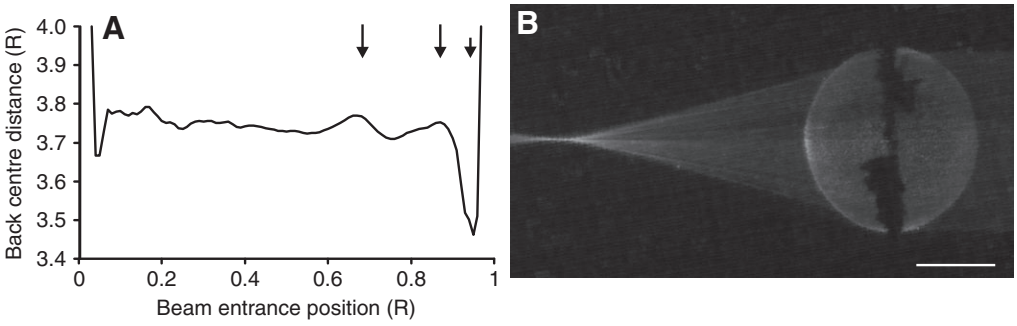

Fig. 7. (A) Mean LSA curve of all lenses except for the neonate's lenses. Two peaks at 0.67 R and 0.87 R are visible (long arrows). Note the steep decline in back centre distance in the periphery (short arrow). (B) An example picture of a spherical harbour seal lens deflecting the laser beams. Scale bar, 5 mm.

The nature of the sharp ring in the periphery seen only in fully dilated pupils remains speculative. To clarify whether it mirrors a functional feature in the optically relevant part of the lens or reflects the lens's very periphery that is normally shaded by the iris, more data from the lens periphery are needed.

Optical quality of extracted lenses

All lenses studied were extracted from very young seals with potentially immature lenses. If they were indeed immature, which means not fully developed, the results of this study might not be representative for the optical status of the adult seal's eye. The variation in the results might suggest that the lenses were still under developmental fine-tuning. However, due to the short mother–pup relation in phocid seals (Bowen, 1992; Atkinson, 1997) there might be a strong selection pressure on developing fully mature sensory organs shortly after birth enabling the juvenile animals to hunt and navigate independently. The juvenile lenses we studied were therefore most likely already optically similar to adult lenses.

The lenses were free of cataracts that could have been induced by the overall weakness or illness of the seals (see Lens measurements, above), nutritional deficiencies due to raising on milk replacer (Bunce, 1979), or as post-mortem changes. Furthermore, the lenses may have absorbed some water in the periphery because it turned out that the osmolarity in seal eyes is approximately 53 mosmol higher compared with the PBS used in the present study, which was iso-osmotic to the body fluids of humans and other terrestrial mammals. However, the difference in osmolarity is overestimated to some extent, as some water has probably evaporated from the frozen samples of aqueous and vitreous humours.

Schlieren photography and laser scanning

Some vitreous humour remained attached to the posterior sides of the lenses (see Preparation of the lenses, above). However, as the vitreous has a refractive index similar to the solution in the immersion baths, its influence was probably minor.

On schlieren photographs of the lenses of seal 1, being the youngest, the coloured part is smaller than the lens itself. The laser scanning results show that the dark part has a considerably shorter focal length than other regions of the lens (Fig. 6A), which suggests that it focused IR-wavelengths on the pinhole in the schlieren setup. This in turn suggests that the periphery of very young seal lenses does not contribute to vision.

Because of their apparent immaturity, we excluded the lenses of seal 1 from the analysis of the laser scanning data. The remaining results from laser scanning are still variable, probably reflecting some residual immaturity. Another source of error is the low accuracy of the laser scanning method for BEPs smaller than 0.3 R (Malkki and Kröger, 2005). However, this central region of a lens covers a small area and has long depth of focus, such that it contributes little to retinal illumination and blur. Damage to the lens, inflicted during excision, and the lens sutures are other sources of variability (Kuszak et al., 1991; Sivak et al., 1994) since only one meridian is probed by laser scanning. Results from individual scans are therefore rarely reliable. The LSA curve obtained by averaging all scanning results (Fig. 7A) is more trustworthy and will be discussed further (see Optical status of harbour seal lenses, below), although variation intrinsic to the individual lens will be hidden. The results of laser scanning that are generally susceptible to noise are consistent with the results from the two other methods applied, which leads us to conclude that the

Table 2. Eye and lens dimensions, refractive index and osmolarity of the aqueous and vitreous humours

					Aqueous		Vitreous	
		Eye diameter (mm)	Lens diameter (mm)		Refractive index	Osmolarity	Refractive index	Osmolarity
Seal	Eye	Equatorial	Axial	Equatorial	(22°C)	(mosmol)	(22°C)	(mosmol)
1	Left	32	10.74	12.04	1.33527	343	no samples available	
	Right	32	10.89	11.56	no samples available			
2	Left	33	11.08	11.34	1.33508	314	1.33444	337
	Right	33	10.54	11.21	1.33489	346	1.33444	322
3	Left	33	11.13	11.52	1.33545	319	1.33470	351
	Right	33	10.89	11.84	1.33483	369	1.33464	356
4	Left	34	10.95	11.99	1.33490	373	1.33449	348
	Right	34	10.96	12.04	1.33445	348	1.33452	342
Means		33	10.90	11.69	1.33498	344.571	1.33454	342.667
(±s.d.)		±0.08	±0.19	±0.33	±0.00032	±22.397	±0.00010	±12.127

complex LSA observed in the mean LSA curve represents not just regular spherical aberration, which is typical for a spherical lens but serves to compensate for LCA.

Optical status of harbour seal lenses

There are visible rings on photorefractive pictures obtained from adult seals (Fig. 4). Furthermore, there are two peaks in the mean LSA curves at approximately 0.67 R and 0.87 R (Fig. 7A). These peaks correspond well to bluish rings on schlieren photographs (Fig. 5), the outer one of these being narrow in the lenses of seal 3 (Fig. 5C). In total, our results from three different methods strongly suggest that harbour seals have multifocal lenses. Even if the refractive zones of seal lenses are not as well defined as in many fish lenses (Kröger et al., 1999; Karpestam et al., 2007), the optical principle seems to be the same, i.e. compensation for LCA by complexly shaped LSA. The method of focal-area imaging, which we did not apply because up to now no portable setup is available (Malkki and Kröger, 2005), could clarify whether seal lenses are indeed able to concentrate different wavelengths of light on small focal areas by directly analyzing the shape of the cone of light exiting the lens.

Multifocal optical systems are present in various vertebrate species that are active under low light conditions, have thick and almost spherical lenses with the lens centre close to the centre of curvature of the cornea, and with eyes with small *f*-numbers (Kröger et al., 1999; Malkki and Kröger, 2005; Malmström and Kröger, 2006; Karpestam et al., 2007; Gustafsson et al., 2008). All of these characteristics are present in harbour seal eyes as well. A number of diurnal birds also have multifocal lenses despite not meeting the above mentioned criteria; however, these species are sensitive to short and very short (ultraviolet) wavelengths. Since LCA increases almost exponentially at the short-wave end of the spectrum (Hecht, 2002), birds seem to need multifocal optical systems to compensate for the strong LCA in the blue to ultraviolet region of the spectrum (Lind et al., 2008).

Refractive zones of the lens and pupil dynamics

Animals can only profit from multifocal lenses if the different refractive zones are not covered by the iris. This problem is solved if the pupil is insensitive to light, as in many fishes (Kröger et al., 1999), or if there are slit-shaped pupils, which are considered to be adaptations to multifocal lenses (Malmström and Kröger, 2006) because even if the pupil is constricted to a narrow slit under high ambient light intensities all refractive zones of a multifocal lens can be used for imaging. Harbour seal irises exhibit a large range of pupillary area covering all stages from circular to vertical slits and a pinhole at maximum constriction (Levenson and Schusterman, 1997). However, according to our own unpublished measurements in harbour seals, vertical pupil diameter decreases by approximately a factor of two if light intensity increases from 0.1 cd m^{-2} (pupil fully dilated and almost circular) to 80 cd m^{-2} (pupil constricted to a small vertical slit). The two peaks, observed in the laser scans, occur at a beam entrance position of 0.67 R and 0.87 R and both contribute to the image if the pupil is fully dilated (radius 1 R). However, at 80 cd m^{-2}, where the pupil's vertical diameter is reduced by a factor of two, none of the distinct refractive zones focuses light on the retina, and the multifocal lens is dysfunctional under these intermediate light intensities. Harbour seals can thus take advantage of the different refractive zones of the lens only if the pupil is widely dilated, which means if ambient light intensity is low.

Functional significance of multifocal lenses in harbour seals

Multifocal lenses are described as a solution to the problem of chromatic defocus (Kröger et al., 1999). Animals equipped with several spectral types of cone photoreceptor in the retina profit from multifocal lenses as each focal length of the lens is used to create a well-focused image for one of the cone types.

Harbour seals are incapable of cone-based colour vision because the animals are L-cone monochromats as far as morphological (Jamieson and Fisher, 1971), electroretinographic (Crognale et al., 1998; Levenson et al., 2006), genetic (Newman and Robinson, 2005; Levenson et al., 2006) and immunocytochemical (Peichl and Moutairou, 1998; Peichl et al., 2001) analyses indicate. Surprisingly, multifocal optical systems are also present in some other cone monochromats (Malmström and Kröger, 2006). These authors speculated that undiscovered different spectral types of rod might explain the presence of multifocal lenses in cone monochromats.

Harbour seals could obtain some colour information by comparing the outputs of rods and cones under mesopic light conditions (Crognale et al., 1998). Consistent with mesopic colour vision, experiments on colour discrimination tested in psychophysical experiments in four pinniped species so far [Bering sea spotted seal, *Phoca largha* (Wartzok and McCormick, 1978); two species of fur seals, *Arctocephalus pusillus* and *Arctocephalus australis* (Busch and Dücker, 1987); California sea lion, *Zalophus californianus* (Griebel and Schmid, 1992)] have revealed some colour vision in the blue–green range of the spectrum. However, except for the study on colour vision in the Bering sea spotted seal (Wartzok and McCormick, 1978), the seals might have used brightness instead of colour cues if the seals' sensitivity for brightness differences had been underestimated. This hypothesis is supported by the low brightness discrimination thresholds assessed in Bering sea spotted seals (Wartzok and McCormick, 1978) and in harbour seals (Scholtyssek et al., 2007). Mesopic colour vision, shown in owl monkeys (Jacobs et al., 1993) and human blue-cone monochromats (Reitner et al., 1991), is currently under investigation in our lab in harbour seals using the lower border of mesopic colour vision in humans as a reference luminance because mesopic light conditions are not clearly defined for harbour seals. If the pupil is wide under lighting conditions that are mesopic for seals (see Photorefractive measurements in live animals, above), the lens might be optimized for mesopic colour vision in the blue–green range of the spectrum where the rods and cones have their λ_{max}. The rod's λ_{max} lies at 495–501 nm (Lavigne and Ronald, 1975; Fasick and Robinson, 2000; Levenson et al., 2006), whereas the cone's λ_{max} is assumed to be approximately 550 nm (Newman and Robinson, 2005; Levenson et al., 2006). The deviating results from Crognale et al. (Crognale et al., 1998) for the cone's λ_{max}, assessed as 510 nm, might be explained by an overlap of the weak cone signal, which is expected to be weak due to the sparse population of cones (Peichl and Moutairou, 1998), by the rod signal. This overlap would have shifted the spectral sensitivity peak to 510 nm if measurements were unintentionally conducted under mesopic conditions. Thus, assuming the λ_{max} of harbour seal rods and cones to be 496 nm (Lavigne and Ronald, 1975) and 550 nm, respectively, the difference in focal length is 1.4% [calculated as in Kröger and Campbell (Kröger and Campbell, 1996)], which would make multifocal optical systems beneficial. Harbour seal lenses therefore solve the problem of chromatic aberration in dim light. In bright light, where the pupil is almost completely or fully constricted, depth of focus is increased and there is little chromatic blur. The difference in focal length would be 0.6% in the case of the rod and cone λ_{max} at 496 nm and 510 nm (Crognale et al., 1998), respectively. Such a small amount of LCA would probably deny the benefit of multifocal lenses.

One may wonder how colour vision can be beneficial to harbour seals. Colour is helpful in the detection and identification of objects if they cannot be distinguished on the basis of intensity. In

environments with fast variations in light intensity, colour offers a reliable cue because the ratio of the signals from two receptor types is little affected by changes in illumination (Maximov, 2000; Kelber et al., 2003). Harbour seals indeed experience rapid changes in light intensity by wave-induced flicker when close to the surface and large variations in brightness while diving. However, their visual systems are highly sensitive to brightness cues (Jamieson and Fisher, 1972; Scholtyssek et al., 2007), and brightness cues as well as contrast might be sufficient for the tasks the animals have to perform. Furthermore, the appropriate ambient light intensity for relying on colour cues concerning the essential number of photons (Lythgoe and Partridge, 1991) might be rarely met as harbour seals also forage at night or in dark waters at great depth. Nevertheless, there may be an unknown advantage of colour vision because the visual ecology of seals is largely unknown.

In addition to a possible function for colour vision, a multifocal lens can be used to increase depth of focus that is short in seal eyes with small f-numbers when the pupils are dilated. It may be advantageous if objects at various distances are simultaneously well-focused without a need for extensive accommodative abilities (Hanke et al., 2006). However, increased depth of focus comes at the cost of reduced contrast because the in-focus image created by the multifocal lens is overlaid by light from its out-of-focus images. The highest possible acuity and contrast for small objects would be obtained with an eye focused on the point of interest using a monofocal lens. According to the presented data, harbour seals view the world through a status close to monofocal if the pupil is constricted to such an extent that light is focused on the retina by just the central parts of the lens and cornea (see Refractive zones of the lens and pupil dynamics, above).

Conclusions

Harbour seals seem to possess multifocal lenses as are present in many other vertebrates. Interestingly, the seal's slit pupil cannot be considered an adaptation to a multifocal lens. Rather pupil constriction changes the function of the lens from multifocal in dim light to a status close to monofocal in bright light. Multiple focal lengths could be beneficial to seals using mesopic colour vision and by increasing depth of focus in dim light when the pupil is dilated.

LIST OF ABBREVIATIONS

BCD	back centre distance
BEP	beam entrance position
IR	infrared
LCA	longitudinal chromatic aberration
LED	light emitting diode
LSA	longitudinal spherical aberration
PBS	phosphate buffered saline
R	lens radius
TIFF	tagged image file format
λ_{max}	wavelength of maximum absorbance

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