Fish (*Oreochromis niloticus*) as a Model of Refractive Error Development

by

Wei Shen

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Doctor of Philosophy

in

Vision Science and Biology

Waterloo, Ontario, Canada, 2008

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be electronically available to the public.

Abstract

Myopia is a common ocular condition worldwide and the mechanism of myopia is still not clear. A number of animal models of myopia and refractive error development have been proposed. The fact that form deprivation myopia could be induced in tilapia fish, as shown previously in my research, suggests the possibility that tilapia could be a new animal model for myopia research. In the first part of this thesis the tilapia model was perfected and then, based on this model, the effect of systemic hormones (thyroid hormones) associated with eye and body development was investigated during refractive error development. Lastly, the physiological and morphological changes on the retina were further studied with optical coherence tomography (OCT).

In these experiments, significant amounts of myopia, and hyperopia were induced within two weeks using goggles with lens inserts as in other higher vertebrate animal models, e.g. chicks. The results from form deprivation treatment also show that the sensitivity of tilapia eyes may be an age related effect during the emmetropization process. The larger the fish, the less hyperopic the fish eye, though the small eye artefact may be a factor. The susceptibility of the refractive development of the eye to the visual environment may be also linked to plasma hormone levels. It was found that induced refractive errors could be shifted in the hyperopic direction with high levels of thyroid hormones. Also, after 2 weeks of treatment with negative or positive lens/goggles, the tilapia retina becomes thinner or thicker, respectively. When the goggles are removed, the thickness of the retina changes within hours and gradually returns to normal.

However, the circadian retinomotor movement is a complicating factor since it affects the retinal thickness measurement with OCT at some time points.

In conclusion, tilapia represent a good lower vertebrate model for myopia research, suggesting a universal mechanism of myopia development, which may involve systemic hormones and immediate, short term retinal responses.

Acknowledgements

I would like to thank my supervisor, Dr. Jacob Sivak, who has supported and guided me in scientific work and with respect to my career. His support has been crucial to myself and my family in regard to our adjustment to life in Canada.

Special thanks to Dr. Mathilakath M. Vijayan for his valuable guidance and all the students in his lab in the biology department for their generous help. I also would like to thank my committee member, Dr Irving for her guidance, help and inspiration.

I would like to acknowledge all the graduate students from all over the world who I interacted with in the school of optometry over the past six years for providing a very warm, friendly, open-minded and supportive research environment

This research was funded by NSERC.

Table of Contents

List of Figures

List of Tables

General Introduction

Refractive errors, especially myopia, are common eye conditions worldwide. The development of refractive errors is thought to be the result of interaction between visual environment and heredity[1, 2].

Refractive errors, myopia or hyperopia, may be the both sides of one coin. Many epidemiological researchers have pointed out that myopia is most common in Asian countries[3]. The highest prevalence rate exists in East Asia, especially in areas of Chinese culture, where teenagers in schools have to study hard and read for a long time to obtain good marks and where there is a lack of outdoor activities. Several studies have provided evidence of relationship between myopia development and relative amount of outdoor/indoor activity[4-6]. In fact, it has been found that myopes are more successful in school work[7, 8] and that the myopia rate is higher in well educated people, such as students in law schools who spend much time on reading[9]. It is uncertain whether poor indoor illumination levels or different signal inputs from near objects play a role in myopia development. Nevertheless, accommodation associated with near work and longitudinal chromatic aberration may not be the signals involved[10, 11]. Monochromatic light is found no effect on the compensation for spectacle lenses[10, 12, 13].

The hyperopic and myopic blurred image on the retina could be discerned by the eye, but blur itself seems not to induce myopia. In fact, when put a Jackson Crossed Cylinders with net spherical power zero in front of a chick eye, it will develop a small amount of hyperopia and it still compensates the minus or plus lenses in combination with the Jackson Crossed Cylinders[14]. In a recent study, it points out that the chick eye

may be able to determine the sign of highly defocused images on the retina by the asymmetric astigmatism or aberration[15].

Investigations in epidemiology provide clues on how refractive errors develop. However, more information as to the actual mechanism is provided by experimental animal research models.

As far back as 1965, the effect of atropine was studied by Young on monkeys[16]. In 1971, Wiesel found that form deprivation induces elongated eyeballs in cats[17], and since then many animal models have been developed, including monkey[16, 18], tree shrew[19], chick[20], American kestrel[21], marmoset[22], guinea pig[23, 24], mouse[25] and recently in this thesis, tilapia[26], a lower vertebrate. These animal models cover species from lower vertebrates to birds and mammals. Also the form deprivation effect is found in the human eye with congenital eye diseases[27, 28]. As a result, it is assumed that the mechanism controlling refractive error development is universal and very basic. A feedback loop has been found in almost all animal experiments, whereby myopia or hyperopia can be induced dependent on the type of the lenses (positive lens induces hyperopia and negative lens induces myopia) placed in front of the eyes, though the positive lens has much stronger effect, over 5 times, than the negative lens in the chick experiment[29, 30]. The eye can recover from the induced myopia/hyperopia after the lenses are taken away, if the animal is young enough. This mechanism may be a local retinal mechanism as supported by the evidence from the chick model with optic nerve sectioning[11].

The eye is able to distinguish whether the input visual signal is over or under focused and adjust the ocular growth accordingly. The eyeball is reshaped, changing the

axial length of the vitreous chamber to match the new focus length of the visual signals. Molecules, especially those with bidirectional property, such as Zenk[31], retinoic acid[32], dopamine[33, 34], GABA[35] are possibly involved in the signal process and the growth factor TGFβ plays a key role in reshaping the eyeball[36]. Antimuscarinic drugs may be helpful for the prevention of myopia.[16, 37, 38]

However, this feedback mechanism does not work well throughout life time, and only the eyes of young animals show enough plasticity in respond to altered visual signals and adjust to the new focal length, though this may not be strictly/linearly linked to age[39, 40]. In fact, human studies suggest that the susceptible period is after 5 years. Most children begin to develop myopia during 9 to 12 years period, and through puberty, with an increasing prevalence rate[41, 42]. Nevertheless, this age related effect brings up another question: is susceptibility of myopia development under control of the growth hormone level? Actually, in a couple of investigations, the refractive development of children with growth defects indicates shifts to hyperopic direction and normal emmetropization could be obtained by appropriate replacement treatment with the growth hormone[43, 44].

Thyroid hormones are secreted by the thyroid gland and are involved in controlling the metabolism of the body and influencing physical development, especially during early eye development. In fish, it is also associated with the metamorphosis process[45]. Ultraviolet photosensitivity and related cone development may be under its control as well[46, 47]. The fish model, tilapia, used in the research on refractive error development described in this thesis has been used for studying the interaction between

thyroid hormone[48, 49] and growth hormone and maybe an appropriate model for hormone related effect.

Refractive error development is accompanied by a series of physical and/or pathological changes in the eye. When the image on the retina is blurred, changes are found both in the choroid and in retina. Choroidal thickness may change in minutes as compensation for the focal length difference. Possibly, nitric oxide innervating both vascular and nonvascular smooth muscle of the choroid is related to the thickness control[50, 51]. Choroids become thinner or thicker, dependent on how the visual signal is defocused[52-54]. Also, edema is found through all the layers in the retina during the early period of recovery from form deprivation myopia in chickens, which is assumed to be related to choroidal expansion [55]. Gradually, the sclera undergoes more permanent morphological change. In myopia, the layer of scleral fibres becomes thinner[56]. As a result of the remodelling of the posterior sclera, the axial length of the vitreous chamber is changed. The defocused visual signal is eventually corrected and a clear image is formed on the retina. Usually myopia is associated with axially elongated eyeballs, deeper anterior chambers and thinner retinae[57].

In addition to the change in choroidal thickness responding to altered visual signals, retinal thickness change may also play a role in the immediate response of the eye. Only recently, RPE has been suspected be involved in growth regulation of the eye and in the signal transportation[58]. RPE cells affect the growth of scleral chondrocytes[59]. At the mean time, they are under modulation of dopamine/apomorphine[60, 61], glucagon[62] and VIP[63]. In most teleost fish retina, the retinal pigment epithelium is relatively thick and occupies a large part of the retina. Not only is it related to photoreceptor metabolism

as it is in other animal eyes, but it also controls light perception by photoreceptors through retinomotor movement. This is a circadian photomechanical movement of photoreceptors and pigment granules in the RPE[64]. In addition, the choroid of the fish eye is distinctive from other animals'. Almost the whole posterior half of the tilapia globe is filled with choroid and tissue together with choroidal glands rich in blood vessels. These unique features of the fish eye may provide more information as to interactions between different retinal layers and the choroid. The immediate, small changes of these layers might be revealed by OCT, an apparatus that has been recently developed and widely used in retinal disease diagnosis with live, optical dissection images of high definition (10-15μm).

In this thesis, I have shown that form deprivation myopia could be induced in the eye of a lower vertebrate (tilapia). Positive and negative lenses are employed to investigate how the fish eye responds to different defocused (hyperopic/myopic) visual signals. Other projects are based on this newly developed animal model. These include study of a possible role by growth hormones, such as thyroxine, and an effort to non-invasively evaluate possible changes in retinal thickness.

*Note: The references for general introduction and conclusion are on page 124.

Chapter 1

Eyes of a lower vertebrate are susceptible to the visual environment

1.1 Introduction

Myopia is a common ocular refractive condition found worldwide that is of increasing concern due to its high prevalence in youth. In Singapore, for example, the incidence of myopia in high school students is over 70%.[1] Intensive research over the past few decades has revealed that there is a relationship between the visual environment and myopia development as well as with heredity.[2-5] However, the precise cause of myopia is still not clear.

Experiments dealing with refractive error development are frequently based on animal models such as chick,[2] monkey,[6, 7]and tree shrew.[8] In fact, various species of animals have been used to induce form deprivation myopia including: grey squirrel,[9] mouse,[10] guinea pig,[11, 12] cat,[13-15] and the American kestrel.[16] They are without exceptions, higher vertebrates. Typically, experimental myopia is induced in young animals and often the younger the animal the higher the induced refractive error .[17, 18] In addition to form deprivation myopia, positive and negative lenses have been used to induce myopia and hyperopia,[19, 20] and efforts to induce astigmatism with cylindrical lenses have been carried out as well.[21-23] Experiments such as those in which the chick optic nerve is cut show that refractive error development is determined by a local retinal mechanism.[24] Somehow the eye distinguishes whether the input visual signal is over or under focused,[19] and, in chicks at least, rapidly adjusts retinal position and focal length of the eye, by thinning or thickening its choroid,[25-27] Eye growth then accelerates or slows down for a more permanent change in refractive state resulting from an eye that is either too long or too short. The growth factor TGFβ may play a key role in regulating scleral synthesis and in reshaping the globe in this process.[28, 29]

Almost all experimental animals are able to recover from the induced refractive errors and the contralateral differences in eye length after removal of the cause of the visual interference during the early stage of ocular development. However, it appears that monkeys are not always capable of recovering fully from form deprivation myopia.[30]

Recently, it was found that form deprivation myopia can also be induced in fish, the largest group of vertebrates, and that complete recovery is possible.[31] This common susceptibility to the visual environment in the vertebrate world suggests that there exists a basic or universal mechanism controlling refractive error development in nature, regardless of differences in habitats, genetics and ocular anatomy and physiology. Also, since fish can continue to grow and develop through life, the eye remains in a lifelong plastic state, both in terms of dimensions and retinal development.[32] This suggests that the fish eye may represent an interesting new animal model to use to study the effect of the visual environment on the refractive state of the eye. This study investigated the susceptibility of the fish eye to the effect of positive and negative lenses as well as to the effect of fish weight on form deprivation myopia.

1.2 Materials and Methods:

1.2.1 Fish

Tilapia (*Oreochromis niloticus*), a commonly cultured species of cichlids, were obtained from a local fish farm, Northern Tilapia Inc., (Box 37, Bondhead, Ont. L0G 1B0). Fish were kept in aquariums with cycled and filtered water at 28 degrees centigrade and fed with Tilapia Fish Food (3PT Regular, Martin Mills INC. Elmira, ON Canada). The size of fish used was chosen for handling ease, with most of them between 15 and 30 grams. However, in some experiments, fish of weights ranging from 9.4 grams to 154.2 grams were selected on the basis of availability and to study the effect of weight on form deprivation myopia. Fluorescent lighting in the aquarium room was set to a 12 hour light/12 hour dark photoperiod schedule. All fish were cared for according to the Guidelines of the Canadian Council on Animal Care and in accordance with the policies of the University of Waterloo and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Table 1 summarizes treatments and numbers of the 10 groups of fish that were studied in this research.

Table 1. Summary of the numbers, weights and treatments received by the groups of tilapia used in this research.

Group	No. of Fish	Treatment	Average Weight (g)
1	25	Untreated, freeze sectioned	26 to 101
2	6	Open goggle	33.3
3	7	+15D, sacrificed+freeze sectioned	30.4
4	7	-12D, sacrificed+freeze sectioned	15.8
5	8	-12D lens-goggle+recovery	25.5
6	5	+15D lens-goggle+recovery	13.9
7	8	+15D lens-goggle+recovery	26.9
8	7	form deprivation goggle+ recovery	16
9	8	form deprivation goggle+ recovery	57.4
10	8	form deprivation goggle+ recovery	98.4

Twenty five untreated fish (group 1) ranging in weight from 26 to 101 grams and in eye axial diameter from 4.96 to 7.34 mm were weighed, sacrificed, and their eyes measured to provide normative data as to the relationship between fish eye size and body weight as well as between refractive state and fish weight. An additional 64 fish were used in the convex and concave lens and form deprivation studies which followed. All treated fish were weighed at the start of treatment and two weeks later at the end of treatment. Pre-treatment measurements of weight and refractive state of these fish were added to those of the untreated fish of group one to generate the weight-refractive state relationship described in Figure 3. Fish were weighed by being placed in a beaker, with water, of known weight and the additional weight measured, to the nearest 0.1 g, represented that of the fish. The weights refer to weight at the start of treatment. Fish were sacrificed with an overdose of 2-phenoxythanol anesthetic (3ml/l), and then decapitated and the eyes enucleated.

1.2.2 Experimental Protocols

1.2.2.1 Lens induced Myopia and Hyperopia

Specially designed goggles with central convex (positive) and concave (negative) lens inserts were used to induce myopia or hyperopia (Fig. 1). The inserted lenses consisted of custom produced intraocular lenses 6.0 mm in diameter with powers, in water, of minus 12 dioptres (D) and plus sphere15 D. The lenses were plastic (polymethyl methacrylate) of 1.49 refractive index, with equal anterior and posterior radii of curvature consisting of 26.175 mm for the negative lenses and 20.801 mm for the positive ones. The lightweight plastic goggles were directly sutured over one eye, the

treated eye, for two weeks (Fig 1). The right eye was always the treated eye while the left eye served as a contralateral control. Sutures were sewn through the skin and soft bones around the orbit of anesthetized fish using nylon stitches through holes in the edge of the plastic goggle. The fish were removed from water for about one minute during the suturing process.

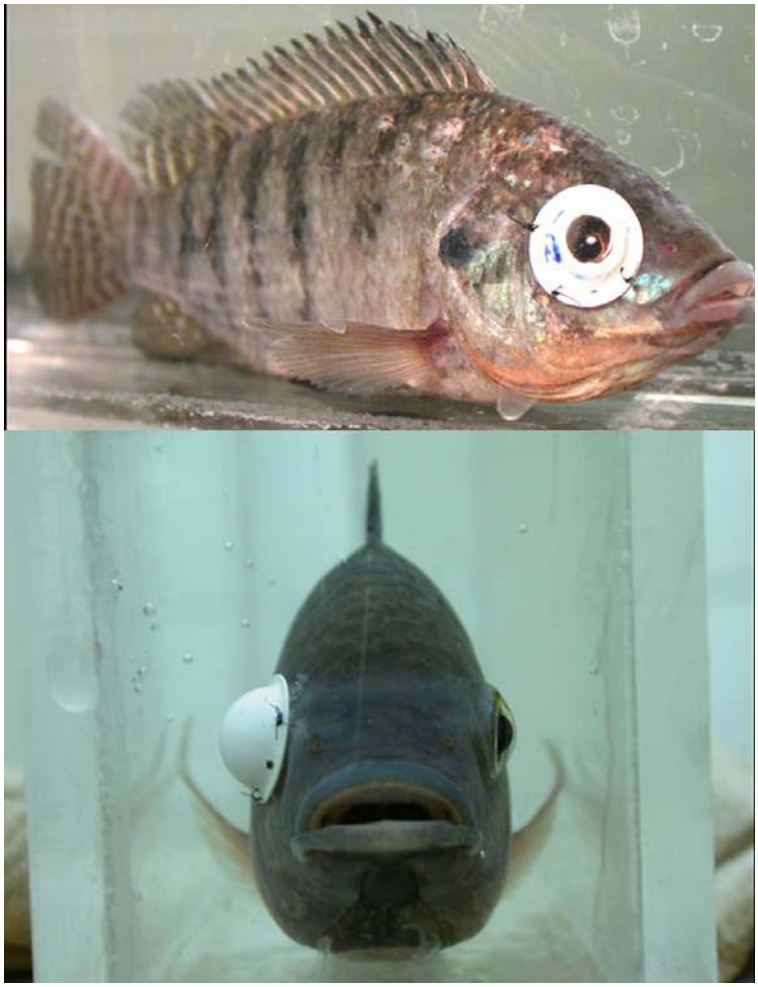

Figure 1. A tilapia wearing a plastic translucent goggle

Photograph of tilapia showing goggle/lens combination (top) and form deprivation goggle (bottom). Fish overall lengths were about 10 cm.

To control for the possible mechanical effect of the goggle, a ring shaped plastic goggle with the central area open was sutured onto the right eye of a group of six fish (group 2, average weight 33.3 g) to evaluate the effect of the suturing and the goggle itself. Two additional groups, each consisting of seven fish, wore goggles with either positive (group 3) or negative (group 4) lenses for two weeks. The average weight of the fish treated with positive lenses and negative lenses was 30.4 and 15.8 grams, respectively. Refractive measurements for these 20 fish were made before and after treatment, after which the fish were sacrificed, the eyes removed and intraocular dimensions measured from frozen sections.

The eyes of both treated and untreated fish were enucleated, immersed in a freeze section medium (Stephens Scientific, Little Rock, Arkansas) and then frozen by being placed on dry ice. Axial lengths were measured by freeze sectioning. Marks made on the eyes with indelible ink when the fish were first sacrificed identified the nasal and temporal limbi, the apex of the cornea and the posterior scleral exit point of the optic nerve. The eyes were freeze - sectioned on a cryostat microtome until a horizontal section through the geometric axis of the eye (pre-marked with indelible ink) was apparent. A loupe with a scale (± 0.10mm) was placed on the section and a photograph was taken with a digital camera. Later, the digital image of the hemi-sectioned eye was transferred to a computer and processed using software Image J (National Institutes of Health, USA). Axial length was measured by placing a cursor on the anterior corneal apex and at the retina-choroid border. An effort to estimate the repeatability of axial dimensions was made by repeating the measurement for several images several times

each. The results indicate that the mean difference in repeated measurements of axial diameter amounts to 0.01 mm with a standard deviation of 0.02mm.

An additional 21 fish were treated with goggles for two weeks after which the goggle was removed and the refractive states of both eyes were measured every day for 6 days (day 19) and then after 28 days. These fish were divided into one group (group 5), wearing negative (-12D) lenses (n=8, average weight 25.5g) and two groups (6 and 7) of differing weights (average weight 13.9g, n=5 and 26.9g, n=8) wearing +15D lenses during the treatment period.

While the untreated contralateral eye in this study and in others involving animal models of refractive error development is considered to be a control eye, it has been pointed out that the contralateral eye can be affected by the treatment as well. For example, Wildsoet and Wallman reported that the refractive states of the untreated eyes of chicks in which one eye is treated with either positive or negative lenses show small shifts in either the hyperopic or myopic directions.[33] In the current study, we examined this point by pooling refractive states before and after treatment for the groups treated with positive lenses (groups 3, 6 and 7) and then those with negative lenses (groups 4 and 5).

1.2.2.2 Form deprivation

Lightweight translucent plastic goggles were directly sutured over the treated eye (the right eye), for two weeks (Fig 1). The fish were divided into three groups: group 8, consisting of fish (n=7) weighing from 12 grams to 20.5 grams and averaging 16.0g (about 4 months old), group 9, consisting of fish (n=8) weighing from 51.7g to 61.6grams

and averaging 57.4g (about 7 month old), and group 10, consisting of fish (n=8) ranging in weight from 60.2 grams to 154.2 grams and averaging 98.4g (about 10 months old). These ages are estimates based on information provided by fish farm personnel when the fish were obtained added to the time the fish were maintained in holding aquaria during experimentation. All fish were treated with goggles for two weeks after which the goggle was removed and the refractive states of both eyes were measured each day for 6 days (to day 19) and then after 28 days.

1.2.2.3 Ocular Measurements

Refractive states were measured with a streak retinoscope and trial lenses at a working distance of 25 cm through the glass wall of a specially designed narrow aquarium while the fish were anesthetized with 0.6ml 2-phenoxythanol/L to fix the direction of gaze and to minimize accommodation. The results are an over-estimation of the refractive error due to the difference in refractive index of water and glass. The true refractive error is obtained dividing by 1.33 and all values reported here were corrected in this manner.[33] The retinoscopic values are estimated to be accurate to within ±0.50 D. Means and standard deviations of the results are given to one decimal. The retinoscopic measurements were made along the direction perpendicular to the plane of the pupil and the results are expressed as the difference between the refractive state, in diopters, of the treated and untreated eyes. While the values are given as diopters of myopia or hyperopia, they represent shifts in refractive state in the myopic or hyperopic directions.

The retinoscopic measurements were carried by an experienced refractionist (W. Shen). These were verified periodically and independently on a limited number of fish

by a second experienced individual using an alternative instrument, a photorefractor, (PowerRefractor, Multi channel Systems Co. Reutlingen, Germany) with which the retinoscopic reflection was neutralized with trial lenses, as in retinoscopy. Since the vast majority of the findings were made by retinoscopy, the results presented are retinoscopic measurements.

1.3. Results

1.3.1 Normal fish eye development (group 1)

It was found that the axial length of the tilapia eye is proportional to weight, showing a strong correlation ($R = 0.91$, $n = 25$, $P < 0.001$) within the weight range (26.5g to 101g) measured. Axial length ranged from 5.86mm to 7.16mm (Fig. 2). At the same time, the refractive state of the eye gradually shifts from hyperopia of about +15D for fish about 10g in weight toward emmetropia and levelling off at about four dioptres of hyperopia (Fig. 3).

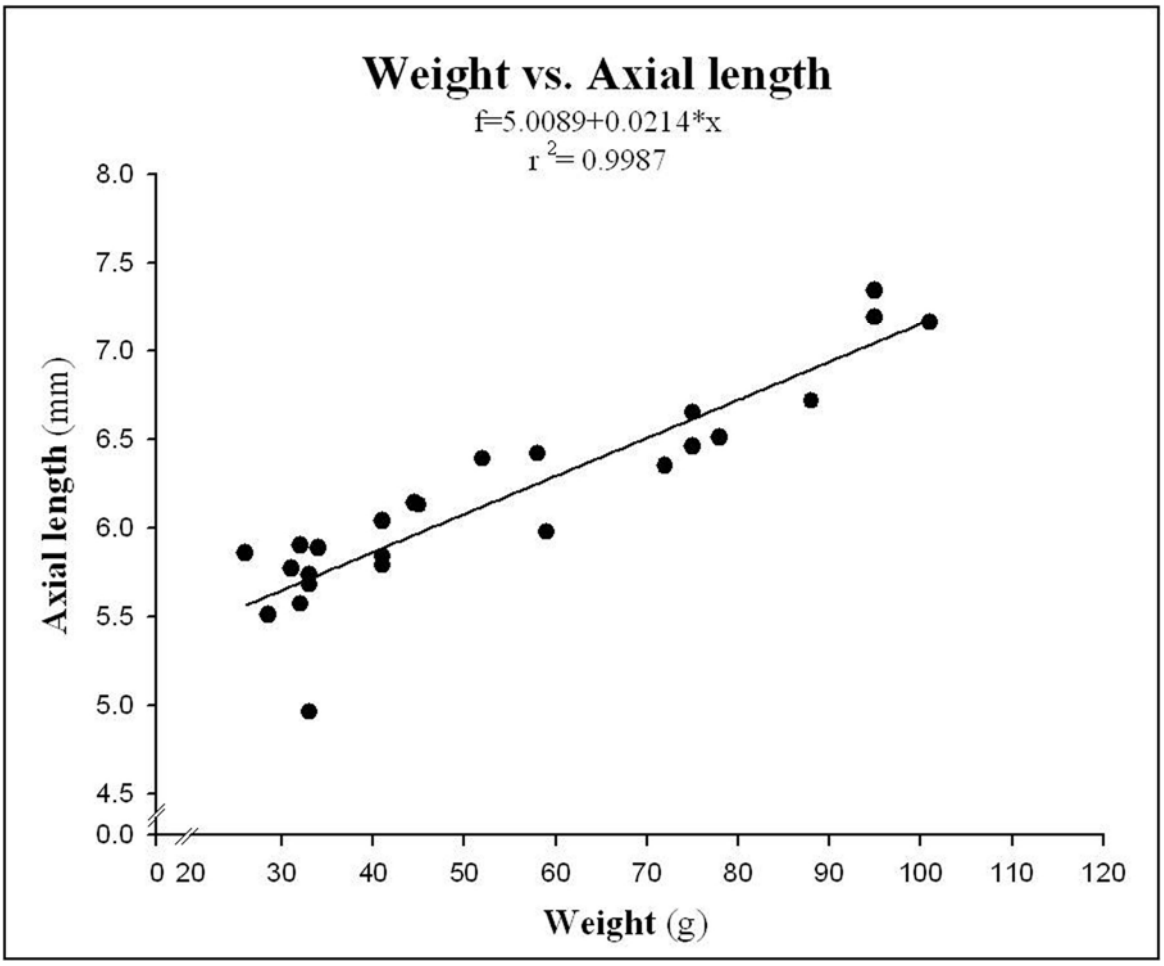

Figure 2. The correlation between the fish eye size (mm) and body weight (g). Fish eye size is measured from the cornea to the retina (mm). There is a significant linear correlation between fish eye size and body weight during the early growth stage.

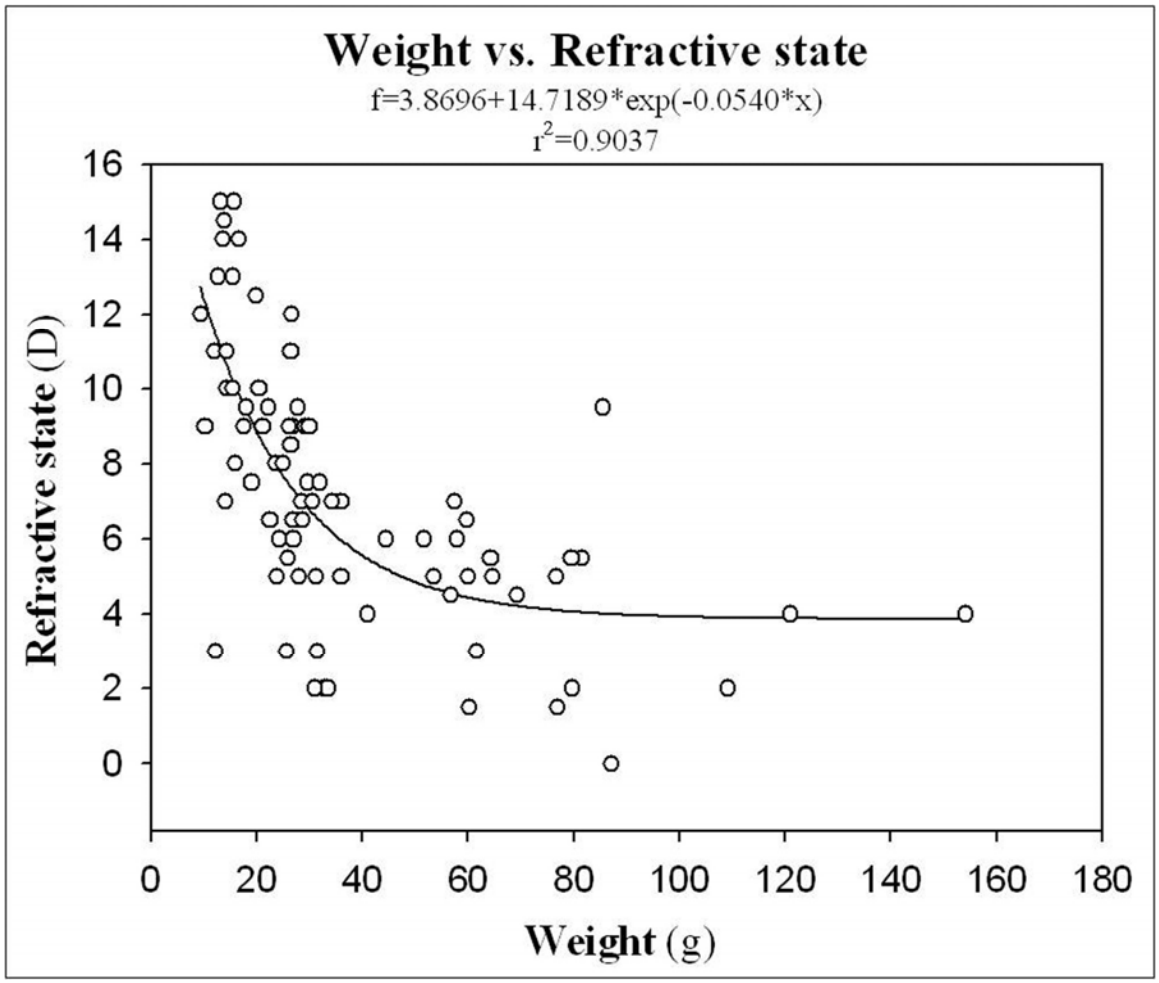

Figure 3. The relationship between refractive state and weight of the fish indicates that the tilapia eye becomes less hyperopic with growth. The data were collected from measurements made on the group of 25 untreated fish (group 1) and from pre-treatment measurements made for fish of the remaining groups and fitted to the inverse exponential function providing the best correlation.

1.3.2 Lens Induced Myopia and Hyperopia

In the fish with goggles having no central lens inserts (group 2), no significant change in refractive state (refractive state change, plus or minus the standard deviation, = 0.2 ± 0.2 D before treatment and 0.6 ± 0.2 D after) was noted (Fig 4). Similarly, the axial dimensions of the treated and control eyes were not significantly different (average axial diameters of 4.33mm, and 4.34mm, respectively) The + 15 D lens - treated fish eye (group 3) became significantly hyperopic relative to the control eye (+7.7 ± 1.6 D, n = 7, paired t-test, $P<0.0001$) while the -12 D lenses (group4) induced significant myopia (-8.4 ± 0.8 D, n = 7, $P < 0.0001$), within two weeks (Fig. 4). The hyperopic eyes were shorter than the contralateral eye (treated eye = 4.16±0.11mm vs. untreated eye = 4.28±0.06mm, paired t test, $P<0.05$), and the myopic eyes longer (treated eye = 3.96±0.36mm vs. untreated eye = 3.84±0.27mm, $P<0.01$). There were no significant differences in nasal-temporal dimensions between the eyes of the two groups In group 2 these dimensions were, 7.41±0.11mm vs.7.41±0.0.08mm ($P = 0.95$); while in group 3 they were 6.39±0.49mm. vs. 6.41±0.51mm ($P = 0.73$).

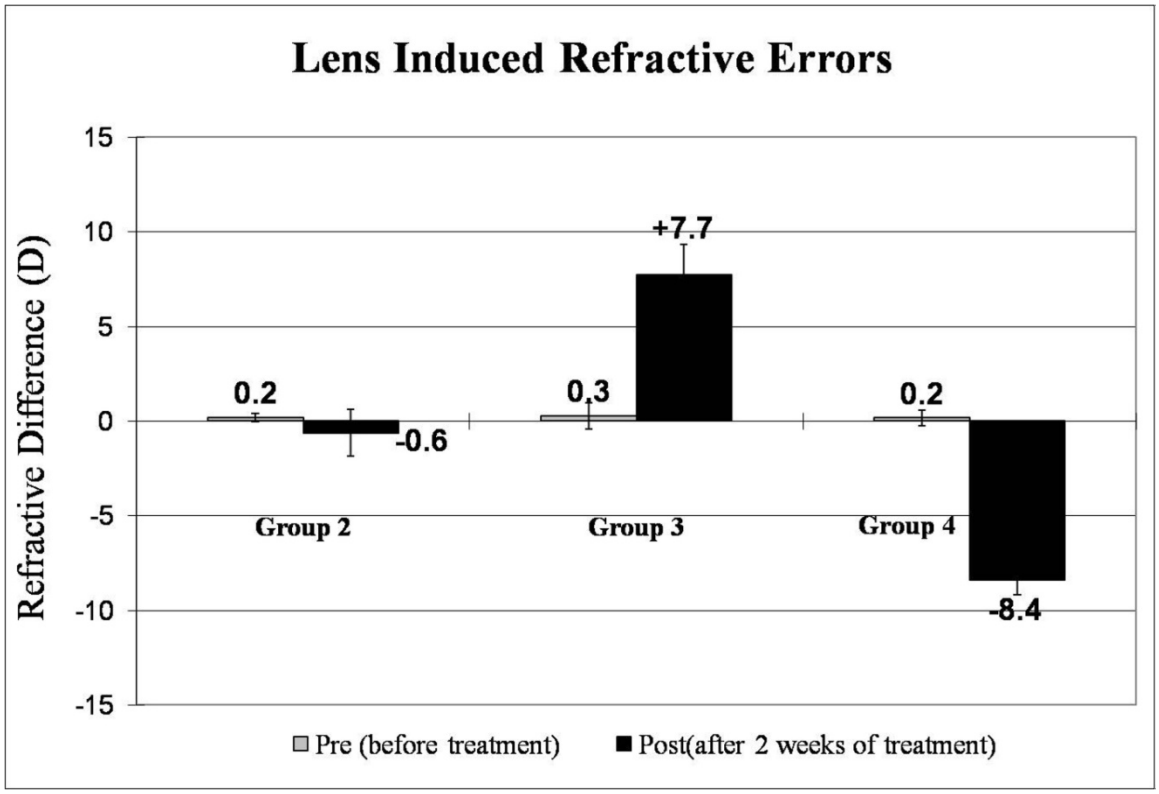

Figure 4. Fish eyes were treated with lenses of positive or negative power over one eye. In addition, a ring-shaped goggle with the central lens absent was used as a control for the mechanical effect of the goggle (group 2, n = 6). In group 3 (n=7), +15D lenses induced +7.7D of hyperopia, while in group 4 (n=7) the -12D goggle induced -8.5D of myopia. The values given are in dioptres (D) and represent the differences between refractive states of treated eyes as compared with untreated contralateral eyes. The error bars in this figure and in the remaining ones represent the standard deviation.

As noted earlier, treated fish were weighed before and two weeks later, after treatment. A small increase in weight, generally one to three grams, was noted. However, there is no correlation between weight change and induced ametropia, a point also noted in our earlier study.[31] In the additional groups of fish treated with lenses, both plus and minus lenses were worn for two weeks, after which the goggle was removed and recovery from induced refractive errors was observed. The fish treated with minus lenses (group 5) developed -9.8±1.9D of myopia (n=8, $P<0.001$) (Fig. 5). After two weeks of treatment with a +15D lens, the treated eyes developed hyperopia (treated eye compared to control eye) of +8.05±1.35D (group 6, n=5, $P<0.001$) and +6.25±2.87D (group 7, n =8, $P<0.001$) (Fig. 5). Also, Table 2 shows the change of refractive states of individual fish in group 5 before and after treatment with -12D lenses while Table 3 shows results for individual fish wearing +15D lenses (groups 6 and 7). After lens removal, the induced refractive errors decreased gradually ($P<0.001$, ANOVA) (Fig5). Refractive states measured six days after recovery were close to the baseline in all groups. All three groups showed no statistical difference between the two eyes two weeks after lens removal.

Table 2. Refractive data in dioptres (D) for treated and contralateral untreated eyes for fish in group 5, as well as means and standard deviations before and after two weeks of treatment with a -12D lens**.** The refractive measurements are corrected for the effect of the air/water interface, but not for the small eye artifact. Refractive errors are for hyperopic unless preceded with a minus (-) sign.

Fish	**Pre-treatment (D)**		**Post-treatment (D)**		**Interocular difference**
	treated	**control eye**	**Treated eye**	**control eye**	**after treatment (D)**
1	7.5	6.8	-3.8	4.5	8.3
2	7.5	7.1	-4.1	5.3	9.4
3	9.0	9.0	-0.8	5.3	6.0
4	5.3	3.8	-7.1	3.8	10.9
5	6.4	6.0	-6.8	4.5	11.3
6	7.1	6.0	-6.8	4.5	11.3
7	5.3	4.9	-7.1	4.1	11.3
8	9.4	9.4	-3.8	6.4	10.2
Mean	7.2± 1.5	6.6 ±1.9	-5.0 ± 2.3	4.8 ± 0.8	9.8 ± 1.9

Table 3. Refractive data in dioptres (D) for treated and contalateral control eyes for fish in groups 6 and 7, as well as means and standard deviations, before and after treatment of one with +15D lens goggle groups 6 and 7**.** The refractive measurements are corrected for the effect of the air/water interface, but not for the small eye artifact and indicate hyperopia.

Fish	**Pre-treatment (D)**		**Post-treatment (D)**		**Interocular difference**
	treated	**control**	**treated**	**control**	**after treatment (D)**
1	11.3	10.9	18.8	10.5	8.3
2	9.8	9.8	20.3	10.5	9.8
3	10.9	10.5	18.8	10.5	8.3
4	10.5	11.3	19.6	11.7	7.9
5	12.	11.3	18.8	12.8	6.0
6	8.3	8.3	14.3	4.9	9.4
7	6.8	6.0	14.3	6.0	8.3
8	5.3	4.9	11.3	5.3	6.0
9	8.3	6.8	15.8	7.52	8.3
10	5.6	5.6	7.1	4.9	2.3
11	6.4	5.6	7.5	2.6	4.9
12	6.8	6.8	13.5	4.9	8.7
13	5.6	4.9	7.5	5.3	2.3
Mean	8.3±2.4	7.9 ±2.5	14.4±4.8	7.5 ± 33	6.9±2.5

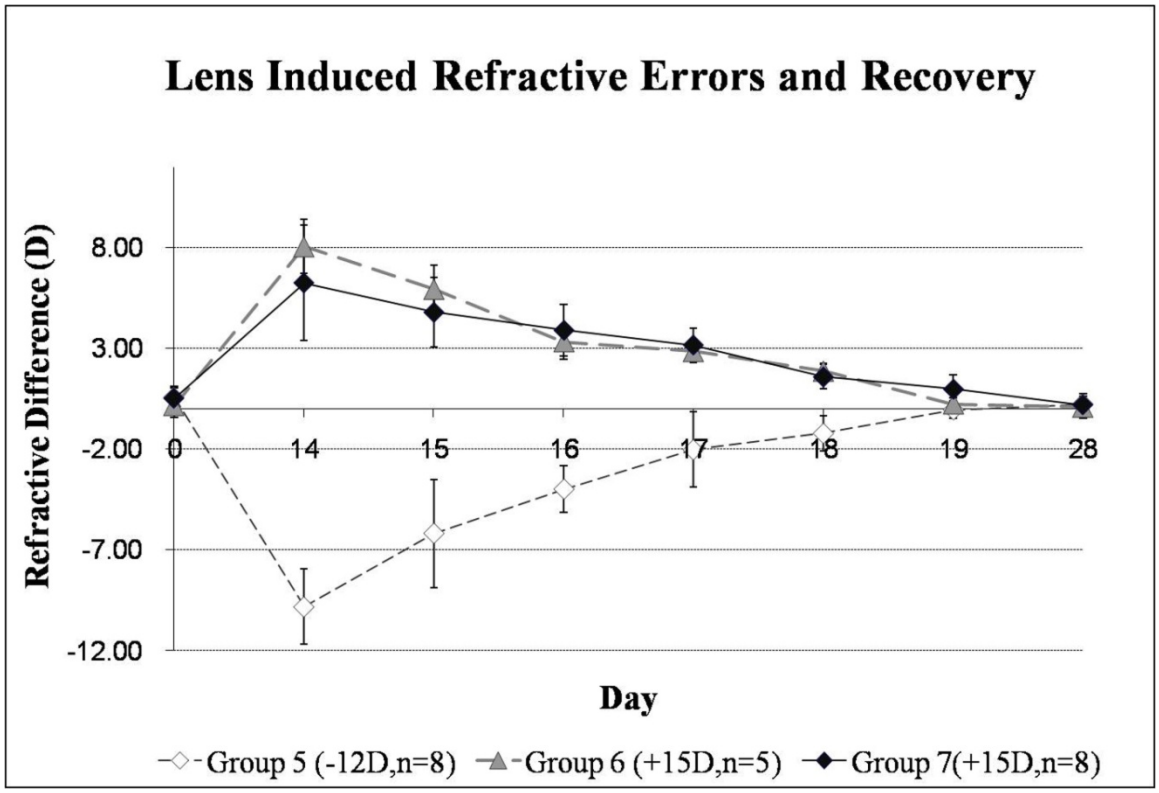

Figure 5. One group of tilapia (group 5, n=8) were treated with -12dioptre lenses over one eye for two weeks and allowed to recover. Two additional groups of tilapia of average weight 13.9grams (g), (group 6) and 26.9g (referred to as group7) were treated with +15diopter (D) lenses for two weeks and allowed to recover.

1.3.3 Form Deprivation Myopia and Weight

After two weeks of treatment with translucent goggles, form deprivation myopia was produced in all three groups (groups 8, 9 and 10) to significantly varying degrees ($P < 0.001$, ANOVA) (Fig. 6). Expressed as average differences in refractive state between the two eyes, the highest amount was 11.9 ± 2.9 D and was found for group 8. In group 9, the myopia averaged 6.3 ± 2.5 D while in group 10, the heaviest fish, the average was 2.3 ± 1.0 D. The treated eyes completely recovered to pre-treatment refractive state levels within two weeks of goggle removal and showed no differences between the three groups.

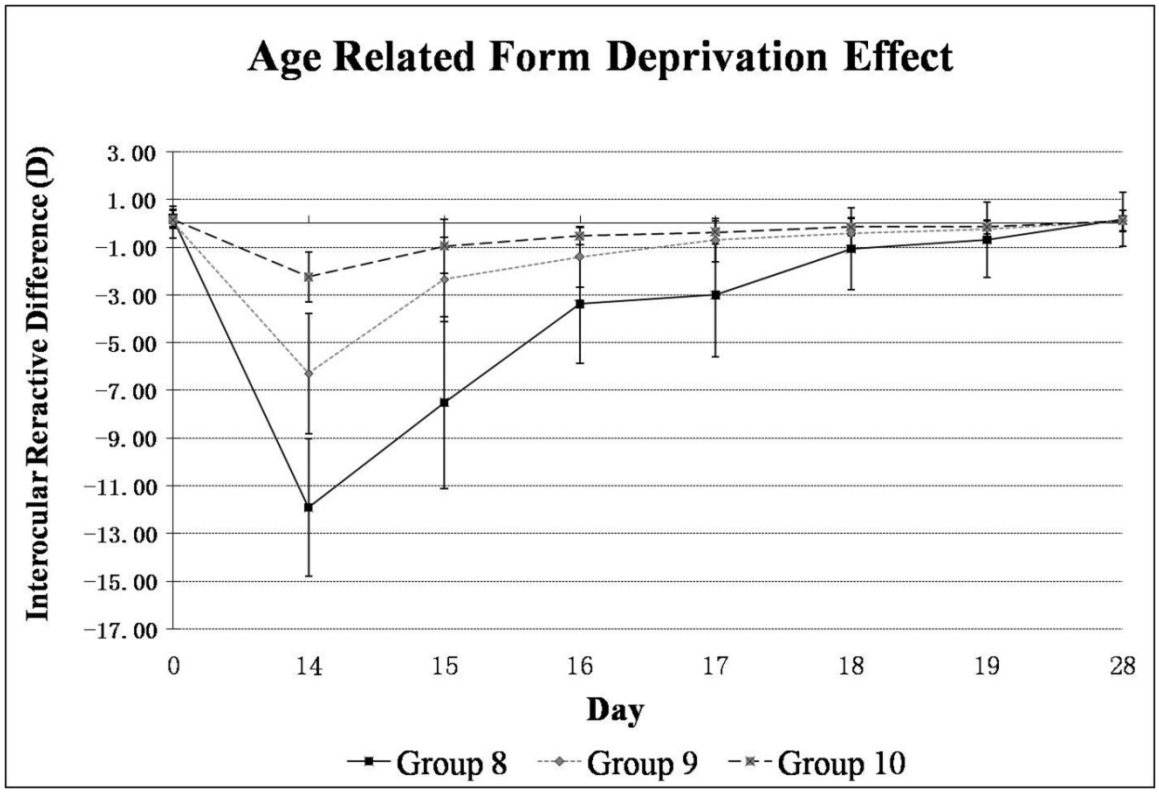

Figure 6. Form deprivation myopia and recovery for three different weight (age) groups of tilapia. The smallest fish (group 8, n=7, average weight 16.2g) were estimated to be 4 months of age while the fish labeled group 9 (n=9, average weight 57.4g) and group 10 (n=8, average weight 98.4g) were estimated to be 7 months and 10 months of age, respectively. All tilapia were treated with a translucent goggle over one eye for two weeks. The results are given in dioptres (D) for the average difference in refractive state between the treated and the untreated contralateral eyes.

1.3.4 Effect on Contralateral Control Eyes

The results (Fig. 7) indicate a small but insignificant contralateral eye effect, with the treatment eyes of the negative lens groups dropping slightly below the horizontal while those of the positive groups remain more or less horizontal. This is also seen in the means of the individual data of Tables 2 and 3. It is possible that these changes are in part due to the normal change in refractive state as a function of weight indicated by the graph shown in Figure 3, particularly for the minus lens results. However, we feel that this is an unlikely explanation given that fish weights increased marginally (1 to 3 grams, as noted earlier) during the two week treatment period and given the difference between the positive and negative lens treatments. Small contralateral effects were noted in the case the form deprivation results as well. For example, while the treated eyes of the lightest fish (group 8) showed an average myopic refractive state change of almost 12 D (Fig. 6), the contralateral eyes changed about 1 D (from an average of +8.1D to +7.0D) in the same direction. These contralateral changes may be another example of the agreement between results for higher species such as chicks and lower ones such as tilapia.

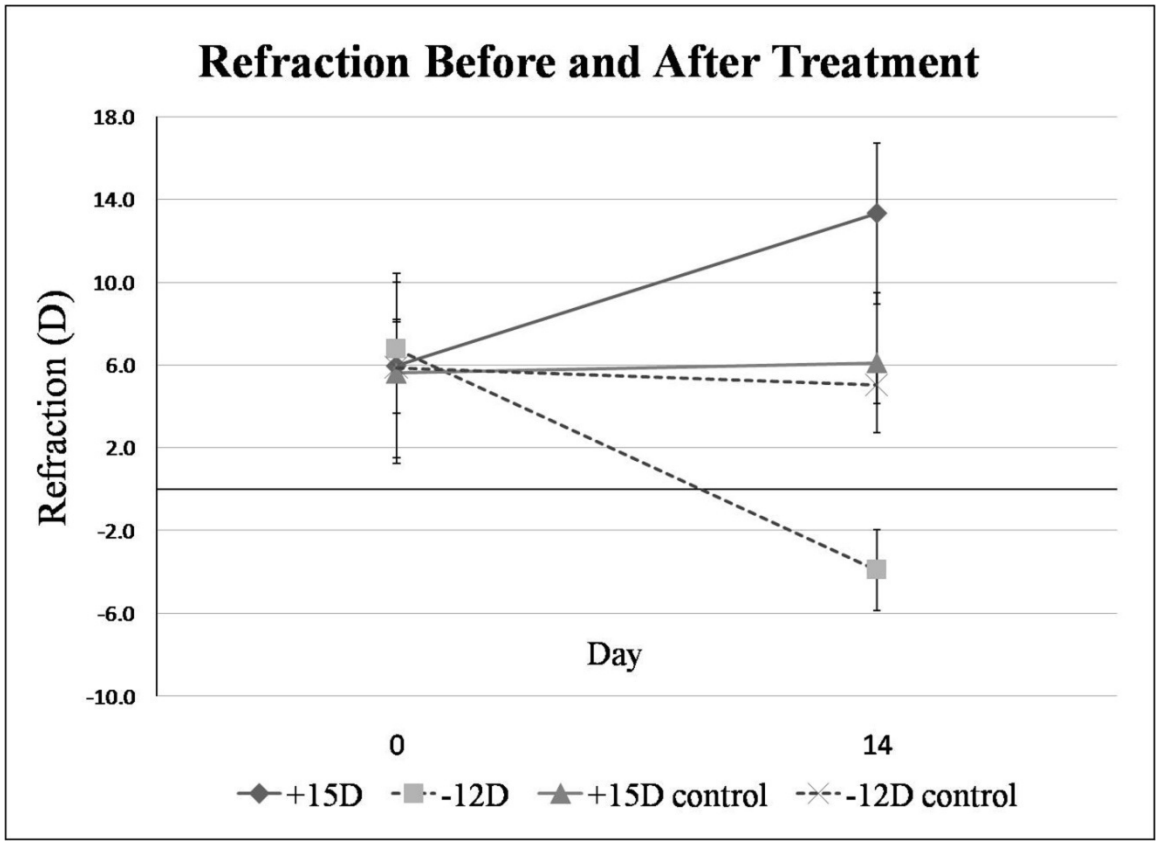

Figure 7. The two graphs show the change in refractive states in dioptres of positive and negative lens treated eyes and the untreated contralateral eyes before and after treatments to induce hyperopia and myopia.

1.4 Discussion

This study demonstrates for the first time that in addition to form deprivation myopia, fish eye refractive development can be manipulated toward myopia and hyperopia with the use of positive and negative lenses and the refractive changes are a result of change in the axial length of the eye. Thus, early eye growth and refractive development of the eyes of both lower and higher vertebrates, including primates.[34] appear to be guided by the visual environment. We suggest that the same mechanism(s) are involved and likely evolved at an early point in vertebrate evolutionary history.

While the results for the groups of fish treated with positive and negative lenses show significant change in refractive state in the hyperopic and myopic directions, the changes do not fully compensate for the +15 and -12 D lenses used. Possibly the lens powers used exceeded the eye's compensatory ability and further testing with other lens powers is needed to clarify this point. However, we feel that it is more likely that a longer treatment period would produce greater compensation, a point also made by Wildsoet and Wallman[35] with respect to chicks. In fact, a recent study by Kisilak et al[36] demonstrates that chicks treated with -30D lenses can fully compensate for the lens treatment if the lens is applied to the eye for a longer period. Unfortunately, due to reflections from the goggle surface it was not possible to measure refractive state consistently with the lenses on the eye in order to monitor the level of compensation prior to the end of each experiment. The two week period of treatment was based on concern as to the ability of the sutures to keep the goggles on the fish.

The normal refractive state of tilapia in the 14g group is around +11D (+10.9±0.8D) along the axis perpendicular to the iris plane. In fact, refractive state often varies with the axis of measurement in fish and it is illusory to refer to a specific refractive state.[37] The direction perpendicular to the iris plane is generally the most hyperopic direction. Moreover, fish often inhabit relatively monochoromatic (blue) environments and thus chromatic aberration is a factor to consider. A refractive state measured under white light conditions may be considerably less hyperopic at wavelengths that are closer to the blue end of the visible spectrum.[38] Nevertheless in this study, and with respect to the axis perpendicular to the iris plane, the hyperopia measured decreases with age and fish weight.

It is possible that the small eye artefact of retinoscopy may be a significant factor in the change of refractive state measured during fish eye growth.[39] The rationale for this point is that the retinoscopic reflection used in measuring refractive state emanates from the retina-vitreous border rather than from the receptor plane of the retina, thus biasing the finding in the hyperopic direction by an amount equal to the dioptric value of the thickness of the retina. Since retinal thickness varies little with eye size (the tilapia retina is about 200 µ thick [31], the smaller the eye is, the larger the artefact. The small eye artefact in relation to retinoscopy has been studied mainly in relation to small – eyed mammals[38, 40] but there is no reason to rule out the possibility that it is a factor in the study of the fish eye as well. Thus, the refractive states of the untreated heavier fish of group 1, which appear to level off at about four dioptres of hyperopia (fig. 3), may in fact represent emmetropia or near emmetropia in the direction perpendicular to the plane of the pupil. For the 25 to 30 gram fish studied with eye diameters of seven to eight

millimetres the artefact could amount to about five dioptres while for the smaller ones, approximately 14 grams in weight with smaller eyes approximately five mm in diameter, the artefact can be substantially greater.[38] Thus, fish in group 6 that were treated with a +15D lens showed +11D of hyperopia on average before treatment. In fact, it has been noted in other species that the response to positive defocus is quite variable, sometimes even producing a change in the opposite direction with an elongated eye after treatment.[41] The tilapia model may be a useful one for further research into the accuracy of retinoscopic and other refractive measurements of small – eyed species.

Though fish eye size keeps increasing throughout life, the susceptibility of the tilapia eye to manipulation of the visual environment is strongly related to weight. Thus, the heavier the fish, the lower the amount of form deprivation myopia produced. When fish weight is about 100 grams (group 10), it is much more difficult to induce form deprivation myopia and only -2.3±1.0D is induced on average in this group, compared with -11.9±2.9D in the lightest fish (group 8). In addition, a goggle with positive power induced higher levels of hyperopia in smaller fish (groups 6 and 7). There may exist a critical period of eye development in fish, as in other animals such as tree shrews, in which induced myopia is not strictly dependent on the growth rate of the eye.[17]

It is still not known how susceptible the fish eye is to visual manipulation during the earliest period from hatching to about 10g and an additional study of this period is warranted. Furthermore, sexual maturation takes place earlier in aquaculture conditions when the fish are in tanks than in fish from ponds and lakes.[42] Growth rate and therefore body weight can vary greatly depending on conditions such as water temperature, availability of food, etc.[43] In fact, in our earlier study it was found that there is no

correlation between the increased weight of the fish during a treatment period of four weeks and the amount of form deprivation-induced myopia.[20] However, this may not be the case at the earlier period of development.

In the recovery process, the refractive error of the treated eye always stops changing when there is no difference between it and the untreated contralateral eye, regardless of the starting point. Fish are not exceptional from other animal models in this regard,[17, 18, 41, 44]

As noted, the tilapia eye recovers completely, not only from form deprivation myopia but also from lens induced myopia and hyperopia, regardless of the weight of the fish. The recovery from form deprivation myopia resembles that found in a guinea pig study, in which about half of myopia disappeared in the first day.[11] However, generally, young animals completely recover in about a week.[11, 18, 41, 45] By comparison, primates (monkeys), only recover to a limited extent from diffuser induced myopia.[30, 34] Though recovery varies with species, it still may be part of visually guided regulation of eye growth. The choroid of the fish eye may also play a role in this process, as found in other higher vertebrates.[26, 46-48] In fact, the choroidal stroma of the teleost fish eye is rich in blood vessels, mainly veins, which make up the choroidal gland and the lentiform body. The role of these structures in short term changes in ocular focal length remains to be investigated.

Chapter 2

Thyroid Hormone and Induced Refractive Error Development in Tilapia (*Oreochromis niloticus*) Fish

2.1 Introduction

Myopia is becoming more common worldwide, especially in Asian countries and is of increasing concern due to its high prevalence in youth, and Chinese populations. A relationship between the visual environment and myopia development as well as with heredity has been explored.

Experiments dealing with refractive error development are frequently based on animal models such as chick [1], monkey [2, 3] and tree shrew [4]. In fact, various species of animals have been used to induce form deprivation myopia including: grey squirrel [5], mouse [6], guinea pig [7, 8], cat [9, 10], and American kestrel [11].

It has been recently demonstrated that form deprivation myopia as well as myopia and hyperopia can be induced in a lower vertebrate, such as tilapia, by treating one eye with either a translucent goggle or positive and negative lenses.[12] This work provides a new animal model to study the effect of the visual environment on the eye in a species in which the eye continues to grow through life.

Evidence derived from studies based on animal models has shown that the effect of the visual environment is age related as well.[13-16] Typically, experimental myopia is induced in young animals; the younger the animal the higher the induced refractive error.[13, 16] Almost all experimental animals are able to recover from the induced refractive errors to their normal refractive state after removal of visual interference during the early stage of ocular development. However, monkeys are only capable of recovering from form deprivation myopia to some degree, probably as the result of the relatively slow rate of ocular development characteristic of primates.

In humans, the prevalence of myopia varies with age. There is almost no myopia in children less than 5 years old.[17] Most children start to develop myopia only after 9 to 12 years of age and the prevalence rate increases through puberty.[17-19] These age-related effects suggest a possible connection between the sensitivity to visual signals and to hormone levels at different developmental stages.

Experiments such as those in which the chick optic nerve is cut show that refractive error development is largely determined by a local retinal mechanism.[20] Somehow the eye distinguishes whether the input visual signal is over or under focused , and, in chicks at least, rapidly adjusts retinal position and focal length of the eye, by thinning or thickening its choroid.[21, 22] Eye growth then accelerates or slows down for a more permanent change in refractive state.

The growth factor TGFβ may play a key role in regulating scleral synthesis and in reshaping the globe in this process.[23, 24] Neurotransmitters such as dopamine and GABA have been found to be involved in this process[25-28]. Also the hormone melatonin is under investigation. It was found the systematic administration of melatonin affects ocular components in response to induced form deprivation myopia, though no refractive state difference was reported.[29] However, in a earlier study, it was assumed that melatonin is not involved in the retinal signalling pathway.[30]

Among all hormones, thyroxine is involved in controlling the metabolism of the body and influencing physical development, especially during early eye development.[31] Thyroid hormone (T4) is secreted by the thyroid gland and works as a prohormone and a reservoir of the more physiologically active form, T3 (3,5,3'-triiodo-L-thyronin). In

some fish, T4 and T3 are associated with the metamorphosis process.[32] Also, thyroxine may alter ultraviolet photosensitivity and the loss or generation of ultraviolet-sensitive cones in the rainbow trout[33-36] as well as cause visual pigment changes in salmonid fishes.[37, 38]

Tilapia have long been used to study the interaction between thyroid hormone and growth hormone or somatostatin.[39, 40] Another study also suggests thyroid hormones may delay tilapia hatching.[41] In the present study, we investigate how thyroxine affects refractive error development of the tilapia eye in response to altered visual stimuli.

2.2 Materials and Methods

2.2.1 Fish

Tilapia (*Oreochromis niloticus*) (about 3 months old), a commonly cultured species of cichlids, were obtained from a local fish farm, North America Tilapia Inc., (Box 37, Bondhead, Ont. L0G 1B0), kept in aquariums with cycled and filtered water at 28 degrees centigrade and fed with Tilapia Fish Food – 3PT Regular (Martin Mills INC. Elmira, ON Canada). The size of fish used was between 11.6 and 26.8 grams. Fluorescent lighting in the aquarium room was set to a diurnal (12 hour light, 12 hour dark) schedule. All fish were cared for according to the Guidelines of the Canadian Council on Animal Care and in accordance with the policies of the University of Waterloo and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2.2 Fish feeding

The vehicle for thyroid hormones delivery is the fish food, which is prepared as described by Tagawa and Iwata in 1991. Thyroxine (T4) (0.25mg/g) and Triiodothyronine (T3) (0.25mg/g, 1.25mg/g, respectively) were dissolved in 1ml 1N NaOH solution and then mixed with 500ml ethanol. Granular fish food (200g) was immersed in the solution and kept in a fume hood till the food completely dried out. The same protocol was applied to make the food for fish in the control group, except no thyroid hormone was added. In order to obtain high thyroid hormone levels in blood serum, fish were fed with these foods about 2% of their weight each day for 5 days before the eye is goggled. Five fish were taken out respectively from both the tank with T4 or

T3 treated fish and those with the control fish. These fish were sacrificed and Thyroid hormone levels analyzed.

2.2.3 Induction of refractive errors

In some fish lightweight translucent plastic goggles were directly sutured over one eye for two weeks to induce form deprivation myopia[42]. In others, goggles with centrally placed convex lenses of plus power (15 diopters in water) to induce hyperopia[12] The right eye was always the treated eye while the left eye served as a contralateral control. Sutures were sewn through the skin and soft bones around the orbit using nylon stitches through holes in the edge of the plastic goggle.

In the control group A for the T4 experiment fish were treated with translucent goggles and fed with regular fish food (average weight was 18.9±1.6g, n=8). Fish in the T4 treatment group (B) were treated with a both translucent goggle over one eye and T4 (thyroxine) fortified food, (average weight was 20.36±2.5g, n=8). Group C (16.48±3.83g, n=6) was the control group for the T3 experiment. These fish were treated with translucent goggles and fed with regular fish food. Fish treated both with the translucent goggle and T3 fortified food were divided into two subgroups in terms of different T3 levels in order to provide some information as to the effect of dose. Group D, dose level 0.25mg/g, n=7, weighed on average 16.24±1.92g. Group E, dose level 1.25mg/g, n=6, weighed on average 16.38±1.74g. The fish of Group F (n=6) which were treated with +15D lens-goggle but with regular food, weighed on average 14.62±1.73g. Fish in group G (n=5) were treated both with a +15D lens-goggle and with food with T3 (0.25mg/g), and weighed on average 20.84±5.30g. The T4 experiments were carried out earlier,

when only form deprivation myopia had been demonstrated in tilapia. Therefore, the T4 results do not include data for plus lens induced changes.

2.2.4 Ocular Measurements

Refractive states were measured with a streak retinoscope and trial lenses at a working distance of 25 cm through the glass wall of a specially designed narrow aquarium while the fish were anesthetized with 0.5ml 2-phenoxythanol/L to fix the direction of gaze and to minimize accommodation. The results are an over-estimation of the refractive error in the hyperopic direction, due to the difference in refractive index of water and glass, and the true refractive error is obtained dividing by 1.33.[43] The retinoscopic measurements were made along the direction perpendicular to the plane of the pupil. The results are expressed as the difference between the refractive state, in diopters, of the treated and untreated eyes. The retinoscopic measurements were verified periodically and independently on a limited number of fish by a second experienced individual using an alternative instrument, a photorefractor (PowerRefractor, Multi channel Systems Co. Reutlingen, Germany) with which the retinoscopic reflection was neutralized with trial lenses, as in retinoscopy.

2.2.5 Measurement of Thyroid Hormone Levels

The thyroid hormone levels were measured before and after the experiment. Approximately at the same time in the morning, fish were anaesthetized by being immersed into water with 2-phenoxythanol (0.5ml/L), and then blood samples were collected from the caudal vein by using a syringe with heparin coated (10,000^{ü}/l) syringe and centrifuged for 10 minutes at 6,000 rpm. The serum was collected and stored at -80

°C before the thyroid hormone levels were analyzed by RIA (radioimmunoassay). The T3 and T4 – I^{125} RIA kit used is from MediCorp Inc., Montreal, Canada. After the final blood collection, fish were sacrificed with a high dose of 2-phenoxythanol (3ml/l).

2.3. Results

During the two weeks of T4 experiments, the weight of fish in both the control and treated groups increased steadily. However, neither the differences in weight change between the two groups (ANOVA, P=0.395, F=0.994), nor the difference in growth rate (19±6% in the thyroxine treated group vs. 25±15% in the control group, p=0.31) was found to be significant (Fig 1).

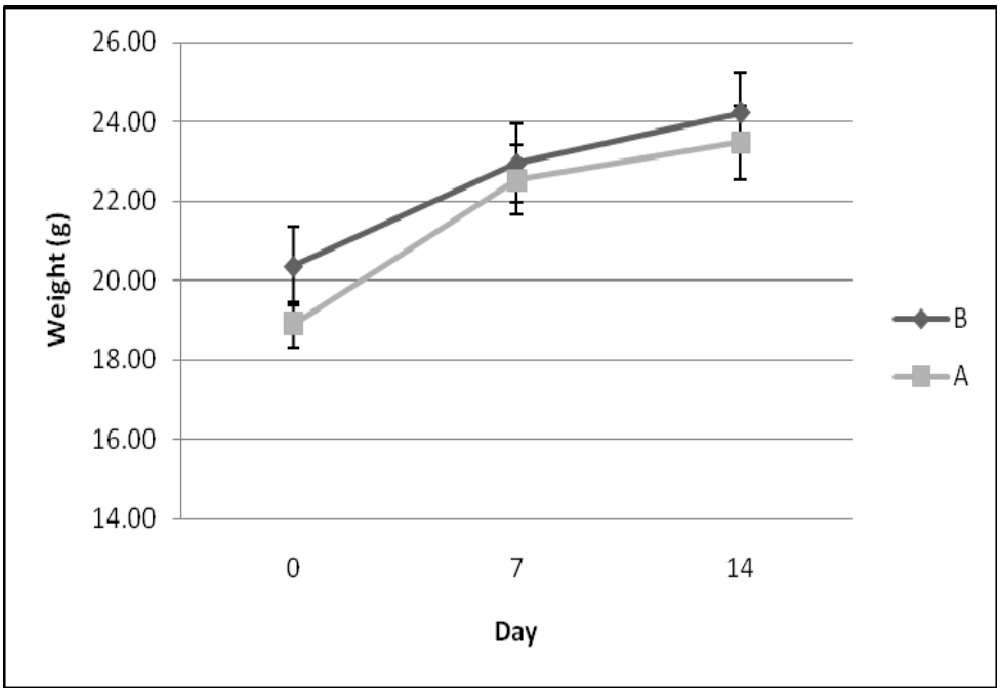

Figure 1. Weight (g) change in tilapia over two weeks for groups A (T4 treated group) and B (T4 control group)

On the day the fish were goggled, T4 levels of fish in both tanks were measured. In the control group, the plasma T4 level of fish was 8.61±1.77nmol/l (n=5), which is significantly lower than the level for the fish treated with thyroxine for 5 days, (139.19±27.63nmol/l, n=5). Two weeks after the treatment, the thyroxine level in the thyroxine treated group decreased to 36.99±8.15nmol/l (n=8) while the level in the control group was 7.59±1.16nmol/l (n=8) (ttest, $p < 0.0001$) (Fig 2.).

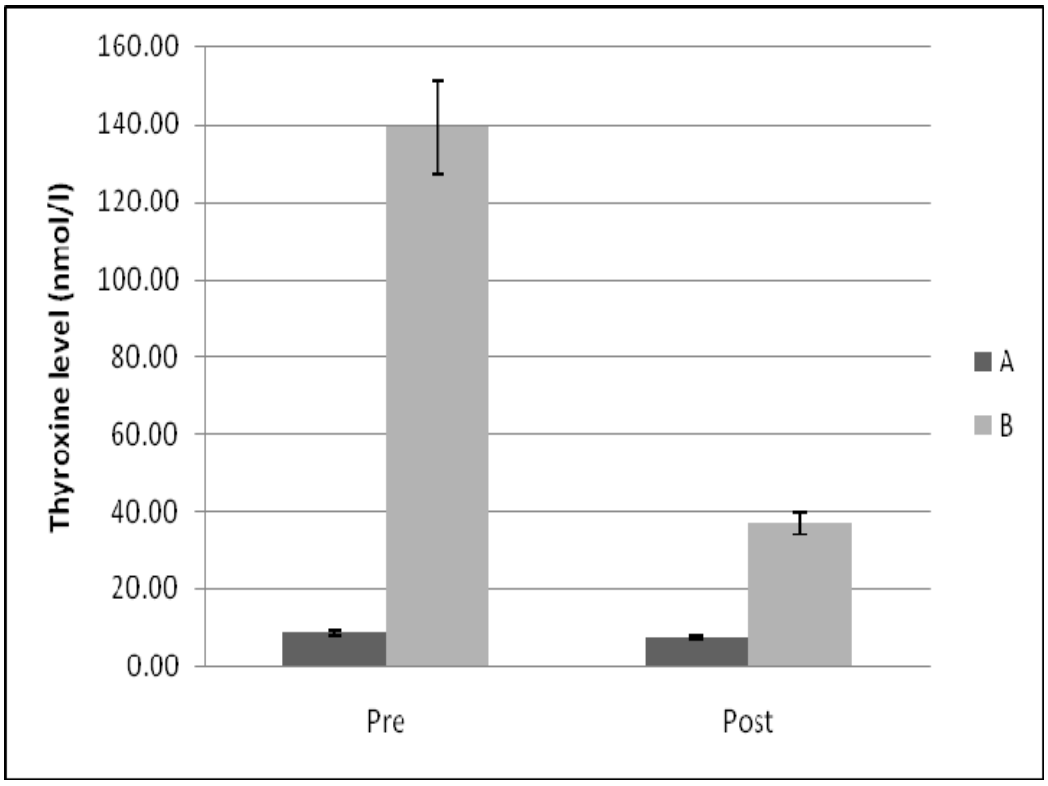

Figure 2. Plasma thyroxine levels were measured before and after experiment. The first measurement was made 5 days before the fish eye was goggled.

Before treatment, the inter-ocular difference in refractive state in control group A was 0.25±0.76D and 0.06±0.32D in treated group B. After two weeks of treatment, fish in group A developed an average level of myopia of -22.13±3.75D while the T4-treated fish in group B developed an average amount of 10.95±5.23D of myopia, a significant over time (ANOVA, $F = 18.78$, $P<0.001$). The induced myopia increased with time (ANOVA, $F=19.3$, $P<0.001$). Fig 3 shows the change in refractive states of fish both in two groups over two weeks. The individual data show that the refractive status of the contralateral control eye in control group A changed non-significantly from +6.48±0.89D at the beginning to +5.31±0.74D after two weeks (paired ttest, $n=8$, $P<0.05$), and in group B, from +6.91±1.17D to +5.17±0.94D (paired ttest, $n=8$, $p<0.05$). There is no significant difference in refractive change between the two groups in this regard (ANOVA, $P = 0.78$, $F=0.25$).

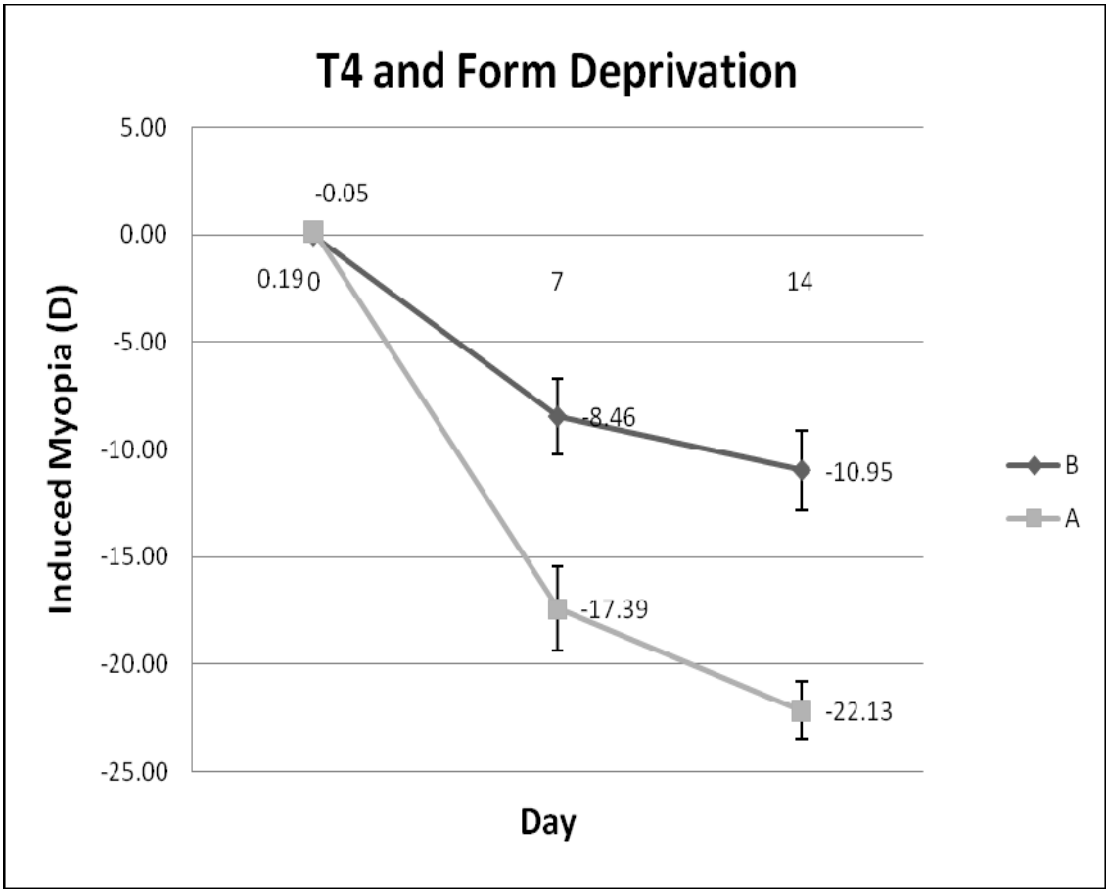

Figure 3. Tilapia eyes were refracted at the first day, 7th day and the last day of the experiment. The data indicate the interocular difference in diopters (D) between the goggled eye and the contralateral control for groups A and B.

In the T3 experiment, T3 levels in group D and E were enormously increased when measured after two weeks of treatment, but only the T4 level of group E showed significant increase (22.23nmol/l compared with the untreated 5.5±3.49nmol/l and lower food T3 level group 6.76±4.32nmol/l)(See Fig 4).

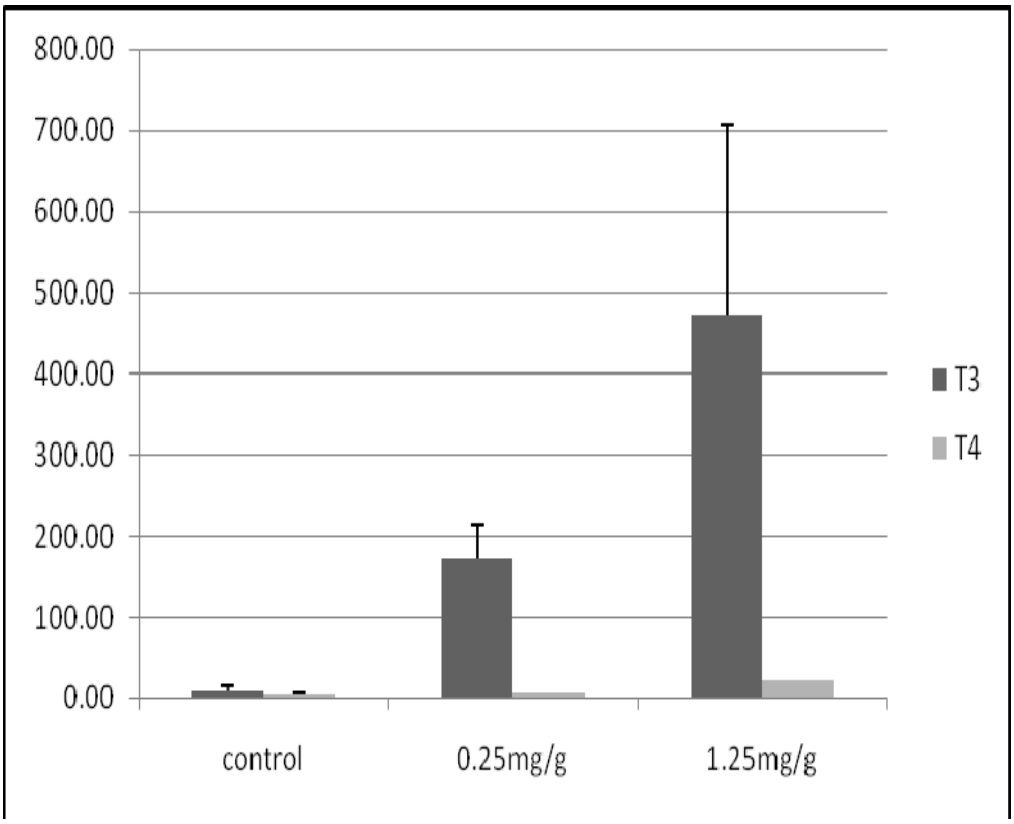

Figure 4. T3 and T4 levels (nmol/l, y axis) measured after two weeks of feeding in the control group and other two groups (group C, D, E) fed with T3 fortified food with different dosages (0.25mg/g and 1.25mg/g, respectively).

The treated eyes showed significant form deprivation myopia after 1 week and the induced myopia is significantly higher after 2 weeks than after 1 week (ANOVA, $F=48.46$, $P<0.001$). The form deprivation effect caused by translucent goggle appears to be significantly suppressed in both group D and E by T3 treatment of different dosages when compared with the control group over time (ANOVA, $F=9.03$, $P<0.001$). However, the difference between these two treated groups is not significant ($F=2.07$, $P=0.15$), though the higher dosage group seems to be more affected and developed less myopia after two weeks (Fig 5). Fish fed regular food developed 8.21±2.61D of myopia after one week compared with, 2.26±1.55D for group D and, 1.00±1.94D for group E. However after two weeks, higher myopia was induced in group E (5.08±4.03D) than group D (3.33±2.55D). At the mean time, 10.40±3.72D of myopia was induced in control group C (Fig 5).

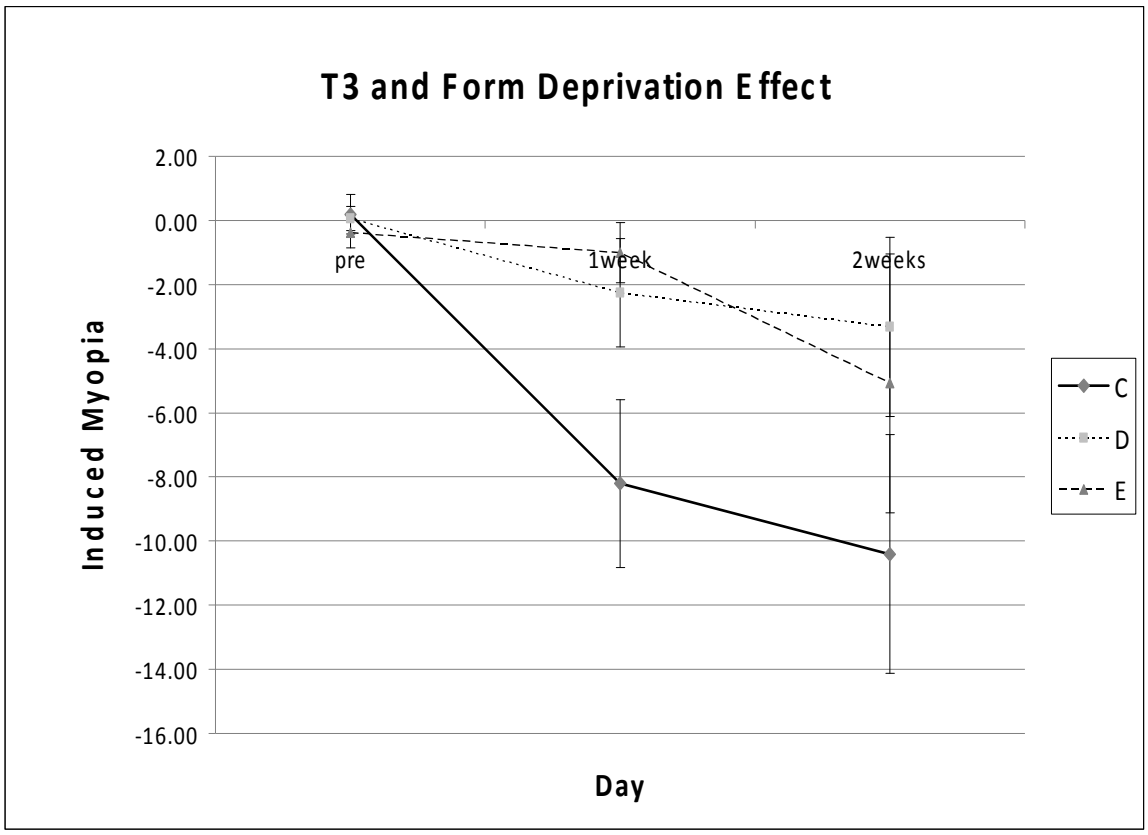

Figure 5. T 3 and form deprivation effect. Induced myopia increased almost linearly with time in control group (C) in two weeks, while T3 treated fish showed a much lower level of response, especially after the first week (groups D and E). However the difference between the dosages is not significant.

Generally, the T3 treated group gained more weight than the control group, though it is not statistically significant. During this two-week period, fish grew to 23.6±4.76g in group C, and 22.67±4.16g in group D, 23.57±3.68g in group E; 14.62±1.73, group F; 29.48±8.77g, group G. The corresponding growth rates are 44±16%, 39±19% and 62±19%, 18.07±8.61%, 40±14.82%, respectively.

The eye treated by +15D lens-goggle in the control group F appeared to be 8.02±1.69D more hyperopic than the contralateral eye treatment. When fed with T3 fortified food, the goggled eye of the fish in group G developed significantly higher hyperopia (10.38±0.78D) than in group F (8.02±1.60D) after two weeks, but not after 1 week (9.70±1.37D vs.8.83±2.21D) and the refractive change between groups following time is marginally significant (ANOVA, $F=3.5$, $P=0.05$).

2.4 Discussion

This study suggests that refractive error development is associated with systemic hormone level. The induced refractive errors of the fish eye during early developmental stage may be shifted to more hyperopic direction by high serum level of the thyroid hormones (Fig. 6).

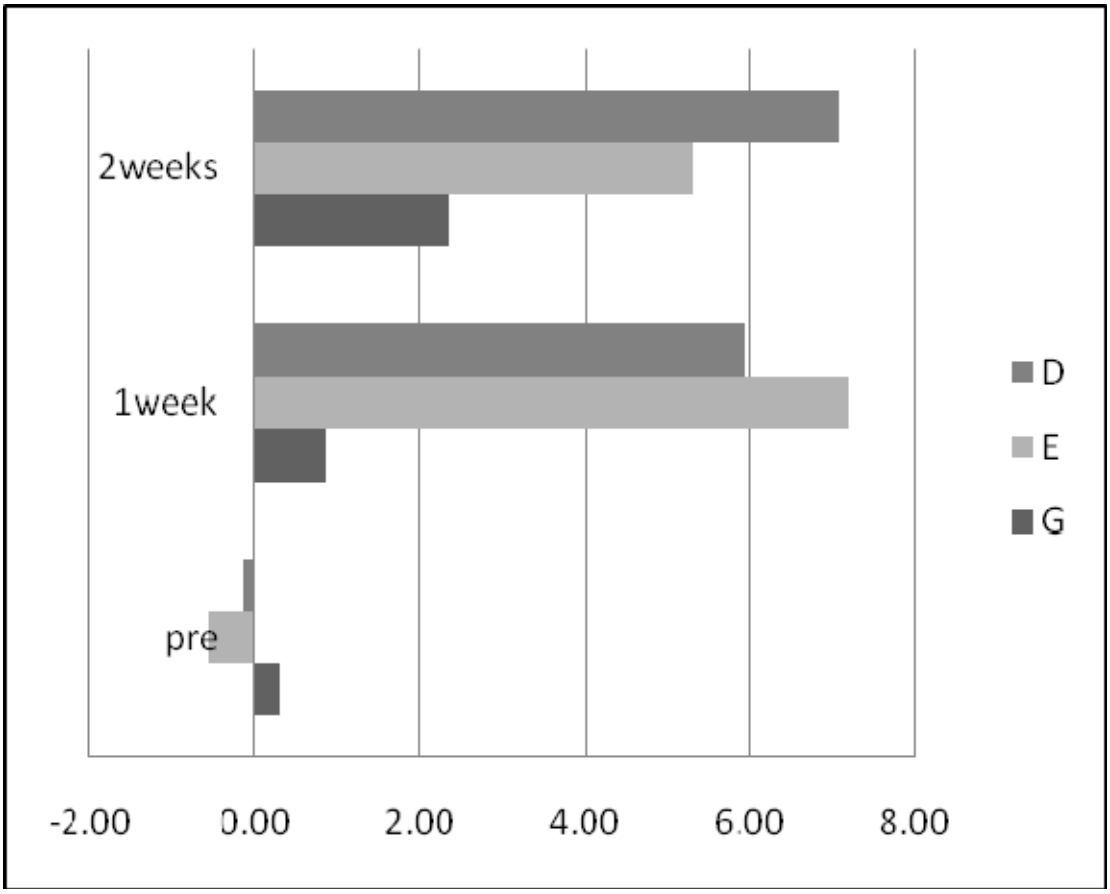

Figure 6. T3/T4 and hyperopic shift of the induced refractive errors

T3/T4 causes less form deprivation myopia and more lens induced hyperopia. This graph compares the T3 effect on refractive error development by showing the difference in induced refractive errors between the T3 treated groups (D, E or G) and the control group (C or F) after treatment. For example, D = induced myopia (mean) in group D – induced myopia (mean) in group C. Hyperopic shift is seen in all three groups.

As mentioned earlier, the response to altered visual stimuli which induces refractive errors is mainly a local retinal mechanism,[20] although the exact pathway is still unknown. Somehow the eye regulates its growth rate to result in an elongated or shortened eyeball to match the optical input and produce a clear retinal image. Though several studies have been done on the relationship between glucagon and form deprivation myopia development,[44-46] it is not well understood whether the growth of the eye in different visual conditions is under control of growth hormone or other hormones associated with ocular development. Thyroxine is vital for central nervous system development, and retinal cell differentiation and the receptors for thyroid hormones are located in many tissues of the body. The transporting polypeptide of thyroid hormone (for T3) has been found in the rat eye, from the cornea to the retina[47]. The retinal pigment epithelium (RPE) has high levels of expression of organic anion transporting polypeptide [47].

In the present study, the form deprivation effect is partially suppressed by the increased T4 level resulting in about half the amount of myopia that would be normally produced. On the contrary, high level of T3 appears to increase the amount of induced hyperopia. As it is known the induced refractive errors are results of altered axial length of vitreous chamber[12, 48] as other animals,[1, 3, 4, 49] these results may suggest that eye growth is delayed rather than accelerated by a high level of the thyroid hormones, although thyroid hormones potentiate the development of the brain.

In this study, involving thyroxine fortified fish food, the thyroxine level of the thyroxine treated fish is over 10 times higher than that of the control group, which may satiate all transport proteins and result in much higher levels of free hormone. After two

weeks, the thyroxine level decreased to a much lower level. Change in T3/T4 levels may further affect the release of TSH (thyroid stimulating hormone) and TRH (thyrotropin releasing hormone) through a feedback loop.

An overdose of thyroxine generally will not cause severe side effects in a short period in children [50, 51] and the fish showed no obvious signs of the altered morphology or behavior as observed in other species during thyroxine induced transformation[52]. In fact, no apparent side effects of high dosages of thyroxine received as supplement were observed in tilapia [53]. Nevertheless, the possibility of side effects with still higher dosages than those of the current study still exists. However, normal refractive development appears not to be interrupted in the current experiments, judging from the individual refractive data collected for the contralateral control eyes in both the T4 treated group and the control group. In fact, the small changes in refractive states found here for the contralateral eyes (referred to as the fellow eye effect), demonstrate the same tendency for a decrease in hyperopia as found in our previous tilapia study [12].

Form deprivation is an age related effect and generally younger animals show larger ocular plasticity though the period of susceptibility varies in species.[12, 13, 15, 54] During the early rapid growth stage, the eye is more sensitive to visual manipulation showing disrupted normal process of emmetropization usually from hyperopia to emmetropia with an enlarged eyeball. In fact, fish growth as well as eye size can be affected by many factors like temperature, feeding, salinity, photoperiod, and oxygen concentration [55]. In fish farms, tilapia could grow from fingerlings to over a pound in 8 months, much faster than in wild field or in lab aquariums. In this study, the increase in weight of the T4 treated group is lower than that of the control (Fig. 1), although the

difference is not significant. This might be the result of accelerated metabolism caused by high T4 levels in the blood.[56] It is not known if the susceptibility could be size related (different age but the same size) or purely growth hormone related. Indeed, growth hormone does have some effect on the emmetropization process found in children [57, 58] Several fish studies suggest that there are interactions between growth hormone and thyroid hormone [39, 40, 59] and many animal studies have found that T3 increases growth hormone (GH) synthesis and secretion. Also, the influence of thyroid hormone on GH action is strongly involved in body growth.[60] However, eye growth in the treated groups seems to be slowed by high levels of T3/T4. The mechanism behind this effect is not known.

In animal experiments of refractive development, the eye is able to find the correct growth direction to match the new focus plane of the blurred image, and it knows when to stop as they both (eye size and focal plane) are matched. This mechanism may be based on the transcription factor Zenk[61] which is expressed by glucagon and which may act as the stop signal for refractive development[46]. Further, there exists interaction between thyroxine and glucagon and it is assumed that the elevated thyroxine level stimulates excess secretion of glucagon[62, 63](Wolf 1981, Mitchell 1986). Thus, thyroxine may affect the ocular response to altered visual signals by changing plasma glucagon levels. Another possible molecular signal with bidirectional features in response to visual signals is retinoic acid[64-66] which functions through the nuclear receptors belonged to the same steroid/thyroid hormone receptor superfamily as thyroid hormone. In addition, an auxiliary protein, retinoic X receptor, interacts with both thyroid hormone and retinoic retinoic acid receptors[67]. Thyroid hormone action on gene transcription is selectively

antagonized by retinoic acid [68] and the interaction of thyroid hormone and retinoic acid receptors may regulate the GH promoter regulation[69].

Many components or factors may affect the final refractive state of the eye as an optical system. In fish, the crystalline lens plays a very important role since the fish cornea does not contribute in a refractive sense to the refractive state of the eye. However, in a previous study we showed that the accelerated eye growth which results in form deprivation myopia does not influence tilapia lens growth and optics after 4 weeks of goggle treatment[42]. While there has been concern related to cataract formation and thyroxine level in trout[70], the thyroxine treatment of this study did not cause obvious lens opacification.

More research is needed to investigate the pathway by which thyroxine has affected refractive development, not only in fish but also in higher vertebrates. The retina should be given more attention in thyroxine related studies because of its role in processing optical signals and as a part of the central nervous system, its development is strongly tied to thyroxine.

Chapter 3

Posterior Segment Thickness Change of the Eye of Tilapia (*Oreochromis niloticus*) in Response to Induced Refractive Errors

3.1 Introduction

In addition to genetic factors[1, 2], evidence has shown that the visual environment plays an important role in the refractive development of the eye [3]. For example, it has been found that: 1) myopia can be induced in many vertebrate species by covering the eye with a transluscent goggle to deprive it of clear form vision [4-12]; 2) the growth of the eye can be either accelerated or slowed, thereby changing the direction of refractive development to myopia or hyperopia, by applying a positive or negative lenses to the eye [13-16]. Moreover, the eye recovers from the induced refractive error when the inducing lens or goggle is removed [12, 17-19]. The main effect of these manipulations is an alteration in the depth of the vitreous chamber of the eye.

Patients with high myopia usually have axially elongated eyeballs, deeper anterior chambers and thinner retinae [20-23]. Similar ocular changes have been found in animal models involving form deprivation myopia, though the anatomy and physiology of the ocular structures involved may be different from that of the human eye [4, 6, 12, 24]. In the case of chicks, as well as primates, it has been shown that when the image on the retina is blurred, the choroid becomes thinner or thicker, depending on whether the eye is being treated with a positive or negative lens. The effect is more meaningful in dioptric value in chicks [15, 25-27]. Subsequent to the choroidal thickness changes, the growth rate of the eye is altered to produce a new focal length as compensation for the visual signals produced by the blur from the treatment. These effects are believed to be due to a local retinal mechanism [27].

The retinal pigment epithelium (RPE), the cellular monolayer between the retina and choroid, is critical to the transportation of signals controlling eye growth between the

neural retina and the choroid. As a result, and in response to signals that are initially visual, the sclera undergoes morphological changes [28, 29]. In form deprivation myopia, the scleral fibrous layer becomes thinner, but total protein content increases [30, 31]. As a result of the remodelling of the posterior sclera, eye shape and vitreous chamber depth are changed.

Change in retinal thickness during refractive error development may also be a part of the immediate response of the eye to altered optical signals. For example, retinal oedema was induced by goggle removal in form deprivation myopia in chicks, and this is assumed to be related to choroidal expansion [29].

It has recently been demonstrated that the refractive development of a fish eye, tilapia, is very sensitive to visual manipulation, both with regard to form deprivation and to positive and negative lenses [12]. In addition, the retina of the fish eye is relatively thick with respect to eye size [32] and the thickness of the fish eye choroid, including the choroidal gland, can be markedly thickened, tilapia being an example [12]. This study involves the use of standard histology along with optical coherence tomography (OCT), a non-invasive approach that has been studied in humans [33], mammals [34-36]. It represents an effort to evaluate retinal and choroidal changes that may be associated with induced refractive change in the eye of tilapia fish.

3.2 Methods and Materials

3.2.1 Fish

Tilapia (*Oreochromis niloticus*), a commonly cultured species of cichlids, were obtained from a local fish farm, Northern Tilapia Inc., (Box 37, Bondhead, Ont. L0G 1B0), kept in aquariums with cycled and filtered water at 28 degrees centigrade and fed with Tilapia Fish Food (3PT Regular (Martin Mills INC. Elmira, ON Canada). The size of fish used was chosen for handling ease, with most of them between 20 and 30 grams (average weight, 23.4±6.2g). Fluorescent lighting in the aquarium room was set to a diurnal (12 hour light, 12 hour dark) schedule. All fish were cared for according to the Guidelines of the Canadian Council on Animal Care and in accordance with the policies of the University of Waterloo and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

3.2.2 Induction of refractive errors

Specially designed goggles with central convex (positive) and concave (negative) lens inserts were used to induce myopia or hyperopia. The inserted lenses consisted of custom produced intraocular lenses 6.0 mm in diameter with powers, in water, of minus 12 dioptres (D) and plus15 D. The lenses were plastic (polymethyl methacrylate) of 1.49 refractive index, with equal anterior and posterior radii of curvature consisting of 26.18 mm for the negative lenses and 20.80 mm for the positive ones. The lightweight plastic lens/goggles were directly sutured over one eye, the treated eye, for two weeks. Sutures were sewn through the skin and soft bones around the orbit of anesthetized fish (0.6ml 2-phenoxyethanol/L) using nylon stitches through holes in the edge of the plastic goggle.

The fish were removed from water for about two minutes during the suturing process. The fish were divided into two groups, positive or negative lens treatment, and there were 12 fish in each group.

3.2.3 Measurement of refractive state

Refractive states of the fish eyes were measured during the experiment with a streak retinoscope and trial lenses at a working distance of 25 cm through the glass wall of a specially designed narrow aquarium while the fish were anesthetized with 0.6ml 2-phenoxyethanol/L to fix the direction of gaze and to minimize accommodation. The results are an over-estimation of the refractive error due to the difference in the refractive index of water and glass. The true refractive error is obtained dividing by 1.33 and all values reported here were corrected in this manner. The retinoscopic values are estimated to be accurate to within ±0.50 D. Means and standard deviations of the results are given to one decimal. An earlier comparison to results obtained with an alternative refractive method, photorefraction, indicated good agreement with retinoscopic findings (Shen and Sivak, 2007). The retinoscopic measurements were made along the direction perpendicular to the plane of the pupil and the results are expressed as the difference between the refractive state, in diopters, of the treated and untreated eyes. While the values are given as diopters of myopia or hyperopia, they represent shifts in refractive state in the myopic or hyperopic directions.

3.2.4 OCT scanning

An optical coherence tomography system (OCT 2000 Humphrey Instruments, Carl Zeiss Inc., Jena, Germany) was utilized to scan the posterior segments of the fish eye

in intact animals, providing axial resolution 10-15μm. Fish were hand held in a damp cloth in air and positioned in front of the objective lens of the instrument. Since measurement errors might be induced by eye position during OCT, measurements for eyes on same side of the fish, either right or left, were grouped together. The fish were out of water for about two minutes or less and then placed back into water. When scanning the fish eye, the right or left eye was placed in position with the iris plane perpendicular to the scanning light beam. The scan was set up as group circle scan with a scan length of 2.50mm. The center of the circle scan always coincided with the pupil center. Each eye was scanned by OCT for 3 to 4 times and one best image was selected from each scan. Images showing the thickness and curvature of the retina were then exported to a computer and thickness was read using a Matlab (Mathworks Inc., Natick, MA. USA) program to obtain the average retinal thickness of each scan (Fig 2). Finally, the results read from all images were averaged to provide a single value of retinal thickness for each eye.

In the distal (bottom) band (right picture in Fig. 3) in the OCT scanning images, there is a high reflective band, marked as the pigment granule layer in this study, which shows up as red and yellow pixels. Its thickness was obtained using a Matlab program which sets up a color scale with values from 0 – 100, from dark to white, based on the built-in color scale. The value of color for pixel picking is set at 70 with a 2-pixel gap along the scanning axis of the image.

To investigate the effect of circadian retinomotor movement on the measurement of retina thickness with OCT, 18 fish weighing from 22 to 45 grams were sorted into 3 weight groups: 20, 30, 40 grams and then divided into 6 weight matched groups for 6

time points: 6am, 9am, 12pm, 4pm, 6pm, 12am. At each time point, fish eyes were scanned, and then fish were sacrificed immediately and enucleated for histological analysis. During the scanning at darkness (6am and 24pm), fish were always kept in dark conditions only with minimum red light illumination when necessary, until the eye was fixed for histological preparation.

3.2.5 Histology

After fish were sacrificed by decapitation, fish eyes were enucleated and immediately fixed with 4% paraformaldehyde with 0.2M Sorensen's solution for 20 minutes and then washed and put into 30% sucrose for 5 days of cryoprotection. The fish eyes were freeze sectioned (8μm in thickness) and stained with Hematoxylin and Eosin. Photos were taken through the microscope and analyzed by Image J. (the National Institute of Health). Retinal thickness was measured from the inner limiting membrane to the interface between pigment granules and the transparent portion of the retina in the anterior direction. The thickness of the pigment granule layer was also measured using the same software.

3.2.5 Statistical analysis

All data were processed using the statistical program: STATISTICA. Data from circadian retinomotor experiments were analyzed with the student t-test or by linear regression (OCT vs. histology) and other data obtained from goggle treatment experiments were analyzed by ANOVA.

3.3 Results

3.3.1 OCT image and its histological correspondence of the fish eye

The OCT image of the tilapia retina mainly showed two broad bands of strong signals, with the distal (bottom) one being more reflective and reddish (Fig 1, right). This resembles what is seen in human eye scanning images. However the corresponding histological structures may be quite different at different time points due to the retinomotor movements of the fish retina. Nevertheless and despite the circadian change, for example during daylight, the basic anatomical structure of the tilapia retina resembles that of humans in the basic appearance of retinal layers. This is also true of the portion of the choroid adjacent to the retina.

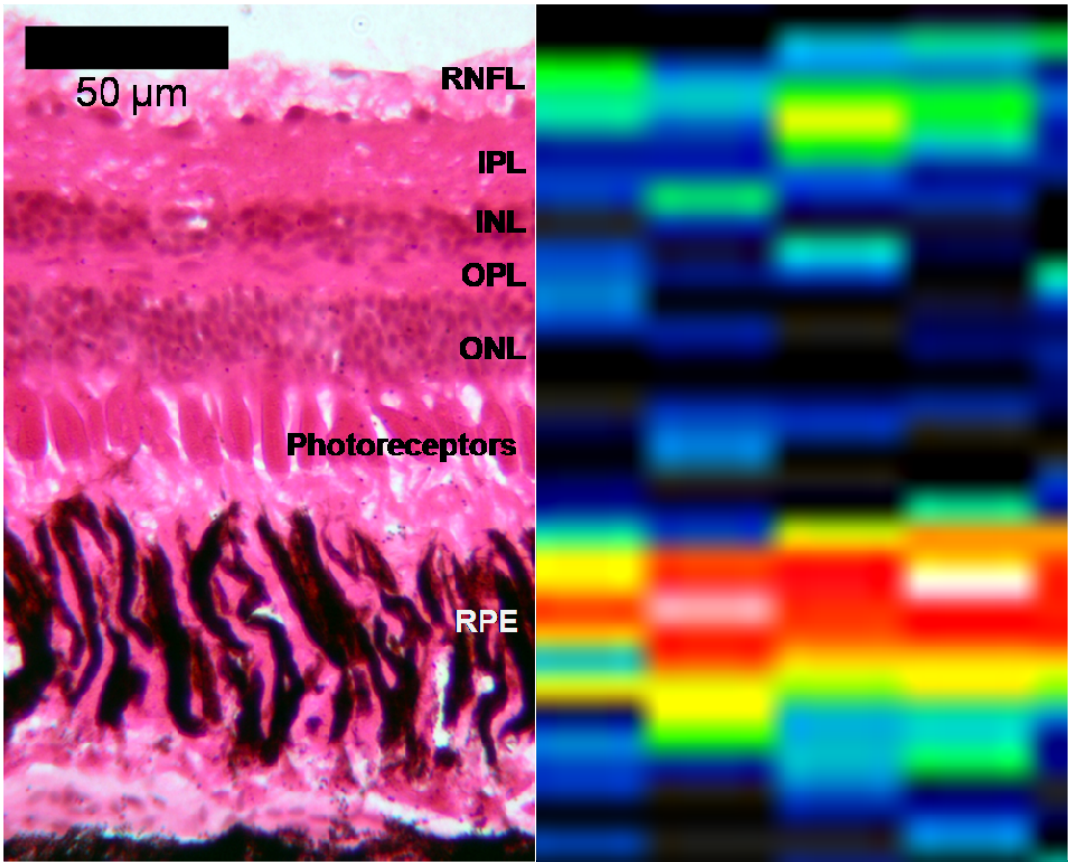

Figure 1. Histology vs. OCT

A histological section is taken from the mid-peripheral area of the fish retina and roughly matched and compared with the optical section scanned by OCT presumably at the same area from the same fish at 6pm. The two main bands with high reflectivity are RNFL and RPE in the OCT image. Between them, IPL and OPL are barely visible. The interface between the inner and outer segments of the photoreceptors is also identifiable, especially at dark adapted retina, see Fig 3. Anatomical aberrations: RNFL, retinal nerve fiber layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium.

The OCT image has been shown to correlate well with histological ocular morphology [33, 37]. In this thesis, the distal (bottom) band may represent a special layer (marked as the pigment granule layer) with accumulated pigment granules in the RPE rather than the RPE/choriocapillaris complex of the human eye, when the penetration ability of the infrared laser beam (800 nm) is considered. The distance between the two high reflective bands is the measured retinal (neural retina) thickness (Fig 2). The thickness of the fish RPE during the daytime period varies from 60 - 90μm. Not many details are seen between these two main bands at higher signal levels. However, in the case of OCT images obtained in the dark, another narrow band appears between the two main bands. This may represent the outer limiting membrane since the pigment granules aggregate towards Bruch's membrane in the dark adapted retina, thus lowing the reflectivity of the RPE previously occupied by pigment granules.

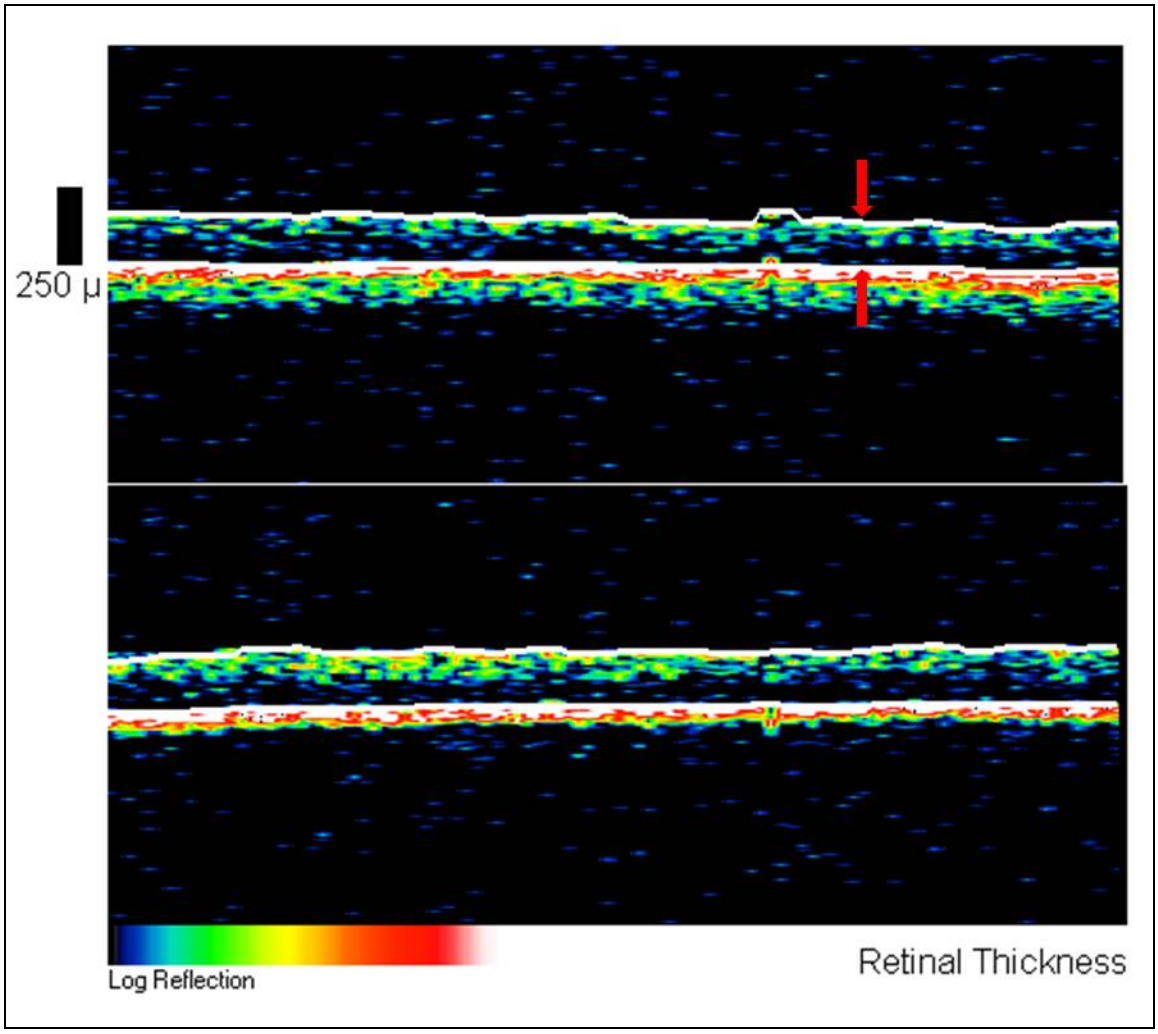

Figure 2. Retinal thickness changes in induced myopia

The picture at top is an OCT scanning image of one fish eye that is scanned when the minus lens goggle is just taken off from the fish eye. The bottom one is taken before treatment. Retinal thickness (the neural epithelium layer of the retina) obtained by OCT is the distance between the two white normalization lines (red arrows).

3.3.2 Circadian change of the fish retina

Circadian retinomotor movement of the tialpia retina was observed both by histology (Fig 3, top) and by OCT (Fig 3, bottom). Before dawn - two hours before the light was on, the outer segments of the photoreceptors (cones) were found histologically spread between the external limiting membrane and the layer of pigment granules that accumulated close to Bruch's membrane. In the morning, before 12pm, the cones moved close to the inner limiting membrane, followed by the rods surrounded by pigment granules. At noon, all pigment granules were much closer to the cones than at 9am. In the afternoon, the pigment granules moved toward Bruch's membrane followed by the photoreceptors. The pigment granules aggregate at Bruch's membrane at midnight. The RPE now became a very thin black band.

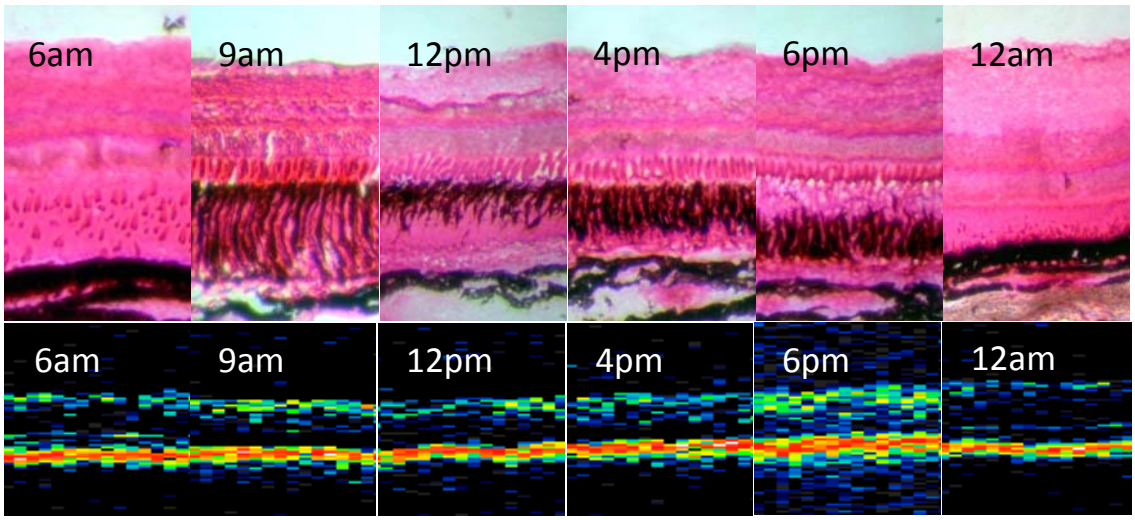

Figure 3. Circadian retinomotor movement: histology vs. OCT

A series of matched histological sections (up) and OCT images (down) taken from 6 time points during a day shows the dynamic change in the retina during a circadian cycle. The measured retinal thickness with OCT depends on the relative position of the pigment granules in the RPE with respect to the RNFL. The interface between the inner and outer segments of the photoreceptors is most visible and of highest reflectivity at 12am and 6am.

Retinal thickness measurement with OCT varies at different time points. However, the results are not affected by the circadian retinomotor movements during the daytime (Fig 4). There is no significant difference neither the measurements at various time points in the daytime or between midnight and before down. However, measurements are significantly different between any time point during daytime and any time point in the darkness (t-test, Bonferroni, $p<0.05$). Comparing 9am with 4pm and 6pm, no significant differences are found (t-test, Bonferroni, $P=1$; 4pm vs. 6pm, $p=0.70$). The pattern of change in the OCT measurement in a daily cycle is consistent with the distance change between the RPE and the inner limiting membrane found in retinal histology (Fig 4). The correlation between measurements made histologically and with the OCT method is significant ($R=0.72$, $n=36$, $P<0.001$) (Fig 5).

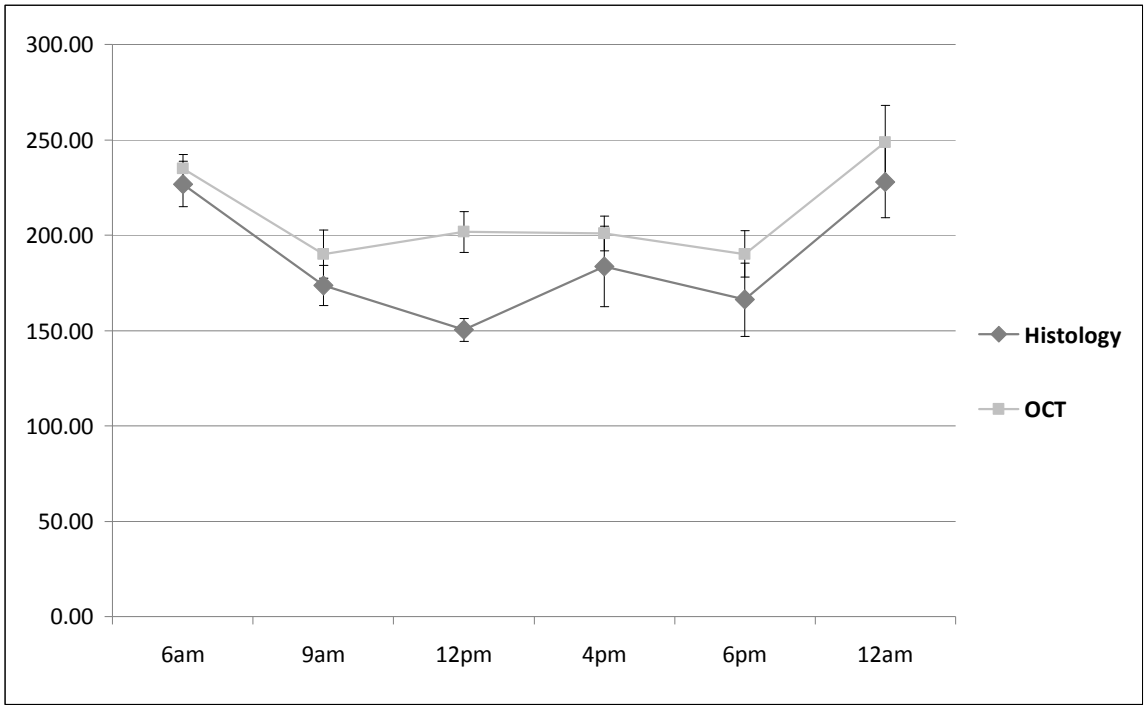

Figure 4. Circadian retinal thickness: histology vs. OCT

Retinal thickness measured at different time points by histology and OCT respectively. During the day time, not much variation was observed by OCT.

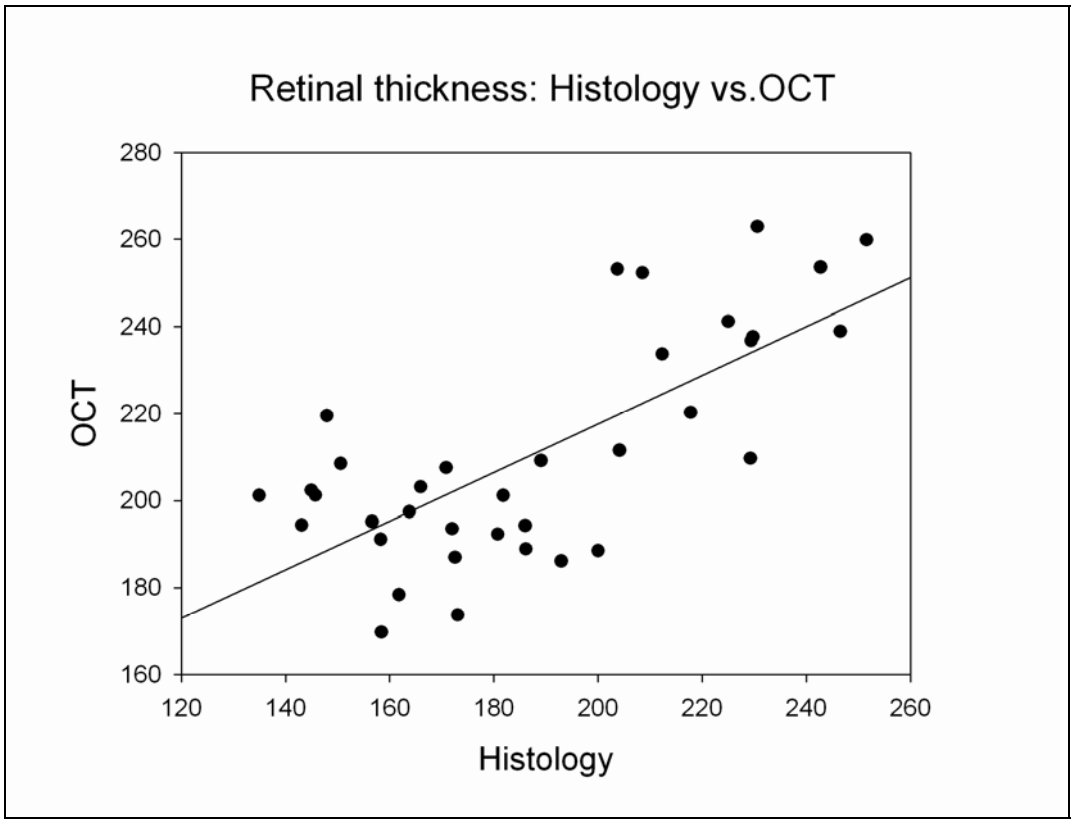

Figure 5. Histological method vs. OCT measurement.

It shows strong correlation between these two methods (R = 0.72), but generally histological values are smaller as shown in Fig 4.

3.3.3 Induced refractive errors and recovery

Before goggle treatment, the average refractive state of the right eye of the negative lens treatment group was + 6.5±1.8D (right eye), and + 6.1±1.9D (left eye). The interocular difference, 0.4±0.5D, is not significant. After two weeks of treatment with a -12D lens over the right eye, the treated eye (right eye) developed significant myopia that averaged 9.1±2.5D in terms of the average difference between the treated and untreated eyes (paired t-test, n=12, $P<0.001$). The treated eye recovered from the induced myopia one week after removal of the treatment lens (Fig. 6, top).

In the positive lens treatment group, no significant difference between the right and left eyes was found before treatment (+8.3±2.4D right eye vs. +7.9±2.5D left eye). After two weeks of treatment, the treated eye became hyperopic (average difference between the two eyes of 6.94±2.5D) and was significantly different from the untreated eye (paired t-test, n=12, $p<0.001$). The recovery process is similar to that found for induced myopia, although in the opposite direction (Fig. 6, bottom).

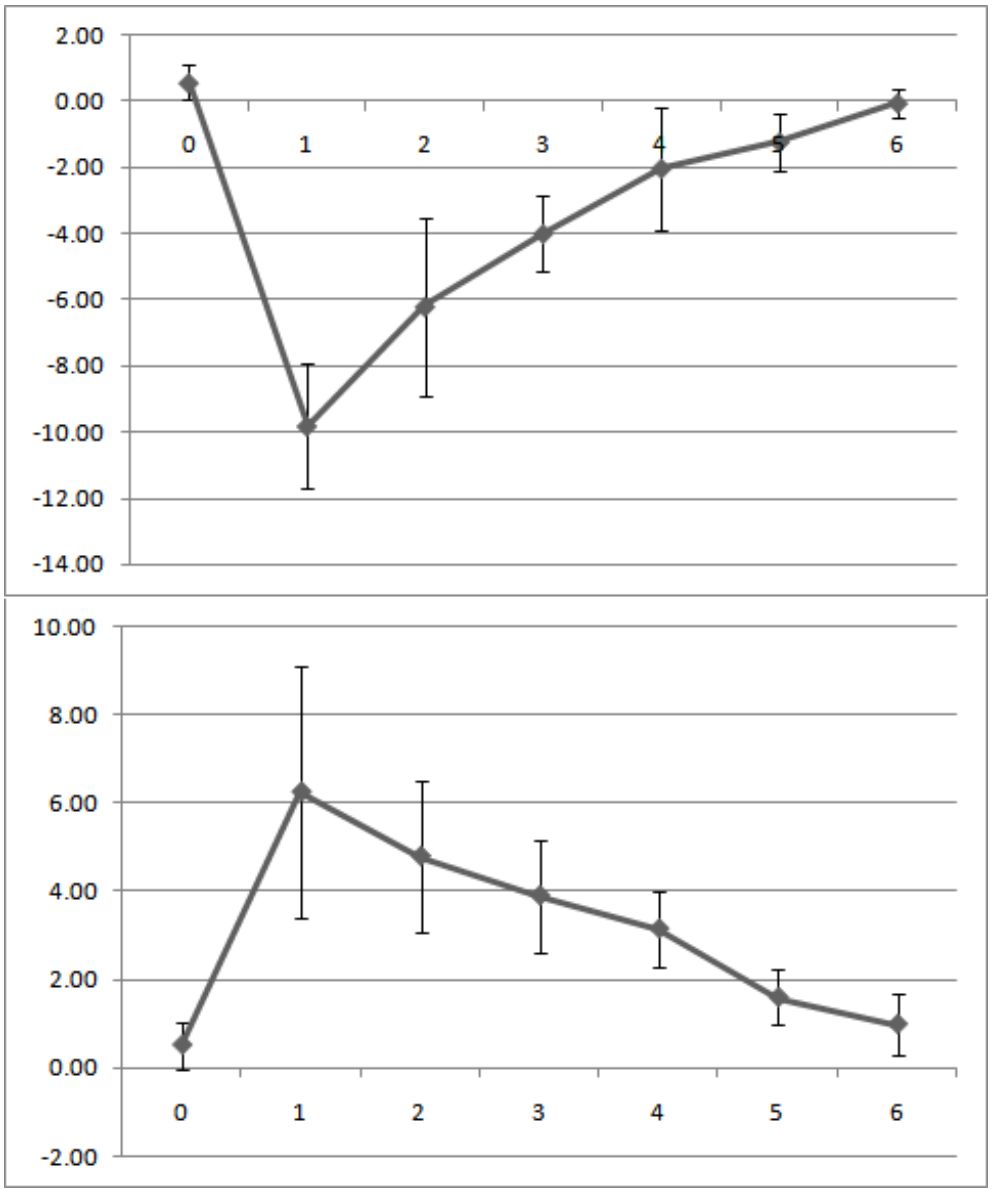

Figure 6. Induced myopia (top), hyperopia (bottom) and their recovery.

The Y axis shows the change of refractive state in diopters, and X axis, time change. 0 is when the fish eye was first treated with goggles. Day 1 repents the first day when the goggle was removed after 14 days of treatment from day 0.

3.3.4 Thickness changes (measured by OCT) of posterior segments of the fish eye in response to induced refractive errors

The thickness change in the retina is presented as the interocular difference between the treated and the contralateral control eye. The changes in retinal thickness of the negative lens treated eyes over time is significant (ANOVA, $F=3.56$, $P<0.005$), compared with the contralateral control eyes. Retinal thickness of the treated group measured by OCT was on average 186±3μm (mean ± standard error) before treatment. After wearing the lens/goggle for 2 weeks, the treated eye developed myopia, as mentioned earlier, and the retina became thinner (166±3μm) (Fig 2). During the two day period after lens/goggle removal, retinal thickness gradually increased, hourly, and then stabilized after 32 hours. Its thickness then increased slightly to average 188±2μm one week later (Fig 7, up). This change in retinal thickness with lens/goggle removal coincides with a rapid recovery from induced myopia.

In the positive lens goggle group, pre-treatment baseline retinal thickness for the eyes that were to be treated was on average 174±3μm. After two weeks of treatment, the hyperopia was induced and retinal thickness increased significantly to an average of 185±2μm In this case, retinal thickness did not change significantly after lens/goggle removal (ANOVA, $F=1.35$, $P=0.26$) (Fig 7, bottom), although the treated eye recovered from hyperopia within a week.

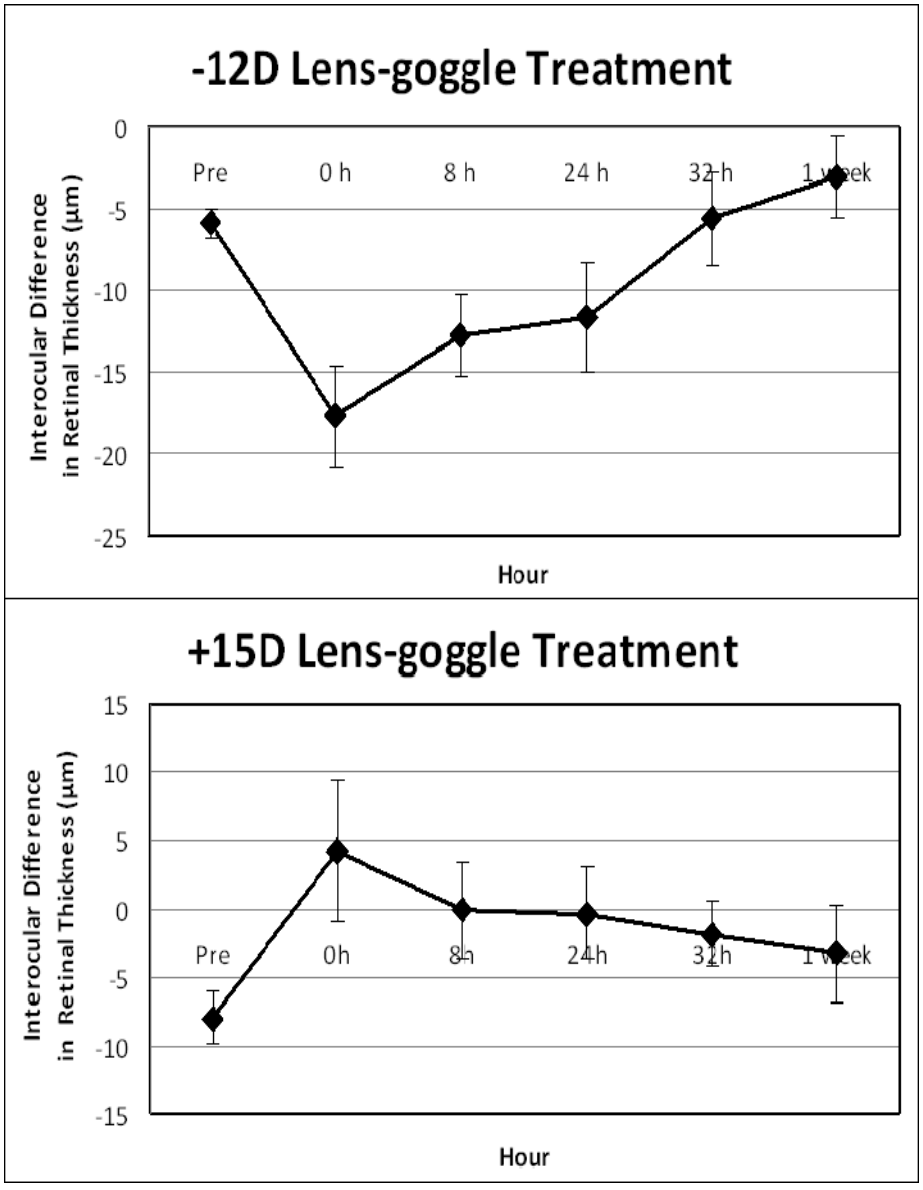

Figure 7. Change in interocular retinal thickness before and after -12D (upper) and +15D (lower one) lens goggle treatment. 0 represents the time point when the goggle is just removed and h, hour. +15D lens goggle.

From histology, the circadian thickness change of the RPE appeared to vary in daytime and darkness (Fig 8). Changes were found, but were not significant between any two daytime points (t-test, Bonferroni, $P<0.05$), but significant differences between any two points in daytime and darkness, for example, at 6am or 24pm (t-test, Bonferroni, $P<0.05$) were found. However, the OCT results did not show any significant circadian change (Table 1 and Fig 8). The correlation between OCT and histology was low ($R=0.32$). OCT measures showed that the lens/goggle treatemnet did not significantly alter the thickness of the pigment granule layer in the RPE in that there was no significant difference between the treated and contralateral eyes, neither before nor after lens /goggle treatment and during the recovery period, Table 1. OCT is likely not sensitive enough to measure the RPE thickness change.

Table 1. Change of RPE thickness before and after lens goggle treatment

	Eye	**Pre**	**0h**	**8h**	**24h**	**32h**
+15D	OD	45.85±6.53	49.76±5.05	50.22±4.48	49.10±5.01	50.05±4.43
	OS	42.56±10.35	48.20±2.12	48.53±3.96	47.05±6.22	47.83±5.76
-12D	OD	41.63±12.79	35.39±7.41	39.32±9.42	40.21±14.10	26.78±3.46
	OS	38.05±13.05	33.73±5.95	38.07±7.92	41.61±12.66	28.01±2.82

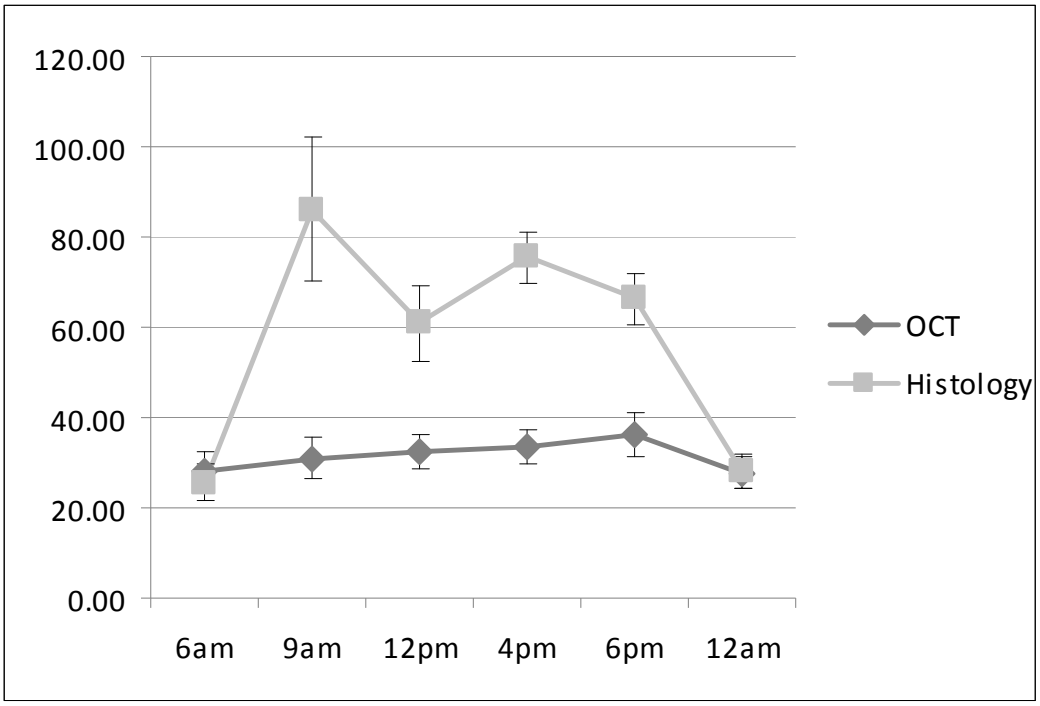

Figure 8. Circadian change of RPE thickness: Histology vs. OCT. Not like retinal thickness measurement, these two methods are not comparable. Except in the dark adapted retina, in which the RPE is very thin and condensed with all the pigment granules aggregate together, all measures during daytime are different from each other. OCT results did not show any significant change, but histology did.

Discussion

To my knowledge, it is the first time that OCT of a lower vertebrate eye was studied. Although the OCT image looks similar to that of the human eye, its histological foundation is quite different. The most important difference is the retinal motor dynamics of the fish eye. To cope with this, both OCT and histological methods were employed and resulted in retinal thickness measurements that are comparable. Non-invasive observations of retinal thickness in living fish with induced refractive errors with OCT may provide new clues as to how the eye responds to altered visual input.

The pupil size of tilapia is fixed in diameter, regardless of retinal illumination. Indeed, in most fish, the amount of light reaching the photoreceptors is controlled by local excursions of the retinal pigment and photoreceptors. In fact, photomechanical retinal mechanisms have been found not only in fishes, but also in many other species including amphibians, reptiles and birds [32]. In dim light conditions, the retinal pigment granules of the fish eye migrate in the scleral direction in the RPE cells, while the cones elongate. The retinomotor movements initiated by light are under control of melatonin and dopamine [38-40]. Sometime these activities persist despite the absence of environmental cues [38, 41].

It is reported the speed of the retinomotor movement is 3.4-3.5μm/min in *Haemulon sciurus* [42]. The relative position change of pigment granules may affect the retinal thickness measurement with OCT in the circadian cycle, especially early in the morning and at midnight when pigment granules aggregate at the bottom. However, during the daytime, the results for fish weighing between 20 and 45 grams are consistent with OCT (190 - 200μm) and with histology (150-185μm).

Myopic and hyperopic eyes showed opposite retinal thickness change after treatment and during recovery (Fig 7). The thinner retina found in the eye with lens induced myopia resembles the high myopia situation found in humans [22]. The retina and choroid in myopic eyes may simply be stretched due to the mechanical effect produced by the elongated eyeball, with retinal photoreceptor degeneration as shown in research based on the chick eye model [43]. However, tilapia retinae became relatively thicker when recovering from induced myopia and after 2 weeks of positive lens goggle treatment. Studies have shown retinal and choroidal thinning, thicker cone inner segments and damage to outer segment lamellae damaged in induced myopia [29]. Edema was produced while treated eyes recovered [29]. But in tilapia, and many other teleost fish, the retina is avascular. *O. niloticus*, the species used for these experiments, there is a lack of cytoplasmic fins in the inner segment of the cones, which might be related to fluid transportation. The mechanism behind the retinal thickness change in the tilapia eye may be different. Nevertheless, one result of the retinal thickness change is that the measured refractive status during recovery from myopia could be more hyperopic due to the edematous, thicker retina than it is supposed to be.

It is generally believed that the light from a retinoscope projected into the eye is reflected back from the inner limiting membrane of the retina. If this is the case, any possible change in the retinal or choroidal thickness may contribute to refractive alterations. In a typical tilapia eye with a lens have a back vertex distance around 2mm, as in this study, 15 - 20μm change in retinal thickness may cause about 3 diopter difference in refraction and this may partially explain the quick recovery from induced myopia in the first two days. The pattern of change of measured retinal thickness seems

to match that of focal length. However, the small eye effect could be exaggerated in a small fish eye, as small back vertex distance change may result in a large effect on retinoscopy. A VEP based rat study also suggests a lower correction factor of the artifact of small eyes[44].

The RPE may be involved in growth regulation of the eye and it is assumed to play a role in refractive error development [45]. In the case of chick eyes, it was found that the RPE undergoes some morphological changes when myopia is induced. RPE expansions occur more generally across the epithelium but was less pronounced in the temporal region compared with control eyes [28]. The fish RPE itself occupies a much larger portion of the tilapia retina, over 1/3 total thickness from histological sections (maximum about 86μm), but thinner with OCT (maximum around 36μm), in comparison to human retina [37]. The retinomotor movement of the RPE may cause a maximum difference of over 60μm in retinal thickness measurements between the histology and OCT. However, OCT was not able to detect significant change in the RPE thickness itself during a circadian cycle, as it may only pick up the peak of back scattering of the pigment granules in the RPE.

Nevertheless, it would be interesting to know whether the RPE retinal pigment granules move in response to visual signals in that this is possibly valuable in understanding the mechanism related to how the retina judges a defocused signal and compensates for the difference. Although the relative position of the inner limiting membrane may not necessarily change during retinomotor movement, would the refractive results obtained with retinoscopy be affected by the retinomotor movement?

This, together with the retinal thickness consideration, may help understand the small eye size effect in fish.

Choroidal thickness has been found to be vision-dependent in many species from birds to mammals and the range varies from 40μm to 400μm depending on the species [15, 26, 27, 46]. In contrast with the lymphatic vessel-like choroidal structure found in chicks [47] and monkeys [26], tissues posterior to the neural retina in the tilapia eye (which is a thick plastic complex composed of the choroid rete, choriocapillaris and RPE producing high oxygen pressure to supply the avascular fish retina) occupy over 1mm in an eye about 7mm in axial length at the posterior pole. Thus, the regular OCT laser beam (wavelength about 800nm) cannot fully penetrate the choroid. In further studies, a high resolution OCT equipped with a longer wavelength light source allowing deeper penetration through the RPE [48] might be able to detect choroidal reactions in response to various visual stimuli.

General Conclusion

The study in chapter 1 demonstrates for the first time that in addition to form deprivation myopia, fish eye refractive development could be manipulated, and a large amount of myopia or hyperopia induced. Together with our knowledge about other higher vertebrate models, including primates, this result suggests that early eye growth and refractive development are guided by the visual environment in a wide range of vertebrates. A universal mechanism may have developed from an early stage of vertebrate evolutionary history, possibly with growth related hormones, such as thyroid hormones, involved. Immediate and long term physiological changes of the retina, revealed with OCT, may help understand the compensatory response and local control of the retina in refractive error development.

Generally, manifest refractive errors are observed in the tilapia fish model within two weeks of visual manipulations. The form deprivation myopia induced varies from 6 to 29D, with an average 12.73D. This can be compared with the myopia induced with a -15D lens (10D). The induced hyperopia is around 8D. The time of treatment is possibly not long enough for the fish eye to fully compensate for the negative lens power or for the form deprivation effect or for the +15D lens power. However, the -15D or +12D lens power used may have exceeded the tilapia eye's plasticity. Longer term treatment could induce greater amount of myopia. One experiment showed that the amount of myopia could be as high as -30D[65]. It is not clear whether there is any form deprivation effect from the peripheral portion of the goggle during lens defocus experiments. However, the hyperopia induced did not increase significantly higher after the first week. Although

large defocus effects are produced after only one week, two weeks produce more reliable outcomes.

The small eye effect[66] might be a concern in retinoscopy of the fish eye. In my study, the normal refractive status of the fish eye is about +11D in the 14g group and the older/larger the fish, the lower hyperopic the uncorrected hyperopia. With the OCT, it is found that the retinal thickness of the tilapia eye is about 200μm and that it varies with fish weight or eye size. The back vertex distance of the fish lens from fish around 10-20g in weight is 2-2.5mm for lens diameters 2.5 to 3mm. Thus, to a small fish of around 20g, 200μm may result in a large small eye effect.

The other factor is the chromatic aberration effect. The habitat of the fish is close to blue monochromatic environment[67]. Therefore, the real refractive status is more myopic than that measured by retinoscopy with white light. However the small eye artifact has never been directly measured.

In Chapter 3, OCT was used to measure fish retinal thickness precisely, actually the distance from the inner limiting membrane to the inner boundary of the pigment granules in the RPE, including the circadian cycle of the retinomotor movement. It would be interest to learn in future work how retinoscopy is affected during the daily photomechanical movement of photoreceptors and pigment granules. One study compares the refractive error measured by retinoscopy with that by visually evoked potentials (VEP) and suggests that light may not be reflected from the inner limiting membrane and the correction factor for small eyes is much smaller than previously assumed[68]. An optical model of the tilapia eye also could be helpful in solving this problem.

Fish keep growing and increasing in size and weight through life and there appears to be an age related effect on refractive error development. The susceptibility of the tilapia fish eye to usual manipulation is strongly related to the weight or age. The heavier or the older the fish, the smaller amount of induced form deprivation myopia. When the fish are over 100g or 1 year old, it is very difficult to induce myopia. In this group, fish only developed about 2D myopia. There may exist a susceptible period (less than 60g and younger than 6 months old), but it may not be strictly correlated with age as that found in tree shrew research[39]. However, fish weighing from 10 to 30g are very sensitive to visual manipulations and used in most experiments. Although, the refractive states of the fish eye become less hyperopic and less sensitive to visual environment when aging, it is not clear whether this is a real emmetropization process or just a small eye artifact.

An age related susceptible period in response to the visual environment is found both in humans[3] and animals[39, 40]. This fact suggests that systemic hormones controlling the body and especially eye development, like growth and thyroid hormone, play an important role. In fact, growth hormone is found to be involved in emmetropization in children, but is the susceptible period under control of the growth hormone? On the other hand, thyroid hormone is vital for central nervous system development and retinal cell differentiation[47] and there is interaction between thyroid hormone and the growth hormone[69]. In this thesis, high levels of thyroid hormones partially block the form deprivation and negative lens effects and the induced refractive errors shift to more hyperopic direction. The growth rate of the eye seems to be slowed down, in spite of the fact that T3 may increase growth hormone synthesis and secretion.

Two molecules with bidirectional features useful in controlling eye growth in response to defocused visual signals, glucagon and retinoic acid, interact with thyroid hormone as well[70, 71]. Thyroid hormone action on gene transcription is selectively antagonized by retinoic acid through the retinoid X receptor[72], or the other way around. Also elevated thyroxine level stimulates excess secretion of glucagon and thus higher levels of glucagon may act as a stop signal for the eye growth[73].

The plasma thyroid hormone levels used in my research were elevated over 10 times physiological amounts. In terms of research on humans, high levels of thyroid hormone usually don't cause severe side effect[74, 75]. However, this may not be the case in tilapia.

When the image on the retina is blurred, the defocused signal is detected by the retina and the eye will undergo a series of immediate, temporary or more permanent physiological and morphological changes that could be monitored by OCT. When OCT is applied to the fish eye to observe those changes on the retina, timing is a concern due to the retinomotor movement. However, in term of retinal thickness measurement, no significant differences were found during the daytime period. As expected, the tilapia retina becomes thinner after 2 weeks of treatment with negative lens goggle, which is similar to that seen in the myopic eyes of other animals and human, possibly as the result of a stretched and degenerated retina. But the retina becomes relatively thicker in the eye of induced hyperopia, it is not understood.

Almost all fish eyes with induced refractive errors recovered within one week. Immediate change in the retinal thickness is observed as the recovery process begins. In the myopic eyes, the relatively thinner retina gradually gets thicker in the first two days

until it reaches the normal level. The reverse is found in the hyperopic eyes. This may be part of an immediate response to altered optical signals and related to the fluid exchange in different layers in the retina and choroid, as happens in the chick retina[55].

As retinal thickness changes, the retinoscopy measurement maybe also vary if the light is reflected from the inner limiting membrane. In this study, all the induced refractive errors were neutralized within a week. This is more rapid than recoveries found for other animal models, except the guinea pig[24]. The thick fish choroid rich in blood vessels may be mainly responsible for this, together with the effect of retinal thickness, change as mentioned.

One possibility can not be excluded, is that the RPE itself also undergoes morphological change as well in response to the defocused optical signal. This possibility creates a new complication but may provide a new clue as to the mechanism of myopia.

Detecting choroidal change is beyond the ability of the OCT used in my research. The movement of the pigment granules is only shown by OCT when they are in extreme positions. Nevertheless, the very thick fish choroid and choroidal rete and glands and their interaction with the RPE is still of interest and the detailed morphological changes of the choroid remain to be revealed with higher resolution OCT instruments with the light sources of longer wavelength, such as 1050nm or longer.

Compared with other animal models, the fish model can readily to produce large refractive effects when visual input is manipulated. It provides unique information about the mechanism of myopia in terms of evolutionary history and age related effects. Also,

the fish retina might be a good model to use to study visual signal processing in myopia research.

References

References to Chapter 1 :

1. Quek TP, CG C, CS C, et al. Prevalence of refractive errors in teenage high school students in Singapore. *Ophthalmic Physiol Opt* 2004;24:47-55.

2. Wallman J, Turkel J, Trachtman J. Extreme myopia produced by modest change in early visual experience. *Science* 1978;201:1249-1251.

3. Richler A, Bear JC. Refraction, nearwork and education. A population study in Newfoundland. *Acta Ophthalmol (Copenh)* 1980;58:468-478.

4. Yap M, Wu M, Liu ZM, Lee FL, Wang SH. Role of heredity in the genesis of myopia. *Ophthalmic Physiol Opt* 1993;13:316-319.

5. Mutti DO, Mitchell GL, Moeschberger ML, Jones LA, Zadnik K. Parental myopia, near work, school achievement, and children's refractive error. *Invest Ophthalmol Vis Sci* 2002;43:3633-3640.

6. Young FA. The Effect of Atropine on the Development of Myopia in Monkeys. *Am J Optom Arch Am Acad Optom* 1965;42:439-449.

7. Wiesel TN, Raviola E. Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature* 1977;266:66-68.

8. Sherman SM, Norton TT, Casagrande VA. Myopia in the lid-sutured tree shrew (Tupaia glis). *Brain Res* 1977;124:154-157.

9. McBrien NA, Moghaddam HO, New R, Williams LR. Experimental myopia in a diurnal mammal (Sciurus carolinensis) with no accommodative ability. *J Physiol* 1993;469:427-441.

10. Tejedor J, de la Villa P. Refractive changes induced by form deprivation in the mouse eye. *Invest Ophthalmol Vis Sci* 2003;44:32-36.

11. Howlett MH, McFadden SA. Form-deprivation myopia in the guinea pig (Cavia porcellus). *Vision Res* 2006;46:267-283.

12. Ouyang CH, Chu RY, Hu WZ. [Effects of pirenzepine on lens-induced myopia in the guinea-pig]. *Zhonghua Yan Ke Za Zhi* 2003;39:348-351.

13. Kirby AW, Sutton L, Weiss H. Elongation of cat eyes following neonatal lid suture. *Invest Ophthalmol Vis Sci* 1982;22:274-277.

14. Sommers D, M. I. Kaiser-Kupfer and C. Kupfer. Increased axial length of the eye following neonatal lid suture as measured with A-scan ultrasonography. *Ophthalmol Vis Sci* 1978;17(Suppl):295.

15. Wilson JR, Sherman SM. Differential effects of early monocular deprivation on binocular and monocular segments of cat striate cortex. *J Neurophysiol* 1977;40:891-903.

16. Andison ME, Sivak JG, Bird DM. The refractive development of the eye of the American kestrel (Falco sparverius): a new avian model. *J Comp Physiol [A]* 1992;170:565-574.

17. Siegwart JT, Jr., Norton TT. The susceptible period for deprivation-induced myopia in tree shrew. *Vision Res* 1998;38:3505-3515.

18. Wallman J, Adams JI. Developmental aspects of experimental myopia in chicks: susceptibility, recovery and relation to emmetropization. *Vision Res* 1987;27:1139-1163.

19. Schaeffel F, Glasser A, Howland HC. Accommodation, refractive error and eye growth in chickens. *Vision Res* 1988;28:639-657.

20. Irving EL, Sivak JG, Callender MG. Refractive plasticity of the developing chick eye. *Ophthalmic Physiol Opt* 1992;12:448-456.

21. Irving EL, Callender MG, Sivak JG. Inducing ametropias in hatchling chicks by defocus--aperture effects and cylindrical lenses. *Vision Res* 1995;35:1165-1174.

22. Schmid K, Wildsoet CF. Natural and imposed astigmatism and their relation to emmetropization in the chick. *Exp Eye Res* 1997;64:837-847.

23. McLean RC, Wallman J. Severe astigmatic blur does not interfere with spectacle lens compensation. *Invest Ophthalmol Vis Sci* 2003;44:449-457.

24. Troilo D, Gottlieb MD, Wallman J. Visual deprivation causes myopia in chicks with optic nerve section. *Curr Eye Res* 1987;6:993-999.

25. Wallman J, Xu A, Wildsoet C, et al. Moving the retina: a third mechanism of focusing the eye. *Invest Ophthalmol Vis Sci* 1992;1053.

26. Wallman J, Wildsoet C, Xu A, et al. Moving the retina: choroidal modulation of refractive state. *Vision Res* 1995;35:37-50.

27. Lin LL, Shih YF, Tsai CB, et al. Epidemiologic study of ocular refraction among schoolchildren in Taiwan in 1995. *Optom Vis Sci* 1999;76:275-281.

28. Rohrer B, Stell WK. Basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-beta) act as stop and go signals to modulate postnatal ocular growth in the chick. *Exp Eye Res* 1994;58:553-561.

29. Seko Y, Tanaka Y, Tokoro T. Influence of bFGF as a potent growth stimulator and TGF-beta as a growth regulator on scleral chondrocytes and scleral fibroblasts in vitro. *Ophthalmic Res* 1995;27:144-152.

30. Qiao-Grider Y, Hung LF, Kee CS, Ramamirtham R, Smith EL, 3rd. Recovery from form-deprivation myopia in rhesus monkeys. *Invest Ophthalmol Vis Sci* 2004;45:3361-3372.

31. Shen W, Vijayan M, Sivak JG. Inducing form-deprivation myopia in fish. *Invest Ophthalmol Vis Sci* 2005;46:1797-1803.

32. Lyall A. The growth of the trout retina. *Q J Microsc Sci* 1957;101-110.

33. Hueter RE, Gruber SH. Retinoscopy of aquatic eyes. *Vision Res* 1980;20:197-200.

34. Smith EL, 3rd, Hung LF, Harwerth RS. Effects of optically induced blur on the refractive status of young monkeys. *Vision Res* 1994;34:293-301.

35. Wildsoet C, Wallman J. Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. *Vision Res* 1995;35:1175-1194.

36. Kisilak M. H, J.J., Huang L., Campbell, M.C.W. and Irving, E.L. In chicks wearing high powered negative lenses, spherical aberration is compensated and oblique astigmatism is induced. J. of Modern Optics. *J of Modern Optics (in press)* 2007.

37. Sivak J, Howland HC. Accommodation in the northern rock bass (Ambloplites rupestris rupestris) in response to natural stimuli. *Vision Res* 1973;13:2059-2064.

38. Millodot M, Sivak J. Hypermetropia of small animals and chromatic aberration. *Vision Res* 1978;18:125-126.

39. Glickstein M, Millodot M. Retinoscopy and eye size. *Science* 1970;168:605-606.

40. Hughes A. The artefact of retinoscopy in the rat and rabbit eye has its origin at the retina/vitreous interface rather than in longitudinal chromatic aberration. *Vision Res* 1979;19:1293-1294.

41. McBrien NA, Lawlor P, Gentle A. Scleral remodeling during the development of and recovery from axial myopia in the tree shrew. *Invest Ophthalmol Vis Sci* 2000;41:3713-3719.

42. Al Hafedh YS. Effects of dietary protein on growth and body composition of Nile tilapia, Oreochromis niloticus L. *Aquaculture Research* 1999;30:385-393.

43. El-Sayed A-FME-G, A ; Al-Amoudi, M Effects of pond depth and water temperature on the growth, mortality and body composition of Nile tilapia, Oreochromis niloticus (L.). *Aquaculture Research* 1996;27:681-688.

44. McBrien NA, Norton TT. The development of experimental myopia and ocular component dimensions in monocularly lid-sutured tree shrews (Tupaia belangeri). *Vision Res* 1992;32:843-852.

45. Guggenheim JA, McBrien NA. Form-deprivation myopia induces activation of scleral matrix metalloproteinase-2 in tree shrew. *Invest Ophthalmol Vis Sci* 1996;37:1380-1395.

46. Troilo D, Nickla DL, Wildsoet CF. Choroidal thickness changes during altered eye growth and refractive state in a primate. *Invest Ophthalmol Vis Sci* 2000;41:1249-1258.

47. Hung LF, Wallman J, Smith EL, 3rd. Vision-dependent changes in the choroidal thickness of macaque monkeys. *Invest Ophthalmol Vis Sci* 2000;41:1259-1269.

48. Gentle A, McBrien NA. Modulation of scleral DNA synthesis in development of and recovery from induced axial myopia in the tree shrew. *Exp Eye Res* 1999;68:155-163.

References to Chapter 2:

1. Wallman J, Turkel J, Trachtman J. Extreme myopia produced by modest change in early visual experience. *Science* 1978;201:1249-1251.

2. Young FA. The Effect of Atropine on the Development of Myopia in Monkeys. *Am J Optom Arch Am Acad Optom* 1965;42:439-449.

3. Wiesel TN, Raviola E. Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature* 1977;266:66-68.

4. Sherman SM, Norton TT, Casagrande VA. Myopia in the lid-sutured tree shrew (Tupaia glis). *Brain Res* 1977;124:154-157.

5. McBrien NA, Moghaddam HO, New R, Williams LR. Experimental myopia in a diurnal mammal (Sciurus carolinensis) with no accommodative ability. *J Physiol* 1993;469:427-441.

6. Tejedor J, de la Villa P. Refractive changes induced by form deprivation in the mouse eye. *Invest Ophthalmol Vis Sci* 2003;44:32-36.

7. Howlett MH, McFadden SA. Form-deprivation myopia in the guinea pig (Cavia porcellus). *Vision Res* 2006;46:267-283.

8. Ouyang CH, Chu RY, Hu WZ. [Effects of pirenzepine on lens-induced myopia in the guinea-pig]. *Zhonghua Yan Ke Za Zhi* 2003;39:348-351.

9. Kirby AW, Sutton L, Weiss H. Elongation of cat eyes following neonatal lid suture. *Invest Ophthalmol Vis Sci* 1982;22:274-277.

10. Sommers D, M. I. Kaiser-Kupfer and C. Kupfer. Increased axial length of the eye following neonatal lid suture as measured with A-scan ultrasonography. *Ophthalmol Vis Sci* 1978;17(Suppl):295.

11. Andison ME, Sivak JG, Bird DM. The refractive development of the eye of the American kestrel (Falco sparverius): a new avian model. *J Comp Physiol [A]* 1992;170:565-574.

12. Shen W, Sivak JG. Eyes of a lower vertebrate are susceptible to the visual environment. *Invest Ophthalmol Vis Sci* 2007;48:4829-4837.

13. Wallman J, Adams JI. Developmental aspects of experimental myopia in chicks: susceptibility, recovery and relation to emmetropization. *Vision Res* 1987;27:1139-1163.

14. Troilo D, Nickla DL. The response to visual form deprivation differs with age in marmosets. *Invest Ophthalmol Vis Sci* 2005;46:1873-1881.

15. Smith EL, 3rd, Harwerth RS, Crawford ML, von Noorden GK. Observations on the effects of form deprivation on the refractive status of the monkey. *Invest Ophthalmol Vis Sci* 1987;28:1236-1245.

16. Siegwart JT, Jr., Norton TT. The susceptible period for deprivation-induced myopia in tree shrew. *Vision Res* 1998;38:3505-3515.

17. Zhao J, Pan X, Sui R, Munoz SR, Sperduto RD, Ellwein LB. Refractive Error Study in Children: results from Shunyi District, China. *Am J Ophthalmol* 2000;129:427-435.

18. Chua WH SS, Wu HM, Hong CY, Chan WY, Wong A, Chia KS, Tan D. . Refractive errors in schoolchildren: The Singapore Myopia Cohort Study. . *Proceedings of the 7th International Conference on Myopia; Boston, United States United States: Conference on Myopia 2000* 2000 July.

19. Norton TT, Gamlin PD. The near response, emmetropia and myopia. *Ophthalmic Physiol Opt* 1999;19:79-80.

20. Troilo D, Gottlieb MD, Wallman J. Visual deprivation causes myopia in chicks with optic nerve section. *Curr Eye Res* 1987;6:993-999.

21. Wallman J, Wildsoet C, Xu A, et al. Moving the retina: choroidal modulation of refractive state. *Vision Res* 1995;35:37-50.

22. Lin LL, Shih YF, Tsai CB, et al. Epidemiologic study of ocular refraction among schoolchildren in Taiwan in 1995. *Optom Vis Sci* 1999;76:275-281.

23. Rohrer B, Stell WK. Basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-beta) act as stop and go signals to modulate postnatal ocular growth in the chick. *Exp Eye Res* 1994;58:553-561.

24. Seko Y, Tanaka Y, Tokoro T. Influence of bFGF as a potent growth stimulator and TGF-beta as a growth regulator on scleral chondrocytes and scleral fibroblasts in vitro. *Ophthalmic Res* 1995;27:144-152.

25. Stone RA, Lin T, Laties AM, Iuvone PM. Retinal dopamine and form-deprivation myopia. *Proc Natl Acad Sci U S A* 1989;86:704-706.

26. Guo SS, Sivak JG, Callender MG, Diehl-Jones B. Retinal dopamine and lens-induced refractive errors in chicks. *Curr Eye Res* 1995;14:385-389.

27. Pendrak K, Nguyen T, Lin T, Capehart C, Zhu X, Stone RA. Retinal dopamine in the recovery from experimental myopia. *Curr Eye Res* 1997;16:152-157.

28. Stone RA, Liu J, Sugimoto R, Capehart C, Zhu X, Pendrak K. GABA, experimental myopia, and ocular growth in chick. *Invest Ophthalmol Vis Sci* 2003;44:3933-3946.

29. Summers Rada JA, Shelton S, Norton TT. The sclera and myopia. *Exp Eye Res* 2006;82:185-200.

30. Hoffmann M, Schaeffel F. Melatonin and deprivation myopia in chickens. *Neurochem Int* 1996;28:95-107.

31. Hoskins SG, Grobstein P. Development of the ipsilateral retinothalamic projection in the frog Xenopus laevis. III. The role of thyroxine. *J Neurosci* 1985;5:930-940.

32. Miwa S, Tagawa M, Inui Y, Hirano T. Thyroxine surge in metamorphosing flounder larvae. *Gen Comp Endocrinol* 1988;70:158-163.

33. Browman HI, Hawryshyn CW. Thyroxine induces a precocial loss of ultraviolet photosensitivity in rainbow trout (Oncorhynchus mykiss, Teleostei). *Vision Res* 1992;32:2303-2312.

34. Browman HI, Hawryshyn CW. The developmental trajectory of ultraviolet photosensitivity in rainbow trout is altered by thyroxine. *Vision Res* 1994;34:1397-1406.

35. Hawryshyn CW, Martens G, Allison WT, Anholt BR. Regeneration of ultraviolet-sensitive cones in the retinal cone mosaic of thyroxin-challenged post-juvenile rainbow trout (Oncorhynchus mykiss). *J Exp Biol* 2003;206:2665-2673.

36. Allison WT, Dann SG, Helvik JV, Bradley C, Moyer HD, Hawryshyn CW. Ontogeny of ultraviolet-sensitive cones in the retina of rainbow trout (Oncorhynchus mykiss). *J Comp Neurol* 2003;461:294-306.

37. Beatty DD. Visual pigment changes in salmonid fishes in response to exogenous L-thyroxine, bovine TSH and 3-dehydroretinol. *Vision Res* 1972;12:1947-1960.

38. Beatty DD. Visual pigment changes in juvenile kokanee salmon in response to thyroid hormones. *Vision Res* 1969;9:855-864.

39. de Luze A, Leloup J. Fish growth hormone enhances peripheral conversion of thyroxine to triiodothyronine in the eel (Anguilla anguilla L.). *Gen Comp Endocrinol* 1984;56:308-312.

40. Byamungu N, Mol K, Kuhn ER. Somatostatin increases plasma T3 concentrations in Tilapia nilotica in the presence of increased plasma T4 levels. *Gen Comp Endocrinol* 1991;82:401-406.

41. Reddy PK, Lam TJ. Effect of thyroid hormones on hatching in the tilapia, Oreochromis mossambicus. *Gen Comp Endocrinol* 1991;81:484-491.

42. Shen W, Vijayan M, Sivak JG. Inducing form-deprivation myopia in fish. *Invest Ophthalmol Vis Sci* 2005;46:1797-1803.

43. Hueter RE, Gruber SH. Retinoscopy of aquatic eyes. *Vision Res* 1980;20:197-200.

44. Vessey KA, Rushforth DA, Stell WK. Glucagon- and secretin-related peptides differentially alter ocular growth and the development of form-deprivation myopia in chicks. *Invest Ophthalmol Vis Sci* 2005;46:3932-3942.

45. Vessey KA, Lencses KA, Rushforth DA, Hruby VJ, Stell WK. Glucagon receptor agonists and antagonists affect the growth of the chick eye: a role for glucagonergic regulation of emmetropization? *Invest Ophthalmol Vis Sci* 2005;46:3922-3931.

46. Feldkaemper MP, Schaeffel F. Evidence for a potential role of glucagon during eye growth regulation in chicks. *Vis Neurosci* 2002;19:755-766.

47. Ito A, Yamaguchi K, Tomita H, et al. Distribution of rat organic anion transporting polypeptide-E (oatp-E) in the rat eye. *Invest Ophthalmol Vis Sci* 2003;44:4877-4884.

48. Siegwart JT, Jr., Norton TT. Selective regulation of MMP and TIMP mRNA levels in tree shrew sclera during minus lens compensation and recovery. *Invest Ophthalmol Vis Sci* 2005;46:3484-3492.

49. Smith EL, 3rd, Maguire GW, Watson JT. Axial lengths and refractive errors in kittens reared with an optically induced anisometropia. *Invest Ophthalmol Vis Sci* 1980;19:1250-1255.

50. Litovitz TL, White JD. Levothyroxine ingestions in children: an analysis of 78 cases. *Am J Emerg Med* 1985;3:297-300.

51. White JD, Litovitz TL. Levothyroxine poisoning. *Pediatrics* 1985;75:129-130.

52. hutchison MJ IM. Effect of thyroxine on the decrease of aggressive behavior of four salmonids during the parr-smolt transformation. *Aquaculture* 1998;168:169-175.

53. Walpita CN, Grommen SV, Darras VM, Van der Geyten S. The influence of stress on thyroid hormone production and peripheral deiodination in the Nile tilapia (Oreochromis niloticus). *Gen Comp Endocrinol* 2007;150:18-25.

54. McBrien NA, Norton TT. The development of experimental myopia and ocular component dimensions in monocularly lid-sutured tree shrews (Tupaia belangeri). *Vision Res* 1992;32:843-852.

55. Dutta H. Growth in fishes. *Gerontology* 1994;40:97-112.

56. Renauld A, Sverdlik RC, Andrade LL, Rodriguez RR. Experimental hyperthyroidism in dogs. Evaluation and effects on blood sugar, serum insulin and free fatty acids during glibenclamide infusion test. *Horm Metab Res* 1975;7:382-385.

57. Parentin F, Tonini G, Perissutti P. Refractive evaluation in children with growth defect. *Curr Eye Res* 2004;28:11-15.

58. Parentin F, Perissutti P. Congenital growth hormone deficiency and eye refraction: a longitudinal study. *Ophthalmologica* 2005;219:226-231.

59. Farchi-Pisanty O, Hackett PB, Jr., Moav B. Regulation of fish growth hormone transcription. *Mol Mar Biol Biotechnol* 1995;4:215-223.

60. Cabello G, Wrutniak C. Thyroid hormone and growth: relationships with growth hormone effects and regulation. *Reprod Nutr Dev* 1989;29:387-402.

61. Fischer AJ, McGuire JJ, Schaeffel F, Stell WK. Light- and focus-dependent expression of the transcription factor ZENK in the chick retina. *Nat Neurosci* 1999;2:706-712.

62. Wolf E, Eisenstein AB. Portal vein blood insulin and glucagon are increased in experimental hyperthyroidism. *Endocrinology* 1981;108:2109-2113.

63. Mitchell MA, Raza A. The effects of glucagon and insulin on plasma thyroid hormone levels in fed and fasted domestic fowls. *Comp Biochem Physiol A* 1986;85:217-223.

64. McFadden SA, Howlett MH, Mertz JR. Retinoic acid signals the direction of ocular elongation in the guinea pig eye. *Vision Res* 2004;44:643-653.

65. Bitzer M, Feldkaemper M, Schaeffel F. Visually induced changes in components of the retinoic acid system in fundal layers of the chick. *Exp Eye Res* 2000;70:97-106.

66. Seko Y, Shimizu M, Tokoro T. Retinoic acid increases in the retina of the chick with form deprivation myopia. *Ophthalmic Res* 1998;30:361-367.

67. Kliewer SA, Umesono K, Mangelsdorf DJ, Evans RM. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. *Nature* 1992;355:446-449.

68. Davis KD, Lazar MA. Selective antagonism of thyroid hormone action by retinoic acid. *J Biol Chem* 1992;267:3185-3189.

69. Garcia-Villalba P, Au-Fliegner M, Samuels HH, Aranda A. Interaction of thyroid hormone and retinoic acid receptors on the regulation of the rat growth hormone gene promoter. *Biochem Biophys Res Commun* 1993;191:580-586.

70. van Doorn KL, Sivak JG, Vijayan MM. Optical quality changes of the ocular lens during induced parr-to-smolt metamorphosis in Rainbow Trout (Oncorhynchus mykiss). Ocular lens optical quality during induced salmonid metamorphosis. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2005;191:649-657.

References to Chapter 3:

1. Yap M, Wu M, Liu ZM, Lee FL, Wang SH. Role of heredity in the genesis of myopia. *Ophthalmic Physiol Opt* 1993;13:316-319.

2. Mutti DO, Mitchell GL, Moeschberger ML, Jones LA, Zadnik K. Parental myopia, near work, school achievement, and children's refractive error. *Invest Ophthalmol Vis Sci* 2002;43:3633-3640.

3. Richler A, Bear JC. Refraction, nearwork and education. A population study in Newfoundland. *Acta Ophthalmol (Copenh)* 1980;58:468-478.

4. Wallman J, Turkel J, Trachtman J. Extreme myopia produced by modest change in early visual experience. *Science* 1978;201:1249-1251.

5. Wiesel TN. Effects of monocular deprivation on the cat's visual cortex. *Trans Am Acad Ophthalmol Otolaryngol* 1971;75:1186-1198.

6. Wiesel TN, Raviola E. Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature* 1977;266:66-68.

7. Sherman SM, Norton TT, Casagrande VA. Myopia in the lid-sutured tree shrew (Tupaia glis). *Brain Res* 1977;124:154-157.

8. McBrien NA, Moghaddam HO, Reeder AP. Atropine reduces experimental myopia and eye enlargement via a nonaccommodative mechanism. *Invest Ophthalmol Vis Sci* 1993;34:205-215.

9. Tejedor J, de la Villa P. Refractive changes induced by form deprivation in the mouse eye. *Invest Ophthalmol Vis Sci* 2003;44:32-36.

10. Howlett MH, McFadden SA. Form-deprivation myopia in the guinea pig (Cavia porcellus). *Vision Res* 2006;46:267-283.

11. Andison ME, Sivak JG, Bird DM. The refractive development of the eye of the American kestrel (Falco sparverius): a new avian model. *J Comp Physiol [A]* 1992;170:565-574.

12. Shen W, Vijayan M, Sivak JG. Inducing form-deprivation myopia in fish. *Invest Ophthalmol Vis Sci* 2005;46:1797-1803.

13. Schaeffel F, Glasser A, Howland HC. Accommodation, refractive error and eye growth in chickens. *Vision Res* 1988;28:639-657.

14. Irving EL, Callender MG, Sivak JG. Inducing ametropias in hatchling chicks by defocus--aperture effects and cylindrical lenses. *Vision Res* 1995;35:1165-1174.

15. Hung LF, Wallman J, Smith EL, 3rd. Vision-dependent changes in the choroidal thickness of macaque monkeys. *Invest Ophthalmol Vis Sci* 2000;41:1259-1269.

16. Ouyang CH, Chu RY, Hu WZ. [Effects of pirenzepine on lens-induced myopia in the guinea-pig]. *Zhonghua Yan Ke Za Zhi* 2003;39:348-351.

17. Wallman J, Gottlieb MD, Rajaram V, Fugate-Wentzek LA. Local retinal regions control local eye growth and myopia. *Science* 1987;237:73-77.

18. Norton TT. Experimental myopia in tree shrews. *Ciba Found Symp* 1990;155:178-194; discussion 194-179.

19. Qiao-Grider Y, Hung LF, Kee CS, Ramamirtham R, Smith EL, 3rd. Recovery from form-deprivation myopia in rhesus monkeys. *Invest Ophthalmol Vis Sci* 2004;45:3361-3372.

20. Mei Q, Rong Z. Early signs of myopia in Chinese schoolchildren. *Optom Vis Sci* 1994;71:14-16.

21. Mrugacz M, Bakunowicz-Lazarczyk A, Sredzinska-Kita D. Use of optical coherence tomography in myopia. *J Pediatr Ophthalmol Strabismus* 2004;41:159-162.

22. Mrugacz M, Bakunowicz-lazarczyk A. [Measurement of retinal thickness using optical coherence tomography in patients with myopia]. *Klin Oczna* 2005;107:68-69.

23. Lo PI, Ho PC, Lau JT, Cheung AY, Goldschmidt E, Tso MO. Relationship between myopia and optical components--a study among Chinese Hong Kong student population. *Yan Ke Xue Bao* 1996;12:121-125.

24. Marsh-Tootle WL, Norton TT. Refractive and structural measures of lid-suture myopia in tree shrew. *Invest Ophthalmol Vis Sci* 1989;30:2245-2257.

25. Wallman J, Xu A, Wildsoet C, et al. Moving the retina: a third mechanism of focusing the eye. *Invest Ophthalmol Vis Sci* 1992;1053.

26. Wallman J, Wildsoet C, Xu A, et al. Moving the retina: choroidal modulation of refractive state. *Vision Res* 1995;35:37-50.

27. Troilo D, Nickla DL, Wildsoet CF. Choroidal thickness changes during altered eye growth and refractive state in a primate. *Invest Ophthalmol Vis Sci* 2000;41:1249-1258.

28. Shih YF, Fitzgerald ME, Norton TT, Gamlin PD, Hodos W, Reiner A. Reduction in choroidal blood flow occurs in chicks wearing goggles that induce eye growth toward myopia. *Curr Eye Res* 1993;12:219-227.

29. Liang H, Crewther SG, Crewther DP, Junghans BM. Structural and elemental evidence for edema in the retina, retinal pigment epithelium, and choroid during recovery from experimentally induced myopia. *Invest Ophthalmol Vis Sci* 2004;45:2463-2474.

30. Kusakari T, Sato T, Tokoro T. Visual deprivation stimulates the exchange of the fibrous sclera into the cartilaginous sclera in chicks. *Exp Eye Res* 2001;73:533-546.

31. Marzani D, Wallman J. Growth of the two layers of the chick sclera is modulated reciprocally by visual conditions. *Invest Ophthalmol Vis Sci* 1997;38:1726-1739.

32. Walls G. The vertebrate eye and its adaptive radiation. *Cranbrook Institute of Science, Bloomfield Hills, Michigan* 1942.

33. Toth CA, Narayan DG, Boppart SA, et al. A comparison of retinal morphology viewed by optical coherence tomography and by light microscopy. *Arch Ophthalmol* 1997;115:1425-1428.

34. Li Q, Timmers AM, Hunter K, et al. Noninvasive imaging by optical coherence tomography to monitor retinal degeneration in the mouse. *Invest Ophthalmol Vis Sci* 2001;42:2981-2989.

35. Ducros MG, de Boer JF, Huang HE, et al. Polarization sensitive optical coherence tomography of the rabbit eye. *Ieee Journal of Selected Topics in Quantum Electronics* 1999;5:1159-1167.

36. Bizheva K, Pflug R, Hermann B, et al. Optophysiology: depth-resolved probing of retinal physiology with functional ultrahigh-resolution optical coherence tomography. *Proc Natl Acad Sci U S A* 2006;103:5066-5071.

37. Chauhan DS, Marshall J. The interpretation of optical coherence tomography images of the retina. *Invest Ophthalmol Vis Sci* 1999;40:2332-2342.

38. Dearry A, Burnside B. Dopaminergic regulation of cone retinomotor movement in isolated teleost retinas: II. Modulation by gamma-aminobutyric acid and serotonin. *J Neurochem* 1986;46:1022-1031.

39. Stenkamp DL, Iuvone PM, Adler R. Photomechanical movements of cultured embryonic photoreceptors: regulation by exogenous neuromodulators and by a regulable source of endogenous dopamine. *J Neurosci* 1994;14:3083-3096.

40. Bumside B DA. Cell motilitv in the retina. In: The retina: a model for cell biology studies (Adler-R, Farber D, eds)

Orlando: Acadamic 1986;152-206.

41. Kremser B, Troger J, Baltaci M, Kralinger M, Kieselbach GF. Retinal thickness analysis in subjects with different refractive conditions. *Ophthalmologica* 1999;213:376-379.

42. Burnside B, Basinger S. Retinomotor pigment migration in the teleost retinal pigment epithelium. II. Cyclic-3',5'-adenosine monophosphate induction of dark-adaptive movement in vitro. *Invest Ophthalmol Vis Sci* 1983;24:16-23.

43. Hirata A, Negi A. Lacquer crack lesions in experimental chick myopia. *Graefes Arch Clin Exp Ophthalmol* 1998;236:138-145.

44. Mutti DO, Ver Hoeve JN, Zadnik K, Murphy CJ. The artifact of retinoscopy revisited: comparison of refractive error measured by retinoscopy and visual evoked potential in the rat. *Optom Vis Sci* 1997;74:483-488.

45. Rymer J, Wildsoet CF. The role of the retinal pigment epithelium in eye growth regulation and myopia: a review. *Vis Neurosci* 2005;22:251-261.

46. Siegwart JT, Jr., Norton TT. The susceptible period for deprivation-induced myopia in tree shrew. *Vision Res* 1998;38:3505-3515.

47. De Stefano ME, Mugnaini E. Fine structure of the choroidal coat of the avian eye. Vascularization, supporting tissue and innervation. *Anat Embryol (Berl)* 1997;195:393-418.

48. Bizheva K, Povazay B, Hermann B, et al. Compact, broad-bandwidth fiber laser for sub-2-microm axial resolution optical coherence tomography in the 1300-nm wavelength region. *Opt Lett* 2003;28:707-709.

References to General Introduction and Conclusion:

1. Yap M, Wu M, Liu ZM, Lee FL, Wang SH. Role of heredity in the genesis of myopia. *Ophthalmic Physiol Opt* 1993;13:316-319.

2. Saw SM, Chua WH, Wu HM, Yap E, Chia KS, Stone RA. Myopia: gene-environment interaction. *Ann Acad Med Singapore* 2000;29:290-297.

3. Saw SM, Katz J, Schein OD, Chew SJ, Chan TK. Epidemiology of myopia. *Epidemiol Rev* 1996;18:175-187.

4. Jones LA, Sinnott LT, Mutti DO, Mitchell GL, Moeschberger ML, Zadnik K. Parental history of myopia, sports and outdoor activities, and future myopia. *Invest Ophthalmol Vis Sci* 2007;48:3524-3532.

5. Rose KA, Morgan IG, Ip J, et al. Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology* 2008;115:1279-1285.

6. Rose KA, Morgan IG, Smith W, Burlutsky G, Mitchell P, Saw SM. Myopia, lifestyle, and schooling in students of Chinese ethnicity in Singapore and Sydney. *Arch Ophthalmol* 2008;126:527-530.

7. Young FA. Reading, measures of intelligence and refractive errors. *Am J Optom Arch Am Acad Optom* 1963;40:257-264.

8. Baldwin WR. A review of statistical studies of relations between myopia and ethnic, behavioral, and physiological characteristics. *Am J Optom Physiol Opt* 1981;58:516-527.

9. Loman J, Quinn GE, Kamoun L, et al. Darkness and near work: myopia and its progression in third-year law students. *Ophthalmology* 2002;109:1032-1038.

10. Wildsoet CF, Howland HC, Falconer S, Dick K. Chromatic aberration and accommodation: their role in emmetropization in the chick. *Vision Res* 1993;33:1593-1603.

11. Troilo D, Gottlieb MD, Wallman J. Visual deprivation causes myopia in chicks with optic nerve section. *Curr Eye Res* 1987;6:993-999.

12. Rohrer B, Schaeffel F, Zrenner E. Longitudinal chromatic aberration and emmetropization: results from the chicken eye. *J Physiol* 1992;449:363-376.

13. Schaeffel F, Howland HC. Properties of the feedback loops controlling eye growth and refractive state in the chicken. *Vision Res* 1991;31:717-734.

14. McLean RC, Wallman J. Severe astigmatic blur does not interfere with spectacle lens compensation. *Invest Ophthalmol Vis Sci* 2003;44:449-457.

15. Kisilak ML, Campbell MC, Hunter JJ, Irving EL, Huang L. Aberrations of chick eyes during normal growth and lens induction of myopia. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2006;192:845-855.

16. Young FA. The Effect of Atropine on the Development of Myopia in Monkeys. *Am J Optom Arch Am Acad Optom* 1965;42:439-449.

17. Wiesel TN. Effects of monocular deprivation on the cat's visual cortex. *Trans Am Acad Ophthalmol Otolaryngol* 1971;75:1186-1198.

18. Wiesel TN, Raviola E. Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature* 1977;266:66-68.

19. Sherman SM, Norton TT, Casagrande VA. Myopia in the lid-sutured tree shrew (Tupaia glis). *Brain Res* 1977;124:154-157.

20. Wallman J, Turkel J, Trachtman J. Extreme myopia produced by modest change in early visual experience. *Science* 1978;201:1249-1251.

21. Andison ME, Sivak JG, Bird DM. The refractive development of the eye of the American kestrel (Falco sparverius): a new avian model. *J Comp Physiol [A]* 1992;170:565-574.

22. Troilo D, Judge SJ. Ocular development and visual deprivation myopia in the common marmoset (Callithrix jacchus). *Vision Res* 1993;33:1311-1324.

23. Ouyang CH, Chu RY, Hu WZ. [Effects of pirenzepine on lens-induced myopia in the guinea-pig]. *Zhonghua Yan Ke Za Zhi* 2003;39:348-351.

24. Howlett MH, McFadden SA. Form-deprivation myopia in the guinea pig (Cavia porcellus). *Vision Res* 2006;46:267-283.

25. Tejedor J, de la Villa P. Refractive changes induced by form deprivation in the mouse eye. *Invest Ophthalmol Vis Sci* 2003;44:32-36.

26. Shen W, Vijayan M, Sivak JG. Inducing form-deprivation myopia in fish. *Invest Ophthalmol Vis Sci* 2005;46:1797-1803.

27. Rabin J, Van Sluyters RC, Malach R. Emmetropization: a vision-dependent phenomenon. *Invest Ophthalmol Vis Sci* 1981;20:561-564.

28. Vadot E, Turcat JP, Pellat B. [Unilateral congenital cataract makes the eye myopic]. *Bull Soc Ophtalmol Fr* 1983;83:965-966.

29. Winawer J, Wallman J. Temporal constraints on lens compensation in chicks. *Vision Res* 2002;42:2651-2668.

30. Zhu X, Winawer JA, Wallman J. Potency of myopic defocus in spectacle lens compensation. *Invest Ophthalmol Vis Sci* 2003;44:2818-2827.

31. Fischer AJ, McGuire JJ, Schaeffel F, Stell WK. Light- and focus-dependent expression of the transcription factor ZENK in the chick retina. *Nat Neurosci* 1999;2:706-712.

32. Seko Y, Shimokawa H, Tokoro T. In vivo and in vitro association of retinoic acid with form-deprivation myopia in the chick. *Exp Eye Res* 1996;63:443-452.

33. Stone RA, Lin T, Laties AM, Iuvone PM. Retinal dopamine and form-deprivation myopia. *Proc Natl Acad Sci U S A* 1989;86:704-706.

34. Guo SS, Sivak JG, Callender MG, Diehl-Jones B. Retinal dopamine and lens-induced refractive errors in chicks. *Curr Eye Res* 1995;14:385-389.

35. Stone RA, Liu J, Sugimoto R, Capehart C, Zhu X, Pendrak K. GABA, experimental myopia, and ocular growth in chick. *Invest Ophthalmol Vis Sci* 2003;44:3933-3946.

36. Rohrer B, Stell WK. Basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-beta) act as stop and go signals to modulate postnatal ocular growth in the chick. *Exp Eye Res* 1994;58:553-561.

37. Iuvone PM, Tigges M, Stone RA, Lambert S, Laties AM. Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. *Invest Ophthalmol Vis Sci* 1991;32:1674-1677.

38. Cottriall CL, Truong HT, McBrien NA. Inhibition of myopia development in chicks using himbacine: a role for M(4) receptors? *Neuroreport* 2001;12:2453-2456.

39. Siegwart JT, Jr., Norton TT. The susceptible period for deprivation-induced myopia in tree shrew. *Vision Res* 1998;38:3505-3515.

40. Wallman J, Adams JI. Developmental aspects of experimental myopia in chicks: susceptibility, recovery and relation to emmetropization. *Vision Res* 1987;27:1139-1163.

41. Zhao J, Pan X, Sui R, Munoz SR, Sperduto RD, Ellwein LB. Refractive Error Study in Children: results from Shunyi District, China. *Am J Ophthalmol* 2000;129:427-435.

42. Norton TT, Gamlin PD. The near response, emmetropia and myopia. *Ophthalmic Physiol Opt* 1999;19:79-80.

43. Parentin F, Tonini G, Perissutti P. Refractive evaluation in children with growth defect. *Curr Eye Res* 2004;28:11-15.

44. Parentin F, Perissutti P. Congenital growth hormone deficiency and eye refraction: a longitudinal study. *Ophthalmologica* 2005;219:226-231.

45. Inui Y, Miwa S. Thyroid hormone induces metamorphosis of flounder larvae. *Gen Comp Endocrinol* 1985;60:450-454.

46. Browman HI, Hawryshyn CW. Thyroxine induces a precocial loss of ultraviolet photosensitivity in rainbow trout (Oncorhynchus mykiss, Teleostei). *Vision Res* 1992;32:2303-2312.

47. Hawryshyn CW, Martens G, Allison WT, Anholt BR. Regeneration of ultraviolet-sensitive cones in the retinal cone mosaic of thyroxin-challenged post-juvenile rainbow trout (Oncorhynchus mykiss). *J Exp Biol* 2003;206:2665-2673.

48. Walpita CN, Grommen SV, Darras VM, Van der Geyten S. The influence of stress on thyroid hormone production and peripheral deiodination in the Nile tilapia (Oreochromis niloticus). *Gen Comp Endocrinol* 2007;150:18-25.

49. Byamungu N, Mol K, Kuhn ER. Somatostatin increases plasma T3 concentrations in Tilapia nilotica in the presence of increased plasma T4 levels. *Gen Comp Endocrinol* 1991;82:401-406.

50. Schrodl F, De Laet A, Tassignon MJ, et al. Intrinsic choroidal neurons in the human eye: projections, targets, and basic electrophysiological data. *Invest Ophthalmol Vis Sci* 2003;44:3705-3712.

51. Nickla DL, Wildsoet CF. The effect of the nonspecific nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester on the choroidal compensatory response to myopic defocus in chickens. *Optom Vis Sci* 2004;81:111-118.

52. Wallman J, Wildsoet C, Xu A, et al. Moving the retina: choroidal modulation of refractive state. *Vision Res* 1995;35:37-50.

53. Hung LF, Wallman J, Smith EL, 3rd. Vision-dependent changes in the choroidal thickness of macaque monkeys. *Invest Ophthalmol Vis Sci* 2000;41:1259-1269.

54. Troilo D, Nickla DL, Wildsoet CF. Choroidal thickness changes during altered eye growth and refractive state in a primate. *Invest Ophthalmol Vis Sci* 2000;41:1249-1258.

55. Liang H, Crewther SG, Crewther DP, Junghans BM. Structural and elemental evidence for edema in the retina, retinal pigment epithelium, and choroid during recovery from experimentally induced myopia. *Invest Ophthalmol Vis Sci* 2004;45:2463-2474.

56. Marzani D, Wallman J. Growth of the two layers of the chick sclera is modulated reciprocally by visual conditions. *Invest Ophthalmol Vis Sci* 1997;38:1726-1739.

57. Troilo D, Xiong M, Crowley JC, Finlay BL. Factors controlling the dendritic arborization of retinal ganglion cells. *Vis Neurosci* 1996;13:721-733.

58. Rymer J, Wildsoet CF. The role of the retinal pigment epithelium in eye growth regulation and myopia: a review. *Vis Neurosci* 2005;22:251-261.

59. Seko Y, Tanaka Y, Tokoro T. Scleral cell growth is influenced by retinal pigment epithelium in vitro. *Graefes Arch Clin Exp Ophthalmol* 1994;232:545-552.

60. Gallemore RP, Steinberg RH. Effects of dopamine on the chick retinal pigment epithelium. Membrane potentials and light-evoked responses. *Invest Ophthalmol Vis Sci* 1990;31:67-80.

61. Seko Y, Tanaka Y, Tokoro T. Apomorphine inhibits the growth-stimulating effect of retinal pigment epithelium on scleral cells in vitro. *Cell Biochem Funct* 1997;15:191-196.

62. Koh SW, Chader GJ. Elevation of intracellular cyclic AMP and stimulation of adenylate cyclase activity by vasoactive intestinal peptide and glucagon in the retinal pigment epithelium. *J Neurochem* 1984;43:1522-1526.

63. Seltner RL, Stell WK. The effect of vasoactive intestinal peptide on development of form deprivation myopia in the chick: a pharmacological and immunocytochemical study. *Vision Res* 1995;35:1265-1270.

64. Walls G. The vertebrate eye and its adaptive radiation. *Cranbrook Institute of Science, Bloomfield Hills, Michigan* 1942.

65. Kisilak M. H, J.J., Huang L., Campbell, M.C.W. and Irving, E.L. In chicks wearing high powered negative lenses, spherical aberration is compensated and oblique astigmatism is induced. J. of Modern Optics. *J of Modern Optics (in press)* 2007.

66. Glickstein M, Millodot M. Retinoscopy and eye size. *Science* 1970;168:605-606.

67. Judd DB, Macadam, D.L., and Wyazecki, G. Spectral distribution of typical daylight as a function of correlated color temperature. *Journal of the Optical Society of America* 1964;54:1031-1040.

68. Mutti DO, Ver Hoeve JN, Zadnik K, Murphy CJ. The artifact of retinoscopy revisited: comparison of refractive error measured by retinoscopy and visual evoked potential in the rat. *Optom Vis Sci* 1997;74:483-488.

69. de Luze A, Leloup J. Fish growth hormone enhances peripheral conversion of thyroxine to triiodothyronine in the eel (Anguilla anguilla L.). *Gen Comp Endocrinol* 1984;56:308-312.

70. Mitchell MA, Raza A. The effects of glucagon and insulin on plasma thyroid hormone levels in fed and fasted domestic fowls. *Comp Biochem Physiol A* 1986;85:217-223.

71. Farchi-Pisanty O, Hackett PB, Jr., Moav B. Regulation of fish growth hormone transcription. *Mol Mar Biol Biotechnol* 1995;4:215-223.

72. Fischer AJ, Wallman J, Mertz JR, Stell WK. Localization of retinoid binding proteins, retinoid receptors, and retinaldehyde dehydrogenase in the chick eye. *J Neurocytol* 1999;28:597-609.

73. Feldkaemper MP, Schaeffel F. Evidence for a potential role of glucagon during eye growth regulation in chicks. *Vis Neurosci* 2002;19:755-766.

74. Litovitz TL, White JD. Levothyroxine ingestions in children: an analysis of 78 cases. *Am J Emerg Med* 1985;3:297-300.

75. White JD, Litovitz TL. Levothyroxine poisoning. *Pediatrics* 1985;75:129-130.