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ADAPTATION TO ENVIRONMENTAL LIGHT CONDITIONS IN MYSID SHRIMPS

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ACADEMIC DISSERTATION

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in lecture hall 2402, Viikinkaari 1 (Biocenter 3), on 9.3.2018 at 12 o'clock noon.

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Author's contribution

I The author participated in the collection of animals and performed MSP recordings for one of the study species. She also contributed to the development and conduction of MSP analyses and the creation of graphs.

II The author was responsible for collecting samples of different *Mysis* populations and determining their λ_{max} by MSP, as well as the measurements and calculations related to light environments at the study sites. She provided most of the modelling and illustrations and participated in writing the manuscript.

III The author participated in developing the experimental design and was responsible for light measurements and calculations. She performed all the MSP recordings and the analyses of MSP, ERG and EM data. The author wrote the first draft of the manuscript and created all illustrations.

Contents

List of Abbreviations

λ_{max}	Wavelength of absorption maximum
A1	Retinal
A2	3,4-didehydroretinal
ADAR	RNA specific adenosine deaminase
COI	Cytochrome oxidase I
ELI-III	Extracellular loops I-III
ERG	Electroretinography
INAD	Inactivation no afterpotential D -protein
LWS	Long-wavelength-sensitive
MII	Metarhodopsin II
MSP	Microspectrophotometry
MWS	Middle-wavelength-sensitive
PCR	Polymerase chain reaction
R	Rhodopsin
SNP	Single nucleotide polymorphism
SNR	Signal to noise -ratio
TEM	Transmission electron microscopy
TMI-VII	Transmembrane helices I-VII
TRP	Transient receptor potential
TRPL	Transient receptor potential like
UV	Ultraviolet
WLMT	Wavelength of maximal transmission

Abstract

Adaptation to environmental light conditions at different time scales and biological levels was studied using the visual system of opossum shrimps (genus *Mysis*) as model. The focus of the study was on two aspects of visual adaptation: 1) mechanisms behind spectral tuning which enables effective photon catch in different light environments and 2) photo-induced damage and protective mechanisms in the eyes arising as a trade-off from tuning the visual system to be highly sensitive.

For spectral adaptation studies mysids representing 12 species were collected from different water bodies around circumpolar and Caspian areas. Their opsin genes were sequenced and compared with phylogenetic relationships. Spectral sensitivities were determined for 15 populations representing four species by recording single-rhabdom absorption spectra with microspectrophotometer. Water transmission spectra were measured and the wavelength of maximal transmission of light and the attenuation coefficient was determined to quantify the light conditions in the respective habitats. Animals originating from different environments were also bred in carefully controlled laboratory conditions to observe possible effects of ambient salinity on spectral sensitivity.

The photoprotective mechanisms were studied by subjecting animals from populations with intrinsically different vulnerability to light-induced damage to an ultra-slow light acclimation procedure before exposing them to a bright light. The effects of this procedure were examined structurally by transmission electron microscopy and functionally by electroretinography. The equilibrium between rhodopsin and metarhodopsin was studied by microspectrophotometry. The acclimation protocol was conducted at different speeds to investigate the time scale of light acclimation.

The studies of spectral tuning show that the spectral sensitivity of different *Mysis* populations generally correlates in an adaptive manner with the light conditions in their living environment. However, neither opsin gene sequence nor water light transmission could fully explain the observed differences in spectral sensitivities between study populations. Neither were there differences in chromophore use. The findings indicate that there are

two opsin genes which are expressed in different proportions following a reaction norm triggered by an yet unidentified environmental factor. This hypothesis still requires more investigation.

The study on light damage shows that very slow light acclimation can prevent structural and functional deterioration of photoreceptors caused by bright light exposure. The time scale of successful acclimation corresponds to the tempo of seasonal changes of light levels in the natural habitat. One key player in this phenomenon seems to be the amount of native visual pigment.

1 Introduction

Earth is a dynamic, constantly changing system. This is sensed by living organisms as environmental changes, shaping species and creating the vast biodiversity around us. On the other hand, human impact today accelerates environmental change at a pace that challenges the ability of many species to cope. Thus understanding the tempo and limits of adaptation of biological systems at many levels is more crucial than ever. The more we know about the diverse mechanisms of adaptation, the better we are able to predict the effects of environmental changes on organisms and ecosystems.

In 1929 Danish physiologist August Krogh published his famous statement *"For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied"*. In the present study the animals of choice are glacial-relict opossum shrimps of the genus *Mysis*, which spend large parts of their lives in very dim light, some populations living at the absolute sensitivity limit of vision. It is hard to imagine a better choice for the study of visual adaptation at multiple levels and time scales. The Baltic Sea and the many Fennoscandian lakes housing mysid populations constitute a natural and accessible laboratory for studying adaptation on well-calibrated time scales of postglacial isolation, ranging from ten thousand years downwards – an experimental design that would be difficult to set up artificially. On the other hand, the biogeography and speciation history of these species is reasonably well known over time scales of millions of years.

In these species, vision is a highly rewarding system for investigations of adaptation, evolutionary as well as physiological. They have well-developed eyes and rely on vision in key behaviours like feeding and predator avoidance. Optimal use of the light information is an important fitness factor, and adaptedness in terms of visual performance in a given environment can be expressed in strictly quantitative terms and related to theoretical measures of optimality.

In an evolutionary or ecological sense, however, adaptation is not just a matter of perfect matches to mean values of some environmental variable, but the capacity to cope with variation in that variable. This leads on to the

examination of the ability to adapt as an adaptive trait, at the crossroads of evolutionary, epigenetic and physiological adaptation. These kinds of approaches can be messy but rewarding. In the present study the focus is thus widened from means and "what is now" to ranges and "what has been or will be".

2 Background and literature review

2.1 Light in aquatic environments

2.1.1 Spectral transmission of light in different water bodies

To understand how aquatic animals are adapted to the light in their living environment and what kind of requirements it sets on their visual system, it is essential to know basics about the physics of light and how illumination changes as light propagates through the water column.

So called visible light is a band of the spectrum of electromagnetic radiation covering the wavelengths roughly between 400 and 700 nanometres. The actual range of wavelengths perceived as light depends on species and types of visual receptors. There are fundamental physical reasons based on the properties of the photoreceptor molecule rhodopsin why vision is restricted to photon energies roughly corresponding to this wavelength band (Ala-Laurila et al., 2004). In addition, life and photoreceptors originally evolved in water, and visible light encompasses wavelengths which penetrate most effectively through clear water. Wavelength composition determines the colour of light.

Pure water is blue, partly due to absorption and partly due to Rayleigh scatter. The transmission of light in water is dependent on wavelength and the transmission spectrum in a certain water body is determined by the combination of the following factors: attenuation of light in pure water, scattering and absorption of light due to non-chlorophyllous particles of organic or inorganic origin and absorption caused by dissolved organic matter and phytoplankton. The details may vary between studies and water bodies, but as a rule two things change in the aquatic light milieu when

shifting from open ocean to estuarine regions: the total amount of light is decreased and the proportion of short wavelengths is reduced. The same changes become even more pronounced in nutrient-rich fresh waters with high concentrations of dissolved organic matter (Lythgoe, 1984; Prieur and Sathyendranath, 1981; Smith and Baker, 1981). Figure 1 shows a schematic representation of light transmission in different water environments.

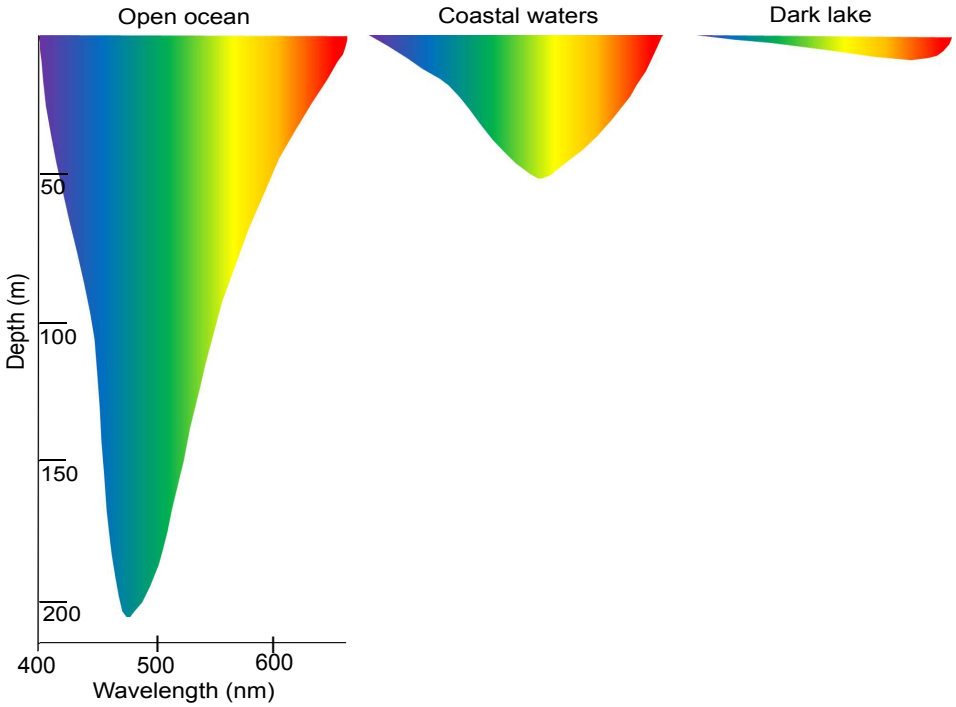

Figure 1: Spectral transmission of light in different water bodies, schematic representation after Levine and McNichol (1982). The total amount of light is reduced and the wavelength of maximal transmission (WTML) shifted towards longer wavelengths when going from open ocean to coastal waters. These phemonena are even more pronounced in dark lakes.

2.1.2 Polarized light in the water

In addition to intensity and wavelength there is a third biologically interesting feature of light: polarization. A ray of light consists of an electric field and a magnetic field which oscillate perpendiculary to each other. The electric field component is relevant for vision, since it can effectively activate the photosensitive rhodopsin molecule. This e-vector can be divided into two orthogonal components oriented along the x- and y-axes. Light can be polarized a) linearly (or plane-polarized), if these components have identical phases b) circularly, if the phases of these two differ by 90 degrees or c) elliptically, if the phase difference is something in between (Wehner and Labhart, 2006). Any plane-polarized light stimulus can be defined by its e-vector orientation (polarization angle), degree of polarization and intensity (Bernard and Wehner, 1977; Labhart, 2016).

Sunlight, the primary source of almost all natural light, is unpolarized i.e. there is no preferred e-vector orientation. Scattering in the atmosphere creates a celestial polarization pattern. Scattering from water molecules and reflection from body surfaces like fish scales and arthropod cuticles make the underwater light partially plane-polarized. The degree of polarization under water depends on the viewing direction, but it is always higher in the direction of open water, which is used for shore-flight by some zooplankton (Schwind, 1999). Many aquatic animals can detect and use the information provided by polarized light for example for habitat selection, contrast enhancement, detecting polarizing biological structures, intra-specific communications and orientation by using celestial e-vector pattern (reviewed in Wehner and Labhart (2006) and Labhart (2016)).

2.1.3 Temporal changes in the aquatic light environment

Ambient light conditions in water environments change as a result of regular fluctuations and sporadic events. This applies to both light intensity and spectral composition within the water column. Some of these changes are cyclic on different time scales. The illumination levels decrease dramatically after sunset every day, and at high latitudes there can be several

months virtually without light, especially if a thick cover of ice and snow prevents the light from reaching even the surface layer of water. Even if there is some sunlight present in winter, its highest intensity may be some ten times higher in the summer (Lindström, 2000b). Seasonal fluctuations can also affect the spectrum of down-welling light. The absorption by green phytoplankton and its yellow decay products during algae bloom reduces the amount of especially short wavelengths shifting the water colour towards green in estuarine regions and even further towards red in heavily stained fresh waters (Lythgoe, 1984). In lakes and estuaries the amount and colour of water flowing in from their drainage basins changes seasonally. For example increased currents during annual floods in many regions can bring extra soil or dissolved organic matter with them. Also irregular extreme weather conditions may affect light conditions in these kind of water bodies through changes in precipitation in the drainage basin. Other unpredictable factors changing the amount of light in the water are local weather conditions on a smaller scale: cloudy weather may reduce the light on the water surface by an order of magnitude compared to a bright day (Lindström, 2000b). In contrast to these fast events, some changes in the underwater light milieu happen on a time scale of centuries or millenia as a result of geological processes.

Human activity may have a great influence on the underwater light milieu on multiple time scales. Eutrophication is one of the most typical human-induced phenomena causing changes in light conditions in lakes and estuaries. Excess of nutrients originating from fertilizers and industrial or domestic run-offs accelerate the growth of phytoplankton which together with its decay products affect light transmission in water. Changes in the water colour due to eutrophication may have severe biological consequences. A well-known example is the increased turbidity in Lake Victoria, which constrains colour vision in cichlids and thus blocks the mechanisms of reproductive isolation based on colour-dependent mate choice (Seehausen et al., 1997). Besides eutrophication land use issues such as draining of peats have had a massive effect on water colour, and the actions to restore the situation have not been successful (Worrall et al., 2007). During the last 30 years an increase in the humic acid concentration and colouration has

been observed in Fennoscandian lakes, and the trend is estimated to be continuing. No unambiguous cause for this phenomenon has been identified, but increased greenhouse impact and precipitation have been suggested as candidates (Forsberg, 1992; Hongve et al., 2004).

2.2 Visual adaptation in crustaceans

2.2.1 General concepts of adaptation

Adaptation is a crucial term in multiple fields of biology, but as with many other central concepts, its definition is not unambiguous. In ecology and evolutionary biology the term "adaptation" is used to describe either traits in animals that are the result of natural selection or a process or mechanism by which selection adjusts the frequencies of genes affecting these traits. Adaptation is typically a slow process, which occurs over generations. In physiology the term adaptation is also used for a situation where more rapid changes in the environment cause changes in the expression of pre-existing potentials. This kind of adaptation, which happens during an animal's life span, is referred to as phenotypic plasticity.

Phenotypic plasticity or developmental plasticity means development of different phenotypes from the same genotype. These different phenotypes may arise dependent on the influence of some environmental factor. If there is a continuum of phenotypes expressed by the same genotype across a range of environmental conditions, the term reaction norm (or norm of reaction) is used (Gilbert, 2001). Environmental factors driving a reaction norm can be very different. In crustaceans it has been shown that the presence of fish cairomones (Mikulski et al., 2004), temperature (Giebelhausen and Lampert, 2001) and light (Cronin et al., 2001) can lead to varying phenotypes via reaction norms. Traits which show phenotypic plasticity in this manner range from ontogeny (Mikulski et al., 2004) to behaviour (Shuster and Arnold, 2007). Sometimes phenotypic plasticity occurs via epigenetic mechanisms (Burdge et al., 2007).

Two concepts, acclimation and acclimatization, are used to describe phenotypic shifts induced by the environment. The key difference between

these two is that acclimatization happens as a response to conditions in the natural environment whereas acclimation is a response to controlled manipulation of an environmental variable. In animals acclimation and acclimatization can become manifest as different kind of changes: a) structural, encompassing changes in histology, morphology, anatomical relationships and body composition b) functional, meaning changes in organ system function and c) psychobehavioural, including changes of complex neural functioning (Mazess, 1975). Mazess (1975) also emphasizes that adaptations may occur at all biological levels of organization:

1. Physicochemical
2. Cellular
3. Organ systems
4. Organisms (individual)
5. Population and
6. Ecosystem.

Also time scales vary: the effects of acclimation or acclimatization can occur in seconds or minutes, but genetic adaptation happens in the course of generations. On a conceptual level it is important to remember that the ability to adapt can in itself be seen as adaptive trait.

2.2.2 Visual systems in Crustacea

Most crustaceans are primarily aquatic, with a few exceptions like some terrestrial isopods, and thus the focus of this chapter is on visual systems in aquatic environments. It is good to bear in mind, though, that the majority of species belonging to the Pancrustacean clade are terrestrial hexapods (Regier et al., 2005). Crustacean visual systems are amazingly versatile both in form and function: some species may have the most elaborate compound eyes within the animal kingdom whereas others lack eyes entirely and functional properties encompass features such as double retinas, up to 16-channel colour vision and ability to detect circular polarization (Protas and Jeffery, 2012; Nilsson and Modlin, 1994; Porter et al., 2009; Chiou et al., 2008).

Eye types Crustaceans have various types of eyes and other light sensitive structures ranging from intra-cerebral ocelli and the caudal photoreceptors of some decapods to highly specialized compound eyes. Compound eyes consist of several hundred to several thousand sensory units known as ommatidia. Morphologically they can be divided into two main categories, the apposition and the superposition type, the form reflecting their function (see figure 2). Apposition eyes have long rhabdoms (the photosensitive structures of the eye, see below) extending longitudinally through the optically isolated ommatidia. This makes them basically well-suited for daylight vision, favouring high acuity. Superposition eyes have short rhabdoms located at the basal ends of the ommatidia and there is an optically clear zone between the rhabdoms and dioptric structures. This clear zone enables gathering light coming through several ommatidial lenses to a single rhabdom, meaning that they basically trade visual acuity for higher sensitivity. In superposition eyes the accessory pigments lining the ommatidia can migrate, controlling the sensitivity of the eye and adjusting it to environmental illumination (Goldsmith, 1972; Meyer-Rochow, 2001). The eye type may also change during ontogeny. In decapods the pelagic juveniles have appostion eyes, which transform to superposition eyes in the more benthic adults (Land, 1980).

Rhabdoms Photoreceptors in the animal kingdom fall into two classes, named after the cellular structure from which the membranes housing the visual pigment is derived: ciliary and microvillar (rhabdomeric). Traditionally the former were thought to be exclusive to vertebrates and the latter to invertebrates, but later both kind of receptors have been found in vertebrates as well as in invertebrates. The two classes of photoreceptors also differ in all basic aspects of phototransduction, although the molecules involved are derived from common ancestors (Nilsson and Arendt, 2008). As far as it is known, only rhabdomeric photoreceptors are found in crustacean eyes.

The individual photoreceptor cells in crustacean eyes are called retinular cells, and the light-absorbing visual pigment molecules are packed in microvilli, which form a structure named the rhabdomere. The rhabdomeres of 6-8 retinular cells form a rhabdom surrounded by the other parts of the

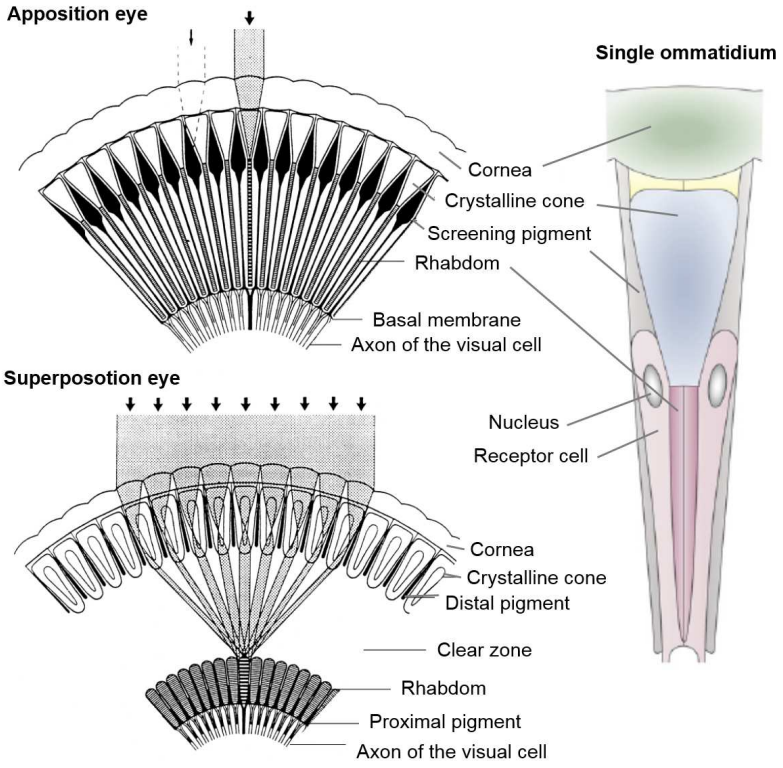

Figure 2: Simple graphic models of apposition and superposition eyes, together with general ommatidial structure of decapod crustaceans. The shaded areas indicated by black arrows describe the light path. The ommatidia of the apposition eye are optically separated, which improves visual acuity, whereas in the superposition eye there is an optically clear zone between the dioptic apparatus and photoreceptor cells, which enables light coming through several ommatidial lenses to reach a single rhabdom thus enhancing sensitivity at the cost of acuity. Modified after Meyer-Rochow (2001) and Nilsson and Kelber (2007)

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retinular cells. The visual pigment molecules floating in the microvillar membranes orient themselves on average so that the light-absorbing chromophores are preferentially aligned with the microvillar axes. Moreover, the microvilli within a rhabdomere are aligned (Goldsmith, 1972). Rhabdomeres often face the neighbouring rhabdomere at a 90 degree angle. This arrangement forms the basis for polarization-selective light responses differing between different retinular cells, since the probability that a visual pigment molecule gets activated is highest for linearly polarized light with the e-vector oriented parallel to the chromophore (Goldsmith and Wehner, 1977).

Visual pigments The visual pigments belong to the large class of G-protein coupled receptors and they consist of two parts (see figure 3): a transmembrane protein, opsin, and a chromophore, some form of retinaldehyde (retinal) bound to a lysine residue of the opsin via a protonated Schiff base. Opsins consist of 7 transmembrane helices (TMI-VII), three extracellular loops (ELI-III) together with the N-terminus outside the cell and three intracellular loops (ILI-III) together with the C-terminus inside the cell. Certain amino acid residues of the opsin form a chromophore binding pocket in the 3D-structure of the protein. The gene sequence has been resolved for hundreds of opsins and multiple important amino acids for chromophore binding have been identified. Although opsin structure is very conserved throughout the animal kingdom, there has been a split into different lineages early in the opsin evolution. The opsins of crustacean eyes are R-type or r-opsins (letter R referring to rhabdomeric photoreceptors) and they activate Gq type G-proteins (Porter et al., 2006; Cronin and Porter, 2014). An ancestral crustacean presumably had four r-opsin genes, which were the progenitors of the opsin clades present in extant crustaceans. These opsin clades are called arthropod SW, MW1, MW2 and LW2 (short-, middle-, and long-wavelength) based on the maximal spectral sensitivity of visual pigments of corresponding clades in extant species. The evolution of (pan)crustacean opsins has been very dynamic including losses and duplications of opsin clades (Henze and Oakley, 2015; Cronin and Porter, 2014). Opsins are not the only light sensing pigments in animals, but according to present knowledge they are the only ones involved in vision (Cronin and

Porter, 2014).

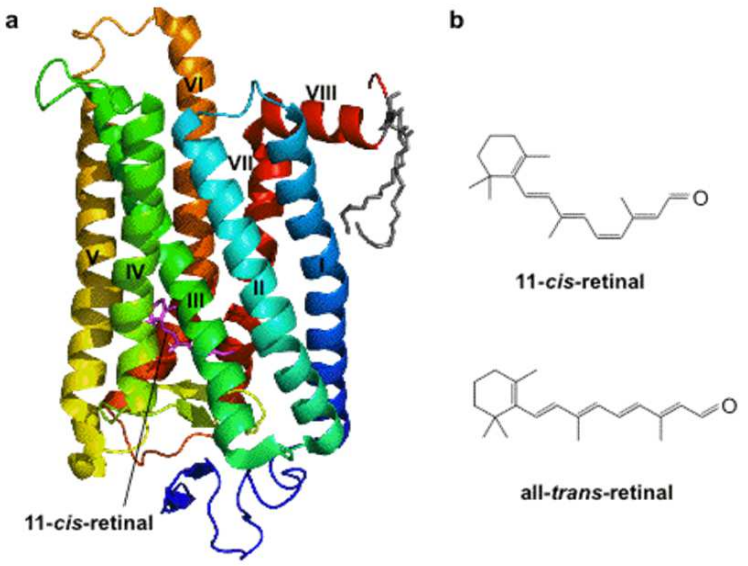

Figure 3: A. Opsin 3D-structure with chromophore, in this case retinal. The chromophore lies in the middle of the barrel formed by the seven transmembrane helices. B. Absorption of a photon changes the conformation of the chromophore from 11-*cis* to all-*trans*. Adopted from Nagata et al. (n.d.).

The chromophore is the actual light-absorbing component of the visual pigment. Visual pigment chromophores are vitamin A derivatives, which occur in two conformations. The chromophore of native visual pigments is in 11-*cis* conformation, but changes its conformation to all-*trans* as a result of photon absorption (3). If the chromophore of a visual pigment is retinal (also referred as A1) the pigment is called rhodopsin and if the chromophore is 3,4-didehydroretinal (or A2) it is called porphyropsin. Pigments using the A2 chromophore are red-shifted by even tens of nanometers compared with pigments consisting of the same opsin coupled to the A1 chromophore (Dartnall and Lythgoe, 1965). Although the visual pigment of most crus-

taceans is rhodopsin, porphyropsin is also found in some species (Suzuki et al., 1984). In some insects visual pigments using 3-hydroxyretinal, xanthopsins, are found (Vogt and Kirschfeld, 1983).

Photoreceptor classes Species living near the water surface where light is abundant have more photoreceptor classes than related species living in deeper waters, a phenomenon common to both invertebrates and vertebrates (Cronin et al., 1994; Lythgoe, 1984). Well-studies aquatic crustaceans whose vision is adapted to bright light conditions are the cladocerean *Daphnia magna* which has as many as four spectrally different photoreceptor types and stomatopods, many species of which have more than ten photoreceptor types (Smith and Macagno, 1989; Cronin et al., 1994). On the other hand, photoreceptors are metabolically expensive and animals from many different taxa have lost functional photoreceptors or even their eyes as an evolutionary response to living in total darkness (Protas and Jeffery, 2012). In crustaceans this has happened in parallel multiple times (Aspiras et al., 2012; Henze and Oakley, 2015).

Photoreceptors representing different classes are often not evenly distributed across the retina, but there are regional differences in the concentration or identity of visual pigments (Smith and Macagno, 1989). In stomatopods the regional differences have been shown to correspond to spatial differences in the illumination in the habitat (Marshall and Land, 1993), which is likely to apply to other crustaceans as well. Since the spectral composition as well as the absolute intensity of down-welling light in water environments differs markedly from the light coming from below, it can be very beneficial for an aquatic animal to have different kinds of photoreceptors in the dorsal and ventral retina. Regional specialization of photoreceptors is well described for insects and vertebrates (White et al., 2003; Temple, 2011).

Phototransduction and bistable photopigment The phototransduction cascade and machinery differ markedly between the two main types of photoreceptors (ciliary and rhabdomeric). The rhabdomeric photoreceptors utilized by crustaceans are generally faster and their dynamic ranges are wider (Hardie and Postma, 2008). The mechanisms of phototransduction in rhabdomeric photoreceptors have been studied most extensively in

the fruitfly *Drosophila melanogaster*, but phototransduction mechanisms in invertebrates exhibit considerable variation and are far from completely known (Cronin and Porter, 2014). In *Drosophila*, the very fast phototransduction is based on several specializations: many components of the phototransduction machinery are assembled into a signaling complex by the scaffolding protein INAD. The very small microvillar compartments allow extremely fast calcium dynamics, and there is an ultrafast contraction of the photoreceptor serving to open the TRP channels faster than any secondary messenger could. The canonical phototransduction cascade in arthropods starts with the absorption of a photon by the visual pigment leading to conversion of native rhodopsin to metarhodopsin. This activates a Gq-protein coupled phospholipase C cascade leading to the opening of TRP and TRPL channels and Ca^{2+} and Na^{+} influx and thus depolarization of the photoreceptor membrane (Hardie and Juusola, 2011).

The photopigments of rhabdomeric photoreceptors are generally thought to be bistable, which means that the chromophore does not detach from the opsin after conversion of rhodopsin to metarhodopsin. The meta II state of the visual pigment is thermostable, and can be photoreconverted back to rhodopsin by absorption of a photon. This bistable system can help to keep the rhodopsin levels adequate even in bright light, if the absorption properties of both pigments are favourable. Some diurnal flies possessing a blue-absorbing rhodopsin, orange-absorbing metarhodopsin and red screening pigments provide a pancrustacean example of this. In this combination metarhodopsin is converted into the rhodopsin state by long-wavelength stray light filtering through the red screening pigments (Stavenga and Hardie, 2011). This kind of photochemical reconversion system would not work in many crustaceans, since they have a green absorbing rhodopsin and a blue absorbing metarhodopsin. Dark regeneration of visual pigment resulting from addition of newly synthesized rhodopsin associated with membrane turnover is more important for these species (Goldsmith and Bernard, 1985; Cronin and Goldsmith, 1984). Light-depended visual pigment degradation combined with rhodopsin biosynthesis at constant rate is one mechanism for adjusting light sensitivity (Moon et al., 2014).

2.2.3 Spectral tuning

Importance When light is scarce it is important to get the most out of it. Animals living in very dim light conditions tend to have visual pigments with absorption spectra roughly overlapping with the light spectra in their living environment. This applies especially to animals living in deep waters, where not only the amount of light but also its spectral composition is limited. Modelling in figure 4 shows how photon catches in an underwater light environment can be affected by spectral tuning. It has been noticed already long ago in fishes that the more light there is in the environment and the wider its spectral distribution, the greater is the number of visual pigments they have, and the wider the spectral range they cover. On the other hand, in deep sea fishes the spectral sensitivity of their only visual pigment coincides quite well with the peak transmission of ocean water and the spectral sensitivity of deep living coastal water species is shifted towards longer wavelengths, with freshwater species extending this shift to even longer wavelengths (Lythgoe, 1984).Yet, the long-wavelength shifts of spectral sensitivities are lesser than the shifts in light spectra, which may be explained by the increase in thermal noise associated with red-shifting pigments (Ala-Laurila et al., 2004).

Mechanisms The spectral sensitivity of an animal can be tuned at different levels. Absorption of a photon by a visual pigment is always the basis of visual perception, and the absorption spectrum of the pigment gives the probability of absorption as a function of the wavelength of the light (or photon energy). The absorption spectra of visual pigments with a given chromophore have basically constant shape; therefore, the differences in curve width and position between between pigments absorbing maximally at different wavelengths can be captured by a single parameter, the wavelength of maximal absorption λ_{max} (Govardovskii et al., 2000).

Shifting the visual pigment chromophore between A1 and A2 or changing their proportions in the eye is a relatively fast way to tune spectral sensitivity, e.g. as a response to seasonal changes in the light environment or repeated habitat shifts during an animal's life span. The phenomenon is well documented both in aquatic vertebrates and some crustaceans (Tem-

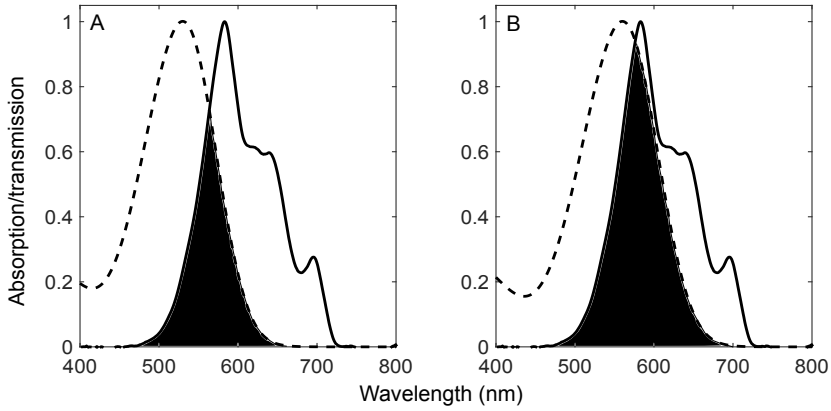

Figure 4: The effect of spectral tuning on photon catch. Solid lines represent a transmission spectrum typical for clear-water lakes and dashed lines the absorption spectrum of rhodopsin with λ_{max} at either 530 nm (A) or 560 nm (B). Shifting λ_{max} 30 nm towards longer wavelengths increases the overlap of the curves (visualized with black colour), which improves the calculated photon catch by 67 % in these circumstances.

ple et al., 2006; Suzuki et al., 1984). Changing the chromphore from A1 to A2 shifts the λ_{max} of the visual pigment towards longer wavelengths, since the activation energy is lower in the A2 due the longer chain of conjugated double-bonds. The magnitude of the shift depends on the λ_{max} of the A1 based pigment. Referred to the properties of the A1-based pigment, it ranges from very small for the most short-wavelength-sensitive pigments to some 60 nm for the most red-sensitive pigments (Dartnall and Lythgoe, 1965). However, using this mechanism requires that the animal possesses the enzyme converting retinal to 3,4-didehydroretinal (Enright et al., 2015).

The opsin amino acid sequence can be modified on an evolutionary time scale through mutations in the opsin gene sequence. Even single nucleotide mutations may result in spectral changes, if they affect the amino acid residues interacting closely with the chromophore. In addition, multi-

ple single nucleotide polymorphisms can have additive effects (Hunt et al., 1996). Since opsin structure is very conserved, convergent or comparable amino acid substitutions may control visual pigment sensitivities in very distant taxa like in butterfly and primate opsins in the 530–560 nm range (Osorio and Vorobyev, 2008). The amino acid sites in opsins are traditionally numbered after bovine rhodopsin, which was the first opsin where amino acid and gene sequences were resolved (Hargrave et al., 1983). Maybe the most important and extremely conserved amino acid in rhabdomeric visual pigments is the glutamate at position 181 acting as counter ion to the chromophore-binding lysine at position 296 (Lamb et al., 2007; Cronin and Porter, 2014).

A wide study of pancrustacean opsins by Porter et al. (2006) suggests that although polarity and charge of the amino acid residues are important in fine-tuning the spectral absorbance, structural aspects like compressibility are more important for opsin function in a broad-scale evolutionary context. And presently, little is known about possible effects of substitutions in the long extra cytoplasmic tail thought to be a distinguishing feature of r opsins compared with c opsins (Murakami and Kouyama, 2008).

If a crustacean has multiple opsins, their expression pattern can vary both spatially and temporally. Differential opsin expression has been observed between eye types in species with both medial and compound eyes as well as across the retina within compound eyes (Oakley and Huber, 2004; Porter et al., 2009). Different opsins expressed within a single ommatidium can be found both segregated into adjacent retinular cells and coexpressed in some retinular cells, as observed in the fiddle crab *Uca pugilator* (Rajkumar et al., 2010). Regional differences in opsin expression patterns have been well described in the highly specialized eyes of stomatopods, where different regions of the eyes correspond to separate visual tasks (Cronin et al., 2010; Bok et al., 2014). The expression pattern of opsins may also change during ontogeny. Many juvenile crustaceans are more sensitive to short wavelengths than adults of the same species and changing the relative amounts of opsins expressed is one way to achieve this (Fanjul-Moles and Fuentes-Pardo, 1988; Frank et al., 2009).

There are also external components which may affect the λ_{max} of a visual pigment. The vertebrate long wavelength sensitive pigments (including human green and red cone pigments) possess a chloride binding site, and the presence of chloride at physiological concentrations shifts their absorption maximum to 20-50 nm longer wavelengths (Ebrey and Koutalos, 2001). This system seems to be restricted to vertebrate LWS pigments, but serves as an example of the influence of external environment on the function of visual pigments. Since opsin is a membrane protein, the constitution of the lipid bilayer can also modulate structural changes in rhodopsin. However, the effects observed in vertebrate photoreceptors occur after phototransduction and thus can not affect spectral properties of native rhodopsin (see for example Jastrzebskal (2011)).

Spectral sensitivity can be tuned also by controlling what kind of light reaches the visual pigment. This is generally done with various kind of filtering structures in the eye. Coloured oil droplets acting as cut-off filters in cones of various vertebrates may be the best known example, but coloured lenses and corneas are also well-known (Bowmaker, 1977; Arnold and Neumeyer, 1987; Walls and Judd, 1933). In the crustacean world, the remarkable eyes of stomatopods have coloured filters even within the rhabdoms (Marshall, 1988). The filtering systems in the stomatopod eyes can be complicated and dynamic. One species, *Haptosquilla trispinosa*, living at various depths uses rhabdomal filter structures to tune long-wavelength photoreceptors, shifting their functional λ_{max} even more towards red. A remarkable feature in this system is that the filtering properties can vary between individuals depending on the light conditions in their juvenile stage (Cronin et al., 2001). Life-stage can affect functional spectral sensitivity in crustaceans via screening pigments like in the lophogastrid *Gnathopausia ingens*, the juveniles having similar visual pigment λ_{max} as adults but being significantly more sensitive to short-wavelength light (Frank et al., 2009). It should be noticed, however, that spectral tuning by filters always acts by reducing light and thus cannot be used to enhance absolute photon catch in any wavelength range.

Limitations The fundamental limits to the spectrum of visible light are set by the molecular physics of the visual pigment, although the lack of certain wavelengths e.g. in aquatic environments further limit what is biologically useful. The high energy content of photons at short wavelengths can cause damage to DNA, proteins and membrane lipids that will in turn compromise the physiology, biochemistry and organismal performance (Lesser et al., 2001). Despite the potentially deleterious effects of short-wavelength light, seeing UV is widespread in aquatic vertebrates and UV-sensitive photoreceptors are present in many crustaceans as well (Salcedo et al., 2003; Cronin et al., 1994; Smith and Macagno, 1989). In some cases these receptors may not be used for wavelength discrimination in a traditional sense, but may be coupled to wavelength-dependent key behaviours (Goldsmith, 1994).

Since visual pigments with λ_{max} at longer wavelengths have lower activation energy, they are also more prone to thermal activation. This means that the visual pigment molecule is activated without photon absorption by thermal energy alone. Because signal transduction proceeds identically independent of the initiator of the activation, thermal activations cause randomly occurring signals that are identical to those due to single photons. These constitute a noise that decreases the signal-to-noise ratio (SNR) and sets an absolute limit to the detection of dim light. If the absorption spectrum of a pigment is pushed towards longer wavelengths to absorb more photons of the red end of the light spectrum, at some point the decrease in SNR due to increasing noise will be greater than the increase in SNR due to improved photon catch even in light environments strongly dominated by long wavelengths. This may explain why the visual pigment λ_{max} values (especially in fresh water environments) tend to fall short of the actual transmission maximum of the water (Aho et al., 1988; Ala-Laurila et al., 2003, 2004; Bowmaker et al., 1994; Lythgoe, 1984).

In addition, there may be constraints against tuning λ_{max} continuously to any wavelength even within these limits. The clustering observed among vertebrate rod pigment λ_{max} across species is assumed to be due to limitations set by the physical interaction between opsin and its chromophore (Dartnall and Lythgoe, 1965).

2.2.4 Spectral sensitivity as a measure of adaptation

While studying adaptation researchers often encounter the problem of how to measure adaptedness. Either finding properties which describe adaptation or quantifying these properties often causes difficulties. The spectral sensitivity of animals living underwater in dim light conditions allows an attractively reductionistic approach to these challenges.

As explained in 2.2.3, photoreceptors can be tuned to be sensitive to different wavelengths to maximize the relevant visual information gained from the environment. Spectral tuning is especially important if the total amount of photons in the environment is low and their spectral distribution is narrow. These are the prevailing conditions in deep aquatic environments, since water both acts as a monochromator and cuts down the intensity of light (see 2.1.1). For an animal which relies on vision in essential tasks like feeding or predator avoidance it is critical to catch as many photons as possible if the light is scarce. With reservation for the trade-off between signal and thermal noise at the absolute sensitivity limit of vision (see 2.2.3), there is no doubt that tuning the visual pigment absorption spectrum to match the light spectrum in the environment is both beneficial and adaptive.

The λ_{max} of a visual pigment is the best feature to describe spectral adaptation, since using filtering structures for spectral tuning inevitably leads to loss of photons before they reach the photoactive pigment and can trigger phototransduction. Visual pigment λ_{max} is also a convenient variable since it is possible to reduce the information on spectral absorption to a single parameter that fully characterizes both the position and shape of the absorption spectrum according to a standard template (Govardovskii et al., 2000). Visual pigment templates based on the absorption spectrum of bovine rhodopsin can also be applied to invertebrate visual pigments with $\lambda_{max} > 400$ nm (Stavenga, 2010). The parameter λ_{max} can be measured both reliably and simply, and can be compared in a straightforward manner with the transmission properties of the surrounding water. Although less accurate, the latter property is often reduced to the wavelength of maximal transmission in a similar manner.

2.2.5 When adaptive mechanisms fail

Sometimes adaptive mechanisms are not capable of reacting to the changes in the light environment. This can happen due to abnormalities in the dynamics of ambient light conditions or as a consequence of evolutionary trade-offs; for example evolving a receptor to be maximally sensitive in dim light environments may cause trouble if light suddenly becomes abundant (the duplex retinas and cone-rod transmutations in vertebrates are well-known examples of tinkering with this problem). Structural and functional impairment as well as behavioural changes following exposure to bright light has been observed in many crustaceans (Meyer-Rochow, 2001; Nilsson, 1982; Lindström and Nilsson, 1988; Attramadal et al., 1985). Not only excess of light but also lack of it can cause changes in the structure of eyes (Bloom and Atwood, 1981).

Typical harmful effects of bright light on crustacean eyes include reduced sensitivity and disturbances in the arrangement of rhabdomal microvilli (Nilsson, 1982; Meyer-Rochow, 2001). There is a continuum from sensitivity reduction associated with normal physiological light-adaptation to increasing light damage. Both can be seen as a threshold rise or even loss of behavioural light responses and decreased amplitudes and altered kinetics of light responses in electrophysiological recordings, whereas pronounced morphological effects such as swelling of the microvilli, vesicle formation at the microvillar base, membrane whorl formations and increase of the number and size of Golgi complexes are clearer signs of damage (Attramadal et al., 1985; Nilsson, 1982; Meyer-Rochow, 2001). In crustaceans living naturally in extremely dim light conditions, a short exposure to normal daylight can be enough to cause damage to the visual system (Lindström, 2000a; Lindström and Nilsson, 1988). Sometimes the suppression of vision is permanent, but usually crustacean eyes are able to recover from the damage in the course of a few days or weeks (Loew, 1976; Meyer-Rochow, 2001).

The exact mechanisms behind the light-induced damage in crustacean eyes are still not known, but reactive oxygen species released as a consequence of massive activation of phototransduction are a good candidate.

There seems to be a connection between thermal and photic stress implicating the role of membrane properties in the emergence of photodamage, but the interaction of these two is complicated. Decreased temperature has been observed to lead to accumulation of metarhodopsin and in different cases both slow down and speed up the recovery process (Bruno et al., 1977; Lindström and Nilsson, 1988).

2.3 Visual ecology of the *Mysis relicta* species group

2.3.1 From marine to fresh-water dwellers

Mysid shrimps are small aquatic malacostracan crustaceas, commonly called opossum shrimps due their brood pouch. The focus of this thesis is on the genus *Mysis* and more specifically on the *Mysis relicta* species group.

The phylogeography of the genus *Mysis* has been studied in considerable detail (Audzijonytė et al., 2005b,a; Audzijonyte and Väinölä, 2006; Väinölä and Rockas, 1990; Väinölä, 1998). While belonging to the primarily marine order Mysida, several species have invaded brackish- and fresh waters as well. The shift from marine to lacustrine habitats has set many challenges to these animals' physiology, including the visual system. The transition from sea to brackish or fresh water has also changed the light environment, although salinity itself does not significantly affect light transmission (see 2.1.1). Analyses based on both morphological and molecular data sets divide the *Mysis* species into two monophyletic branches: basal marine species and continental species derived from the marine branch. The continental group is further divided into Caspian species and the *M. relicta* species group (Audzijonytė et al., 2005b).

The *M. relicta* species group consists of four so-called glacial relict species (*M. relicta sensu stricto*, *M. salemaai*, *M. segerstralei* and *M. diluviana*), which have a broad circumpolar distribution encompassing boreal and subarctic lakes of the previously glaciated continental areas of Europe and North America, and estuarine and coastal regions of the arctic seas (Audzijonytė and Väinölä, 2005). During the Pleistocene the Fennoscandian species have experienced repeated switches between marine, estuarine

or coastal and fresh water environments (Eronen et al., 2001). Within the mysids of this area the genetically and ecologically closely related, euryhaline *M. salemaai* and *M. segerstralei* diverged from the stenohaline *M. relicta sensu stricto* already over 2 million years ago, but the isolation of true glacial relict populations in Fennoscandian lakes happened within the last ten thousand years (Väinölä, 1986; Audzijonytė et al., 2005b,a).

2.3.2 Light guided behaviour

The eyes are maybe the most prominent feature of the *M. relicta* species. The sheer size of the eyes compared to the rest of the body suggests that vision must be important for these animals, otherwise it would not be worthwhile to allocate so much resources to them. Indeed *M. relicta* use their eyes for many tasks crucial for survival, like feeding, predator avoidance and navigation (Ramcharan and Sprules, 1986; Næsje et al., 1991; Viherluoto and Viitasalo, 2001). Both positive and negative phototaxis has been observed, depending on the light levels and exposure history (Beeton, 1959; Bauer, 1908).

Although *M. relicta* is omnivorous and able to catch its prey items even in darkness, the presence of light multiplies its feeding rates (Ramcharan and Sprules, 1986). Mysids have a complex role in aquatic ecosystems, since they act at multiple trophic levels and in many water bodies form a very substantial part of the animal biomass (Rudstam et al., 2008). They show life-history omnivory, typically consuming algae as juveniles and becoming generally zooplanctivorous as they mature (Branstrator et al., 2000). This together with predator avoidance defines their light-guided behaviour over their life span. Juveniles generally occur nearer the surface than adults (Horppila et al., 2003; Lasenby and Langford, 1972).

There is a complex interplay between light and other environmental factors affecting *Mysis* behaviour. *M. relicta* species exhibit clear diurnal vertical migration, which is primarily guided by light levels. Even as subtle changes in the illumination as the presence of moonlight affect how near the water surface they ascend (Beeton and Bowers, 1982). In some populations only some of the animals migrate, which may widen their ecological

niche (Euclide et al., 2017). Temperature is one of the key factors affecting habitat choice within a certain location. The vertical movements and the distribution of animals are strongly influenced by absolute temperature, but the rate of temperature change is less significant. In laboratory experiments *M. relicta* preferred waters between 6 and 8 °C, but if prey was present they invaded temperatures as high as 16 °C(still never 18 °C) (Boscarino et al., 2007). Besides temperature, unfavorable seasonal changes in other physical and chemical conditions like limited oxygen availaibity may restrict vertical migration and essential functions like feeding and predator avoidance (Horppila et al., 2003).

2.3.3 Characteristics of the visual system

Earlier studies on the vision of *M. relicta* species have shown that there are considerable differences in the properties of the eye and vision between populations and species. These differences encompass absolute visual sensitivity, spectral sensitivity, susceptibility to light induced damage and relative amounts of carotenoids and retinoids in the eyes (Jokela-Määttä et al., 2005; Audzijonytė and Väinölä, 2005; Lindström and Nilsson, 1988; Belikov et al., 2014; Feldman et al., 2010).

M. relicta species have refracting superposition compound eyes typical of arthropods living in dim light conditions. Although the vision of these species has been studied for a long time, no comprehensive structural description of their eyes has been published, and much of the information on eye morphology has been extrapolated from related species. The basic eye structure closely resembles the spherical, stalked eye of another mysid, *Praunus flexuosus* described in detail by Hallberg (1977). In this species the eye contains approximately 2000 ommatidia, each of which consists of a corneal lens, a crystalline cone, eight retinular cells and pigment cells (Hallberg, 1977). Three kinds of non-visual pigments are found in the eyes of mysids: there are dark pigments in retinular and distal pigment cells, reflecting pigment both distally and proximally, and red basal pigment around the ommatidial bases. Some of these pigments are migratory and contribute to light and dark adaptation (Hallberg and Elofsson, 1989).

M. relicta species were traditionally thought to have just one opsin, and the differences in visual pigment λ_{max} were interpreted to arise from varying proportions of A1 and A2 chromophores (Jokela-Määttä et al., 2005). However, later studies have shown that there are two visual pigments located in different photoreceptor cells and A1 is the only chromophore present in *M. relicta* eyes (Zak et al., 2013; Belikov et al., 2014), which suggest that there must be two opsins.

Some of the *M. relicta* populations are living in habitats where there is very little light available, thus it is extremely important to catch the scarce photons effectively. This emphasizes the importance of spectral tuning in order to adapt to their light environment. Spectral sensitivities of several species and populations of the *M. relicta* group have been studied at multiple levels of the visual system and with different methods. Behavioural action spectra of north American specimens of the *M. relicta* group were determined already in 1958 and the spectral sensitivity maximum was estimated to be around 515 nm (Beeton, 1959). Based on the geography these animals may be assumed to represent the species now named *M. diluviana*, and the results are in line with visual pigment spectral sensitivities later determined for that species by microspectrophotometry (Jokela-Määttä et al., 2005).

Data based on whole-eye electroretinography from two *M. relicta sensu stricto* populations with different spectral sensitivities show a consistent long-wavelength shift of eye spectral sensitivity compared to visual pigment absorption spectra (Lindström, 2000a; Jokela-Määttä et al., 2005). This shift is probably caused by absorption of short wavelengths by screening pigment granules containing xanthommatins, which can protect the eye against harmful effects of short-wavelength light (Khamidakh et al., 2010).

Another group of protective screening pigments found in considerable quantities in *M. relicta* eyes are the ommochromes. The concentration of ommochromes is twice as high in the eyes of a bright-light tolerant *M. relicta sensu stricto* population than in the eyes of a population from a deep dark lake (Dontsov et al., 1999). The eyes of the latter population are very sensitive to light and susceptible to light induced damage. Even a brief exposure to daylight has been shown to reduce the responsiveness

of their eyes for several days (Lindström, 2000a; Lindström and Nilsson, 1988). However, differing concentrations of photoprotective pigments can explain no more than part of the differences in light tolerance (Feldman et al., 2010).

The visual system of many crustaceans is capable of detecting cues based on the polarization of light. Several species with polarization vision are known within the class Malacostraca (where also mysids belong) (Roberts et al., 2011). Some stomatopod crustaceans (mantis shrimps) have been proven to be cabable of detecting even circular polarization (Chiou et al., 2008). The arrangement of the microvilli in mysid eyes would enable polarization sensitivity, and in the present thesis it is shown that the *M. relicta* eye gives polarization-selective light responses, but no behavioural evidence of polarization vision in mysid shrimps has been reported.

3 Aims of the study

In 1959 Alfred Beeton published a pioneer study on photoreception in *M. relicta* stating in the introduction: *"Little information is available on the physiology of photoreception in the Mysidacea other than a few studies of phototaxis. Practically nothing is known of their spectral sensitivity, dark adaptation, or lower limits of vision."*. Since then the mysid visual system has proven to be a convenient model system for ecophysiological studies, especially for questions related to dim-light vision. Although mysid vision has been in the focus of intensive research already for several decades and our understanding has developed a lot, answering one scientific question has raised a number of others. The general aim of this thesis is to extend the knowledge of evolutionary and physiological adaptations in mysid vision, building on the classical studies, and to gain a deeper understanding of the mechanisms, using state-of-the-art technology.

Two closely linked main themes concerning different aspects of visual adaptation are addressed. One is about adaptations to maximize sensitivity in a certain light environment, addressing specifically the mechanisms underlying spectral sensitivity differences observed between and within *Mysis*

species. The aims are to test more comprehensively the empirical finding of a lake/sea dichotomy in the spectral sensitivities of glacial-relict Mysis populations and to clarify the underlying mechanisms of spectral tuning. The specific questions are:

1. How general is the dichotomy between lake and sea populations in absorption spectra (characterized by λ_{max}) recorded from single rhabdoms?

2. To what extent does λ_{max} correlate with, on one hand, opsin genetics, on the other hand, light conditions in each habitat?

3. To the extent that neither factor alone can explain the variation pattern in λ_{max} , how can this be understood in terms of environmental factors acting on a constant genetic background?

These form the subject of papers I and II. In addition, some recent experiments illuminating question 3 are reported in the present thesis summary.

The second main theme concerns the other side of the coin: a system evolved for extreme light sensitivity is, as a trade-off, also highly vulnerable to the large amounts of energy liberated when it is exposed to higher light intensities. Again, *M. relicta* offers a promising model for studying mechanisms of light damage, as the dark-adapted eyes of two carefully studied populations differ in their susceptibility to damage from sudden bright-light exposures. Those of a population living constantly in very dim light have been shown to be more easily damaged than those of a population living in more varying light conditions. The general hypothesis is that this could depend on differences in long-term physiological states of light/dark adaptation (acclimation) rather than genetic differences. The specific questions are:

4. Can very slow light acclimation protect the eyes against light-induced damage?

5. If so, is the crucial protective effect a shift in the rhodopsin-metarhodopsin equilibrium?

6. Are there differences between the two intrinsically different populations in the effects of very slow light acclimation?

These questions form the subject of paper III.

4 Materials and Methods

4.1 Study animals and housing conditions

4.1.1 Natural populations and study sites

For the genetic analyses 12 *Mysis* species falling into four zoogeographical and ecological groups were collected. The samples were collected mainly during a two-decade period between 1984-2004 and preserved deep-frozen or in ethanol before the analyses. Details of the collection sites and populations for genetic analyses are described in paper I.

For the physiological characterization of spectral sensitivity, specimens of five of these species were used. The focus was on the four fresh or brackish water species forming the *M. relicta* species group: *M. relicta sensu stricto*, *M. salemaai*, *M. segerstralei* and *M. diluviana*. The fifth species was the slightly more distantly related marine *M. mixta*. The collection sites were chosen based on literature about the distribution of *M. relicta* species and water properties. After capture the animals were housed in complete darkness at +7-8°C before the physiological measurements were conducted. Details of animals and study sites can be found in paper II.

In the centre of this study were two *M. relicta sensu strito* populations, for these populations and their habitats have been in the focus of visual and ecological studies for decades. One of these populations lives in the very deep and dark Lake Pääjärvi in Southern Finland and the other in a narrow bay of the Gulf of Finland, Pojoviken. Although the Pojoviken population represents sea environments in this study, the salinity at the study site changes both in space and time, varying between 0‰and 6‰(Niemi, 1973).

4.1.2 Laboratory experiments

In the laboratory experiments with living animals the goal was to compare effects on the visual physiology of animals from a lake and a brackish water population under different experimental conditions. Two kinds of laboratory experiments were conducted: light acclimation experiments and rearing mysids in different salinities. For both experiments only specimens

of *M. relicta sensu stricto* were used.

Light acclimation experiments Animals from two well-studied *M. relicta* populations (Pojoviken and Pääjärvi) were kept in aquaria under very slowly increased background illumination before exposing some of the animals to bright light to investigate whether the acclimation procedure could mitigate light-induced damage in the eyes. The populations were chosen because they had been shown to differ in the light tolerance of their eyes (Lindström and Nilsson, 1988). In the study both long- and short-wavelength background lights were increased very slowly over four to eight weeks to induce changes in the equilibrium of rhodopsin and metarhodopsin of the bistable visual pigments. After each acclimation procedure eye morphology and physiology were examined with electron microscopy, electroretinography and microspectrophotometry. For details, the reader is referred to paper III.

Developmental experiments Specimens of *M. relicta* were also raised at different salinities in laboratory conditions in order to find out whether water salinity has any effect on the λ_{max} of their visual pigments. Animals from the Pojoviken population were used to represent sea-type spectral sensitivity and animals from Pääjärvi or Lake Kukkia to represent lake-type sensitivity. The Kukkia population originates from the same drainage system and had similar λ_{max} as the Pääjärvi population both in MSP (paper II) and ERG measurements. In the first stage of the experiments adult animals from Pääjärvi and Pojoviken were raised in the laboratory both in the salinity of their natural living environment and in the salinity of the other population's living environment. The λ_{max} was determined by microspectrophotometry (see below) from batches of animals a) right after capture b) after 1 month c) after 6 months in the laboratory. In the second phase λ_{max} was measured from second-generation animals which had been hatched in different salinities in the laboratory.

4.2 Light measurements

Information on ambient light environments was obtained by field measurements or in some cases based on literature. Irradiance spectra (W m^{-2}

nm^{-1}) were measured by OceanOptics JAZ-spectrometer and converted to photon fluxes. The light measurements at the collection sites of the natural populations were used to estimate the spectral composition and attenuation coefficient for light in the water column, which is described in paper II. In the light acclimation experiments photon fluxes of the stepwise increased housing lights and the bright exposure lights were measured as explained in paper III. For this study the calibration of the light stimuli (photon fluxes) in the ERG rig was also carefully checked by measurements with the same spectrometer.

4.3 Measurements of functional properties of the eye and visual cells

4.3.1 Microspectrophotometry

Microspectrophotometry (MSP) was used to characterize the identity and to some extent also quantity of visual pigments. Single-rhabdom absorption spectra were recorded with a single-beam, computer-controlled, fast wavelength-scanning microspectrophotometer. The general method and equipment are described earlier in Govardovskii et al. (2000) and Jokela-Määttä et al. (2005) among others, and specifics related to this study in papers I, II and III. General bleaching or spectrally selective bleaching by the measuring beam was used to evaluate the photoactivity and possible presence of multiple visual pigments.

When absorption spectra of a natural population were being determined, animals were fully dark adapted before the measurements were conducted. The dark acclimation period was at least 24 hours, usually longer. The weather conditions (amount of light) at the time of capture and the habitat water colour were taken into account when determining the length of dark acclimation. For animals captured from very dark lakes the dark acclimation was extended to several weeks to ensure that the eyes had recovered from potential light damage.

4.3.2 Electroretinography

ERG was used to determine the light sensitivity of the intact, excised eye in the light acclimation experiments (paper III). ERG was also used in paper II to study the polarization sensitivity of the eye and the possible presence of two distinct visual pigments. The method and equipment is described in the respective papers and earlier for example in Lindström *et al* (1988) and Pahlberg *et al* (2005). Compared to some of the earlier studies with the same ERG set up particular attention was paid to quantification of the estimates of eye sensitivity. Absolute stimulus intensities were measured and absolute photon fluxes used instead of relative intensities, and a fit of the Michaelis-Menten equation with the Naka-Rushton modification was used to determine visual parameters from intensity-response data.

4.4 Genetic analyses

In the genetic analyses of paper I a major part of the opsin gene was amplified from the genomic DNA with nested PCR and sequenced. For the specimens of *M. relicta* populations with the most interesting visual properties, longer sequences of the opsin genes were sequenced and the functionality of the opsin coding sequences were confirmed with reverse transcriptase PCR. However, the analysis of the coding sequences did not cover the last seven amino acids from the seventh transmembrane helix nor the long cytoplasmic and extracellular domains (ca 180 and 35 residues, respectively).

To study the opsin evolution in relation to speciation, nucleotide and haplotype diversity indices were calculated and the opsin gene diversity compared with the diversity of mitochondrial cytochrome oxidase I (COI). Phylogenetic relationships among emerged opsin haplotypes were constructed and signatures of possible directional selection as well as positive and negative selection on amino acid changes were investigated. The exact methods used in these analyses can be found in paper I.

4.5 Histology

Histological methods were used in the light acclimation experiments of paper III to characterize the general eye morphology and especially the integrity of the rhabdoms. The general structure of the eye was examined by light microscopy from semi-thin sections and the arrangement of rhabdomal microvilli with transmission electron microscopy (TEM). The methods are explained more comprehensively in paper III.

5 Results and Discussion

5.1 Light environment at the study sites

Based on the wavelength of maximal transmission and attenuation coefficients calculated from the light transmission spectra measured at several depths, the water bodies at the study sites could be divided into distinct categories. The attenuation coefficients went hand in hand with the spectral properties. There were three classes of lakes (clear/greenish, intermediate and dark/brownish) and two Baltic sea classes (coastal and open water). Even though the water bodies in this study fell quite nicely in distinct categories, their boundaries were somewhat arbitrary and investigating additional study sites would probably have led to a more continuous distribution of determined properties. The light conditions in coastal Baltic sea areas were similar to those in the clear lakes. Effects of seasonal changes could not completely be ruled out, but light measurements during special conditions like algae blooms were avoided. Even though no comprehensive study across seasonal and diel illuminations was conducted, the current results provide substantially better quantification of ambient light at the study sites than was available before. For more details see paper II.

5.2 Visual physiology of the studied *Mysis* populations

5.2.1 Spectral sensitivities

In general there seemed to be a bimodal distribution in the λ_{max} of single-rhabdom absorption spectra among the study populations, which was in line with earlier ERG- and MSP-results. This bimodality corresponded mainly to the division into sea and lake populations regardless of the light conditions or species and seems to be caused by different concentrations of medium- and long wavelength sensitive visual pigment (MWS and LWS). The presence of two visual pigments was established by selective bleaching experiments as described in paper II (technically, initially selective photoconversion of native pigment to MII). These two visual pigment types are likely due to different opsins, since when chromophore identity was determined for one lake and one sea population with different spectral sensitivities, both were found to have only A1 in their eyes (see paper II). The presence of two visual pigments is in line with Zak et al. (2013) but does not correspond to recent interpretations of opsin genetics in *Mysis*. According to the measured transmission spectra and estimates based on literature, the light conditions at the study sites showed no strict correlation with the observed differences in the visual pigment λ_{max}.

Since the sea/lake dichotomy in λ_{max} values was the most striking generalization from these studies, the effect of ambient salinity during the animals' life span on visual pigment λ_{max} appeared interesting, but the results of rearing experiments were mainly negative. When adult sea animals and lake animals were housed at different salinities in the laboratory for up to six months, no effect on their visual pigment λ_{max} was observed. The lake animals did not show any difference in their λ_{max} even if they had been hatched in the foreign salinity (unpublished data). The number of sea animals hatched in the laboratory was too small, however, to allow definite conclusions regarding possible developmental control of visual-pigment expression by salinity (unpublished data).

Although the λ_{max} was red-shifted in the lake populations it fell short of the actual transmission maxima of the waters in dark lakes. The gap

between visual pigment λ_{max} and WLMT was so wide that even taking the thermal noise into account (see 2.2.3) could not explain the mismatch. This is consistent with the idea that the lake populations in this study are at the limit set by the LWS pigment, which cannot be pushed further by changing pigment proportions. It should be noted that the spectral red-shift seen in whole-eye ERG compared with single-rhabdom MSP is evidently due to screening pigments taking up a light-adapted position, which will decrease rather than increase quantum catch (Jokela-Määttä et al., 2005; Khamidakh et al., 2010)).

5.2.2 Visual sensitivity and susceptibility to damage by light

Vulnerability to light-induced morphological damage in the rhabdoms and loss of responsiveness of the eye were significantly different in a *M. relicta sensu stricto* population from a dark lake (Pääjärvi) than in a population from a coastal Baltic sea location (Pojoviken) where the ambient light levels are higher. As judged by the saturated response amplitudes and stimulus intensities needed to elicit the half-saturated response in ERG, the initial visual sensitivities were similar in both study populations. Ultra-slow ramping up of background light from darkness in the aquaria where the animals were kept provided significant protection of the eyes against functional and morphological impairment caused by subsequent exposure to bright light. This effect was more pronounced in the lake population. A calibration of the time scale of the physiological response was obtained by comparing the effects of a faster and a slower ramping-up protocol: if the procedure was run in the course of 6 weeks instead of 12, the acclimating light itself was harmful to the eyes.

The shift of λ_{max} of single rhabdom absorption spectra indicated that concentration of native visual pigment decreased during light acclimation and the equilibrium of the bistable photopigment shifted towards metarhodopsin II. Contrary to expectations, however, the effects of short and long wavelength light were unidirectional and additive. This indicates that dark regeneration may be more important than photoregeneration in the renewal of the native visual pigment in *M. relicta*.

5.2.3 Variation in visual parameters

When comparing different populations or treatments it is customary to focus on the mean values of measured parameters, and small variances are often regarded as sign of high data quality. This has been the traditional way also in the visual studies of *Mysis*. However, during this study the importance of intrapopulation variation became evident, and thus the focus of comparison has been widened from just species and populations to include lower-level sources of variance: individual animals, rhabdoms and retinular cells.

Variation in λ_{max} between species was surprisingly small compared to variation between populations within species. Among the populations from the Baltic Sea, even *M. mixta*, which belongs to the more distantly related marine branch in the *Mysis* phylogeny, had similar λ_{max} as the more euryhaline *M. salemaai* and *M. relicta sensu stricto* populations. Most of the *Mysis*-populations from the Baltic sea had single-rhabdom λ_{max} roughly between 520 and 530 nm with quite small variation within populations. *M. mixta* had λ_{max} at slightly shorter wavelengths than *M. relicta* sensu stricto and *M. salemaai*. Of the latter species pair the former had λ_{max} relatively red-shifted by ca. 5 nm.

With just one exception, the single-rhabdom λ_{max} of all lake populations in this study was around 560 nm regardless of the species. These results cover populations of three species: *M. relicta sensu stricto*, *M. salemaai* and *M. segerstralei*. Even though no general correlation between water colour and spectral sensitivity was observed in the lake populations, the only lake population which had λ_{max} at markedly shorter wavelengths lived indeed in a clear-water lake. The exact parameters for the light conditions in the water at the study sites and the population means of λ_{max} are given in Table 1 of paper II.

There were two *M. relicta sensu stricto* populations in the Baltic sea which had particularly interesting spectral sensitivities. One of these was a well-studied population from Pojoviken and the other one was a coastal population from the Gulf of Finland. Only two specimens of the latter were measured, but both of them had λ_{max} around 550 nm. Although no sig-

nificant variation between individuals from the Pojoviken population had been observed in the older studies reported in paper I, in more recent measurements individuals with intermediate or even almost lake-type spectral sensitivities were found (unpublished data, see also the large SEM value for that population in Table 1 of paper II). The ecologically relevant common factor of these two study sites is that the water in both locations was intermediate between fresh and brackish. These estuarine sites were also the ones with the strongest seasonal and other variations in environmental conditions, including illumination. Thus the variation in the parameters of visual physiology within populations seems to correlate with the variability of the living environment of the population.

No estimates of variation in light tolerance or visual pigment concentrations and eye sensitivity between species could be made, since these were characterized in only one species (*M. relicta sensu stricto*). Between the two study populations there was a significant difference in these properties. An interesting finding was that within the Baltic sea (Pojoviken) population there was markedly more variation in the visual parameters measured in the light acclimation experiments than in the lake (Pääjärvi) population. Thus regarding both eye sensitivity and vulnerability as well as spectral sensitivity of *M. relicta*, there seems to be more variation within the sea populations than lake populations. In all lake populations the variation both between individuals and between rhabdoms within one individual was small.

In the Pojoviken population of *M. relicta sensu stricto* there was not only great variation in the λ_{max} of single rhabdom absorption spectra between individuals, but also between rhabdoms of the same animal. Although no comprehensive analysis of the spatial distribution of λ_{max} values of single rhabdoms in the eye was made, neighbouring rhabdoms always had similar λ_{max} . This might indicate that there are regional differences in the proportions of MWS and LWS visual pigments in the rhabdoms across the eye, but could also be due to the fact that the pigments are located in separate retinular cells within the rhabdoms and contribute differently to the measured absorption depending on the path of the measuring beam (or both). Interestingly, the ERG results showing that the selectivity for

the plane of polarization of linearly polarized light is different for long-wavelength ("red") and short-wavelength ("blue") light may be consistent with both possibilities. In paper II, the results were primarily taken to indicate that different retinular cells within single rhabdoms have at least different proportions of MWS and LWS pigments. The assumption then is that the polarization selectivity arises in rhabdoms with axial incidence of the stimulus light. However, regional differences in overall proportions of the two pigments between rhabdoms could also cause differential responses to orthogonally polarized red and blue light, since the angle between the polarization plane and microvilli will depend both on the geometry of rhabdoms and the geometry of the whole eye. As an extreme example, a rhabdom receiving the stimulus light from the side will respond maximally to light polarized at right angles to its axis and minimally to light polarized parallel to its axis. It is hard to judge to what extent such a situation is optically possible in the excised but otherwise mainly intact *Mysis* eye as mounted in the ERG chamber, but the possibility cannot be excluded.

5.3 Genetics

The results of genetic analyses presented in paper I were somewhat confusing and not in line with the results describing visual physiology. All studied opsins were monophyletic and belonged to the crustacean LWS opsin group described by Porter *et al.*(2007). As usual in crustacean opsins, no introns were found. Levels and patterns of intraspecies diversity varied much among species. The levels of intraspecific diversity in the opsin gene only partially correlated with the variability of the mitochondrial COI marker, and the geographical pattern of opsin differentiation was unrelated to that in the allozyme set at this level (studied only in *M. salemaai*). Opsins of the study species were generally forming two lineages, I and II. Lineage II was found in three species of the fresh and brackish water taxa only: exclusively in *M. relicta*, and along with lineage I in the *M. salemaai–M. segerstralei* cluster. Two sequences of *M. nordenskioldi* were intermediate between I and II.

The finding that opsin gene sequences did not match with visual pigment absorption spectra is strictly against the hypothesis of mechanisms behind spectral tuning in *Mysis* (see below). Because the opsin lineages do not correlate with the spectral sensitivity or phylogeny, they seem to be of questionable relevance for explaining the observed diversity in the visual pigment λ_{max} in the *M. relicta* species group. One explanation could be that there have been methodological deficiencies either in identifying the amino-acid changes responsible for spectral tuning or in detecting opsin types present in the eyes. Possible caveats in genetic analyses are a) that haplotypes are too large units to focus on, if SNP:s are enough to cause shifts in λ_{max} b) there is crucial polymorphism in the unsequenced domains, which are quite substantial (Viljanen, Paulin and Donner, unpublished results), c) the primers happen to catch just one of two (or several) opsin genes. Other explanations are discussed further in 5.4.1.

5.4 Possible mechanisms underlying the differences in visual physiology

5.4.1 Spectral sensitivity may be set by a reaction norm controlling differential expression of two pigments

The large data set in this study confirmed the observation made by Jokela-Määttä *et al* (2005) that the λ_{max} of visual pigments of *M. relicta* populations living in the Baltic sea is at ca. 30 nm shorter wavelengths than the λ_{max} of the lake populations of the same species. This is ecologically beneficial, for the WLMT of water is generally at shorter wavelengths in sea than in lake waters. It is good to remember, though, that the measured λ_{max} values are from single rhabdoms, not individual visual pigments. Since this constant shift towards longer wavelengths in the λ_{max} in lake populations compared to sea populations occurs in species separated long before the lake populations separated from the corresponding sea populations, an adjustable mechanism affecting the spectral sensitivity must have emerged before the speciation of the *M. relicta* group. The other relevant fact is that they possess two visual pigments positioned at ca 520-530 and 560

nm, respectively. One explanation could be that a reaction norm controlling the expression ratio of these pigments and thus allowing rapid shifts of λ_{max} evolved while their progenitors living in the Baltic Sea area experienced repeated habitat shifts between marine and freshwater conditions and these shifts were on average associated with changes in light conditions. The mysids apparently lack the option serving this purpose in many fish species, as well as in some crayfish, that of switching between chromophores A1 and A2.

The most obvious scenario would be that the expression of two opsins in different proportions depends on the light conditions. Unfortunately, the results of this study do not totally support this scenario for two reasons: first, the light conditions at the study sites did not unambiguously correlate with the measured spectral sensitivity and second, only one opsin gene has been found so far. This does not mean that the reaction norm does not exist, merely that the mechanism is not the most obvious one.

The exact molecular regulatory mechanism behind the suggested reaction norm is beyond the scope of this study, but some speculations can be made. If there are two versions of the opsin gene, their differential expression could be achieved by environmentally sensitive transcription factors, or selective methylation of the genes themselves. Since the data from natural populations and preliminary experiments of housing *M. relicta* at different salinities suggest that the spectral sensitivity is stable during an animal's life time and not affected directly by ambient light conditions, it is possible that there are epigenetic tuning mechanisms acting during ontogeny. Environmentally guided ontogenetic regulation of spectral sensitivity has been observed in the stomatopod crustacean *Haptosquilla trispinosa*, but in this case it is not the visual pigment themselves which are affected. In this species the light environment during juvenile development affects the properties of filtering structures in the rhabdoms, which are used to modify the spectral sensitivity of long-wavelength visual pigments especially in shallow waters (Cronin et al., 2001).

The result in paper I that *M. relicta* has only one opsin gene must be considered as uncertain for at least two reasons. First, the two haplotypes could possibly represent two opsin genes present in all populations,

although for unknown reasons differentially and seemingly randomly amplified in the DNA studies. Second, there could be important differences in the cytoplasmic and extracellular tails that were not sequenced. Recent work (Viljanen, Paulin and Donner, unpublished results) has shown firstly that these parts comprise no less than 215 amino acids in all, secondly that for some reason the amplification of opsin genes is erratic especially in sea *Mysis relicta*.

On the other hand, if there are no differences in the opsin gene explaining the differences could there be some post-translational modification of the amino acid sequence? Lack of introns in the opsin gene takes splice variants out of consideration, but there are other mechanisms which could be involved. For example ADARs (RNA specific adenosine deaminases) have been shown to catalyze the site-specific conversion of adenosine to inosine in primary mRNA transcripts in a G-protein coupled receptor in *Drosophila* eyes (Stapleton et al., 2006). Even though this protein is not directly involved with photreception, similar mechanisms could post-translationally modify opsin amino acid sequence as well. There are also other possible factors that might modify the spectral sensitivity of visual pigments even when based on a single opsin, including membrane lipids and ion concentrations (see 2.2.3).

In principle the reaction norm hypothesis is plausible whether the target is opsin genes or not. Even though ambient light conditions alone cannot be the driver of this reaction norm, there can well be some other critical environmental factor (see figure 5). The most promising candidate for that factor is salinity, since it is predictive of the light conditions in water. Actually, due to temporal fluctuations in illumination described in section 2.1.3 salinity may be a more stable indicator of future light conditions than a light level experienced at a certain time during ontogeny.

5.4.2 The amount of native visual pigment affects light tolerance

Against predictions, the lake and sea populations were quite similar in their responses to light acclimation and exposure to bright light after different acclimation procedures. The main difference between the two populations

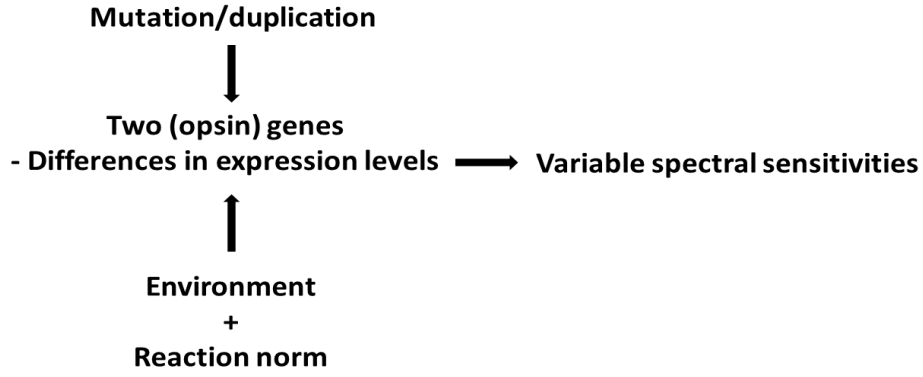

Figure 5: Schematic representation of the reaction norm hypothesis. Some environmental factor drives a reaction norm, which causes differences in spectral sensitivities trough differential gene expression. The system could have emerged via duplication and mutation(s) of some gene(s) affecting the visual pigment absorption spectrum, the gene in question being most likely opsin.

was not on the average responses, but in the variation within populations. Especially intriguing is the possible connection between variability in the spectral sensitivity and light-induced damage. However, this could not be established at the individual animal level.

Results of the light acclimation experiments partially confirmed and partially rejected the original hypothesis that the susceptibility to light induced damage in mysid eyes could be reduced by shifting the rhodopsin-metarhodopsin equilibrium towards MII. The hypothesis seemed to hold when the acclimation was done slowly enough, since as a result the R-MII equilibrium indeed shifted and eye resilience to bright light increased. Unexpectedly, short-wavelength acclimating light could not drive the R-MII equilibrium back towards rhodopsin, but instead led to further reduction of native rhodopsin and enhanced protective effect. These new results imply that the photoregeneration of the native visual pigment is not a very

effective mechanism of visual pigment regeneration in *M. relicta*, and they must rely more on dark regeneration of the visual pigment.

The time scale on which the protective effect developed was extremely interesting, since too fast an increase in the background illumination was harmful itself. This gives some guidelines for estimating the time scale of adaptive physiological changes in mysid eyes. The speed with which the eyes can adapt to changing levels of ambient illumination seems to correlate with the tempo of seasonal fluctuations in environmental light levels. The incapacity to adapt to more rapid changes may reflect the reduced need for fast adaptation, as vertical migration effectively keeps the light levels *M. relicta* encounter in a desired range.

Although a high concentration of native rhodopsin enabling excess phototransduction in bright light seems to be critical in the development of photodamage in mysid eyes, changes in rhodopsin concentration did not fully explain the slow acclimation. Identifying other factors must await further study, but there are some obvious candidates. For example changes in visual membrane properties modifying the rate of phototransduction or differences in the amount or identity of screening pigments may also play important roles in seasonal light acclimation.

5.5 Levels and time scales of visual adaptation

5.5.1 Biological levels of adaptation

The adaptive mechanisms investigated in this study were mostly characterized by their effects on functional traits. It is likely that they are closely connected to structural and behavioural effects as well, but these could not be proven with the methods used. Different biological levels of adaptation as defined by Mazess (1975) encompass six categories (see 2.2.1), most of which were represented in this study. The division into adaptive levels is of course artificial, and the levels interact with each other. In one way or another, genetic adaptation lies behind adaptive mechanisms at all levels.

Adaptation at the **physicochemical level** includes the shifts in visual pigment λ_{max} by a yet undefined mechanism. The molecular characteristics

of protective visual screening pigments reported by Feldman et al. (2010) and Dontsov et al. (2004) also fall in this category. Somewhere between the physicochemical and **cellular** levels of adaptation are the mechanisms controlling the regeneration and amount of native visual pigment. The visual pigment renewal relying on slow dark-regeneration rather than photoregeneration can be seen as an adaptation to dim light conditions for two reasons: if the amount of light and its spectral composition in the environment is restricted, there is no fuel for photoregeneration and because the rate of photoactivations is low in these conditions, there is also no need for fast visual pigment regeneration.

The spectral tuning and acclimation to current light levels is further modified at the level of **organ systems** by the migration of screening pigments. The structural arrangement which enables polarization sensitivity can also be seen as adaptive at this level, especially if there really is adequate neural coding involved. Another finding connecting structure to organ function is the possible segregation of different visual pigments into different photoreceptor cells, as suggested in paper II. One of the most prominent mechanisms functioning at the level of organ systems is the slow acclimation to ambient illumination. Although the roots of the acclimation may be at lower hierarchical levels, the acclimation is primarily a property of eyes as functional unities.

Different spectral sensitivities and light tolerances between individuals affect the adaptation of **individual** animals to their environment at **organism** level. Variation in the visual physiology between individuals can be seen also as adaptation at the **population** level. Even if individual animals are not able to set up an adaptive response to the spectral composition of light during their life time, variation increases the population's ability cope with changes in environmental light conditions. Other results falling into this category are the differences in light tolerance and spectral sensitivities between sea and lake populations correlating with the amount and colour of light in their habitats.

The hierarchically highest level is the **ecosystem** level of adaptation, which could not directly be seen in this study, but is discussed in 5.6.

5.5.2 Time scales of adaptation

Regarding the time scales of adaptation, signs of mechanisms acting in evolutionary time which affect the capability to adapt on much shorter time scales were detected. The potential to have quite different spectral sensitivities has presumably evolved about 2 million years ago, but the spectral sensitivities can be adjusted as a response to environmental demands most likely between generations. Thus the adaptive mechanism acting on a physiological time scale, the ability to adapt, has emerged as a result of genetic adaptation on an evolutionary time scale.

Animals from brighter light environments probably have intrinsically faster regeneration of the native visual pigment associated with recycling of the membrane of rhabdomal microvilli. At what time scale the adjustment of this speed is done, remains still unclear. The renewal of the visual pigment, or dark acclimation, seems to be quite a slow process in *M. relicta*.

It is unknown if there are some developmental time windows for setting the gene expression levels which determine the proportions of proposed MWS and LWS pigments in *Mysis* and if such time windows exist on what time scale they operate.

5.6 Ecological effects of Mysis vision

Since *M. relicta* species have a significant role in many freshwater ecosystems and in turn vision is important for them, it is logical that the visual capacities of these mysids may have profound ecological effects. Specimens of *M. relicta* were introduced mainly during the 1970's into several fresh waters around Northern Europe and North America in order to serve as nutrition to valuable fish stocks. This was done without sufficient knowledge of their ecology though, and the desired ecological impact was often not achieved. On the contrary, in many cases either the introductions failed or the ecological effects were so detrimental that *M. relicta* is now regarded as harmful invasive species (Næsje et al., 1991; Spencer et al., 1991).

The failure of introduction may be directly related to vision, if the light conditions in the target site or during transfer have been too different from

the source site. The light acclimation experiments in paper III confirmed the earlier observation that sudden exposure to bright light disrupts eye function in *M. relicta* and showed that acclimatization to changing ambient light conditions is very slow. Thus it is likely that the changes in light intensities during transfer and release have been too fast to retain the integrity of vision in the introduced animals, which has made them ineffective feeders and easy targets for predators.

It is easy to understand how vision can hamper successful transplantation, but the connection to invasiveness is not as straightforward. Water transparency has been shown to be a key factor in explaining the effect of *M. relicta* introduction on fish stocks in Norwegian lakes. The competition for zooplankton has reduced the amount of arctic char in these lakes. In more transparent lakes the effect is milder, since mysids do not exploit the surface layer of the water, which creates a refuge for the zooplankton. High transparency also expands the habitat of char where they can capture *M. relicta* as prey (Langeland and Moen, 1992). If mysids are able to adjust their spectral sensitivity as flexibly as suggested in 5.4.1 they may achieve high photon catch at very low light levels in water bodies with different WLTM, a visual adaptation which may widen their ecological niche and give a significant competitive advantage. In any case visual plasticity is just one factor behind the - sometimes excessive - success of *M. relicta*.

As explained in 2.1.3, waters in the study area are becoming darker which may have surprising effects on food webs and the balance of the lake ecosystem via changes in the niches of animals relying on vision. Based on the results of this study it seems that *M. relicta* is capable to adapt visually to these kinds of changes in the light environment happening over the course of a few generations. Predicting the actual consequences of *M. relicta* vision in the ecosystems would require multidimensional ecological modelling beyond the scope of this study, but the presents results can help to create parameters for such modelling.

6 Conclusions

The results concerning spectral tuning in *Mysis* led to the following conclusions:

1. The division of spectral sensitivities into sea and lake types was present among the study populations across species. The habitat (fresh water vs. brackish water) was the single factor that best predicted single-rhabdom absorption spectra (λ_{max}).

2. There were minor differences in λ_{max} between the species, associated with differences in opsin gene sequences. Within species, the differences in λ_{max} between populations could not be explained by observed differences in the opsin gene sequence. Although the lake/sea dichotomy as such entails a degree of correlation with the transmission properties of the water bodies, these could not comprehensively explain the differences in λ_{max} between populations.

3. The variation in single-rhabdom λ_{max} observed between populations as well as between individuals in some populations could be explained by two visual pigments with different λ_{max} expressed in different proportions in response to some environmental factor.

The experiments designed to study whether slow light acclimation can protect against damage from exposures to bright light allowed the following conclusions:

4. Slow light acclimation very significantly mitigated the deleterious effects of bright-light exposures. The time required for acclimation corresponded roughly to the time scale of seasonal changes in illumination in the natural habitats of *M. relicta*.

5. The shift of the rhodopsin-metarhodopsin equilibrium (R:MII) towards MII correlated with the emergence of the protective effect but could not explain it fully.

6. The two study populations, which originated from different light environments, exhibited similar responses to acclimation procedures, but the effects were clearer in the animals living naturally in very dim light conditions.

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