

VISUAL SENSITIVITY OF LAKE SUPERIOR FISHES

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BY

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## **Dedication**

In this venture of successfully moving half way across the country and completing a master's degree, I have many people to thank. Without the support system I have been lucky enough to gather, I would not have been able to transition as well as I did.

With that being said, I would like to dedicate this thesis to my friends and family who have listened to my rants and revelations in the pursuit of this degree. My utmost appreciation goes to my parents as well as my significant other, Christopher Bruhn, for their unending support and love. For keeping me happy and sane throughout this journey, I would like to dedicate this thesis to them.

## **Abstract**

Within this study, fish of two ecosystems are investigated: 1) shallow water fishes in waters located in proximity to Duluth-Superior Harbor and 2) open water fishes. Investigation of the former includes a comparison of the invasive round goby to the logperch and black bullhead to surmise any physiological visual advantage for the intruding species. The latter investigation compares the siscowet, kiyi, and deepwater sculpin visual sensitivity to the downwelling light available at depth.

Electroretinography was used to formulate spectral sensitivity curves for interspecific comparison. In both studies, fish visual sensitivity matched that of downwelling light available in the respective environments. Logperch showed greater visual depth than the round goby. Siscowet and kiyi visual depth profiles overlapped and deepwater sculpin retained vision at greater depths. Diurnal vision for all species is possible in shallow depths but it is likely that deepwater fishes rely on other sensory modalities during most predator-prey interactions.

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## **Foreword**

This master's thesis is submitted as partial fulfillment of the degree of Master of Science in Integrated Biosciences at the University of Minnesota. Chapter one includes a review of background literature and introductory information pertinent to the project. Chapters two and three are papers prepared for publication in PLOS ONE and the Journal of Great Lakes Research, respectively. Coauthors for Chapter two include Thomas Hrabik and Allen Mensinger. Allen Mensinger is also the coauthor of Chapter three.

## **Chapter 1: Introduction**

### **1.1 Open water food web of Lake Superior**

The open water of Lake Superior is host to complex interactions among species. Siscowet, (*Salvelinus namaycush siscowet*) the most abundant predator in the lake, prey primarily on deepwater sculpin (*Myoxocephalus thompsoni*) and occasionally on kiyi (*Coregonus kiyi*) (Isaac 2010). Mysis (*Mysis diluviana*), a crustacean found at the center of the offshore food web, is the main prey item of deepwater sculpin and kiyi (Figure 1). Mysis are primarily responsible for the recycling of nutrients from benthic waters to the surface via consumption of bottom dwelling organisms and surface plankton (Ahrenstorff *et al.* 2011). Mysis consumption by deepwater sculpin and kiyi must remain at relative equilibrium to preserve the current nutrient cascade. The open lake system is dominated by this simple food web consisting of siscowet, deepwater sculpin, kiyi, and mysis. Drastic changes in the abundance of one species could manifest in the alteration of energy dynamics and nutrient flow within the system.

Overabundance of siscowet is a rising problem in the open waters of Lake Superior (Hansen *et al.* 1995). Although predation of kiyi by siscowet appears to be sustainable, siscowet are consuming deepwater sculpin at an alarming rate (Gamble 2010). The deepwater sculpin on average constituted 60-79% of the prey weight consumed by the siscowet. Data suggest deepwater sculpin are the sole contributor of the siscowet fall diet (Gamble *et al.* 2011). Diminishing sculpin populations may lead to a rise in mysis populations, changing the flow of nutrients through the system. Additionally, as sculpin abundance continues to decline, siscowet may shift their diet preference, causing a drastic decline in kiyi, and potentially other open water fishes.

The sensory mechanisms used by siscowet, kiyi, and deepwater sculpin in foraging remain undocumented. To predict future trends in fish populations and allow for optimal management of lake organisms, better understanding of these mechanisms must be achieved.

## **1.2 Diel vertical migration in Lake Superior fish**

Movement of the siscowet in response to diel vertical migration (DVM) by its prey subjects these organisms to low intensity light environments (Thurston 1962). DVM is a phenomenon involving the upward movement of organisms in the water column at night and downward movement during the day. The exact motive of this behavior is unknown, but predator avoidance, prey capture, and competition may influence the pattern (Ahrenstorff *et al.* 2011). Some migration patterns shift with ontogenetic changes. Juvenile siscowet (< 225 mm) in the Apostle Island region of Lake Superior, undergo diel bank migration (DBM), moving from deep water during the day to shallow banks at night (Gorman *et al.* 2012). Juvenile siscowet in this region occupy shallower habitats, indicating a preference for increased depth with age (Figure 2). Adult siscowet, however, remain in open waters and perform DVM. In the western arm of Lake Superior however, juvenile siscowet exhibit normal DVM behavior and do not undergo DBM (Ahrenstorff *et al.* 2011). This may suggest differences in behavior regionally within Lake Superior.

Siscowet can be found at the bottom of Lake Superior, as low as 407 m during the day. Diet studies found terrestrial insects in the siscowet stomach contents suggesting their ability to reach surface waters at night (Sitar *et al.* 2008). Despite differing depths ontogenetically and diurnally, it remains evident that siscowet dwell in minimal light intensity areas.

Night trawls at 30 m caught kiyi, a fish traditionally thought to stay in benthic waters (Hrabik *et al.* 2006). This suggests that kiyi are following the migration of mysis (Ahrenstorff *et al.* 2011) and are undergoing DVM for prey capture. Ontogenetic shifts do not manifest in changes in kiyi migration patterns (Figure 2). Kiyi distribution ranges from 25 m to below 325 m and varies between seasons (Hrabik *et al.* 2006; Ahrenstorff *et al.* 2011). As discussed with siscowet behavior, DVM patterns also place kiyi in a constant low light intensity isolume. Deepwater sculpin inhabit depths between 15 and 407 m (Figure 2); this keeps these fish in variable, low light intense environments as well.

### **1.3 Shallow water species**

While the open waters of Lake Superior retain this native dominated food web, shallow water organisms contend with invaders. Introduced to the Great Lakes in 1990 via ship ballast water (Jude *et al.* 1992), the round goby (*Neogobius melanostomus*) population quickly expanded, dominating the near shore waters of Lake Superior by 1995 (Jude 1997). With high fecundity due to superior egg fitness (Kovtun 1978; MacInnis & Corkum 2000) and aggressive behavior, the round goby outcompetes both native and noninvasive colonizers in this aquatic system.

Round goby mating behavior maximizes egg production and viability. The male round goby will lure several females to a self-constructed nest with acoustic, olfactory, and visual cues (Protasov *et al.* 1965; Gammon *et al.*, 2005; Stammer & Corkum, 2005, Rollo *et al.* 2007). After egg deposition, the male round goby will guard the nest until the eggs hatch, enabling higher egg viability (Rollo *et al.* 2007). Round goby courtship occurs multiple times during the year with females spawning up to six times every 18

days between April and September (Jude *et al.* 1992). Sexual maturity is reached between one and two years of age for females and after 2 years for males (Jude *et al.* 1992). These characteristics allow the species to rapidly spread and quickly populate new territories.

The logperch is a native benthic species threatened by the round goby's presence in Lake Superior. Logperch declines seen in other areas of the Laurentian Great Lakes have been correlated with the round goby presence (French & Jude 2001). Both logperch and juvenile round gobies consume dipterans, *Caenis spp.*, and amphipods, causing competition for resources. Although mature round gobies will consume bivalves and ephemeropteran nymphs, absence of these prey will cause the invasive to continue to consume small invertebrates, further limiting logperch resources (Ray and Corkum 1997; French & Jude 2001). Additionally, the consumption of eggs by the round goby is thought to be the main contributor to recent logperch decline within the Great Lakes (French & Jude 2001).

The logperch and round goby generally use similar substrate for habitat. Found commonly on rocky shorelines, both organisms rely on crevices for shelter and predation. The logperch will actively search for food on rubble substrate by turning over small rocks with its protrusive nose (Greenberg 1991). The round goby will also feed in similar territory, and will monopolize substrate space with its nest guarding behavior. Despite this preference for rocky substrate, the logperch has been extirpated from this habitat in Duluth-Superior Harbor, forced to use sandy bottom substrate for habitat (Leino & Mensinger, unpublished). This shift in habitat use is added evidence that the round goby has a deleterious effect on the native logperch.

The black bullhead is a native benthic fish that is seemingly unaffected by the presence of the round goby largely in part due to the rarity with which these two species occupy the same habitat (Kornis *et al.* 2013). MN-DNR has reported declining numbers for the black bullhead since 1993 (2012 report). This decline is unlikely caused by round goby presence. Like the round goby, the black bullhead is active at night. Additionally, the black bullhead commonly resides in turbid waters and captures prey in muddy substrates (Darnell & Meierotto 1965; Johnson *et al.* 2008). This subjects the black bullhead to low light intensity environments while attempting to capture prey. Accustomed to low light intensities, the black bullhead retina may be adapted for visual sensitivity in minimal light conditions. Comparison of black bullhead spectral sensitivities to the sensitivity of the native logperch and invasive round goby may reveal differences in visual perception in these three species.

#### **1.4 Fish vision**

The fish visual system has been investigated for better understanding of optical function in varying light environments (Wald 1936; Denton & Warren 1957; Wald *et al.* 1957; Crescitelli 1991; Guthrie & Muntz 1993; Hrabik *et al.* 2006). The many functions of the fish eye include perception of predators, identification of mates, detection of prey, and sensing contrast of objects against ambient backgrounds. The perception of these complex images is dictated by the highly variable habitats in which each species evolved (Crescitelli 1991; Guthrie and Muntz 1993). Horodysky *et al.* (2010) characterized the light and spectral sensitivities of four piscivores common to coastal and estuarine systems in the western North Atlantic using electroretinography. The benthic species exhibited higher sensitivity to light intensity and had a broader range of wavelength perception

compared to pelagic fishes. Decreased light availability in benthic environments requires bottom-dwelling organisms to have increased sensitivity. Pelagic fishes, acquainted with more variable photic environments, showed larger differences in their day versus night perception when compared to benthic fish (Horodysky *et al.* 2010). This corresponds with the increased variability in light intensity and ambient wavelengths present in pelagic waters compared to benthic waters over the course of a day.

Differences in visual sensitivity are most likely due to difference at the cellular level within the retina. An examination of spectral sensitivity of 17 species of *Cottoids* in Lake Baikal revealed differences in retinal cell organization and size (Bowmaker *et al.* 1994). Surface species (species inhabiting depths of 1-120 m) possessed retinas with double cones, single cones, and rods arranged in a square mosaic. The rods and cones of these fish were thin and long (cone diameter 2-4  $\mu\text{m}$ , cone length 10-15  $\mu\text{m}$ ; rod diameter 2  $\mu\text{m}$ , rod length 90  $\mu\text{m}$ ). More pelagic species (50-450 m) possessed larger cones (cone diameter 9  $\mu\text{m}$ , cone length 45  $\mu\text{m}$ ), with double cones arranged in a row and single cones irregularly spaced. Rods in these species were short but thick (rod diameter 4  $\mu\text{m}$ , rod length 30  $\mu\text{m}$ ). In deeper dwelling species (100-1000 m), rods were long and thick (some lengths up to 165  $\mu\text{m}$ ) while double and single cones were thick and medium in length (cone diameter 5-6  $\mu\text{m}$ , length 20  $\mu\text{m}$ ). Their arrangement was loose and disorganized. Cones were absent in abyssal fish (200-1500 m) and rods were thick (70-120  $\mu\text{m}$ ) (Bowmaker *et al.* 1994). These changes in photoreceptor morphology reflect limited light availability with depth. At great depth, rod photoreceptors alone are present due to cone insensitivity. Decrease in organization of the retinal cells with depth may suggest the inefficiency of structured mosaic at capturing sparse photons; alternatively,

the lack of organization may imply reliance on another sensory modality in prey detection with minimal energy allocated to retinal maintenance. Regardless, the varying shapes and sizes of retinal components with depth reflect differences in the visual systems of fish present in the lake.

### **1.5 The “Sensitivity Hypothesis”**

The “sensitivity hypothesis” proposed by Clarke (1936) discusses the visual capabilities of fish with increasing depth. As light penetrates the water column, scattering and absorption prevent different wavelengths from traveling to equal depths (Jerlov 1968). The sensitivity hypothesis suggests that fish remaining at greater depths need only be sensitive to those ambient wavelengths in order to visually detect their prey and avoid predators. Organisms occupying more than one isolume will be more sensitive to the wider range of wavelengths present in the varying light environments they inhabit (Clarke 1936). Therefore, Clarke’s work suggests a shift in the visual systems of fish corresponding to wavelengths present in the fish’s environment. Munz (1958) and Denton and Warren (1957) continued to build on this hypothesis and noticed a blue shift in bathypelagic organisms compared to marine surface species. Since the properties of light in marine waters favor the relatively slow attenuation of blue wavelengths, their findings supported the “sensitivity hypothesis” for the investigated pigments.

Bowmaker *et al.* (1994) similarly supported the sensitivity shift principle outlined in the “sensitivity hypothesis” by noting a decrease in  $\lambda_{\text{max}}$  for species residing in greater depths. The study of Lake Baikal, the deepest lake in the world at 1600 m, allowed Bowmaker *et al.* (1994) to study a wide variety of species at various depths for



comparison of  $\lambda_{\max}$  values in these differing light conditions. While surface species contained single cones with a  $\lambda_{\max}$  just over 540 nm and a rod  $\lambda_{\max}$  around 515 nm, abyssal fish had a cone  $\lambda_{\max}$  below 500 nm and a rod  $\lambda_{\max}$  at 500 nm. While this shift is expected with the light attenuation in the water column, the data challenge the “sensitivity hypothesis”. The slowest attenuating wavelengths within the system fall between 550-600 nm. According to the sensitivity hypothesis, abyssal fish should have  $\lambda_{\max}$  values adapted for these wavelengths most prevalent at depth. This may support the alternative hypothesis to the sensitivity hypothesis that states some fish utilize a  $\lambda_{\max}$  offset to the predominant ambient environment. With offset sensitivity, fish may have increased ability to detect contrast within the environment, and may be able to better detect predators and prey as a result (McFarland & Munz 1975; Munz 1976; Douglas *et al.* 1995)

## 1.6 Retinal Pigments

Photoreceptors are responsible for initial absorption of light in the retina. Pigments within these photoreceptors are responsible for the absorption of specific photons found in the electromagnetic spectrum. The diversity of these pigments is greater in bony fishes than in all other vertebrates combined (Munz & McFarland 1977). Scotopic pigments can be classified into two groups: rhodopsins and porphyropsins. Rhodopsin, based on vitamin A<sub>1</sub>, is the visual pigment responsible for perception of relatively short wavelengths between 467 and 526 nm, while porphyropsin, based on vitamin A<sub>2</sub>, aids longer wavelength perception between 502 and 551 nm (Dartnall & Lythgoe 1965; Munz & McFarland 1977). Rhodopsin forms as a result of the binding of

11-*cis* retinene with an opsin molecule (Wald *et al.* 1957). The *cis* configuration becomes all *trans* upon photobleaching and returns to its original structure upon dark adaptation (Wald *et al.* 1957). Porphyropsin consists of 11 *cis*-3-dehydroretinal chromophore bound to an opsin molecule and undergoes a similar transformation upon bleaching (Bowmaker *et al.* 1988). While rhodopsin has been found extensively in marine teleost species (Wald 1939; Denton & Warren 1957; Munz & MacFarland 1973; Crescitelli *et al.* 1985; Bowmaker *et al.* 1988), porphyropsin is often used alone or in conjunction with rhodopsin in freshwater species (Dartnall & Lythgoe 1965). The proportions with which these pigments are found within organisms vary among individuals. Changes in the ratio of rhodopsin to porphyropsin in individuals containing both pigments can change based on hormonal fluctuations, temperature, and light regime (Munz & McFarland 1977; Levine & MacNichol 1979; Beatty 1984)

Jokela-Määttä *et al.* (2007) studied the evolutionary divergence of these pigments in fish species found in both marine and freshwater systems. Their findings supported modification of the rod structure over thousands of years of isolation from conspecifics. Microspectrophotometry revealed differences between chromophore components in Baltic fish (fish subjected to longer wavelengths) compared to conspecifics of the east coast of Scotland, the west coast of Norway, and the Adriatic Sea (truly marine fish subjected to shorter wavelengths). While fish in long wavelength environments exhibited porphyropsin alone or both porphyropsin and rhodopsin chromophores, marine fishes strictly possessed rhodopsin. No divergence was seen between Baltic fish and fish from Lake Vesijärvi, Lake Päijänne, and Lake Tuusulanjärvi as these waters are all shifted towards longer wavelengths (Jokela-Määttä *et al.* 2007).

Through these studies, it is evident that fish populations are able to adapt to their environmental settings over time indicating the importance of the visual system in daily function. While the sensitivity hypothesis for vitamin A<sub>1</sub> pigments has been supported extensively (Clarke 1936; Munz 1956; Denton & Warren 1957; Jokela-Määttä *et al.* 2007) with few criticisms (Bowmaker *et al.* 1994), evidence for its application in regard to vitamin A<sub>2</sub> pigments is not well studied (Jokela-Määttä *et al.* 2007).

### **1.7 Particles Bending Light**

Visibility underwater is made difficult by the inherent properties of light. Even in pure water, light bends as the photons transfer from a gaseous to a liquid median. As the angle of the sun changes, and the intensity of light changes throughout the day, variation in light angles and attenuation change (Guthrie and Muntz 1993). Air quality, cloud cover, and time of year are also factors that affect light levels in the water column (Bukata *et al.* 1995; Jerlov 1968). Additionally, the line of sight of the fish's eye will change the perceived image (Levine *et al.* 1979). Longer optical paths will be subjected to greater spectral shifts as photons are more likely to scatter over a longer distance. Thus proximity of the fish to the light source, as well as the ocular orientation of the species, changes the ability to perceive images with high acuity.

Variability of light properties in aquatic environments is not limited to distance. Light absorbing materials are found in all aquatic ecosystems. Scattering and absorption occur as a result of numerous factors including the concentration of chlorophyll a, dissolved organic carbon levels, colored dissolved organic material, and suspended solids (Effler *et al.* 2010; Guthrie and Muntz 1993). To account for the scattering and

absorption of light due to the inherent properties of light as well as particulates in the water, coefficients are empirically gathered to determine the attenuation or extinction of light in the water column (Figure 3). The attenuation of light in a specific aquatic environment can be calculated using Beer's Law:

$$E_{(z, \lambda)} = E_{(0, \lambda)} e^{-k(\lambda) z} \quad (1)$$

where E denotes the downwelling irradiance at depth z for wavelength  $\lambda$  and  $k(\lambda)$  represents the irradiance attenuation coefficient of that wavelength. Lower irradiance attenuation coefficient values correlate with deeper penetration through the water column. Irradiance attenuation coefficients in marine systems exhibit rapid red wavelength attenuation and blue wavelength attenuation occurs more slowly than green shifted light. Marine systems are therefore referred to as blue-shifted. Freshwater systems vary in attenuation based on water clarity. Red light extinction occurs at greater depths in murky fresh waters compared to oligotrophic fresh water systems. Clear freshwater systems allow for the slow attenuation of green light while blue light diminishes at shallower depths (Jerlov 1968; Munz 1976; Walmsley *et al.* 1979). Freshwaters are generally green-shifted based on their light availability at depth.

Different species have developed different adaptations to overcome variability in the intensity of light. Pelagic piscivores, for example, tend to rely on contrast between prey and their backgrounds for predation; plantivores however develop higher visual acuity to capture smaller prey (Hansen *et al.* 2013, Breck 1993). Differences in visual capabilities among species are highly variable due to differing adaptations to changes in light properties.

Lake Superior is an oligotrophic aquatic system with low standing stocks of phytoplankton, zooplankton and fish. The oligotrophic nature of the lake gives rise to high beam transmittance and low beam attenuation (Bukata *et al.* 1995). Light attenuates more slowly than in more eutrophic systems due to the decreased abundance of scattering particles (DOC, chlorophyll a, etc.) (Bukata *et al.* 1995; Jerlov 1968). Lowest lake wide transmission values are seen in the winter, while the highest values are exhibited in mid-summer (Bukata *et al.* 1995).

### **1.8 Electroretinography and Ocular Function**

Electroretinography is a minimally invasive procedure used to characterize the spectral sensitivity of the retina via electrode implantation in live specimen. By measuring electrical potentials of the retina, assessment of photoreceptor (a-wave) and bipolar cell and Müller cell (b-wave) sensitivities can be performed. Two electrodes are required to record retinal information: the recording electrode and the ground electrode. The recording electrode senses the electrical activity of the retina. The ground electrode decreases the level of background noise and acts as an electrical reference; measurement of the electrical difference between the recording electrode and the reference levels detected by the ground electrode reveals retinal cell sensitivity to a specified wavelength of light (Grimnes and Martinsen 2008; Kettenmann and Grantyn 1992; Mensinger and Powers 1999). This data is displayed as an electroretinogram (Figure 4). The initial hyperpolarization indicates photoreceptor activity while the depolarization demonstrates the initiation of bipolar and Müller cells. In darkness, photoreceptors, rods and cones, exhibit a constant influx of sodium accompanied by efflux of sodium and potassium

downstream (Figure 5a). When a photon reaches the photoreceptor, sodium influx is inhibited causing an overall loss in potential with the continued downstream release of sodium and potassium (Figure 5b). This negative potential generates hyperpolarization known as the a-wave (Figure 4). In the absence of light, photoreceptors also release glutamate to inhibit bipolar cell activity. ON bipolar cells continually receive glutamate until the photoreceptor receives a photon of light (Figure 6). Glutamate release is inhibited in the presence of light, and ON bipolar cells depolarize in response to the absence of glutamate (Shiells *et al.* 1981). This depolarization was thought to be the main contributor to the b-wave. Recent studies show that depolarization of the Müller cells via potassium uptake (Wen and Oakley 1990) plays a stronger role in the formation of the b-wave.

While rod and cone photoreceptor physiology is very similar, rod sensitivity is approximately 30 times greater than cone sensitivity (Kingsley 2000). Although light attenuation in water favors specific wavelengths of light (blue in marine systems and green in freshwater systems), ambient light levels are reduced with increasing depth. Dim light environments restrict cone perception due to cone insensitivity. Thus, although water acts as a monochromator, selecting specific wavelengths of light, light intensity dictates organismal perception, initiating rod photoreceptor activity in scotopic environments (Kingsley 2000).

Electroretinography has been used to test visual perception of certain wavelengths at different intensities in fish to detect photoreceptor activation. Mensinger and Powers (1999) used the technique to assess retinal cell function after impairment by the metabolic poison, ouabain. Regeneration of photoreceptor and inner nuclear layer cells

was detected over time by increased a and b-waveforms. Electroretinography improves understanding of ocular function *in situ* whereas procedures involving the removal of the eye and dissection of its components may only shed light on ionic movement or compounds present at the point of tissue fixation. By using live specimen and subjecting them to multiple wavelengths of light at varying light intensities, a more complete understanding of retinal cell activity can be achieved.

### **1.9 Study Objectives and Hypothesis**

As siscowet, kiyi, and deepwater sculpin all survive in minimal light intensities, the objective of this study is to investigate their visual sensitivity using electroretinography to determine if vision is a feasible mechanism of prey detection. Siscowet and kiyi undergo DVM placing them in more variable light intensities than found at the bottom of the lake. It is hypothesized that siscowet and kiyi will have similar spectral sensitivity curves reflecting increased sensitivity to a wider range of wavelengths. The  $\lambda_{\text{max}}$  of deepwater sculpin is expected to be shifted towards the shorter wavelengths available at depth. Deepwater sculpin should also have a higher sensitivity to light intensity as previously observed in benthic fish (Horodysky *et al.* 2010).

This study also investigates the visual sensitivity of shallow water species in Lake Superior. As the round goby is a relatively recent immigrant to the Great Lakes, its visual sensitivity should be adapted to its native location in Caspian Sea. Investigation of black bullhead and round goby spectral sensitivities may reveal adaptation to low light intensities, while comparison to the logperch may reveal differences in visual perception. Calculation of the wavelengths available at depth and comparison of the fish visual

capabilities at depth can help evaluate the accuracy of the visual sensitivity hypothesis. Comparison of the light environment in the Caspian Sea to that of Lake Superior may reveal information of the evolutionary adaptations of the round goby. Additionally, understanding the role of vision in these species will increase accuracy of models of fish populations in Lake Superior. Management strategies can be adapted accordingly.



Figures

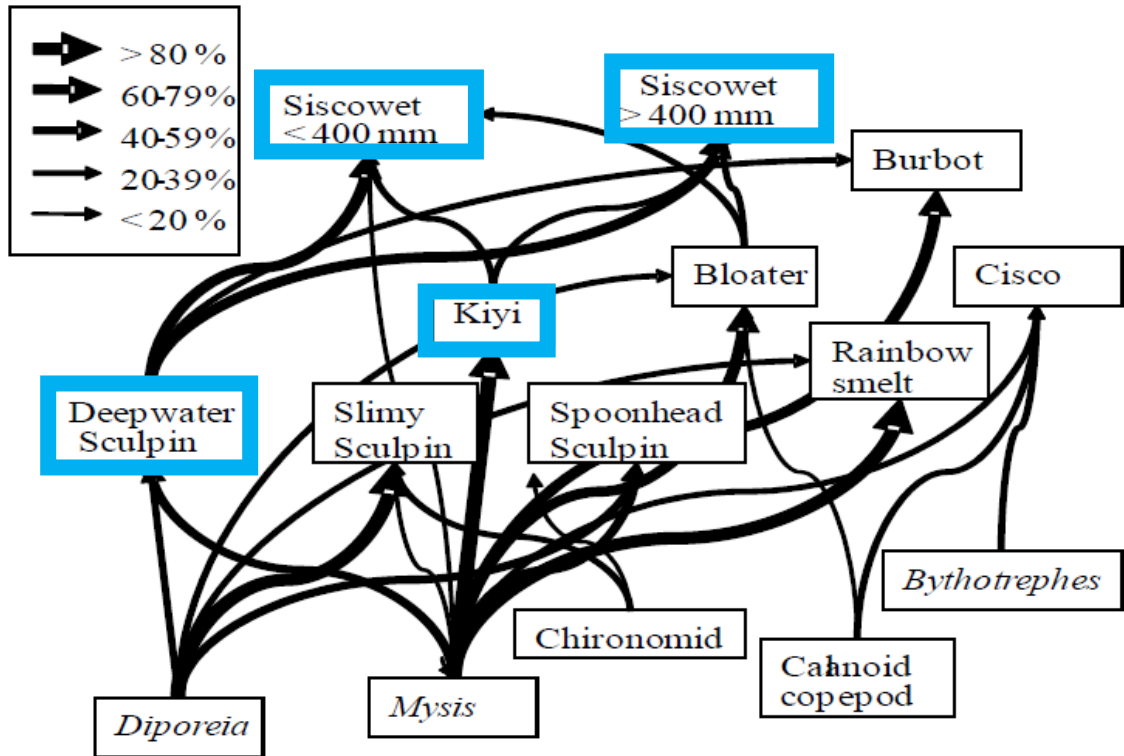


Figure 1: Food web of the open waters of Lake Superior. Thickness of arrow represents significance of prey item in predator diet. Species of interest to this study are highlighted. (Figure adapted from Gamble 2010).

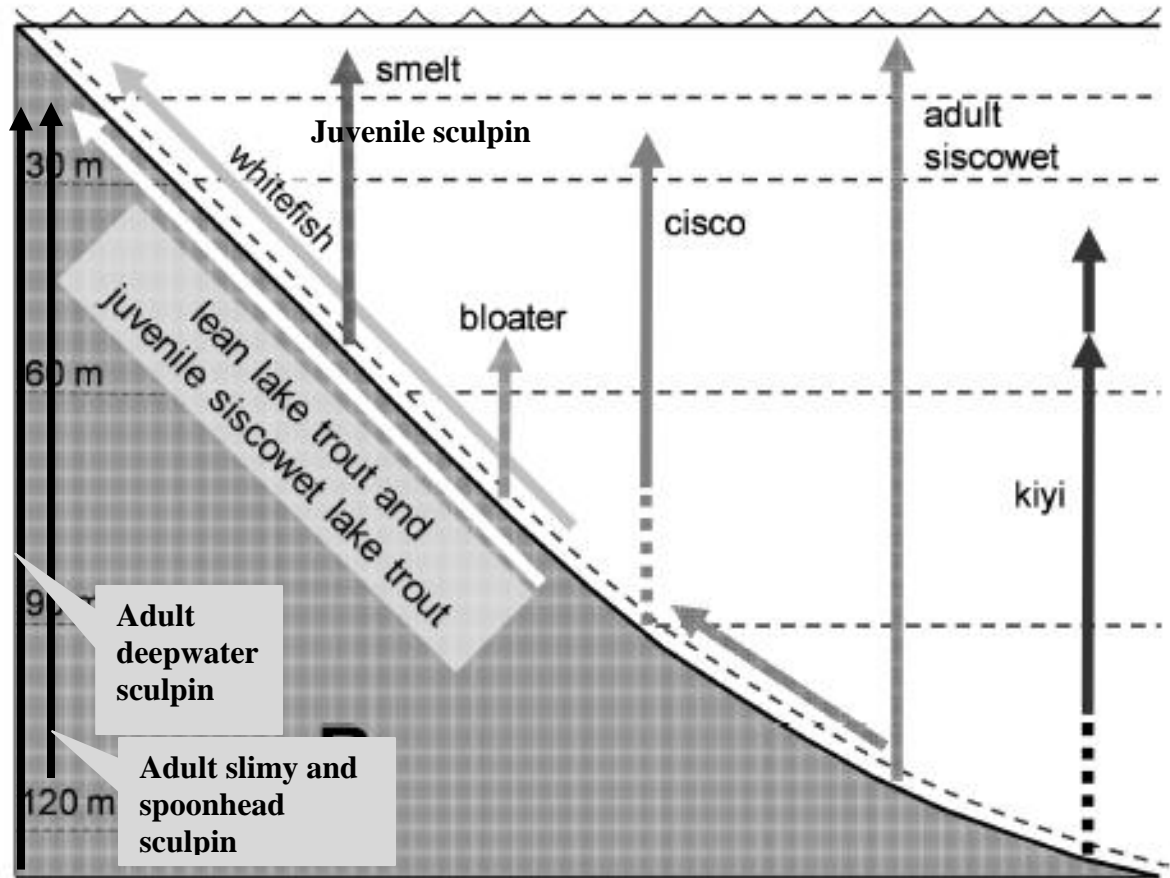


Figure 2: Movement of the open water Lake Superior fishes. Adult siscowet exhibit DVM while juveniles undergo DBM. Kiyi of all age classes perform DVM. Sculpin are pelagic in their first year of life but settle to the lake bottom at varying depths when mature. (Figure adapted from Gorman *et al.* 2012).

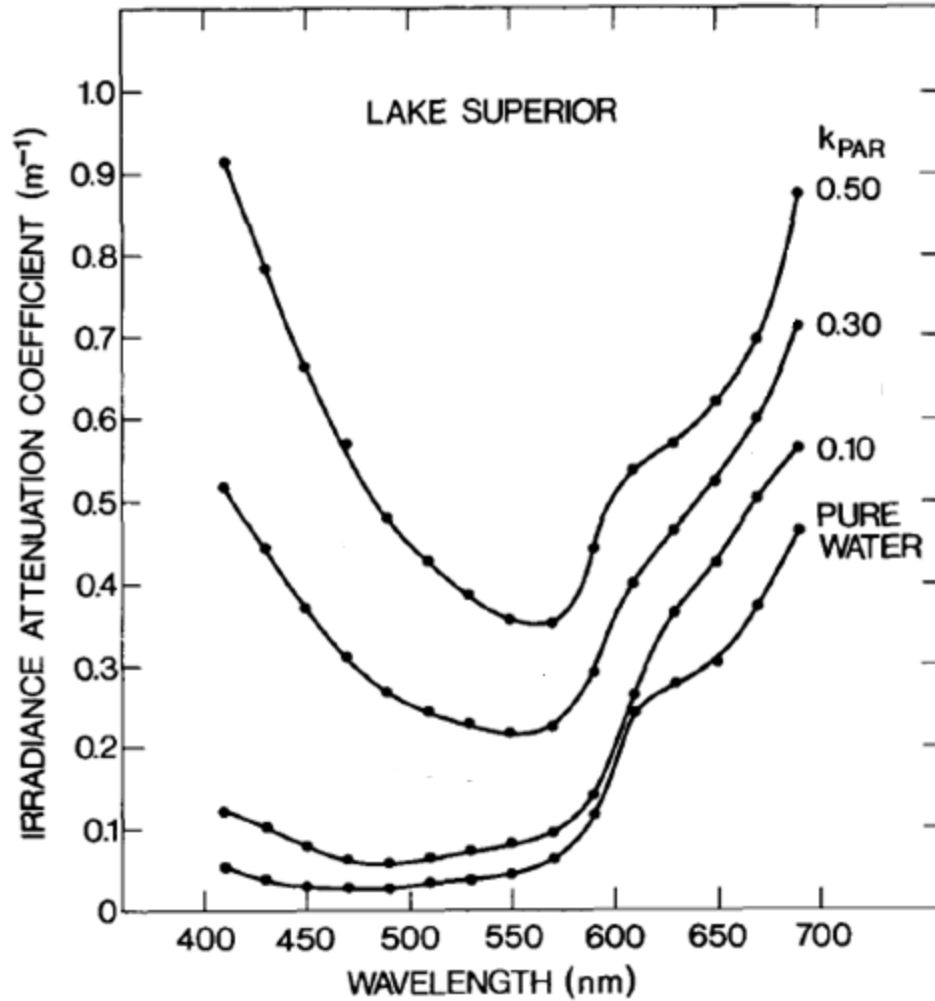


Figure 3: Irradiance attenuation coefficient values per wavelength at differing values of  $k_{PAR}$  (Figure adapted from Jerome *et al.* 1983).  $k_{PAR}$  values 0.3 and 0.5 resemble that of Duluth harbor in summer and fall, respectively (Schertzer *et al.* 1978).

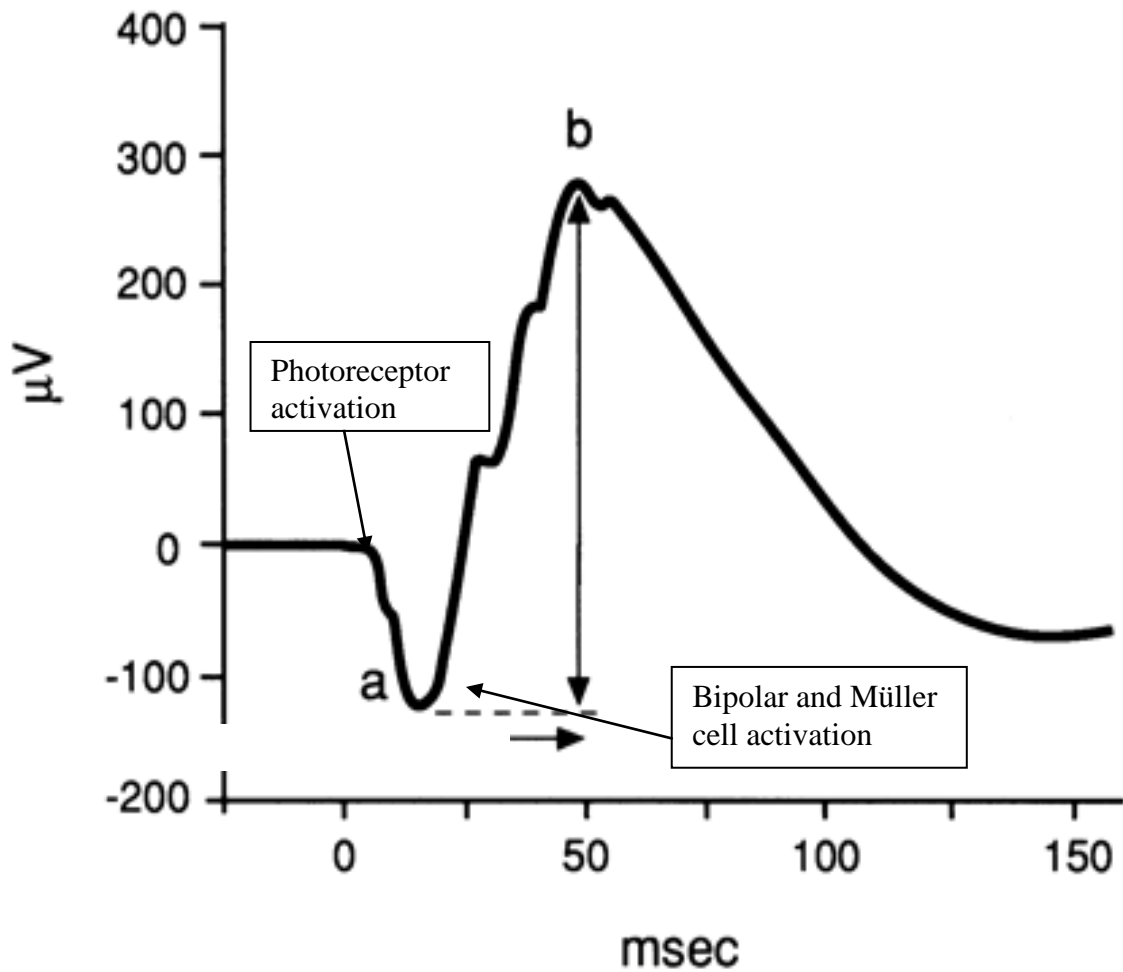


Figure 4: Depiction of and electroretinogram. The a-wave is representative of photoreceptor hyperpolarization while the b-wave demonstrates Müller cell depolarization. Response is measured from the trough of the a-wave to the peak of the b-wave. Adapted from Neuringer *et al.* (1994).

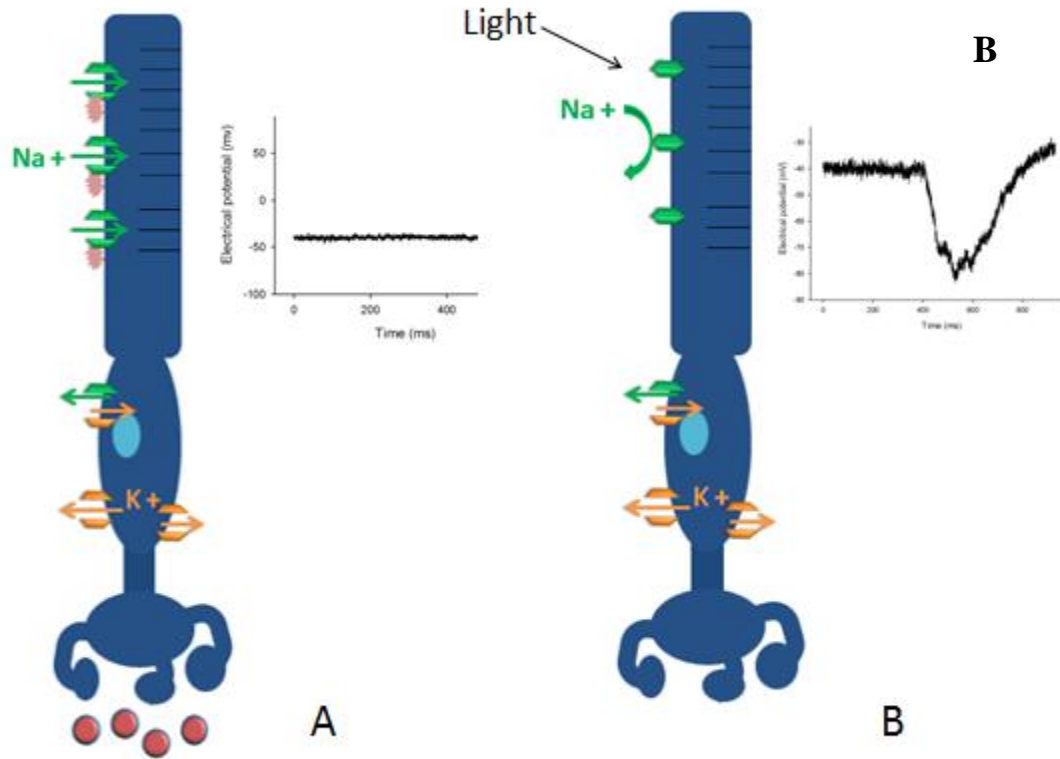


Figure 5: Rod photoreceptors. A) Photoreceptor in darkness receiving sodium influx. cGMP gated ionophores all sodium movement into the cell. Glutamate release occurs to inhibit downstream bipolar cells in the absence of light. B) Photoreceptor in the presence of light. cGMP disassociates from sodium channels and hyperpolarization results with the constant loss of positive potassium ions in the cell body. Glutamate is no longer released. (Figure adapted from Kingsley 2000).

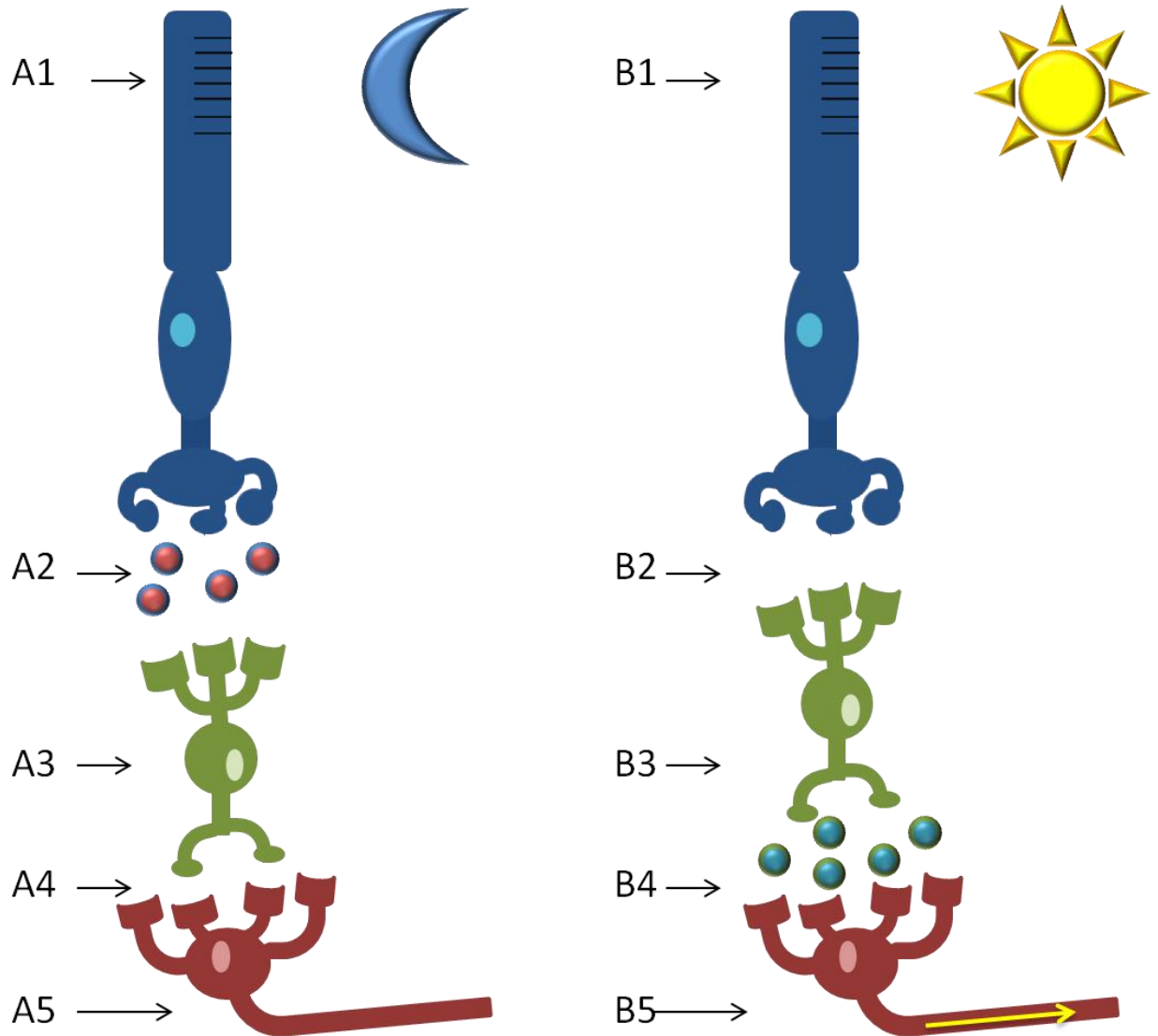


Figure 6: Signaling cascade from photoreceptor to optic nerve. A1) Rod cell in the absence of light. A2) Rod releases glutamate. A3) ON bipolar cell is inhibited (A4) preventing release of downstream neurotransmitter. A5) Ganglion cell fails to receive signal and no signal is relayed to the brain. B1) Rod photoreceptor in the presence of light. B2) Glutamate secretion is prohibited. B3) ON bipolar cell is activated and B4) downstream neurotransmitter is released. B5) Signal is carried from the ganglion cell to the optic nerve (Figure adapted from Kingsley 2000).

## **Chapter two: Comparative Physiology of Round Goby, Logperch, and Black Bullhead Visual Sensitivities**

### **Overview**

The round goby (*Neogobius melanostomus*) is an invasive fish to the Laurentian Great Lakes that originated in the Ponto-Caspian region. It has negatively affected a number of native fish species primarily through its high fecundity and aggressive behavior. The behavioral ecology of the round goby is well documented, however, its sensory physiology remains largely unexplored. Since the round goby evolved in a different optic environment than found in Lake Superior, understanding the capabilities of its visual system is crucial to predict range expansion into turbid tributaries or deeper water. Electroretinography was used to determine the spectral sensitivity of the round goby in comparison to two native benthic species: logperch (*Percina caprodes*) and the black bullhead (*Ameiurus melas*) catfish. The logperch and the round goby demonstrated similar spectral sensitivity with broad sensitivity between 400 and 575 nm. The black bullhead by contrast demonstrated narrow spectral sensitivity shifted to higher wavelengths between 525 and 600 nm. The native fish appear to have visual advantages over the round goby as logperch show a broader spectral sensitivity especially in the prevalent downwelling wavelengths and the black bullhead maintains a visual advantage in murky, red shifted waters. The shallows of the Caspian Sea display a light intensity profile similar to that of Duluth Harbor. Despite the round goby's adaptation to similar aquatic conditions, they do not have a visual advantage when compared to the logperch's

sensitivity as evidenced by the shallower visual depth profiles and narrower spectral sensitivity.

## **Introduction**

Introduced to the Great Lakes in 1990 via ship ballast water (Jude *et al.* 1992), the round goby population quickly expanded, spreading to near shore waters of Lake Superior by 1995 (Jude 1997). Originating in the Ponto-Caspian region, the round goby thrives in the Laurentian Great Lakes (Kovtun 1978; Ricciardi & MacIsaac 2000; MacInnis & Corkum 2000; Balshine *et al.* 2005; Bergstrom *et al.* 2008). With superior egg fitness leading to higher fecundity (Kovtun 1978; MacInnis & Corkum 2000), aggressive behavior, and an opportunistic diet (French & Jude 2001), the round goby outcompetes natives in this aquatic system.

Laboratory studies demonstrated the round goby's ability to outcompete logperch for shelter whether it was the established fish within the tank or an intruder (Balshine *et al.* 2005). But while the round goby can better acquire shelter due to its high level of aggression, round goby reaction and strike distance was decreased in high light intensities compared to the logperch (Bergstrom and Mensinger 2008). Despite this sensory disadvantage, the round goby maintained a competitive advantage over the logperch for resources within an artificial stream. These laboratory studies reflect an ecological trend; the round goby has demonstrated the ability to colonize regions of the Great Lakes, causing declines in native populations and even extirpating species from their original habitat. The round goby's presence has corresponded with dramatic declines in mottled sculpin, johnny darter, and logperch populations (Jude *et al.* 1995; Lauer *et al.* 2004; Riley *et al.* 2008).



Round gobies will also consume the eggs of logperch as well as other species including lake trout (*Salvelinus namaycush*) (Chotkowski & Marsden 1999; Jonas *et al.* 2005), lake sturgeon (*Acipenser fulvescens*) (Nichols *et al.* 2003), and smallmouth bass (*Micropterus dolomieu*) (Steinhart *et al.* 2004). The consumption of eggs by the round goby is thought to be the main contributor to recent logperch decline within the Great Lakes (French & Jude 2001). Egg consumption is also expected to severely threaten the lake trout recruitment rate with the round goby population expansion (Fitzsimons *et al.* 2009).

In the shallow waters of Duluth–Superior Harbor, the native logperch (*Percina caprodes*) competes with the round goby (*Neogobius melanostomus*) for resources. Logperch populations declined in the St. Clair River following the round goby invasion and although the logperch population remains robust in the soft bottom community of the Duluth-Superior Harbor, the fish have been extirpated from near shore rocky areas (Leino & Mensinger 2014). Smaller gobies and logperch both consume small invertebrates such as dipterans, *Caenis* spp., and amphipods and the more aggressive round gobies maintain a competitive advantage over the logperch for these prey resulting in declines of logperch populations (French & Jude 2001). Recent in situ case studies demonstrated the logperch were outcompeted for resources in both rocky and soft-bottom habitats when round gobies were added. The population inhabiting the off shore sandy bottoms of Duluth-Superior Harbor currently remains similar to pre-invasion levels (Leino & Mensinger unpublished).

Integrated pest management approaches such as using models to predict future invasions and locate susceptible areas of invasion hold promise for control or eradication

of round gobies. However, to optimize these strategies, it is imperative to understand the natural history, behavioral ecology, and sensory physiology of the invasive species. The impact of the sea lamprey has been partially mitigated by understanding its olfactory system. Recent work on the behavioral ecology of the common carp, has promoted innovative control measures. Investigating sensory physiology may allow novel controls to exploit invasive species weaknesses. Bergstrom and Mensinger (2009) compared the sensory systems of the logperch to the round goby using behavioral tests in varying light conditions. In individual trials, the logperch demonstrated a visual advantage over the round goby with larger strike distances in higher light intensities. However, when forced to compete interspecifically, the round goby was able to outcompete the native fish for prey. Therefore, while the round goby does not appear to have a visual or mechanosensory advantage, it is still able to acquire prey at levels equaling or exceeding those of native fish. A further comparison of the physiological properties of these fishes is necessary to understand the success of the round goby.

The native benthic black bullhead remains fairly unaffected by the round goby invasion (Kornis *et al.* 2013). Tolerance to low oxygen availability, high carbon dioxide levels, and high pollutants enables the black bullhead to survive in environments inhospitable to many species of fish (Kornis *et al.* 2013). Like the round goby, adult black bullhead exhibit higher levels of activity at night, allowing this species to avoid competition with diurnal feeders (Darnell & Meierotto 1965; Johnson *et al.* 2008). Residing in turbid conditions, the black bullhead has adapted to capturing prey in visually challenging situations.

When foraging at night, the black bullhead and round goby consume prey under minimal light intensities (Darnell & Meierotto 1965; Johnson *et al.* 2008) whereas the diurnal logperch consumes prey in more light intense conditions (Bergstrom *et al.* 2009). In smaller lakes and shallow aquatic environments, the black bullhead experiences a visual disadvantage with increased levels of turbidity (Braig & Johnson 2003). Differences between the visual capabilities of the round goby, the native logperch, and black bullhead may suggest superior visual sensitivity of one species, enabling more successful prey capture, predator avoidance, and mate localization. Electrophysiology was employed to determine if the round goby has a physiological advantage over the declining logperch or the black bullhead.

## **Materials and methods**

Round gobies [66 to 111 mm total length (TL)] were captured using 40.6 cm (0.6-cm square mesh, 3.0 cm diameter opening) galvanized minnow traps baited with thawed fish remnants (Lake Superior Fish Company, Superior, WI) in the Duluth-Superior Harbor in the summer of 2012. Logperch (50 to 60 mm TL) were collected via beach seine (20 m length with 6.4 mm mesh net and 3.2 mm mesh purse) on a sandy shoreline in Duluth-Superior Harbor also during the summer of 2012. Black bullheads were captured via angling in Hartley Pond, Duluth, MN in the summer of 2013. Fish were housed by species in aerated glass aquarium filtered with Tetra Power Filters (model PF10, Tetra Holding, Inc., Blacksburg, VA), Marineland Penguin<sup>®</sup> mini filters (model PF99, United Pet Group, Blacksburg, VA) and Aquaclear 70 Power (model HG10615, Marine Depot, Garden Grove, CA) filtration systems. All tanks were kept at

approximately 20°C. Fish were fed Nelson's Silver Cup™ sinking trout pellets (Harrietta Hills, Harrietta, Michigan) every other day and water quality was monitored according to animal care protocols. Fish were kept on a 12 hour light and 12 hour dark schedule, illuminated by a combination of natural and fluorescent light.

#### *Electroretinogram Preparation*

Fish were anesthetized via submersion in 0.002-0.006% MS-222 in 0.003 M buffer (4.5% sodium phosphate dibasic, 1.06% potassium phosphate monobasic in distilled water, Sigma Chemical Co., St. Louis, MO). Following initial anesthetization, the sedated fish was placed on a sponge in a 44.5 x 11.0 x 9.0 cm acrylic tank and the fish was submerged up to the ventral border of the eyes. The experimental tank was placed in an opaque metal Faraday cage (77.0 x 67.0 x 96.0 cm) and the front door of the cage was lowered after electrode insertion to block instrument lights in the dark room. A 15°C solution of 0.002-0.007% MS-222 was circulated over the gills through an intraoral tube for the duration of the experiment. Water was chilled to the average temperature of Lake Superior in late August and early September (GLERL 2011) using the 420 W Teco SeaChill® Aquarium Chiller (Teco® model SCTR20, Ravenna, Italy).

#### *Electroretinogram Collection*

A constant current power supply (Newport® model 68938) powered the 100 W quartz tungsten-halogen lamp (Newport® model 6333, Stratford, CT) to provide the light stimulus for the experiments. The light stimulus duration was regulated by an Oriel® Electronic Shutter (model 76994, Stratford, CT) in conjunction with the Oriel® Shutter Controller (model 76995). The wavelength of light per stimulus was selected using a

monochromator (1.56 mm slit) (Newport<sup>®</sup> model 77250) and neutral density filters (0.1 to 3.0) were used to diminish the intensity of the light stimulus. A fiber optic light pipe (Newport<sup>®</sup> model 77632) delivered the light to the fish's eye. The intensity of the light was measured using a radiant power energy meter (Ophir<sup>®</sup> model 70260) and probe (Ophir<sup>®</sup> model 70268).

A small incision was made in the limbus and with a 3 mm surgical stab knife and a 200  $\mu\text{m}$  silver-silver chloride recording electrode was inserted into the vitreous. The reference electrode was placed on the epidermis on the frontal bone midway between the eyes. ERGs were amplified using World Precision Instrument, Inc. amplifier (1000x, 1 Hz low pass, 3 kHz high pass, model DAM50; Sarasota, FL), filtered using a digital 60 Hz notch filter, recorded with PowerLab 4SP (AD Instruments, Castle Hill, Australia), and stored using Lab Chart<sup>®7</sup> (AD Instruments, Castle Hill, Australia) software on a portable computer. Data were collected between 1015 and 1715 to minimize affect of circadian rhythms. Fish were dark adapted for 30 minutes after electrode insertion, prior to testing. Flashes of monochromatic light (200 ms duration) were presented at 30 s intervals and wavelengths were presented in 10 or 25 nm intervals from 400 to 700 nm with wavelength presentation order randomly determined for each trial.

#### *Minimum Criterion Response*

Minimal b-wave amplitudes were consistently encountered in response to short wavelengths ( $\leq 425$  nm) and therefore the b-wave amplitude at 400 nm was set as the minimal criterion response for each fish. Longer wavelengths were reduced in intensity by neutral density filters until the b-wave amplitude equaled the criterion amplitude established at 400 nm, and the irradiance needed to reach the criterion amplitude was

used to generate spectral sensitivity curves for each species. B-wave amplitudes were calculated from baseline and criterion responses ranged from 6 and 167 mV by measuring from the baseline to the top of b-wave. Neutral density filters were used to decrease light intensity to match the criterion response. The irradiance needed for the criterion response was calculated for each wavelength to produce the spectral sensitivity curves.

#### *Light Attenuation Calculation*

Beer's Law:

$$E_{(z, \lambda)} = E_{(0, \lambda)} e^{-k_{(\lambda)} z} \quad (1)$$

where E denotes the downwelling irradiance at depth z for wavelength  $\lambda$  and  $k(\lambda)$  represents the irradiance attenuation coefficient of  $\lambda$  was used to calculate light irradiance at depth. For the western arm of Lake Superior,  $k_{PAR}$  ranges between 0.3 and 0.5 were used to estimate downwelling irradiance and a  $k_{PAR}=1.0$  was used for Duluth-Superior Harbor=0.3 and  $k_{PAR}=0.5$  (Schertzer *et al.* 1978). The depth at which light of each wavelength is present at one percent surface irradiance was then calculated using Beer's Law.

Estimates of light transmission in the Caspian Sea were obtained from shallow near shore, offshore (3km), and open water (15km offshore) (Gholamalifard *et al.* 2013). Based on this data, the Caspian Sea has  $k_{PAR}$  values of approximately 0.3, 0.5, and 0.7 for the shallow water, offshore water, and deep water, respectively. To find the depth of penetration for each wavelength to one percent of the surface irradiance, the attenuation coefficient corresponding to wavelength ( $\lambda$ ) in the specific aquatic location was used.

### *Visual Depth Profiles*

To estimate the depth at which fish can see, the minimum energy required to illicit an ERG was substituted for  $E_{(z, \lambda)}$  in Beer's Law (Equation 1) for each wavelength tested. Crater Lake solar irradiance values (Tyler and Smith 1970) were used in visual depth calculations as this lake at similar latitude to Lake Superior and thus experiences similar irradiances. Since lunar values were not available for Crater Lake, the lunar to solar irradiance ratio was calculated using values from Eniwetok Atoll (Munz and McFarland 1973; McFarland and Munz 1975) and applied to Crater Lake solar values to estimate lunar irradiance. The minimum irradiance needed to evoke a b-wave in the dark adapted retina was used to determine visual sensitivity.

### **Results**

Round goby spectral sensitivity peaked between 450 and 525 nm with slightly reduced sensitivity to 400 to 425 nm. There was sharp decline in sensitivity to wavelengths greater than 550 nm. Logperch displayed similar broad spectral sensitivity however sensitivity was relatively unchanged from 400 to 550 nm before dropping sharply to higher wavelengths. In contrast, the black bullhead showed broad sensitivity between 500 and 600 nm with reduced sensitivity to both shorter and longer wavelengths (Figure 1).

### *Light Attenuation in Duluth waters and the Caspian Sea*

To illustrate the differences in underwater light environments, the depths that at which surface irradiance is reduced to one percent was calculated for three areas in Lake Superior and the Caspian Sea. Maximum transmission was by 550 to 575 nm light with

rapid attenuation of shorter and longer wavelengths. The offshore sites in both Lake Superior and the Caspian Sea provided the deepest light penetration.

At its clearest ( $k_{PAR}=0.3$ ), Duluth Bay allowed deepest penetration of light with one percent of 550 nm surface irradiance occurring deeper than 20 m. The speed of light extinction increased in more turbid waters ( $k_{PAR}=0.5$ ) causing the depth of attenuation to decrease to approximately 13 m. Duluth-Superior Harbor waters ( $k_{PAR}=1.0$ ) only allowed one percent of 575 nm light to penetrate to 7 m. One percent of the surface irradiance of wavelength 575 nm attenuated to 18 m in the deep waters of the Caspian Sea. In the Caspian shallows, one percent of 575 nm light reached less than 8 m. Offshore Caspian water light attenuation profiles resembled that of Duluth Bay ( $k_{PAR}=0.5$ ). Similarly, the Caspian shallows closely resembled that of Duluth-Superior Harbor.

#### *Visual Depth Profiles*

Based on ERG measurement and light attention, the maximum depth at which sufficient irradiance is available to mediate fish visual interactions were calculated under three transmission conditions: Duluth Bay ( $k_{PAR}=0.3$  and  $k_{PAR}=0.5$ ) and an estimate of Duluth-Superior Harbor ( $k_{PAR}=1.0$ ) were analyzed for each species. Diurnal conditions allowed for visual depth profile calculations while insufficient surface irradiance under nocturnal conditions prevented the construction of nocturnal visual depth profiles.

The logperch displayed marginally greater sensitivity than the round goby under all conditions with both fish more sensitive than the black bullhead. Irradiance was sufficient for vision at 550 nm light under sunny, summer conditions to 37 and 40 m depths for the round goby and logperch, respectively with the black bullhead estimated to retain vision to approximately 24 m. However, at shallower depths the black bullhead



was more sensitive to long wave length light than the round goby. Higher  $k_{PAR}$  values and nocturnal conditions reduced the depth at which sufficient light is available for vision accordingly.

## **Discussion**

The round goby and logperch have similar broad spectral sensitivity curves that show high sensitivity to 400 nm to 550 nm and are tuned to prevailing available irradiance in the water column. The black bullhead shows red shifted peak sensitivity with relatively low sensitivity to short wavelength light. The logperch vision is very similar to the round goby and neither species appears to have a significant advantage based on spectral sensitivity curves.

The ERG has long been used to assess spectral sensitivity by determining the electrical potential of the retina. In light adapted retinas, ERG waveforms include an a-wave generated by the photoreceptor hyperpolarization upon initiation of a light stimulus, and a b-wave, originating from Müller cells and bipolar cells depolarization (Wen & Oakley 1990). When dark adapted the a-wave is absent and allows a more precise determination of the b-wave amplitude. To assess species visual advantage in high turbidity environments, dark adapted retinae were tested.

The ERG provides a mechanism for minimally invasive sampling. Since the ERG only detects electrical differences within the eye, a caveat to this procedure is the inability to monitor brain activity and subsequently image formation. Thus since the central visual pathway and neural activity is not analyzed, ERGs cannot be directly correlated with image perception. However, b-wave formation is required to initiate the ionic cascade leading to neuronal image formation. Therefore if sufficient light is

absorbed by the photoreceptors and the bipolar and Müller cells are stimulated, the electrical potential of the retina changes; this change suggests that fish can centrally detect light and therefore, may be able to perceive an image. Additionally, the position and distance between the recording and ground electrodes insures that current will be lost before detection of retinal electrical potential. Therefore, it is likely that visual sensitivity reported here is reduced compared to the actual sensitivity of the organism. For the purposes of this paper, visual sensitivity is defined as the minimal irradiance sufficient to elicit a b-wave in a dark adapted retina.

### *Fish Sensitivity*

Previous studies on predator-prey interactions showed that the logperch have greater reaction and strike distances in comparison to the round goby, suggesting that logperch possess a sensory advantage. However, these were done under white light conditions and did not accurately recreate the spectral composition of the water column at depth. If the logperch and round goby have different spectral sensitivities, it could reflect an additional advantage for the logperch. Additionally, it could allow predictions of where the logperch could flourish and where the round goby may be limiting. Long term studies in the Duluth-Superior Harbor demonstrated that although logperch have been extirpated from the rocky community, they are still present in pre-round goby numbers on the soft sediment and their enhanced sensory system may partially offset round goby aggressiveness.

The spectral sensitivities of logperch, round goby, and black bullhead can be compared based on the shape of the data curves. While increasing along the y axis indicates higher sensitivity, these data should not be compared interspecifically in the

context of individual wavelengths; an incorrect interpretation of this data would assert that logperch display greater retinal sensitivity as this data set appears higher on the sensitivity axis (Figure 1). Comparing the sensitivities in this manner is misleading because of differences in MCRs between species resulting from variability in signal strength and noise levels. Instead, the shapes of the curves shed light on the visual capabilities of each species.

Both the round goby and logperch exhibit relatively high sensitivity in the lower wavelengths (400-500 nm) compared to their sensitivity to the longer wavelengths between 600 and 700 nm, suggesting possible UV light sensitivity. The black bullhead demonstrates insensitivity to these short wavelengths suggesting insensitivity to UV light in this species. Round goby sensitivity began decreasing after 525 nm while logperch sensitivity remained relatively high until 575 nm. Black bullhead sensitivity did not decrease until after 600 nm. Due to the broader spectral sensitivity, the logperch may have the most versatile visual sensitivity of the three species, capable of perception of a broad range of light environments. The longer wavelength shift in visual perception seen in the black bullhead may be the result of the high turbidity environment commonly inhabited by the species. As more turbid, freshwater conditions favor higher wavelength attenuation (Jerlov 1968; Walmsley *et al.* 1979; Shaoying *et al.* 2009;), the black bullhead may have an advantage in murkier environments as it is able to better perceive longer wavelengths than the other two species.

Poor sensitivity to the longest wavelengths of these three species corresponds with the isolume where they dwell. Benthic fish are subjected to a wide array of wavelengths depending upon their depth. Typically, shallow water species are likely to

encounter the entire visual spectrum, however the intensity of red shifted light representative of the longer wavelengths is diminished closer to the water's surface (Jerlov 1968; Munz 1976; Walmsley *et al.* 1979). Murky waters with increased concentrations of suspended particulate matter, however, allow some red wavelengths to penetrate, and filter the lower wavelengths (Walmsley *et al.* 1979; Shaoying *et al.* 2009). As a result, these turbid waters enable red light to attenuate more slowly than blue and green light. The logperch and round goby are appropriately insensitive to red wavelengths as these are not predominant in shallow clear waters (Jerlov 1968; Jerome *et al.* 1983; Schertzer *et al.* 1978; Walmsley *et al.* 1979; Fahnenstiel *et al.* 1984; Shaoying *et al.* 2009). The black bullhead sensitivity is shifted towards the red wavelengths corresponding with those present in the murky waters of its habitat.

*Fish Sensitivity and Light Attenuation in Duluth Bay, Duluth Harbor, and the Caspian Sea*

As the round goby evolved in the Ponto-Caspian basin, it is important to compare the optical environment with Lake Superior. The similar properties in both environments may be attributable to why the round goby has flourished. The offshore waters of the Caspian Sea and the waters of Duluth Bay in the fall ( $k_{PAR}=0.5$ ) have similar light transmission properties. The Caspian shallows and Duluth-Superior Harbor also have very similar spectral properties. Given the spectral environment, it is somewhat surprising that both species show relatively strong sensitivity to shorter wavelengths as these are rapidly attenuated in the deeper, clear offshore waters.

The light environments of Duluth-Superior Harbor and the Caspian shallows are similar in their estimated depth profiles (Figure2) with Duluth-Superior Harbor showing

slight depth limitation in the red-shifted wavelengths greater than 575 nm. The round goby should be equally adapted to conditions in Duluth-Superior Harbor compared to native fishes since the species evolved in a similar light environment.

Comparison of visual sensitivity to light depths reveals inconsistencies.

Interestingly, the round goby and logperch visual sensitivity curves do not resemble the depth of light attenuation at one percent surface irradiance depth curves (Figure 5). The six aquatic systems reveal fast attenuation of the shorter wavelengths (400-500 nm). Round goby and logperch vision however does not correspond with this diminished light intensity. The visual sensitivity of these two fishes remains fairly high at the lower wavelengths; peak sensitivity is only slightly higher than the sensitivity at shorter wavelengths. Visual sensitivity should be closely related to the prevailing wavelengths at depth to maximize visual perception and minimize energy allocated to the visual system according to the sensitivity hypothesis proposed by Clarke (1936). Since the slope of one percent surface irradiance decreases in these shorter wavelengths with increases in  $k_{PAR}$  values, the fish may be adapted to waters with  $k_{PAR}$  values higher than 1.0. Their insensitivity to longer, red-shifted wavelengths prevalent in high turbidity waters, however, is inconsistent with adaptation to such high turbidity.

Black bullhead sensitivity corresponds with wavelengths below 500 nm in the six aquatic systems shown. Although the bullhead's sensitivity peak is shifted slightly higher than the most predominant wavelengths in these waters, the diminishment of sensitivity at the longest wavelengths corresponds with the extinction of the longer wavelengths in these aquatic systems. This might suggest that the black bullhead is more closely specialized to the predominant wavelengths in these systems, and possibly those with

higher turbidity, while the logperch and round goby utilize a generalized visual perception.

The discrepancy between logperch and round goby visual sensitivity and predominant wavelengths available at depth may be an adaptation to living in varying light environments. Marine ecosystems allow the slowest attenuation of blue light (Jerlov 1968). The increased sensitivity in the short wavelengths may be an adaptive relic of a marine ancestor. Visual perception of light is made possible by pigments in the retina. These variable pigments are sensitive to a particular wavelength,  $\lambda_{\max}$ . Bony fish have a higher diversity of visual pigments than all other vertebrates combined, likely due to the highly variable light environments of aquatic ecosystems. Rhodopsin visual pigments are responsible for perception of shorter wavelengths and can have  $\lambda_{\max}$  from 467 to 526 nm (Munz & McFarland 1977). Many marine fishes rely solely on rhodopsin pigments for scotopic perception (Wald *et al.* 1957; Dartnall & Lythgoe 1965; Jokela-Määttä *et al.* 2007). Freshwater fishes possess porphyropsin visual pigments whose  $\lambda_{\max}$  falls between 502 and 551 nm (Munz & McFarland 1977). Freshwater fishes may rely solely on porphyropsin or implement both porphyropsin and rhodopsin pigments for scotopic perception (Dartnall & Lythgoe 1965; Bowmaker *et al.* 1988; Jokela-Määttä *et al.* 2007). In fishes that use two different pigment types, ratios of the two pigments vary between species (Jokela-Määttä *et al.* 2007). The relatively broad spectral perception of the logperch and the round goby may suggest use of rhodopsin pigments as well as porphyropsin, while the black bullhead sensitivity may be dependent only on porphyropsin activity.

*Visual depth profiles*

Both the round goby and the logperch are able to perceive the majority of the visual spectrum (425 to 575 nm) past 16 m in full sunlight in Duluth Bay's clear water ( $k_{PAR}=0.3$ ). As these two species are shallow water dwellers, their vision is likely minimally diurnally in waters with  $k_{PAR}=0.3$ . Residing in Duluth-Superior Harbor, fish experience reduced visual capabilities at depth diurnally. All three fishes are able to see slowly attenuating wavelengths past 8 m, but vision is reduced to shallow waters towards the ends of the visible spectrum. The logperch retains an advantage over the round goby and black bullhead with the capability to perceive light up to 13 m in this turbid environment; the round goby and black bullhead experience restriction to vision at approximately 12 and 8 m, respectively. Although active nocturnally, neither the black bullhead nor the round goby is sensitive enough to perceive the minimal surface irradiance provided by the moon. Vision is not feasible for any of these three shallow dwellers, nocturnally. The round goby migrates to deeper waters in the winter reaching depths greater than 120 m (Walsh *et al.* 2007). These results indicate that the round goby would experience visual impairments at these depths. Residing at 75 m in abundance, (Walsh *et al.* 2007), the round goby would be unable to perceive even peak wavelengths. Other sensory modalities would be necessary for predator avoidance at these depths in winter conditions.

Interspecific comparison of the round goby and the logperch visual sensitivity reveals an advantage for the logperch. Logperch are able to perceive light at greater depths under each condition suggesting a higher visual sensitivity throughout the visual spectrum. Occupying the rocky shoreline of Duluth-Superior Harbor at shallow depths above 8 m, both the logperch and the round goby face some limitation to their visual

sensitivity in sunny conditions, but vision in the peak wavelengths is retained. The increased sensitivity of the logperch, specifically between wavelengths 475 and 575 nm, suggests a visual advantage at depth compared to the round goby allowing the logperch to see slightly deeper in the varying conditions of Duluth-Superior Harbor. The behavioral aggression exhibited by the round goby may drive logperch to further depths where they will experience less competition for resources with the visually insensitive round goby.

Both the round goby and the logperch demonstrate a visual sensitivity at greater depths compared to the black bullhead, although this visual depth advantage diminishes in the longer wavelengths. This is most likely the result of the black bullhead's adaptation to murkier habitats. While black bullheads have been found in Duluth-Superior Harbor, their preferred habitat lies in still streams with soft bottoms (Braig & Johnson 2003). These environments are highly turbid, containing a higher concentration of suspended particulate matter, resulting in low light intensity (Shaoying *et al.* 2009; Walmsley *et al.* 1979). Shorter wavelengths are quickly extinguished and therefore not used by the black bullhead in visual perception. Longer wavelengths are absorbed by the black bullhead visual pigments instead.

The round goby's success in invading and proliferating in Lake Superior cannot be attributed to a physiological advantage in terms of vision; no visual advantage over the logperch in Duluth waters is apparent. Conversely, the native logperch demonstrates a visual advantage based on the broader spectral range of perception as well as deeper estimated visual depth profiles. Based on electroretinograms, both fish appear to have similar visual sensitivity profiles to each other throughout the visual spectrum. Deeper visual depth profiles seen in the logperch indicate versatile functionality to varying



aquatic conditions compared with the other two fishes. Both the logperch and round goby have sensitivity spectrums shifted in favor of shorter wavelengths compared to the red shifted spectral sensitivity of the black bullhead.

## Figures

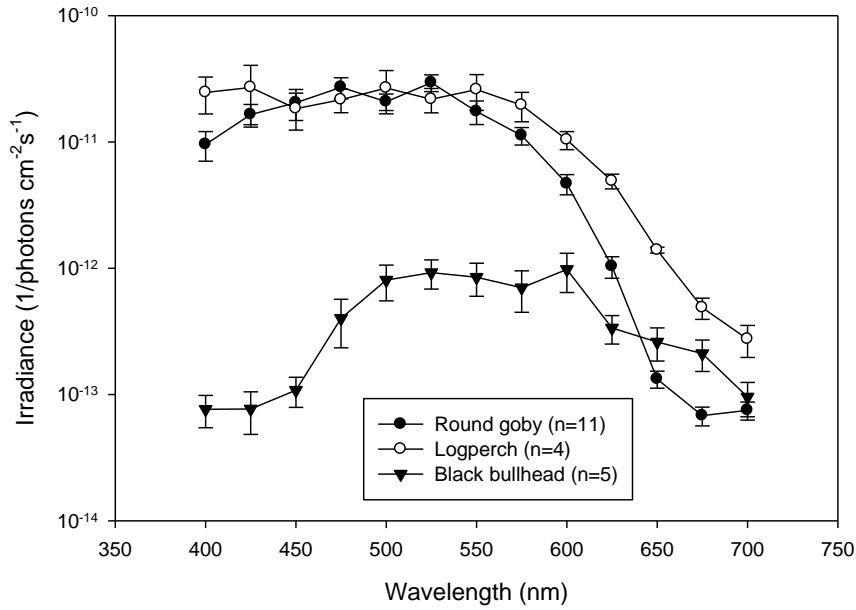


Figure 1: Comparison of round goby, logperch, and black bullhead spectral sensitivity curves. Round goby (n=11) data is represented by black circles, logperch (n=4) data is represented by white circles and black bullhead (n=5) data is shown with black triangles. Data are represented with 25 nm increments of wavelength. Standard error bars are presented.

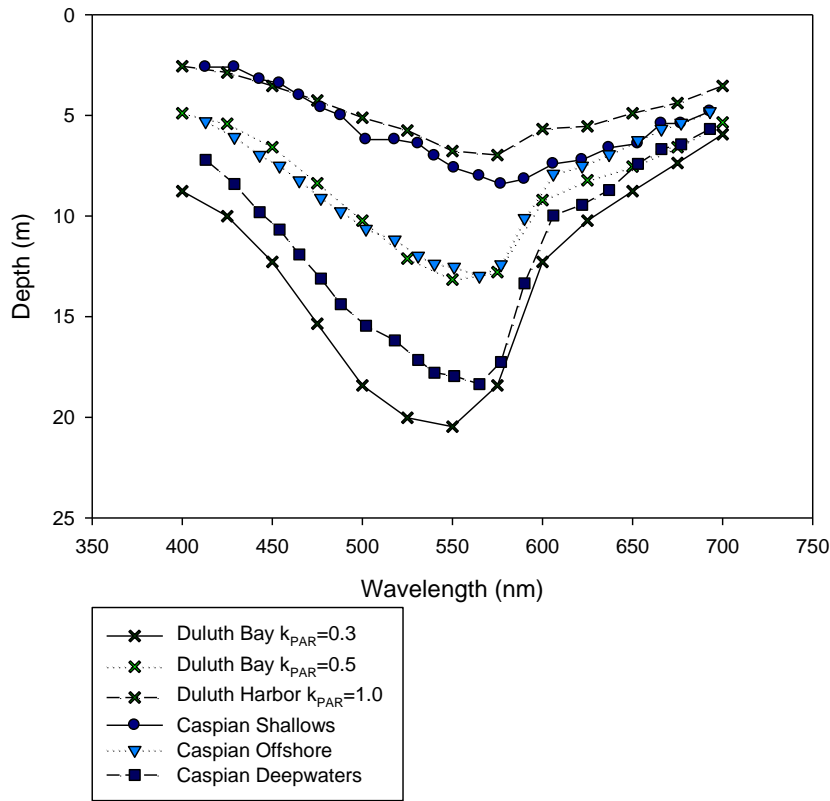


Figure 2: Comparison of the depth of one percent surface irradiance in two different turbidities of Duluth Bay, one turbidity of Duluth-Superior Harbor, and three sites in the Caspian Sea. Duluth waters are represented as crosses and Caspian Sea waters are circles (shallows), triangles (offshore) and squares (deepwater). Less turbid Duluth Bay ( $k_{PAR}=0.3$ ) uses a solid line while a more turbid Duluth Harbor ( $k_{PAR}=0.5$ ) is uses a dotted line. Duluth-Superior Harbor uses a dashed line. Depth decreases with an increase on the y-axis.

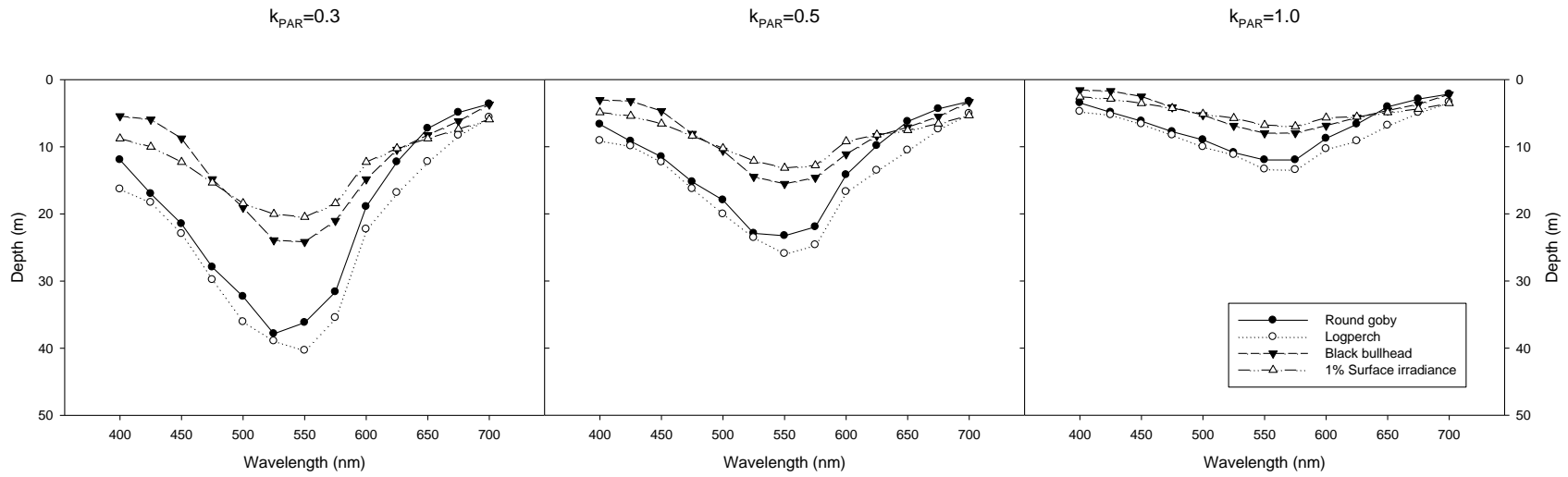


Figure 3: Visual depth profiles of round goby (black circles), logperch (white circles), and black bullhead (black triangles) with 1% surface irradiance for varying levels of  $k_{PAR}$  under diurnal conditions. A) Profiles for  $k_{PAR}=0.3$  representative of clear Duluth Bay conditions. B) Profiles for  $k_{PAR}=0.5$  representative of turbid Duluth Bay conditions. C) Profiles for  $k_{PAR}=1.0$  representative of Duluth Harbor. Wavelength is represented on the x axis and depth decreases with increase along the y axis.

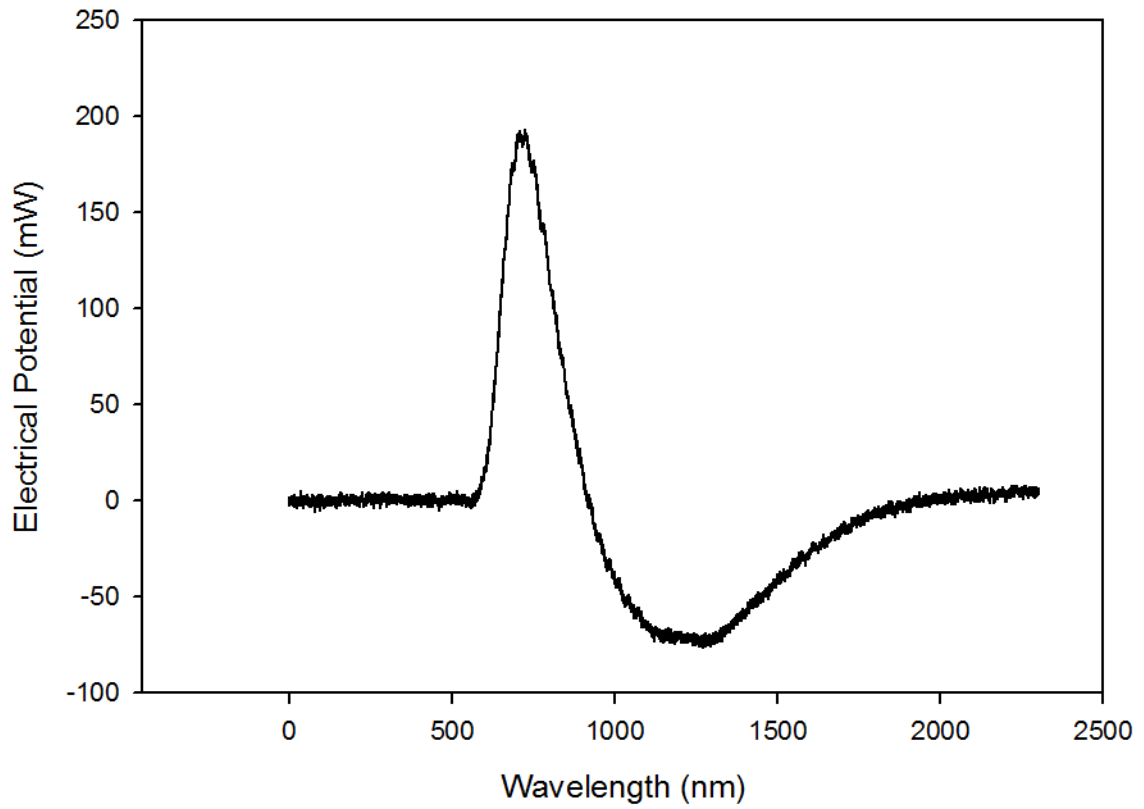


Figure 4: An electroretinogram of a dark adapted round goby in response to 700 nm light. The a-wave is not present in dark adapted fish. The b-wave seen at 500 ms is representative of the depolarization of the Muller cells. This wave is reduced in experimentation to reach a minimum criterion response.

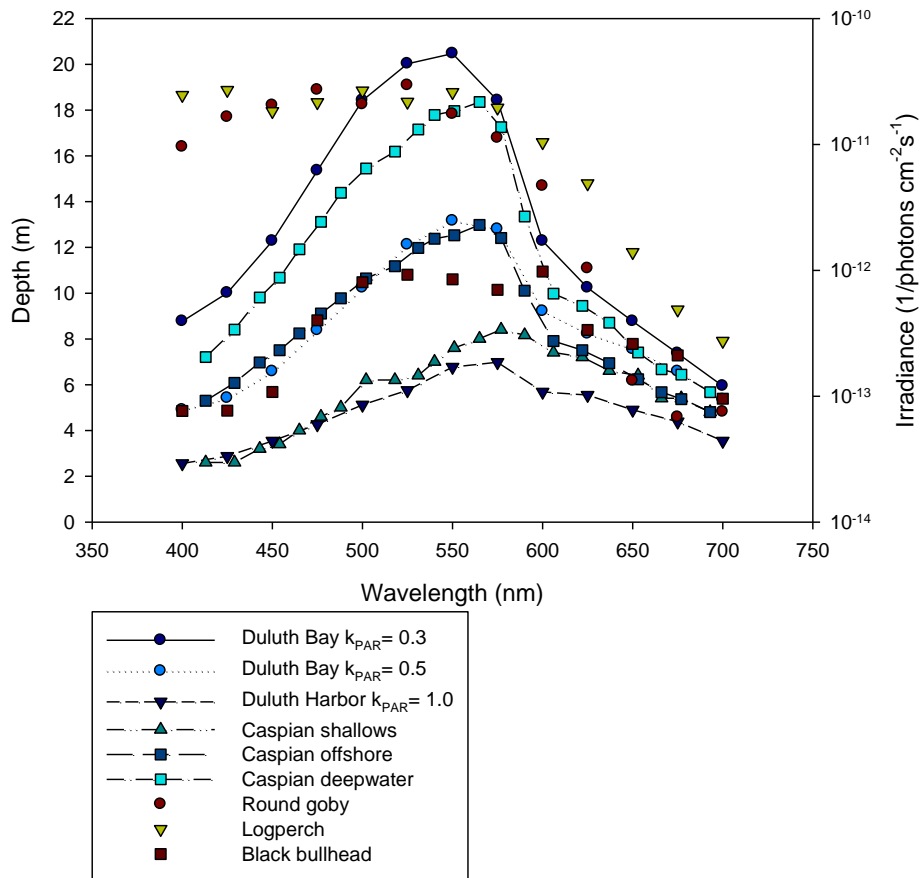


Figure 5: Fish spectral sensitivity plotted against one percent light profile calculations. Duluth Bay conditions are represented circles with solid and dotted lines corresponding with  $k_{PAR}=0.3$  and  $k_{PAR}=0.5$ , respectively. Duluth-Superior Harbor is represented as inverted triangles. Caspian shallows and offshore waters are plotted as triangles and squares, respectively. Caspian deepwater is represented by squares with dotted and dashed lines. Round gobies are represented as circles without lines, logperch are represented as inverted triangles without lines, and black bullheads are squares without lines. Depth is plotted increasingly on the left y-axis, irradiance is shown on the right y-axis, and wavelength is found increasing along the x-axis. Spectral sensitivity values are for shape comparison rather than interspecific intensity comparison.

## **Chapter 3: Visual Sensitivity of Deepwater Fishes in Lake Superior**

### **Overview**

The predator-prey interactions in the offshore food web of Lake Superior have been well documented but the sensory systems mediating these interactions remain unknown. The deepwater sculpin, (*Myoxocephalus thompsoni*), siscowet (*Salvelinus namaycush siscowet*), and kiyi (*Coregonus kiyi*) inhabit low light level environments. To investigate the potential role of vision in predator-prey interactions, electroretinography was used to determine visual sensitivity for each species. Spectral sensitivity curves revealed peak sensitivity at 525 nm for each species. To determine if sufficient light is available to mediate predator prey interactions at depth, visual sensitivity was correlated with the intensity of downwelling light in Lake Superior to construct visual depth profiles for each species. Sufficient daytime irradiance exists for visual interactions to approximately 100 m depth for siscowet and kiyi and 140 m for the deepwater sculpin during summer months. Nocturnal vision, however, is not feasible at any depth, for any of the three offshore fish due to insufficient surface irradiance. Visual interactions are therefore limited to daytime interactions at depths that are relatively shallow to the fish depth distributions. Thus, it is likely that all three organisms utilize another sensory modality for nighttime predation and visual daytime predation is limited to relatively shallow depths (<100 m).

### **Introduction**

Lake Superior is the largest of the Laurentian Great Lakes and home to 38 fish species, including 19 nonnative species (Habermann *et al.* 2012), with the majority of these fishes inhabiting the shallow, nearshore waters or surrounding watersheds. The cold deep, oligotrophic offshore waters of Lake Superior are depauperate with fish densities less than 1.14 kg/ha (Gorman *et al.* 2012). Although many invasive aquatic species have been introduced and have disrupted and/or become integrated into shallow water community, the deep waters of Lake Superior remain dominated by native species (Gamble *et al.* 2011). Piscivorous fish including burbot (*Lota lota*) and siscowet lake trout (*Salvelinus namaycush siscowet*) dominate the highest trophic levels and prey predominately on deepwater sculpin (*Moxocephalus thompsonii*) and/or kiyi (*Coregonus kiyi*) (Isaac *et al.* 2012). The deepwater sculpin and kiyi, along with the cisco (*Coregonus artedi*) form the second trophic level, and consume various zooplankton such as mysis (*Mysis relicta*), scuds (*Diporeia* spp.), cladocerans, and copepods (Gamble *et al.* 2011; Auer *et al.* 2013).

This relatively simple food web dominates the deep, oligotrophic water of Lake Superior. The diel vertical migrating (DVM) zooplankton, *Mysis relicta*, is the primary conduit for energy flow from benthic waters to the surface, as it feeds diurnally on benthic detritus and then consumes surface phytoplankton and zooplankton during its nightly ascent (Beeton & Bowers 1982; Ahrenstorff *et al.* 2011). Two planktivorous fish, deepwater sculpin and kiyi prey primarily on the mysis, however the deepwater sculpin will also consume benthic amphipods (*Diporeia* spp) (Auer & Kahn 2004; Gamble *et al.* 2011). The siscowet is the most abundant piscivore in the lake (Sitar *et al.* 2008; Gorman



*et al.* 2012) and its feeding habits are dictated by diurnal vertical migrations of the planktivores (Ahrenstorff *et al.* 2011). During the day, the siscowet remain in deepwater (>140 m) and prey primarily on the benthic sculpin, while at night, they vertically migrate to consume kiyi which in turn are following the migration of mysis (Hrabik *et al.* 2006; Gamble *et al.* 2011; Gorman *et al.* 2012; Isaac *et al.* 2012; Ahrenstorff *et al.* 2012).

However, little is known about the nature of the predatory-prey interactions and the role that vision plays in mediating these interactions. While olfactory and auditory cues may be used for long range detection of prey, short range interactions usually are mediated by the mechanosensory lateral line or visual input (Pitcher 1993). Vision is often the main sensory modality in shallow, sun lit waters while the lateral line may dominant in turbid and/or low light environments. To understand the role of vision, it is important to know both visual and spectral sensitivity of the organisms and the intensity and spectral composition of downwelling irradiance. Predator-prey or population models often contain little to no sensory information and by incorporating sensory physiology into these traditional fisheries models, more accurate models can be developed to better predict population structure and dynamics.

The fishes that comprise the deep water food web spend the majority of their time in a dimly lit or dark environment. Following a pelagic larval stage, deepwater sculpin become benthic fish and remain at depths ranging from 15 to 407 m (Wells 1968; Mansfield *et al.* 1983; Selgeby 1988; Geffen & Nash 1992) with the majority of the Lake Superior population inhabiting depths below 70 m. In contrast, siscowet (surface to 407

m) and kiyi (25 to 325 m) are midwater water fish that undergo diel vertical migration (DVM) (Hrabik *et al.* 2006; Stockwell *et al.* 2010; Ahrenstorff *et al.* 2011). However, as they inhabit deep waters during the day and only ascend into shallow waters at night, they spend the majority of time in light limited environments.

The visual pigment sensitivity hypothesis (Clarke 1936) suggests that fish visual sensitivity corresponds with the light environment inhabited by the fish due to the adaptation of visual pigments. Many marine fishes provide support for this hypothesis (Denton & Warren 1957; Munz 1958; Munz & McFarland 1973; McFarland & Munz 1975; Crescitelli *et al.* 1985; Partridge *et al.* 1988; Jokela-Määttä *et al.* 2007; Horodysky *et al.* 2010) and exhibit peak sensitivity to wavelengths in the blue range of the visible spectrum because oceanic water filters out both shorter and longer wavelengths (Jerlov 1968). The rhodopsin visual pigment, based on vitamin A<sub>1</sub>, allows for the detection of these blue shifted wavelengths. However, freshwater systems favor the transmittance of green shifted light due to the high concentration of chlorophyll and other particulate matter (Jerlov 1968; Lythgoe & Partridge 1989). The visual pigment porphyropsin, based on vitamin A<sub>2</sub>, is present in freshwater fish allowing visual perception in these green shifted waters (Bridges 1972). Freshwater fish utilize porphyropsin exclusively, or in conjunction with rhodopsin for visual perception (Munz & McFarland 1973; Hunt *et al.* 1996).

The visual sensitivity hypothesis has less supporting evidence for fish in freshwater systems compared to those in marine systems (Munz 1976; Crescitelli *et al.* 1985); historically, deep sea fishes received more attention for their visual ability at depth

than did freshwater fish, causing a gap in the knowledge between marine and freshwater fishes (Lythgoe & Partridge 1989). The clear, offshore water of Lake Superior provides light attenuation approaching that of the open ocean and offers an opportunity to examine deep water fishes in a freshwater system. Given the similarity between Lake Superior and marine environments, fishes may exhibit spectral sensitivities similar to marine fishes based on the use of rhodopsin. Conversely, porphyropsin may shift visual sensitivity to green-shifted wavelengths predominant in freshwater systems (Wald 1939; Crescitelli 1991).

The goal of the current study was to determine the potential role of the visual system in mediating predator-prey interactions. Electroretinography was performed on three species of deep water fish found in Lake Superior to determine dark adapted spectral sensitivity to compare each visual system to the prevailing light environment. The fishes' visual sensitivity was combined with estimates of the transmission of light in Lake Superior to model the depths at which vision may mediate predator prey interactions.

## **Materials and Methods**

### *Fish Collection*

Siscowet, deepwater sculpin, and kiyi were collected via daytime bottom trawls in the Apostle Islands region of Lake Superior, east of Stockton Island (Lat: 6° 54.751 Long: 90° 30.611) on November 13, 2012 and June 26, 2013. Fish were collected at depths ranging from 100 to 117 m during 10 minute bottom trawls using a 12 m Yankee

bottom trawl. Immediately after removal from the net, fish were submerged in a solution of lake water containing of 0.0024 % tricaine methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, MO), 0.026 % Stresscoat<sup>®</sup> (Mars Fishcare North America Inc., Chalfont, PA), and 0.5 % Instant Ocean<sup>®</sup> (Aquarium Systems Inc., Mentor, OH) in 570 L plastic holding tanks in 6°C water. After 2 minutes, kiyi and siscowet swim bladders were deflated using 14 gauge veterinary needles (QC Supply, Schuyler, NE), the incisions treated with betadine (Purdue Products L.P., Stamford, CT), and the fishes placed back in the holding tanks. After an additional five minutes, fish were transferred to two 285 L transportation tanks at 6°C containing lake water solutions of 0.0002 % MS-222, 0.026 % Stresscoat<sup>®</sup>, and 0.5 % Instant Ocean<sup>®</sup>. These tanks were then transported to the University of Minnesota Duluth. Throughout the entire capture and transport process, the water was aerated with compressed O<sub>2</sub> via 5" Deluxe Bubble Disks<sup>™</sup> (Penn Plax<sup>®</sup>, Hauppauge, NY).

At the University of Minnesota Duluth, the sculpin, kiyi, and siscowet were placed into 40 L, 575 L, and 1900 L aquaria, respectively, equipped with mechanical, chemical and biological filtration using Penn-Plax Cascade<sup>™</sup> 1500 canister filters. Prior to arrival, all tanks were aerated with compressed O<sub>2</sub> with for three days. Instant ocean<sup>®</sup> was added to all tanks to achieve 0.5% salt concentration. Carbon filtration was used during oxygen treatment, but was removed upon Stresscoat<sup>®</sup> treatment. Tanks were treated with 0.026 % Stresscoat<sup>®</sup> one day prior to fish arrival and were aerated with pure oxygen for four days after arrival, and carbon filtration resumed seven days post trawl. Water temperatures were maintained between 3 and 6°C, and all tanks were illuminated

indirectly by dim red light (Sunbeam 40 W red light bulb) when experimenter vision was necessary (cleaning tanks and selecting fish). Water quality (pH, temperature, ammonia, nitrate, nitrite, and oxygen concentration) was monitored twice daily for the first 2 weeks, daily for weeks 3 and 4, and twice weekly thereafter. Fish were illuminated by red light (15 W bulb with Kodak GBX-2 dark red safelight filter), consistent with the minimal light conditions of their natural habitat. Feeding was initiated 48 hours after arrival and fishes were provided frozen mysis, with kiyi and siscowet supplemented with live mysis when available. Food was provided every other day and uneaten food was removed from tanks the next day. Procedures for animal care and handling conformed to institutional animal care protocols (Protocol 1205A13881).

#### *Electroretinogram Preparation*

All experimental procedures were conducted in a dark room illuminated by dim red light (15 W light bulb with Kodak GBX-2 dark red safelight filter). Siscowet, kiyi, and deepwater sculpin were anesthetized with buffered (4.5 % sodium phosphate dibasic, 1.1 % potassium phosphate monobasic in  $d_4H_2O$ , Sigma Chemical Co., St. Louis, MO) 0.002 % MS-222. Fish were immobilized by an intramuscular injection of pancuronium bromide (0.001 to 0.1 %; 0.0004 - 0.0030 % of body weight) dissolved in 0.9 % NaCl. Fish then were placed on a moist sponge in a 45 x 11 x 9 cm experimental tank and submerged up to the ventral border of the eyes. The experimental tank was housed within an opaque metal Faraday cage (77 x 67 x 96 cm) to eliminate instrumentation light from interfering with dark adaptation. Buffered 0.002 % MS-222 maintained at 4°C (420 W Teco SeaChill® Aquarium Chiller, Teco® model SCTR20, Ravenna, Italy) was

circulated continuously over the gills through an intraoral tube to maintain the surgical plane of anesthesia throughout the experiments.

#### *Electroretinogram Collection*

Light stimulus was provided by a 100 W quartz tungsten-halogen lamp (Newport® model 6333, Stratford, CT) powered by a constant current power supply (Newport® model 68938). The stimulus duration was regulated by an Oriol® Electronic Shutter (model 76994) and Controller (model 76995). The light was passed through a monochromator (Newport® model 77250) with a 1.56 mm slit. Neutral density filters (0.1 to 3.0) were used to regulate intensity. A fiber optic light pipe (Newport® model 77632) was used to transmit the light to the eye. Light intensity was measured using a radiant power energy meter (Ophir® model 70260) and probe (Ophir® model 70268). A 0.20 mm diameter silver-silver chloride recording electrode was inserted into the vitreous of the eye through an incision at the limbus, and a reference electrode was placed in the center of the frontal bone between the eyes. ERGs were amplified using World Precision Instrument, Inc. amplifier (1000x, 1 Hz low pass, 3 kHz high pass, model DAM50; Sarasota, FL), filtered using a 60 Hz notch filter, recorded with PowerLab 4SP (AD Instruments, Castle Hill, Australia), and stored using Lab Chart®7 (AD Instruments, Castle Hill, Australia) software on a portable computer.

All fishes were dark adapted for 30 minutes prior to testing. A 200 ms flash of monochromatic light was used to elicit the ERG. Wavelengths tested were from 400 to 700 nm at 25 nm intervals with the presentation order randomly determined. Stimulus intervals were determined for each species by presenting consecutive flashes to control

fish to determine the delay required to produce the same response amplitude to minimize photobleaching. Interflash intervals ranged from 30 s for the kiyi to 190 s for the deepwater sculpin.

The b-wave amplitude of the ERG was used as the response criterion (Figure 1). Minimal b-wave amplitudes were consistently encountered in response to short wavelengths ( $\leq 425$  nm) and therefore the b-wave amplitude at 400 nm was set as the minimal criterion response for each fish. Longer wavelengths were reduced in intensity by neutral density filters until the b-wave amplitude equaled the criterion amplitude established at 400 nm, and the irradiance needed to reach the criterion amplitude was used to generate spectral sensitivity curves for each species.

#### *Light Attenuation Calculation*

Beer's law was used to estimate light attenuation and intensity at depth. Seasonal changes in water clarity result in different  $k_{PAR}$  values for Lake Superior with clearer water present in the spring and summer ( $k_{PAR}=0.1$ ) and fall months characterized by a reduction in water clarity ( $k_{PAR}=0.3$ ) (Jerome *et al.* 1983). Data for winter months were not available; however ice and snow cover can limit surface irradiance (Leppäranta *et al.* 2003) and combined with low sun angles and short days, is probably the period of minimal light availability at depth. Crater Lake solar irradiance values (Tyler & Smith 1970) were used in visual depth calculations as this lake at similar latitude to Lake Superior and thus experiences similar irradiances. Since lunar values were not available for Crater Lake, the lunar to solar irradiance ratio was calculated using values from Eniwetok Atoll (Munz & McFarland 1973; McFarland & Munz 1975) and applied to

Crater Lake solar values to estimate lunar irradiance. The minimum irradiance needed to evoke a b-wave in the dark adapted retina was used to determine visual sensitivity.

## **Results**

### *Spectral sensitivity*

Visual spectral sensitivity curves for dark adapted siscowet, kiyi, and deepwater sculpin were constructed using ERG responses to monochromatic light. All three fishes exhibited maximum sensitivity at 525 nm with relatively broad sensitivity from 500 to 550 nm with markedly decreasing sensitivity to wavelengths <475 nm and >575 nm (Figure 2).

### *Visual depth profiles*

To illustrate the spectral composition of downwelling irradiance under different aquatic conditions, depth profiles were generated for one percent surface irradiance in Lake Superior. The clearer spring and summer water ( $k_{PAR}=0.1$ ) showed maximum transmission of 500 nm light (Figure 3A) while the increase in the fall attenuation coefficient ( $k_{PAR}=0.3$ ) shifted the predominant downwelling irradiance to 550 nm (Figure 3B).

Visual depth profiles were created to approximate the maximum depth at which fish can respond to downwelling irradiance. All three species had the capability of detecting 500 nm light to depths greater than 90 m during the day in the off shore waters of Lake Superior ( $k_{PAR}=0.1$ ). Longer wavelengths ( $\geq 600$  nm) were rapidly attenuated and not detectable deeper than 20 m (Figure 3A). Deepwater sculpin displayed greater



sensitivity between 475 to 550 nm but outside of these wavelengths, all three species had approximately equal sensitivity.

Visual depth profiles changed shape and depth with decreased water clarity ( $k_{PAR}=0.3$ ). Downwelling daytime irradiance would be sufficient to elicit ERGs to approximately 24 m for the siscowet and kiyi and 32 m for deepwater sculpin (Figure 3B). Again, all species demonstrated similar profiles, with the deepwater sculpin retaining a slight advantage in detection of wavelengths between 475 and 550 nm. While deepwater sculpin and siscowet profiles reach greatest depth at 525 nm, the kiyi depth profile was greatest at 550 nm.

Profiles could not be generated for nocturnal conditions in either turbidity as the surface irradiance was less than that required to illicit a physiological response in all three species of fish.

## **Discussion**

The fishes occupying the offshore waters of Lake Superior exhibited similarity in their spectral sensitivities curves with peak sensitivity at 525 nm that were correlated to the predominant downwelling wavelengths. Based on visual sensitivity and light attenuation estimates in Lake Superior, sufficient daytime irradiance exists to mediate visual interactions in the upper layers of the lake (<100 m). Sufficient downwelling irradiance is not available under nocturnal conditions to elicit ERG responses in any of the fish tested for any depth.

The fishes were trawled from 100 to 115 m during both day and night and thus were exposed to sunlight or deck lights upon capture. Marine midwater fish and

crustaceans can suffer eye damage when exposed to these light conditions (Loew 1976; Frank *et al.* 2012) and the effects of light on the visual sensitivities of the Lake Superior fishes are uncertain. However, every effort was made to maintain fish under dim red light conditions following capture, and all animals displayed a robust ERG when tested. Control sculpins and siscowet were maintained months in captivity without detectable changes in visual or spectral sensitivity so any light damage that may have occurred to the retina probably was minimal. However, the kiyi were less robust and were unable to regulate buoyancy resulting in short survival times (generally less than one week). Due to their compromised state, they were tested first and also exhibited strong response to the light. However, given their compromised physiology and the shorter recovery period under dim light, their data should be treated with a degree of caution.

Due to both specialized morphological retina adaptation and the clarity of open ocean water, it has been estimated that mid water fish can detect downwelling light to 1000 m (Dartnall 1975; Guthrie 1986). However, most lakes contain more particles in the water, such as non-algal particulates and colored dissolved organic matter (Guthrie & Muntz 1993; Effler *et al.* 2010) that decrease light attenuation and shifts the downwelling spectral irradiance to longer wavelengths than in salt water. Estimates of fish visual sensitivity at depths greater than 100 m are rare for freshwater fishes. The deep, oligotrophic Lake Superior provides an excellent venue to understand fish visual capabilities in clear freshwater systems.

The ERG has long been used to assess spectral sensitivity by determining the electrical potential of the retina. In light adapted retinas, ERG waveforms include an a-

wave generated by the photoreceptor hyperpolarization upon initiation of a light stimulus, and a b-wave, originating from Müller cell and bipolar cell depolarization (Wen & Oakley 1990). When dark adapted, the a-wave is absent and allows a more precise determination of the b-wave amplitude. As the Lake Superior fish inhabit minimal light environments, the dark adapted retina was more consistent with environmental conditions and was used to assess visual sensitivity.

The ERG provides a mechanism for minimally invasive sampling and allows the fish to be used also in behavioral studies. While it is an effective tool to measure spectral sensitivity, it does not assess the central visual pathways and brain centers involved in image formation, and therefore cannot be directly correlated with image formation. However, for the b-wave to be induced, sufficient light must be absorbed by the photoreceptors to stimulate the bipolar and Müller cells to allow the electrical potential of the retina to be detected, strongly suggesting that fish can centrally detect these light levels. Additionally, the current path to the extracellular electrodes must travel through the vitreous and epidermis and the position and distance between the electrodes insures that current will be lost before detection. Therefore, it is highly probable that visual sensitivity may be greater than values reported. However, given the challenge of capturing and maintaining these deep water species, the ERG provides the best proxy to assess visual sensitivity in these fishes. Therefore, for the purposes of this paper, visual sensitivity is defined as the minimal irradiance sufficient to elicit a b-wave in a dark adapted retina.

The spectral sensitivity curves showed all three species had broad spectral sensitivities that correlate with prevailing downwelling light in Lake Superior. The spring and summer water column is very clear containing less particulate matter than fall and allows greater light transmission to depth with 500 nm reaching maximal depths. The optic properties of the water column shift in the fall with a greater suspension of particulate matter that increases light absorbance and changes the peak spectral transmission shifts to 550 nm. Thus the spectral sensitivity for all three species at 525 nm is well adapted to the light environment.

The reduced visual sensitivity in all fishes in the longer wavelengths is consistent with their deep water environment because red light is quickly attenuated in the water column (Jokela-Määttä; Jerlov 1968). Similarly, shorter wavelengths are absorbed relatively close to the water's surface in freshwater systems, although attenuation of 400 to 450 nm light occurs more slowly than longer wavelength red light (Clarke 1936; Jerlov 1968; Jokela-Määttä *et al.* 2007). Thus the visual pigments in the fish are most sensitive to the prevailing spectrum and are consistent with Clarke's sensitivity hypothesis.

These findings offer a unique investigation into the utility of vision within a local environment through comparison of spectral sensitivities to the calculated light available within the system. Many studies of fish vision have investigated spectral sensitivity using electroretinography to understand whole retina function (Horodysky *et al.* 2008) or other procedures such as microspectrophotometry to understand the maximum wavelength of perception for retinal components (Denton & Warren 1957; Dartnall & Lythgoe 1965; Crescitelli *et al.* 1985; Bowmaker *et al.* 1988; Bowmaker *et al.* 1994;

Utne-Palm & Bowmaker 2006). Few studies, however, compare the spectral sensitivities of fishes to a detailed profile of the light available in the natural environment (Douglas & Partridge 1997; Marshall *et al.* 2003; Warrant 2004).

Deep water marine fishes are often physiologically compromised or blinded by deck or sun light which destroys the retina so the maximum depths at which midwater fishes can see is based on retina histology rather than determined by *in situ* experiments. This has resulted in a wide knowledge base of evolutionary adaptation of the eye to low light levels at depth, but information about the maximum depth of vision is lacking for most species.

Clarke (Clarke 1936) estimated the depths at which *Lepomis* retained vision in various aquatic environments and postulated that these estimates serve as accurate estimates for other fish with similar visual sensitivity. In these estimations, Clarke combined light information of several aquatic ecosystems (Juday & Birge 1931; Birge & Juday 1932; Erikson 1933; Oster & Clarke 1935) with data regarding the visual capability of *Lepomis* (Grundfest 1932). The peak sensitivity of *Lepomis* (Grundfest 1932) is approximately 10 log units less than diurnal irradiance found by Oster and Clarke (1935). Clarke (1936) thus calculated the average depth required to reduce light by 1 log unit in each aquatic ecosystem and multiplied by 10 to find the total depth of vision for each body of water for *Lepomis*. The Sargasso Sea revealed the greatest estimation of depth of vision for fish similar to *Lepomis* for the estimated aquatic bodies; vision is possible for these fish at depths up to 430 m. Using Clarke's methods, the maximum depth *Lepomis* detects light in the open waters of Lake Superior 110 m.

Although the wavelength detected differs (*Lepomis* is most sensitive to 540 nm while peak depth of vision for the kiyi, siscowet, and deepwater sculpin is 500 nm under clear conditions), this places *Lepomis* vision at a maximum depth equal to siscowet, with a slight advantage of approximately 10 m over kiyi, and a disadvantage of approximately 30 m to deepwater sculpin. While our calculations utilize spectral sensitivity and light attenuation factors to find maximum depth of vision per wavelength, the results are comparable with peak depth values that are based on more general calculations.

Kiyi and siscowet visual profiles suggest a broad range of perception from 450 to 550 nm allowing for vision deeper than 60 m during the day. However, since these species typically reside in deeper waters during the day, the visual range may be limited to between 475 and 500 nm light. Insufficient nocturnal surface irradiance exists to allow for visual capabilities for any of the three species.

Deepwater sculpin diurnal vision varies slightly from those of the diel vertical migrants between 475 and 550 nm light; the sculpin is able to perceive light up to 40 m deeper than the two pelagic fish in this wavelength band. This correlates with a specialized spectral sensitivity to these wavelengths of light with diminished visual sensitivity in the wavelengths at the extremes of the visible spectrum. The deepwater sculpin likely has a visual adaptation to wavelengths between 475 and 550 nm because these wavelengths are predominant at depth. Remaining at the lake bottom, deepwater sculpin need not expend energy on a broadly sensitive visual system as wavelengths at the extremes of the visible spectrum are unlikely to penetrate to greater depths. With this specialized visual system and visual advantage at depth, the deepwater sculpin may be

able to avoid predation by the siscowet. This advantage may diminish at the greatest depths of Lake Superior where the deepwater sculpin's eye is insensitive; individuals may be vulnerable to predation at night as the visual system is not sensitive to low nocturnal illumination and individuals dwelling below 145 m during the day may also experience difficulty in visual perception.

Calculations for the visual depth profiles of Lake Superior fishes and the irradiance profile of Lake Superior used solar irradiance values from Crater Lake, Oregon (Tyler & Smith 1970). These data were used as they represented a complete spectral irradiance data set for diurnal illumination at latitude similar to that of Lake Superior. However, as nocturnal irradiances at this location were not available, a ratio of nocturnal to diurnal irradiance (Munz & McFarland 1977) was used to calculate approximate nocturnal illumination for this location.

Under diurnal conditions, much of the population of these offshore fishes has insufficient light to utilize visual cues for predator avoidance and prey capture and minimal nocturnal irradiance prevents the use of the visual system. While other sensory modalities may be important for long range detection, most short range predator prey interactions are mediated by the mechanosensory lateral line and/or vision. Teleosts are certainly capable of finding prey in complete darkness, although, at best range, the mechanosensory lateral line range is estimated to be one or two body lengths; neurophysiological studies with free swimming fish feeding on natural prey suggest even shorter distances of less than a body length (Palmer *et al.* 2005). Vision can extend this range; however, optical conditions in the aquatic environment can be highly variable and

limiting to the visual system. Future studies implementing these spectral sensitivity findings can be incorporated into laboratory studies investigating fish reaction distance under diminishing availability of light. By simulating environmental conditions, foraging mechanisms for offshore fish can be further understood.

### **Acknowledgements**

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## Figures

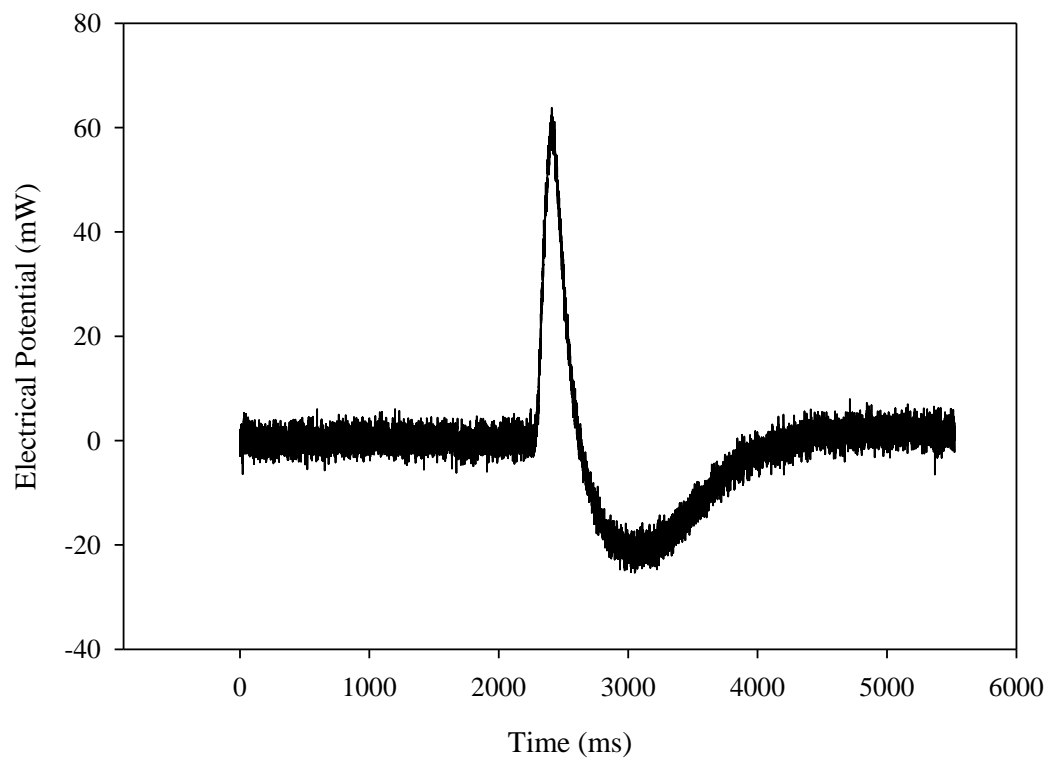


Figure 1: An electroretinogram recorded from a kiyi in response to 550 nm light. The b-wave seen at 500 ms is representative of the depolarization of the Müller cells. This wave is reduced in experimentation to reach a minimum criterion response.

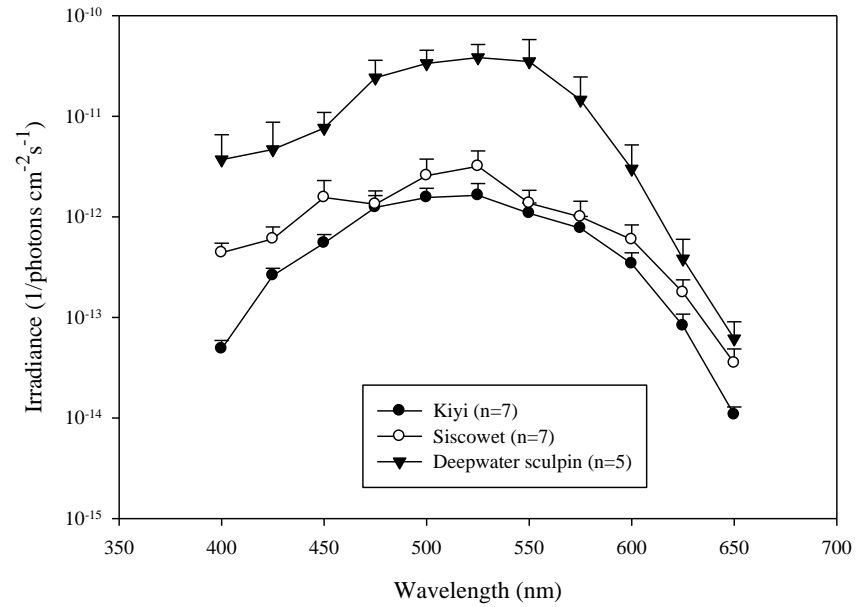


Figure 2: The average irradiance (units) needed to invoke the criterion response is plotted versus wavelength. Spectral sensitivity curves of kiyi (black circles), siscowet (white circles), and deepwater sculpin (black triangles).

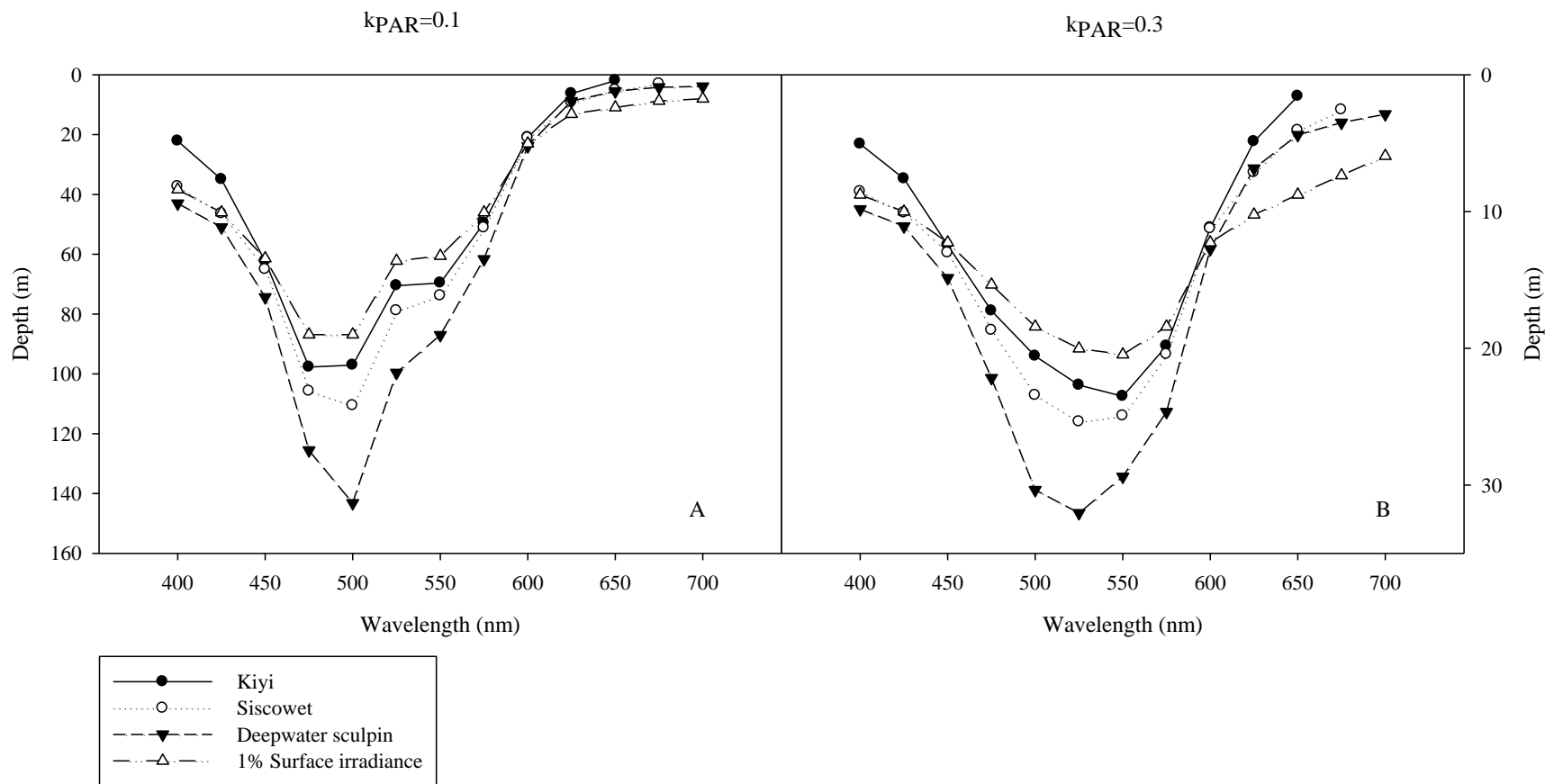


Figure 3: Visual depth profiles of kiyi (black circles), siscowet (white circles), and deepwater sculpin (black triangles) in comparison to one percent surface irradiance calculations (white triangles) in diurnal conditions with (A)  $k_{PAR}=0.1$  and (B)  $k_{PAR}=0.3$ . Depth decreases with increases along the y-axis. Increase on the x-axis corresponds with increasing wavelength.

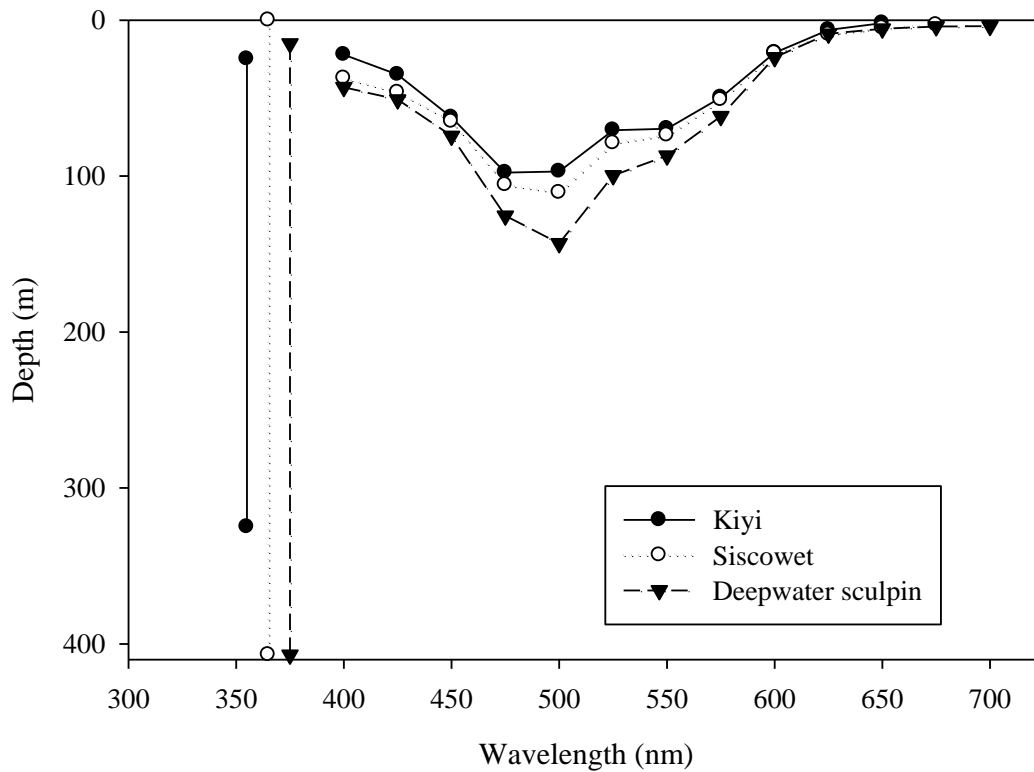


Figure 4: Comparison of fish visual depth profiles to depth ranges. Diurnal values for  $k_{PAR}=0.1$  are used for the visual depth profiles. Vertical lines represent the depth ranges of fish throughout day and nighttime and are presented here independent of wavelength for comparison to visual depth profiles. Lines for kiyi (black circles), siscowet (white circles), and deepwater sculpin (black triangles) demonstrate visual depth profiles (horizontally) and depth range (vertical).

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