J Comp Physiol (1983) 150:329–332

Journal of Comparative Physiology · A

Changes in Optical Properties of the Eye During Metamorphosis of an Anuran, *Pelobates syriacus*

J.G. Sivak[1] and M.R. Warburg[2]

[1] School of Optometry and Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3Gl
[2] Department of Biology, Technion – Israel Institute of Technology, Haifa 32000, Israel

Accepted November 4, 1982

Summary. Change in optical anatomy of the eye of an anuran, *Pelobates syriacus*, was monitored during metamorphosis using a freeze-sectioning technique. Concurrent refractive state measurements in air and water were carried out by retinoscopy. The eye of *Pelobates* changes dramatically from a fish-like eye with a spherical lens in young tadpoles to a terrestrial apparatus with a markedly flattened lens in the juvenile. The gradual reduction in lens refractive power and the absence of corneal refraction in water means that *Pelobates* becomes extremely hyperopic during the long (20 weeks) metamorphic period when the eye is still in water. The large rostralward change in eye position during metamorphosis indicates that the terrestrial form is much more binocular.

Introduction

One of the most important environmental factors to consider in the adaptive radiation of the vertebrate eye is the effect of air and water on visual optics. Whereas the cornea is an important refractive structure in air, it is of little or no refractive consequence when the eye is in water (Sivak 1978). As a result, the lens of the aquatic eye is spherical in shape and high in refractive index while that of terrestrial species is flattened in shape and softer in consistency. The aquatic cornea is usually flattened for streamlining (Walls 1942).

With the exception of a few relatively recent publications (e.g. Moller 1951; Himstedt 1967; de Jongh 1967; Manteuffel et al. 1977) the study of visual optics of the eye during amphibian metamorphosis has been largely ignored. Recently, we monitored the changes in gross ocular structure associated with metamorphosis of a salamander, *Salamandra salamandra* (Sivak and Warburg 1980). Briefly, the larval eye is typically acquatic with a spherical lens which closely approximates the flattened fish-like cornea. During metamorphosis, the lens assumes a flattened shape while moving away from a more prominent cornea. A large proportion of the change takes place during a brief critical phase just before and after movement onto land. In addition to lens and corneal changes, the angle formed by the two ocular axes diminishes with metamorphosis so that the terrestrial form is more binocular.

The present study monitors the ocular metamorphosis of the eye of an anuran, *Pelobates syriacus*. Interest in this species stems from the fact that *Pelobates* undergoes a much longer metamorphic period (5 months) than *Salamandra* (Degani 1982). In addition, the relatively large size of the eye of the larval and juvenile forms of this species means that it is possible to monitor refractive states in air and water during metamorphosis by convential refractive methods.

Materials and Methods

A total of 17 animals of the species *Pelobates syriacus*, all from Sasa pond in Northern Galilee, Israel, were used in the study. Of this total, four were legless tadpoles and four were completely metamorphosed juveniles. The rest represented various metamorphic stages in between and could roughly be divided into two, three and four legged tadpoles. The latter could be subdivided further on the basis of disappearance of the tail.

Refractive states of the eyes of each animal were determined with a retinoscope and trial lenses before the anatomical portion of the study. Refractive states of the tadpoles were measured with the animals in water, through the flat side of a small aquarium. All refractive values are reported after subtraction for working distance and for the effect of the air-water interface (Sivak 1982). A loose, 28 mm positive aspheric lens was used for estimates of very high refractive errors. Juvenile refractive states were measured in air.

Each animal was subsequently sacrificed by decapitation. The head was rapidly frozen in liquid air or liquid nitrogen and placed on the freezing plate of a sliding bench microtome. The head was kept frozen through the release of pressurized CO_2.

As sections of the head were removed, photographs of the remaining block of tissue were taken with a single lens reflex camera and bellows mounted above the preparation. Sections through the central portions of the eye were about 25 μm apart. The photographs of sections with maximum axial lens thickness were assumed to represent horizontal sections through the geometric axis of the eye. Since the horizontal placement of the head on the microtome was often imperfect, a single section did not usually include central sections of both eyes. Care was taken to ensure that central sections of both eyes were sectioned. Photographs of a millimeter rule provided a control for magnification.

Intraocular dimensions were measured by projection of the negatives representing axial sections. Dimensions measured include corneal curvature, corneal-lens distance, axial and equatorial lens thickness and the distance between the lens and the retina. Curvatures (r) were calculated from the formula $r = (y^2/2S) + (s/2)$, where y = one half of any chord to a curved surface which is assumed to be spherical and S = the sagittal depth of that chord.

In addition to changes in ocular structures, the freeze sectioning procedure makes it possible to also monitor changes in bilateral eye position during metamorphosis. The angular separation of the two eyes was determined as follows: A straight line joining the nasal and temporal roots of the iris was drawn through the lens on the projection of the appropriate negative. A perpendicular was then drawn from the center of that lens chord toward the retina and cornea. This line was assumed to represent the axis of the eye. The interocular angle was measured from extensions of these axes. The eyes were assumed to occupy positions of rest (the positions taken up in the absence of extraocular muscle innervation) after decapitation.

Results and Discussion

The eye of *Pelobates* undergoes marked structural change during metamorphosis (Table 1). Furthermore, the change observed is directly related to the change in visual optics associated with movement from water to air (Fig. 1). Thus, at the tadpole stage (legless) the eye resembles the fish eye in that the crystalline lens is spherical in shape, it protrudes through the pupil, and the cornea is relatively flat. In the juvenile animal, the lens is substantially flattened, anteriorly-posteriorly, as expected in the terrestrial situation where the cornea becomes an additional refractive element. In fact the flattening of the lens of *Pelobates* is even more dramatic than the change noted for *Salamandra* (Sivak and Warburg 1980). The equatorial thickness of the lens is 13.6% larger than its axial thickness in juvenile *Salamandra* and 27.5% larger in juvenile *Pelobates*. In *Salamandra* the lens continues to flatten into the adult stage. However, adult *Pelobates* specimens were unavailable for study.

While *Pelobates* lens changes are greater than those of *Salamandra*, change in corneal shape is not as obvious. In the latter, the cornea is markedly flat at the tadpole level and becomes more prominent in the juvenile.

The cornea of *Pelobates* is not as flat to begin with. In legless tadpoles corneal radius of curvature is about 1.8 mm while the radius of curvature of the whole eye (one half axial eye diameter) is about 1.5 mm. In juveniles, the radius of curvature of the cornea is reduced relative to that of the whole eye (approx. 1.7 mm vs 2.1 mm), but not very much. As a consequence, the lens of *Pelobates* does not appear to recede toward the retina as much as in *Salamandra* and the relative change in depth of the anterior chamber of the eye (cornea to lens) is less.

The most striking difference between *Salamandra* and *Pelobates* concerns the rate of change in visual optics.

In *Salamandra* metamorphosis takes place in about 6–8 weeks. Ocular change appears to be saltatory, with a major proportion of the optical rearrangement of the eye taking place during the brief period just before movement on to land. *Pelobates*' metamorphosis is much slower (about 20 weeks). Ocular change takes place at a slower and regular pace. This means that the animal spends a long time in an aquatic environment while the optical

Table 1. Sample intraocular dimensions (to ±0.05 mm) of the eye of *Pelobates syriacus* at various stages during metamorphosis. Eye diameter measured to retina-choroid border

Stages of metamorphosis	Eye diameter	Cornea to ant. lens	Corneal radius	Lens thickness	Lens diameter	% diff. diameter vs thickness	Post. lens to retina
1. Tadpole – legless	2.80	0.35	1.80	1.15	1.15	0	1.30
2. Tadpole – 2 legs	3.30	0.45	1.90	1.30	1.40	7.7	1.55
3. Tadpole – 3 legs	3.45	0.55	2.0	1.45	1.50	11.1	1.45
4. Tadpole – 4 legs – with tail	3.50	0.60	2.0	1.30	1.50	17.0	1.60
5. Tadpole – 4 legs – 2/3 tail resorbed	3.75	0.65	1.75	1.45	1.75	20.7	1.60
6. Juvenile – 1 month after metamorphosis	4.20	0.65	1.70	1.80	2.30	27.8	1.75

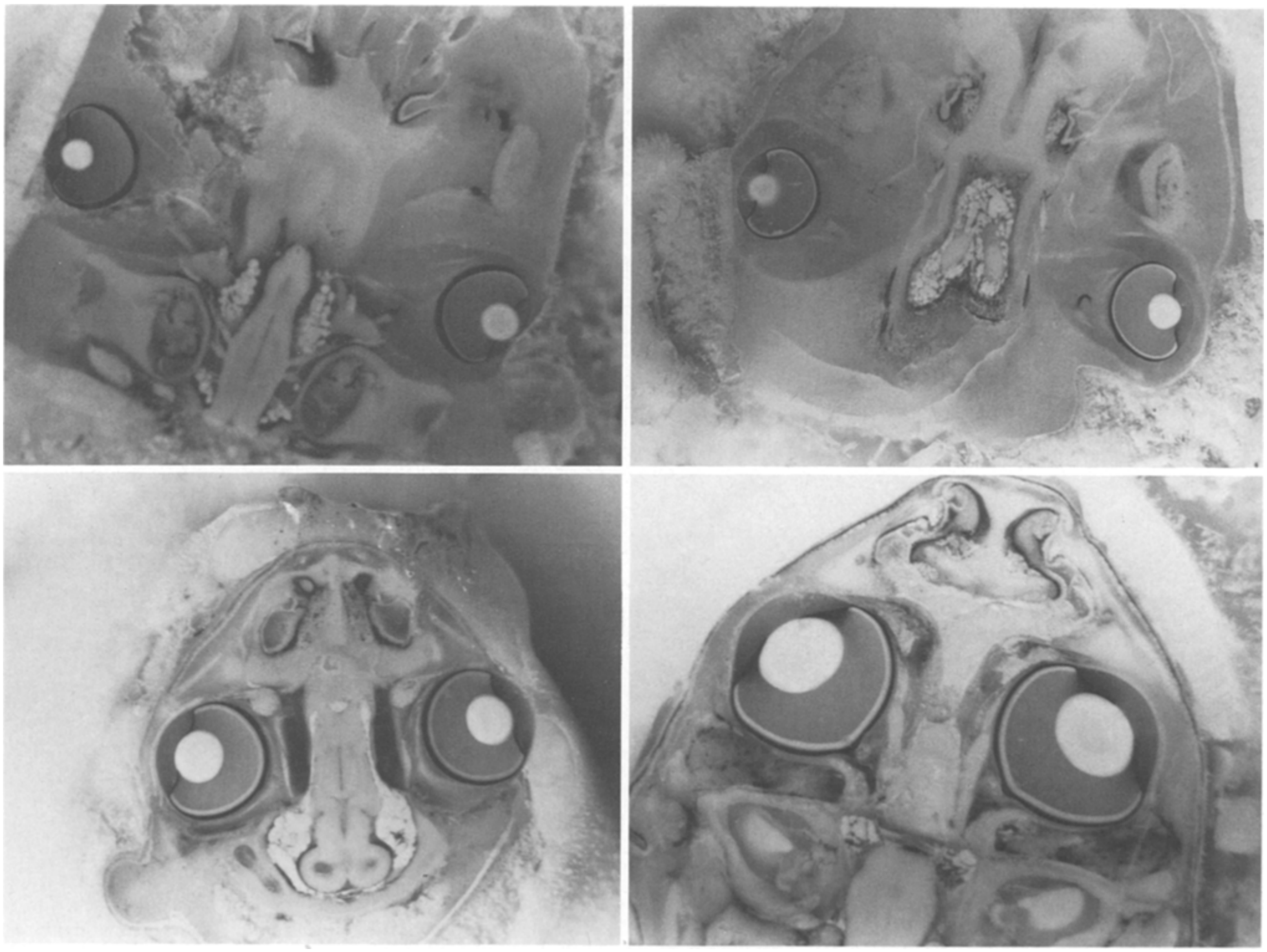

Fig. 1. Frozen sections of head of *Pelobates* at various stages of metamorphosis. Legless tadpole (×4,6), *upper left*. Two-legged tadpole (×3,4), *upper right*. Four legged tadpole (×4,3), *lower left*. Juvenile (×5,1), *lower right*

structure of the eye is not appropriate for vision in water (Table 2). Moreover, this situation gets progressively worse as the eye becomes more and more terrestrial during the lengthy aquatic metamorphic period. During this period the refractive contribution of the lens is gradually lessened by its assumption of a flattened shape. Change in corneal curvature is of little or no consequence while the eye is in water. This gradual loss of refractive power is verified by the refractive measurements which show a gradual increase in refractive error in the hyperopic direction in water (Table 2). That is, the eye is undercorrected and cannot focus entering rays of light on the retina.

The fact that some hyperopia already exists at the early tadpole stage should not detract from its progressive increase with metamorphosis. It has been pointed out that retinoscopy refractive measurements are based on reflection from the vitreous surface of the retina rather than from the epithelial or receptor surface and that this artifact produces a bias toward hyperopia in small eyes (Glickstein and Millodot 1970; Hughes 1977). This view is supported by the finding of less hyperopia in the case of juveniles (measured in air) where the eye is larger.

The significance of the gradual rate of ocular metamorphosis in *Pelobates* is that vision must deteriorate and play a relatively minor role in the animal's behavioral repertoire, particularly during the latter portion of the metamorphic period. The refractive nature of this dilemma is best exemplified by the example of a four-legged tadpole at an advanced stage of metamorphosis (Tables 1 and 2, Fig. 2) as indicated by the resorption of two-thirds of the tail. Here the eye has assumed the optical proportions of a fully-terrestrial juvenile (Table 1, Fig. 2). In water, the eye is immeasurably hyperopic while in air it is very slightly so. The existence of a corneal radius of curvature of

Table 2. Refractive states (diopters) measured retinoscopically in air and water and angles between interocular axes for the same *Pelobates* individuals listed in Table 1. + indicates hyperopia

Stage of metamorphosis	Refractive state		Inter-ocular angle (°)
	Air	Water	
1. Tadpoles – legless		+ 9.00	170
2. Tadpole – 2 legs		+16.00	165
3. Tadpole – 3 legs		+26.00	150
4. Tadpole – 4 legs – with tail		+40.00 approx.	140
5. Tadpole – 4 legs -2/3 tail resorbed	+3.00	⩾ +50.00	
6. Juvenile – 1 month after metamorphosis	+3.00		95

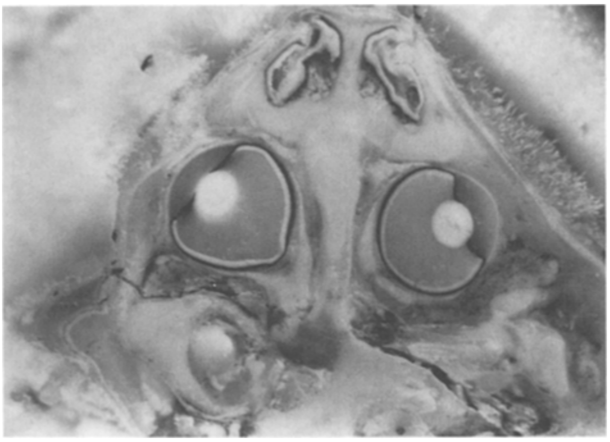

Fig. 2. Frozen section of head of *Pelobates* tadpole with four legs and with most of tail resorbed (×4,3). Note similarity of lens shape to that of juvenile of Fig. 1

1.8 mm in this individual means that the refractive power of the eye is altered by roughly 200 diopters, depending on whether it is in air or water. Despite the obvious visual advantage of an aerial life, movement to land cannot take place until resorption of the tail is completed. At this stage the tadpole does not feed.

As in *Salamandra*, the change in angle between the axes of the two eyes of *Pelobates* shows a rostralward movement with metamorphosis. In fact, the difference is even more dramatic in *Pelobates* since the angle between the ocular axes is more obtuse (170° vs 123°) in the larval form of *Pelobates* than in larval *Salamandra*. By the juvenile stage the angle decreases to 95° in *Pelobates* and 103° in *Salamandra*. This suggests that binocular vision is much more important when *Pelobates* is on land than when it was in water. The functional significance of this change could be related to a shift from herbivorous to carnivorous feeding, but a detailed behavioral examination of the visual performance of *Pelobates* is necessary.

The results of this study emphasize the importance of the relationships between ocular morphology and environment in amphibians. We believe that this study provides an example of a relatively simple method of monitoring this relationship and that it should be applied to a number of amphibians on a widespread basis.

Acknowledgements. This research was supported in part by the Natural Sciences and Engineering Research Council of Canada and the Technion – Israel Institute of Technology. The first author occupied the I. Taylor Chair during the study. The authors are grateful for the assistance of Dr. G. Degani.

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