

**OPSIN EXPRESSION IN YELLOW PERCH (*Perca flavescens*)
AND WALLEYE (*Sander vitreus*)**

A Thesis

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by

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ABSTRACT

Vision arises from the absorption of photons by visual pigments located in retinal photoreceptor cells. Visual pigments are composed of a protein moiety (opsin) and a bound molecule of vitamin A aldehyde. The spectral sensitivity of an animal is determined by the absorbance characteristics of its visual pigments. The ancestral vertebrate has five visual pigment families, an RH1 found in rod photoreceptors for low light vision, and four visual pigments found in cone photoreceptors for bright light vision. The cone pigment types are a long to middle wavelength sensitive (LWS, red), a middle wavelength sensitive (RH2, green), and two short wavelength sensitive classes (SWS1, ultraviolet and SWS2, violet). The spectral region over which a particular visual pigment absorbs is determined both by the amino acids present at the opsins specific 'tuning sites' as well as the form of vitamin A used. In fish, there are morphologically distinct photoreceptor types, common ones being rods and double/twin, single and short single cones with visual pigments often associated with specific cone types. In percids such as the yellow perch (*Perca flavescens*) and walleye (*Sander vitreus*), each member of the twin cones contains the same LWS pigment; single cones contain a RH2 pigment and short single cones contain one of the SWS pigments. In the yellow perch, the short single cones are found only in the larval stage, and disappear at the time of transition in habitat

and prey. Microspectrophotometry (MSP) has identified the pigment in the short single cones as being SWS; however the specific SWS family is unknown. For the closely related walleye histological and microspectrophotometric examination has found no evidence for an SWS cone at any developmental stage.

Retinas of larval yellow perch and walleye were used for RNA extraction and cDNA synthesis. Opsin specific primers were used to amplify and then identify visual pigment sequences. Retinas of the juveniles of both species express LWS, RH2 and SWS2 and both have a genomic copy of SWS1 that does not appear to be expressed. Interestingly, SWS2 was also found in slightly larger fish retinas suggesting that there may be small population of short single cones not previously found, or that there is co-expression of SWS2 and another visual pigment in either one or both of the other cone classes.

These findings suggest the presence of all five opsin classes genomically with differential expression being used temporally to adapt the fish for the specific visual tasks needed for the photic environment. In the case of the yellow perch, the planktonic prey of larval fish scatter short wavelength light which may make them most easily identified with violet visual pigment sensitivity. Larval walleye should be examined histologically to determine the location of SWS2 expression.

BIOGRAPHICAL SKETCH

June Eillenberger was born on June 10, 1973 in Newburgh, NY. She attended mostly Onteora High School in Boiceville, New York. She received a Bachelor of Science Degree from Fairleigh Dickinson University in Teaneck, NJ with a major study in Biology and a minor study in Chemistry. She has been a graduate student of the Department of Biomedical Sciences at Cornell University since 2001.

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CHAPTER ONE
**Adaptations of Vision in Fish – A Review of Short
Wavelength Sensitivity**

Visual ecologists hold the primary tenet that the visual system of an organism will be adapted for performance of relevant visual tasks within the organism's environment (Walls 1967, Lythgoe 1979). This should operate at all levels – from the absorption of photons by photosensitive pigments to the neural algorithms used to extract meaningful information from the continual stream of neural impulses reaching the brain's analytical centers. This thesis concentrates on adaptations affecting the absorption of photons by the photoreceptor cells. Since vision starts with absorption of photons by visual pigments (which are members of the G-coupled protein family of signaling molecules specialized for photon absorption), the boundary conditions for vision will depend directly on the absorptive characteristics of the visual pigment.

Vision

There is a distinction to be made between simple photosensitivity and vision. All cells are photosensitive in the sense that their molecules can absorb photons over some region of the electromagnetic spectrum. In some cases, photon absorption increases their vibrational state and, ultimately, temperature (see Gates 1980). For example, the absorption of infrared radiation (IR) by skin is sensed as heat.

In other cases, the photon energy can lead to specific photochemical reactions such as the breaking of chemical bonds. For example, ultraviolet (UV) radiation can damage DNA. (e.g. Giese 1976) In the context of sensation, the term *photoreception* is usually reserved for a process that uses photon absorption to affect the behavior of an organism (see Fraenkel and Gunn 1961 and Thomas 1965). For example, it might respond to an increase in photon absorption by moving toward or away from the photon source (phototaxis), or changing its locomotor speed (photokinesis). Vision is a specialized sense associated with a specific organ (an eye) that uses an array of photosensitive cells to encode two-dimensional photon flux (light) and enables the extraction of environmental information impressed by the photon field through the use of neural circuits.

The Eye

The organ for vision is the eye which is used to collect and extract visual information from the light field. Diffractive and refractive elements of the eye focus an image of the environment onto an array of photoreceptive cells in the retina.

The Retina

The retina is the neural layer at the back of the eye (see Dowling 1987). The outermost layer is the pigment epithelium, made up of a single layer of cells usually

containing melanin pigment (see Figure 1). These cells do not directly engage in the process of vision, but are supportive of the next inner layer of cells, the photoreceptors that absorb photons and begin the process of vision. Interior to the photoreceptors is the outer plexiform layer which is the site of synapses between the photoreceptors and the bipolar and horizontal cells that receive their signals. The inner nuclear layer is composed mainly of the nuclei of the horizontal and bipolar cells. The inner plexiform layer is the site of connections between the bipolar, amacrine and ganglion cells. The ganglion cell layer contains the cell bodies of the ganglion cells. Finally, the innermost layer is the nerve fiber layer, which is composed of the axons of the ganglion cells which converge on the optic disk where they leave the eye as the optic nerve.

The Photoreceptors

The photoreceptive cells stand parallel to the direction of incident light (see Figure 2). The synaptic terminal is the innermost part of the cell, followed by the axon, then the nucleus and finally two portions specific to these nerve cells, the inner and outer segments. The inner segment contains many mitochondria and sometimes an oil droplet for light filtering as seen in some non-mammals (Dowling 1987, Douglas and Marshall 1999). The outer segment contains the visual pigment molecules embedded in stacked membranes. The visual pigment molecules are sensitive to incoming

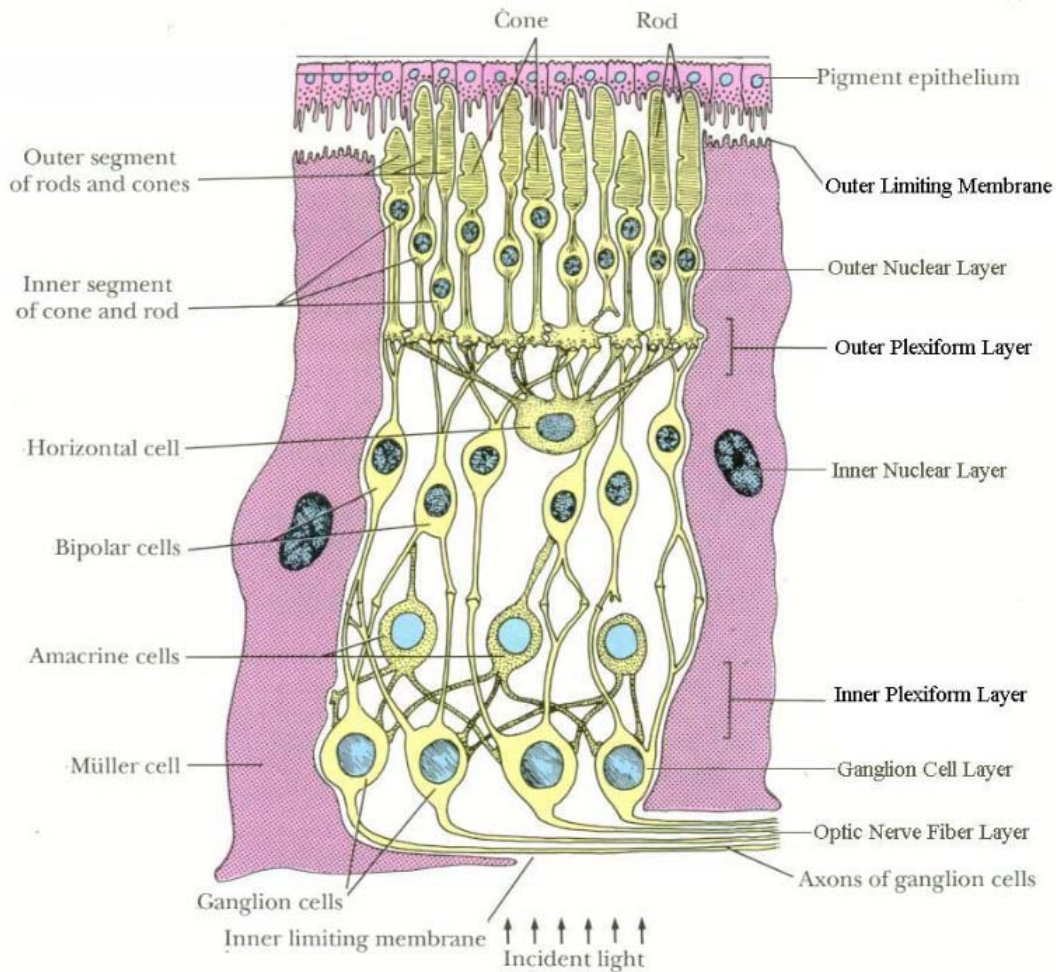


Figure 1 Cross Section of a Typical Vertebrate Retina: Light enters at the bottom of this figure and passes through all of the other cellular layers before reaching the photoreceptor cells for absorption. Neural impulses travel in the opposite direction from the photoreceptors to the bipolar cells to the ganglion cells with some forms of two dimensional analyses occurring with the horizontal and amacrine cells. See text for discussion. Modified from Ross and Romrell, 1989.

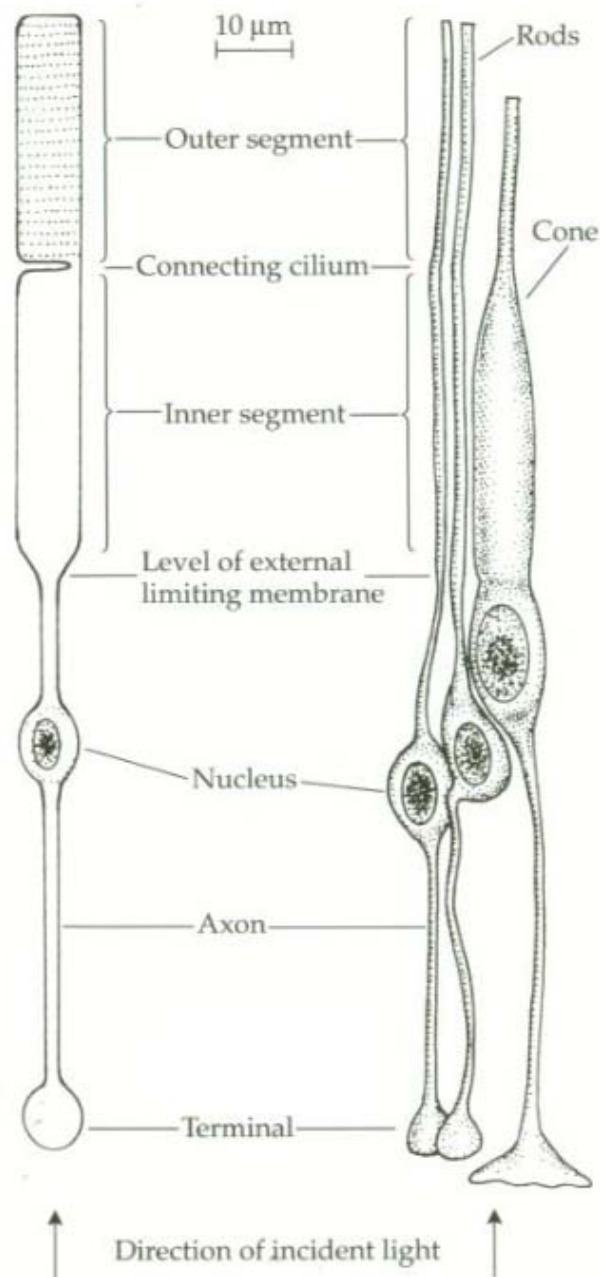


Figure 2 Typical Vertebrate Photoreceptors: Light approaches from the bottom of this figure and passes through the photoreceptors to be absorbed in the outer segment. Photoreceptors are named for their outer segment shape with cones generally being more tapered than rods. Oyster, 1999

photons and initiate the photoreceptor cell's signal to other cells in the visual pathway.

Classes of Photoreceptors

Two classes of photoreceptor cells can be identified in most vertebrate retinas using structural and/or physiologic criteria: rods and cones, which were originally named for the shape of their outer segments. The rods are specialized for detecting light in scotopic (low light levels) conditions, the cones are specialized for detecting light in photopic (high light levels) conditions (e.g. Baylor 1987). Some cones may be joined together at the inner segments creating twins, doubles or triples (Figure 3).

Retinal Mosaics

In some vertebrates such as fish, photoreceptors are frequently arranged in regular patterns known as mosaics (Ryder 1895, Lyall 1957) (Figure 4). The most common types found are square and row patterns, consisting of both double and single cones. A frequently found mosaic pattern in teleosts is the simple square mosaic in which double cones form a square around a central single cone. Short single cones may also be found at all or some of the corners of the square forming complete or incomplete squares, respectively.

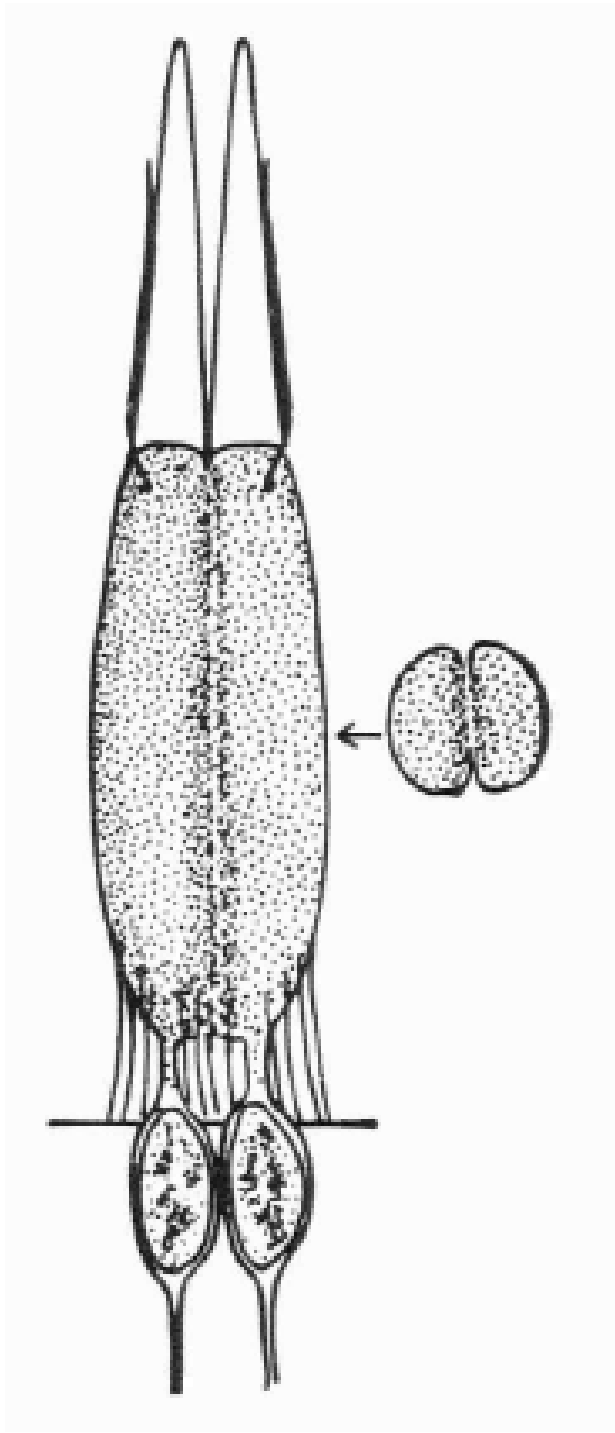
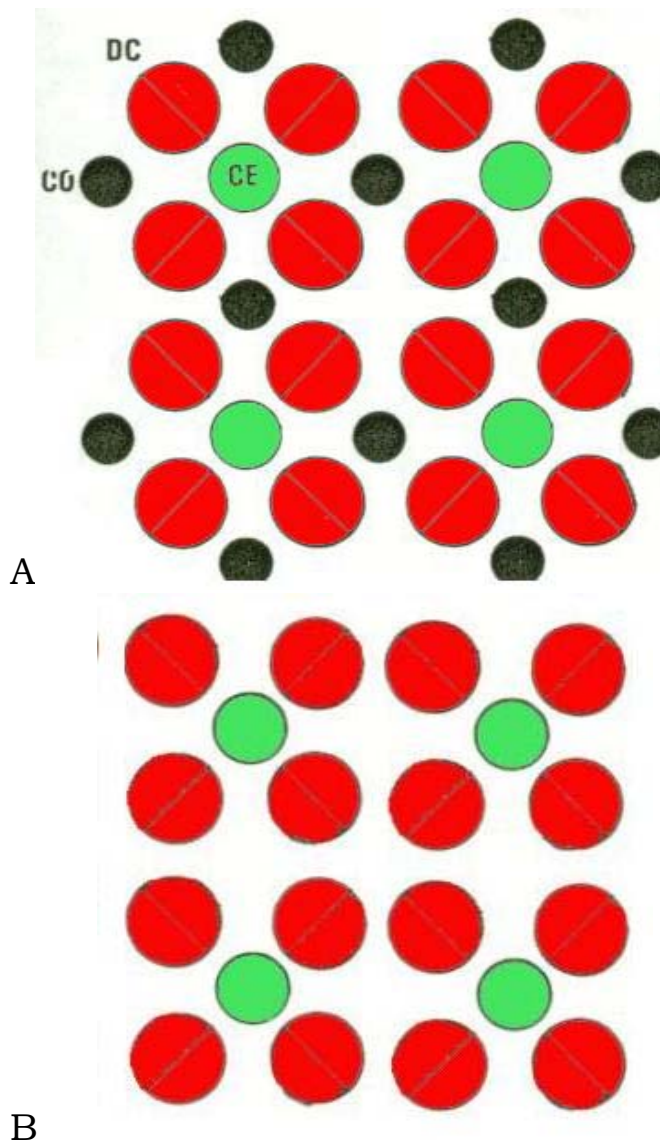


Figure 3 Double Cones: Cones may be joined at the level of the inner segment making double, triple or quadruple cones found in vertebrate groups other than placental mammals. Shown is a double cone pair as seen in *Perca fluviatilis* by Engström, 1963.



B

Figure 4 Typical Mosaics: Several types of mosaics are present in fish retinas. A- A common type is a complete squares in which the 'sides' of the square usually consists of double cones sensitive to long wavelengths (red), the 'center' of the square is a single cone sensitive to medium wavelengths (green), and the 'corner' of the square is a single cone sensitive to short wavelengths (ultraviolet). B- Simple square mosaics are missing the corner cones of the complete square mosaics. Modified from Bowmaker and Kunz, 1987.

Visual Pigments

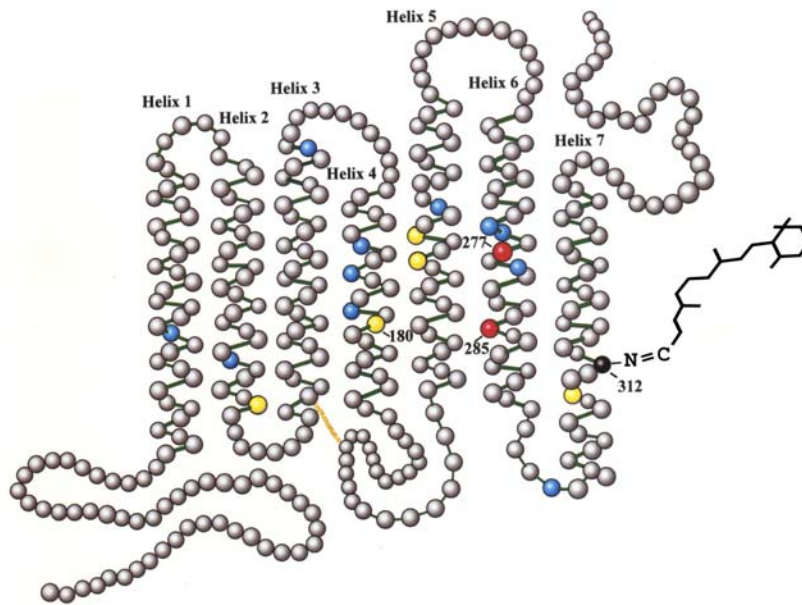
The outer segment of all cone photoreceptors are composed of folded membranes made of a phospholipid bilayer similar to a plasma membrane. Embedded within them is a specialized molecule composed of two moieties, a protein, opsin, and a molecule of vitamin A aldehyde, retinal, that together form the visual pigment.

Opsin

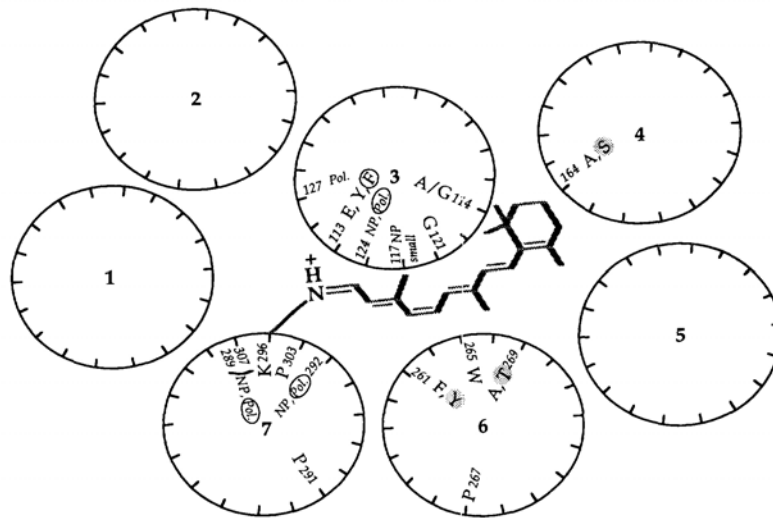
Opsins are members of the G-protein coupled receptor family used in the detection of physical, biological and chemical signals and begin signal transduction cascades affecting cellular processes. All G protein receptors have seven helical transmembrane segments with a variable number of amino acids making up the extra- and intra-cellular tails (Figure 5). Opsins are ancient – genes have coded for them for about 700 million years (see Trezise and Collin 2005).

Retinal

At site 296 on the opsin is a lysine residue that binds a molecule of retinal, which as stated before is an aldehyde of vitamin A. The retinal is called a chromophore because when bound to opsin which is colorless, the resulting molecule appears colored. The alternating double-bond structure of the tail of retinal permits a number of stereoisomeric forms, but all functional visual pigments use the 11-cis form (see Abrahamson and Wiesenfeld 1972) (Figure 6). Two similar



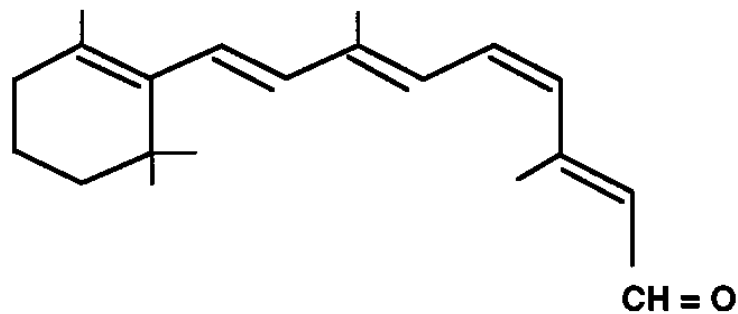
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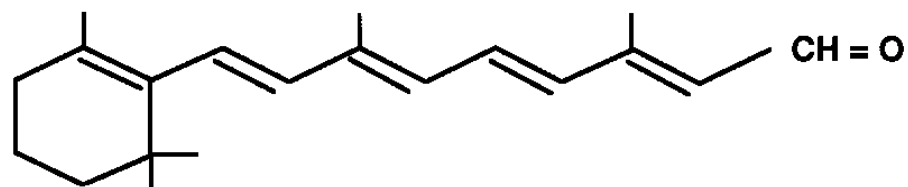
B.

Figure 5 Opsin molecule with bound retinal: Opsin (shown open in A.) has seven transmembrane segments consisting of α helices, the retinal attaches to a lysine in the seventh of these segments which is shown in black. The tuning sites are yellow. B. Opsin as seen from the top, the retinal is confined within the pocket created by the transmembrane segments. See explanation in text. A. Modified from Jacobs 1998; B. Chang *et al* 1995.

11 - *cis* retinal (vitamin A1 aldehyde)



all -*trans* retinal



3 - dehydroretinal (vitamin A2 aldehyde)

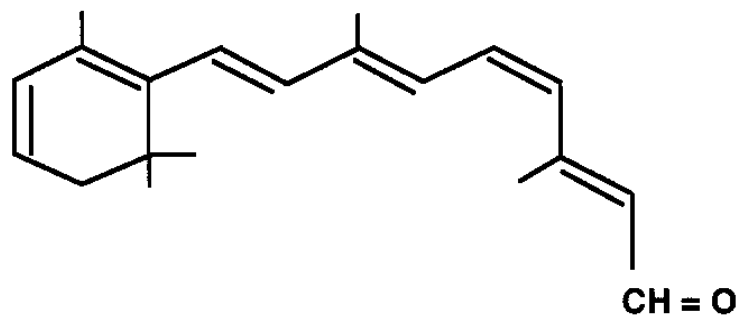


Figure 6 Retinal: Active retinal is an aldehyde of vitamin A and is in the 11-*cis* form until a photon causes isomerization to the all-*trans* form. The vitamin A1 form has one double bond in the ring while the vitamin A2 form has a second double bond in the ring. See text for explanation. (Yokoyama and Yokoyama 1996)

forms of retinal are used for visual pigment formation. Vitamin A₁ has a single double bond in the polyene ring and forms the rhodopsin family. Vitamin A₂ has two double bonds in the ring and forms the porphyropsin family of pigments (Figure 6). For the same opsin, the substitution of vitamin A₂ for vitamin A₁ shifts the absorption spectrum to longer wavelengths (see below).

Photon Absorption

The absorption of a photon by retinal causes isomerization of the 11-cis-retinal to all-trans retinal. The isomerization of retinal causes the opsin to activate due to restructuring and a newly exposed charged site. It can then interact with other molecules and begin a transductional cascade of reactions that starts the visual process. Regardless of the initiating photon's wavelength, once isomerization of the chromophore molecule occurs, the opsin has a uniform transductional response (see Wald 1955, Dartnall 1957).

Spectral Sensitivity

Visual pigments are characterized by their absorbance spectrum, which represents the number of photons absorbed as a function of wavelength or frequency (Figure 7). The absorption spectrum of a visual pigment represents the probability of absorption and therefore its spectral sensitivity (see Dartnall 1957). The peak of the main absorption band (alpha) is called the λ_{\max} .

Absorbance of Photo Pigment

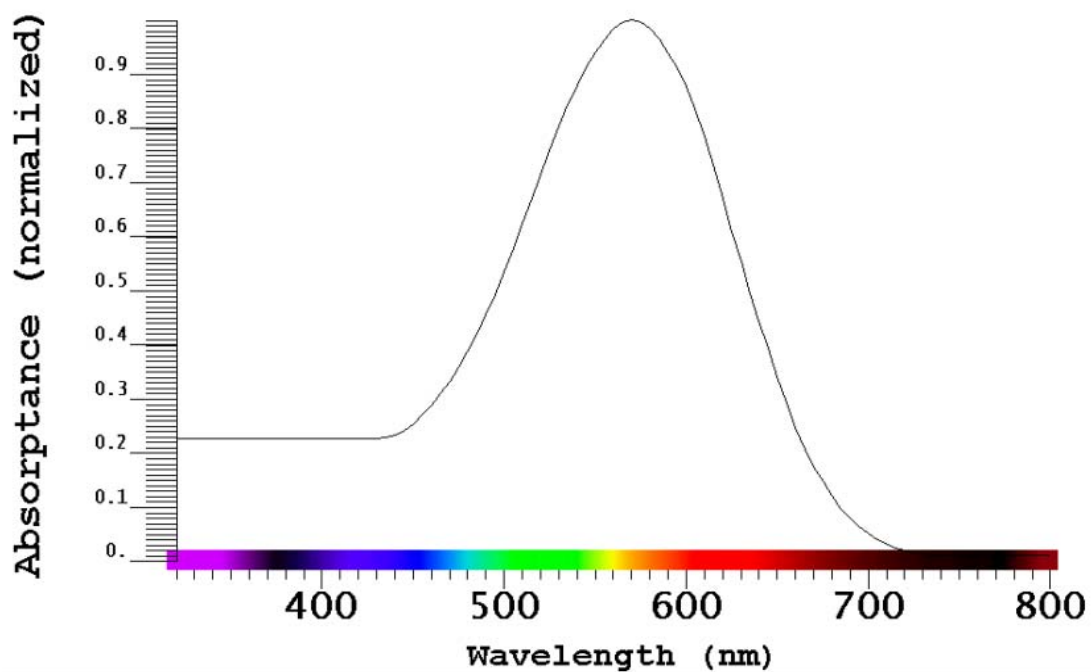


Figure 7 Absorbance Spectra: The absorbance probability of a photon by a visual pigment is dependent on the wavelength of the photon. The maximum point of absorption is the λ_{max} (565nm shown) and there are β -bands present in the short wavelength region due to protein absorption (not shown).

Visual Pigment Tuning

While the overall shape of visual pigment absorbance spectra are similar (see Dartnall 1957), it is the position of the λ_{\max} that determines the spectral sensitivity or region of the spectrum over which the pigment is most sensitive. The position of λ_{\max} is shifted or ‘tuned’ by altering the biophysical environment around the chromophore. The amino acid residues at specific locations along the opsin’s transmembrane segments in proximity to the chromophore can alter the energy state of the bonds of the chromophore slightly, producing a λ_{\max} shift (see Nathans, *et al* 1986a and Kochendoerfer *et al* 1999) (Figure 8). Some opsins also use anion binding near the chromophore to modify the local environment for tuning (Kleinschmidt and Harosi 1992). These changes yield visual pigments with their λ_{\max} es in discrete clusters along the visual spectrum. For example, there are visual pigments maximally sensitive to the red/yellow region of the spectrum forming the long wavelength sensitive (LWS) family of opsins. Others exist in the green region forming the medium wavelength sensitive family (called RH2 or MWS) and in the violet to ultraviolet region are the short wavelength sensitive (SWS) families (see Bowmaker and Hunt 2006). The amino acid differences at just a few tuning sites near the center of the opsin can, together with the type of chromophore employed (see below), explain the range of λ_{\max} positions for most visual pigments (Kochendoerfer *et al.* 1999).

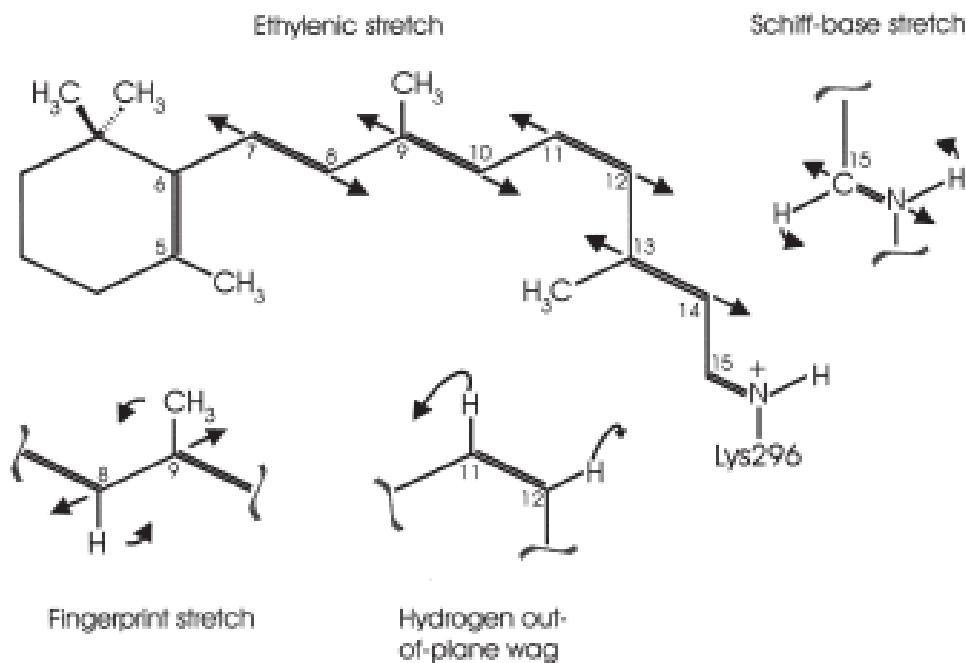


Figure 8 Tuning of Visual Pigments: Visual pigments are tuned by modification of the amino acids near the binding pocket of the opsin which affect the energy state of the retinal causing more or less energy needed for isomerization to all-trans retinal.

Hunt *et al* 2001

Color Discrimination

Generally, a photoreceptor expresses a single opsin, which along with the chromophore used, determines its spectral sensitivity. Thus, classes of photoreceptors can be defined by their spectral sensitivity that depends on the expressed opsin and chromophore used. In some cells, there can be more than one visual pigment present for a short or long time period, causing the cell's spectral sensitivity to be determined by the combination of opsins expressed.

However, a single photoreceptor cannot give unequivocal information about the spectral position of an absorbed photon. As seen in Figure 7, more than one spectral location along the absorption curve can have the same probability of absorbance. In addition, since the photoreceptor is simply a photon counter, the absorption of any wavelength photon causes a uniform transductional response. Therefore, in order for an animal to discriminate color, more than one spectral class of photoreceptor is needed so that their outputs can be compared neurally to extract chromatic information. The ensemble of expressed opsins defines the overall chromatic spectral sensitivity of the animal.

Almost all diurnal vertebrates have several spectral classes of cones along with one rod. However, just having multiple visual pigments does not necessarily indicate that there is discriminatory color vision. Simple photoreception does not require color perception, the ability to detect the widest range of wavelengths possible would be ideal.

Discriminatory color vision can only be confirmed by behavioral experiments. (Reeves 1919, see Northmore & Yager 1975)

Visual Ecology

As already stated, the visual system of an organism will be adapted for performance of relevant visual tasks within the organism's environment (Walls 1967, Lythgoe 1979). While this 'rule' defining visual ecology should apply to any visual organism, much of the work in visual ecology has been done on fish. This is due in large part to the wide range of photic environments fish inhabit. Aquatic environments vary not only in spectral properties, but also in average intensity and turbidity. (see Lythgoe 1988, Munz 1965). These parameters may be relatively constant, or may change daily, seasonally or over longer time scales. Most if not all diurnal fish are visual predators at some or all developmental stages making the adaptations of their visual systems essential to their survival – meaning they must be able to detect prey under a range of photic conditions. There must also be adaptations for predator detection. The goal of any visual adaptation is to ensure that there is adequate contrast between the visual target and its background to make detection possible.

Opsin Expression

One way in which fish adapt to their photic environment is by utilizing visual pigments that maximize contrast potential

for relevant predators or prey. This can involve expression of different opsin classes and spectral tuning of the opsins expressed. The ancestral vertebrate had four classes of opsins: Rh, SWS1, SWS2 and LWS with the division of Rh1 and Rh2 occurring after the evolution of jaws (Trezise and Collin 2005) (Figure 9). Common features of diurnal, freshwater fish are a rod class with RH1 pigment, at least one LWS cone class and at least one RH2 cone class. The short wavelength end of the spectrum, below 460nm, appears to have wide variability of expression. Some adult fish such as goldfish (*Carassius auratus*) have short wavelength sensitivity with both SWS1 (Hisatomi *et al* 1996) and SWS2 (Johnson *et al* 1993) pigments, other fish have ultraviolet expression as juveniles and violet expression as adults such as rainbow trout, *Oncorhynchus mykiss* (Cheng and Novales Flamarique 2007) or the opposite case of violet sensitivity as juveniles and ultraviolet sensitivity as adults such as in the Blacksmith, *Chromis punctipinnis* (McFarland and Loew 1994) and still others do not show expression of either short wavelength sensitive opsin such as the pikehead, *Luciocephalus pulcher* and pickerel, *Esox americanus* (Lythgoe 1984). The most notable example of differential opsin expression comes from the rapidly evolving African cichlids. These fish have the usual five opsins found in the teleosts, but through duplication have formed subclasses for a total of seven opsin types. Some individual species of cichlids have identical sets of opsin genes, but each expresses only select subsets of those

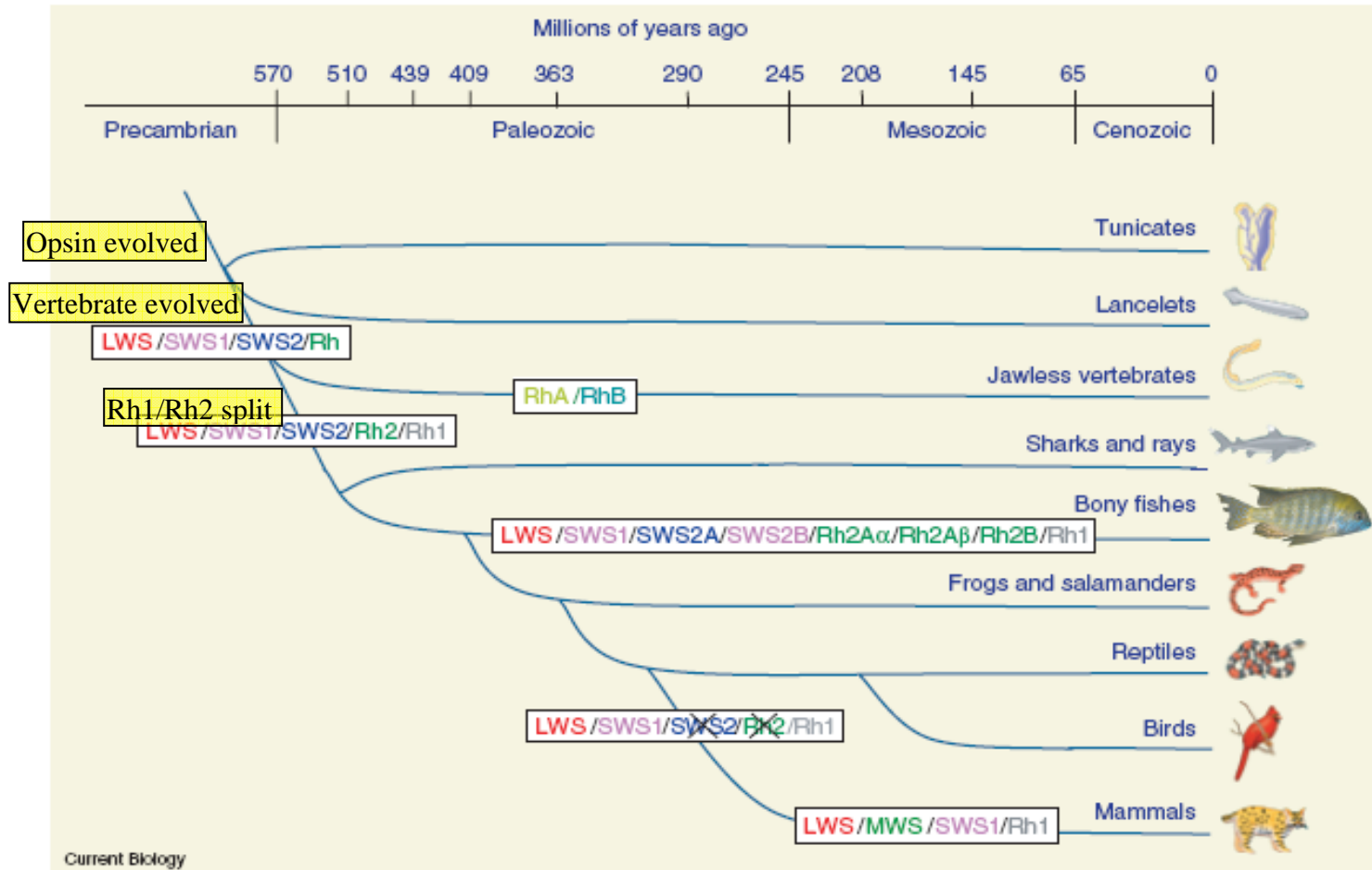


Figure 9 Evolution of Opsins: Opsins evolved early in evolution, approximately 600 MYA. Through duplications and deletions of functional genes, extant fish species have a variety of opsins present genomically which may be expressed as needed. Modified from Trezise and Collin 2005

based on environmental and lifestyle needs (Carleton and Kocher 2001). This shows the ability for variation in spectral sensitivity based on gene regulation instead of amino acid substitution within the opsin genes.

Short wavelength sensitivity is variable across fish species indicating that it may not be necessary for basic functions but may be specifically adaptive for functions relative to the fish. For example, some planktonic species of zooplankton scatter short wavelength light making them easier to detect for fish that prey on these species (Loew and McFarland 1990). The short wavelength cells may be used for orientation and navigation utilizing polarization patterns (Hawryshyn 1987), there may be intraspecies-specific coloration patterns or communication signals (see Losey *et al* 1999), or the short wavelength sensitivity may just extend color vision for greater ability to detect both predators and prey (Coughlin and Hawryshyn 1994).

Adaptations of Fish

To the extent that the visual niche of an organism can change during its lifetime, the visual system should change to ensure that the organism remains adapted for relevant visual tasks. For a fish that progresses through its life stages and occupies different photic habitats that require different visual tasks, then the visual system should adapt for those tasks requiring spectral sensitivity remodeling of the retina. For example, larvae in a different photic environment with

different prey than adults should have different adaptations in its visual system compared to adults.

Photoreceptor Loss

In addition to changes in opsin expression, Bowmaker and Kunz (1987) found that photoreceptor cells can disappear from the retina with age. Using brown trout that lose the ability to detect ultraviolet light as the fish undergo smoltification; they found ultraviolet sensitive photoreceptors by microspectrophotometry in yearling retinas absorbing maximally at about 355nm but did not find these same cells in two-year-old retinas. The mosaic pattern of juvenile and adult trout retina showed that adult trout were missing the corner cones in the square mosaic pattern, which exist in the juvenile. Therefore, they hypothesized that these short corner cones are the photoreceptors responsible for the ultraviolet detection. Allison *et al* (2003) used *in situ* hybridization to label the photoreceptors sensitive to ultraviolet light in the rainbow trout and confirmed that the ultraviolet sensitive cells are in fact the corner cones of the mosaic.

Yellow Perch

The loss of photoreceptors by fish as they advance past the larval stage may be widespread. The yellow perch, *Perca flavescens*, is an example of a diurnal fish that has twin cones absorbing maximally at 630nm, single cones absorbing at 535nm and rods absorbing at 540nm (Loew, personal

communication). Juvenile perch have these same cells as well as another single cone maximally absorbing at 403nm (Loew and Wahl 1991) (Figure 10). The mosaics of adult yellow perch are simple squares while the juveniles have incomplete square mosaics with corner cones completing some of the squares (Wahl 1990) (see figure 4). Juvenile yellow perch also have short wavelength sensitivity which is lost in the adult (Loew *et al* 1993, Cameron, 1982). As these fish age, their corneas become yellow which blocks most short wavelength light (Otten, 1981, Kennedy, Milkman and Sigman 1971). These changes support a short wavelength sensitive cell being located in the corner position of the mosaic of the juvenile fish which disappears in the adult fish. This shortwave sensitivity may be an adaptation for planktivory in the juvenile fish (Loew and McFarland 1990). Their first choice of prey is primarily copepod nauplii, such as *Diaptomus sp.* which highly scatter short wavelength light below 550nm (Loew and McFarland 1990) (Figure 11). At the time of the short wavelength cone loss, the fish are approximately 30mm total length (TL) (Wahl *et al* 1993), move to near shore habitats (personal observation) and their prey preference changes to *Daphnia pulicaria* (Mills *et al* 1984). *Daphnia* are transmissive to short wavelength light down to at least 400nm for which short wavelength sensitivity may not provide sufficient contrast. The differential scattering of short wavelength light by these prey species in the surface waters where these fish feed (Hairston *et al* 1982) may be the driving force for the cell loss. Therefore, the short

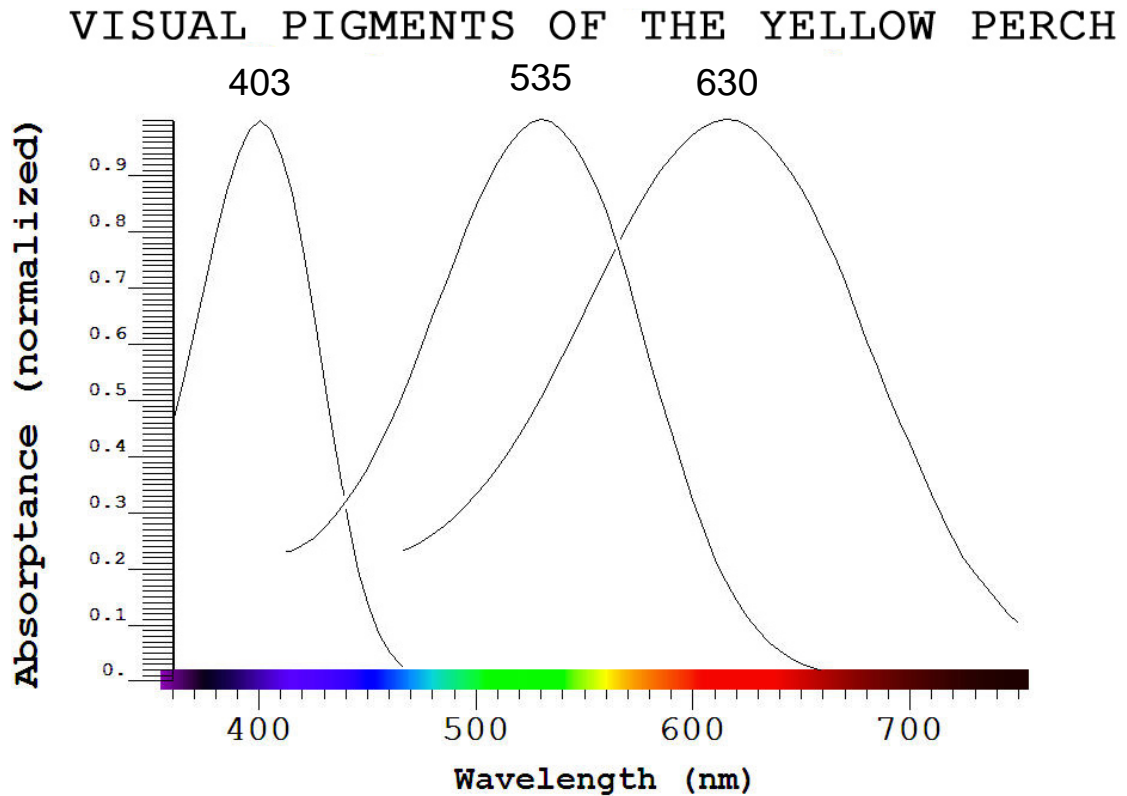


Figure 10 Visual Pigments of the Yellow Perch: Visual pigments absorb light over a region of the spectrum, maximally at the λ_{\max} . Yellow perch have cones with 403, 560, 630nm visual pigments (data from Loew personal communication and Loew and Wahl 1991).

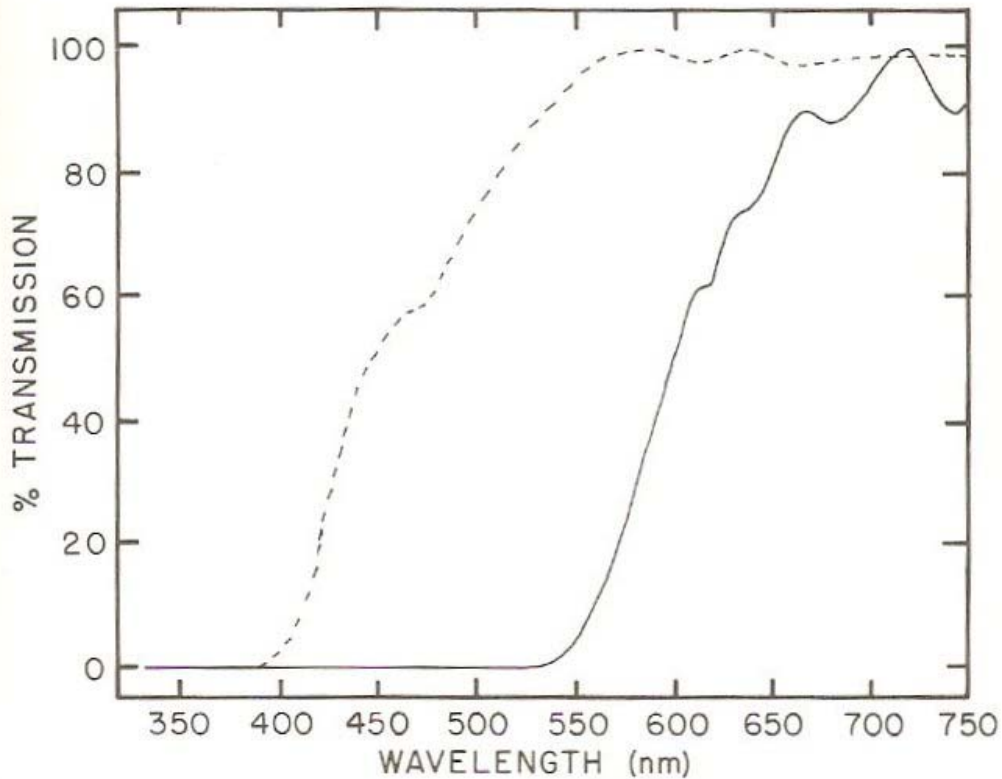


Figure 11 Light transmission of prey species: The first prey species of yellow perch, *Diaptomus sp.* reflects light below 550nm which may make them conspicuous to fish with short wavelength sensitivity. Once these cones are lost, yellow perch feed primarily on *Daphnia pulicaria* which is transmissive to light above 400nm. Loew and McFarland 1990, figure 1.9

wavelength sensitivity may be utilized for prey discrimination via contrast enhancement.

SWS Cone Class Review

Microspectrophotometry (MSP) can identify the spectral characteristics of the expressed visual pigments in isolated cells and therefore locates the λ_{\max} . However, this technique cannot unequivocally allocate a visual pigment to a particular opsin class. The opsin class can only be definitively determined by molecular-biological characterization, which also allows examination of the tuning sites. Loew and Wahl (1991) speculated that the corner cones of yellow perch contain an ultraviolet sensitive pigment, which would make it an SWS1 using current classifications. This was based on analogy to what was known about the corner cones of salmonids (Bowmaker and Kunz 1987, see also Allison *et al* 2003). However, a λ_{\max} at 403nm is beyond the upper end of the tuning range for known SWS1 pigments in teleosts. If the corner cones are expressing an SWS2, the λ_{\max} would be at the very lower limit of this class. In addition, exclusive SWS2 expression in corner cones has not been described previously. In rainbow trout (*Oncorhynchus mykiss*), SWS2 expression in corner cones follows SWS1 expression (Cheng and Novales Flamarique 2007) and again in lingcod (*Ophiodon elongatus*) there may be SWS2 expression after SWS1 expression (Britt as described in Bowmaker and Loew in press) though sequencing has not been completed to confirm this class.

Current Goal

This study extends the MSP work in age-zero yellow perch by using molecular techniques to identify the classes of the expressed opsins and, in particular, to assign the expressed short-wavelength sensitive pigment to its appropriate class. The walleye (*Sander vitreus*, formerly *Stizostedion vitreum vitreum*) was also examined for opsin expression. Wahl (1990) found no evidence of corner cones at any developmental stage and there is not clear evidence of short wavelength sensitivity in the larvae (Burkhardt *et al* 1980).

CHAPTER TWO

Opsin Expression in Yellow Perch, *Perca flavescens* and Walleye, *Sander vitreus*

Fish have been useful in studies of visual ecology due to the wide range of photic environments they inhabit (see Lythgoe 1988 and Munz 1965). Fish adapt their visual system for their photic environment and visual tasks through the use of visual pigments that maximize contrast. This can be done through tuning of the opsins present (Nathans *et al* 1986a) or through differential gene expression (Carleton and Kocher 2001). Since the visual environment and tasks of a fish may change during its lifetime, the visual system should adapt as well (see Chapter One). The retina of fish has the plasticity to change such that a class of cells can disappear from the retina during lifestyle changes as seen in the corner cone loss in brown trout (Bowmaker and Kunz 1987) and other salmonids (see Hawryshyn *et al* 1989) (see Chapter One). These disappearing corner cones are known to express a short wavelength sensitive opsin, specifically SWS1 (Allison *et al* 2003). In some other salmonids such as the rainbow trout, the corner cones do not disappear, but change from SWS1 to SWS2 expression (Cheng and Novales Flamarique 2007). This loss of corner cones or SWS1 expression is temporally aligned with a change in diet, particularly prey species, indicating the SWS1 pigment may be adaptive for increasing the contrast of planktonic prey (see Loew and McFarland, 1990)

The yellow perch (*Perca flavescens*) also have a short wavelength sensitive cell in the corner position of their square mosaic that is present in larvae, but not found in the adult (Wahl 1990). The perch move from off-shore to near shore habitats (personal observation) and their preferred prey species change (Mills *et al* 1984) at approximately the same time that these cells are lost. The loss of these cells may be directly related to the change in feeding as opposed to the change in photic environment. This is because there is a substantial amount of short wavelength photons close to the surface (see Losey *et al* 1999) and the fish are always in the top of the water column whether it is near-shore or off-shore (personal observation, see Methods).

The disappearing corner cones in salmonids belong to the SWS1 class (Allison *et al* 2003) but the opsin class of corner cones in the yellow perch was unknown. The λ_{\max} of these cells is 403nm (Loew and Wahl 1991) which would be beyond the upper end of the range for SWS1 in teleosts or the very lower limit of the range for SWS2 opsins.

This study extends the microspectrophotometry (MSP) on yellow perch (Loew and Wahl 1991) and opsin expression studies in salmonids (Dann *et al* 2004) by using molecular methods to identify the classes of the expressed opsins and, in particular, to assign the short-wavelength pigment to its appropriate class. The walleye (*Sander vitreus* formerly *Stizostedeon vitreum vitreum*) was also examined for opsin

expression. My approach was to utilize retinas from age-zero yellow perch for opsin expression studies.

Methods

Fish Collection

Age-zero yellow perch ranging in size from Standard Length (SL) 19mm to 45mm were collected from Oneida Lake, New York in the summer of 2006 by seining in shallow water (<1 meter deep). Larval walleye were similarly collected in the summer of 2008 (see table 1).

Fish Handling

All fish were dark adapted for a minimum of one hour before anesthetizing with tricaine methanesulfonate (MS-222), decapitating, enucleating the eyes and dissecting out the retinas in deep red or infrared light. Animals were handled in accordance with Cornell University Institutional Animal Care and Use Committee policy under the Animal Use Protocol: 2002-0050.

RNA isolation

Retinas were flash frozen in liquid nitrogen and stored at -80°C. RNA was isolated by homogenizing in TRIzol Reagent (Invitrogen, catalog number 15596-026) and phenol:chloroform extraction. First strand cDNA was synthesized using SuperScript III First-Strand Synthesis

Table 1 Yellow perch and Walleye retinas used for opsin expression. Collection done by seining on the south shore of Oneida Lake, NY.

Species	Date Collected	Size – SL in mm	Used for
Yellow Perch	July 6, 2006	34, 34	LWS
	June 17, 2006	22, 22	RH1
	June 17, 2006	24, 25, 25	RH2
	June 12, 14, 17, 2006	19, 21, 22, 22, 22, 23, 23, 23	SWS2
	July 6, 2006	35, 37, 37, 45	SWS2 – larger
	June 12, 14, 17, 2006	22, 22, 22, 22, 22	SWS1
Walleye	June 12, 2008	Larval, not measured	All opsins

SuperMix (Invitrogen, catalog number 18080-400), according to the manufacturer's instruction.

RH2 amplification

Degenerate primers designed by Helvik *et al* (2001) based on conserved regions of several vertebrate opsins were used to amplify the sequence for RH2 in yellow perch and walleye.

Forward primer: AAG AAG YTC MGT CMA CCT CTY AAY T,

Reverse primer: GGT CAT GAA GAC RTA GAT DAY AGG GTT

RTA, Abbreviations: D: G/A/T, M: A/C, R: G/A, Y: T/C.

LWS amplification

Primers designed to closely related fish LWS opsin (*Dimidiochromis* GenBank number AF247131, *Metriaclima* GenBank number AF247126, *Girella* GenBank number AB158261) were used to amplify LWS pigment sequence for both yellow perch and walleye. Forward primer: ATG GCA GAA GAG TGG GGA AAA, Reverse primer: TTA TGC GGG AGC CAC AGA GGA.

RH1 amplification

Primers designed to closely related fish SWS1 amplified RH1 in yellow perch. Forward primer: ACB CAG AAG GCB GAG AAG GA, Reverse primer: CTG TTT GTT CAT GAA GRC GTA GAT, Abbreviations: B: C/G/T, R: A/G.

SWS2 amplification

PCR product from use of primers designed by Helvik *et al* (2001) above with yellow perch cDNA was restricted with AelI (OliI, Fermentas Life Science, catalog number ER1631) and yielded the SWS2 opsin sequence. Gene specific primers designed to this SWS2 opsin were then used on both the yellow perch and walleye and as control on perch brain and fin tissue. Forward primer: TGT GGT CTC TTG CTG TGG TAG CTT, Reverse primer: GCC CAC GGT TAT TCA CAA CCC AAA.

SWS1 amplification

SWS1 opsin sequence was amplified while performing 3' RACE for SWS2 in yellow perch. The sequence was completed with designed primers and used for both yellow perch and walleye cDNA. Forward primer: GTG CAG GTG CAA TGG TGT GTT TCT, Reverse primer: CTG TTT GTT CAT GAA GRC GTA, Abbreviation: R: A/G.

PCR conditions

PCR to amplify opsins was performed in 25 or 50 μ l reactions with the above primers and Platinum PCR Supermix (Invitrogen, catalog number 12532-016) and used under the following conditions: Lid 95°C, 94°C 2 min, 94°C 20 sec, 53°C 30 sec, 72°C 2 min, repeat for 30 cycles, 72°C 10 min.

3' RACE

Three prime RACE was performed using GeneRacer kit (Invitrogen, catalog number L1502-01) and used according to manufacturer's instructions.

Sequencing Conditions

PCR products were ligated with pGEM-T (Promega, catalog number A1360) into DH5α cells. Plasmids were sequenced on an Applied Biosystems Automated 3730xl DNA Analyzer using the primer: TGT AAA ACG ACG GCC AGT.

Sequence Identification

Sequences were identified by homology searches using BLAST at the National Center for Biotechnology Information (NCBI) website: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. Nucleotide and amino acid sequences were aligned with ClustalW multiple alignment in BioEdit version 7.0.9.0 (NCSU). Phylogenetic tree analyses were based on the neighbor-joining method.

Results

Opsins expressed

Homology searches using the BLAST database showed the following sequences were obtained in yellow perch: RH1, RH2, LWS, SWS1 and SWS2 (see Appendix 1). All of these are expressed except SWS1, where sequence data suggest genomic

contamination due to the inclusion of non-coding regions (see Appendix 2). No change in SWS2 expression was seen in yellow perch with standard lengths 35 to 45mm. Walleye cDNA was synthesized and homology searches showed the following sequences: RH2, LWS, SWS1 and SWS2. Again, SWS1 appears to be from genomic contamination and was not found expressed. The nucleotide sequences for all opsins were aligned with published opsin sequences for the same class from other Perciformes fish and can be found in Appendix 2.

Sequence Portions

From the above nucleotide sequences, the deduced amino acid sequences for yellow perch contain the following portions of the opsins:

- LWS: before transmembrane (TM) segment one to after TM seven
- RH2: from the start of TM two through the end of TM seven as expected with Helvik *et al* (2001) primers
- RH1: TM six and seven
- SWS1: complete coding sequence
- SWS2: beginning of TM two through the end of TM seven

Conserved Residues

These deduced amino acid sequences show several conserved residues required for teleost opsins such as lysine on TM seven for binding the chromophore, glutamate in TM3

and two cystines in the 1st and 2nd extracellular loops for the disulfide bridge. (Helvik *et al* 2001)

Hydrophobicity regions

The deduced amino acid sequence for the SWS2 opsin nucleotide sequence was compared with closely related fish in a hydrophobicity plot which suggests the seven transmembrane segments characteristic of opsins (Figure 12).

Identity within and between Percidae family

The deduced amino acid sequences, when compared to other Teleost opsin families, have identities ranging from approximately 20% (for Walleye SWS1) to 70% (for Yellow Perch LWS). Identities between yellow perch and walleye opsin classes are between 20% (for SWS1) and 80% (for RH2) (Tables 2 and 3).

Phylogenetic Analysis

The yellow perch and walleye deduced amino acid sequences of all cone opsins were compared with other fish, *Girella punctata* (nibbler), *Dimidiochromis compressius* (a cichlid), *Hippoglossus hippoglossus* (halibut), *Salmo salmar* (Atlantic salmon), and *Carassius auratus* (goldfish) to show the phylogenetic relationship (Figure 13, GenBank accession numbers in Table 4). Octopus rhodopsin was used as the outgroup.

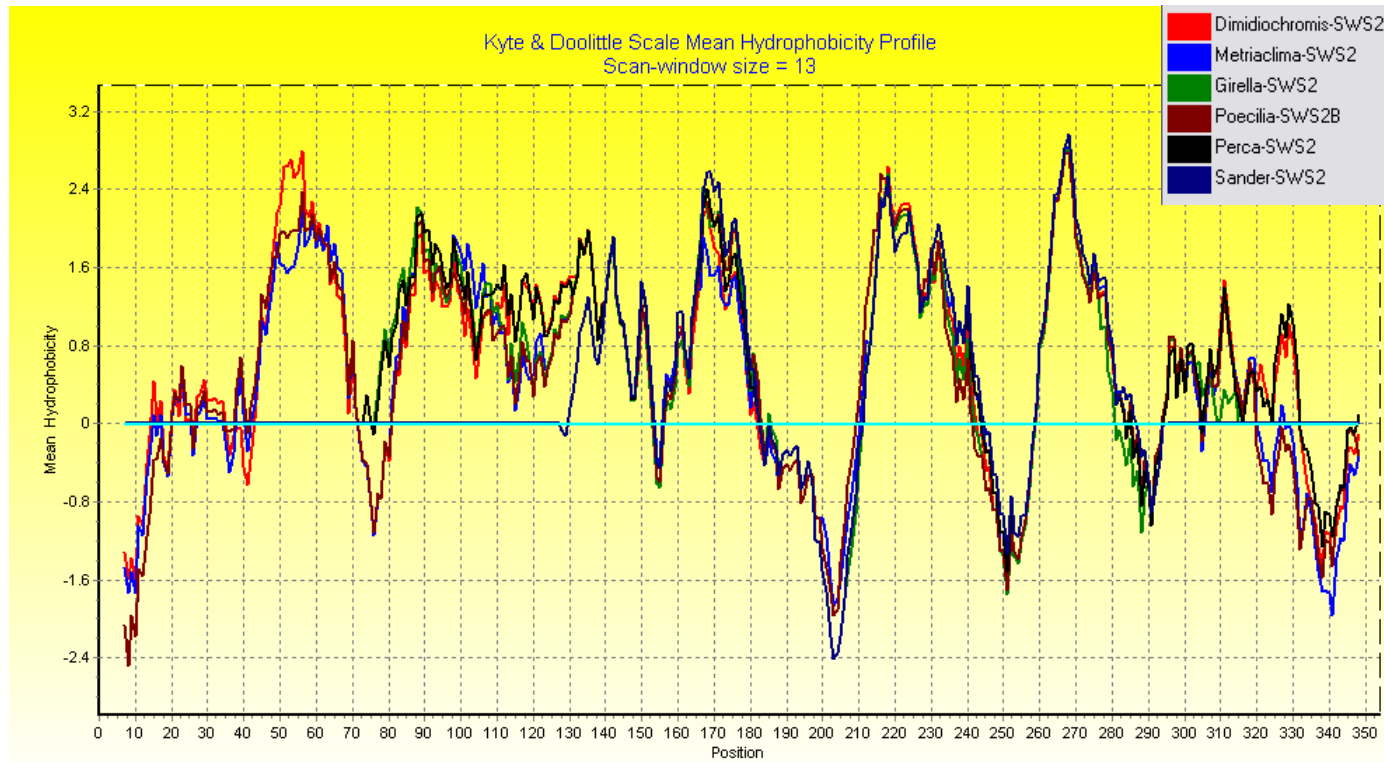


Figure 12 Hydrophobicity of SWS2 opsins: The SWS2 opsin sequences obtained show similar hydrophobicity profiles to known SWS2 opsins indicating the predicted transmembrane segments. Other obtained opsin sequences show similar hydrophobicity profiles (data not shown).

Table 2 Amino acid Identities within representative Teleosts

Seq->	Perca-SWS2	Sander-SWS2	Girella-SWS2	Dimidiochromis-SWS2	Hippoglossus-SWS2	Salmo-SWS2	Carassius-SWS2
Perca-SWS2	ID	0.551	0.66	0.672	0.634	0.589	0.584
Sander-SWS2	0.551	ID	0.548	0.378	0.382	0.344	0.353
Girella-SWS2	0.66	0.548	ID	0.532	0.507	0.497	0.501
Dimidiochromis-SWS2	0.672	0.378	0.532	ID	0.832	0.707	0.713
Hippoglossus-SWS2	0.634	0.382	0.507	0.832	ID	0.683	0.686
Salmo-SWS2	0.589	0.344	0.497	0.707	0.683	ID	0.697
Carassius-SWS2	0.584	0.353	0.501	0.713	0.686	0.697	ID
Seq->	Perca-SWS1	Sander-SWS1	Girella-SWS1	Dimidiochromis-SWS1	Hippoglossus-SWS1	Salmo-SWS1	Carassius-SWS1
Perca-SWS1	ID	0.234	0.548	0.846	0.706	0.716	0.654
Sander-SWS1	0.234	ID	0.279	0.248	0.227	0.221	0.208
Girella-SWS1	0.548	0.279	ID	0.58	0.545	0.559	0.502
Dimidiochromis-SWS1	0.846	0.248	0.58	ID	0.814	0.814	0.755
Hippoglossus-SWS1	0.706	0.227	0.545	0.814	ID	0.743	0.716
Salmo-SWS1	0.716	0.221	0.559	0.814	0.743	ID	0.773
Carassius-SWS1	0.654	0.208	0.502	0.755	0.716	0.773	ID

Table 2 (continued)

Seq->	Perca- RH2	Sander- RH2	Girella- RH2	Dimidiochromis- RH2	Hippoglossus- RH2	Salmo- RH2	Carassius- RH2
Perca-RH2	ID	0.837	0.763	0.619	0.613	0.549	0.535
Sander-RH2	0.837	ID	0.776	0.571	0.565	0.508	0.495
Girella-RH2	0.763	0.776	ID	0.588	0.568	0.531	0.51
Dimidiochromis- RH2	0.619	0.571	0.588	ID	0.9	0.776	0.75
Hippoglossus- RH2	0.613	0.565	0.568	0.9	ID	0.74	0.731
Salmo-RH2	0.549	0.508	0.531	0.776	0.74	ID	0.76
Carassius-RH2	0.535	0.495	0.51	0.75	0.731	0.76	ID
Seq->	Perca- LWS	Sander- LWS	Girella- LWS	Dimidiochromis- LWS	Hippoglossus- LWS	Salmo- LWS	Carassius- LWS
Perca-LWS	ID	0.515	0.587	0.736	0.719	0.694	0.719
Sander-LWS	0.515	ID	0.408	0.563	0.554	0.537	0.546
Girella-LWS	0.587	0.408	ID	0.599	0.568	0.549	0.565
Dimidiochromis- LWS	0.736	0.563	0.599	ID	0.907	0.84	0.871
Hippoglossus- LWS	0.719	0.554	0.568	0.907	ID	0.845	0.857
Salmo-LWS	0.694	0.537	0.549	0.84	0.845	ID	0.843
Carassius-LWS	0.719	0.546	0.565	0.871	0.857	0.843	ID

Table 3 Amino acid Identities within Percids

Seq->	Perca-SWS1	Perca-SWS2	Perca-RH1	Perca-RH2	Perca-LWS				
Perca-SWS1	ID	0.314	0.084	0.331	0.339				
Perca-SWS2	0.314	ID	0.135	0.483	0.38				
Perca-RH1	0.084	0.135	ID	0.143	0.1				
Perca-RH2	0.331	0.483	0.143	ID	0.365				
Perca-LWS	0.339	0.38	0.1	0.365	ID				
Seq->	Sander-SWS1	Sander-SWS2	Sander-RH1	Sander-RH2	Sander-LWS				
Sander-SWS1	ID	0.096	No data	0.049	0.051				
Sander-SWS2	0.096	ID	No data	0.372	0.18				
Sander-RH2	0.049	0.372	No data	ID	0.226				
Sander-LWS	0.051	0.18	No data	0.226	ID				
Seq->	Perca-SWS1	Perca-SWS2	Perca-RH1	Perca-RH2	Perca-LWS	Sander-SWS1	Sander-SWS2	Sander-RH2	Sander-LWS
Perca-SWS	ID	0.317	0.084	0.333	0.334	0.221	0.238	0.288	0.265
Perca-SWS2	0.317	ID	0.135	0.48	0.361	0.149	0.551	0.439	0.192
Perca-RH1	0.084	0.135	ID	0.143	0.1	0.356	0.131	0.103	0.003
Perca-RH2	0.333	0.48	0.143	ID	0.362	0.142	0.348	0.837	0.21
Perca-LWS	0.334	0.361	0.1	0.362	ID	0.124	0.225	0.325	0.521
Sander-SWS1	0.221	0.149	0.356	0.142	0.124	ID	0.129	0.094	0.015
Sander-SWS2	0.238	0.551	0.131	0.348	0.225	0.129	ID	0.372	0.157
Sander-RH2	0.288	0.439	0.103	0.837	0.325	0.094	0.372	ID	0.219
Sander-LWS	0.265	0.192	0.003	0.21	0.521	0.015	0.157	0.219	ID

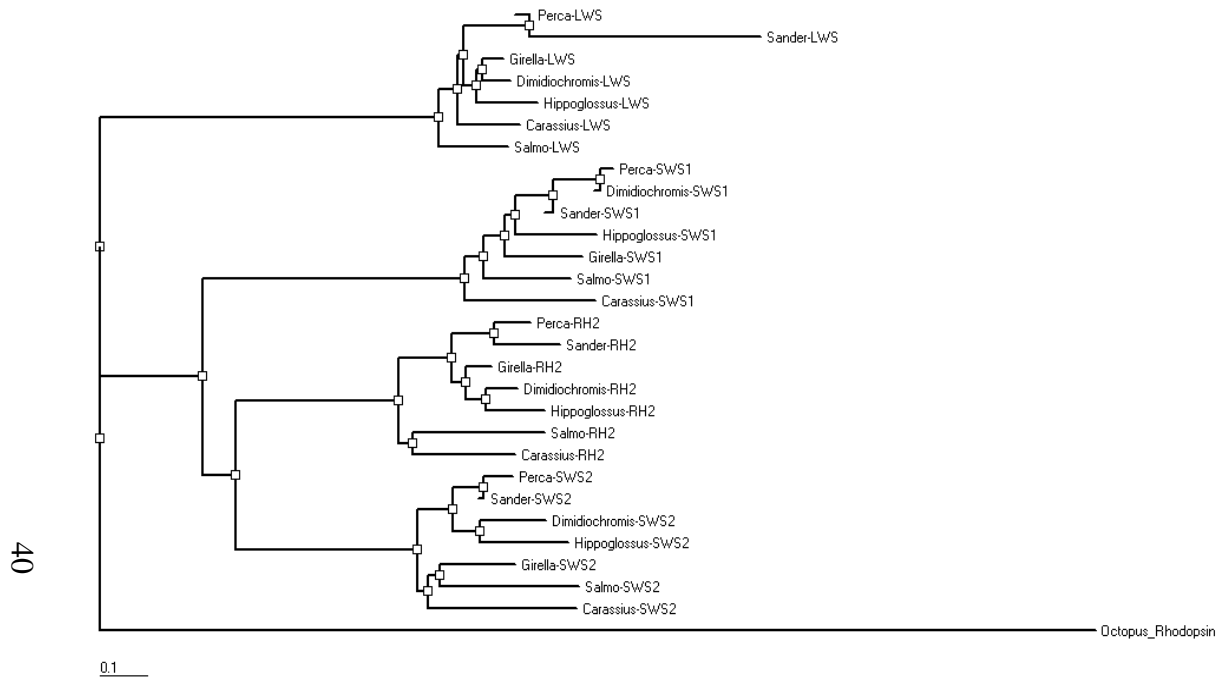


Figure 13 Phylogenetic tree of deduced and known amino acid sequences: Deduced amino acid sequences compared with amino acid sequences from GenBank (accession numbers in table 4) using the neighbor joining method shows the predicted groupings. Octopus rhodopsin was used as the outgroup (Genbank accession number X07797). 1000 bootstrap replicates.

Table 4. Genbank accession numbers used for figure 13.

	SWS1	SWS2	RH2	LWS
<i>Girella punctata</i>	AB158257	AB158256	AB158259	AB158261
<i>Dimidiochromis compressius</i>	AF191220	AF247129	AF247130	AF247131
<i>Hippoglossus hippoglossus</i>	AF156264	AF316497	AF156263	AF316498
<i>Salmo salmar</i>	AY214133	AY214134	AY214132	AY214131
<i>Carassius auratus</i>	D85863	L11864	L11866	L11867

The above analyses clearly support the previously presented compliment of opsin genes present and their expression patterns.

Discussion

Yellow perch expresses SWS2 pigment

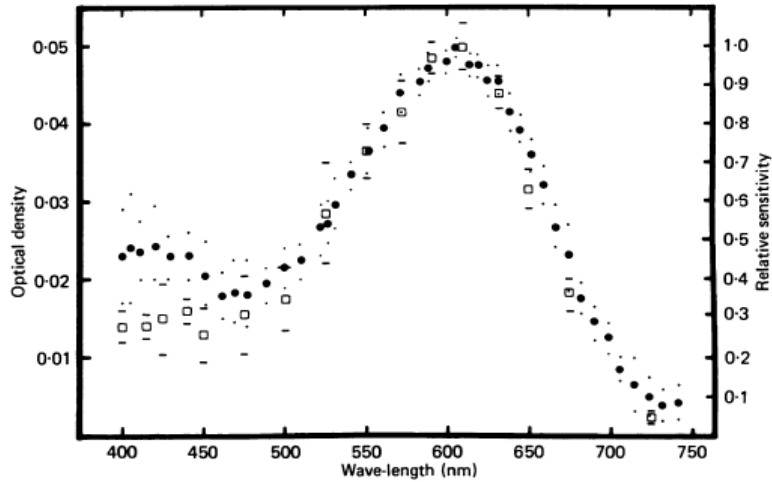
In the juvenile yellow perch, Loew and Wahl (1991) found a short-wavelength sensitive cell by MSP containing a 403nm A₂-based visual pigment. These cells are most likely the corner cones of the mosaic that are lost when the fish undergoes a significant change in prey preference and moves to near-shore waters (Wahl 1990, personal observation) which changes both the contrast needs and spectral environment of the fish. Behavioral studies show loss of short wavelength sensitivity at this same time (Loew *et al* 1993). However, the class of opsin in these short wavelength sensitive cells was not known. Based on extension from the salmonids (Bowmaker and Kunz 1987), Loew and Wahl suggested that these cells have an ultraviolet pigment, but the molecular evidence presented above indicates it is a violet (SWS2) pigment. Assigning this opsin to its appropriate class was the main objective of this study.

Walleye expresses SWS2 pigment

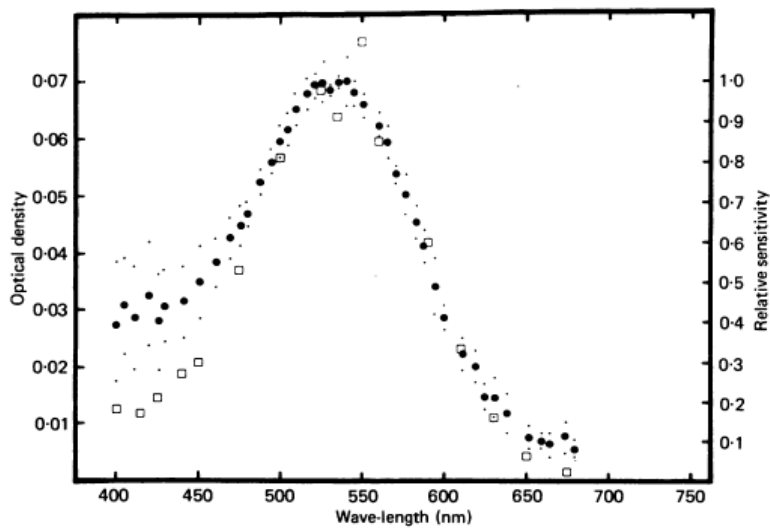
Expression of SWS2 in larval walleye in this study was unexpected as there is no histological evidence for corner cones or short wavelength sensitivity at any age (Wahl 1990, personal communication). Burkardt *et al* (1980) describes unexpected variability in the short wavelength end of the spectrum between measuring the absorbance spectrum using MSP versus the action spectrum of both the long and medium wavelength sensitive cones (see Figure 14). The SWS2 opsin may be coexpressed in these cells leading to this observation. Or it may be expressed in a class of cones that have not been identified histologically, developing cells at the periphery which are rapidly lost or in the large accessory outer segments of the walleye. An *in situ* study utilizing a probe of the SWS2 sequence would locate the expression within the cells of the Walleye mosaic.

SWS2 tuning

The yellow perch have tuned their SWS2 pigment to the shortest known λ_{\max} of this class in teleosts at 403nm (Figure 15) while utilizing an A₂ based retinal. No teleost has a described SWS1 pigment with λ_{\max} higher than 374nm although other vertebrates have tuned the SWS1 pigment to higher wavelengths. The form of retinal used with the opsin can shift the λ_{\max} slightly as noted previously. The yellow perch are not utilizing an A₁ based retinal or expressing the



A.



B.

Figure 14 Walleye cone MSP and action spectra: The MSP values (large circles) from walleye double (A) and single (B) cones both show peaks in the short wavelength region of the spectrum which are above those recorded for action spectra (squares) in the same cell types. Burkhardt 1980.

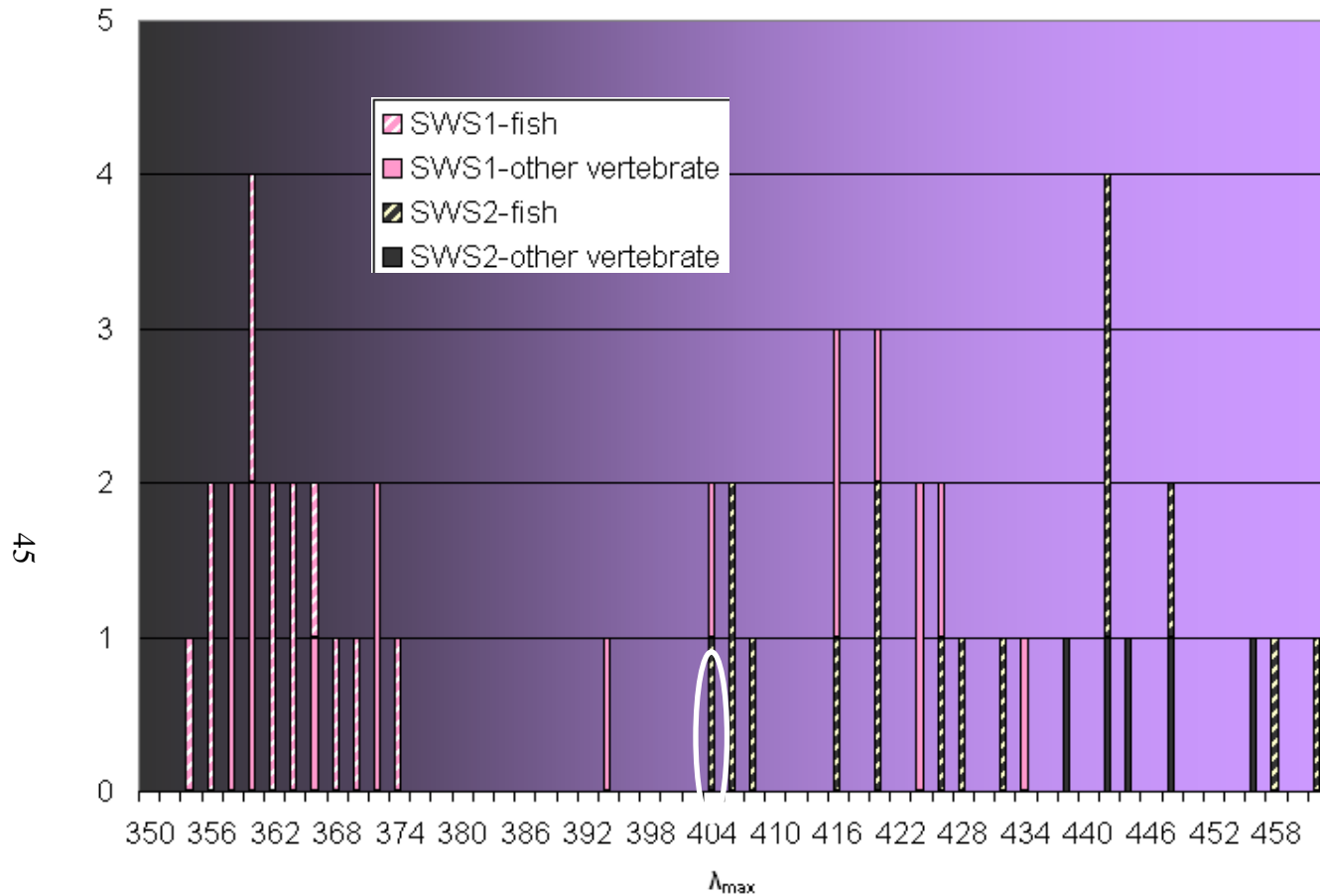


Figure 15 λ_{max} distribution of SWS pigments: The λ_{max} of both SWS1 and SWS2 shows that fish have not increased the λ_{max} of their SWS1 pigments above 374nm although other vertebrates have. Fish have greater variability of their SWS2 pigment λ_{max} . Yellow perch have the shortest λ_{max} value for an SWS2 pigment described (circled).

SWS1 pigment present in their genome indicating that violet sensitivity may be more adaptive than ultraviolet sensitivity for planktivory in their environment. If only the SWS2 can be functional due to stop codons or a non-functional promoter region of the SWS1 gene, then moving the SWS2 to be sensitive to as short of wavelengths as possible may be the best for adaptation. There may also be physiological differences or properties of the opsins that make SWS1 and SWS2 cells different. According to Hawryshyn (1992) and Deutchlander, *et al* (2001), the SWS1 cones may be sensitive to polarized light just like the red and green sensitive cells whereas SWS2 cones are not. For a review of mammalian SWS cone differences (versus LWS and MWS) see Petry and Murphy (1995).

SWS2 in related species

Since both the yellow perch and walleye express SWS2 opsin, it may be ubiquitous in the superfamily Percoidea since *Girella punctata* express it as well (Miyazaki *et al* 2005). The entire percidae family, including darters and saugers, should be examined from the standpoint of determining what genes are present in the genome, where their short wavelength sensitive expression is occurring by *in situ*, when exactly it turns off as well as behavioral experiments to determine if it is used for color vision.

Corner cone loss

Some fish such as goldfish (*Carassius auratus*) keep short wavelength sensitivity with both SWS1 (Hisatomi *et al* 1996) and SWS2 (Johnson *et al* 1993) expression throughout their lifetime. The yellow perch loses the cells associated with SWS2 expression (Loew and Wahl 1991) at approximately 30mm SL and do not show SWS1 expression at any examined age. This may be due to the fact that the retina is a high energy consumer and keeping cells which are no longer needed may be maladaptive. Or these cells may not disappear, they may become more difficult to identify due to their low frequency. The loss of these cells may make room for other cells such as the rods often found in the position previously used by these cells. Lyall (1957) presumed that these cells transmuted into rods directly. Also, as these fish age, their corneas become yellow which blocks light of shorter wavelengths (Kennedy and Milkman 1971). Yellow perch up to 35mm SL show expression of the SWS2 opsin indicating that the cell loss may not occur instantaneously given that Wahl *et al* (1993) describe the loss as happening at about 30mm.

Summary/Future Studies

Yellow Perch are similar to other orders of fish (such as Salmoniforms) in that they have mosaics including corner cones containing a short wavelength sensitive opsin and that these cells disappear when no longer needed for planktivory. The yellow perch are distinctive in that the opsin class they

use for short wavelength sensitivity is the SWS2 and not the SWS1 class of cells. The expression of the SWS2 gene and not the SWS1 gene indicates that violet sensitivity may be more adaptive for planktivory in their environment than ultraviolet sensitivity. These findings indicate that fish may be plastic in their opsin expression for adaptation. Yellow perch retinas should be stained with *in situ* using an SWS2 probe to localize the expression to its cell type, mostly likely the corner cones of the mosaic. In addition, quantitative PCR should indicate declining levels of SWS expression as would be expected since the cells are disappearing. A study examining the neural pathway of these cells would indicate what other changes in the retina occur due to the loss of an entire class of photoreceptors.

APPENDIX 1: SEQUENCES

Nucleotide sequences:

	5	15	25	35	5	15
Bos-RH1	-----	-----	-----	-----	ATGAACGGGA	CCGAGGGCCC
Perca-SWS1	-----	-----	-----	-----	-----	-----
Perca-SWS2	-----	-----	-----	-----	-----	-----
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	-----	-----	-----	-----	-----	-----
Perca-LWS	----GCAGAA	GAGTGGGGAA	AACAGGCATT	TGCTGCCAGG	CGGTACAACG	AAGATACAAC
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-RH2	-----	-----	-----	-----	-----	-----
Sander-LWS	TATGGCAGAA	GAGTGGGGAA	AACAGGCATT	TGCTGCCAGG	CGGTACAGTG	ACGATACAAC

	25	35	45	55	65	75
Bos-RH1	AAACTTCTAC	GTGCCTTTCT	CCAACAAGAC	GGGCGTGGTG	CGCAGCCCCT	TCGAGGCCCC
Perca-SWS1	-ATGGGAAAA	CACTTCCACC	TGTACGAGAA	CATCTCCAAA	ATCAGTCCCT	TTGAGGGGCC
Perca-SWS2	-----	-----	-----	-----	-----	-----
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	-----	-----	-----	-----	-----	-----
Perca-LWS	A---AGCGGT	GGCTTTGCTT	ACACAAACAG	CAATCATACC	AAAGGTCCTT	TTGAGGGTCC
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-RH2	-----	-----	-----	-----	-----	-----
Sander-LWS	AGCGAGTGGT	GGCTTTGTTT	ACGTAAACAG	CAATAATACC	AGAGGTCCTT	TTGAGGGTCC

	85	95	105	114	124	134
Bos-RH1	GCAGTACTAC	CTGGCGGAGC	CATGG-CAGT	TCTCCATGCT	GGCCGCCTAC	ATGTTCTCTGC
Perca-SWS1	CCAGTATTAC	CTGGCCCCTG	TCTGG-GCCT	TTTACCTGCA	GGCCGCCTTT	ATGGGTTTTG
Perca-SWS2	-----	-----	-----	-----	-----	-----
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	-----	-----	-----	-----	-----	-----

Perca-RH2	GCTTGTTCCG	ATTCACAATC	ACCATCATAT	CTGCTTTCAG	CGGCTACTTC	ATTCTCGGAG
Perca-LWS	CCATTCTTGC	CAGCACCATC	AGCGTATGCA	ACCAGTTTTT	TGGTTACTTC	ATTCTGGGAC
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-RH2	GCCTGTTTGG	GTTCACTATA	ACCATCATAT	CTGCTTTCAG	TGGCTACTTC	ATTCTTGGAG
Sander-LWS	CAATTCTCGC	CAGCACCATC	AGTGTATGCA	ACCAGTTCTT	TGGTTACTTC	ATTCTGGGAC

	324	334	344	354	364	374
Bos-RH1	CCACGGGCTG	CAACCTGGAG	GGCTTCTTTG	CCACCTTGGG	CGGTGAAATT	GCACTGTGGT
Perca-SWS1	ACACGCTGTG	TGCTCTGGAG	AGTGCTGTGG	GCTCCGTGGC	AGGTCTGGTG	ACCGCCTGGT
Perca-SWS2	CACTAGCATG	CAAGATTGAA	GGTTTCCTGG	CAACACTTGG	CGGTATGGTA	AGCTTGTGGT
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	CCACTTCCTG	CGCTATTGAG	GGATTCATGG	CCACACTTGG	AGGTGAAGTT	GCTCTATGGT
Perca-LWS	ACCCAATGTG	CATCTTTGAG	GGCTACACTG	TCGCATGTTG	TGGTATTGCT	GGTCTCTGGT
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	-----	-----	-----	-----	-----	----TGTGGT
Sander-RH2	CCACTTCCTG	CGCTATTGGG	GGATTCATGG	CCACACTTGG	AGGTGAAATT	TCTCTATGGT
Sander-LWS	ACCCAATGTG	CGTCTTTGAG	GGCTACACTG	TCGCATGTTG	TGGCATTGCT	GGTCTCTGGT

	384	394	404	414	424	434
Bos-RH1	CCTTGGTGGT	CCTGGCCATC	GAGCGGTACG	TGGTGGTGTG	CAAGCCCATG	AGCAACTTCC
Perca-SWS1	CTTTGGCGGT	GCTTTCCTTT	GAGAGGTACC	TGGTCATTTG	TAAACCGTTC	GGAGCCTTTA
Perca-SWS2	CTCTTGCTGT	GGTAGCTTTT	GAAAGATGGC	TGGTCATCTG	CAAGCCACTT	GGAAACTTTA
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	CCCTTGTAGT	CCTGGCTGTT	GAGAGATACG	TTGTCGTCTG	CAAACCCATG	GGAAGCTTCA
Perca-LWS	CCCTCACTAT	CATCTCCTTT	GAGAGATGGA	TAATTGTGTG	CAAACCTTTT	GGAAATGTCA
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	CTCTTGCTGT	GGTAGCTTTT	GAAAGATGGC	TAATCATCTG	CAAGCCACTT	GGAAACTTTA
Sander-RH2	CCCTTGTAGT	CCTGGCTGTT	GAGAGATACA	TTGTCGTCTG	CAAACCCATG	GGAAGCTTCA
Sander-LWS	CCCTCACTAT	CATCTCCTTT	GAGAGATGGA	TAATTGTGTG	CAAACCTTTT	GGAAACGTTA

	444	454	464	474	484	494
Bos-RH1	GCTTCCGGGA	GAACCACGCC	ATCATGGGCG	TCGCCTTCAC	CTGGGTCATG	GCTCTGGCCT
Perca-SWS1	AGTTTGGCTC	AAATCACGCT	CTGGCTGCAG	TGGCCTTCAC	CTGGTTCATG	GGCATCGGCT
Perca-SWS2	TTTTCAAGCC	TGACCATGCT	GTCGCTTGCT	GTGTATTTAC	CTGGGTGTTT	GGACTGATTG

Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	AGTTTCAGTGG	AACTCATGCA	GGAGCTGGAG	TGCTTTTTCAC	CTGGATCATG	GCTTTTGCAT
Perca-LWS	AGTTTGATTC	CAAAATGGCC	ATAGCTGGAA	TAGTGTTCTG	CTGGGTTTGG	GCAGCGGCTT
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	TTTTCAAGCC	TGACCATGCT	GTCGCTTGCT	GTGTATTTAC	CTGGGTGTTT	GCACTGATTG
Sander-RH2	AGTTTCAGTGG	AACTCATGCA	GGAGCTGGAG	TGGCTTTTTCAC	CTGGATCATG	GCTCTTGCAT
Sander-LWS	AGTTTGATGC	CAAAATGGCC	ATAGCTGGAA	TAGTGTTCTG	CTGGGTTTGG	GCAGCGTTTT

	504	514	524	534	544	554
Bos-RH1	GTGCCGCGCC	CCCCCTCGTC	GGCTGGTCCA	GGTACATCCC	GGAGGGCATG	CAGTGCTCGT
Perca-SWS1	GTGCCTGCC	ACCTTTCTTT	GGCTGGAGCA	GGTACATCCC	TGAAGGTCTG	GGCTGCTCCT
Perca-SWS2	CTTCACTTCC	TCCACTGTTC	GGATGGAGCA	GGTACATCCC	AGAAGGCTTG	CAGTGCTCCT
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	GTGCTGGACC	CCCACTGTTT	GGCTGGTCCA	GGTACATTCC	TGAGGGCATG	CAGTGCTCCT
Perca-LWS	GCACTGCTCC	CCCCGTCTTT	GGATGGAGCA	GGTACTGGCC	TCATGGACTG	AAGACTTCCT
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	TCTCACTTCC	TCCACTGTTC	GGATGGAGCA	GGTACATCCC	AGAAGGCCTG	CAGTGCTCCT
Sander-RH2	GCGCTGCACC	CCCACTGTTT	GGCTGGTCCA	GGTACATTCC	TGAGGGCATG	CAGTGCTCCT
Sander-LWS	GGACTGCTCC	CCCCGTCTTT	GGATGGAGCA	GGTACTGGCC	TCATGGACTG	AAGACTTCCT

	564	574	584	594	604	614
Bos-RH1	GCGGGATTGA	CTACTACACG	CCCCACGAGG	AGACCAACAA	TGAGTCGTTT	GTCATCTACA
Perca-SWS1	GTGGACCCGA	CTGGTACACT	CACAATGAGC	AGTACAACAC	GACCAGCTAC	ACACACTTCC
Perca-SWS2	GTGGTCCAGA	CTGGTACACC	ACAAACAACA	AATACAACAA	TGAATCCTAC	GTGATCTTTC
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	GTGGACCTGA	CTACTACACT	CTGGCTCCAG	GCTACAACAA	TGAATCATAT	GTCATCTACA
Perca-LWS	GTGGACCTGA	TGTATTTCAGT	GGAAGTACAG	ACCCTGGAGT	CCAGTCCTAC	ATGATTTTTT
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	GTGGTCCAGA	CTGGTACACC	ACAAACAACA	AATACAACAA	TGAATCCTAC	GTGATCTTTC
Sander-RH2	GTGGACCTGA	CTACTACACT	TTGGCTCCAG	GCTTCAACAA	TGAATCATAT	GTCATCTACA
Sander-LWS	GTGGACCTGA	TGTATTTCAGT	GGAAATACAG	ACCCTGGAGT	CCAGTCCTAC	ATGATTACGC

	624	634	644	654	664	673
Bos-RH1	TGTTTCGTGGT	CCACTTCATC	ATCCCCCTGA	TTGTCATATT	CTTCTGCTAC	-GGGCAGCTG
Perca-SWS1	TCATGGTCAC	CTGCTTCATC	ATACCTCTCT	CCATCATCAT	CTTCTGCTAC	-TCCCAGCTG

Perca-SWS2	TCTTCGGCTT	CTGCTTTGCT	GTTCCATTCA	CCACCATTAT	TTTCTGCTAT	-GGTCAGCTG
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	TGTTTTGCTGT	CCACTTCTTC	ACTCCCGTCT	CCATCATTTT	CTTCAGTTAT	-GGATGCCTT
Perca-LWS	TTATGATTAC	ATGTTGTTTC	ATTCTCTGG	GCATCATCTT	TGTTTTGCTAC	CTTGCAGTT-
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	TCTTTGGCTT	CTGCTTTGCT	GTTCCATTCA	CCACCATTAT	CTTCTGCTAT	-GGTCAGCTG
Sander-RH2	TGTTTTGCTGT	CCACTTCTTC	ACTCCCGTCT	TCATCATTTT	CTTCAGTTAT	-GGATGCCTT
Sander-LWS	TTATGATTAC	ATGTTGCTTC	ATTCTCTTTA	GCATCATCAT	TGTTTTGCTAC	CTTGCAGGTA

	683	693	703	713	722	732
Bos-RH1	GTGTTACCG	TCAAGGAGGC	GGCTGCCAG	CAGCA-GGAG	TCGGCCACCA	CTCAGAAGGC
Perca-SWS1	CTGGGTGCTC	TGAGAGCAGT	GGCAGCCAG	CAGGC-TGAG	TCAGCCTCCA	CCCAGAAGGC
Perca-SWS2	CTCATTGTGA	TGAAAATGGC	AGCAAAGGCC	CAAGC-AGAG	TCTGTCTCCA	CCCAGAAAGC
Perca-RH1	-----	-----	-----	-----	-----ATTA	CTCAGAAGGC
Perca-RH2	GTGCTGACAG	TCAAAGCTGC	TGCCGCCAAG	CAGCA-GGAG	TCAGAGTCCA	CCCAGAAGGC
Perca-LWS	TTTATGGCTA	TCCATTCAGT	TGCTATGCAG	CAGAA-GGAA	TCAGAGTCAA	CCCAGAAAGC
Sander-SWS	-----	-----GT	TGCAGCCAG	CAGGC-TGAG	TCAGCCTCCA	CCCAGAAGGC
Sander-SWS	CTCATTGTGA	TGAAAATGGC	AGCAAAGGCC	CAAGT-AGAG	TCTGTCTCCA	CCCAGAAAGC
Sander-RH2	GTGCTGACAG	TCAAAGCTGC	CGCAGCCAAG	CAGCA-GGAG	TCAGAGTCCA	CCCAGAAGGC
Sander-LWS	TTTATGGCTA	TCCATTCAGT	TGCTATGCAG	CAGAAAGGAA	TCAGAGTCAA	CCCAGA----

	742	752	762	772	782	792
Bos-RH1	CGAGAAGGAG	GTCACCCGCA	TGGTGATCAT	CATGGTCATC	GCTTTCCTAA	TCTGCTGGCT
Perca-SWS1	GGAGAAGGAG	GTGTCAAGAA	TGATCATCGT	TATGGTGGGG	TCCTTCGTCA	CCTGCTATGG
Perca-SWS2	AGAGAAGGAG	GTGACCAAGA	TGGTGGTCCT	CATGGTGATT	GGCTTCCTGG	TGTGCTGGTT
Perca-RH1	SGAGAAGGAA	GTCTCCCRYA	TGGTAGTGAT	GATGGTCGTC	TCCTACCTGG	TGTGTTGGTT
Perca-RH2	AGAGAGGGAA	GTGACACGCA	TGTGTTCTT	GATGGTTATG	GGCTTCCTAA	TAGCCTGGGT
Perca-LWS	TGAGAGAGAA	GTATCCAGAA	TGGTCGTGGT	CATGATTGGC	GCATTTTGTT	TCTGCTGGGG
Sander-SWS	GGAGAAGGAG	GTGTCAAGAA	TGATCATCGT	TATGGTGGGG	TCCTTCGTCA	CCTGCTATGG
Sander-SWS	AGAGAAGGAG	GTGACCAAGA	TGGTGGTCCT	CATGGTGATT	GGCTTCCTGG	TGTGCTGGTT
Sander-RH2	AGAGAGGGAA	GTGACACGCA	TGTGCTTCTT	GATGGTTATG	GGCTTCCTAG	TAGCCTGGGT
Sander-LWS	-----	-----	-----	-----	-----	-----

	802	812	822	832	842	852
Bos-RH1	GCCCTACGCT	GGGGTGGCGT	TCTACATCTT	CACCCATCAG	GGCTCTGACT	TTGGCCCCAT

Perca-SWS1	CCCGTACGCC	CTGGCAGCTC	TTTAC-TTTG	CCTACTCCAC	AGATGAAAAC	AAAGACTATC
Perca-SWS2	GCCTTATGCC	TCCTTTGCTC	TTTGGGTTGT	GAATAACCGT	GGGCAACCAT	TCGATCTTCG
Perca-RH1	ACCCTATGCC	AGTGTGGCCT	GGTTTATCTT	TACAAATAAG	GGCTCTGAGT	TTGGACCACT
Perca-RH2	GCCATATGCC	TCTTTTCGCCG	GTTGGATCTT	CCTGAACAAA	GGAGCTTACT	TCTCCCCCCT
Perca-LWS	ACCTTACACT	GTTTTTGCCT	GCTATGCCGC	GGCTAACCCCT	GGATATGCCT	TCCATCCTCT
Sander-SWS	CCCGTACGCC	CTGGCAGCTC	TTTAC-TTTG	CCTACTCCAC	AGATGAAAAC	AAAGACTATC
Sander-SWS	GCCTTATGCC	TCCTTTGCTC	TTTGGGTTGT	GAATAACCGT	GGG-----	-----
Sander-RH2	GCCATATGCC	TCTTTTCGCCG	GTTGGATCTT	CCTGAACAAAG	GGAGCTTACT	TCTCCCCCCT
Sander-LWS	-----	-----	-----	-----	-----	-----

	861	871	881	891	900	910
Bos-RH1	C-TTCATGAC	CATCCCGGCT	TTCTTTGCCA	AGACTTCTGC	CGTC-TACAA	CCCCGTCATC
Perca-SWS1	GACTTGTCAC	CATCCCGGCG	TTTTTCTCTA	AGAGCGCCTG	CGTC-TACAA	CCCACTCATC
Perca-SWS2	A-CTGGCGAC	CATACCTTCC	TGCTTCTCAA	AGGCCTCTGC	AGTC-TACAA	CCCTGTCATC
Perca-RH1	C-TTCATGAC	CCTCCCGGCC	TTCTTTGCCA	AGACTGCAGC	AGTC-TACAA	CCCAGTGATC
Perca-RH2	GACAGCA-GC	TGTACCCGCA	TTCTTTGCAA	AGAGCTCA-G	CATTGTATAA	CCCTGTCATC
Perca-LWS	GGCTGCTTCC	ATGCCTGCAT	ACTTTGCAAA	AAGCGCCACC	ATCTGGAACC	CAGTTATATA
Sander-SWS	GACTTGTCAC	CATCCCGGCG	TTTTTCTCTA	AGAGCGCCTG	CGTC-TACAA	CCCACTCATC
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-RH2	GACAGCAAGC	TATACCCGCC	TTCTTTGCAA	ANAGCTCAAG	CGTTGTACAA	CCCTGTTATC
Sander-LWS	-----	-----	-----	-----	-----	-----

	920	930	940	950	960	970
Bos-RH1	TACATCATGA	TGAACAAGCA	GTTCCGGAAC	TGCATGGTCA	CCACTCTCTG	CTGTGGCAAG
Perca-SWS1	TACGTCTTCA	TGAACAAACA	GTTTAATGGC	TGTATCATGG	AGACAGTGTT	TGGGAAG---
Perca-SWS2	TATATTTTCT	TCAATAAACA	GTTCCGTTCA	TGCATAATGA	CAATGCTGGG	GATAGGTGGA
Perca-RH1	TACG-CTTCA	TGA-----	-----	-----	-----	-----
Perca-RH2	TATGTCTTCA	TGAACAATCA	CCAGTCGAAT	TCGCCGCCGC	CTGCAGGTCG	A-----
Perca-LWS	TGTCTTCATG	AACCGGCAGT	TCCGTTTCATG	TATCATGCAA	CTCTTTGGCA	AACAAGGGGA
Sander-SWS	TACGCCTTCA	TGAAC-----	-----	-----	-----	-----
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-RH2	TACG-----	-----	-----	-----	-----	-----
Sander-LWS	-----	-----	-----	-----	-----	-----

	980	990	1000	1010	1020	1030

Bos-RH1	AACCCGCTGG	GTGACGACGA	GGCCTCCACC	ACCGTCTCCA	AGACAGAGAC	CAGCCAAGTG
Perca-SWS1	-----	-----	-----	-----	-----	-----
Perca-SWS2	GGTGAGGAGG	AATCCTCAAC	AACATCATCA	GTGACTGAAG	TCTCCAAAGT	TGGGCCTGCT
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	-----	-----	-----	-----	-----	-----
Perca-LWS	TGATGGCTCT	GAGGTATCCA	CATCAAAGAC	AGAGGTCTCC	TCTGTGGCTC	CCGCA-----
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-SWS	-----	-----	-----	-----	-----	-----
Sander-RH2	-----	-----	-----	-----	-----	-----
Sander-LWS	-----	-----	-----	-----	-----	-----

.....|.....| ..
1040

Bos-RH1	GCGCCTGCCT	AA
Perca-SWS1	-----	--
Perca-SWS2	TAG-----	--
Perca-RH1	-----	--
Perca-RH2	-----	--
Perca-LWS	-----	--
Sander-SWS	-----	--
Sander-SWS	-----	--
Sander-RH2	-----	--
Sander-LWS	-----	--

Deduced amino acid sequences:

Counting per Bovine (*Bos taurus*) Rhodopsin

Highlights: Transmembrane segments per Carelton 2000

Rectangles: conserved sites (Helvik 2001)

Purple circles: tuning sites for SWS1 (Yokoyama 2000)

Pink circles: tuning sites for SWS2 (Yokoyama 2003)

	-8	2	12	22	32	42
Bos-RH1	-----	---MNGTEGP	NFYVPFSNKT	GVVRSPEAP	QYYLAEPWQF	SMLAAYMFLI
Perca-SWS1	-----	-----	MGKHFHLYEN	ISKISPFEGP	QYYLAPVWAF	YLQAAFMGFV
Perca-SWS2	-----	-----	-----	-----	-----	-----
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	-----	-----	-----	-----	-----	-----
Perca-LWS	-AEEWGKQAF	AARRYNEDTT	S-GGFAYTNS	NHTKGPFEAP	NYHIVRDGVS	XISTLWMSMV
Sander-SWS1	-----	-----	-----	-----	-----	-----
Sander-SWS2	-----	-----	-----	-----	-----	-----
Sander-RH2	-----	-----	-----	-----	-----	-----
Sander-LWS	MAEEWGKQAF	AARRYSDDTT	ASGGFVYVNS	NNTRGPFEGP	NYHIAPRWVY	HVATLWMSVY

	52	62	72	82	92	102
Bos-RH1	IMLGFPINFL	TLYVTVQHKK	LRTPLNLYILL	NLAVADLFMV	FGGFTTTLYT	SLHGYFVFGP
Perca-SWS1	FFAGTPLNFI	VLVATMKYKK	LRVPLNFILV	NISFSGFIEV	TFSVSQVFLA	SMRGYYFLGH
Perca-SWS2	-----	-----	-----LKYILV	NLAVANLLVS	AVGSEIACCS	FAFRYFIFGA
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	-----	-----IKK	LSQPLNYILV	NLAVAGLIMC	LFGFTITIIS	AFSGYFILGA
Perca-LWS	VVASLFTNGL	VLVATAKFKK	LRHPLNWILV	NLAVADILET	ILASTISVCN	QFFGYFILGH
Sander-SWS1	-----	-----	-----	-----	-----	-----
Sander-SWS2	-----	-----	-----	-----	-----	-----
Sander-RH2	-----	-----KK	LSQPLNYILV	NLAVAGLIMC	LFGFTITIIS	AFSGYFILGA
Sander-LWS	VVASVFTNGL	VLVATAKFKK	LRHPLNWILV	NLAFADIMET	ILASTISVCN	QFFGYFILGH

	112	122	132	142	152	162
Bos-RH1	TGCNLEGFFA	TLGGEIALWS	LVVLAIERVY	VVCKPMSNFR	FGENHAIMGV	AFTWVMALAC
Perca-SWS1	TLCALESAVG	SVAGLVTAWS	LAVLSFERYL	VICKPFGAFK	FGSNHALAAV	AFTWFMGIGC
Perca-SWS2	LACKIEGFLA	TLGGMVSLWS	LAVVAFERWL	VICKPLGNFI	FKPDHAVACC	VFTWVFLIA
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	TSCALEGFMA	TLGGEVALWS	LVVLAVERYV	VVCKPMGSFK	FSGTHAGAGV	LFTWIMAFAC
Perca-LWS	PMCIPEGYTV	ACCGIAGLWS	LTIISFERWI	IVCKPFGNVK	FDSKMAIAGI	VFCWVWAAAC
Sander-SWS1	-----	-----	-----	-----	-----	-----
Sander-SWS2	-----	-----WS	LAVVAFERWL	IICKPLGNFI	FKPDHAVACC	VFTWVFALIV
Sander-RH2	TSCALEGFMA	TLGGEISLWS	LVVLAVERYI	VVCKPMGSFK	FSGTHAGAGV	AFTWIMALAC
Sander-LWS	PMCIPEGYTV	ACCGIAGLWS	LTIISFERWI	IVCKPFGNVK	FDKMAIAGI	VFCWVWAAFW

	172	182	192	202	212	222
Bos-RH1	AAPPLVGWSR	YIPEGMQCSC	GIDYYTPHEE	TNNESFVIYM	FVVHFIIPLI	VIFFCYGQLV
Perca-SWS1	ACPPFFGWSR	YIPEGLGCSC	GPDWYTHNEQ	YNTTSYTHFL	MVTCFIIPLS	IIIFCYSQLL
Perca-SWS2	SLPPLFGWSR	YIPEGLQCSC	GPDWYTTNKK	YNNESYVIFL	FGFCFAVPFT	TIIFCYGQLL
Perca-RH1	-----	-----	-----	-----	-----	-----
Perca-RH2	AGPPLFGWSR	YIPEGMQCSC	GPDYITLAPG	YNNESYVIYM	FAVHFFTPVS	IIFFSYGCLV
Perca-LWS	TAPPVFGWSR	YWPHGLKTS	GPDVFSGSTD	PGVQSYMIFL	MITCCFIPLG	IIFVCYLAVF
Sander-SWS1	-----	-----	-----	-----	-----	-----
Sander-SWS2	SLPPLFGWSR	YIPEGLQCSC	GPDWYTTNKK	YNNESYVIFL	FGFCFAVPFT	TIIFCYGQLL
Sander-RH2	AAPPLFGWSR	YIPEGMQCSC	GPDYITLAPG	FNNESYVIYM	FVVHFFTPVF	IIFFSYGCLV
Sander-LWS	TAPPVFGWSR	YWPHGLKTS	GPDVFSGNTD	PGVQSYMITL	MITCCFIPLS	IIIVCYLAGI

	232	242	252	262	272	282
Bos-RH1	FTVKEAAQQ	QESATTQKAE	KEVTRMVLIM	VIAFLICWLP	YAGVAFYIFT	HQGSDFGPIF
Perca-SWS1	GALRAVAAQQ	AESASTQKAE	KEVSRMIIVM	VGSFVTCYGP	YALAALYFAY	STDENKDYRL
Perca-SWS2	IVMKMAAKAQ	AESVSTQKAE	KEVTKMVVLM	VIGFLVCWLP	YASFALWVVN	NRGQPFDLRL
Perca-RH1	-----	----ITQKXE	KEVSMVVM	VVSYLVCWLP	YASVAWFIFT	NKGSEFGPLF
Perca-RH2	LTVKAAAQ	QESASTQKAE	REVTRMCFLM	VMGFLIAWVP	YASFAGWIFL	NKGAYFSPLT
Perca-LWS	MAIHSVAMQQ	KESESTQKAE	REVSVMVVVM	IGAFCFCWGP	YTVFACYAAA	NPGYAFHPLA
Sander-SWS1	-----VAAQQ	AESASTQKAE	KEVSRMIIVM	VGSFVTCYGP	YALAALYFAY	STDENKDYRL
Sander-SWS2	IVMKMAAKAQ	VESVSTQKAE	KEVTKMVVLM	VIGFLVCWLP	YASFALWVVN	NRG-----
Sander-RH2	LTVKAAAQ	QESASTQKAE	REVTRMCFLM	VMGFLVAWVP	YASFAGWIFL	NKGAYFSPLT
Sander-LWS	YGYP-----	-----	-----	-----	---FSCYAAE	RN-----

	292	302	312	322	332	342
Bos-RH1	MTIPAFFAKT	SAVYNPVIYI	MMNKQFRNCM	VTTLCCKGNP	LGDDEASTTV	SKTETSQVAP
Perca-SWS1	VTIPAFFSKS	ACVYNPLIYV	FMNKQFNGCI	METVFGK---	-----	-----
Perca-SWS2	ATIPSCFSKA	SAVYNPVIYI	FFNKQFRSCI	MTMLGIGGGE	EESSTTSSVT	EVSKVGPA--
Perca-RH1	MTLPAFFAKT	AAVYNPVIYA	S-----	-----	-----	-----
Perca-RH2	AAVPAFFAKS	SALYNPVIYV	FMNNHQSNP	PPAGR-----	-----	-----
Perca-LWS	ASMPAYFAKS	ATIWNPVIYV	FMNRQFRSCI	MQLFGKQGDD	GSEVSTSKTE	VSSVAPA---
Sander-SWS1	VTIPAFFSKS	ACVYNPLIYA	FMN-----	-----	-----	-----
Sander-SWS2	-----	-----	-----	-----	-----	-----
Sander-RH2	ASYTRLLCKX	LKRCTTLLST	-----	-----	-----	-----
Sander-LWS	QSQPR---	-----	-----	-----	-----	-----

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Bos-RH1	A
Perca-SWS1	-
Perca-SWS2	-
Perca-RH1	-
Perca-RH2	-
Perca-LWS	-
Sander-SWS1	-
Sander-SWS2	-
Sander-RH2	-
Sander-LWS	-

APPENDIX 2: ALIGNMENTS

SWS1 Nucleotide Alignment:

Perca	ATGGGAAAACACTTCCACCTGTACGAGAACATCTCCAAAATCAGTCCCCTTGAGGGGCCAGTATTACCTGGCCCTGTCTGGGCCCTTTACCTGCAGG
Sander	-----
Girella	-----
Metria	ATGGGAAAACACTTCCACCTGTACGAGAACATCTCCAAAATCAGTCCCCTTGAGGGGCCAGTATTACCTGGCCCTGTCTGGGCCCTTTACCTGCAGG
Dimidio	---GGAAAACACTTCCACCTGTACGAGAACATCTCCAAAATCAGTCCCCTTGAGGGGCCAGTATTACCTGGCCCTGTCTGGGCCCTTTACCTGCAGG
Stigmat	ATGGGAAAACACTTCCACCTGTACGAGAACATCTCCAAAATCAGTCCCCTTGAGGGGCCAGTATTACCTGGCCCTGTCTGGGCCCTTTACCTGCAGG
Hippog	ATGGGAAAACACTTCCACCTGTATGAGAACGTTCTAAATGTGAGTCCCCTTCGATGGGGCCAGTATTACCTGGCCCTCAATGGGCCCTTTACCTGCAGG
Salmo_	ATGGGAAAGACTTCCATCTGTACGAGAACATCTCTAAGATCAGCCCAATTGAGGGGCCAGTATTACCTGGCCCTCAATGTGGGCCCTTTACCTGCAGG
Carassius	ATGGACGCGTGGACCTAATCAATTTGGTAACCTCTCCAAAATCAGTCCCCTTGAGGGGCCAGTATTACCTGGCCCTCAAGTGGGCCCTTTACCTGCAGG

Perca-SWS1	CCGCCCTTATGGGTTTTGTCTTTTCTGCTGGCACTCCTTTAAACTTTCATGTCTCTCGTGGCGACGATGAAGTACAAGAACTCAGAGTCCACTCAACTT
Sander-SWS1	-----
Girella-SWS1	-----
Metriaclima	CCGCCCTTATGGGTTTTGTCTTTTCTGCTGGCACTCCTTTAAACTTTCATGTCTCTCGTGGCGACGATGAAGTACAAGAACTCAGAGTCCACTCAACTT
Dimidiochromis	CCGCCCTTATGGGTTTTGTCTTTTCTGCTGGCACTCCTTTAAACTTTCATGTCTCTCGTGGCGACGATGAAGTACAAGAACTCAGAGTCCACTCAACTT
Stigmatochomis	CCGCCCTTATGGGTTTTGTCTTTTCTGCTGGCACTCCTTTAAACTTTCATGTCTCTCGTGGCGACGATGAAGTACAAGAACTCAGAGTCCACTCAACTT
Hippoglossus	CGATCTTCATGGGCTCCGTCCTGTTCTGCTGGCACTCCTTTAAACTTTTATGTCTCTCTGTGACACTCAAGTACAAGAACTCAGAGTCCCTCTCAATTA
Salmo_salar	CTGCTTTCATGGGCTTTTGTCTTTTCTGCTGGAAACCCCTTAAACTTTTAAATCTCTGTTGACAGTGAAGTACAAGAACTGAGACAACTGTAACCT
Carassius	CAGCTTTCATGGGCTTTTGTCTTTTCTGCTGGCAACCTTTGAATGCCATCTCTCTCTTTTACAATGAAGTACAAGAACTCAGACAGCCTCTCAACTA

Perca-SWS1	CATCTCTCTCAACATCTCCTTCTCCGGCTTCATCTTCTCACCCTTCTCTCTCAGTCAGGTGTTCTTAGCTCCATGCGAGGTACTACTTCTTGGGTAC
Sander-SWS1	-----
Girella-SWS1	CATCTCTCTCAACATCTCCTTCTCCGGGATTCATCTTCTCACCATTCTCTCTCTCAGTCAGGTGTTCTTAGCTCCATGCGAGGTACTACTTCTTGGGTAC
Metriaclima	CATCTCTCTCAACATCTCCTTCTCCGGCTTCATCTTCTCACCATTCTCTCTCTCAGTCAGGTGTTCTTAGCTCCATGCGAGGTACTACTTCTTGGGTAC
Dimidiochromis	CATCTCTCTCAACATCTCCTTCTCCGGCTTCATCTTCTCACCATTCTCTCTCTCAGTCAGGTGTTCTTAGCTCCATGCGAGGTACTACTTCTTGGGTAC
Stigmatochomis	CATCTCTCTCAACATCTCCTTCTCCGGCTTCATCTTCTCACCATTCTCTCTCTCAGTCAGGTGTTCTTAGCTCCATGCGAGGTACTACTTCTTGGGTAC
Hippoglossus	CATCTCTCTCAACATCTGCTTCTCAGGATTAATCTTTTGTGGTCTCTCAGTCAGGTGTTTGTCTCACCATGAGGGGTACTTCTCTCTTGGGTCCC
Salmo_salar	CATCTCTCTCAACATCTCTTAGCAGGTTCATCTTTTGTGACGTTTCTCTCTGAGTCAAGTGTGTTTCTAGCGGAGAGGATACTACTTCTTGGGTAC
Carassius	CATCTCTCTCAACATCTCCTTAGGAGGCTTATTTTCTCAGACTTTTCTCTTAAGCCAAGTATTCTTTTCTGCTCTTAGAGGTATTACTTCTCTCGGTTAT

Perca-SWS1	ACGCTGTGTCTCTGGAAGAGTGCTGTGGGCTCCGTGGCAGGTAACCACCCAAATCCATCGGCAGGATGTTACAGGTGTTGTCAGGTGCAATGGTGTGTT
Sander-SWS1	-----
Girella-SWS1	ACCTGTGTCTCTGGAAGGCGCCATGGGATCTATAGCAGGA-----
Metriaclima	ACGCTGTGTCTCTGGAAGAGTGCTGTGGGCTCCGTGGCAGGT-----
Dimidiochromis	ACGCTGTGTCTCTGGAAGAGTGCTGTGGGCTCCGTGGCAGGTAACCACCCAAATCCATCGGCAGGATGTTACAGGTGTTGTCAGGTGCAATGGTGTGTT
Stigmatochomis	ACGCTGTGTCTCTGGAAGAGTGCTGTGGGCTCCGTGGCAGGTAACCACCCAAATCCATCGGCAGGATGTTACAGGTGTTGTCAGGTGCAATGGTGTGTT

Hippoglossus ACAC**TGTGT**GCAC**TGGA**ATCTGCCAT**TGGGTC**GATAGCAGGT-----
 Salmo_salar ACCT**TGTGT**GCAAT**TGGA**AGCTTGCA**TGGGTC**AAATAGCAGGG-----
 Carassius ACAC**TGTGT**GCAAT**TGGA**AGCTGCAA**TGGGTC**GATTGCGGGA-----

Perca-SWS1 **TCTCCCTCCTCCTCAGGTC****TGGTGACCGCCTGGTCTTTGGCGGTGCTTTCTTTGAGAGGTACCTGGTCATTTGTAAACCGTT**CGGAGC**TTTAAGTTTG**
 Sander-SWS1 -----
 Girella-SWS1 -----CT**GGTGACATCC****TGGTCTTTGGCTGTCTGTCTTTGAGAGATACCTGGTCATCTGTAAACCTTT**CGGAGC**TTCAAGTTTG**
 Metriaclima -----CT**GGTGACCGCCTGGTCTTTGGCGGTGCTTTCTTTGAGAGGTACCTGGTCATTTGTAAACCGTT**CGGAGC**TTTAAGTTTG**
 Dimidiochromis **TCTCCCTCCTCCTCAGGTC****TGGTGACCGCCTGGTCTTTGGCGGTGCTTTCTTTGAGAGGTACCTGGTCATTTGTAAACCGTT**CGGAGC**TTTAAGTTTG**
 Stigmatichomis **TCTCCCTCCTCCTCAGGTC****TGGTGACCGCCTGGTCTTTGGCGGTGCTTTCTTTGAGAGGTACCTGGTCATTTGTAAACCGTT**CGGAGC**TTTAAGTTTG**
 Hippoglossus -----CT**GGTCACGTCGTTCCTTTGGCGGTTCCTCTCTTTGAAAGATACCTGGTCATTTGTAAACCGTT**CGGAGC**TTTAGGTTTG**
 Salmo_salar -----TT**GGTGT**CAGCT**TGGTCCCTGGCTGTCCTTTGCTTTGAGAGATACCTGGTCATCTGCAAACCGTT**CGGAGC**TTCAAATTTTG**
 Carassius -----CT**TGTGACAGGATGGTCTCTGGCAGTTCTGGCTTTGAGAGATACCTGGTTATCTGTAAACCTTTT**GAAAG**CTTCAAGTTTG**

Perca-SWS1 GCTCAAAT**CAGC**TCTGGCT**GCAGTGGCCTTCACCTGGTTCATGGGCATCGGCTGTGCC**TGC**CCACCTTTCTTTGGCTGGAGCAGGTACTGTAGGCATCA**
 Sander-SWS1 -----
 Girella-SWS1 GCAATAAT**CAGC**TCTGGCT**GCAGTGGCCTTCACCTGGTTCATGGGCATCGGCTGTGCC**ACC**CCGCGTTCTTCGGCTGGAGCAGGTAC**-----
 Metriaclima GCTCAAAT**CAGC**TCTGGCT**GCAGTGGCCTTCACCTGGTTCATGGGCATCGGCTGTGCC**TGCC**CCACCTTTCTTTGGCTGGAGCAGGTAC**-----
 Dimidiochromis GCTCAAAT**CAGC**TCTGGCT**GCAGTGGCCTTCACCTGGTTCATGGGCATCGGCTGTGCC**TGCC**CCACCTTTCTTTGGCTGGAGCAGGTACTGTAGGCATCA**
 Stigmatichomis GCTCAAAT**CAGC**TCTGGCT**GCAGTGGCCTTCACCTGGTTCATGGGCATCGGCTGTGCC**TGCC**CCACCTTTCTTTGGCTGGAGCAGGTACTGTAGGCATCA**
 Hippoglossus GCAGTAAT**CAGC**CGCAGCGCT**GTGCTTCACCTGGTTCATGGGCATCGGCTGTGCC**ATA**CCACCTTTCTTTGGATGGAGCAGGTAC**-----
 Salmo_salar ACAACA**ACCAGGCTCTGGCGGCTGTGCTTCACCTGGTTCATGGGCATCGGATGTGCC**ACC**CCACCTTTCTTTGGCTGGAGCAGGTAT**-----
 Carassius GACAAAGC**CAGC**ATTGG**CGTTCGCTTCACCTGGATCATAGGTATCGGTTGTGCC**ACT**CCTCCATTTCTGGGATGGAGCAGATAC**-----

Perca-SWS1 **GGTGATTCTGGCCGTGTGTCTCTGCACAGGTGAGCTGACTGTCTGTGTTGGTAGGTACATCCCTGAAGGCTCTGGGCTGCTCCTGTGGA**CC**CGACTGGTA**
 Sander-SWS1 -----
 Girella-SWS1 -----AT**CCCTGAGGTC**TGGGCTG**CTCCTGTGGA**CC**CGACTGGTA**
 Metriaclima -----AT**CCCTGAAGGTC**TGGGCTG**CTCCTGTGGA**CC**CGACTGGTA**
 Dimidiochromis **GGTGATTCTGGCCGTGTGTCTCTGCACAGGTGAGCTGACTGTCTGTGTTGGTAGGTACATCCCTGAAGGCTCTGGGCTGCTCCTGTGGA**CC**CGACTGGTA**
 Stigmatichomis **GGTGATTCTGGCCGTGTGTCTCTGCACAGGTGAGCTGACTGTCTGTGTTGGTAGGTACATCCCTGAAGGCTCTGGGCTGCTCCTGTGGA**CC**CGACTGGTA**
 Hippoglossus -----AT**CCCTGAAGGTC**TGGGTTG**CTCCTGTGGA**CC**CGACTGGTA**
 Salmo_salar -----AT**CCCTGAGGTC**TGGGTTG**CTCCTGTGGA**CC**CGACTGGTA**
 Carassius -----AT**CCAGAGGTA**TGGCACC**CTGCGG**ACC**CGACTGGTA**

Perca-SWS1 **CACTCACAATGAGCAGTACAA**CACGACC**AGCTACACACACTTCCTCA**TGG**TCACTGCTTCATCATACCTCTCTCCATCATCATCTTCTGCTACTCCCAG**
 Sander-SWS1 -----
 Girella-SWS1 **CACCCACAACGAGGAGTATAA**CTGCACC**AGCTACTTATACTTCCTGA**-----
 Metriaclima **CACTCACAATGAGCAGTACAA**CACGACC**AGCTACACACACTTCCTCA**-----
 Dimidiochromis **CACTCACAATGAGCAGTACAA**CACGACC**AGCTACACACACTTCCTCA**TGG**TCACTGCTTCATCATACCTCTCTCCATCATCATCTTCTGCTACTCCCAG**
 Stigmatichomis **CACTCACAATGAGCAGTACAA**CACGACC**AGCTACACACACTTCCTCA**TGG**TCACTGCTTCATCATACCTCTCTCCATCATCATCTTCTGCTACTCCCAG**
 Hippoglossus **CACGCACAACGAGGAGTTT**CACTGCAGC**AGCTACACAACTTCCTGA**-----
 Salmo_salar **CACAAACAACGAGGAGTACC**ACTGTGCC**AGCTACACCAAACTTCCTTA**-----

Perca-SWS1 CTTTCATGAACAAACAGGTTGAGCACACTCAGATAAACACAGTGCAGAACGCAGGGTTTAGTTTTTCGCAGCTTTTCTCTGACAGTTTGAGTTGTCCCTGCTA
 Sander-SWS1 CTTTCATGAACAA-----
 Girella-SWS1 -----
 Metriaclima CTTTCATGAACAAACAG-----
 Dimidiochromis CTTTCATGAACAAACAGGTTGAGCACACTCAGATAAACACAGTGCAGAACGCAGGGTTTAGTTTTTCGCAGCTTTTCTCTGACAGTTTGAGTTGTCCCTGCTA
 Stigmatochomis CTTTCATGAACAAACAGGTTGAGCACACTCAGATAAACACAGTGCAGAACGCAGGGTTTAGTTTTTCGCAGCTTTTCTCTGACAGTTTGAGTTGTCCCTGCTA
 Hippoglossus CTTTCATGAACAAACAG-----
 Salmo_salar CTTTCATGAACAAACAG-----
 Carassius CTTTCATGAACAAACAG-----

Perca-SWS1 CTGAAGTGATGATGAGTTCTTCTGTTCTGTTCTCAGTTTAAATGGCTGTRTCATGGAGACAGTGTGTTGGGAAGA-----
 Sander-SWS1 -----
 Girella-SWS1 -----
 Metriaclima -----TTTAAATGGCTGTATCATGGAGATGGTGTGTTGGAAAGACAATGGACGAATCCTCCGAAGTTTCCA
 Dimidiochromis CTGAAGTGATGATGAGTTCTTCTGTTCTGTTCTCAGTTTAAATGGCTGTATCATGGAGATGGTGTGTTGGAAAGACAATGGACGAATCCTCCGAAGTTTCCA
 Stigmatochomis CTGAAGTGATGATGAGTTCTTCTGTTCTGTTCTCAGTTTAAATGGCTGTATCATGGAGATGGTGTGTTGGAAAGACAATGGACGAATCCTCCGAAGTTTCCA
 Hippoglossus -----TTTAAAGCCTGTATCATGGAAACAGTGTGTTGGAAAGAAAATGGACGAATCATCTGAAGTTTCTT
 Salmo_salar -----TTTAAAGCCTGCATCATGGAGACTGTGTTTCGGAAAGCAGATTGAGGAGACTTCAGTTTCCAGCTT
 Carassius -----TTCAATGCCTGCATCATGGAGACTGTATTTGGCAAGAAGATTGATGAGAGCTTCAGAGTTTCCA

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Perca-SWS1 -----
 Sander-SWS1 -----
 Girella-SWS1 -----
 Metriaclima CAAAGACTGAAGTGTCACAGCTTCT----TAATGCTCACACCAAAGTGACACAAAGAACTTTAAGAGGGCGTGACTGAGCTGAAGAGGACCTTCACA
 Dimidiochromis CAAAGACTGAAGTGTCACAGCTTCT----TAATGCTCACACCAAAGTGACACAAAGAACTTT-----
 Stigmatochomis CAAAGACTGAAGTGTCACAGCTTCT----TAATGCTCACACCAAAGTGACACAAAGAACTTT-----
 Hippoglossus CAAAACTGAGGCATCTTCAGTTCCACAGTTAATTAATGTT-----TGA-ACAAA-----TTAGAAGTGATGCCGGATGAAAAAGATTTAATAGCT
 Salmo_salar CCAAGACTGAGGTCTCCACAGCA-----TAA-----
 Carassius GCAAGACTGAACCCTCTCTGCTGCA-TAAATATATCCACAATTTCTATTCCCTATTACAACCTGTCTGGACCTGTTTTTCATGTTATCCCCTTGCC

Perca-SWS1 -----
 Sander-SWS1 -----
 Girella-SWS1 -----
 Metriaclima GGTCCGAGTTTGGAGGACGAATGTTTTCTGTAATCTCATCGTTATCTAAATAAAGTTATACGTACACACGATCAGCTGATATCTATGTTCTGTAGAAC
 Dimidiochromis -----
 Stigmatochomis -----
 Hippoglossus ACATTTTTGTCAAATA---CAAGCA---AACCTGTTCAATTAACCTGATAGAT-----TTTTTTAGATAAAAATAAAAAATAAAATGTTATATAACTAAAA
 Salmo_salar -----
 Carassius TGATTGTTCAAATCCTGACCTTTACACTTGCTGAGGGCTGAGGGATCTCCATGCTCAAGAAGACACAGCCCGCTGGATGGCCAACCAGTGGGTGAT

Perca-SWS1 -----
 Sander-SWS1 -----


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Girella-SWS1 -----
Metriaclima ATACGTGTTAGATAAAAGGCAAAATAAACATTAAAAACAGTGAAGTGTCTTTCTTTAGACTTTTGAATAAAACGTTCACGAACCAAAAAAAAAA
Dimidiochromis -----
Stigmatochomis -----
Hippoglossus AAAAAAAAAAAAAAAAAAAAAA-----
Salmo_salar -----
Carassius ATCCTACCATCCTTTCTGTGCAGTACTGGTACACCTTCAAATACTACTATTTTTTTTATTTTTTTGTAATTGCTGTCTAATGTTTACAATGC

Perca-SWS1 -----
Sander-SWS1 -----
Girella-SWS1 -----
Metriaclima AAAAAAAAA-----
Dimidiochromis -----
Stigmatochomis -----
Hippoglossus -----
Salmo_salar -----
Carassius ATTATGCATGACGATGAATGTTAATTGTTAACTTGCAACA

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SWS1 Protein Alignment:

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Perca-SWS1      -MGKHFHLYENISKISPFEGPQYYLAPVWAFYLAQAFMGFVFFAGTPLNFIVLVATMKYKLRVPLNFILVNISFSGFIFVTF SVSQVFLASMRGYYFLG
Sander-SWS1    -----
Girella-SWS1   -----ILVNISFAGFIFAIFSVSQVFLASVKGYYFLG
Dimidiochromis --GKHFHLYENISKISPFEGPQYYLAPVWAFYLAQAFMGFVFFAGTPLNFIVLVATMKYKLRVPLNFILVNISFSGFIFVTF SVSQVFLASMRGYYFLG
Hippoglossus  MMGKHFHLYENSVNSVSPFDGPQYYLAPQWAFHLQTI FMGSVLFAGTPLNFIVLLVTLKYKLRVPLNYILVNICFAGLIFVVF SVSQVFLVSTMRGYFFLG
Salmo-SWS1     -MGKDFHLYENISKISPFEGPQYHLASMWAFYLAQAFMGFVFFAGTPLNF IILVVTVKYKLRQPLNFILVNISLAGFIFVTF SVSQVFLVSSARGYYFLG
Carassius-SWS1 -MDAWTYQFGNLSKISPFEGPQYHLAPKWAFFYLAQAFMGFVFFVGTPLNAIVLVFVTMKYKLRQPLNYILVNISLGGFIFDTF SVSQVFFSALRGYYFFG

Perca-SWS1     HTLCALESVAGSVAGLVTAWSLAVLSFERYLVI CKPFGAFKFGSNHALAAVAFTWFMGIGCACPPFFGWSRYIPEGLGCSCGPDWYTHNEQYNTTSYTHF
Sander-SWS1    -----
Girella-SWS1   YTLCALEAAMGSIAGLVTWSLAVLSFERYLVI CKPFGAFKFGNNHALAAVAFTWVMGICCATPPFFGWSRYIPEGLGCSCGPDWYTHNEEYNCTSYLYF
Dimidiochromis HTLCALESVAGSVAGLVTAWSLAVLSFERYLVI CKPFGAFKFGSNHALAAVAFTWFMGIGCACPPFFGWSRYIPEGLGCSCGPDWYTHNEQYNTTSYTHF
Hippoglossus  PTLCALESAMGSIAGLVTWSLAVLSLERYLVIC KPFGAFRFGSNHAAGAVAFTWFMGISC AIPFFGWSRYIPEGLGCSCGPDWYTHNEEFHCSSYT NF
Salmo-SWS1     YTLCAMEACMGSIAGLVSAWSLAVLAFERYVVI CKPFGTFKFDNNQALAAVAFTWVMGIGCATPPFFGWSRYIPEGLGCSCGPDWYTNNEEYHCASYTKF
Carassius-SWS1 YTLCAMEAAMGSIAGLVTGWSLAVLAFERYVVI CKPFGSFKFGQSQALGAVALTWIIGIGCATPPFFGWSRYIPEGIGTACGPDWYTKNEEYNTESYTYF

Perca-SWS1     LMVTCFIIPLSIIIFCYSQLLGALRAVAAQQAESASTQKAEKEVSRMIIVMVG SFVTCYGPYALAALYFAYSTDENKDYRLVTIPAFFSKSACVYNPLIY
Sander-SWS1    -----VAAQQAESASTQKAEKEVSRMIIVMVG SFVTCYGPYALAALYFAYSTDENKDYRLVTIPAFFSKSACVYNPLIY
Girella-SWS1   LITTCFISPLSIIIFSYSQLLGALRAVAAQQTESASTQKAEKEVSRMIIVMVG SFVTCYAPYALAGLYFAFSTNENKDYRLVTVPAYFSKSSCV-----
Dimidiochromis LMVTCFIIPLSIIIFCYSQLLGALRAVAAQQAESASTQKAEKEVSRMIIVMVG SFVTCYGPYALAALYFAYSTDENKDYRLVTIPAFFSKSACVYNPLIY
Hippoglossus  LMVTCFIIPLSIIIFSYTQLLSSLRAVAAQQTESVSTQKAEKEVSRMIIVMVG SFVTCYGPYALAALYFAHSSDTNKDYRLVTIPAFFSKSSCVYNPLIY
Salmo-SWS1     LIVTCFLMPMSIIFFSYSQLLGALRAVAAQQAESASTQKAEKEVSRMIIVMVG SFILCYGPYALAGLYFAYTTSENKDYRLVTIPAFFSKSSCVYNPLIY
Carassius-SWS1 LLVSCFMMPIMIITFSYSQLLGALRAVAAQQAESASTQKAEKEVSRMVVMVG SFVVCYGPYAITALYFSYAEDSNKDYRLVAIPSLFSKSSCVYNPLIY

Perca-SWS1     VFMNKQFNGCIMETVFGK-----
Sander-SWS1    AFMN-----
Girella-SWS1   -----
Dimidiochromis VFMNKQFNGCIMEMVFGKTMDESSEVSTKTEVSTAS---
Hippoglossus  VFMNKQFKACIMETVFGKMDDESSEVSSKTEASSVSTVN
Salmo-SWS1     AFMNQFNACIMETVFGKQIEETSVSASKTEVSTA----
Carassius-SWS1 AFMNQFNACIMETVFGKIDESSEVSSKTETSSVSA--

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SWS2 Nucleotide Alignment:

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis AA TAAAGACAAGAGACGATAGAA TT CCTAACCGCACAGCGTTCATGTCTGCTCCTTAAATTTA TTA TTTTGGCAGAGCCTGCGCGATACCTAAATTGAGCC
 Hippoglossus AGAGACACGGGAATTCC TCCATCA CATGAAAG
 Salmo-SWS2
 Carassius-SWS2

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis GTAACA T CAGCAGGAGTACGTT GTT CGAAATAAGCAAAATGAAGG ---GTAAA -CGTGATATGGAGCTTGCAGAAAGACTTCTGGATACCGTTCAGCCTGG
 Hippoglossus GCAAATTTT GATTTTTCTTCTTTG CCGTGGGGGAAAAATGAAGC ---ACGGC -CGGGTCATGGAGCTTCCAGAGGATTTCTGGATCCCGTCCCTTTGG
 Salmo-SWS2 ---ATGAACA CAATGAGGT CGAATGCT -CGCCCCGTGGAGCTCCAGGAGGGA TTTCTACATCCCTATCGCGCTGG
 Carassius-SWS2 ---GATGAAGCAAGATGAAGCAAGTACCAGAGTTTACAGAGGACTTCTACATCCCATCCCTTTAG

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis ACACAAACAAATCACGTC ACTYAGCCCTTTCTGGTTCCACAGGACCCTTGGGGGATACTGCCACCCTTTTACGCCATGGCAGTTTTCATGTTATTCA T
 Hippoglossus ATACGAACAAACATCAGACTCTCAGCCCCCTTCC TTGTCCCCAGGACCATCTAGCAAGCTCAGCCACCCTTCTACGCCATGGCCATATACATGTTTTTTGT
 Salmo-SWS2 ATACCAACAAACATCACTTCACTCAGCCCCCTTCC TTGGTTCCCTCAGGACCACCTGGCGGGCAGTGTGTCTTCTATGGCATGTTCATTTTTCATGTTCTTCC T
 Carassius-SWS2 ATATCAACAAACCTCTCAGCTACAGCCCTTTTTTGGTCCCCAGGACCACCTGGGAAACCAAGGCATA TTTATGTCATGTCCTGTTTATGTTCTTTCAT

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis
 Hippoglossus
 Salmo-SWS2
 Carassius-SWS2

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis
 Hippoglossus
 Salmo-SWS2
 Carassius-SWS2

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis
 Hippoglossus
 Salmo-SWS2
 Carassius-SWS2

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis
 Hippoglossus
 Salmo-SWS2
 Carassius-SWS2

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis
 Hippoglossus
 Salmo-SWS2
 Carassius-SWS2

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Perca-SWS2 GTTTCTGCAACACTTGGCGGTATGGTAAGCTTGTGGTCTCTTGGTGTGGTAGCTTTTGAAAGATGGCTGGTCACTCTGCAAGCCACTTGGAAACTTTAT
 Sander-SWS2 -----TGTGGTCTCTTGGTGTGGTAGCTTTTGAAAGATGGCTAAATCATCTGCAAGCCACTTGGAAACTTTAT
 Girella-SWS2 GATTTACAGCAACTGTGTGGTATGGTAAGCCCTTGTGGTCTCTTGGTGTGGTAGCTTTTGAGAGATGGCTTGTGCTCTGCAAGCCACTTGGAAACTTTAT
 Dimidiochromis GTTTCATGCTAACACTCGGTGGTATGGTAAGCCCTTGTGGTCTCTTGGTGTGGTAGCTTTTGAGAGATGGCTTGTGATTTGCAAGCCGCTCGGTAACTTTAT
 Hippoglossus GTTTCATGCTGACACTTGGTGGTATGGTCAAGCCCTTGTGGTCTCTTGGTGTGGTAGCTTTTGAAAGATGGCTGGTCACTCTGCAAGCCATTAAGTAAGTTTGT
 Salmo-SWS2 GATTTACAGCTACTCTTGGCGGTATGGTGAAGCTTATGGTCTCTCTAGTAGTGGCGTTTGAAAGATGGTGGTATTTGTAAGCCAGTCCGTAAGTTTCA
 Carassius-SWS2 GCTTCTTGCACAGCTCGGAGGAATGGTGGTGTGTGGTCTCTTGGTGTGGTAGTGGCAATTTGAAAGATGGCTGGTCACTTGCAGAACCCCTTGGAAACTTTAC

Perca-SWS2 TTTCAAGCCTGACCATGCTGTTCGCTTGGTGTGTATTTACCTGGGTGTTTGGACTTGATTGCTTCACTTCCCTCCACTGTTCCGGATGGAGCAGGTACATCCCA
 Sander-SWS2 TTTCAAGCCTGACCATGCTGTTCGCTTGGTGTGTATTTACCTGGGTGTTTGGACTTGATTGCTTCACTTCCCTCCACTGTTCCGGATGGAGCAGGTACATCCCA
 Girella-SWS2 CTTCAAGCCTAACACGCTATAGCTTGTTCGCGCATTTAGACTTGGGTGATTGCTTTGACGGCTGCAAATCCCTCCCTGTTGGATGGAGTAGGTATATCCCA
 Dimidiochromis TTTCAAGCCSGACACGCTATAGCCTGCTGTGCATTCACTTGGTGTGTTTGGCAGTATTTGCTCAGCTCCCTCCACTGTTCCGGCTGGAGCAGGTATATCCCA
 Hippoglossus TTTCAAGCCTGACCATGCTATAGCTTGGTGTGCGCATTTACCTGGGTGTTTGGACTTATTTCCGCACTTCCCTCCCTGTTGGATGGAGCAGGTACATCCCA
 Salmo-SWS2 ATTTCAAGAGCACACATGCAATACTTGGCTGTGCAATCACTTGGGTGTTTGGGTGGCTCCAGTCTTCCCTCCCTGTTGGCTGGAGTAGATACATCCCA
 Carassius-SWS2 CTTCAAGACCCCTCATGCTATAGCTGGCTGCATACTTCCCTGGATAAGTGCATGGCACTTCACTCCCTCCACTGTTGGCTGGAGCCGGTACATACCT

Perca-SWS2 GAAGGCTTGCAGTGCTCCTGTGGTCCAGACTGGTACACCAAAACAACAATAACAACAATGAATCCCTACCTGATCTTTCTTTGGGTTTGTGTTTGTCTG
 Sander-SWS2 GAAGGCTTGCAGTGCTCCTGTGGTCCAGACTGGTACACCAAAACAACAATAACAACAATGAATCCCTACCTGATCTTTCTTTGGGTTTGTGTTTGTCTG
 Girella-SWS2 GAAGGCTTGCAGTGCTCCTGTGGGCCAGACTGGTACACCAAAACAACAATAACAACAATGAGTCCCTACCTGATGTTCTTTCTGCTTTTGTGTTTGTCTG
 Dimidiochromis GAAGGCTTGCAGTGCTCCTGTGGGCCAGACTGGTACACCAAAACAACAATAACAACAATGAGTCCCTACCTGATGTTCTTTCTGCTTTTGTGTTTGTCTG
 Hippoglossus GAGGGTCTGCAGTGCTCCTGTGGACCGACTGGTACACCAAAACAACAATAACAACAATGAGTCCCTACCTGATATTTCTTTCTGCTTTTGTGTTTGTCTG
 Salmo-SWS2 GAAGGTCTCCAGTGCTCCTGTGGACCGACTGGTACACCAAAACAACAATAACAACAATGAGTCCCTACCTGATGTTCTTTCTTTCTGCTTTGTGTTTGTCTG
 Carassius-SWS2 GAAGGTTTGCAGTGCTCCTGTGGACCTGACTGGTATACCACTAACACAACAATAACAACAATGAATCCCTACCTGATGTTTGTGTTTGTCTGTTTGTCTG

Perca-SWS2 TTCCATTCACCACCATTATTTTCTGCTATGGTCAGCTGCTCATTTGTGATGAAAATGGCAGCAAAGGCCAAGCAGAGTCTGCTTCCACCAGAAAGCAGA
 Sander-SWS2 TTCCATTCACCACCATTATTTTCTGCTATGGTCAGCTGCTCATTTGTGATGAAAATGGCAGCAAAGGCCAAGTAGAGTCTGCTTCCACCAGAAAGCAGA
 Girella-SWS2 TCCCTTCTCTACCATCGTTTCTGCTACTCAGCTCCTTTTATGCTGAAAATCGGCAGGGAAGGCCAAGCTGAGTCTGCCCTCCACCAGAAAGCAGA
 Dimidiochromis TTCCCTTGACCACGATTATCTTTTGTACTCCAGCTGCTCATCACACTGAAAATGGCAGCAAAGGCCAAGCTGAGTCTGCCCTCCACCAGAAAGCAGA
 Hippoglossus TTCCCTTCACCACCATCCTCTTTCTGCTACTCACAGCTGCTCATCACACTGAAAATGGCGCCGGAAGGCTCAAGCGGAGTCTGCCCTCCACCAGAAAGCAGA
 Salmo-SWS2 TCCCATTCAGTGTATTGTTTCTGCTATGCCCAGCTGCTCTTCATGATGAAAAGGCCGCGCCGAGACTCTGCCCTCCACCAGAAAGCAGA
 Carassius-SWS2 TTCCCTTCCGCACCATCGTGTCTGTTATAGGCCAACTACTCATCACACTCAAATTAGCAGCAAAGGCTCAAGCAGATTCTAGCTTCGACCAGAAAGCAGA

Perca-SWS2 GAAGGAGGTGACCAAGATGGTGGTCCCTCATGGTGAATGGCTTCTGGTGTGCTGGTTCCTTATGCCTCCTTTGCTCTTTGGGTTGTGAATAACCGTGGG
 Sander-SWS2 GAAGGAGGTGACCAAGATGGTGGTCCCTCATGGTGAATGGCTTCTGGTGTGCTGGTTCCTTATGCCTCCTTTGCTCTTTGGGTTGTGAATAACCGTGGG
 Girella-SWS2 GAGGGAGGTGACCAAGATGGTGGTCCCTCATGGTGAATGGCTTCTGGTGTGCTGGTTCCTTACGCCCTCCTTTGCTCTTTGGACTATCAACAATCGTGGG
 Dimidiochromis GAGGGAGGTGACCAAGATGGTGGTCCCTCATGGTGAATGGCTTCTGGTGTGCTGGTTCCTTACGCCCTCCTTTGCTCTTTGGGTTGTGAACAACCGTGGG
 Hippoglossus AAGGGAGGTGACCAAGATGGTGGTCCCTCATGGTGAATGGCTTCTGGTGTGCTGGTTCCTTACGCCCTCCTTTGCTCTTTGGGTTGTGAACAACCGTGGG
 Salmo-SWS2 GAAGGAGGTGACCAAGATGGTGGTGGTGAATGGTGGTTCCTTAGTGTGCTGGATGCCCTACGCCCTCCTTTGCTGTCTGGGTTGTGAACAACCGCGGT
 Carassius-SWS2 GAGGGAGGTGACCAAGATGGTGGTGGTGAATGGTGGTTCCTTAGTGTGCTGGGCGCCATATGCTAGCTTTTCTCTCTGGATAGTTTCCCAACCGTGGT

Perca-SWS2 CAACCAATTCGATCTTCGACTGGCGACCAATACCTTCCTGCTCTCAAAAGCCTCTGCAGTCTCAAAACCTCTCATCTATAATTTCTTCAATAAACAGTTCC

Sander-SWS2
 Girella-SWS2 CAGACTTTTGACCTGAGACTGGCCACCAATACCTTCCTGTCTGTCTAAAGCCTCCACAGTCTC
 Dimidiochromis CAGTCAATTCGATCTGAGACTGGCCGACTATACCGTCCTGTCTTCTCAAAGGCTTCAGCAGTCTACAAACCCCTGTATCTATGTGGTCTTTAATAAAACAGTTCC
 Hippoglossus CAAACGTTTGACCTGAAATTTGCTACTCTGCCTTCTGTCTTCTCAAATCTCTGCGTCTACAAACCCCTCATCTATGTCTTACTCAATAAACAGTTCC
 Salmo-SWS2 GCACCCTTTGATCTCCGATGGCCCTCCATACCTTCCTGTCTTCTCAAAGGCTCCACAGTCTACAAACCCCTCATCTATGTCTTCAATGAATAAGCTGTTCC
 Carassius-SWS2 GAAGAAATTTGATCTGAGAAATGGCAACTATACCATCCTGCCTTCTCAAAGCCTCTACAGTGTATAAACCCCTGTATCTACGTCTTAATGAACAAAACAGTTCC

Perca-SWS2 GTTCATGCATAATGACAAATGCTGGGGATAGGTGGAGGTGAGGA--GGAAATCCCAACACAT--CATCAGTGACTGAAGTCTCCAAAGTTGGCCCTGC
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis GTACATGTATGTTAGCGATGATGGGGATGGGAGGAGGTGAGGA--GGAGAGCTCAACCAACACA--ATCGGTGACTGAAGTTTCCAAAGTCGGCCCTGC
 Hippoglossus GTTCATGCATGATGAAGATGATGGGAATGGGTGGAGGTGATGATGAAGAATCTCAACATCACAGACCTCAGTCACCCGAGTCTCCAAAGTCGGCCCTGC
 Salmo-SWS2 GCTCATGCATGATGAATTTGCTGGGATTTGAAGTCCGGAGATGATGAGGAAGCATCATCAACAT--CCTCAGTCACTCAAGTGTCTTCTGCTGGTTAA--
 Carassius-SWS2 GTTCCTGTATGATGAAGATGGTCTGTGGCAAAAATATTGAGGAAGATGAGGCCCTACTTCAT--CTCAGTCAACCAGCTCTCTCCCTTGACCACA

Perca-SWS2 TTAG
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis TTAGGCAACACGTTCAATGCTCGCTGATTCTGAACTGTAAATACAAAGAATGTACATGTATTTTATCATATGTAAAATGTGATGATTTAAGTAGGAAT
 Hippoglossus TTAGACTGAACATCCACAACGTTTCCACAGTGTCTTCTTTTATTGTCAAAAATACCTCTGCACITGGCGACTGACAAATTTGTGTAATGTAAAATATGTAAA
 Salmo-SWS2
 Carassius-SWS2 GAAATAAACCTATTTTCAATG--TAACCTCATACTGTGGCAGTGAAAAACAATTTTACAACGATTTGAAATAATAAACAGACACAATAGGAATTTCTTGCT

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis AATCTGCAAAATGAAAATGCAATAAAAAACAGACACAAAATCAAAAAAATAAAAAAATAAAAAAATAAAAAA
 Hippoglossus CATGATGATGTTTTGTGCATATAAATTGCATTAAGTGAATAAGAGGGCTATAGTGAATAATTAACACATCTTTTACCAGCAAAATATAAATAAAAAATAAAA
 Salmo-SWS2
 Carassius-SWS2 TTTAATGTCAACATTTTTCTAACTTTATTTTGGCTTGCTGAACCAGTAGAATTAGTATAAGTTCAGTGTATCATTAAAAATGTACAAATAAATGCAATG-

Perca-SWS2
 Sander-SWS2
 Girella-SWS2
 Dimidiochromis
 Hippoglossus TGTTTGGATCAAGGATTAGCTAAAAAAAAAAAAAAAAAAAA
 Salmo-SWS2
 Carassius-SWS2

SWS2 Protein Alignment:

Perca-SWS2	-----LKYILVNLAVANLLVSAVG
Sander-SWS2	-----
Girella-SWS2	-----ILVNLAVANLLVSTVG
Dimidiochromis	----MKGKRMELPEDFWIPVSLDTNNITSLSPFLVPQDHLGDTATFYAMAVFMLFIFIFGTFINALTIACTIQYKKLRSHLNYILLNLAAANLLVSTVG
Hippoglossus	----MKHGRVMELPEDFWIPVPLDTNNITTLSPFLVPQDHLASSATFYAMAIYMFVFLGTSINTLTIILCTVKHKKLRSHLNYILLNLAVGNLLVSCVG
Salmo -SWS2	MNTMRSNARPVELQEGFYIPIALDTNNITSLSPFLVPQDHLGSAVFGMSFFMFFLVAGTAINVLTIVCTIQFKKLRSHLNYILVNLAIANLLVSMFG
Carassius-SWS2	-----MKQVPEFHEDFYIPIPLDINNLSAYSPFLVPQDHLGNQGI F MAMSVFMFFIFIGGASINILTILCTIQFKKLRSHLNYILVNLSIANLFVAIFG
Perca-SWS2	SFTACCSFAFRYFIFGALACKIEGFLATLGGMVSLWLSLAVVAFERWLVI CKPLGNFIFKPDHAVACCVFTWVFGLIASLPPLFGWSRYIPEGLQCSCGPD
Sander-SWS2	-----WSLAVVAFERWLIICKPLGNFIFKPDHAVACCVFTWVFALIVSLPPLFGWSRYIPEGLQCSCGPD
Girella-SWS2	SFTCCFCFGARYMILGPLGCKIEGFTATVGGMVSLWLSLAVVAFERWLVI CKPLGNFAFKPNHAIACCALTWVIALTAIIPPLVFGWSRYIPEGLQCSCGPD
Dimidiochromis	SFTACCTFSFRYFIFGALACKIEGFMVTLGGMVSLWLSLAVIAFERWLVI CKPLGNFIFKPDHAIACCAFTWFFAVFASAPPLFGWSRYIPEGLQCSCGPD
Hippoglossus	SFVGCCAFSVKYFFFGPLACRIEGFMVTLGGMVSLWLSLAVIAFERWLVI CKPLGNFVFKPDHAIACCAFTWVFALIAALPPLFGWSRYIPEGLQCSCGPD
Salmo -SWS2	SSTACLYFGNRYFIMGSVACQIEGFTATLGGMVSLWLSLVVAFERWLVI CKPVGNFQFKSTHAILGCAITWVFGLAASLPPLFGWSRYIPEGLQCSCGPD
Carassius-SWS2	SPLSFYSFFNRYFIFGATAACKIEGFLATLGGMVGLWLSLAVVAFERWLVI CKPLGNFTFKTPHAIAGCILPWISALAASLPPLFGWSRYIPEGLQCSCGPD
Perca-SWS2	WYTTNNKYNNESYVIFLFGFCFAVPFTTIIFCYQQLLIVMKMAAKAQAESVSTQKAEKEVTKMVVLMVIGFLVCWLPYASFALWVVNNRGQPFDLRLATI
Sander-SWS2	WYTTNNKYNNESYVIFLFGFCFAVPFTTIIFCYQQLLIVMKMAAKAQVESVSTQKAEKEVTKMVVLMVIGFLVCWLPYASFALWVVNNRG-----
Girella-SWS2	WYTTNNKYNNESYVMFLFCFCFAVPFSTIVFCYSQLLITLKMAAKAQAESASTQKAEREVTRMVVVMVLGFLVCWMPYASFALWTINNRRGQTFDLRLATI
Dimidiochromis	WYTTNNKYNNESYVMFLFCFCFAVPLTTIIFCYSQLLITLKMAAKAQAESASTQKAEREVTRMVVIMVLGFLVCWMPYASFALWVVNNRGQSFDLRLATI
Hippoglossus	WYTTNNKYNNESYVIFLFGFCFAVPLTTIIFCYSQLLITLKMAAKAQAESASTQKAEREVTRMVVVMVVGFLVCWLPYASFALWVVNNRGQTFDLKFIATL
Salmo -SWS2	WYTTNNKYNNESYVMFLFFFVFPVSVIVFCYQQLLIMMKAQADASTQKAEKEVTKMVVVMVVGFLVCWMPYASFVWVVQNRGAPFDLRLASI
Carassius-SWS2	WYTTNNKYNNESYVMFLFCFCFAVPFGTIVFCYQQLLITLKLAAKAQADASTQKAEREVTKMVVVMVLGFLVCWAPYASFSLWIVSHRGEFDLRLMATI
Perca-SWS2	PSCFSKASAVYNPVIYVFNKQFRSCIMTMLGIGGGE-EESSTSS-VTEVSKVGP-
Sander-SWS2	-----
Girella-SWS2	PSCLSKASTV-----
Dimidiochromis	PSCFSKASAVYNPVIYVFNKQFRSCMLAMMGMGGE-EESSTQS-VTEVSKVGP-
Hippoglossus	PSVFSKSSAVYNPVIYVLLNKQFRSCMMKMMMGGGDDEESSTSQTSVTEVSKVGP-
Salmo -SWS2	PSCFSKASTVYNPLIYVFMNKLFRSCMMNLLGLKSGDDEEASSTSS-VTQVSSAG---
Carassius-SWS2	PSCLSKASTVYNPVIYVLMNKQFRSCMMKMCVKGKNI EDEEASTSSQ-VTQVSSVAPEK

RH2 Protein Alignment:

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Perca-RH2      -----IKKLSQPLNYILVNLAVAGLIMCLFGFT
Sander-RH2    -----KKLSQPLNYILVNLAVAGLIMCLFGFT
Girella-RH2   -----ILVNLAVAGLIMCSFGFT
Dimidiochromis MAWEGGIEPNGTEGKNFYIPMSNRTGIVRSPFEYTYQYYLADPIFFKLLAFYMFFLICTGTPINSLTLFVTAQNKKLRQPLNYILVNLAVAGLIMCCFGFT
Hippoglossus  MVWDGGIEPNGTEGKNFYIPMSNRTGIVRSPFEYYPQFYMVDSMMFKFLAFYMFFLVCTGTPINGLTLFVTAQNKKLRQPLNYILVNLAVAGLIMCCFGFT
Salmo-RH2     -----MQNGTEGSNFYIPMSNRTGLVRSPLYQYYLAPPWQYHGLAVYMFFLICFGFPINGLTLVYVATNKKLRQPLNFIILVNLAAAGMIMVLFGFT
Carassius-RH2 -----MNGTEGNNFYVPLSNRTGLVRSPLYQYYLAEPWQFKLLAVYMFFLICLGLPINGLTLICTAQHKLRQPLNFIILVNLAVAGAIMVCFGFT

Perca-RH2     ITIISAFSGYFILGATSCAIEGFMATLGGEVALWSLVLAVERYVVVCKPMGSFKFSGTHAGAGVLFWTWIMAFACAGPPLFGWSRYIPEGMQCSCGPDYY
Sander-RH2    ITIISAFSGYFILGATSCAIGGFMATLGGEISLWSLVLAVERYIVVCKPMGSFKFSGTHAGAGVAFTWIMALACAAPPLFGWSRYIPEGMQCSCGPDYY
Girella-RH2   ITITSAVNGYFILGPTACAVEGFMATLGGEVALWSLVLAIERIYIVVCKPMGSFKFTGTHAGAGVLFWTWIMALACAAPPLFGWSRYLPEGMQCSCGPDYY
Dimidiochromis ITITSAFNGYFILGSTFCAIEGFMATLGGEVALWSLVLAIERIYIVVCKPMGSFKFSGAHAGAGVLFWTWIMAMACAAPPLFGWSRYIPEGMQCSCGPDYY
Hippoglossus  ITITSAFNGYFILGATFCTIEGFMATLGGEVALWSLVLAVERYIVVCKPMGSFKFSGTHAGIGVLFWTWMAFACAGPPLFGWSRYIPEGMQCSCGPDYY
Salmo-RH2     ITITSAVNGYFIWGPLGCAIEGFMATLGGEVALWSLVLAVERYIVVCKPMGSFTFTSTHAGAGVAFTWIAAMTCAAPPLLGWSRYIPEGMQCSCGPDYY
Carassius-RH2 VTFYTAINGYFALGPTGCAVEGFMATLGGEVALWSLVLAIERIYIVVCKPMGSFKFSSTHASAGIAFTWVMAMACAAPPLVGWSRYIPEGIQCSCGPDYY

Perca-RH2     TLAPGYNNESYVIYMFVHFFTPVSIIFFSYGCLVLTVKAAAAAQQSESTQKAEREVTRMCFMLVMGFLIAWVPYASFAGWIFLNKGAYFSPLTAAVPA
Sander-RH2    TLAPGFNNESYVIYMFVHFFTPVFIIFFSYGCLVLTVKAAAAAQQSESTQKAEREVTRMCFMLVMGFLVAVVPYASFAGWIFLNKGAYFSPLTASYTR
Girella-RH2   TLAPGFNNESYVYMFVHFFIPVFLIFFTYGSLVLTVKAAAAQQQDSESTQKAEREVTRMCFMLVMGFLVAVVPYASFAGWIFLNKGASFSALTAIPA
Dimidiochromis TLAPGFNNESYVIYMFVHFFVFPVFIIFFTYGSLVLTVKAAAAQQQDSESTQKAEREVTRMCFMLVMGFLIAWTPYASFAGWIFMKNKGASFSALTAIPA
Hippoglossus  TLAPGFNNESYVIYMFVHFFLPVFIIFFTYGSLVLTVKAAAAQQQSESTQKAEREVTRMCFMLVMGFLFAWTPYATFAGWIFMKNGAAFTALTASIPA
Salmo-RH2     TLAEGFNNESYVIYMFSCHFIPVCLIAFTYGSLVLTVKAAAAQQQDSESTQKAEREVTRMCFMLVCGFMIAWTPYATLAAYIFFNKGIAFSAQSMIPA
Carassius-RH2 TLNPEYNNESYVLYMFICHFILPVTIIFFTYGRVLTVKAAAAQQQDSESTQKAEREVTRMCFMLVCGFLVAVWTPYATVAAWIFFNKGAAFSAQFMAIPA

Perca-RH2     FFAKSSALYNPVIYVFMNNHQSNSPPPAGR-----
Sander-RH2    LLCKXLKRCITLLST-----
Girella-RH2   FFAKSSAL-----
Dimidiochromis FFAKSSALYNPVIYVLLMNKQFRNCMLSTIGMGG---MVEDETSVSTSKTEVSSVS--
Hippoglossus  FFAKSSALYNPVIYVLLNKQFRNCMLSTIGMGG---MVEDESSVSASKTEVSSVS--
Salmo-RH2     FFSKSSALFNPVIYVLLMNKQFRGCM LATVGMK----AEDETSVSTSKTEVSSVGP
Carassius-RH2 FFSKTSALYNPVIYVLLNKQFRSCMLTTLFCGKNPLGDEESSTVSTSKTEVSSVSPA

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LWS Protein Alignment:

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Perca-LWS      -AEEWGKQAF AARRY NEDTTS -GGFAYTNSNHTKGFEGPNYHIVRDGVSXISTLWMSMVVVASLFTNGLVVLVATAKFKKLRHPLNWILVNLAVADILET
Sander-LWS     MAEEWGKQAF AARRYSDDTTASGGFVYVNSNNTRGPFEGPNYHIAPRWVYHVATLWMSVVVVASVFTNGLVVLVATAKFKKLRHPLNWILVNLAFADIMET
Girella-LWS    -----ILVNLAIADLGET
Dimidiochromis MAEEWGKQSFAARRYHEDSTRGSFAFYTNSNNTRDPFEGPNYHIAPRWIYNLATLWMFVVVLSVFTNGLVVLVATMKFKKLRHPLNWILVNLAIADLGET
Hippoglossus  MAGAWGKQAF AARRYHEDTTRGSFAFVYTNSNHTRDPFEGPNYHIAPRWVYNMATLWMFFVVIASVFTNGLVVLVATAKFKKLRHPLNWILVNLAIADLGET
Salmo-LWS     MAERWGSAA YAARRQNQD TTRESSFTTNSNNTKDPFEGPNYHIAPRWVYNLSTLWMVIVVILSVFTNGLVVLVATAKFKKLQHPLNWILVNLAIADIGET
Carassius-LWS MAEQWGD AIFAARRRGDETTRESMFVYVYVNSNNTRDPFEGPNYHIAPRWVYNLATVWMFVVVASTFTNGLVVLVATAKFKKLRHPLNWILVNLAVADLAET

Perca-LWS      ILASTISVCNQFFGYFILGHPMCIFEGYTVACCGIAGLWLSLTIIISFERWIVCKPFGNVKFDKMAIAGIVFCWVWAAACTAPPVFGWSRYWPHGLKTSC
Sander-LWS     ILASTISVCNQFFGYFILGHPMCVFEGYTVACCGIAGLWLSLTIIISFERWIVCKPFGNVKFDKMAIAGIVFCWVWAAFWTAPPVFGWSRYWPHGLKTSC
Girella-LWS    VFASTISVCNQFFGYFILGHPMCVFEGFTVSTCGIAALWLSLTIIISWERWIVVCKPFGNVKFDKAKWATGGIVFSWVWSAAWCAPPVFGWSRYWPHGLKTSC
Dimidiochromis VFASTISVCNQFFGYFILGHPMCIFEGYVVSVCGIAALWLSLTIIISWERWIVVCKPFGNVKFDKAKWATAGIVFSWVWAAVWCAPPVFGWSRYWPHGLKTSC
Hippoglossus  VFASTISVCNQFFGYFILGHPMCIFEGYTVSVCGIAALWLSLSIIISWERWVVVCKPFGNVKFDKAKWATGGILFSWIWSAVWCAPPVFGWSRYWPHGLKTSC
Salmo-LWS     LLASTISVCNQFFGYFILGHPMCVFEGYTVSVCGIAALWLSLAVISWERWVVVCKPFGSVKFDKAKWAMGGIIFSWVWAAFWCAPPVFGWSRYWPHGLKTSC
Carassius-LWS LLASTISVTNQFFGYFILGHPMCIFEGFTVVSVCGIAAGLWLSLTVISWERWVVVCKPFGNVKFDKAKWASAGIIFSWVWSAIWCAPPVFGWSRFWPHGLKTSC

Perca-LWS      GPDVFSGSTDPGVQSYMIFLMITCCFIPLGIIIFVCYLAVFMAIHSVAMQQKESESTQKAEREVS RMVVVMIGAF CFCWGPYTFVFCYAAAANPGYAFHPLA
Sander-LWS     GPDVFSGNTDPGVQSYMITLMITCCFIPLSIIIVCYLAGIYGYP-----FSCYAAERN-----
Girella-LWS    GPDVFSGSEDPGVQSYMIVLMLITCCIIPLAIIILCYLAVWLAIRAVAMQQKESESTQKAEREVS RMVVVMI IAYCVCWGPYTF FACFAAANPGYAFHPLA
Dimidiochromis GPDVFSGSEDPGVQSYMIVLMLTCCILPLAIIILCYLAVWMAIRAVAMQQKESESTQKAEREVS RMVVVMIVAYCVCWGPYTF FACFAAANPGYAFHPLA
Hippoglossus  GPDVFSGSGDPGVQSYMIVLMTCCFLPLSVIILCYLAVWMAIHSVALQQKESESTQKAEREVS RMVVVMIVAYCVRWGPYTF FACFAAANPGYAFHPLA
Salmo-LWS     GPDVFGGNEDPGVKSYMITLMITCCFFPLFVIIFCYIFVWLAIRAVAAQQKDSESTQKAEREVS RMVVVMI IAYCVCWGPYTF FACFAAANPGYAFHPLA
Carassius-LWS GPDVFSGSEDPGVQSYMIVLMLITCCIIPLAIIILCYIAVWLAIRTVAAQQKDSESTQKAEREVS RMVVVMI FAYCFCWGPYTF CACFAAANPGYAFHPLA

Perca-LWS      ASMPAYFAKSATIWNPVIYVFMNRQFRSCIMQLFGKQVDDGSEVSTSKTEVSSVAPA
Sander-LWS     QSQPR-----
Girella-LWS    AAMPAYFAKSATI-----
Dimidiochromis AAMPAYFAKSATIYNPIIYVFMNRQFRSCIMQLFGKQVDDGSEVSTSKTEVSSVAPA
Hippoglossus  AAMPAYFAKSATIYNPVIYVFMNRQFRSCIMQLFGKEVDDGSEVSTSKTEVSSVAPA
Salmo-LWS     AAIPAYFAKSATIYNPVIYVFMNRQFRSCIMQLFGKAEDDGTEVSTSKTEVSSVAPA
Carassius-LWS AAMPAYFAKSATIYNPIIYVFMNRQFRVCIMQLFGKQVDDGSEVSTSKTEVSSVAPA

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