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Adaptation of vision in deep-water fish: a comparison of freshwater and marine species

Adaptace zraku u ryb z hlubokých vod: porovnání sladkovodních a mořských systémů

Bachelor's thesis

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## **Prohlášení**

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Při tvorbě práce jsem využila také nástroje umělé inteligence pro jazykovou korekturu. Tato práce, ani její podstatná část, nebyla předložena k získání jiného nebo stejného akademického titulu.

## **Poděkování**

Tímto bych chtěla poděkovat Mgr. Zuzaně Musilové, Ph.D., za odborné vedení a cenné rady při psaní mé bakalářské práce. Dále děkuji všem členům její laboratoře za podporu a inspirativní prostředí. Velké poděkování patří také mému příteli Filipu Doležalovi za neustálou podporu a motivaci. Mé díky patří i mým rodičům a celé mé rodině, především Ivě Drbalové za to, že při mně vždy stála a vzbudila ve mně zájem o přírodní vědy. V neposlední řadě děkuji svým přátelům, kteří mě podporovali a motivovali na této cestě.

## **Abstrakt**

Tato bakalářská práce je rešerší na téma zrakových adaptací hlubokovodních ryb. Ryby žijící ve velkých hloubkách čelí výzvě ve formě omezeného množství světla, jehož intenzita s rostoucí hloubkou klesá a kolem 1000 metrů zcela mizí. Aby se těmto světelným podmínkám přizpůsobily, vyvinula se rybám řada adaptací na molekulární úrovni. Mezi tyto adaptace patří duplikace a následná mutace opsinových genů, jejich ztráta, nebo flexibilní regulace jejich exprese. Pomocí těchto mechanismů, společně s využitím derivátů vitamínu A jako chromoforů, dokážou ryby posouvat absorpční maximum opsinových pigmentů směrem ke kratším vlnovým délkám, které ve větších hloubkách převažují. Závěrečná část práce se zaměřuje na srovnání mezi hlubokomořskými a hluboko-sladkovodními druhy. Ačkoli se jejich prostředí liší, světelné podmínky jsou často velmi podobné a následně i adaptace na ně. Hlubokomořské druhy běžně využívají bioluminiscenci a mívají sítnice složené pouze z tyčinek. Sladkovodní druhy, jako jsou cichlidy a vranky, sice některé opsinové geny neexprimují, ale na rozdíl od mořských druhů je neztratily zcela, což ovšem souvisí s mladším evolučním stářím kolonizace hlubin. Tyto paralelní strategie naznačují přítomnost konvergentní evoluce u ryb přizpůsobených životu v extrémně tmavých podmínkách.

## **Klíčová slova**

vizuální adaptace, hlubokovodní ryby, opsinové geny, spektrální ladění, konvergentní evoluce

## **Abstract**

This bachelor's thesis is a review on the topic of visual adaptations in deep-water fish. Fish living at great depths face the challenge of limited presence of light, the intensity of which decreases with increasing depth and completely disappears around 1000 meters. To adapt to these light conditions, fish have developed a range of molecular-level adaptations. These adaptations include the duplication and subsequent mutation of opsin genes, their loss, or flexible regulation of their expression. Through these mechanisms, along with the use of vitamin A derivatives as chromophores, fish can shift the absorption peak of opsin pigments towards shorter wavelengths, which dominate at greater depths. The final part of the thesis focuses on a comparison between deep-sea and deep-freshwater species. Although their environments differ, the light conditions are often very similar, and consequently, the adaptations are also similar. Deep-sea species commonly utilize bioluminescence and have retinas composed solely of rods. Freshwater species, such as cichlids and cottoids, do not express certain opsin genes, but unlike marine species, they have not completely lost them, which is related to the younger evolutionary age of their deep-water colonization. These parallel strategies suggest the presence of convergent evolution in fish adapted to life in extremely dark conditions.

## **Keywords**

visual adaptation, deep-water fish, opsin genes, spectral tuning, convergent evolution

### **Abbreviations used:**

A1	11-cis retinal
A2	11-cis 3,4-didehydroretinal
GPCR	G-protein-coupled receptor
LWS	long wavelength-sensitive opsin
Mya	million years ago
RH1	rhodopsin
RH2	rhodopsin like 2
RH2A $\alpha$	rhodopsin like 2A alpha
RH2A $\beta$	rhodopsin like 2A beta
RH2B	rhodopsin like 2B
SWS1	short wavelength-sensitive opsin 1
SWS2	short wavelength-sensitive opsin 2
SWS2A	short wavelength-sensitive opsin 2A
SWS2B	short wavelength-sensitive opsin 2B

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## **1. Introduction**

Deep-water environments, both marine and freshwater, present extreme conditions for life, including low temperatures, high hydrostatic pressure, and near or total darkness (Shen et al., 2019). While deep-sea habitats extend on average to depth of 3,800 meters (Partridge et al., 1988), the deepest freshwater environment, Lake Baikal, reaches approximately 1,600 meters (Sideleva, 2006). Despite these differences in scale, both environments impose similar visual challenges on the species that inhabit them (Bowmaker et al., 1994).

Vision remains a vital sensory system for fish in both environments, influencing their ability to detect prey, avoid predators, and navigate their surroundings. The extreme light limitations in deep waters have driven significant molecular adaptations in fish vision, particularly in the evolution and regulation of opsin genes, which determine spectral sensitivity. These adaptations allow fish to optimize their visual systems for the specific wavelengths of light available in their environment (Musilová et al., 2021).

This thesis examines the molecular adaptations of fish vision in deep-water environments, comparing freshwater and marine species. By focusing on cichlids and cottoids from deep lakes alongside their marine counterparts, this study explores how different species adapt to similar selective pressures. While both groups exhibit convergent evolution in some aspects of visual adaptation, key differences arise due to the distinct optical properties of freshwater and marine habitats. Through this comparison, this research aims to provide a deeper understanding of how vision evolves under extreme low-light conditions.

## **2. The visual system of fish**

The eyes of fish have evolved a variety of adaptations to serve two main functions: capturing light and forming a sharp image for retinal processing (Douglas and Djamgoz, 1990). Since light behaves differently underwater than on land, fish vision is heavily influenced by environmental factors such as water depth, turbidity, and the spectral composition of available light (Spady et al., 2005). These conditions have shaped the anatomy and physiology of fish eyes, resulting in diverse adaptations that enhance visual sensitivity and acuity across different habitats (Lythgoe, 1988).

### **2.1. The underwater light environment**

Before light enters the water, it is affected by the atmosphere, where it may be scattered or absorbed by particles. Once it reaches the water surface, its properties are further modified

due to the inherent optical characteristics of water. The most significant factor affecting underwater visibility is light scattering, which causes a fog-like effect, reducing clarity (Carleton, 2009). The absorption and scattering characteristics depend on multiple factors, including phytoplankton content, dissolved organic matter, and suspended particles (Douglas and Djamgoz, 1990).

As depth increases, light intensity decreases significantly. Both absorption and scattering play crucial roles in determining how much light penetrates deeper layers. Water acts as a spectral filter, allowing only a narrow band of wavelengths to reach deeper. In clear waters, shorter to middle wavelengths (blue and green) penetrate deeper beyond 200 m, while the shorter wavelengths (UV) and longer wavelengths (red) are absorbed quickly, reaching just up to 50 m. The residual light in the deep waters primarily consists of wavelengths between 460 and 490 nm (Carleton, 2009). At depths beyond 1,000 meters, there is insufficient light to support vision, and organisms in deep-sea environments must rely on bioluminescence for illumination. In turbid waters, however, light intensity and spectral composition shift toward longer wavelengths, allowing red light to penetrate deeper compared to clearer waters. This occurs due to the increased scattering and absorption of shorter wavelengths (Bowmaker et al., 1994).

The penetration of light differs between marine and freshwater environments due to their varying levels of clarity and particulate content. Open ocean waters allow for greater light penetration compared to coastal waters, which are rich in suspended sediments and organic matter (Atema et al., 1988). In contrast, freshwater bodies can exhibit a wide range of optical properties. In freshwater environments, the optical properties of water vary based on the ecosystem. Rift lakes, such as Lake Tanganyika and Lake Malawi, exhibit high clarity, allowing deeper light penetration. In contrast, Lake Victoria, which is much shallower, experiences turbid, red-shifted waters due to sediment and eutrophication, which significantly alter the available light spectrum (Carleton, 2009). Similarly, in Lake Barombi Mbo, species residing in the deep-water zone have adapted to reduced light intensity and a narrower spectral range, with a lack of red and UV wavelengths (Musilova et al., 2019b).

## **2.2. Anatomy and physiology of the fish eye**

The fish eye has the same basic structure as other vertebrate eyes, with one key difference in the lens. The fish lens is spherical and lacks accommodation, meaning it moves from back to front to focus (Fernald and Wright, 1985). On the retina, we can find specialized

cells called photoreceptors, which are capable of capturing photons, and then through a specific cascade of signaling and neural processes send information to the brain (Bowmaker, 2008).

There are two main types of photoreceptors in the retina. The first are rods, which are used for vision in dim (scotopic) light. They are composed of a specialized protein called opsin, and in this instance, they use only one kind of opsin: the rhodopsin (RH1), which has its max absorbance around 500 nm wavelengths. Opsins are covalently bound to a specialized chromophore, 11-cis retinal, a vitamin A derivative, which together create a visual pigment. The second type of photoreceptor are cones, which are responsible for color (photopic) vision. Vertebrates have evolved four classes of cone opsins for color vision: short wavelength-sensitive 1 (SWS1) ( $\lambda_{\max} \approx 360\text{--}440$  nm), short wavelength-sensitive 2 (SWS2) ( $\lambda_{\max} \approx 400\text{--}450$  nm), rhodopsin like 2 (RH2) ( $\lambda_{\max} \approx 450\text{--}530$  nm) and long wavelength-sensitive (LWS) ( $\lambda_{\max} \approx 510\text{--}560$  nm). Many lineages have lost one or more of them over evolutionary time. Teleostei have undergone two rounds of gene duplication which has led to broadening their opsin repertoire. Teleosts usually have more opsin genes. As an example, in Nile tilapia (*Oreochromis niloticus*; *Cichlidae*) there are seven opsin genes coding for the following opsin proteins: for short-wavelength light the short wavelength-sensitive opsin 1 (SWS1), which detects the UV light ( $\lambda_{\max} \approx 360$  nm), followed by short wavelength-sensitive opsin 2B (SWS2B) ( $\lambda_{\max} \approx 425$  nm) and short wavelength-sensitive opsin 2A (SWS2A) ( $\lambda_{\max} \approx 456$  nm) for the blue light. For medium length wavelengths, they have rhodopsin like 2B (RH2B) ( $\lambda_{\max} \approx 472$  nm), rhodopsin like 2A beta (RH2A $\beta$ ) ( $\lambda_{\max} \approx 517$  nm) and rhodopsin like 2A alpha (RH2A $\alpha$ ) ( $\lambda_{\max} \approx 528$  nm) for green light. For the long wavelengths they have long wavelength-sensitive opsin (LWS) ( $\lambda_{\max} \approx 560$  nm) for red light (Fig. 1). Not all fish express all the cone opsins at once. They vary depending on numerous factors like water depth and light intensity. The various opsins they do express reflect the environment they live in and what wavelengths of light are relevant for them. Fish standardly express two to four opsins making them dichromatic, trichromatic or tetrachromatic (Spady et al., 2006; Yokoyama, 2008; Schweikert et al., 2018; Musilova et al., 2021, p. 2023; Ricci et al., 2023).

Cones can be found on the retina either as single cones or double cones, which can enhance phototransduction (Fig. 1) (Pignatelli et al., 2010). In single cones we strictly find opsins for shorter wavelengths meanwhile in double cones we exclusively find pairs of different longer wavelength opsins. Though the double cones are joined at the top half, they work as a separate channel forming different information (Marchiafava and Boycott, 1997). In teleosts they serve as an enhancing mechanism for color vision, unlike in birds, where double cones

consist of principal and accessory cone, which are used for achromatic vision (Günther et al., 2024).

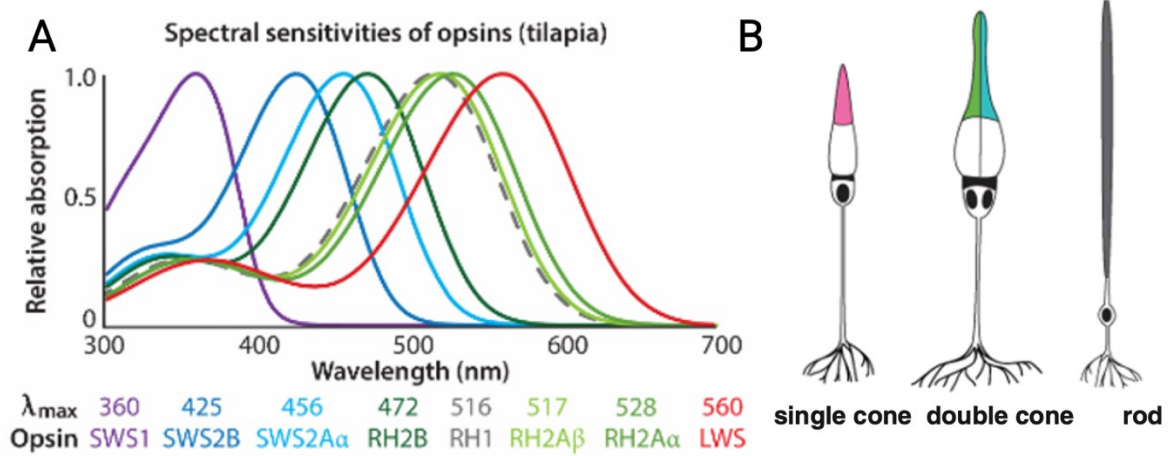


Figure 1: (A) Spectral sensitivities of visual opsin in cichlid (tilapia), Borrowed from (Musilova et al., 2021), (B) Retinal photoreceptor types: single cones, double cones, and rods, Borrowed from (Carleton et al., 2020)

Teleosts form specific patterns on the retina, where the single and double cones occur in an organized mosaic. In the zebrafish, the model organism for fish, we can find rows of double cones expressing two opsins sensitive to longer wavelengths followed by a row next to them formed by single cones expressing opsins sensitive to shorter wavelengths (Fernald, 1981). In cichlids, fish inhabiting various depths of lakes ranging from shallow to deep waters around 100 m, we can find a specific square mosaic where 4 double cones surround a single cone (Dalton et al., 2014). This specific mosaic structure was found to be lost in deepwater cichlids. It remains unknown for what reason this loss is happening (Fig. 2) (Musilova et al., 2021).

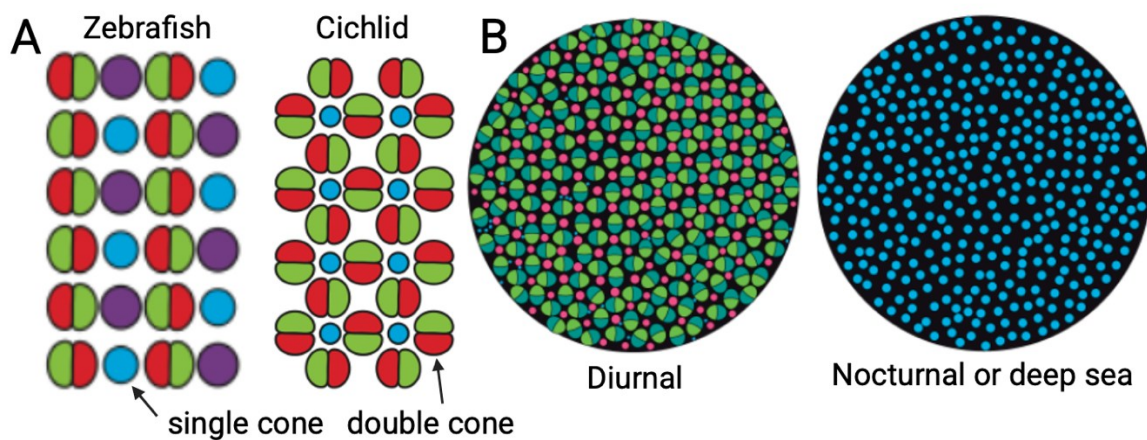


Figure 2: (A) Comparison of cone mosaic in zebrafish and square mosaic in cichlids, Borrowed from, (Musilova et al., 2021) (B) Comparison between diurnal species and nocturnal or deep-sea species, which have lost the mosaic and double cones, Borrowed from (Carleton et al., 2020)

Teleosts living in deep environments, whether in freshwater or marine habitats, have undergone various anatomical adaptations to improve their ability to see in low light. For example, many species have enlarged their eyes to capture as much light as possible, and some have specialized structures like tubular eyes, which help to optimize the light-gathering process. Additionally, the presence of the tapetum lucidum, a reflective layer in the eye, helps to reintroduce light by reflecting photons that have passed through the retina, thus enhancing the likelihood of photoreceptor activation (de Busserolles et al., 2020).

### **3. Molecular adaptation of the visual system of deep-water fish**

Teleosts have evolved a diverse array of molecular adaptations to optimize vision in deep waters, where light is extremely limited (Lythgoe, 1988). These adaptations range from older evolutionary mechanisms, such as opsin gene duplication and loss and spectral tuning through opsin mutations, to more recent innovations like the dynamic regulation of opsin expression, which can vary within the same species depending on environmental conditions (Parry et al., 2005). By modifying their visual systems at genetic and molecular levels, teleosts have achieved remarkable flexibility in detecting and interpreting light, enhancing their ability to survive and thrive in diverse ecological niches (Muntz, 1976).

#### **3.1. Opsin gene mutation**

##### **3.1.1. Mechanism of spectral tuning**

One of the oldest evolutionary mechanisms for adapting to a specific habitat, in this case, the deep waters, is through gene mutation (Bowmaker, 2008). Opsin genes located within the fish genome, consists of specific nitrogenous bases that, through transcription and translation, encode a chain of amino acids forming the opsin protein (Yokoyama, 2000). Mutations in these genes can lead to alternative versions of opsins, modifying their spectral sensitivity (Musilova et al., 2021).

Opsins are membrane-bound proteins, belonging to the G-protein-coupled receptor (GPCR) family (Palczewski et al., 2000). They consist of seven transmembrane helices and contain specific amino acid positions, known as key tuning sites, located within the retinal binding pocket (Fig. 3) (Hunt et al., 2001). Mutations at these sites can shift the wavelength of

light absorption, fine-tuning visual sensitivity. These shifts may result from single nucleotide substitutions or more substantial changes, such as frameshift mutations, although not as common, which alter the amino acid composition of the final protein (Nagai et al., 2011). Additionally, structural modifications in opsins, such as changes in glycosylation sites or interactions with chromophores, can further refine visual capabilities (Sugawara et al., 2005).

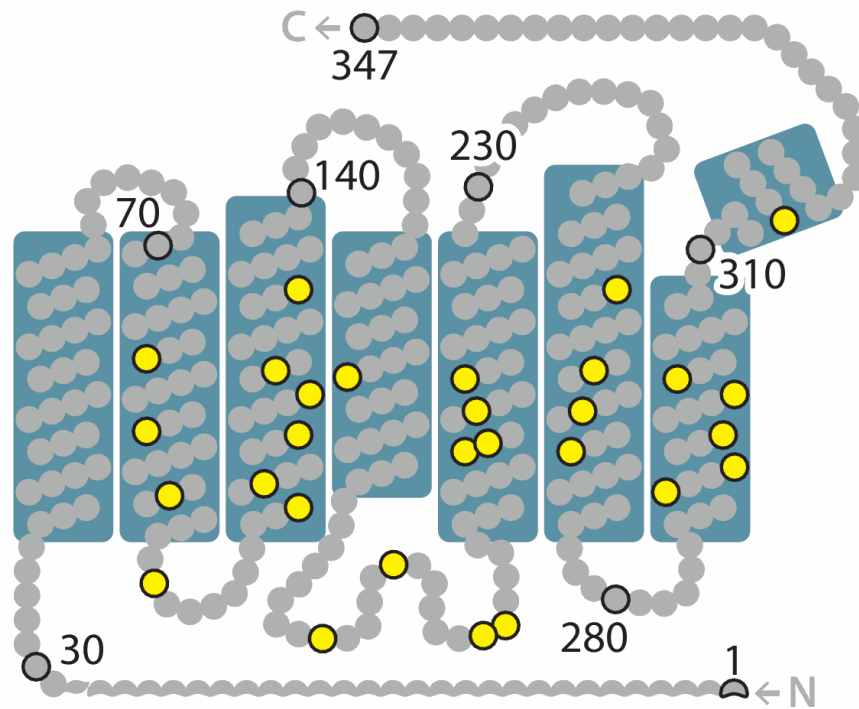


Figure 3: A graphical representation of the rhodopsin molecule, illustrating its seven transmembrane  $\alpha$ -helices and key amino acid sites in yellow, Borrowed from (Musilova et al., 2021)

Key tuning sites play a critical role in determining opsin spectral properties (Douglas et al., 1998b). Changes at these sites can lead to either shortwave shifts (toward blue/UV light) or longwave shifts (toward red/infrared light), depending on the selection pressures imposed by the environment. In deep-water fish, these mutations frequently result in spectral shifts toward shorter wavelengths, since that's the composition of the remaining downwelling light and bioluminescence (Carleton and Kocher, 2001). Depending on the amino acid background, these shifts can range from 0 to 75 nm (Yokoyama, 2002). The primary opsins affected include rod opsins RH1 and cone opsins, typically shifting the absorbencies by 6–8 nm (Hunt et al., 1996). Some opsin mutations are conserved across multiple deep-sea lineages, suggesting convergent evolution in response to similar selective pressures. This phenomenon highlights the power of natural selection in shaping molecular adaptations (Hunt et al., 2001). Notably, amino acid

substitutions at tuning sites have been identified even between closely related species (Spady et al., 2005).

### 3.1.2. Key tuning sites in opsins

Among vertebrates, the most extensively studied opsin gene is RH1. Rhodopsin in fish is completely intronless, in contrast to the four to five introns typically found in other vertebrates (Fitzgibbon et al., 1995). The key spectral tuning sites of RH1 have been well described, illustrating how specific amino acid substitutions can shift the wavelength of peak absorbance. For comparison in bovine rhodopsin, six most important tuning sites have been identified at positions 83, 122, 134, 135, 211, and 292 (Nathans, 2002). However, a broader range of tuning sites across different opsin classes has been described in comparative studies, particularly in teleosts and other vertebrates (Yokoyama, 2008). Among fish, the substitution of alanine with serine at position 292, referred to as A292S, is commonly observed when species adapt from shallow to deeper waters. This specific substitution has independently evolved at least four times in lineages transitioning to deep water and has also been reversed at least three times in species adapting back to shallow environments (Nagai et al., 2011). In cichlids, the shift from serine to alanine causes an 11 nm red-shift (from 487 nm to 498 nm), while the reverse shift to deep-water from alanine to serine results in a 14 nm blue-shift (from 501 nm to 491 nm). Other commonly substituted RH1 sites include positions 83, 118, 124, 168, 292, and 299, which are known to influence spectral tuning due to changes in charge or the gain/loss of hydroxyl groups (Hope et al., 1997).

As for cone opsins, those at the edges of the light spectrum (SWS1 and LWS) exhibit more variability than the middle-wavelength opsins (SWS2 and RH2) (Carleton et al., 2020). In LWS opsins, the key tuning sites include S164, H181, T269, and A292. A well-documented example is the S164A substitution in cichlids, which causes a significant blue shift in peak sensitivity. Other substitutions associated with blue shifts in LWS include Y261F in *Mola mola*, S164P and H181Y in guppies, A292S in duplicated LWS opsins of *Cyprinodon variegatus* and in salmonids the substitutions A164S and A269T. Similar blue-shifting substitutions have been observed across multiple lineages including Cyprinodontiformes, Cypriniformes, Salmoniformes, Gobiiformes, Osteoglossiformes, and Characiformes. This confirms a widespread evolutionary trend toward shorter wavelength sensitivity. In the SWS2 opsin the substitutions A94C, S97C, T118G and W265Y serve as major tuning sites linked to shifts in spectral sensitivity. For SWS1 the substitution at F86 is essential for enabling ultraviolet sensitivity. Lastly, for the RH2 opsin, primary tuning sites include E122, M207, and S292.

These substitutions enable fine-scale adjustments in spectral sensitivity, allowing species to adapt to varying photic environments (Fig.4) (Lin et al., 2017).

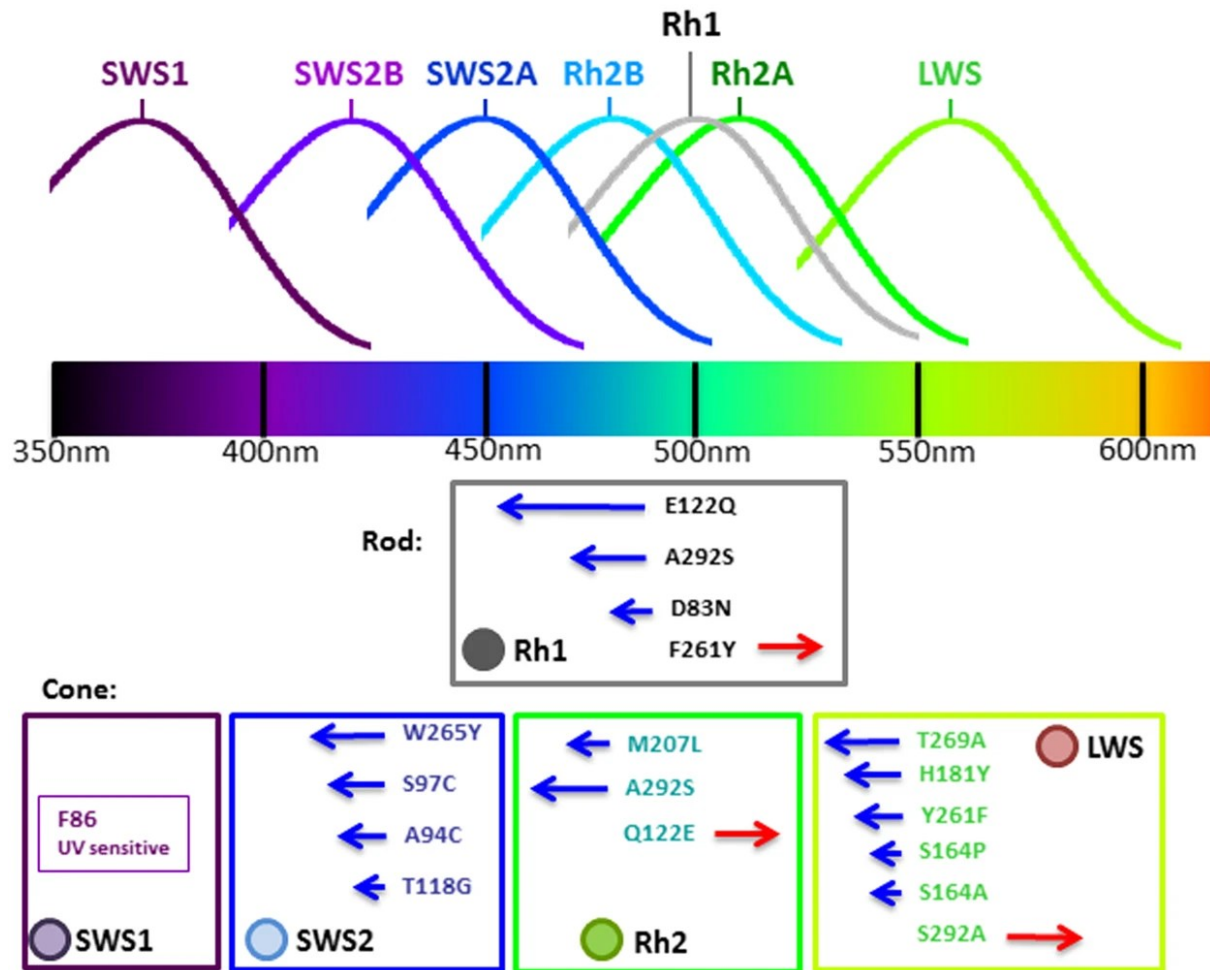


Figure 4: A graphical summary of key tuning sites of opsins and the corresponding spectral shifts following amino acid substitutions, Borrowed from (Lin et al., 2017)

### 3.2. Opsin gene duplication and loss

Beyond individual amino acid substitutions, gene duplications and losses also play a crucial role in spectral tuning (Hunt and Collin, 2014). Duplication events can lead to the diversification of opsin genes, allowing fish to express multiple opsins with different absorption spectra. This diversification increases visual flexibility, providing an advantage in environments with variable lighting conditions. Some species retain duplicated opsins with slightly different spectral properties, enabling them to detect a broader range of light wavelengths (Lupše et al., 2022).

In contrast, opsin gene loss can also be an adaptive response, particularly in environments where certain wavelengths of light are absent. In deep-water, mainly deep-sea environments with limited spectral diversity, some species have lost specific opsins that no

longer provide a functional advantage. This streamlining of the opsin repertoire reflects evolutionary pressures that optimize vision for the prevailing light conditions (Musilova et al., 2019b).

Both duplication and loss contribute to the fine-tuning of visual sensitivity, allowing fish to adapt to their specific photic environments through evolutionary modifications in their opsin gene repertoires (de Busserolles et al., 2020).

### 3.2.1. Significance of opsin gene duplication

Opsin gene duplication has occurred multiple times across vertebrate lineages (Matsumoto et al., 2006) and has been especially prominent in cone opsins, where it plays a central role in the diversification and spectral tuning of visual systems (Carleton and Kocher, 2001). Among cone opsins, those sensitive to middle wavelengths (RH2 and SWS2) tend to duplicate more frequently than those at the spectral edges (SWS1 and LWS), enabling neofunctionalization and fine-tuned adaptation to varying photic environments (Bowmaker, 2008). For instance, in Osteoglossiformes and Characiformes, the RH2 gene was lost, but a duplication of the LWS gene compensated for this loss. One of the duplicated LWS paralogs underwent spectral tuning to shorter wavelengths, effectively replacing RH2 function through neofunctionalization (Musilova and Cortesi, 2021). A similar process has been observed in SWS2, where duplications can shift sensitivity into the violet range (Cortesi et al., 2015).

The RH2 has independently duplicated multiple times, particularly within ostariophysan and acanthopterygian lineages around 230 million years ago (Mya). The SWS2 underwent a duplication event roughly 200 Mya in the acanthopterygian lineage, while LWS has experienced several rounds of duplication across different teleost groups (Hofmann and Carleton, 2009). In contrast, SWS1 remains a single-copy gene in nearly all lineages, suggesting evolutionary constraints on its diversification (Bowmaker, 2008).

Although RH1 is usually found as a single-copy gene in most vertebrates, several deep-sea fish lineages have duplicated it. These duplications provide additional RH1 paralogs that can accumulate mutations, enabling spectral tuning and enhanced sensitivity to dim-light conditions (Musilova and Cortesi, 2021). For example, certain eel species express two RH1 opsins: RH1fwo, associated with freshwater habitats, and RH1dso, associated with marine habitats. These are differentially expressed during the eel's migration (Morrow et al., 2017). In the pearleye (*Benthallbella spp.*), an additional RH1 gene (RH1b) is expressed specifically in adults that inhabit deeper waters, serving as an adaptation to the deep-sea environment (Pointer

et al., 2007). In contrast to marine examples, such duplications have not been found in freshwater deep-living cichlids (Ricci et al., 2022).

### 3.2.2. Loss of opsin genes

In the process of adapting to dim-light environments, many deep-water fish have lost certain opsin genes. The loss of opsin genes is particularly common among species living in habitats where light is scarce, such as the deep (Musilova et al., 2019b) or turbid waters (Escobar-Camacho et al., 2017). These environments often lack UV and red wavelengths of light, which are typically detected by SWS1 and LWS cone opsins. As a result, many deep-sea fish species have lost these genes and the ability to perceive these wavelengths entirely, relying instead on the detection of shorter or middle wavelengths, particularly in the blue-green part of the light spectrum (440-520 nm). The loss of these opsins can be understood as a form of photoreceptor simplification, driven by evolutionary pressures that remove the need for costly or unnecessary genetic material in environments, where such wavelengths are no longer relevant (Lupše et al., 2021; Musilova and Cortesi, 2021).

Complete opsin loss has been observed in extreme environments such as in aphotic caves. For example, *Typhlichthys subterraneus* is a species with severely degenerated eyes, which retains only one functional RH2 opsin gene due to the total absence of light (Aardema et al., 2020). While cavefish serve as an illustrative extreme, the majority of opsin gene loss in deep-water systems is selective, rather than total, and aligned with the prevailing light conditions (Musilova and Cortesi, 2021).

### 3.3. Opsin gene regulation and expression

Opsin gene regulation plays a crucial role in visual adaptation, enabling fish to respond to diverse environmental light conditions without relying solely on genetic mutations or duplications. This dynamic regulation is influenced by ecological variables, developmental stage, and evolutionary history (Hofmann et al., 2009). Unlike gene duplications, which accumulate over evolutionary time, changes in gene expression can occur rapidly within an individual's lifespan (O'Quin et al., 2010).

Across most teleosts, seven cone opsin genes and one rod opsin (RH1) are present in the genome. Shallow-water species often utilize all cone opsins, functioning as tetrachromats (Parry et al., 2005), whereas deep-water species tend to simplify their visual system, expressing only one or two cone opsins or relying exclusively on RH1 for scotopic vision (Musilova et al.,

2019a). However, this reduction does not necessarily indicate gene loss; rather, many opsins are simply not expressed (Musilova and Cortesi, 2021).

Gene regulation occurs through both cis- and trans-regulatory elements, allowing for fine-tuning of opsin expression to adapt to specific environments (Carleton et al., 2010). In rods, expression is mainly controlled by cis-regulation, whereas cones use both regulatory mechanisms (Tsujimura, 2020). Expression patterns can also vary seasonally in freshwater environments, responding to environmental shifts such as daylength, temperature, or phytoplankton density, an effect which is less commonly observed in marine systems (Musilova and Cortesi, 2021).

### 3.3.1. Interspecies variation

Opsin expression patterns differ widely between species and are often linked to the ambient light environment. Comparative expression studies reveal that closely related species occupying different light environments (shallow vs. deep habitats) may express different subsets of opsin genes (Hofmann et al., 2009). This indicates that adaptive visual tuning in cichlids is achieved largely through modulation of gene expression, rather than structural genetic changes. Such plasticity allows populations to adjust rapidly to their visual environments, contributing to speciation (Carleton and Yourick, 2020).

For example, deep-living cichlids in Lake Tanganyika or Lake Barombi express a reduced set of cone opsins, favoring shorter to middle wavelengths, while silencing opsins that detect UV and red light (Hofmann et al., 2009; Musilova et al., 2019b). In Barombi cichlids, this includes the downregulation of LWS and SWS2B and a shift towards the expression of RH2A paralogs, especially RH2A $\alpha$  in double cones and SWS2A in single cones (Musilova et al., 2019b).

In deep-sea species, opsin expression is similarly tuned to environmental light conditions. A study of 200 deep-sea fish species showed that 176 had retinas with only a single visual pigment, highlighting a widespread trend toward monochromatic vision adapted for low-light conditions (Douglas et al., 2003).

### 3.3.2. Developmental changes

Opsin expression is not static throughout life. Instead, it changes dynamically from larval to juvenile to adult stage. This pattern is observed in a variety of teleosts including salmon, zebrafish, flounder, and tilapia (Spady et al., 2006). This developmental modulation

allows fish to match their visual sensitivity to the changing photic environments they inhabit as they grow (Temple et al., 2008).

In Lake Malawi cichlids, two distinct patterns of opsin expression development have been described: neoteny, where adults retain a juvenile or larval expression pattern, and direct development, where adult-type opsins are expressed from early developmental stages (Carleton et al., 2008). The general developmental pattern involves a decline in expression of short-wavelength opsins and a shift toward longer wavelengths as the fish matures. For example, SWS1 and RH2B are highly expressed in larvae but are turned off by adulthood. SWS2B then peaks in juvenile stage and then declines, while SWS2A expression increases throughout development and is maintained into adulthood. RH2A is expressed at low levels throughout development, and LWS increases steadily during development and is maintained in adults (Lupše et al., 2022).

These shifts in opsin expression are crucial for adapting to ecological transitions, such as moving from shallow nursery habitats to deeper adult territories (Hope et al., 1998) or from freshwater habitats into the deep sea (Temple et al., 2008). In deep-living cichlids, the developmental transition includes the silencing of UV- and red-sensitive opsins (SWS1, LWS, and SWS2B), reinforcing the specialization toward mid-wavelength sensitivity (Musilova and Cortesi, 2021). This transition supports dichromatic vision dominated by SWS2A and RH2A $\alpha$  in species from deeper zones (Musilova et al., 2019b). Interestingly, larval stages of many marine species begin life in epipelagic zones, expressing mid to long wavelength cone opsins (RH2), which are replaced by RH1-only systems in adults after vertical migration (Lupše et al., 2021).

### **3.4. Opsin binding to retinal A1 vs A2**

In many vertebrates, especially fish, there is a specialized molecular mechanism that allows them to adjust their vision based on environmental conditions by utilizing different chromophores. Normally, opsins bind to 11-cis retinal (A1), derived from vitamin A, which triggers a phototransduction cascade leading to a neural response. However, some fish (and also frog tadpoles (Zhong et al., 2012)) possess an additional form of the vitamin A, which is based on 11-cis 3,4-didehydroretinal (A2) (Terai et al., 2017).

The presence of A2 shifts the same opsin spectral sensitivity toward longer wavelengths, enhancing the fish's ability to perceive red light (Escobar-Camacho et al., 2019). This change is mediated by *cyp27c1*, an enzyme from the cytochrome P450 family (Enright et al., 2015), which catalyzes the conversion of A1 to A2 by introducing an additional double bond in the  $\beta$ -

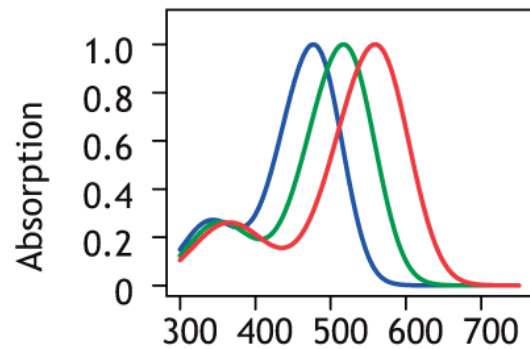
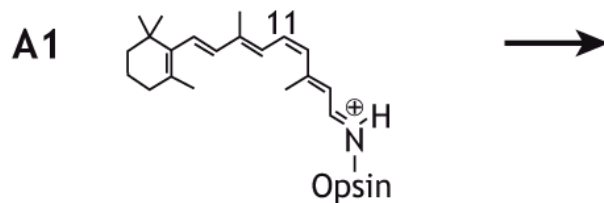
ionone ring (Fig. 5) (Carleton et al., 2020). Importantly, this process is reversible, and fish maintain both A1 and A2, with the ratio shifting in response to environmental factors (Carleton et al., 2005) such as water turbidity (Torres-Dowdall et al., 2017), light availability, seasonal changes, and life stage (Ueno et al., 2005).

Fish living in turbid, shallow waters with red-shifted or highly variable ambient light tend to express more A2 retinal, enhancing their ability to detect red-shifted light (Palczewski and Kiser, 2020). In contrast, species in clear or deep waters, where red light is scarce, predominantly express A1 retinal, optimizing their vision for shorter wavelengths (Kondrashev and Lamash, 2019).

Although this mechanism allows fish to adapt to varying light conditions, it comes with trade-offs. The increased presence of A2 reduces overall photosensitivity and increases thermal noise, potentially affecting vision in low-light environments (Ala-Laurila et al., 2007).

## Chromophore

### 11-*cis*-retinal



### 11-*cis*-3,4-didehydroretinal

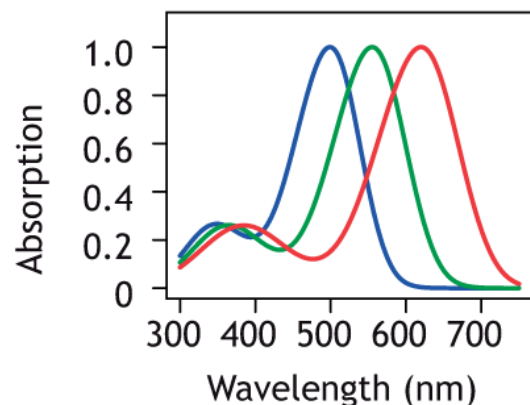
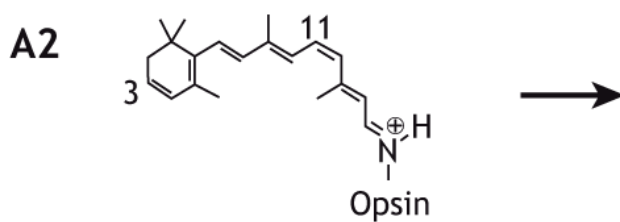


Figure 5: A graphical abstract illustrating how A1 retinal and A2 retinal influence light perception by shifting sensitivity toward longer wavelengths, Borrowed from (Carleton et al., 2020)

### 3.5. Special visual adaptation

Deep-sea fish have evolved numerous molecular adaptations to survive in the harsh conditions of the deep ocean. One of the most remarkable adaptations to the limited light environment, where the remaining downwelling light falls within the 470–492 nm range, has evolved in dragonfish from the Stomiidae family (Douglas et al., 1999).

Three genera, *Aristostomias sp.*, *Pachystomias microdon*, and *Malacosteus niger*, possess a unique ability. In addition to emitting blue bioluminescence, like most deep-sea organisms, they can also emit and perceive red light (Douglas et al., 2016). Since red light is entirely absent in the deep sea, most organisms lack the photoreceptors needed to detect it. This gives dragonfish a private communication channel, allowing them to signal to each other without being detected by most predators or prey (Douglas et al., 2003).

The blue bioluminescence originates from postorbital light organs, while suborbital photophores produce far-red bioluminescence, with spectral emissions peaking sharply at wavelengths beyond 700 nm. The three species have evolved two distinct mechanisms for producing and perceiving long-wavelength light (Douglas et al., 1998a). *Aristostomias sp.* and *Pachystomias microdon* possess three long-wavelength visual pigments, with two formed by opsin bound to A1 and A2, along with an additional mechanism on the third pigment that remains unknown, enabling them to perceive red light. Similarly, *Malacosteus niger* possesses three of these long-wavelength pigments, forming an A1/A2 pigment pair on a single opsin with  $\lambda_{\max}$  values around 520 and 540 nm. To expand its sensitivity towards red spectrum, it utilizes magnesium-free chlorophyll derivative, specifically bacteriochlorophyll, which enhances spectral sensitivity on the third pigment. This adaptation enables *Malacosteus niger* to detect long-wavelength light, with peak sensitivity at 666.8 nm, close to the emission maximum of its red bioluminescence at 705 nm. Its sensitivity to red light is further enhanced by a bright red tapetum, which reflects long-wavelength light (Douglas et al., 1999).

Evidence suggests that *M. niger* acquires bacteriochlorophyll from its prey (Collin et al., 2000). These fish primarily feed on euchaetid and aetideid copepods, which, in turn, consume photosynthetic organisms (Douglas et al., 2016).

A study demonstrated that the evolution of a red visual system from a primitive blue system occurred as a single evolutionary event in dragonfish. The unique shift toward far-red sensitivity in the Stomiidae can be attributed to five amino acid substitutions: M183F, M253L, F261Y, T289G, and S292I. This example further supports the pattern introduced earlier, as the substitution at position 292 is shared with commonly known tuning sites across opsin classes.

These substitutions serve as a strong indicator of far-red phenotypes ( $\lambda_{\max} \approx 520$  nm) (Kenaley et al., 2014).

## 4. Comparison of visual adaptation in deep-freshwater and marine deep-sea fish

### 4.1. Visual adaptations in deep-freshwater fish

Cichlids have emerged as model organisms for studying rapid sympatric speciation and visual evolution, particularly in Africa's Great Lakes (Majtánová et al., 2019). These species display a high diversity of cone opsin gene expression profiles, which are often correlated with ecological variables such as depth and water clarity (Smith et al., 2011). In neotropical cichlids from Central America, variation at site 83 in rod opsin RH1 has been associated with increased photosensitivity in dim environments (Hauser et al., 2017). In Tanganyikan cichlids, the A292S substitution in RH1 is found in species inhabiting deeper waters. Cone opsins in cichlids also exhibit notable variability. Among them, SWS1 has the highest nucleotide variation, followed by SWS2A, SWS2B, and LWS (Spady et al., 2005). Deep-freshwater cichlids from Lakes Malawi and Tanganyika exhibit spectral shifts of 1–15 nm toward blue wavelengths in both RH1 and RH2 opsins (Carleton and Yourick, 2020). Notable substitutions have also been observed in RH2A $\alpha$  at amino acid position V214I in deep-living cichlids such as *Myaka myaka* and *Konia dikume* (Musilova et al., 2019b) as well as additional fine-tuning mutations at sites 83, 183, 214, 292, 299, and 300 in RH1, which correlate with the depth distribution of species (Ricci et al., 2022). Furthermore, deep-living cichlids typically express a combination of SWS2A, RH2B, and RH2A $\alpha$  opsins, tailored for the blue-shifted light environment (Ricci et al., 2023).

In parallel, cottoid fishes from Lake Baikal demonstrate similar depth-related adaptations. These species have also undergone sympatric speciation and exhibit opsin gene modifications like those of cichlids. The A292S substitution in RH1 is again a key spectral tuning site (Bowmaker et al., 1994), accompanied by other substitutions such as D83N and Y261F, which together contribute to blue-shifted absorption by 6–8 nm (Hunt et al., 1996). In their cone photoreceptors, SWS2 pigments, which normally absorb around 467 nm in species like *Cottus gobio*, are shifted toward 450–430 nm due to substitutions at positions 215 and 269, resulting in a 10 nm blue shift (Cowing et al., 2002).

Morphological changes in the retina are also apparent in these deep-freshwater species. In cottoids, cone density is progressively reduced with depth, and deep-dwelling species

ultimately possess rod-only retinas adapted for scotopic vision (Sideleva, 2006). Unlike their marine counterparts, no species in Lake Baikal exhibit bioluminescence suggesting that visual adaptations in fresh water rely solely on maximizing the detection of ambient light rather than integrating light production (Bowmaker et al., 1994).

#### 4.2. Visual adaptations in deep-sea fish

One of the most prominent molecular changes in deep-sea fish is the spectral tuning of RH1 pigments. Amino acid substitutions at sites 194, 195, 292, and 299 are associated with shifts of the absorbance maximum toward shorter wavelengths, better suited for the blue-dominated light environment of deep waters. In particular, the A292S substitution has been widely recognized as an adaptation to these conditions (Varela and Ritchie, 2015). The absorbance maxima of RH1 pigments in deep-sea fishes typically fall between 470–495 nm (Partridge et al., 1989; Bowmaker et al., 1994), aligning with the spectral range of both residual daylight (432–507 nm) and bioluminescent emissions (420–520 nm) (Douglas et al., 1995).

Several species demonstrate the functional effects of such tuning. For example, *Bathylagus bericoides* possesses two RH1 variants with peaks at 466 nm and 500 nm, and *Malacocephalus laevis* has RH1 pigments absorbing at 478 nm and 485 nm (Partridge et al., 1988). Remarkably, three lineages of deep-sea fishes show extreme expansion of RH1 genes. These include the tube-eye (*Stylephorus chordatus*), which has six RH1 genes, the glacier lanternfish (*Benthoosema glaciale*) with five RH1s, and two species of the family Diretmidae: the longwing spinyfin (*Diretmoides pauciradiatus*) with 18 RH1s and the silver spinyfin (*Diretmus argenteus*), which possesses 38 RH1 genes. This expansion is hypothesized to have occurred through repeated single-gene duplications rather than whole-genome duplication events. These multiple RH1 paralogs exhibit varying spectral sensitivities and are differentially expressed during development, likely allowing these species to broaden their range of scotopic vision and improve the detection of both residual daylight and diverse bioluminescent signals in the deep sea (Musilova et al., 2019a).

Morphological adaptations further optimize vision in extreme depths. Tubular eyes, which are often oriented dorsally, increase the field of view for capturing downwelling light (Wagner et al., 1998). The presence of a reflective tapetum lucidum enhances photon capture by reflecting unabsorbed light back onto the retina (de Busserolles et al. 2020). In *Rhynchohyalus natalensis*, a rare dual-optic system combines both refractive and reflective mechanisms to collect light from multiple directions using a mirror-lined diverticulum (Partridge et al., 2014). In addition, color filtering may also play a role in visual discrimination.

Yellow lenses can in some species enhance contrast by filtering out scattered blue light and improving detection of long-wavelength bioluminescent signals (Somiya and Marshall, 1997).

Bioluminescence is a key environmental feature at depth, produced by organisms ranging from bacteria and fungi to invertebrates and fish (Crescitelli, 1990) via a luciferin-luciferase oxygen dependent reaction (Wilson and Hastings, 1998). It serves several ecological functions, including communication, prey detection (Haddock et al., 2010), predator evasion and attracting mates (Martini et al., 2019). The spectral tuning of visual pigments in deep-sea fishes, particularly the RH1 blueshifts, likely optimize photon capture from these bioluminescent sources (Turner et al., 2009).

### **4.3. Convergent and divergent evolution of the visual adaptation**

Despite the phylogenetic distance between deep-freshwater and deep-sea fishes, similar environmental pressures in dim-light habitats have led to cases of convergent evolution in their visual systems (Lupše et al., 2021). One of the clearest examples is the spectral tuning of rod opsin RH1 through amino acid substitutions at conserved sites. The substitution A292S, which causes a shift toward shorter wavelengths, has evolved independently in both deep-living cichlids from Lakes Tanganyika and Malawi, as well as in various marine species (Nagai et al., 2011). Additional tuning mutations such as D83N, V214I, and Y261F also appear repeatedly across unrelated taxa (Lin et al., 2017), resulting in similar shifts in absorbance maxima suited to the blue-dominated spectrum (430–500 nm) prevalent in deep habitats (Douglas et al., 1995).

Similar trends are observed in cone opsins. In both deep-freshwater and deep-sea fishes often show reduced diversity of cone opsins yet consistently retain and express RH2 and SWS2 classes (Lin et al., 2017). These opsins are tuned to the remaining wavelengths of ambient light at depth (Eaton et al., 2021). In addition, many species exhibit rod-dominated or rod-only retinas, a morphological adaptation that enhances scotopic vision under chronically low-light conditions (Hunt et al., 1996).

In contrast, several aspects of visual adaptation reveal divergent evolutionary trajectories between freshwater and marine species. A key difference is the extensive duplication of RH1 observed in deep-sea fishes (Musilova et al., 2019a). While cichlids and cottoids typically retain a single copy of RH1 (Ricci et al., 2022), certain deep-sea lineages have undergone multiple rounds of duplication. This may allow for finer discrimination of both residual daylight and the diverse bioluminescent signals that dominate the deep-sea visual scene; yet the exact mechanism of this visual system remains unknown (Musilova et al., 2019a).

Bioluminescence itself represents another point of divergence, as it is widespread in marine systems but absent from freshwater environments (Bowmaker et al., 1994). Deep-sea fishes have evolved not only to detect bioluminescence, but in many cases to produce it, using specialized photophores for communication, camouflage and predation (Turner et al., 2009). Another major difference lies in the cone opsin gene repertoire. Deep-sea fishes often undergo permanent loss of LWS and SWS1 opsins from their genomes, reflecting long-term adaptation to narrow-band blue light environments (Lin et al., 2017). In contrast, freshwater fishes generally retain these genes, but exhibit strong developmental or environmentally induced downregulation, ceasing expression in adults adapted to deep, dim-light conditions (Musilova and Cortesi, 2021). This is, however, mainly due to the fact that colonization of the deep sea happened on larger evolutionary scale (Priede and Froese, 2013), whereas the studied deep-freshwater species are usually of much younger evolutionary origin (Genner et al., 2007).

Finally, optical specializations further distinguish deep-sea taxa. Structures such as tubular eyes (Wagner et al., 1998), reflective tapetum lucidum (de Busserolles et al. 2020), yellow lenses (Somiya and Marshall, 1997), and even dual-optic systems provide enhanced photon capture and directional sensitivity, enabling survival in extreme photic conditions (Partridge et al., 2014).

## **5. Conclusion**

The visual systems of fish inhabiting deep and dim-light aquatic environments exhibit remarkable diversity shaped by evolutionary pressures at the molecular, developmental, and anatomical levels. Central to this diversity are modifications in visual pigments, achieved through gene duplications, mutations at key tuning sites, differential gene expression, and chromophore shifts. These molecular adaptations enable fish to optimize vision in challenging habitats, whether in the depths of freshwater lakes or of the deep sea.

By comparing deep-freshwater and deep-sea species, this thesis has highlighted both convergent and divergent visual adaptations. One of the most striking examples of convergence lies in the spectral tuning of rod opsins. Both groups independently acquired similar amino acid substitutions in sites 83, 214, 261 and mainly 292, resulting in shifts toward the blue-green wavelengths (430–500 nm) that dominate deep-water environments. Similar trends were observed in cone opsins, particularly RH2 and SWS2, which remain functionally expressed in both systems, when the cones prevail on the retina. The presence of rod-only or rod-dominated retinas are another common trend that further illustrates how low-light conditions repeatedly drive similar visual strategies across distant lineages.

In contrast, divergent evolutionary outcomes emphasize the influence of habitat-specific and phylogenetic factors. Deep-sea fish often show permanent gene losses of LWS and SWS1 opsins and exhibit extensive RH1 duplications, paired with innovatory optical structures and sensitivity to bioluminescence. Meanwhile, deep-freshwater fish retain a broader opsin repertoire but downregulate the expression of certain opsins developmentally. These contrasting strategies reflect different ecological contexts, such as the presence of bioluminescence in the ocean and its absence in lakes and offer valuable insights into the flexibility and constraints of sensory evolution.

Future research could focus on studying lesser-known freshwater lineages and deep-living species from underexplored habitats which may reveal additional cases of adaptation. Investigating the regulation of opsin gene expression throughout development and in response to environmental changes could also provide new insights into plasticity in visual systems. Finally, expanding phylogenetic sampling and combining molecular data with behavioral and ecological observations would help us to understand not just how fish see in the dark, but how vision evolves in response to the wide variety of aquatic light environments.

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