Environmental, population, and life stage plasticity in the visual system of Atlantic cod

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Summary statement

This study clarifies the potential of visual tuning in Atlantic cod through differential opsin usage during changes in environmental light, and considers the influence of developmental pre-programming, and population ecotypes.

Abstract:

The visual system is for many fishes essential in guiding behaviors such as foraging, predator avoidance and mate choice. The marine environment is characterized by large spatiotemporal fluctuations in light intensity and spectral composition. However, visual capabilities are restricted by both space limitations set by eye size, and by the genomic content of light absorbing opsin genes. The rich array of visual opsins in teleosts may be used differentially to tune vision towards specific needs during ontogeny, and to changing light. Yet, to what extent visual plasticity is a pre-programmed developmental event, or is triggered by photic environment, is unclear. Our previous studies on Atlantic cod revealed an evolutionary genomic loss of UV-sensitive *sws1* and red-sensitive *lws* opsin families, while blue-sensitive *sws2* and green-sensitive *rh2* opsins had duplicated. The current study have taken an opsin expression approach to characterize visual plasticity in cod towards; different spectral light during the larval stage, to maturation, and extreme seasonal changes in the Barents Sea. Our data suggest that opsin plasticity in cod larvae is controlled by developmental program rather than immediate light environment. The lack of expressional changes during maturation, suggest a less important role for visual modulation related to mate choice. Although no seasonal effects on visual opsins were detected in migratory North East Arctic cod, the expressed opsin subset differed from the more stationary Norwegian Coastal cod described in previous studies. Interestingly these data provide the first indications of a population difference in actively used visual opsins associated with cod ecotypes.

Introduction

The marine light environment is rapidly changing with depth, mainly due to light being absorbed and scattered by the water and its components, a sharp contrast to life on land (Partridge and Cummings, 1999). The dynamic light environment has put pressure on a variety of visual adaptations that have both genetic and environmental influences (Hofmann and Carleton, 2009, Hofmann et al., 2010, Hunt et al., 2004, Partridge and Cummings, 1999). The eye size sets spatial limits to visual capabilities and demands strict prioritization in time and space (Evans and Browman, 2004, Moran et al., 2015). Consequently many fishes have specialized vision for specific photic environments, and may also change visual capabilities during the course of development correlated to altered light ecology (Evans and Browman, 2004). Comparative studies have indicated that light environment is important for evolution of color vision, yet cannot alone account for mechanisms underlying this correlation (Boughman, 2001, Fuller et al., 2004, Fuller et al., 2010, Lythgoe et al., 1994, Seehausen et al., 2008, Travis and Reznick, 1998).

The signaling process of light clues used for vision is complex and involves light transmission, retinal reception and integration, then higher order processing by the brain, ultimately leading to a response in animal behavior (Endler, 1992, Fuller et al., 2010, van der Sluijs et al., 2011). Environmental light may influence this process in three ways; 1) immediate effects on signal propagation and transmission, 2) induce variation of visual perception due to developmental plasticity, and 3) lead to genetic differences among species and populations due to history of selection in different habitats (summarized by; Fuller et al. 2010, (Fuller et al., 2010)). Interactions may involve developmental plasticity, genetics, rearing environment and immediate environment (Fuller et al., 2010).

Visual perception is largely dependent on the structure and function of retina where rod and cone photoreceptors are the functional units (Reid and Usrey, 2008). While the range of light spectra that is visible to a given species is determined by the variety of cone opsin genes expressed, the ability to sense low intensity light requires rods expressing the rhodopsin pigment (Yokoyama, 2000a). Hence, the visual pigment component, opsin has an essential role of directly translating light information (photons) from the outer environment to generate an image projected to

the brain. The cone opsins used for color vision are distinguished into separate classes based on distinct spectral sensitivities within the: UV (SWS1, λ_{max} 350-440nm), blue (SWS2, λ_{max} 430-470 nm), green (RH2, λ_{max} 460-530 nm) and red (LWS, λ_{max} 520-575 nm) range of the spectra (Yokoyama, 2000a). Based on the opsin sequence and expression patterns one can make assumptions about visual color sensitivity, and in some cases even visual guided behaviors such as foraging, predator avoidance and mate choice (Fuller and Claricoates, 2011, Fuller and Johnson, 2009, Fuller et al., 2010, Hofmann and Carleton, 2009, Horth, 2007).

Although the genomic array of opsins restricts the potential of light discrimination and sensitivity, adaptive phenotypic plasticity may further locally adapt species or populations to different light (Kawecki and Ebert, 2004, Larmuseau et al., 2009, Larmuseau et al., 2010, Spady et al., 2005, Yokoyama, 2000b). Comparative studies have shown that changes in opsin expression may be used to tune visual sensitivity (Carleton and Kocher, 2001, Hofmann and Carleton, 2009, Spady et al., 2006). As a consequence, visual systems are often under strong natural selection, and phenotypic plasticity in visual systems may help organisms adjust to changing conditions (Hofmann and Carleton, 2009). One example is Rainbow trout (*Oncorhynchus mykiss*) that experiences both loss and gain of UV vision through degeneration/regeneration of UV cones timed to sea-river migration (Allison et al., 2006). Phenotypic plasticity has also been shown to have evolutionary consequences as it facilitates colonization of novel habitats, and the synergistic change in environment and sensory systems can promote population differentiation and speciation (Price et al., 2003, Seehausen et al., 2008). A less dramatic change in sensitivity include differential chromophore usage; where switch of vitamin A1 and A2 have been reported in fish migrating between freshwater and marine habitats (Bowmaker et al., 2008, Carleton, 2009, Enright et al., 2015, Temple et al., 2006, Toyama et al., 2008).

The teleost visual system of fishes is particularly diverse, and likely reflects environmental heterogeneity including variety in light (Levine and MacNichol, 1982). In our previous work on Atlantic cod we elucidated the genetic basis and developmental plasticity of opsin expression (Valen et al., 2014, Valen et al., 2016). Interestingly, we found that cod has lost SWS1 and LWS opsins, sensitive to UV and red light, respectively. In contrary, both SWS2 and RH2 have tandem-duplicated

resulting in two and three paralogs of each subfamily, respectively (Valen et al., 2014). Comparative studies have shown that having a wide array of opsin gene sets is a typical teleost feature, which is a result of numerous duplication events and retention of favorable gene paralogs (Lagman et al., 2013, Larhammar et al., 2009, Rennison et al., 2012). Studies in cichlids have shown that different light environments have led to contemporary evolution of visual opsins and expression patterns (Hofmann et al., 2010). Modulation of vision plays a critical role in tuning towards environmental light, and may be achieved through triggering of differential opsin expression (Fuller and Claricoates, 2011). Cichlids show some of the largest known shifts in visual sensitivity that result from modulated expression of seven cone opsin genes (Parry et al., 2005). The mechanisms regulating opsin gene expression is largely unknown and have only recently become more clear (Carleton et al., 2010, O'Quin et al., 2011, Schulte et al., 2014, Takechi et al., 2008). Both genetic architecture and gene regulatory factors are involved in opsin gene regulation (O'Quin et al., 2011, Schulte et al., 2014).

Several fishes undergo natural ontogenetic changes in opsin expression, often suggested to correspond to changes in photic environment (Carleton et al., 2008, Cheng and Flamarique, 2007, Cottrill et al., 2009, Schweikert and Grace, 2017, Shand et al., 2002, Veldhoen et al., 2006). In Atlantic cod, we have shown that *rh1* and *sws2/rh2* opsin gene duplicates are used differentially during development from larval to juvenile transition (Valen et al., 2016). Hence, larval vision is purely driven by color vision, while ability for low sensitivity vision appears later on, typical for indirect developing species (Evans and Browman, 2004, Evans and Fernald, 1990). The difference in sensitivity among cichlid species have been attributed to heterochronic shifts in developmental opsin programs (Carleton et al., 2008). Altogether, indicating that ontogenetic changes in visual opsins are determined by a multitude of factors such as photic environment, ecology, life strategy, and evolutionary history.

Maturation represents a major life event of fishes, and combined with spawning may be linked to dramatic habitat shifts that affect opsin expression (Allison et al., 2006, Archer et al., 1995). Also stickleback, cichlids and guppies change visual sensitivity upon mating, through differential cone opsin expression (Carleton et al., 2010, Laver

and Taylor, 2011, Shao et al., 2014). Still, whether this is developmentally programmed or triggered by environment, or a combination, is unknown.
Efforts to discern apart developmental plasticity of opsin regulation, from plasticity towards light changes in fish, have so far been focused on to a few species (Fuller and Claricoates, 2011, Fuller et al., 2010, Hofmann et al., 2010, Shand et al., 2008). Hence, comparative knowledge including how these operate in separate and may interact in species with different life strategies is lacking. This may turn out to be a key issue as eye development is fundamentally different in most marine species where color- and scotopic vision is introduced stepwise (Evans and Browman, 2004). In contrast, direct developing fish typically have both visual capabilities functional from early on. A model has been put forth where the ecological versus developmental constraints on visual system depend on developmental stage upon hatching (Evans and Browman, 2004). Hence, it may be likely that opsin expression plasticity towards immediate light is restricted by developmental program, but may also include “developmental windows of opportunity” in which tuning towards environment may occur.

Atlantic cod is one of the most important fisheries species in the Northern Atlantic, and has a key role as an ecosystem apex predator (Ottersen et al., 2014). Previous studies on Atlantic cod and response to variation in light environment have mainly focused on foraging, growth, survival and maturation, mostly linked to optimization of aquaculture conditions (Puvanendran and Brown, 2002, Sierra-Flores et al., 2015, Taranger et al., 2006, Vollset et al., 2011). These studies demonstrated that cod responded differently in these traits to various light intensities, wavelength and photoperiod. Still, the underlying molecular mechanism of light reception was only recently described by our group (Valen et al., 2014, Valen et al., 2016). The change of visual capabilities in Atlantic cod is likely linked to changes in ecology from planktonic foraging in the epipelagic to active predatory lifestyle in both deep and shallow waters. Previous light experiments suggested a cod population difference in growth and survival in response to varying light (van der Meeren et al., 1994, Van der Meeren and Jørstad, 2001).

In nature, Atlantic cod display divergent feeding behaviors depending on spawning ground, termed ecotypes (Karlsen et al., 2013). While the Norwegian Coastal cod (henceforth NC cod) remain more or less stationary, the North East Arctic cod (NEA

cod) migrates north of the Arctic Circle, an area characterized by dramatic seasonal changes in photoperiod. Genome analyses have associated cod population differences to certain genomic regions, which includes variation within the Rhodopsin, *rh1* gene itself (Hemmer-Hansen et al., 2013, Pampoulie et al., 2015, Sarvas and Fevolden, 2005). Yet, so far, it is not known whether this may cause a population difference in visual sensitivity due to gene variation, or by differential gene regulation.

Recent advances in genome sequencing have given access to the whole genome of several teleosts. This genomic backbone provides the framework for visual function. To understand how various genes are used functionally in the organism and in response to environment, analysis of gene activity is central. We have taken a gene expression approach focusing on Atlantic cod to gain insight on how a marine teleost uses its opsin gene complement during ontogeny, and in response to environmental changes. Previously we have shown dramatic ontogenetic changes in visual opsin expression profile (Valen et al., 2016). In this study, we attempt to discern apart developmental and life history driven opsin regulation, from environmental driven plasticity, and unravel potential population effects. In summary, we will use our previously published methods on visual opsins to: 1) Characterize potential of phenotypic plasticity in NC cod larvae in response to different light regimes, 2) investigate opsin expression during maturation in NC cod, and 3) characterize expression levels in NEA cod, and compare results with previous data on NC cod. We will also check for potential seasonal tuning in visual opsins in NEA cod.

Material and methods

Biological material

Fertilized NC cod (*Gadus morhua*) used in the current study for characterization of different wavelength light on cone opsins, was obtained from Parisvannet Research station, Institute of Marine Research (IMR), Bergen, Norway. Embryos from one egg batch/group was transported to Bergen High Technology Centre at stage 9 days post fertilization (dpf), and raised in black 15 L tanks (see FigM1 for setup). All tanks had oxygenated sea water running through, set to 6°C (for setup see below). Black tanks have been considered the best for marine larval rearing as it best represents natural conditions in terms of background and light regime (Duray et al., 1996, Monk et al., 2008). Embryos were kept under similar white light conditions until 17 dpf (equals to 2 days post hatching (dph)), then split into three replicate tanks with different light regimes (see Fig. S1). The developmental phase exposed to different light treatments in the current study, corresponds to our previous observations of this stage involving dramatic shifts in cone opsins (Valen et al., 2016). The light regime for all treatments was 14 hours light – 10 hours dark, simulating approximate day length in Bergen, Norway in March/April. When approaching time of natural feeding when yolk sac resources was exhausted (17 dpf), larvae where fed daily natural zooplankton enriched with microalgae **(***Rhodomonas* and *Isocrysis*) of approximately 3000 prey items L^{-1}. The zooplankton where harvested from Department of Biology, field station, Espegrend (University of Bergen, Norway). Prior to sampling, cod larvae where transferred to petri dishes with buffered seawater containing metacaine (MS-222) sedative (Sigma-Aldrich, MO, USA), then into RNA*later*® (Ambion, MA, USA). Samples were stored at 4°C for 24 hours, then transferred to -80°C until further analysis. Necessary permit for the use of larval cod in the current study was obtained from the local IACUC with permit number 6388.

Maturing NC Atlantic cod (~2.5 year) was donated from Austevoll Research station, IMR. Prior to sampling, fish where sedated with buffered MS-222 (Sigma-Aldrich, MO, USA) until movement ceased, then euthanized with a blow to the head and bled out by cutting the main artery. Eyes from 11 fish including 6 females (average length: 57.5 cm) and 5 males (average length: 54.8 cm), were sampled in November. Sex was determined based on gonadal features, and all fish were characterized as

maturing following the gonadal staging index proposed by ICES (Bucholtz et al., 2007). The dissected eyes were transferred to RNA*later*® (Ambion) for real-time quantitative PCR (qPCR). To allow optimal penetration of RNA*later*® through the tissue, incisions were made in the cornea and the lens was carefully removed. Samples were first kept at 4°C (24-48 hours) then stored at -80°C.

The NEA cod used for characterization of seasonal effects on visual opsin expression was obtained as part of research and ecosystem surveillance cruises organized by IMR associated with Norwegian national fisheries management (for further information: http://toktsystem.imr.no/cruises/). Cod was sampled during the winter survey (N=10, length=9.1-25 cm) in the Barents Sea with bottom trawls (Campelen 1800) with the Johan Hjort vessel (February 12th-18th, cruise id: 1395, nr: 2014202), and during early fall (N=10, length=19-26 cm) from the ecosystem survey with G.O. Sars (cruise id: 1414, nr: 2014116). Eyes were sampled and treated in a similar procedure as described for NC cod, except for NEA cod the right eyes were transferred to 4% Paraformaldehyde-Phosphate-buffered saline (PFA-PBS) (Sigma-Aldrich) fixative for *in situ* hybridization studies, in parallel with left eyes being sampled on RNA*later*® (Ambion) for qPCR.

Experimental setup – Effect of different wavelength light on cone opsins

In our light treatment experiment, we used five different light regimes on NC cod larvae: White light (LD), continuous light (LL), blue light (B), green light (G) and red light (R). The LD, B, G, and R groups followed a 14h light 10 hour dark light cycle, while light was kept on (24 hour/day) in the LL group. The light source used was connected LED strips (RGB LED Strip Starter Kit, North Light, Riga, Latvia); see Fig. S1. Both intensity of light (mW m^{-2} nm^{-1}), and wavelength distribution (nm) of each channel (LD, B, G, R) was measured using a RAMSES/SAM-ACC-UV-VIS (350-900 nm wavelength range) irradiance sensor (TriOS GmbH, Rastede, Germany) with associated software MSDA_XE (TriOS, version 8.8.13 2012-06-28). Light measurements obtained from the MSDA-XE software was plotted in STATISTICA (Version 12, Dell Inc., USA). For additional information including spectral distribution, see Fig. S1. Intensity of the LED light could be adjusted in ten steps, and the step corresponding to approximately the same intensity (~ 0.2 mW m^{-2} nm^{-1}) was used in the experiment. In each light treatment larvae where distributed into three tanks

consisting of buckets (15L) with plankton mesh in the bottom to allow water circulation (Fig. S1).

RNA extraction, cDNA synthesis and visual opsin expression studies

For the light experiment, total RNA was extracted from pools of 10 larvae each from three replicate tanks (N = 10x3) at 7 dph and 12 dph, representing 5 and 10 days of light treatment, respectively. In addition, RNA from a pool of 20 larvae at 2 dph was isolated from the white light tank as an opsin expression reference prior to exposure of the various light regimes. RNA isolation was performed on whole larvae using column based Total RNA Purification Kit according to manufactures protocol (Norgene Biotek Corp., ON, Canada).

On retinal tissue from NEA cod and maturing NC cod, total RNA was isolated by phenol-chloroform extraction (removed from sclera) as previously described (Chomczynski, 1993, Valen et al., 2014). RNA from all samples was treated with Turbo DNase free kit (Ambion). Synthesis of cDNA single strand was performed on 700 ng of DNase treated RNA as input, according to Valen et al. (2014). A minus reverse transcription enzyme (minRT) control was included by pooling RNA from all larval samples, and all adult retina samples. In addition to a minRT control, a non-template control (NTC) was also included in the qPCR. No signals were detected in either control, indicating no genomic contamination. Primers used in qPCR for all visual opsins in cod, has previously been published by our group, along with qPCR reaction and cycling conditions (Valen et al., 2016).

Threshold value for qPCR was set manually to a fixed value for all samples, well above baseline fluorescence. Cycle threshold (Ct) values were efficiency corrected and normalized to an internal housekeeping gene; *ubuiqitin*, ranked as best out of three tested (*rpl4* and *ef1a*) by the NormFinder algorithm (MDL, 2004, Denmark (Andersen et al., 2004)). Relative expression of opsins (rather than proportional values) has been suggested to be the best choice for making conclusions concerning which opsins are differentially regulated (Fuller and Claricoates, 2011).

In order to visualize the spatial retinal pattern of opsin expressing photoreceptors in NEA cod, we also performed *in situ* hybridization studies in parallel to qPCR on a subset of eyes from the winter survey. The procedure in cod for analyzing visual opsin expression by sectional *in situ* hybridization, including synthesis of opsin specific probes, have previously been described by our group (Valen et al., 2014). Sections were mounted in 70% glycerol (Sigma, USA) in 1x PBS. Images were taken with a Leica 6000B microscope (Leica Microsystems, Germany), and contrast/brightness adjusted with Adobe Photoshop CS5 (2010, Adobe Systems Inc., USA).

Statistical analysis

All statistical analyses were performed in Statistica 12.0. (StatSoft, Inc., Round Rock USA). For the light exposure experiment, the total number of individuals (N) was for the following stages and treatment: 2 dph: N=20 (20x1 tank), 7 dph (LD/LL/B/G/R): N=30 (10x3tanks) per treatment, 12 dph: LD; N=10 (10x1 tank), LL; N=30 (10x3tanks), B; N=20 (10x2tanks), G; N=10 (10x1tank), R; N=20 (10x2 tanks). Data were tested for normality distribution and homogeneity of variance (see; (Valen et al., 2016)). Analysis of Variance (ANOVA) was used to determine differentially expressed genes between light treatments and stages (one-way: treatment), and main-effects ANOVA: treatment*stage). In case of significant ANOVA ($p<0.05$), Tukey-HSD post-hoc test, and Bonferroni test was used to identify significant differences. As not enough tank replicates were present for all data points (2- and 12 dph), the power of the ANOVA test was reduced, and results were thus interpreted with some caution. It should be emphasized that ANOVA interprets N=1 for 1 tank, that represents a pool of at least 10 larvae. For analysis of the effect of NC cod maturation including different retinal regions on differential expression of visual opsins, a main effects ANOVA was performed (gene*part of retina*gender). A similar analysis was performed on NEA cod, however including seasonal effects (gene*part of retina*season). All of the expressional data was tested for homogeneity of variances using Levene's test, and for normality distribution using Shapiro-Wilks test. In case of significant ANOVA ($p<0.05$), a Tukey HSD post hoc, and Bonferroni test

was followed up. See Table S1-S23 for more detailed information on the statistical analysis.

Results

Visual opsin expression in cod larvae exposed to different spectral light

To investigate the plasticity of the cod retina to various spectral lights during a phase of rapid eye growth, the regulation of visual opsin genes were assesed quantitatively by qPCR. The different spectral light treatments of larvae did not give any significant effect on cone opsin expression after 5 days (7 dph)- or after 10 days of treatment (12 dph) (Fig. 1). Furthermore most light regimes included variation in cone opsin expression, resulting from differences in average gene expression between tanks (Fig. 1, A-D). For blue-sensitive *sws2a* expression, no significant temporal changes were detected from 2 dph to 12 dph (Fig. 1, A). The *rh2a-1* expression increased from 2 dph (LD group) prior to light treatment, to 7- and 12 dph. This trend of increasing *rh2a-1* expression is seen in all light groups, however a significant increase ($p<0.05$) from 7- to 12 dph was seen in the red light treated larvae. *Rh2a-2* expression showed less change from the 2 dph to 12 dph stage, however a significant decrease in expression was found from 7- to 12 dph in the constant light (LL) group. The overall *rh2a-3* expression showed a slight decrease from 2 dph to 12 dph, and in similar to *rh2a-2* a significant decrease was detected from 7- to 12 dph in the LL group. For a clearer visulization of temporal changes of visual opsins within each light treatment, see Fig. S2. The *sws2b* expression was set to 0 in the current study, as mRNA levels were below the detectable range of qPCR.

Expression of visual opsins during maturation in NC cod

In order to unravel potential effects of maturation, including sex-related differences affecting opsin regulation, expression levels of all visual opsins were investigated by qPCR. By analyzing mRNA expression of visual opsins in 2 year maturing NC cod, the highest expressed gene was found to be *rh1* followed by *rh2a-1*, and the least expressed gene was *sws2a* (Fig. 2A). Expression levels of *rh2a-2*, *rh2a-3* and *sws2b*

were all below detectable levels. Our comparisons of visual opsin expression between female and male maturing cod, did not detect any significant differentially expressed opsins (Fig. 2B). However, the expression levels varied slightly more among male cod compared to females. By comparing opsin expression in dorsal and ventral retina, no topographic differences in opsin expression levels were found (Fig. 2C,D). Yet, this analysis revealed that the opsin expression variance observed in the male group could mainly be attributed to ventral retina (Fig. 2D). No such regional difference in visual opsin expression variance was observed for female fish (Fig. 2C).

Effect of population and season on visual opsin expression in Northeast Arctic (NEA) cod from the Barents Sea

As the overall expression pattern of visual opsins in NEA cod have remained unknown, and also to what extent extreme seasonal changes in available light may influence vision through opsin regulation, both quantitate and qualitative analysis were performed. The quantitative assessment of visual opsin mRNA expression by qPCR, revealed that the highest expressed gene in NEA cod was *rh1*, followed by *rh2a-1*, *rh2a-2*, *sws2a,* while the lowest expressed gene was *rh2a-3* opsin *(rh1* > *rh2a-1* > *rh2a2* / *sws2a* >*rh2a3*) (Figure 3A). The *sws2b* opsin was not expressed in high enough levels to be detected by qPCR. By comparing visual opsin expression in NEA cod sampled in February (winter) and September (early fall), we could not detect any seasonal effects on opsin gene expression level (Figure 3B). However, we did detect a regional difference in *rh2a-2* expression, where the ventral retina showed significantly higher expression ($p<0.05$), compared to the dorsal retina (Figure 3C, D). This regional difference was detected in NEA cod sampled both during winter and early fall. The spatial tissue expression patterns of opsins was investigated by in situ hybridization studies (Figure 4A-R), which altogether supported the quantitative estimations performed by qPCR. While *rh1*, *rh2-a1* and *sws2a* are expressed in all retinal regions (4P-R/A-D/J-L) the *rha-2* and *rh2a-3* expressing cones are mostly localized to ventral retina, to a lesser degree in dorsal retina, and almost absent in between (4D-I). Cones expressing *sws2b* could not be detected in any retinal regions (4M-O).

Discussion

To gain insight into the plasticity of the visual photoreceptive system in cod, the current study investigates activity of visual opsin genes in response to: 1) larval rearing under different spectral light (NC cod), 2) to maturation in NC cod and 3) to season in NEA cod. The resulting data suggests limited phenotypic plasticity of visual opsins to the analyzed conditions. These findings may suggest degree limited capacity of visual tuning to photic environmental changes, or during maturation. Surprisingly, our current study on NEA cod reveals a population difference in visual opsin usage compared to our previous studies in NC cod. To our knowledge, this is the first study to demonstrate plasticity in visual opsins linked to different cod population ecotypes. Each of the three conditions will be discussed separately.

Developmental plasticity and effect of different spectral light on opsin expression

Analysis of visual opsin expression in cod larvae reared under different spectral light, did not show any immediate response to light environment. Our data show that the overall temporal changes in opsin expression from 2-12 dph, correlate well with the pattern observed between 4- and 22 dph in a previous study (Valen et al., 2016), where the larvae was developed under broad spectrum light/dark (LD) conditions (Discussion Figure 1)(Karlsen et al., 2015). In both experiments we find upregulation of *rh2a-1* expression, concomitant with a decrease in *rh2a-2* and *rh2a-3* expression. The *sws2a* expression is less regulated, although a slight upregulation from 2- to 12 dph seemed to be present. In the current study, *sws2b* opsin levels proved too low for exact quantification, despite previous successful detection in cod larvae using similar experimental conditions (Valen et al., 2016). Whether this is caused by variation in larval rearing conditions or by intra-population differences is unknown.

The developmental stages of NC cod larvae used in the current study, have previously been shown to include large ontogenetic changes in green-sensitive *rh2a* cone opsin expression (Valen et al., 2016)(see Discussion Figure 1). This, combined with our current lack of response to different spectral light, along with temporal expression patterns, indicate that opsin usage is ontogenetically pre-programmed

during this phase of development. Consequently, ability of adaptive plasticity in cone opsins towards spectral environment is likely limited in larval cod.

Although different spectral light did not alter visual opsin expression under the given conditions, some significant temporal changes were seen in the continuous and red light groups. The more significant increase in *rh2a-1* in red light, and decrease in *rh2a-2* and *rh2a-3* in continuous light, suggest a possible difference in the timing of developmental changes in these light regimes. However as red light had no effect on *rh2a-2* and *rh2-a3*, or continuous light did not affect *rh2a-1,* inconsistency in light effect may indicate involvement of mechanisms other than light alone. Nevertheless, the overall temporal *rh2a-1* expression from 2- to 12 dph, indicate an expressional increase similar to that previously reported (Valen et al., 2016). The overall less apparent change of *rh2a-2* and *rh2a-3*, corresponds to previous findings, including a slight decrease towards the 12 dph stage (Discussion Figure 1). The overall similarities further suggest that opsins were unaffected by spectral and potential intensity differences in the currently used LED light, and previously used Tungsten Halogen light sources (Karlsen et al., 2015, Sierra-Flores et al., 2015, Valen et al., 2016).

The large variation within most treatments is most likely a result of differential larval growth and survival success related to the period after start of feeding (Puvanendran and Brown, 1999). Hence, the slight variation in temporal opsin profiles among light groups may thus represent more and less developed larva. It is likely that a constant light environment allows more hours for visual feeding and as a consequence may increase growth as previously suggested in cod (Puvanendran and Brown, 2002). A recent study showed improved growth and survival of cod larvae reared in blue/green light, compared to red-light (Sierra-Flores et al., 2015). However, effects of various light on growth, was most obvious at 60 dph, indicating more prominent long term effects (Sierra-Flores et al., 2015). The improved performance in these lights correlates well with cod larvae being naturally adapted to blue-green dominated light in the marine environment. It is intriguing to speculate whether the poor performance in red light is related to the genomic loss of LWS cones (Valen et al., 2014).

Role of ontogeny versus environment on opsin plasticity

The lack of rapid light induced effects on cone opsins, combined with dynamic changes during development (Valen et al., 2016), suggest that opsin changes in cod are pre-programmed developmental events. The lower degree of opsin plasticity towards environment may be linked to the continued postembryonic retinal development, characteristic of indirect developing species (reviewed by Evans and Browman (2004)). Typical for indirect developing fish, is a prolonged larval stage with undeveloped pure-cone retina (Evans and Fernald, 1990), which also is the case in cod (Valen et al., 2016). There are examples of changes in cone sensitivity during earlier life stages of other indirect developing fish (Archer et al., 1995, Cheng and Flamarique, 2007, Helvik et al., 2001, Shand et al., 2002, Shand et al., 1988). Yet, these changes have in most cases been attributed to ontogeny, and fewer studies have elucidated the role of light environment independent of ontogeny.

A somewhat exception to this, is studies in black bream, which also has an early pelagic pure-cone larvae that later acquires rods (indirect eye development) (Blaxter and Staines, 1970, Shand et al., 2002). In black bream, cone opsin expression changes both during development, and in response to rearing light environment (Evans and Fernald, 1990, Shand et al., 2008). These observations thus suggest that visual opsin gene activity can be regulated during periods of rapid transformation and eye growth, and according to light environment. In contrast to cod, the more direct developing Bluefin killifish showed rapid light-induced responses in all cone opsins (SWS1, SWS2, RH2 and LWS) (Fuller and Claricoates, 2011, Fuller et al., 2010). Interestingly, it was also found that light condition experienced during development had larger effects on visual behavior (opsins) than immediate light treatments, indicating long lasting developmental plasticity (Fuller et al., 2010). Hence, the studies mentioned above suggest that environmental long-term effects on opsins may occur. Thus we cannot exclude that this may also be the case in cod, however this requires longer time studies.

Furthermore, in contrast to the aforementioned species, cod have lost opsins sensitive to UV and red light, which may genetically restrict the potential of plasticity to various light input. In both killifish and black bream, more natural light situations were mimicked by light treatment, and show that these changes in light are sufficient

to change opsin expression (Fuller et al., 2010, Shand et al., 2008). It is also likely that these species naturally experience larger variation in spectral light than cod, and in combination with more available visual opsins have a greater in-built potential to change. Although, we have used narrower bandwidth light that represent a more extreme situation and perhaps less natural, we hypothesize that visual opsins would be able to change if the ability for adaptive plasticity was present. In addition, we cannot exclude a missed developmental "window-of-opportunity", prior either to sampling or after, along with undetected opsin changes. Altogether, both current and previous data suggest that variation in plasticity towards environment varies among fish, which may, or may not be influenced by life strategy.

In nature, cod embryos and larvae are found in the upper epipelagic with multi-spectral light (Tupper and Boutilier, 1995). Yet, variation in plankton, particulate matter and sediments, changes spectral properties and consequently differs in the selective pressure put on visual adaptation (Partridge and Cummings, 1999). Furthermore, due to life strategy; prey detection and larval growth are critical for increasing survival chances (Meekan and Fortier, 1996). Thus, having a pre-programmed larval vision, may be speculated as a successful adaption towards a variable photic environment. Furthermore, cod seasonal spawning is closely tied to yearly algal- and plankton blooms, which improves larval survival success (Kristiansen et al., 2011). Consequently, having a more constant predictable visual program using all cone opsins present (Valen et al., 2014, Valen et al., 2016), may have proved a successful adaptation reflecting life strategy and ecology.

Visual opsin expression during maturation

Our data on visual opsin expression in maturing 2 year old NC cod shows that the most expressed visual opsin is *rh1*, followed by *rh2a-1* and *sws2a* opsin. This profile is similar to our previous observations for late juvenile NC cod (Valen et al., 2016). These data thus suggest that the adult visual program is established in the juvenile cod and maintained through maturation. Analysis of potential sex differences in visual opsins showed no significant differences between males and females, indicating that opsin expression is not used to tune differential sensitivity during maturation in cod. The male cod did however show higher variation in all opsins compared to females, yet whether this is sex dependent or caused by natural

variation among samples, is not known. However, by comparing male opsin expression in dorsal retina with ventral retina, there is clearly most expressional variation in the ventral region. These data could suggest topographic differences in opsin expression in some males, however this remains speculative at the time. No regional differences in opsin expression were detected in the female group. Altogether, our data suggest that the visual system of males and females are similar, and does not change during maturation. Yet, we cannot exclude a potential tuning of vision during spawning in the spring.

Common for many fishes displaying ontogenetic plasticity of vision, is plasticity of SWS1 and LWS cones sensitive to the most extreme parts of the visible spectrum; UV and red, respectively (Allison et al., 2006, Shao et al., 2014). In fish, examples of both UV and red sensitivity changes during maturation have been reported (Allison et al., 2006, Shao et al., 2014). The *lws* opsin is actively used by Lake Victoria cichlids in response to water depth, coloration and preferences, and is suggested to even mediate speciation through sensory drive (Seehausen et al., 2008, Terai et al., 2002). In guppies *lws* opsin is upregulated in the transition from juvenile to adult (Laver and Taylor, 2011). The female mating preference of male coloration has been hypothesized to favor males that contrast with their visual background (Boughman, 2001, Gray et al., 2008). It is tempting to speculate whether LWS opsin has a special function towards mate selection, and if present, makes it more likely that visual clues are central in courtship behaviors. If this may be the case, the loss of UV and LWS opsins in cod, combined with lack of differential opsin regulation during maturation, may be linked to the lack of sex-differential coloration of cod in general.

Localization and attraction of partners may also be mediated via other sensory systems, such as the olfactory and the auditory system (Andersson, 1994). The natural mating behavior of cod is less known, however studies have shown the involvement of male-male competition, including both acoustic and visual signal display (Bekkevold et al., 2002, Brawn, 1961, Engen and Folstad, 1999, Hutchings et al., 1999, Rowe et al., 2007, Skjaeraasen et al., 2010). Apart from males displaying fin size movement during courtships, no visual color clues are known present (Brawn, 1961). Conclusively, our data combined with previous studies suggest that visual system tuning by opsins may not be a key process involved in mate choice. Likely, the well-documented studies of cod mating calls may represent a more

central mating signal (Engen and Folstad, 1999, Nordeide and Kjellsby, 1999, Rowe and Hutchings, 2006).

A comparison between NC cod and NEA cod reveals population variation in visual opsin usage

The current expression profile of visual opsins in NEA cod reveals that all opsins except *sws2b* are expressed within a quantifiable range, which is supported by qualitative analysis of tissue expression patterns. In contrast to late juvenile and maturing NC cod where retinal *rh2a-2* and *rh2a-3* expression is switched off, late juvenile NEA cod chooses to express all three green-sensitive *rh2* opsins. These data thus indicate population differences in the complement of visual opsins used, and thus suggest population specific visual programs in cod. To the authors' knowledge, this is the first report that documents a difference within the visual system between cod ecotypes resulting from differential opsin usage.

Due to history of selection in different habitats, different lighting environments can lead to genetic variations in sensory-system properties among fish populations (Endler et al., 2001, Fuller et al., 2005, Fuller et al., 2010). Although a number of studies have examined opsin sequence variations related to spectral sensitivity, the extent to which these arise due to variable light environment is less clear (Fuller et al., 2010, Osorio and Vorobyev, 2008, Seehausen et al., 2008). The persistence of Atlantic cod populations through a history of extreme environmental variation, including sea ice, has been suggested to be a result of considerable inherent resilience (Bigg et al., 2008). Furthermore, evolutionary selection of genetic differences in opsins requires a long period of time, and may only cause subtle changes in sensitivity (Hofmann and Carleton, 2009). In contrast, a much more dramatic and rapid mode of changing sensitivity is by differential opsin regulation (Hofmann and Carleton, 2009). Hence, differential *rh2* opsin plasticity may represent local adaptation of vision to different environments among cod. No population genetic differences within any of the cone opsins represented in the current study, have previously been associated with cod ecotypes.

In the initial survey of cod population differences, we used a qPCR assay, and *in situ* probes designed for NC cod. Although primers were placed within less conserved opsin regions, the assay works well on NEA cod, indicating highly similar opsin

genes. Genetic differences between Migratory and stationary cod ecotypes can be differentiated based on variation at the polymorphic pantophysin locus (Pan I), and multiple other genomic regions mostly in linkage group 1 (LG1), through single nucleotide polymorphism (SNP) analysis (Berg et al., 2016, Godø and Michalsen, 2000, Hemmer-Hansen et al., 2013, Karlsen et al., 2013, Kirubakaran et al., 2016, Nordeide and Båmstedt, 1998, Sarvas and Fevolden, 2005). The recent identification of polymorphic differences associated with rhodopsin, suggest genetic differences in visual opsins between stationary and migratory Icelandic cod populations (Pampoulie et al., 2015). However, as these SNPs are not associated with previously reported functional phenotypes (Nakamura et al., 2013), and spectral analysis has not been performed, the functional significance remains uncertain. In our current study, we did not find any significant differences in rhodopsin (*rh1*) expression, when comparing juvenile NC cod with juvenile NEA cod. Still we cannot exclude that such differences may exist, either spectrally or undetected expressional variation.

Interestingly, these data also indicate different levels and possibly combinations of opsin regulatory networks in cod populations. Variations within the *rx1* gene among closely related cichlid species have recently been shown to differentially regulate *sws2a* expression (Schulte et al., 2014). The same study showed that an ancestral polymorphism influenced *rx1* expression levels. Future studies targeting opsin regulatory factors and associated population polymorphisms will be highly relevant to test visual adaption in cod ecotypes. Whether expressing three *rh2* opsins in later life stages of NEA cod is a result of dynamic environmental adaption, and/or functionally improves resolution towards green light, is currently unknown.

Sampled cod material and developmental stage; effect of size and age at maturation

Our opsin expression data on NEA cod was obtained by cod sampled wild in the Barents Sea. On the other hand, both mature NC cod, and the late juveniles of our previous study were raised in captivity (Karlsen et al., 2015, Valen et al., 2016). It has been shown for both populations that age at maturation decrease in captivity, compared to wild conditions (Godø and Moksness, 1987). Furthermore, NEA cod may use around 6-9 years to reach maturation, while NC cod use around 2-4 years (Ajiad et al., 1999, Godø and Moksness, 1987, Svåsand et al., 1996). As we do not

have information concerning gonadal features on NEA cod, we do not know for certain whether the NEA cod have reached the first maturation. However, previous studies report first-time spawning NEA cod from 60 cm and larger, more than triple the length of our averaged length of ~20 cm (Ajiad et al., 1999, Bergstad et al., 1987).Thus the cod used in the current study are most likely late juveniles. Previously we showed that NC cod expresses all visual opsins during the larval stage, while *rh2a-2* and *rh2-3* expression is almost completely lost in the three month juvenile cod (5 cm standard length/SL) (Valen et al., 2016). Hence, we hypothesize that the observed differences is a consequence of population differences, and find it unlikely that the NEA cod opsin pattern is caused by an earlier developmental stage.

Limited visual opsin plasticity to seasonal change in NEA cod

Our overall comparisons on opsin expression between February and early September in the Barents Sea, did not show any significant differences despite extreme seasonal variation in available light. These data thus suggest that visual tuning by opsin plasticity is minimal in cod north of the Arctic Circle despite experiencing dark period. Yet we cannot exclude alternative tuning by chromophore switch that has been shown to vary depending on season in fish (Temple et al., 2006, Ueno et al., 2005). However, most marine fishes display only vitamin A1, and the A1-A2 switch is typically associated with fish migrating between fresh- and seawater (Toyama et al., 2008). In general, very little is known concerning seasonal adaptions in vision of fishes inhabiting areas at high latitudes with large fluctuations in photoperiod. Studies of Antarctic notothenoid fishes initially suggested that LWS opsins were lost in these species, yet subsequent studies detected LWS in some fish (Miyazaki and Iwami, 2012, Pointer et al., 2005). This suggests that *lws* loss is not a common feature at high latitudes.

By comparing regional expression of visual opsins in NEA cod, we found that *rh2a-2* opsin was higher expressed in ventral retina than in dorsal retina, indicating topographic differences in opsin expression. These differences were observed both in material sampled during February and September, indicating regionalization independent of season. In our previous developmental studies on NC cod opsins, we detected more cones expressing *rh2a-2* and *rh2a-3* in ventral retina during metamorphosis (Valen et al., 2016). Thus, the detection of higher levels of *rh2a-2*

opsin in ventral retina of NEA cod, have similarities to our previous findings in transforming juvenile NC cod. Future studies on older and mature NEA cod, will be needed to elucidate whether *rh2a-2* and *rh2a-3* expression is sustained. Still, immature NEA cod does not express sws2b, which is similar to late juvenile NC cod (Valen et al., 2016)., indicating a population difference in regulation of the green opsin locus, while the blue opsins seems to be under similar regulation. It is also possible that differences between coastal and oceanic photic environments within the green part of the light spectra influence the expression of *rh2* genes differently as a visual adaptive mechanism.

Conclusions

Our initial investigations on cod visual opsin expression towards environmental light and to maturation indicate limited plasticity of tuning in cod. The developmental program of visual opsins in larval cod appears to be robust towards immediate photic changes, yet does not exclude long-term effects. The lack of changes in visual opsins during maturation suggest that differential tuning of blue- and green-sensitive opsins play a less important role during cod mating behavior. Interestingly, *rh2* opsins are differently expressed in NEA cod compared to NC cod, indicating phenotypic plasticity in visual systems related to cod ecotypes. Whether this relates to adaptations to different habitats, or to differences in gene regulation, is still unclear. Furthermore, the spatial similarities in cone opsin expression in NEA cod and earlier stages of NC cod, may suggest shared aspects of opsin regulation. However, which factors are involved and how these operate in concert are currently unknown. Our initial survey on visual opsins in NEA cod does not indicate any major visual adaptation to season, despite the extreme variation in available light. The lack of *sws2* and *rh2* opsin plasticity towards different spectral light, maturation and season, suggest that developmental programs of vision prevail in cod. It is intriguing to speculative whether the lack of plasticity may be a consequence of evolutionary genomic loss of UV- and red-sensitive cone opsin genes. Cod may thus have less inherent genomic potential for tuning vision to different spectral light. Although this study have focused on a subset of factors, the method and approach have provided novel knowledge of visual system dynamics with implications extending beyond Atlantic cod.

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Competing interests

No competing interests declared.

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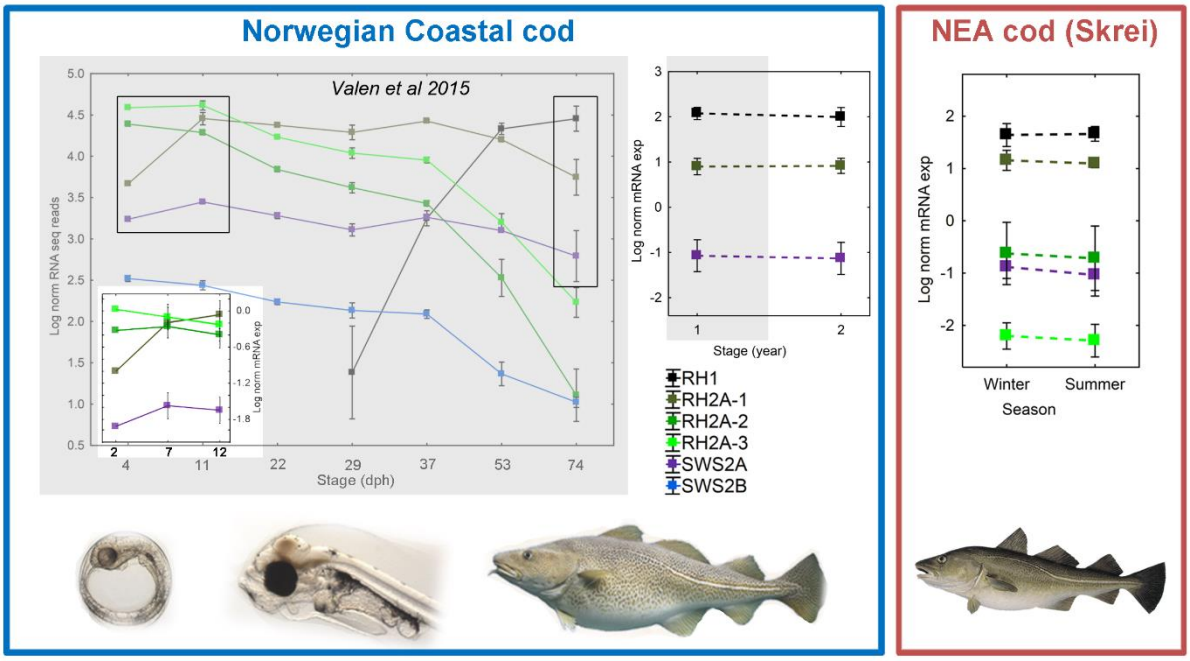
Figures



Discussion Figure 1. Visual opsins in Norwegian Coastal cod (blue frame) and plasticity related to different light and maturation. Grey areas represent previous published results on visual opsins using RNA-Seq and qPCR during development (Valen et al., 2015). Areas in grey marked with black boxes highlights developmental periods where relative opsin profile correlate with current detected profiles marked by white in graphs. A summary of all data representing the overall temporal expression is shown in white covering 2-12 dph (left corner) and of males and females (right hand side). A comparison between current findings and previous studies, suggest similar patterns that indicate less degree of plasticity towards environmental light, or to maturation. See Fig. S2 for detailed information on temporal patterns of visual opsins in response to separate light treatments. The red box shows visual opsin expression in immature Northeast Arctic cod (NEA cod) during winter and early fall. Photo credit adult Norwegian Coastal cod; Joachim S. Müller, and NEA cod; Institute of Marine Research, Bergen, Norway.

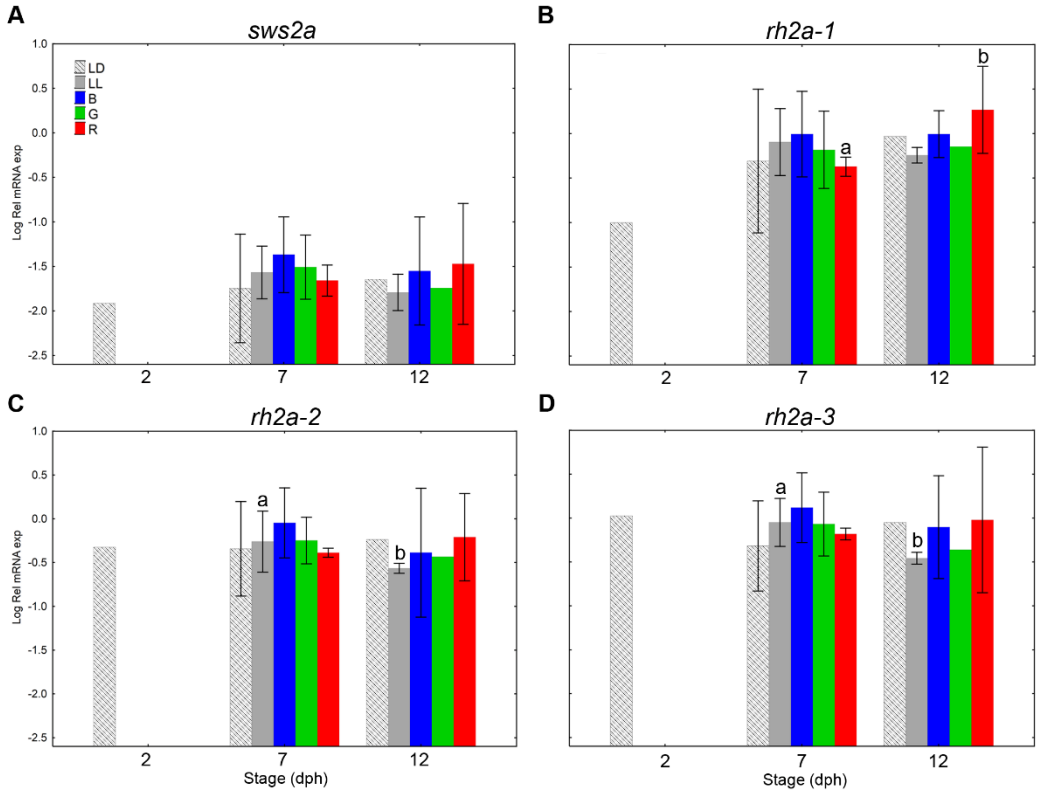


Figure 1. Different spectral light treatment of cod larvae and effect on cone opsin expression. Cone opsin mRNA expression levels were measured by quantitative real-time PCR (qPCR) using SYBR green assay and opsin specific primers. The qPCR cycle threshold (ct) values were efficiency corrected and normalized to an internal housekeeping gene; *ubiquitin*, then plotted as log transformed values (y-axis). To gain a complete picture of potential spectral effects on opsin gene regulation, opsin expression is presented for all cone opsins except *sws2b*, which showed too low expression to be exactly quantified. The 2 days post hatching (dph) stage represent opsin expression in a pool of 20 larvae from a common tank at start feeding, and just prior to light treatment. The 7- and 12 dph stage represent 5 and 10 days of light treatments (x-axis). The LD group represents white light day/night rhythm (see Material and methods section), while LL = constant white light day/night, BD = blue light, GD = green light, and RD = red light. Data are presented as average opsin expression ±SD of a pool of ~10 larvae in 3 tanks for 5 days of light treatment. Due to larval mortality, 10 days of light treatment included N

larvae: LD; N=10 (10x1 tank), LL; N=30 (10x3tanks), B; N=20 (10x2tanks), G; N=10 (10x1tank), R; N=20 (10x2 tanks). Different letters note statistically different expression ($p<0.05$) between stages within a light treatment group, using a main-effects ANOVA (treatment*stage), followed by a Tukey-HSD post-hoc test.

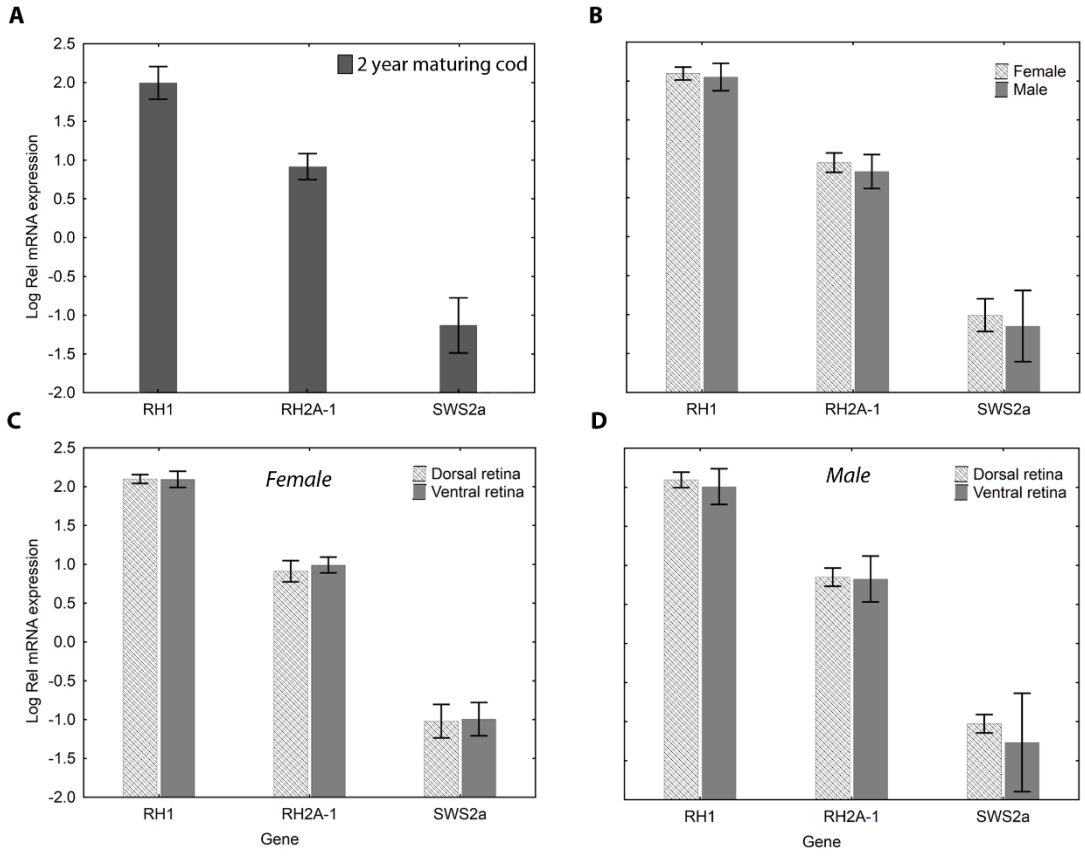


Figure 2. Visual opsin expression during maturation of male and female Atlantic cod. Visual opsin mRNA expression was measured by qPCR on 2-year maturing cod to unravel potential opsin regulation during maturation. Expression levels were plotted as efficiency corrected, relative (housekeeping gene: *ubiquitin*) and log-transformed values (y-axis) for *rh1*, *rh2a-1* and *sws2a* (x-axis). A shows average visual opsin expression for all maturing fish analyzed, while B shows expression values seperated for different sexes. In C and D, regional visual opsin expression is presented for dorsal and ventral retina, in female and male cod, respectively. Data is presented for N=6 females, and N=5 males (total N=11) as mean expression ±SD, and statistical analysis using main effects ANOVA did not reveal any significant effects of sexes, or retinal regions.

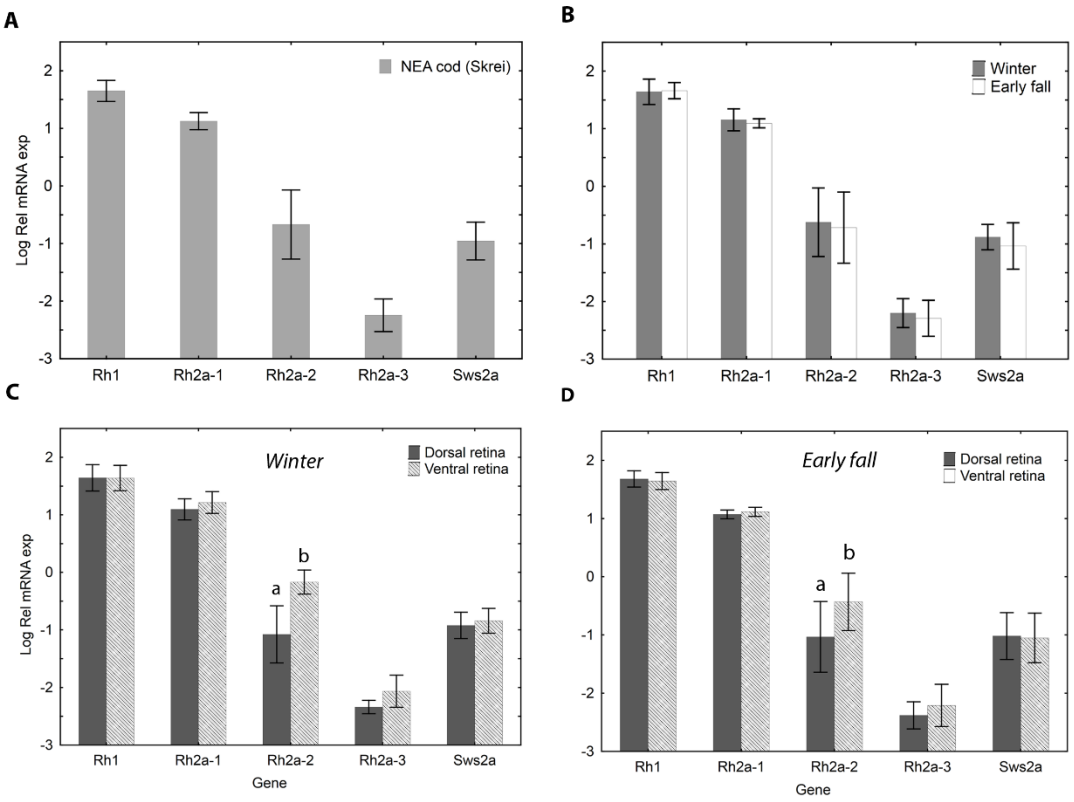


Figure 3. Visual opsin expression in Northeast Arctic (NEA) cod. To obtain an overview of opsin expression and potential seasonal regulation in NEA cod, opsin expression levels were quantified by qPCR from eye samples collected during winter and early fall in the Barents Sea, (A). The bars show efficiency corrected mRNA expression levels, and log transformed values (y-axis) for all visual opsins, except *sws2b* that was not detected by qPCR. B shows seasonal expression profiles of visual opsins, while C and D show regional dorsal/ventral expression during winter and early fall, respectively. Different letters in C and D show significant ($p<0.05$) differentially expressed genes between retinal regions using main effects ANOVA. Expression values are represented as mean ±SD for N=10 fish sampled during winter and during early fall (total N=20).

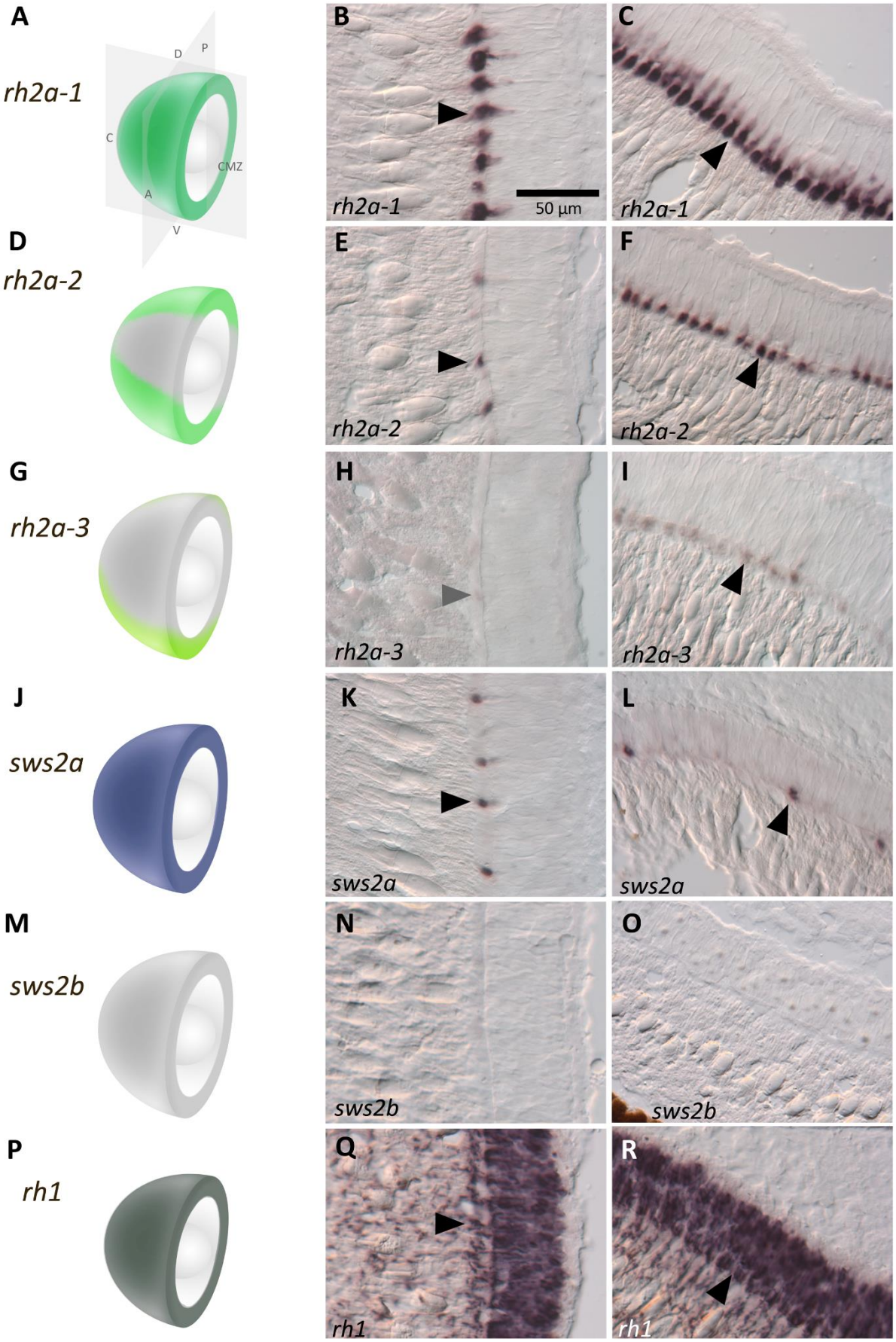
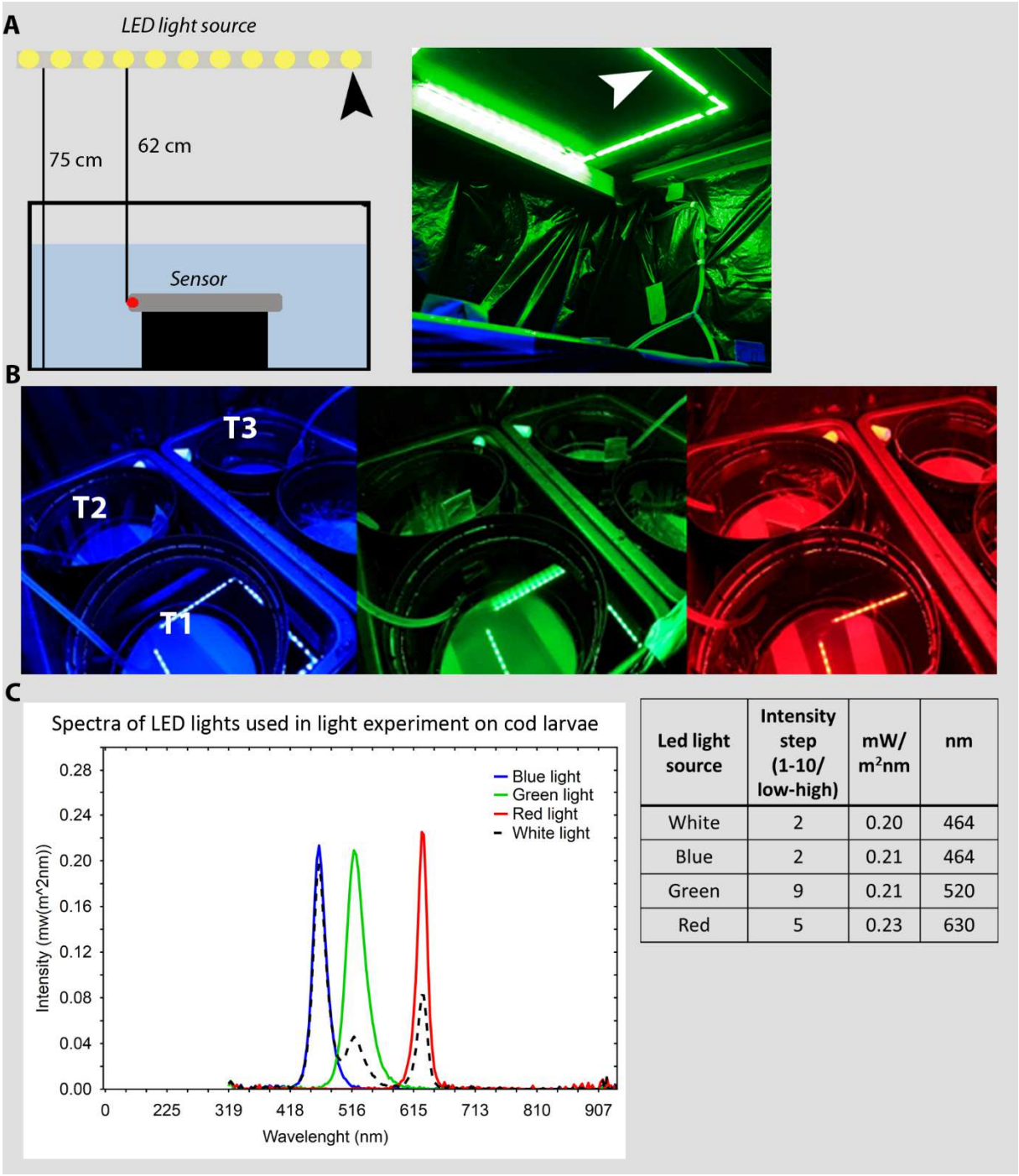


Figure 4. Retinal spatial expression patterns of visual opsins in North East Arctic (NEA) cod. Left hand side: A schematic summary of visual opsin spatial mRNA expression in the NEA cod retina based on the *in situ* hybridization technique using opsin specific DIG-labelled probes. Axis of orientation is illustrated in A, where: D=dorsal, V=ventral, A=anterior, P=posterior, C=central retina, and CMZ=circumferential marginal zone. A, D and G illustrates green-sensitive *rh2a* expression, while J and M summarize blue-sensitive *sws2*, and P illustrate *rh1* expression. Retinal tissue expression of *rh2* opsins are shown in the central retina (B, E, H), and in the ventral retina in proximity to CMZ (C, F, I). While *rh2a-1* is expressed in cones all through retina (A, B, C), *rh2a-2* and *rh2a-3* are predominantly expressed in ventral retina, then in varying degree detected in dorsal retina (D-I). Cones expressing *sws2a* were found in all retinal regions (J), and tissue expression is shown in central (K) and ventral retina (L). No cones expressing *sw2b* could be detected in NEA cod retina (M, N, O). Rods expressing rhodopsin (*rh1)* were present in all retinal regions (P, Q, R). Black arrows indicate cones and rods expressing the respective opsin, while the grey arrow in H indicate possible weak *rh2-3* expression in central retina. Scale bar (50 µm) shown in B is the same for all images.

Supplemental material



Led light source	Intensity step (1-10/ low-high)	mW/ m^2nm	nm
White	2	0.20	464
Blue	2	0.21	464
Green	9	0.21	520
Red	5	0.23	630

Figure S1. Experimental setup light treatments cod larvae. A) Left handside illustrates the position at which the optical recordings were obtained within the fish tanks, and a photo of the LED light source is shown on the right hand side. B) The photos show the setup of the larval rearing tanks under different wavelenght lights of blue, green and red, respectively. C) Optical measurements were plotted for all LED lights used (left hand side), where the y-axis shows LED intensity measured in mW

m^{-2} nm^{-1} over nanometer wavelenght distribution (x-axis). The table on the right hand side shows specter data values for the used LED lights, where the chosen intensity step was normalized across the different light sources.

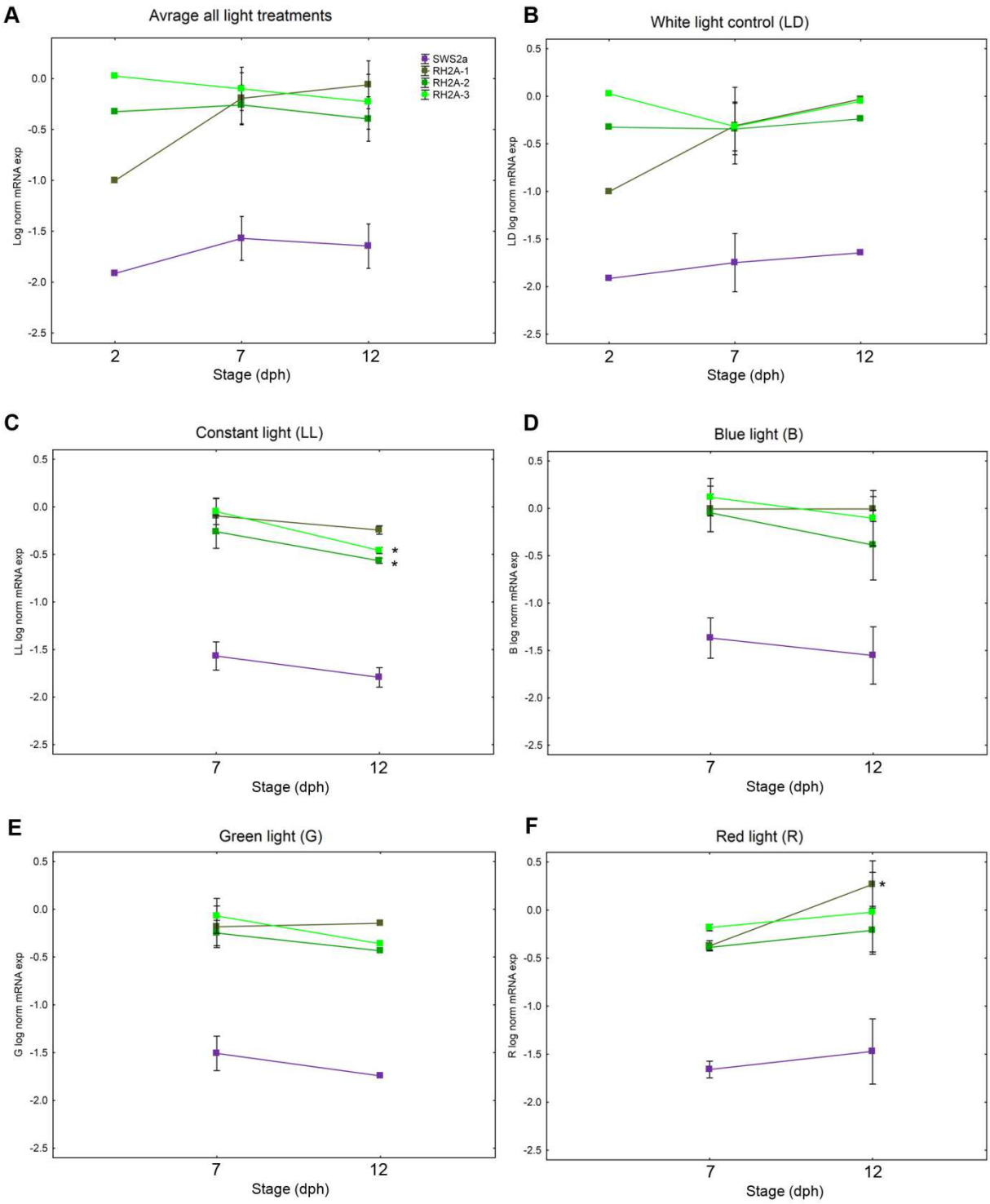


Figure S2. Temporal expression of cone opsins during light treatments (A-F). The different opsins are represented by different colors shown in A. Expression values marked with (*) in C and F represents significantly different ($p<0.05$) expression between 7- and 12 dph, using a one-way ANOVA.

Supplemental material: Statistical analyses

Light experiment NC cod larvae

Table S1. Normality test light experiment. *Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins in all stages and treatments. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 (p<0.05), indicating rejection of the normal distribution hypothesis. Missing cells (-) represent stages where N is too low and test could not be performed.*

Stage (dph)	Treatment	SWS2A	RH2A-1	RH2A-2	RH2A-3
7	LD	W = 0.815	W = 0.943	W = 0.936	W = 0.937
12	LD	W = -	W = -	W = -	W = -
7	LL	W = 0.889	W =0.942	W =0.938	W =0.827
12	LL	W = 0.886	W = 0.875	W = 0.967	W = 0.823
7	B	W = 0.992	W = 0.948	W = 0.970	W = 0.991
12	B	W = -	W = -	W = -	W = -
7	G	W = 0.899	W = 0.876	W =0.999	W =0.999
12	G	W = -	W = -	W = -	W = -
7	R	W =0.958	W =0.991	W = 0.882	W = 1.000
12	R	W = -	W = -	W = -	W = -

Table S2. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Stage (dph)	Treatment	SWS2A	RH2A-1	RH2A-2	RH2A-3
7-12	LD	P = 0.248	P = 0.354	P = 0.344	P = 0.349
7-12	LL	P = 0.421	P = 0.096	P = 0.064	P = **0.048**
7-12	B	P = 0.483	P = 0.356	P = 0.195	P = 0.439
7-12	G	P = 0.230	P = 0.215	P = 0.402	P = 0.405
7-12	R	**P = 0.009**	**P = 0.007**	**P < 0.001**	**P < 0.000**

Table S3. Two-way ANOVA light treatment; stage*treatment.

Effect	Analysis of Variance. Marked effects are significant at p < .050				
	Value	F	Effect df	Error df	p
Intercept	0.005	780.006	4	15.000	0.000
Stage (dpf)	0.121	7.029	8	30.000	0.000
Treatment	0.423	0.944	16	46.463	0.528

Table S5. Tukey HSD post hoc for stage effect *sws2a*.

Cell No.	Tukey HSD test; variable **Log Relative SWS2a express**. Approximate Probabilities for Post Hoc Tests Error: Between MS = .04453, df = 18.000			
	Stage (dph)	{1} -1.915	{2} -1.570	{3} -1.646
1	2		0.2780	0.464
2	7	0.280		0.677
3	12	0.464	0.677	

Table S6.1. Tukey HSD post hoc for stage effect *rh2a-1*.

Cell No.	Tukey HSD test; variable **Log Relative RH2-1 express.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .06455, df = 18.000			
	Stage (dph)	{1} -1.002	{2} -.1932	{3} -.0430
1	2		0.017	0.006
2	7	0.017		0.361
3	12	0.007	0.3617	

Table S6.2. Bonferroni post hoc for stage effect *rh2a-1*.

Cell No.	Bonferroni test; variable **Log Relative RH2-1 express.** Probabilities for Post Hoc Tests Error: Between MSE = .06712, df = 17.000			
	Stage (dph)	{1} -1.002	{2} -.1932	{3} -.0601
1	2		0.023	0.010
2	7	0.023		0.771
3	12	0.010	0.771	

Table S7. Tukey HSD post hoc for stage effect *rh2a-2*.

Cell No.	Tukey HSD test; variable **Log Relative RH2-2 express.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .04333, df = 18.000			
	Stage (dph)	{1} -.3244	{2} -.2580	{3} -.3966
1	2		0.949	0.942
2	7	0.949		0.280
3	12	0.942	0.280	

Table S8. Tukey HSD post hoc for stage effect *rh2a-3*.

Cell No.	Tukey HSD test; variable **Log Relative RH2-3 express.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .05228, df = 18.000			
	Stage (dph)	{1} .02566	{2} -.1001	{3} -.2266
1	2		0.860	0.558
2	7	0.860		0.407
3	12	0.558	0.407	

Table S9. One-way ANOVA light treatment for stage effect.

Effect	Analysis of Variance. Marked effects are significant at p < .050				
	Value	F	Effect df	Error df	p
Intercept	0.005	921.301	4	19	0.00
Stage (dph)	0.106	9.824	8	38	0.000

Significant (p<0.05) Tukey HSD post hoc test for stage effect within treatment

Table S10.1 Tukey HSD post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Tukey HSD test; Variable: **Log Relative RH2-2 express**. Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.2613	{3} M=-.5667
2 {1}			
7 {2}			0.040
12 {3}		0.040	

Table S10.2 Bonferroni post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Bonferroni test; Variable: **Log Relative RH2-2 express**. Marked differences are significant at p < .050. Error: Between MS = ,01562, df = 4,0000		
	{1} M=0.0000	{2} M=-.2613	{3} M=-.5667
2 {1}			
7 {2}			0.040
12 {3}		0.040	

Table S11.1 Tukey HSD post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Tukey HSD test; Variable: **Log Relative RH2-3 express**. Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.0496	{3} M=-.4579
2 {1}			
7 {2}			0.008
12 {3}		0.008	

Table S11.2 Bonferroni post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Bonferroni test; Variable: **Log Relative RH2-3 express**. Marked differences are significant at p < .050. Error: Between MS = ,00992, df = 4,0000		
	{1} M=0.0000	{2} M=-.0496	{3} M=-.4579
2 {1}			
7 {2}			0.008
12 {3}		0.008	

Table S12.1 Tukey HSD post hoc for stage effect within treatment R.

Stage (dph)	**Treatment=R** Tukey HSD test; Variable: **Log Relative RH2-1 express.** Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.3723	{3} M=.26714
2 {1}			
7 {2}			0.018
12 {3}		0.018	

Table S12.2 Bonferroni post hoc for stage effect within treatment R.

Stage (dph)	**Treatment=R** Bonferroni test; Variable: **Log Relative RH2-1 express.** Marked differences are significant at p < .050. Error: Between MS = ,02186, df = 3,0000		
	{1} M=0.0000	{2} M=-.3723	{3} M=.26714
2 {1}			
7 {2}			0.018
12 {3}		0.018	

Maturing NC cod

Table S13. Normality maturing cod. *Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 (p<0.05), indicating rejection of the normal distribution hypothesis.*

Gene	Female	Male
RH1	**W = 0.841**	**W = 0.756**
Rh2a-1	W = 0.903	**W = 0.751**
Sws2a	W = 0.924	**W = 0.565**

Table S14. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Variable Female-Male	Levene Test of Homogeneity of Variances. Marked effects are significant at p < .05							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Rh1 Log Norm mRNA exp	0.030	2	0.015	0.254	25	0.010	1.497	0.243
Sws2a Log Norm mRNA exp	0.052	2	0.026	1.518	25	0.061	0.431	0.654
Rh2a-1 Log Norm mRNA exp	0.033	2	0.016	0.295	25	0.012	1.387	0.268

Table S15. Main effects ANOVA Gene*Part-of-retina*Gender. Significant effect ($p<0.05$)

Effect	Univariate Tests of Significance for Log norm mRNA exp. Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	10.903	1	10.903	279.150	0.000
Gene	110.587	2	55.294	1415.652	0.000
Part of retina	0.009	1	0.009	0.240	0.626
Gender	0.128	1	0.128	3.273	0.076
Gene:*Part of retina	0.050	2	0.025	0.636	0.533
Gene:*Gender	0.027	2	0.013	0.344	0.710
Part of retina*Gender	0.048	1	0.047	1.215	0.275
Gene:*Part of retina*Gender	0.027	2	0.013	0.344	0.710
Error	2.109	54	0.039		

Table S16.1. Tukey HSD post hoc for gene effect.

Cell No.	Tukey HSD test; variable Log norm mRNA exp. Approximate Probabilities for Post Hoc Tests Error: Between MS = .03698, df = 61.000			
	Gene:	{1} 1.8530	{2} -1.293	{3} .67694
1	RH1		P << 0.001	P << 0.001
2	SWS2A	P << 0.001		P << 0.001
3	RH2A-1	P << 0.001	P << 0.001	

Table S16.2. Bonferroni post hoc for gene effect.

Cell No.	Bonferroni test; variable Log norm mRNA exp. Probabilities for Post Hoc Tests Error: Between MSE = ,06607, df = 81,000			
	Gene:	{1} 1.9954	{2} -1.132	{3} .9150
1	RH1		P << 0.001	P << 0.001
2	SWS2A	P << 0.001		P << 0.001
3	RH2A-1	P << 0.001	P << 0.001	

Northeast Arctic cod (NEA cod)

Table S17. Test of normality distribution Northeast Arctic cod. *Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 (p<0.05), indicating rejection of the normal distribution hypothesis.*

Season	Part of retina	RH1	SWS2A	RH2A-1	RH2A-2	RH2A-3
Summer	Dorsal	W = 0.902	W = 0.883	W = 0.926	W = 0.955	W = 0.923
Summer	Ventral	W = 0.945	W = 0.856	W = 0.934	W = 0.954	W = 0.929
Winter	Dorsal	W = 0.946	W = 0.930	**W =0.808**	W = 0.959	W = 0.951
Wintre	Ventral	W = 0.953	**W = 0.731**	**W = 0.893**	W = 0.943	W = 0.929

Table S18. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Gene	Levene's Test for Homogeneity of Variances: Effect: **Season** Degrees of freedom for all F's: 1, 138			
	MS Effect	MS Error	F	p
RH1	0.000	0.025	0.012	0.915
Sws2a	0.007	0.049	0.145	0.704
Rh2a-1	0.230	0.021	18.7	**0.000**
Rh2a-2	0.001	0.10	0.014	0.907
Rh2a-3	0.037	0.030	1.221	0.276

Table S19. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Season	All Groups Levene's Test for Homogeneity of Variances: **Effect: "Part of retina" by gene** Degrees of freedom for all F's: 1, 131			
	MS Effect	MS Error	F	p
Summer Log Norm. mRNA exp (ubiq)	0.247	0.368	0.672	0.414

Table S20. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Season	All Groups Levene's Test for Homogeneity of Variances: Effect: **"Part of retina" by gene** Degrees of freedom for all F's: 1, 138			
	MS Effect	MS Error	F	p
Winter log norm mRNA exp	0.675	0.387	1.742	0.189

Table S21. Main effects ANOVA Gene*Part-of-retina*Season. Significant effect ($p<0.05$)

Effect	Univariate Tests of Significance for Log Norm. mRNA exp. Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	123.368	1	123.368	1268.362	0.000
Gene:	693.768	5	138.754	1426.542	0.000
Part of retina	2.228	1	2.228	22.910	0.000
Season	0.343	1	0.343	3.529	0.062
Gene:*Part of retina	3.990	5	0.798	8.203	0.000
Gene:*Season	0.168	5	0.034	0.344	0.885
Part of retina*Season	0.187	1	0.187	1.925	0.167
Gene:*Part of retina*Season	0.121	5	0.024	0.248	0.940
Error	20.426	210	0.097		

Table S22.1. Tukey HSD post hoc for Gene*Part of retina Winter.

Cell No.	Tukey HSD test; **variable log norm mRNA Winter.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .07208, df = 108.00													
	gene3	Part of retina3	{1} 1.6424	{2} 1.6384	{3} -.9213	{4} -.8416	{5} -3.287	{6} -3.152	{7} 1.0950	{8} 1.2138	{9} -1.077	{10} -.1692	{11} -2.339	{12} -2.064
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.978	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.716	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.993	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.993		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.998	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.998		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	0.978	0.716	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P << 0.001**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P << 0.001**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.489
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.489	

Table S22.2. Bonferroni post hoc test for Gene*Part of retina Winter.

Cell No.	Bonferroni test; **variable log norm mRNA Winter.** Probabilities for Post Hoc Tests Error: Between MS = ,07208, df = 108,00													
	gene3	Part of retina3	{1} 1.6424	{2} 1.6384	{3} -.9213	{4} -.8416	{5} -3.287	{6} -3.152	{7} 1.0950	{8} 1.2138	{9} -1.077	{10} -.1692	{11} -2.339	{12} -2.064
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P << 0.001**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P << 0.001**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	

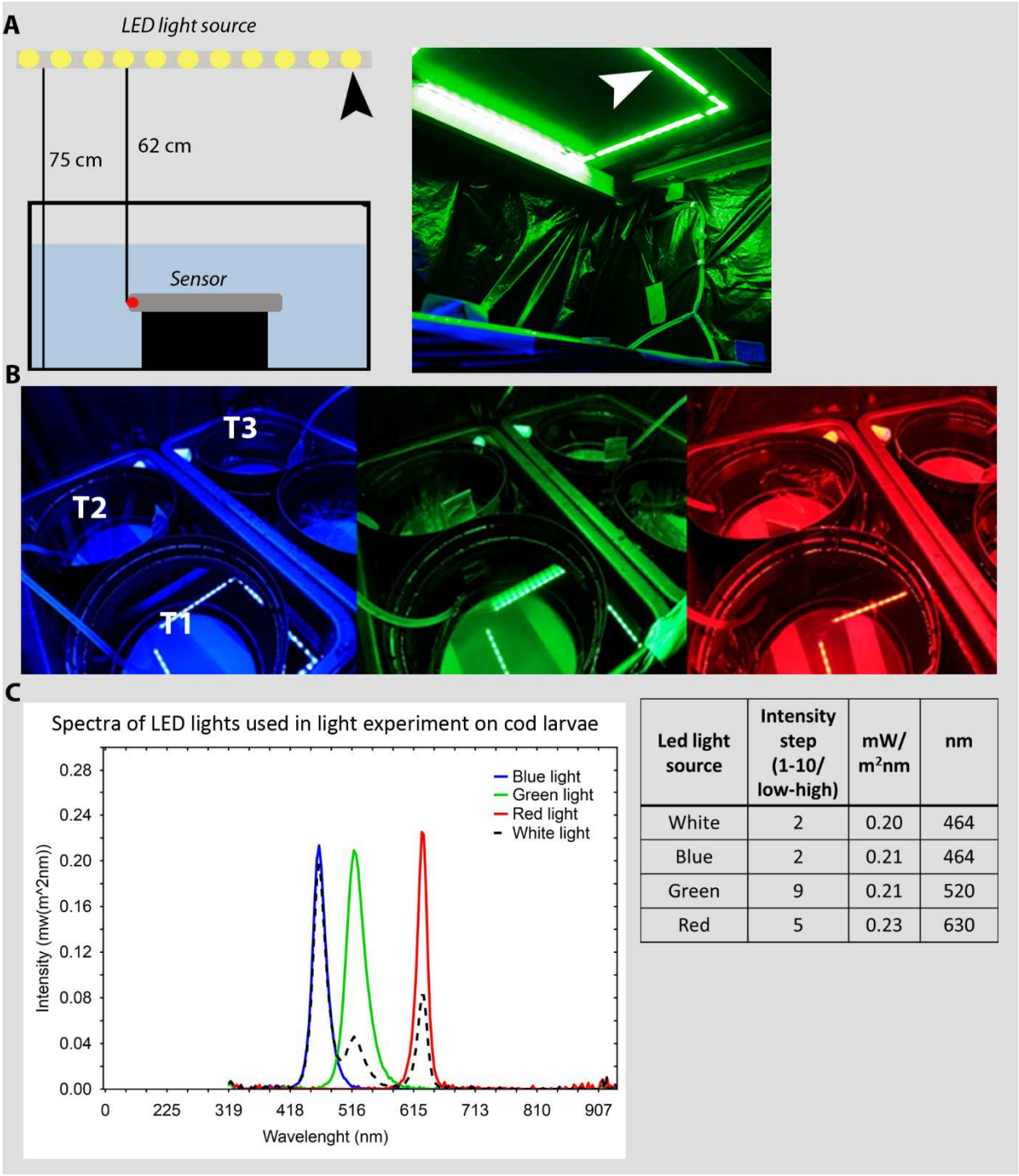
Table S23.1. Tukey HSD post hoc for Gene*Part of retina Early fall.

Cell No.	Tukey HSD test; **variable log norm mRNA exp Early fall.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .12394, df = 102.00													
	Gene	Part of retina	{1} 1.6795	{2} 1.6445	{3} -1.018	{4} -1.051	{5} -3.326	{6} -3.242	{7} 1.0714	{8} 1.1148	{9} -1.033	{10} -.4316	{11} -2.381	{12} -2.210
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	0.000118	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.999996	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.999996		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P < 0.05**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P < 0.05**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.996
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.996	

Table S23.2. Bonferroni post hoc test for Gene*Part of retina Early fall.

Cell No.	Bonferroni test; **variable log norm mRNA exp Early fall.** Probabilities for Post Hoc Tests Error: Between MS = .12394, df = 102.00													
	Gene	Part of retina	{1} 1.6795	{2} 1.6445	{3} -1.018	{4} -1.051	{5} -3.326	{6} -3.242	{7} 1.0714	{8} 1.1148	{9} -1.033	{10} -.4316	{11} -2.381	{12} -2.210
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	0.000118	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P < 0.05**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P < 0.05**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	

Supplemental material



Led light source	Intensity step (1-10/ low-high)	mW/ m^2nm	nm
White	2	0.20	464
Blue	2	0.21	464
Green	9	0.21	520
Red	5	0.23	630

Figure S1. Experimental setup light treatments cod larvae. A) Left handside illustrates the position at which the optical recordings were obtained within the fish tanks, and a photo of the LED light source is shown on the right hand side. B) The photos show the setup of the larval rearing tanks under different wavelenght lights of blue, green and red, respectively. C) Optical measurements were plotted for all LED lights used (left hand side), where the y-axis shows LED intensity measured in mW

m^{-2} nm^{-1} over nanometer wavelenght distribution (x-axis). The table on the right hand side shows specter data values for the used LED lights, where the chosen intensity step was normalized across the different light sources.

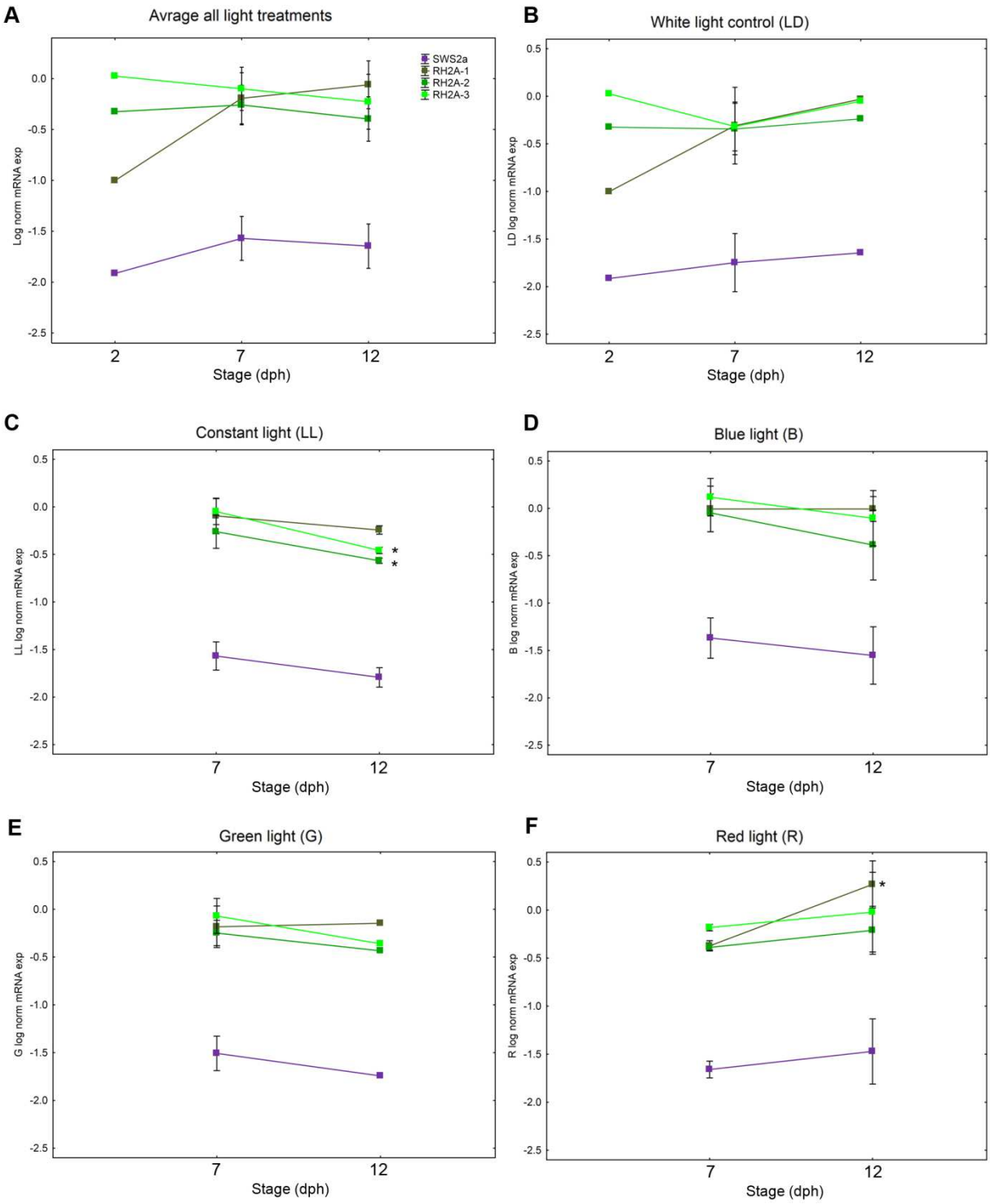


Figure S2. Temporal expression of cone opsins during light treatments (A-F). The different opsins are represented by different colors shown in A. Expression values marked with (*) in C and F represents significantly different ($p<0.05$) expression between 7- and 12 dph, using a one-way ANOVA.

Supplemental material: Statistical analyses

Light experiment NC cod larvae

Table S1. Normality test light experiment. *Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins in all stages and treatments. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 (p<0.05), indicating rejection of the normal distribution hypothesis. Missing cells (-) represent stages where N is too low and test could not be performed.*

Stage (dph)	Treatment	SWS2A	RH2A-1	RH2A-2	RH2A-3
7	LD	W = 0.815	W = 0.943	W = 0.936	W = 0.937
12	LD	W = -	W = -	W = -	W = -
7	LL	W = 0.889	W =0.942	W =0.938	W =0.827
12	LL	W = 0.886	W = 0.875	W = 0.967	W = 0.823
7	B	W = 0.992	W = 0.948	W = 0.970	W = 0.991
12	B	W = -	W = -	W = -	W = -
7	G	W = 0.899	W = 0.876	W =0.999	W =0.999
12	G	W = -	W = -	W = -	W = -
7	R	W =0.958	W =0.991	W = 0.882	W = 1.000
12	R	W = -	W = -	W = -	W = -

Table S2. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Stage (dph)	Treatment	SWS2A	RH2A-1	RH2A-2	RH2A-3
7-12	LD	P = 0.248	P = 0.354	P = 0.344	P = 0.349
7-12	LL	P = 0.421	P = 0.096	P = 0.064	P = **0.048**
7-12	B	P = 0.483	P = 0.356	P = 0.195	P = 0.439
7-12	G	P = 0.230	P = 0.215	P = 0.402	P = 0.405
7-12	R	**P = 0.009**	**P = 0.007**	**P < 0.001**	**P < 0.000**

Table S3. Two-way ANOVA light treatment; stage*treatment.

Effect	Analysis of Variance. Marked effects are significant at p < .050				
	Value	F	Effect df	Error df	p
Intercept	0.005	780.006	4	15.000	0.000
Stage (dpf)	0.121	7.029	8	30.000	0.000
Treatment	0.423	0.944	16	46.463	0.528

Table S5. Tukey HSD post hoc for stage effect *sws2a*.

Cell No.	Tukey HSD test; variable **Log Relative SWS2a express**. Approximate Probabilities for Post Hoc Tests Error: Between MS = .04453, df = 18.000			
	Stage (dph)	{1} -1.915	{2} -1.570	{3} -1.646
1	2		0.2780	0.464
2	7	0.280		0.677
3	12	0.464	0.677	

Table S6.1. Tukey HSD post hoc for stage effect *rh2a-1*.

Cell No.	Tukey HSD test; variable **Log Relative RH2-1 express.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .06455, df = 18.000			
	Stage (dph)	{1} -1.002	{2} -.1932	{3} -.0430
1	2		0.017	0.006
2	7	0.017		0.361
3	12	0.007	0.3617	

Table S6.2. Bonferroni post hoc for stage effect *rh2a-1*.

Cell No.	Bonferroni test; variable **Log Relative RH2-1 express.** Probabilities for Post Hoc Tests Error: Between MSE = .06712, df = 17.000			
	Stage (dph)	{1} -1.002	{2} -.1932	{3} -.0601
1	2		0.023	0.010
2	7	0.023		0.771
3	12	0.010	0.771	

Table S7. Tukey HSD post hoc for stage effect *rh2a-2*.

Cell No.	Tukey HSD test; variable **Log Relative RH2-2 express.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .04333, df = 18.000			
	Stage (dph)	{1} -.3244	{2} -.2580	{3} -.3966
1	2		0.949	0.942
2	7	0.949		0.280
3	12	0.942	0.280	

Table S8. Tukey HSD post hoc for stage effect *rh2a-3*.

Cell No.	Tukey HSD test; variable **Log Relative RH2-3 express.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .05228, df = 18.000			
	Stage (dph)	{1} .02566	{2} -.1001	{3} -.2266
1	2		0.860	0.558
2	7	0.860		0.407
3	12	0.558	0.407	

Table S9. One-way ANOVA light treatment for stage effect.

Effect	Analysis of Variance. Marked effects are significant at p < .050				
	Value	F	Effect df	Error df	p
Intercept	0.005	921.301	4	19	0.00
Stage (dph)	0.106	9.824	8	38	0.000

Significant (p<0.05) Tukey HSD post hoc test for stage effect within treatment

Table S10.1 Tukey HSD post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Tukey HSD test; Variable: **Log Relative RH2-2 express**. Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.2613	{3} M=-.5667
2 {1}			
7 {2}			0.040
12 {3}		0.040	

Table S10.2 Bonferroni post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Bonferroni test; Variable: **Log Relative RH2-2 express**. Marked differences are significant at p < .050. Error: Between MS = ,01562, df = 4,0000		
	{1} M=0.0000	{2} M=-.2613	{3} M=-.5667
2 {1}			
7 {2}			0.040
12 {3}		0.040	

Table S11.1 Tukey HSD post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Tukey HSD test; Variable: **Log Relative RH2-3 express**. Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.0496	{3} M=-.4579
2 {1}			
7 {2}			0.008
12 {3}		0.008	

Table S11.2 Bonferroni post hoc for stage effect within treatment LL.

Stage (dph)	**Treatment=LL** Bonferroni test; Variable: **Log Relative RH2-3 express**. Marked differences are significant at p < .050. Error: Between MS = ,00992, df = 4,0000		
	{1} M=0.0000	{2} M=-.0496	{3} M=-.4579
2 {1}			
7 {2}			0.008
12 {3}		0.008	

Table S12.1 Tukey HSD post hoc for stage effect within treatment R.

Stage (dph)	**Treatment=R** Tukey HSD test; Variable: **Log Relative RH2-1 express.** Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.3723	{3} M=.26714
2 {1}			
7 {2}			0.018
12 {3}		0.018	

Table S12.2 Bonferroni post hoc for stage effect within treatment R.

Stage (dph)	**Treatment=R** Bonferroni test; Variable: **Log Relative RH2-1 express.** Marked differences are significant at p < .050. Error: Between MS = ,02186, df = 3,0000		
	{1} M=0.0000	{2} M=-.3723	{3} M=.26714
2 {1}			
7 {2}			0.018
12 {3}		0.018	

Maturing NC cod

Table S13. Normality maturing cod. *Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 (p<0.05), indicating rejection of the normal distribution hypothesis.*

Gene	Female	Male
RH1	**W = 0.841**	**W = 0.756**
Rh2a-1	W = 0.903	**W = 0.751**
Sws2a	W = 0.924	**W = 0.565**

Table S14. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Variable Female-Male	Levene Test of Homogeneity of Variances. Marked effects are significant at p < .05							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Rh1 Log Norm mRNA exp	0.030	2	0.015	0.254	25	0.010	1.497	0.243
Sws2a Log Norm mRNA exp	0.052	2	0.026	1.518	25	0.061	0.431	0.654
Rh2a-1 Log Norm mRNA exp	0.033	2	0.016	0.295	25	0.012	1.387	0.268

Table S15. Main effects ANOVA Gene*Part-of-retina*Gender. Significant effect (p<0.05)

Effect	Univariate Tests of Significance for Log norm mRNA exp. Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	10.903	1	10.903	279.150	0.000
Gene	110.587	2	55.294	1415.652	0.000
Part of retina	0.009	1	0.009	0.240	0.626
Gender	0.128	1	0.128	3.273	0.076
Gene:*Part of retina	0.050	2	0.025	0.636	0.533
Gene:*Gender	0.027	2	0.013	0.344	0.710
Part of retina*Gender	0.048	1	0.047	1.215	0.275
Gene:*Part of retina*Gender	0.027	2	0.013	0.344	0.710
Error	2.109	54	0.039		

Table S16.1. Tukey HSD post hoc for gene effect.

Cell No.	Tukey HSD test; variable Log norm mRNA exp. Approximate Probabilities for Post Hoc Tests Error: Between MS = .03698, df = 61.000			
	Gene:	{1} 1.8530	{2} -1.293	{3} .67694
1	RH1		P << 0.001	P << 0.001
2	SWS2A	P << 0.001		P << 0.001
3	RH2A-1	P << 0.001	P << 0.001	

Table S16.2. Bonferroni post hoc for gene effect.

Cell No.	Bonferroni test; variable Log norm mRNA exp. Probabilities for Post Hoc Tests Error: Between MSE = ,06607, df = 81,000			
	Gene:	{1} 1.9954	{2} -1.132	{3} .9150
1	RH1		P << 0.001	P << 0.001
2	SWS2A	P << 0.001		P << 0.001
3	RH2A-1	P << 0.001	P << 0.001	

Northeast Arctic cod (NEA cod)

Table S17. Test of normality distribution Northeast Arctic cod. *Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 (p<0.05), indicating rejection of the normal distribution hypothesis.*

Season	Part of retina	RH1	SWS2A	RH2A-1	RH2A-2	RH2A-3
Summer	Dorsal	W = 0.902	W = 0.883	W = 0.926	W = 0.955	W = 0.923
Summer	Ventral	W = 0.945	W = 0.856	W = 0.934	W = 0.954	W = 0.929
Winter	Dorsal	W = 0.946	W = 0.930	**W =0.808**	W = 0.959	W = 0.951
Wintre	Ventral	W = 0.953	**W = 0.731**	**W = 0.893**	W = 0.943	W = 0.929

Table S18. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Gene	Levene's Test for Homogeneity of Variances: Effect: **Season** Degrees of freedom for all F's: 1, 138			
	MS Effect	MS Error	F	p
RH1	0.000	0.025	0.012	0.915
Sws2a	0.007	0.049	0.145	0.704
Rh2a-1	0.230	0.021	18.7	**0.000**
Rh2a-2	0.001	0.10	0.014	0.907
Rh2a-3	0.037	0.030	1.221	0.276

Table S19. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Season	All Groups Levene's Test for Homogeneity of Variances: **Effect: "Part of retina" by gene** Degrees of freedom for all F's: 1, 131			
	MS Effect	MS Error	F	p
Summer Log Norm. mRNA exp (ubiq)	0.247	0.368	0.672	0.414

Table S20. Levene's Test for Homogeneity of Variances. Significant effects (**p<0.05**)

Season	All Groups Levene's Test for Homogeneity of Variances: Effect: **"Part of retina" by gene** Degrees of freedom for all F's: 1, 138			
	MS Effect	MS Error	F	p
Winter log norm mRNA exp	0.675	0.387	1.742	0.189

Table S21. Main effects ANOVA Gene*Part-of-retina*Season. Significant effect ($p<0.05$)

Effect	Univariate Tests of Significance for Log Norm. mRNA exp. Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	123.368	1	123.368	1268.362	0.000
Gene:	693.768	5	138.754	1426.542	0.000
Part of retina	2.228	1	2.228	22.910	0.000
Season	0.343	1	0.343	3.529	0.062
Gene:*Part of retina	3.990	5	0.798	8.203	0.000
Gene:*Season	0.168	5	0.034	0.344	0.885
Part of retina*Season	0.187	1	0.187	1.925	0.167
Gene:*Part of retina*Season	0.121	5	0.024	0.248	0.940
Error	20.426	210	0.097		

Table S22.1. Tukey HSD post hoc for Gene*Part of retina Winter.

Cell No.	Tukey HSD test; **variable log norm mRNA Winter.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .07208, df = 108.00													
	gene3	Part of retina3	{1} 1.6424	{2} 1.6384	{3} -.9213	{4} -.8416	{5} -3.287	{6} -3.152	{7} 1.0950	{8} 1.2138	{9} -1.077	{10} -.1692	{11} -2.339	{12} -2.064
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.978	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.716	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.993	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.993		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.998	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.998		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	0.978	0.716	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P << 0.001**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P << 0.001**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.489
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.489	

Table S22.2. Bonferroni post hoc test for Gene*Part of retina Winter.

Cell No.	Bonferroni test; **variable log norm mRNA Winter.** Probabilities for Post Hoc Tests Error: Between MS = ,07208, df = 108,00													
	gene3	Part of retina3	{1} 1.6424	{2} 1.6384	{3} -.9213	{4} -.8416	{5} -3.287	{6} -3.152	{7} 1.0950	{8} 1.2138	{9} -1.077	{10} -.1692	{11} -2.339	{12} -2.064
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P << 0.001**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P << 0.001**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	

Table S23.1. Tukey HSD post hoc for Gene*Part of retina Early fall.

Cell No.	Tukey HSD test; **variable log norm mRNA exp Early fall.** Approximate Probabilities for Post Hoc Tests Error: Between MS = .12394, df = 102.00													
	Gene	Part of retina	{1} 1.6795	{2} 1.6445	{3} -1.018	{4} -1.051	{5} -3.326	{6} -3.242	{7} 1.0714	{8} 1.1148	{9} -1.033	{10} -.4316	{11} -2.381	{12} -2.210
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	0.000118	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.999996	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.999996		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P < 0.05**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P < 0.05**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.996
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.996	

Table S23.2. Bonferroni post hoc test for Gene*Part of retina Early fall.

Cell No.	Bonferroni test; **variable log norm mRNA exp Early fall.** Probabilities for Post Hoc Tests Error: Between MS = .12394, df = 102.00													
	Gene	Part of retina	{1} 1.6795	{2} 1.6445	{3} -1.018	{4} -1.051	{5} -3.326	{6} -3.242	{7} 1.0714	{8} 1.1148	{9} -1.033	{10} -.4316	{11} -2.381	{12} -2.210
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SWS2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SWS2A	Ventral	P << 0.001	0.000118	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SWS2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SWS2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2A-1	Dorsal	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	**RH2A-2**	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		**P < 0.05**	P << 0.001	P << 0.001
10	**RH2A-2**	Ventral	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	**P < 0.05**		P << 0.001	P << 0.001
11	RH2A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000
12	RH2A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	