Fish Lenses

Anatomy and Optics

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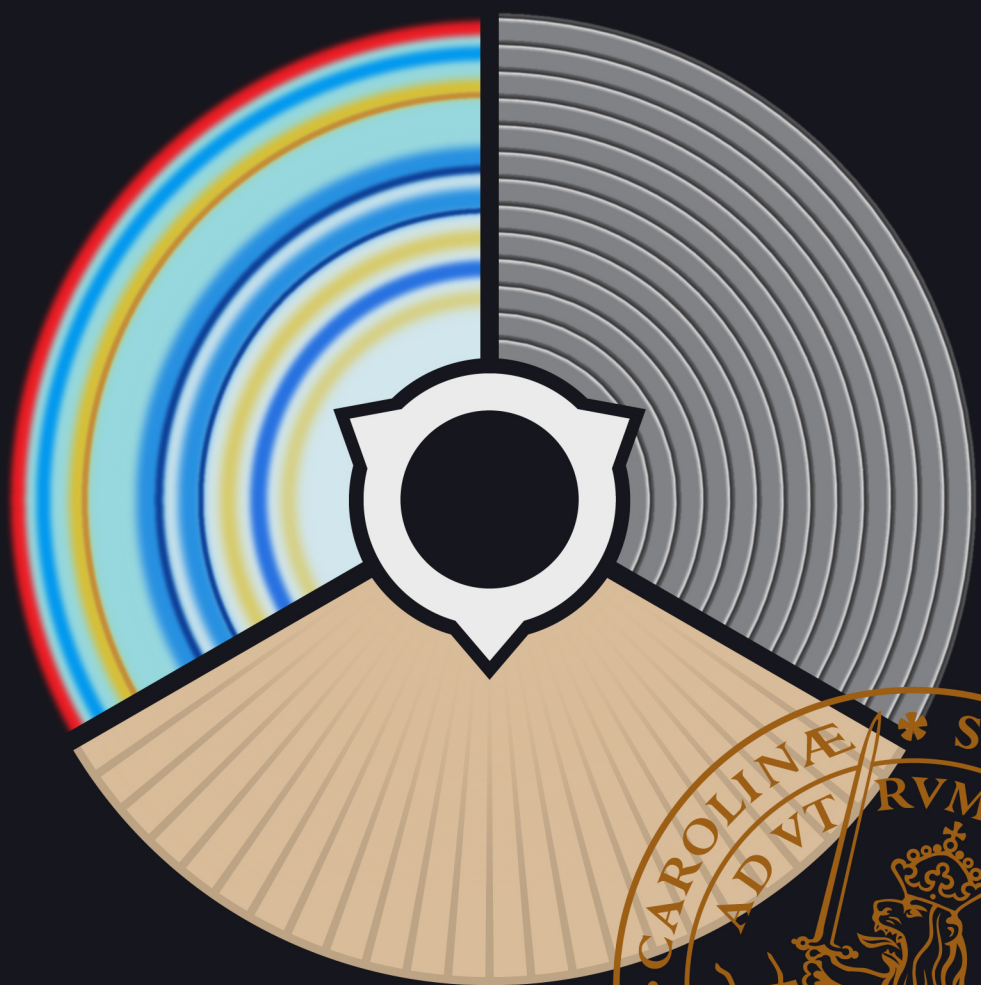

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Anatomy and Optics

Tomasz Kozłowski

LUND
UNIVERSITY

DOCTORAL DISSERTATION
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Fish Lenses: Anatomy and Optics

Abstract:

I have investigated some of the biological regulatory mechanisms governing the development of crystalline lenses. I used fish as model animals because they possess optically interesting lenses, while the geometrical simplicity of fish lenses allows for studies that are difficult or impossible with the lenses of other animals.

First we have investigated lens optical plasticity by measuring longitudinal spherical aberration in light and dark adapted fish of two species, Atlantic and Polar cod. We noticed that Atlantic cod, native to regions with daily light/dark changes responded to light/dark adaptation by changing the optics of its lens, whereas the optics of Polar cod, living in the polar region, was unchanged on a daily basis (**Paper I**). However, we observed that the optics of the Polar cod lens changed annually between seasons corresponding to polar day and night (unpublished data). Our findings can be explained by the existence of two different mechanisms controlling the optics of fish lenses. A short-term one adapting the lenses to daily light/dark cycles (Atlantic cod) and a long-term one evolved for coping with long polar days and nights (Polar cod).

The second project involved investigation of the osmolality of fish larvae body fluids. We tested two levels of osmolality in two different ways. The first one involved measuring the rate of optical deterioration of excised fish lenses placed in different immersion media, the second one the quality of a whole eye fixation. In both cases, lower osmolality gave better results for fish larvae. The optical quality of larval lenses deteriorated slower and fixation preserved the larval eye in a more natural shape (**Paper II**). We concluded that zebrafish larvae have lower osmolality in their bodies than adult fish.

The third project was dedicated to the investigation of the cellular structure of fish lenses. First, we developed a method to visualize an equatorial cross-sections of adult fish lenses. Than we used the method to examine lenses in two size groups of fish of the same species. We measured lens fiber thickness in four relative radial positions in the lens. Our measurements showed that fish lens fiber cells have the same thickness along the radius of the lens and in both size groups. The average thickness was much lower than in other vertebrates (**Paper III**).

We followed up that study by measuring full thickness profile along the lens radius in nine fish species. The thickness of a fiber was independent from its radial position in the lens in all but one species. We observed that the average fiber thickness depends on species. Additionally, we developed a model for calculating historical lens fiber thicknesses necessary for the cells to reach their current refractive indices and thicknesses by cell compaction. The model showed that the cells would have to lose 66% of their volumes to reach their current sizes. This unlikely number and the constancy of cell thickness suggest that a different mechanism is at work. (**Paper IV**). Based on the findings from both papers, we conclude that, at least in fish, protein is transported inwards between denucleated fibers in the growing lens. The cells in the peripheral lens layers have synthetic capabilities and are most likely the source of those proteins.

Key words: plasticity, development, cellular structure, lens fiber cells, modeling, laser scan, schlieren photography, Fast Fourtier Transform

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To my mom and to my wife

"I cannot for the life of me understand why, while people without driver's licenses are not allowed on public roads, in bookstores one can find any number of books by persons without decency – let alone knowledge."

His Master's Voice, Stanisław Lem

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List of figures

Original papers and manuscripts

Paper I

Jönsson, M., Varpe, Ø., **Kozłowski, T.**, Berge, J. and Kröger, R. H. H. (2014).

"Differences in lens optical plasticity in two gadoid fishes meeting in the Arctic."

Journal of Comparative Physiology A - Neuroethology, Sensory, Neural, and Behavioral Physiology **200**(11): 949-957. DOI: 10.1007/s00359-014-0941-z

Paper II

Kozłowski, T. M., Jönsson, M., Ek, F., Olsson, R. and Kröger, R. H. H. (2017).

"Osmotic Concentration of Zebrafish (*Danio rerio*) Body Fluids Is Lower in Larvae than in Adults."

Zebrafish. October 2017, ahead of print. DOI: 10.1089/zeb.2017.1504

Paper III

Kozłowski, T. M. and Kröger, R. H. H. (2017).

"Visualization and thickness measurement of adult fish lens fiber cells."

Manuscript to be submitted to Experimental Eye Research

Paper IV

Kozłowski, T. M. and Kröger, R. H. H. (2017).

"Constant lens fiber cell thickness in fish suggests crystallin transport to denucleated cells."

Manuscript to be submitted to Vision Research

Author's contribution to the papers

Paper I

TMK designed and prepared the data analysis tool and calculated the seasonal variation in light conditions. **All authors** participated in discussing the findings and critical revision of the final version of the manuscript.

Paper II

TMK and MJ designed the optical experiment and collected the data, RHHK proposed the hypothesis that an osmolality mismatch causes the rapid deterioration of larval lenses in vitro, FE and RO prepared larvae for the experiments, and **TMK** prepared the fixations of larvae and analyzed the data from both experiments. **All authors** participated in discussing the findings and critical revision of the final version of the manuscript.

Paper III

TMK developed the sample preparation method, collected and analyzed the data. **TMK** and RHHK designed the experiment and prepared the manuscript.

Paper IV

TMK designed the model, collected and analyzed the data. **TMK** and RHHK designed the experiment and prepared the manuscript.

"[...] humanity is a hunchback who, in ignorance of the fact that it is possible not to be hunchbacked, for thousands of years has sought an indication of a Higher Necessity in his hump, because he will accept any theory but the one that says that his deformity is purely accidental, that no one bestowed it upon him as part of a master plan, that it serves absolutely no purpose, for the thing was determined by the twists and turns of anthropogenesis."

His Master's Voice, Stanisław Lem

Popular science summary

How fish eyes may save yours?

According to the World Health Organization, cataract was responsible for over half of the blindness world-wide in 2010. Getting a cataract means that the lens inside your eye gets clouded, preventing clear, sharp vision. Cataract can be caused by age, physical trauma, genetics, or even skin disease. It can virtually happen to everyone. It is possible to fix cataract by surgically replacing your lens with an artificial one. However, not everyone has access to such an expensive procedure. Replacing the natural lenses is also far from ideal. Artificial lenses have problems with accommodation, which is the ability to focus on objects at different distances, like looking over the horizon and reading a book. Cataract is a serious problem and its cause is often elusive. What can we do about it?

If your car breaks, you go to a mechanic who knows how to fix it. Similarly, you go to a doctor if something is wrong with your body. The major difference is that the mechanic can always ask the designer of the car; “How does it work?” It is much more difficult to ask Nature how it has shaped us. Thanks to science, it is a difficult, but not an impossible task. By unraveling the unknowns piece by piece, we can gain an understanding about how things work. The more pieces we get, the easier it becomes to figure it all out.

My work with other researchers at Lund University unraveled some of those pieces that in the future may be useful for preventing cataract. The lens develops by the same basic mechanisms in the eyes of all vertebrates, including humans. However, it is much easier to experiment on fish than on people.

What exactly have we done?

In our research, we have found out that fish can change the optics of their lenses differently depending on the geographic region they are from. Fish from the polar region, experiencing mainly the annual changes of polar day and night, change their lenses much slower than fish from regions with a daily light/dark cycle!

Another finding involved how tiny fish survive the harsh influence of… water. All animals are made of cells that contain water. Dissolved salts and other molecules keep the cells alive. How much minerals there are in the water is called osmolality. If osmolality inside a cell is higher than in the water outside, the water will rush into the cell to equalize osmolality on both sides, letting the cells swell and eventually killing them. You can imagine that this is a real problem for aquatic animals! Fish have mechanisms to keep osmolality inside their bodies higher than in the water they swim in. However, we found out that fish larvae cannot keep osmolality in their bodies on the same high level as adults, so that their bodies and lenses operate at much lower osmolality.

We also studied how the fish lens is built. As I have mentioned, the mechanisms of creating the lens are similar in all vertebrates. However, fish lenses are very hard and it has therefore been impossible to section and see inside them. There was no method for studying the cells in a fish lens. I have developed such a method and used it to look into the cellular structure of the lenses of nine fish species. We found that the cells, organized in concentric layers, have the same thickness irrespective of lens size. All layers are equally thick and the only difference between lenses of two sizes is the amount of layers. This has also been observed in other vertebrates, which further confirms that studying fish can benefit us humans. Interestingly, the layers in fish lenses were much thinner than in cattle, chicken, rabbit, and mouse. All nine fish species had layers of different sizes, which means that there are not only differences between animal groups, but also among fishes. Through computer modeling, we discovered that fish lenses change their optics by transporting proteins inside the lens. This is a very surprising observation because most of the cells in the lens are “dead”, just as the surface layers of your skin. Yet, lens cells can change their properties anyhow!

An old Chinese proverb says: “A journey of a thousand miles begins with a single step”. I would like to point out that the journey not only begins with a single step, but also consists of single steps. My research and findings, described in the book you are reading, are a few of such steps toward understanding lenses and potentially stop the leading cause of blindness; lens cataract.

Alien? No… just a fish that one day can save your vision!

"[...] ludzkość jest garbusem, który, dla niewiedzy o tym, że można garbatym nie być, od tysięcy lat poszukuje znamion wyższej konieczności w swoim garbie, ponieważ gotów jest na każdą wersję oprócz takiej, że kalectwo to jest przypadkowe po prostu, że nikt go nim z rozmysłu wyższego nie obdarzył, że ono najzupełniej niczemu nie służy, bo tak właśnie ustaliły rzecz skręty i uchyłki antropogenezy."

Głos Pana, Stanisław Lem

Podsumowanie popularnonaukowe

Jak rybie oczy mogą uratować twoje?

Według Światowej Organizacji Zdrowia zaćma (inaczej katarakta) odpowiadała za ponad połowę przypadków ślepoty w 2010 roku. Choroba ta sprawia, że soczewki w twoich oczach stają się zmętniałe, przez co uniemożliwiają wyraźne, ostre widzenie. Zaćma może być spowodowana wiekiem, traumą fizyczną, genetyką, a nawet chorobami skóry. Może się przytrafić praktycznie każdemu. Usunięcie zaćmy jest możliwe poprzez chirurgiczne zastąpienie soczewki jej sztucznym odpowiednikiem, jednak nie każdy ma dostęp do tak kosztownej procedury. Dodatkowo, soczewki zastępcze dalekie są od ideału. Mają problem z akomodacją, czyli możliwością skupienia się na obiekcie w różnych odległościach, takich jak patrzenie na horyzont i czytanie książki. Zaćma stanowi poważny problem i nie jesteśmy pewni, co tak naprawdę ją powoduje i jak ją zatrzymać. Co możemy z tym zrobić?

Jeśli twój samochód zepsuje się, pójdziesz do mechanika, gdy coś z tobą nie tak, pójdziesz do lekarza. Obydwaj rozwiązują problemy korzystając z wiedzy o tym jak coś działa. Główna różnica jest taka, że mechanik może bezpośrednio skonsultować się z konstruktorem samochodu, o wiele trudniej jest zapytać Naturę, jak nas uformowała. Tu wkracza świat nauki, gdzie dzięki badaniom uzyskanie odpowiedzi jest w ogóle możliwe. Odkrywając nieznane - kawałek po kawałku, możemy stworzyć obraz tego jak działa świat. Im więcej kawałków odkryjemy, tym łatwiej będzie nam go zrozumieć.

Badania, które przeprowadziłem wraz z innymi badaczami Uniwersytetu Lund, a które zostały przedstawione w niniejszej pracy, doprowadziły do odkrycia kilku takich nieznanych kawałków. Mam nadzieję, że można będzie je wykorzystać w walce z zaćmą. Mechanizmy, które stworzyły ludzkie soczewki, są takie same u wszystkich kręgowców. Znacznie łatwiej jednak jest się uczyć i przeprowadzać badania na rybach niż na ludziach.

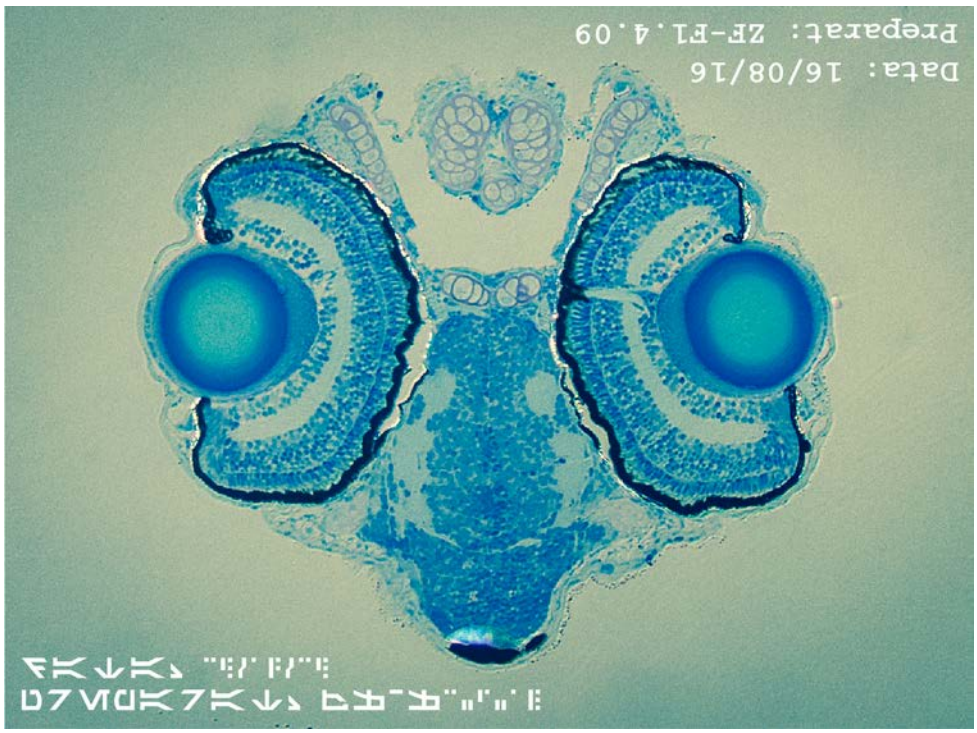
Co dokładnie zrobiliśmy?

W moich badaniach dowiedzieliśmy się, że ryby mogą zmieniać optykę swoich soczewek w różny sposób w zależności od regionu, z którego pochodzą. Ryby z regionu polarnego, gdzie doświadczają polarnych dni i nocy, zmieniają soczewki znacznie wolniej niż ryby z regionów o normalnym cyklu dnia i nocy!

Kolejne odkrycie dotyczyło sposobu, w jaki maleńkie ryby znoszą destrukcyjny wpływ... wody. Wszystkie zwierzęta zbudowane są z komórek zawierających wodę. To woda zawiera sole i inne minerały, by utrzymać komórki przy życiu. Koncentracja takich minerałów w wodzie określana jest jako osmolalność. Jeśli osmolalność komórki jest wyższa niż osmolalność wody na zewnątrz, woda penetruje komórkę, aby wyrównać osmolalność po obydwu stronach. To sprawia, że komórki puchną i umierają. Jak możesz sobie wyobrazić, to prawdziwy problem dla zwierząt wodnych! Ryby mają wiele mechanizmów, aby utrzymać osmolalność na wysokim poziomie. Okazało się jednak, że larwy ryb nie są w stanie utrzymać wysokiej osmolalności, jak ryby dorosłe, dlatego dostosowały swoje ciało i soczewki do znacznie niższej osmolalności.

Ostatnią rzeczą, jaką zrobiliśmy, było sprawdzenie, jak zbudowana jest soczewka ryby. Jak już wspomniałem, mechanizmy tworzenia soczewki są podobne u wszystkich kręgowców. Soczewka ryby jest jednak bardzo twarda, dlatego trudno ją przeciąć i zobaczyć w środku. Do tej pory nie istniały żadne metody badania komórek w rybiej soczewce. Opracowałem taką metodę, co umożliwiło dokładne przyjrzenie się strukturze komórek w soczewkach dziewięciu gatunków ryb. Okazało się , że komórki, ułożone w koncentryczne warstwy, mają tę samą grubość, niezależnie od rozmiaru soczewki. Oznacza to, że każda warstwa jest równie gruba, a jedyną różnicą między soczewkami o dwóch rozmiarach jest licza występujących w nich warstw. Zaobserwowano to u innych kręgowców, co dodatkowo potwierdza, że badanie ryb może przynieść korzyści nam, ludziom. Co ciekawe, warstwy były znacznie cieńsze niż u bydła, kurcząt, królików, a nawet myszy. Wszystkie dziewięć ryb miało warstwy o różnej wielkości, co oznacza, że istnieją różnice nie tylko między grupami zwierząt, ale także między gatunkami ryb. Dzięki modelowaniu komputerowemu odkryliśmy również, że ryby zmieniają swoją optykę, przenosząc do wnętrza soczewki białka, z których soczewki są zrobione. Jest to bardzo zaskakująca obserwacja, ponieważ większość komórek w soczewce jest "martwa" tak jak wierzchnia warstwa skóry. Mimo to ryby potrafią modyfikować soczewkę by utrzymać jej właściwości optyczne w trakcie rozwoju, a co za tym idzie, zwiększania rozmiarów ryby.

Stare chińskie przysłowie głosi, że: "Podróż tysiąca mil zaczyna się jednym krokiem". Chciałbym zauważyć, że ta podróż nie tylko zaczyna się, ale również składa z pojedynczych kroków. Moje badania i odkrycia opisane w książce, którą czytasz, są kilkoma z takich kroków, by pomóc zrozumieć ludzkie soczewki i potencjalnie zatrzymać główną przyczynę ślepoty, zaćmę.



Kosmita? Nie… jedynie ryba, która pewnego dnia może uratować twój wzrok!

"All it takes is one bad day to reduce the sanest man alive to lunacy."

The Joker

What is this thesis about?

All complex organisms are composed of organs that work together to perform basic biological functions. Each organ has a unique and precise purpose, often fulfilling requirements difficult to meet. Organs have specific shapes, sizes, and cellular as well as chemical compositions. Assuming everything goes well with your health, it is irrelevant whether you are a small child or a full-grown adult, your heart, lungs, and eyes function properly during the entire development of the body. It is interesting not only how organs form and function, but also how they grow and maintain their fine-tuned properties throughout life.

One of such fascinating organs is the eye. Vision has demanding requirements, being one of the most important sensory systems in many species. To make things worse, the system has to function within a demanding physical framework– optics. The image formed by an eye is usually corrected for all sorts of optical flaws - aberrations – in order to convey reliable information. Additionally, biological regulatory mechanisms allow the eye to grow without ever losing its amazing function.

I have chosen fishes as model animals for the study of eye development. In particular, I concentrated my effort on investigating fish eye lenses. Fishes have crystalline lenses of a specific shape that allows for investigations that are impossible in other animal groups. Additionally, fish lenses are crystal clear and usually well corrected for spherical and chromatic aberration, while being the sole refracting element in the eye. How such a lens develops is the core question of my study. My thesis consists of three projects and the results are reported in four papers.

Project 1 – Plasticity

First, we have investigated lens optical plasticity by measuring longitudinal spherical aberration in light and dark adapted fish of two species, Atlantic and Polar cod. We noticed that Atlantic cod, native to regions of daily light/dark changes, responded to light/dark adaptation by changing the optics of its lens, whereas the optics of Polar cod, living in the polar region, was unchanged on a daily basis (**Paper I**). However, we observed that the optics of the Polar cod lens changed annually between seasons corresponding to polar day and night (unpublished data).

Project 2 – Osmolality

The second project involved investigation of the osmolality of fish larval body fluids. We tested two levels of osmolality in two different ways. The first one involved measuring the rate of optical deterioration of excised fish lenses placed in different immersion media, the second one the quality of a whole eye fixation. In both cases, lower osmolality gave better results for fish larvae. The optical quality of larval lenses deteriorated slower and fixation preserved the larval eye in a more natural shape (**Paper II**).

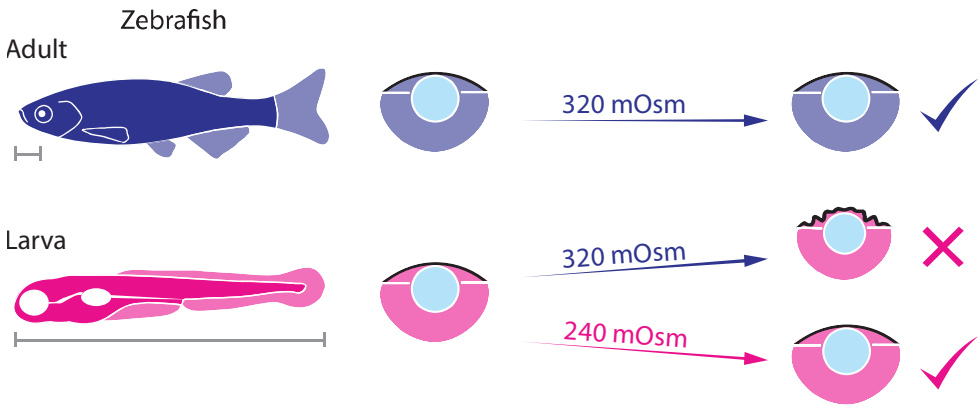


Figure 1 Graphical representation of some of the findings reported in Paper II. Adult fish eyes have to be fixed in the “adult” value of osmolality. The same osomolality is too high for larvae and fixation results are poor. Good fixation of larval eyes requires lower osmolality. Scale bars are for size comparison.

Project 3 – Cell thickness

The third project was dedicated to the investigation of the cellular structure of fish lenses. First, we developed a method to visualize an equatorial cross-section of an adult fish lens. Than we used the method to examine lenses in two size groups of fish of the same species. We measured lens fiber thickness in four relative radial positions in the lens. Our measurements showed that fish lens fiber cells have the same thickness along the radius of the lens and in both size groups. The average thickness was much lower than in other vertebrates (**Paper III**).

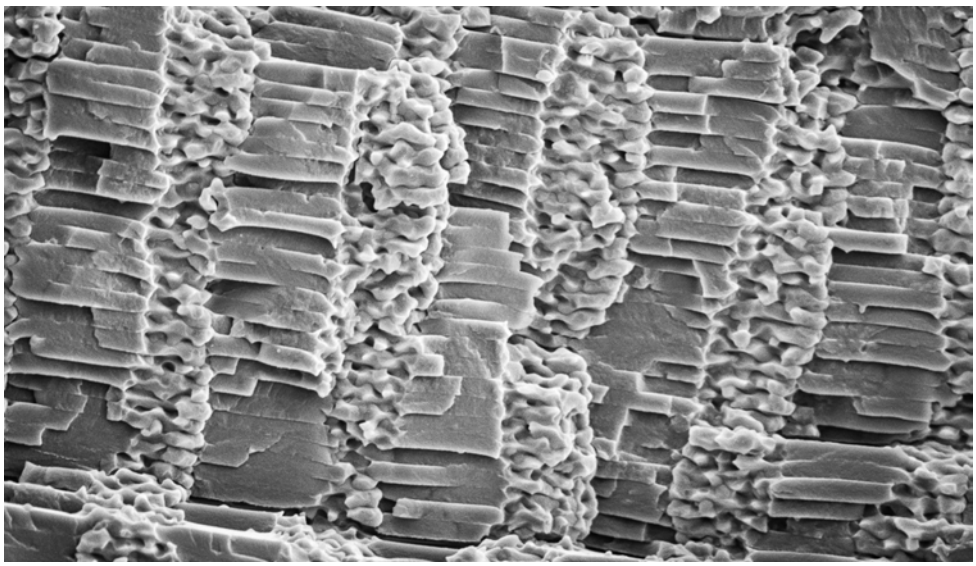


Figure 2 Fish lens fibers. Radial cell columns in a fish lens. Lenses were prepared with our new method and cross sections were visualized with a scanning electron microscope. We used such images to measure cell thicknesses in the equatorial planes of fish lenses.

We followed up on that study by measuring full thickness profiles along the lens radius in nine fish species. The thickness of a fiber was independent from its radial position in the lens in all but one species. We observed that the average thickness depends on species. Additionally, we developed a model for calculating historical lens fiber thicknesses necessary for the cells to reach their current refractive indices and thicknesses by cell compaction. The model showed that the cells would have to lose 66% of their volumes to reach their current sizes. This unlikely number and the constancy of cell thickness suggest that a different mechanism is at work. (**Paper IV**).

"There's always a bigger fish."

Qui-Gon Jinn

Fish and their environment

Why vision?

Just as other animals, fish have certain needs necessary for their survival. In order to pass the genetic material to the next generation, fish have to evade death until producing offspring. To do so, fish have to find food and avoid predators. They can do the latter by early detection of a hunter or by finding a suitable shelter. Avoiding death cannot last forever, so that fish eventually have to mate in order to contribute to the survival of their species.

All of those tasks are made much easier (sometimes possible at all) by the use of vision. Vision relies on light and uses almost every aspect of it: amplitude, wavelength, and in some cases polarization. However, only two properties of light are truly important for vision in general: the high speed of light and its propagation in straight lines (most of the time). Light is the only stimulus modality that guarantees instant information from a distance. The transit time of light is negligible for any distance relevant to animals. Because light travels in straight lines, it provides reliable information about the direction of the stimulus. It is speculated that the utilization of this directionality has kick-started the evolution of motility (Land et al. 2002). Gaining information instantly with reliable directionality seems to be a perfect sensory modality. However, light is strongly affected by the environment.

Visual challenges of aquatic environments

The major problem of aquatic environments is the short viewing distances. It is difficult to benefit from instant directional information if that information is only provided from a short distance. Fish can miss an approaching predator or a promising hideout. The problem occurs already in clear, shallow waters where the viewing distance is heavily limited, mainly by the absorption of light by water (Cronin et al. 2014). Seeing further than 100 meters horizontally is impossible in water, even under the most favorable conditions. Furthermore, aquatic environments rarely fulfil these conditions. Some of them, like turbid waters, allow

for viewing distances of only a few centimeters (Mobley 1994). Under such conditions, rapid attenuation of down welling light creates a narrow visual layer that is available for fish vision. With increasing depth and even in clear ocean water, the level of ambient light gets eventually too low to be utilized for vision. Dedicated visual adaptations may extend that depth somewhat. An example is the mesopelagic region (200 – 1000 m deep) of the oceans where the light comes mainly from bioluminescence (Cronin et al. 2014). In an overwhelming darkness with sparse light sources, every photon counts and intraocular scattering is vision's greatest enemy. However, the difficulties of vision in aquatic habitats range beyond the limitations of viewing distance. Highly illuminated regions are troubled with their own set of problems – seeing color.

Detecting different wavelengths adds another layer of information. Animals can use that extra information channel for intra- and interspecific signaling, predator avoidance, foraging and more. Additionally, comparing the spectral reflections of objects is more robust than the comparison of their brightness (Maximov 2000). However, colorful environments are much more complex to analyze and put extra requirements on eyes, optically in form of chromatic compensation over a larger spectral range. That range may be affected by the tint of the surrounding water.

Having eyes fine-tuned to the environment is advantageous, even if it is energetically expensive (Moran et al. 2015) and requires special adaptations. Fish can be found in almost every aquatic environment and they have to cope with the problems encountered. Deep-sea fishes have crystal clear, optically corrected lenses (Kröger et al. 2009), meaning that the intraocular scattering problem has been solved. Shallow coastal waters are filled with colorful fishes with spectral sensitivity ranges that may stretch from near-UV to near-IR (Bowmaker 1995). Sensitivity to a wide spectral range requires good correction of chromatic aberration caused by unavoidable dispersion. Technical solutions involve specialized optical elements and/or additional lenses. In fish eyes, this problem is solved with only one, specialized lens.

What is a fish?

There are over 33 000 described species of fish (Froese et al. 2018), making them the most diverse group of vertebrates (Bone et al. 2008). They cover a wide range of shapes, sizes, habitats, dietary requirements, behaviors, and visual needs. It is useful to use the umbrella term "fish" only to a certain point. While describing details of fish vision, it is inevitable to exclude some groups or species. To avoid listing each exception of every trait or characteristic, I will define a "typical fish" for the scope of this thesis.

My work involves only teleosts, an infraclass of ray-finned fish. It also limited to species living in the photic zone. In this zone, down welling sunlight is sufficiently bright for photosynthesis. The included species are equipped with a camera-type eye with a large aperture. Typically, the pupil is almost as large as the lens (Fernald et al. 1985b, Dahm et al. 2007) and can therefore be ignored in the optical analysis. Because the fish eye functions underwater, which has a similar refractive index as the aqueous humor inside the eye, the optical power of the cornea is negligible (Matthiessen 1886) and can thus be ignored as well. Fish lenses are sphere-shaped[*] (Matthiessen 1886, Jagger 1992, Kröger 2013) and retain that form even during accommodation to different viewing distances, which is done by moving the lens (Beer 1894, Fernald et al. 1985a). Since adult fish can range from a few millimeters (e.g. male anglerfish) to a few meters (e.g. swordfish), lens size varies to a similar degree. For this reason, all measurements and models presented in this thesis use data normalized to the lens radius, so that all distances and positions are relative. Normalization is useful to compare species and developmental stages. One should note that many species do not nurse their offspring. That means that the larvae have to rely on their own vision already very early in development. Some species show visually guided behavior shortly after hatching (Jackson et al. 2016) and have functional lenses even earlier (Easter et al. 1996, Jackson et al. 2016). They have among the smallest functional vertebrate eyes. Even in small fish like zebrafish, the increase in lens size during development is enormous. Zebrafish larvae have to increase their lens volume by three orders of magnitude to reach the size of an adult lens. That number can be much larger in other species (personal observations). Despite that enormous growth, fish maintain functional lenses throughout life.

[*] It is tempting to call them spherical, however when describing lenses, "spherical" means something else. It means that the optical surfaces are part of spheres. Hence the awkward term "sphere-shaped". This kind of lenses is called ball lenses.

"Your eyes can deceive you. Don't trust them."

Obi-Wan Kenobi

Optics of fish lenses

It is a sphere

What truly makes a lens is the curvature of the border between media of different refractive indices. To increase the power of a lens for a given aperture, one can increase the difference in refractive index (material) and/or decrease the radius of the curvature (geometry). The shorter the radius, the more strongly curved is the surface (Figure 3). That means that the ball lenses of fish are geometrically the most powerful lenses. A focal length that is short in comparison to the size of aperture results in shallow depth of focus. Depth of focus is the range of distances from the lens within which a sensor or retina can be placed and still perceive the sharpest possible image. In case of shallow depth of focus, optical flaws (aberrations) can easily degrade the quality of the image and thus reduce the amount of transferred information. Fish eyes therefore require lenses of high optical quality. However, ball lenses made of homogeneous materials (e.g. glass) have severe spherical aberration. In animal eye lenses (crystalline lenses), this problem is solved by a gradient of refractive index. They are so-called GRIN (gradient-index) lenses.

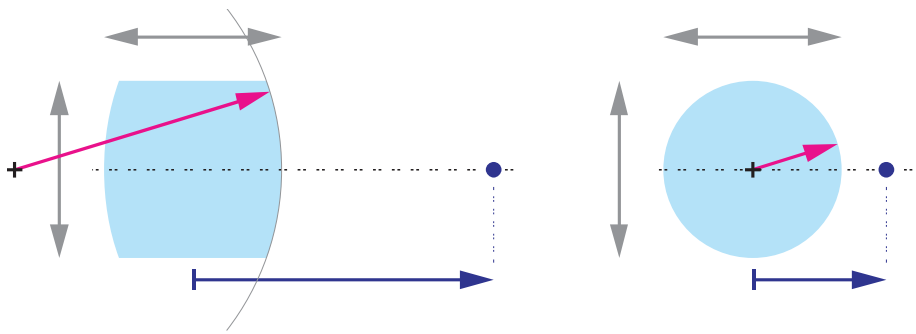


Figure 3 Effect of lens geometry on focal length. In a given set of two lenses made of the same material and with the same diameter and thickness (gray arrows), the one with the shorter radius of curvature (magenta arrows) has the shorter focal length (blue arrows). Thick dashed lines represent the optical axes.

It is a GRIN

The first descriptions of GRIN lenses as a solution to spherical aberration date back to the early XIX century when Young speculated about variation of refractive index in the human lens (Young 1801), and the mid-XIX century when Maxwell developed a more detailed model of fish lenses (Maxwell 1854). GRIN lenses are very common in camera-type eyes.

Typically, refractive index is highest in the center of the lens (Hoshino et al. 2011). In human lenses, the central value of refractive index is approximately 1.41 (Hecht 1987, Augusteyn et al. 2008). Fish have among the highest refractive indices found in animal eyes, with values between 1.53 and 1.57 (Fernald et al. 1983, Axelrod et al. 1988, Kröger et al. 1994, Pierscionek et al. 1995, Jagger et al. 1996). In animal lenses, refractive index usually decreases gradually toward the lens surface, but there are exceptions. In the case of chameleon lenses, the gradient is reversed and creates a diverging lens so that the eye functions as a Galilean telescope (Ott et al. 1995). In the ageing human lens, a refractive index plateau develops by compression of central fiber cells (Al-Ghoul et al. 2001, Augusteyn et al. 2008).

In fish, the refractive index gradient can be approximated by a parabola that drops to about 1.38 at roughly 92% of the lens radius (Gagnon et al. 2012). It is followed by a zone of constant refractive index that ends at the lens capsule of relatively high refractive index (1.40) (Gagnon et al. 2008) (Figure 8).

Study of GRIN

Obtaining detailed information about the refractive index distribution is possible because of the simple geometry of fish lenses. Non-spherical shapes make the mathematical equations unsolvable, preventing the calculation of the inverse Abel transform, a method necessary to acquire the refractive index distribution from the paths of light beams (Chu 1977, Campbell 1984). The unique refractive index profile of a fish lens, if normalized to the radius of the growing lens, is constant throughout the life (Kröger et al. 2001). This is especially remarkable because of the precision with which the profile must be controlled. Even the smallest changes in the GRIN profile result in large effects on the quality of the lens (Gagnon et al. 2012). For example, an almost invisible variation in refractive index is responsible for the difference between a mono- and a multifocal lens. A multifocal lens corrects the eye for another major aberration – chromatic aberration. Because the function of a fish lens is so very sensitive to changes in the refractive index gradient, such lenses can be used as highly sensitive measuring tools.

Optical plasticity (Paper I & II)

Fish lenses exhibit developmental plasticity and can adjust to varying visual needs. Significant optical alterations are induced by, for example, changes in natural light after migration to a different body of water (Gagnon et al. 2011) and by rearing in the lab under various lighting conditions (Kröger et al. 2001). Another form of developmental plasticity is related to osmolality (Paper II). During the early stages, the larvae may be underdeveloped, such that they cannot maintain osmolality of their body fluids at the high adult level in freshwater. However, zebrafish larvae show visually guided behavior when they are still incapable of using their gills for osmoregulation (Rombough 2002, Jackson et al. 2016). Consequently, they need functional lenses that can cope with the low osmolality.

Optical plasticity is also present in adult fish. Lenses showed short-term adjustments between light and dark adapted fish (Schartau et al. 2009). This phenomenon is likely linked to the circadian changes in lighting conditions, because such changes occur only in the lenses of fish native to regions with daily light/dark cycles. In a species native to an area with annual (polar) day and night, the lenses remained unchanged despite several hours of light or dark adaptation (Paper I). Interestingly enough, the polar species exhibited a substantial difference in the lens optics between fish caught during the polar day and during the polar night (Jönsson, unpublished data). This strongly suggests that there is another mechanism of lens plasticity for long term changes suitable for life in the polar region.

“That's no moon.”

Obi-Wan Kenobi

Crystalline lens anatomy

This chapter contains a general description of the cellular structure of crystalline lenses. However, most of the research on crystalline lenses has been performed on tetrapod, more specifically on mammalian lenses. The reason behind is the difficulty in sectioning hard lenses, like the ones of fish. However, it is reasonable to assume that the basic mechanisms of the formation, development, and growth of lenses are common to all vertebrates (Piatigorsky 1981, Shu et al. 2003), especially because of consistent findings in multiple species. Nevertheless, one must be aware of differences and limitations.

General structure

A crystalline lens is an inverted epithelium enclosed in a thick basal membrane called lens capsule. Typical epithelial cells, called the lens epithelium, are lining the anterior, inner surface of the capsule (Piatigorsky 1981, Bassnett et al. 1992, Bassnett et al. 2017). In fish, the lens epithelium stretches across the lens equator and covers also part of the posterior capsule (Dahm et al. 2007). The main volume of the lens is made of specialized lens fiber cells. New fiber cells differentiate from the epithelial cells in the germination zone, located at the edge of the lens epithelium. New fibers are inserted between the epithelium and a “core” of maturating and fully mature fibers. From that layer outwards, structurally speaking, the lens continuously changes, excluding the untouched core, core never changes. There is no cell turnover in the inner fiber cell layers and the oldest cells have been formed in an early embryonic stage (Piatigorsky 1981, Bron et al. 1994).

The lens grows by addition of new layer of fiber cell layers on top of the old ones in concentric single-cell-thick growth shells (Kuszak et al. 1985, Kuszak et al. 2004a, Kuszak et al. 2006, Dahm et al. 2007). The fibers of each growth shell are stretched between the poles of the lens (Kuszak et al. 2004a). All fibers are connected radially (layer-to-layer connection) by ball-and-socket-type connections (Figure 6), and laterally (connection of cells within one growth shell) by means of membrane edge protrusions (Figure 6) (Dahm et al. 2007).

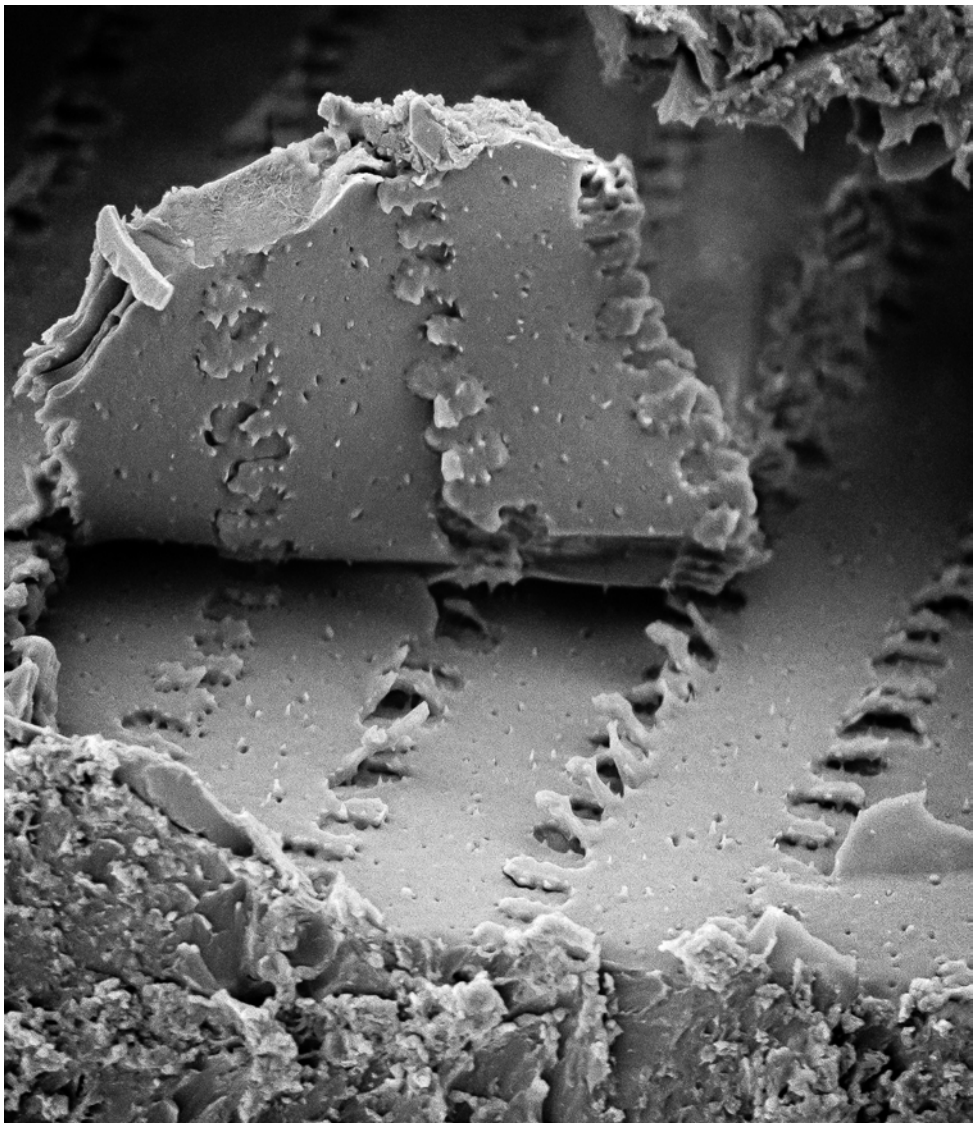


Figure 4 Scanning electron micrograph of the connections between lens cells. Several fibers are peeled off, revealing ball-and-socket connections between fibers in the radial direction. Along the fibers' edges, there are membrane protrusions connecting the fibers laterally.

Sutures

Crystalline lenses are sometimes considered “mathematical constructs” (Kuszak et al. 2004a) because of their highly organized fiber arrangement. Constructing a lens by systematically fitting in exactly formed elements poses the problem of how to fill the surface of a sphere without the fibers overlapping. If you lay equally wide ribbons from one pole of a ball to the other, the ribbon ends cannot meet without overlap (Figure 5, left). In lenses, the problem is solved by differently shaped sutures – structures where all the fiber ends in a shell meet.

Figure 5 The problem of covering a sphere with fibers. Left: Equally wide straight fibers (stripes), laid from one pole to the other, cannot cover the poles without overlapping. They would leave gaps. Center: Example of organizing the fiber ends in a ‘line’ suture. Left: Umbilical sutures require fibers to reduce their widths before reaching the pole.

Three types of suture are common in fish. Umbilical sutures are formed by fibers laid straight between two opposing points of the anteroposterior axis (e.g. zebrafish (Greiling et al. 2012)) (Figure 5, right). Other suture types are called branched sutures and feature fibers that do not reach the second point of the anteroposterior axis. They are typically referred to by the name of the shape they are forming. The simplest suture of the branched kind is the ‘line’ suture (Figure 5, center) and it is common in fish (Bantseev et al. 2004). More complex ‘Y’ suture can also be found in piscine lenses (Bantseev et al. 2004).

Irrespective of the type, each new growth shell will form a pair of sutures. In consequence, the branched sutures from all shells form suture planes that affect the optical quality of the lens by increasing focal length variability (Kuszak et al. 1991, Kuszak et al. 1994, Sivak et al. 1994, Priolo et al. 1999). To minimize the effect and the risk of light scattering, the planes are oriented parallel to the optical axis. However, some light will be scattered, making the sutures visible. We used them as landmarks in orienting excised lenses (Paper I).

Fiber cells (Paper III & IV)

Lens fiber cells are hexagonal in cross-section. They have two broad, parallel faces and four short (Rabl 1900, Taylor et al. 1996, Kuszak et al. 2004a). Fiber thickness is the distance between the two broad faces of the fiber (Figure 6). The broad faces are perpendicular to the radius of the lens. The combination of concentric growth shells and the specific shape of the fibers creates radial cell columns in cross-section (Figure 2) (Rabl 1900, Kuszak et al. 2004a). As lens radius increases, new columns are introduced by occasionally adding special cells with pentagonal cross-sections (Kuszak et al. 1985, Kuszak et al. 2004a). To avoid discontinuities, fiber widths vary slightly. However, in general, fiber width in the equatorial plane is constant along the entire lens radius (Kuszak et al. 2004a), meaning that larger, newer shells consist of more cells side-by-side instead of wider cells.

The width of a fiber may vary along its length, typically by getting smaller close to the sutures. The magnitude of the change depends on the suture type of the lens. In a lens with an umbilical type suture, fiber width decreases basically to zero when it reaches the suture, whereas the fibers narrow down to 1/3 – 1/2 of their equatorial width in a lens with 'line' suture (Kuszak et al. 2004a, Kuszak et al. 2004b).

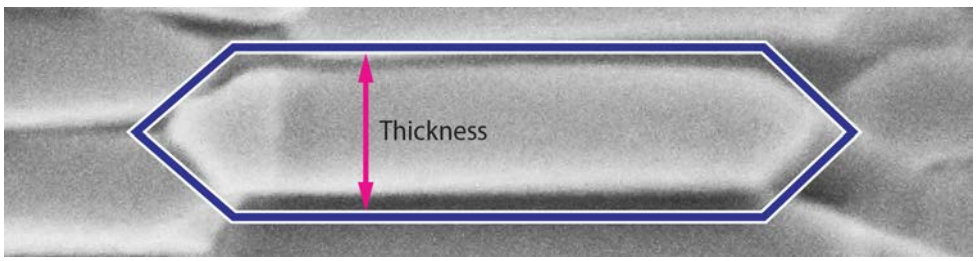


Figure 6 Lens fiber in cross-section. The blue offset highlights the shape of the cross-section of one fiber. The measured dimension is labeled for clear reference. The tissue was visualized with a scanning electron microscope.

Human, cattle, and chicken lenses have different fiber shapes, and consequently fiber thicknesses, along the lens radius. The cells' cross-sections are rounder close to the lens core. Closer to the lens surface, the cells are flatter, regular, and closely packed (Rae et al. 1982, Taylor et al. 1996, Al-Ghoul et al. 1997, Bassnett et al. 2003). At approximately 250 – 500 µm from the lens center, the cells obtain a hexagonal shape and start forming regular growth shells. This corresponds to radial distances between 1% and 8% of the lens radius (cattle and chicken, respectively), which could explain why in our study on fish lenses (Paper IV), there were only regular fiber cells in all investigated areas. The innermost measurement we have performed on a fish lens was at 7% of lens radius. Beyond the irregular zone, the

thickness of growth shells is constant both in terrestrial animals and fishes (Kuszak et al. (2004a), Paper III & IV).

Surprisingly, the average fiber thickness in fish was considerably lower than in other vertebrates (Figure 7). Additionally, fishes exhibited different averages that were independent from lens size. It is likely that the thickness of a lens fiber cell is species-specific.

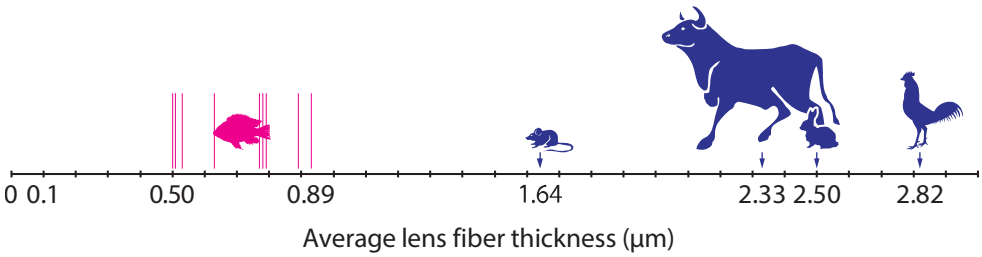


Figure 7 Lens fiber cell thickness in various vertebrates. Multiple magenta lines indicate average cell thicknesses in different fish species as reported in Paper IV. Values for the other animals (mouse, cattle, rabbit, chicken, respectively) are taken from Kuszak et al. (2004a)

Because of the relative consistency in fiber width and thickness, there is a simple relationship between lens diameter and shape. The diameter strictly depends on the number of concentric growth shells and the shape (from a sphere to spheroid) depends on the difference in fiber thickness between the lens equator and fiber endings at the lens poles (Kuszak et al. 2004a).

Transparency

The main cause of light being scattered by cells are cytoplasmic organelles. To become as transparent as possible, lens fiber cells have to remove their organelles from the path of incoming light (Bassnett et al. 1992, Bassnett 2002). Only cells in the lens periphery have organelles, located in the equatorial plane of the lens. Nuclei and mitochondria are clustered together to take less space. In many species, the iris covers this zone of the lens, so that it does not contribute to image formation (Bassnett et al. 1992). In fish, the zone with nucleated cells is restricted to about the outer 3% of lens radius. In the rest of the lens, the cells are devoid of nuclei and organelles (Bassnett 2002, Bantseev et al. 2004, Dahm et al. 2007). Organelle degradation is rapid and occurs shortly after the ends of a developing, stretching fiber cell have reached the sutures (Bassnett et al. 2003). There is no transition zone with partially decomposed organelles at the edge of the organelle free zone (Costello et al. 2016).

"I got a bad feeling about this."

Han Solo

Anatomy meets Optics

Typically, in studies on lens anatomy there is only a broad and simple optical context provided, for example focal length variation. On the other hand, anatomical studies of fish lenses were impossible due to difficulties with sectioning the hard lenses of adult fish. That is why the anatomical context was lacking in detailed optical investigation of fish lens optics (refractive index distribution, multifocality, plasticity). Thanks to the development of a method that allows for the dissection of adult fish lenses (Paper III), this gap can now be filled.

What makes refractive index?

Beyond the central organelle free zone, the young growth shells of a fish lens are constant in refractive index and consists of metabolically active cells (Gagnon et al. 2008). Inside of the zone, each shell has a specific refractive index depending on the relative radial distance from the lens center. That refractive index is the direct consequence of concentration of proteins dissolved in the cytoplasm (Barer et al. 1954, Barer 1957, Zhao et al. 2011b).

Lens fiber cells contain specialized proteins called crystallins. The proteins are water-soluble and have higher refractive index increments ($\Delta n/\Delta c$) than other proteins (Pierscionek et al. 1987). That means that the same change in protein concentration causes a higher change in the refractive index of the cell. Vertebrate lenses are dominated by three types of crystallin: α-, β- and γ-crystallin (van Kamp et al. 1973, Pierscionek-Balcerzak et al. 1985, Pierscionek et al. 1988). In addition, there are some other, taxon-specific crystallins (Williams et al. 1979, Zhao et al. 2011a). Each crystallin type has a particular refractive index increment (Pierscionek et al. 1987, Zhao et al. 2011a). Crystallins can be packed closer than other proteins without the molecules aggregating, so that scattering of light is avoided (Slingsby 1985, Jaenicke 1999). If such aggregation of proteins occurs, it causes cataract (Moreau et al. 2012).

Among the crystallins, γ-crystallin has the highest refractive increment (Pierscionek et al. 1987, Zhao et al. 2011a) and can be packed most densely (Slingsby 1985). High amounts of γ-crystallin lead to high refractive index and make the lens hard (Pierscionek et al. 1991, 1995). In contrast, bird lenses are soft and strongly deformed during accommodation. They contain little or no γ-crystallin (de Jong et al. 1989) and have considerably lower maximum refractive index than fish lenses (Avila et al. 2010).

In fish lenses, there is a large span in refractive index between the periphery (ca. 1.4) and the center (ca. 1.55). Consequently, crystallin concentration is much higher in the center than in the periphery. However, the central cells are devoid of nuclei and organelles. How, then, can mature lens fiber cells, incapable of protein synthesis, substantially increase refractive index during growth of the lens?

Increasing refractive index

Lens fiber cells located close to the lens center in a large, old lens used to be at the lens periphery when the lens was young and small. The fibers did not move, only their relative positions changed because of the addition of further growth shells (Figure 8, left). To maintain the optical properties of the lens and thus its refractive index profile (normalized to lens radius), the refractive indices of central growth shells must increase as the lens increases in size (Figure 8, right). The necessary increase in crystallin concentration may be achieved in two ways. Water could be expelled from the central fiber cells, causing them to shrink in size and crystallin concentration to rise or more crystallin could be added to the cytoplasm, in which case cell size may remain unchanged. Lack of organelles prevents protein synthesis, so the only feasible solution in the second case is the transport of crystallin from the metabolically active cells in the lens periphery. These two scenarios will be referred to as the compaction and transport hypotheses, respectively.

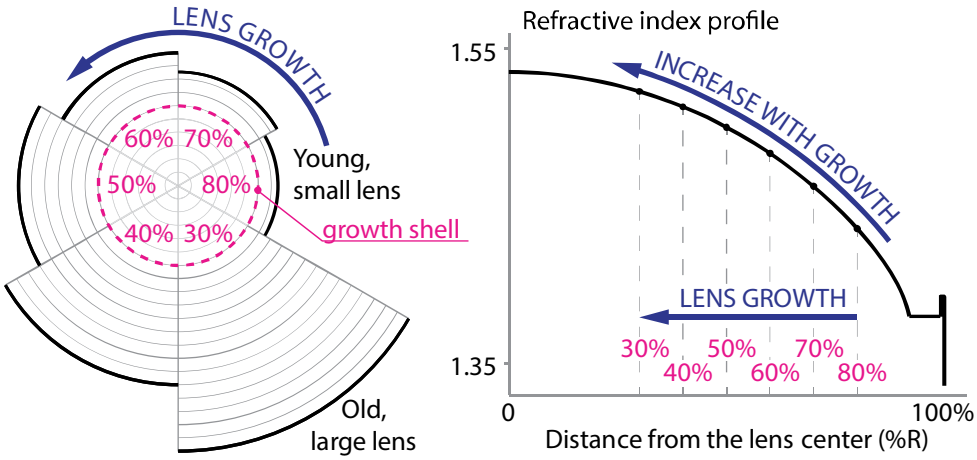


Figure 8 Refractive index increase with lens growth. Left: During lens growth, a certain growth shell (dashed line) stays in place, but changes its relative distance from the lens center (magenta numbers) while new shells are added on top of it. Right: Refractive index profile of a fish lens normalized to lens radius. With increasing lens size, the cells in the growth shell have to increase refractive index in order to maintain the shape of the normalized profile.

Compaction vs. Transport (Paper III & IV)

In research on the lenses of terrestrial species, it is typically assumed that the central cells compact to increase refractive index (Fernald et al. 1983, Pierscionek et al. 1988, Pierscionek et al. 1992). The natural consequence of compaction - alteration in cell anatomy - is often overlooked. If the difference in refractive index comes from a change in cell size, the profile of cell thickness as a function of radial position in the lens should reflect that change. Central cells should be thinner than peripheral ones. The shape of gradient in cells size should furthermore reflect the gradient in refractive index, being steepest in the periphery of the lens (Figure 8, right). However, lens fiber cell thickness profiles are flat (see “Fiber cells” on page 37). In contrast to prediction from the compaction hypothesis, the central cells are larger and more irregular, rather than compressed (Rae et al. 1982, Kuszak et al. 1985, Al-Ghoul et al. 1997, Bassnett et al. 2003).

In a comparative study on nine fish species (Paper IV), we modeled the change in cell thickness predicted by the compaction hypothesis. We measured cell thickness in a central growth shell in an adult fish lens and reversely followed lens growth, so that in the end the shell was much closer to the surface in a younger and smaller lens. The model calculated the initial size of the growth shell if it were reduced by compaction to the size measured. For all nine species, the model predicted an unrealistic shrinkage of 66% in cell thickness caused be compaction. Such large shrinkage of a young growth shell would be necessary to achieve the measured

thickness of the older growth shell (Figure 10). This large effect has not been observed in our study involving comparison of fiber thickness in two lens size groups (Paper III). On the contrary, average fiber thickness was the same in both groups (Figure 9).

Our model is based on the most conservative assumptions. We assumed that only γ-crystallin is present in the lens fibers. Because of the high refractive increment of γ-crystallin, the least change in concentration, and thus the least change in cell thickness, would be required to achieve a certain increase in refractive index. We are therefore confident that the compaction hypothesis has to be rejected.

It is more likely that the required changes in refractive index in growing lens are achieved by transporting crystallin inwards from the metabolically active cells in the lens periphery. It is too early to speculate about the involved mechanism(s).

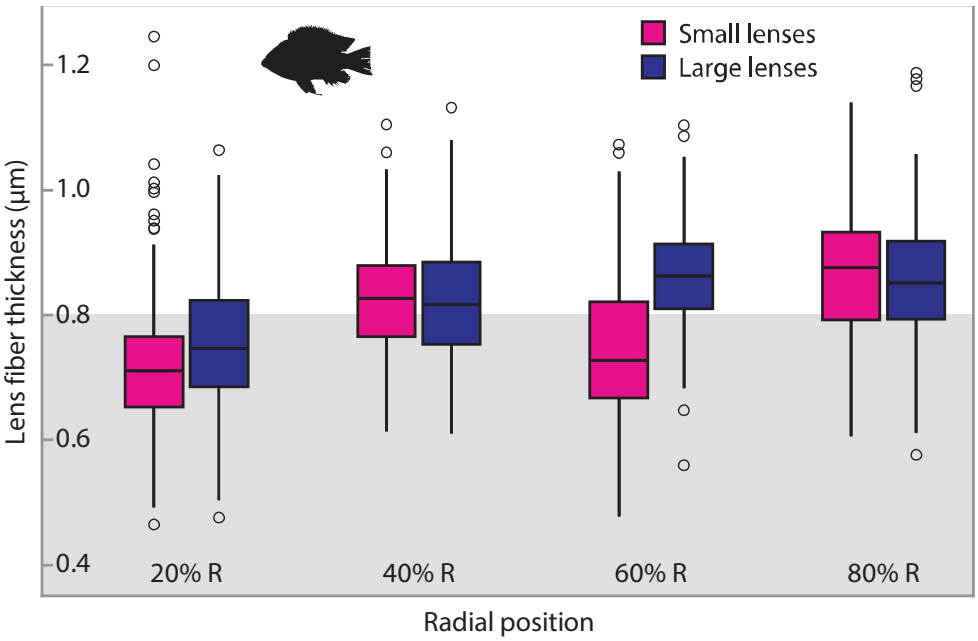


Figure 9 Lens fiber cell tickness measured in the Nile tilapia. The figure shows distributions of measured thickesses at four radial distances from the lens center in % lens radius (Paper III). Labeled in magenta are small lenses (Average diameter 2.4 mm, S.D.: 0.2 mm, n = 11), labeled in blue are large lenses (Average diameter 3.5 mm, S.D.: 0.4 mm, n = 14). The black lines across the boxes indicate the medians. The gray region spanning across the figure and between 0.35 and 0.80 µm represents the range of spectral sensitivities in fish.

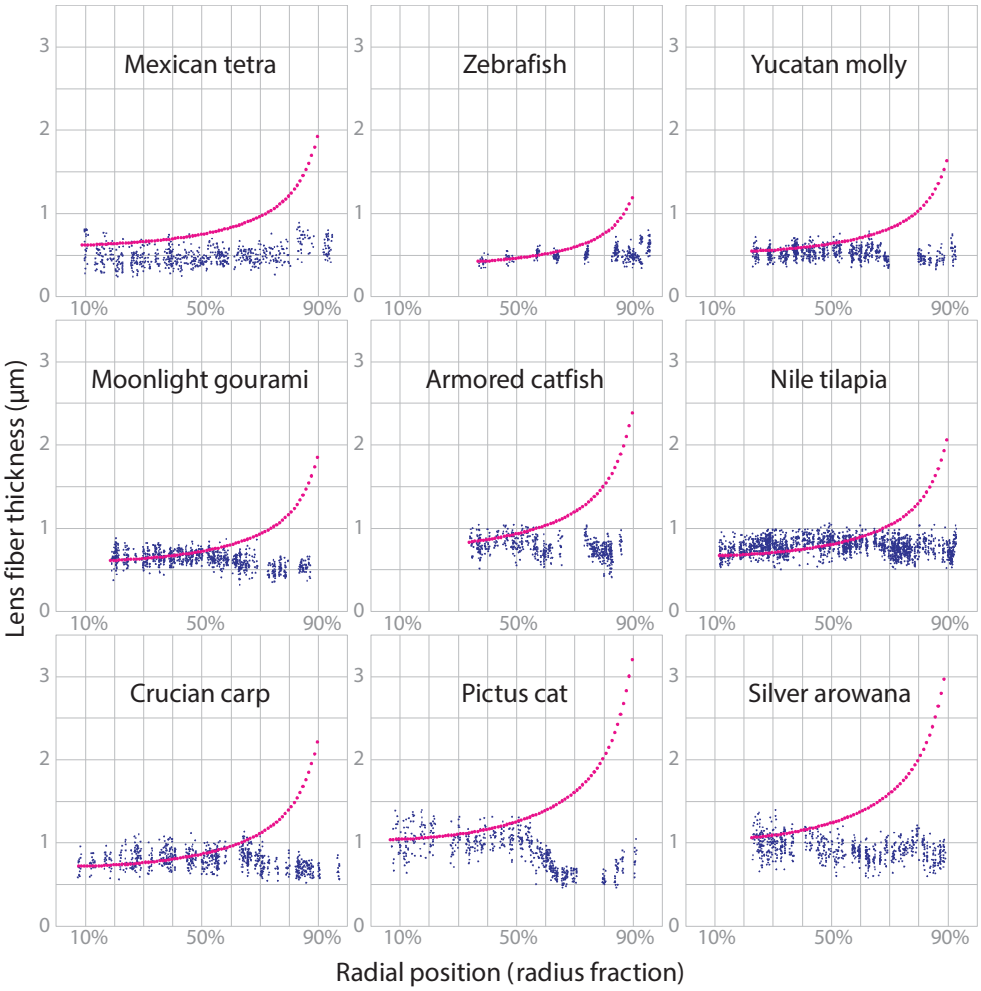


Figure 10 Lens fiber cell thickness profiles of nine fish species. Measured thickness (blue dots) is plotted against relative radial distance from the lens center (100% is the lens surface). Marked in magenta are the results from modeling (Paper IV). The dots represent historical thicknesses of the most central fibers measured in each species. Each relative radial position indicates the time when the lens was so small that the central fibers were at that relative radial position (i.e. 90% is a point in time when the lens was so small that the now central fibers were close to the lens surface, but had already degraded their nuclei).

"Been there. Seen that. Got the scars."

Marcus, sheriff of Broken Hills

Conclusions

I have investigated some of the biological regulatory mechanisms governing the development of crystalline lenses. I used fish as model animals because they possess optically interesting lenses, while the geometrical simplicity of fish lenses allows for studies that are difficult or impossible with the lenses of other animals.

Project 1 – Plasticity (Paper I)

This project concentrated on the comparison of optical plasticity in two fish species. Because of global warming, Atlantic cod has moved northward into the territory of the closely related Polar cod native to the region. We investigated fish from around Svalbard, Norway, where they experience annual polar days and nights. Evolution has not adapted Atlantic cod to such a light regime and we hypothesized therefore that the light/dark adaptive mechanisms may be different between the species. To test this hypothesis, we light adapted fish of both species caught locally during the polar night and compared the optical properties of their lenses, using dark adapted fish as controls. The lenses of Polar cod did not respond, whereas there were light adaptive changes in the lenses of Atlantic cod. We repeated the experiment during the polar day and observed that the lenses of Polar cod now were optically different (unpublished data). Our findings can be explained by the existence of two different mechanisms controlling the optics of fish lenses. A short-term one adapting the lenses to daily light/dark cycles (Atlantic cod) and a long-term one evolved for coping with long polar days and nights (Polar cod).

Project 2 – Osmolality (Paper II)

During a pilot study on the focal lengths of larval zebrafish lenses, we observed that the optical quality of the lenses deteriorated rapidly and some even burst after being placed in an immersion medium of adult-level osmolality (320 mOsm). That value is well established for work on the lenses of adult fish of various species, but it

seemed to be too high for the larval lenses. We designed an experiment to systematically study our preliminary observations. We submerged larval lenses in media of different osmolalities and designed a method for the quantification of the speed of optical deterioration. The results showed that lower osmolality (240 mOsm) cultivated larval lenses much better. To further test the hypothesis that the body fluids of fish larvae have lower osmolalilty than in adults, we fixed whole larval eyes in three fixatives. Again, we found that 240 mOsm gave the best results. The eyes were fixed in more natural shapes than in fixatives of higher osmolalities. We concluded that zebrafish larvae have lower osmolality in their bodies than adult fish. It is reasonable to believe that the larvae of other species have similar properties because the body surface to volume ratio is unfavorable for the maintenance of an osmolality difference to the environment in such tiny animals. We have shown that the optical properties of their crystalline lenses can be used as indicator for the match in osmolality. The optimal osmolality value has to be determined experimentally in each species and age group.

Project 3 – Cell thickness (Paper III & IV)

The key interest of the third project was the investigation of the cellular structure of adult fish lenses. Because fish lenses are very hard, standard histological sectioning methods fail. We developed a new technique for the visualization of the cellular structure of fish lenses and used it for looking into the thicknesses of fish lens fiber cells. We observed that (1) the average cell is much thinner than the lens fiber cells in other vertebrates, (2) cell thickness remains constant throughout a fish lens, and (3) the average value is independent from the size of the lens (Paper III). We followed up on our findings with a more detailed analysis, measuring continuous cell thickness profiles across lenses. In that study, we included nine species of fish. All of them had considerably thinner lens fibers than other vertebrates, with species-specific differences (Paper IV). This work allowed us to address the question of how mature lens fibers achieve the necessary increase in refractive index in a growing lens. The cells lack nuclei and organelles, so that they are incapable of protein synthesis. It had generally been assumed that the cells increased cytosolic protein concentration by compaction, i.e. that they simply expel water. However, with our new method, we observed that fiber thickness was constant within a lens and between lenses of different sizes of the same species. Modeling of fiber thickness during lens growth confirmed that compaction cannot be the main mechanisms. Based on the findings from both papers, we conclude that, at least in fish, protein is transported inwards between denucleated fibers in the growing lens. The cells in the peripheral lens layers have synthetic capabilities and are most likely the source of those proteins.

***

This work has unraveled some aspects of crystalline lens development and its regulatory mechanisms. We have learned about larval developmental plasticity and found a new form of long term optical plasticity. We managed to visualize the cellular anatomy of fish lenses and gained a better understanding of how lenses grow. Our results demonstrate that mature lens fiber cells are not as passive as one might think.

However, the answers we found lead to new questions. What are the principles behind short and long term optical plasticity? When does a fish larva become “osmotically adult” and how do its lenses cope with the osmotic changes in the body? Why are the lens fiber cells thinner in fish than in other vertebrates and why are there differences between fish species? How is the transport of proteins in a lens controlled so precisely that crystalline lenses can meet high optical requirements throughout life?

New issues are identified to be addressed. I have provided a tool and this thesis as a guide. I can only hope that someone after me will show fish lenses the appreciation they deserve.

"Nobody exists on purpose, nobody belongs anywhere,
everybody's gonna die."

Morty Smith

Acknowledgements

I will start from an umbrella. There is a chance that I have missed you or didn't credited you properly. I apologize. There are three possible causes: (1) I have a terrible memory, (2) I am really bad with words and in expressing my feelings, and (3) I was writing these acknowledgements in a hurry shortly before the submission deadline! Have mercy!

Thank you, **Ronald**, for being an amazing supervisor, mentor, and friend. You made it possible for us to work together despite my difficult character. I could freely express myself, be independent, and get away with all my shenanigans. All this did not stop you from guiding my education and personal development. You made an engineer appreciate biology! You were pushing when I needed to be pushed, and you were supportive when I was lost. You treated me, and your other students, almost like your own children. I will never forget that! A million thanks, Boss. Slave out ;)

Big thanks to my Supervisory Committee: my co-supervisor **Eric**, my scientific mentor **Pål-Axel**, and my examiner **Dan**. Thanks for making sure I was going the right direction and kicking my butt when I was behind schedule.

When I was young, I fell in love with the TV series "Friends". It is a comedy show portraying a group of friends of vastly different characters. Despite those differences, they were great friends. Thank you, **Andrea**, **Atticus**, **Cara**, **Cynthia**, **Jochen**, **Johanna**, **Kevin**, **Peter**, and **Pilar**, for being the Friends of mine. Thank you for all the movies we have watched, for all the TV series we have discussed, and for all the board games we have played. Thank you for being a nursing ground for my nerdiness. Bonus thanks to da boyz for sucking at games with me!

Giant thanks to people who shared office with me: **Micke**, **Nicola**, **Andrea**, **Julia**, **Sandra**, and **Mikael**. You deserve medals and should visit an otologist and a psychologist to make sure that long-term exposure to me didn't leave any permanent damage. Special thanks to **Micke** for discussing all that we hate about things and people! I miss those talks, man!

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People come and go, yet **the Vision Group** remains unchanged. Home-like atmosphere, lots of fun, joy of doing science, and hectoliters of alcohol. Thank you all individually and as the group you are forming. You guys are absolutely marvelous!

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[*] I do pay taxes. You are missing the metaphor!!!

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"Page-turners they were not."

Master Yoda

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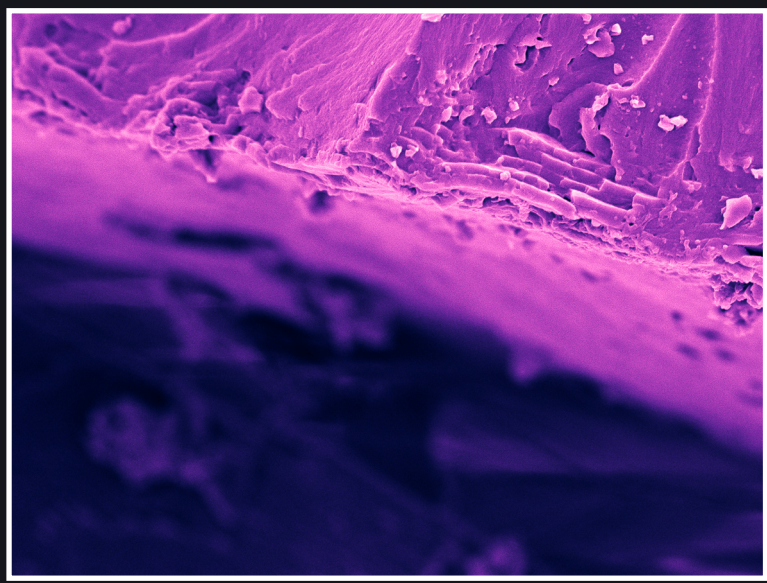

This work has unraveled some aspects of crystalline lens development and its regulatory mechanisms. We have learned about larval developmental plasticity and found a new form of long term optical plasticity. We managed to visualize the cellular anatomy of fish lenses and gained a better understanding of how lenses grow. Our results demonstrate that mature lens fiber cells are not as passive as one might think.

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