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Spectral sensitivity and contrast potentials for four species of cleaner fish

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SPECTRAL SENSITIVITY AND CONTRAST POTENTIALS FOR FOUR SPECIES OF CLEANER FISH

Parasitization is a big problem faced by fish farmers throughout the world. While chemical treatment is an option, a biological solution based on predation of the relevant parasite by cleaner fish is a more environmentally friendly solution and in many cases more cost effective. Salmon lice, *Lepeophtheirus salmonis,* are a particularly costly problem for penned salmon (see Costello, 2009).

For cleaner fish to be effective, they must be able to see the parasites attached to the fish and recognize them as potential food. The most important metric here is **contrast** between the fish and the parasite it is attached to. This may be brightness contrast (i.e. luminosity contrast) where the parasite is either brighter or darker than the flank of the fish (i.e. the surround), or colour contrast (chromaticity or hue contrast) where there is a spectral reflectance difference between the parasite and the background leading to a detectable colour difference. Anything that can be done to maximize either contrast type should improve predation and, therefore, decrease infestation. As a start in analyzing the visual situations faced by cleaner fish, their visual systems need to be characterized. To this end, microspectrophotometry (MSP) has been used to measure the absorbance spectrum of the photoreceptor cells in the retinas of four species of cleaner fish of importance to Norwegian fish farmers. It is the contained visual pigments characterized by MSP that determine what colours will produce a response in the photoreceptor cells. Only photons of light absorbed by the visual pigments can stimulate a visual response. Light NOT absorbed is invisible. With these MSP data and information about the spectral reflectance of parasites and fish along with measurements of the available light, it is possible to model contrast situations with a goal of identifying those metrics that could be used to maximize the contrast. These data are also useful for maximizing the contrast of feed in culture tanks through selection of lighting and tank wall colour. A word about contrast. In general, the brighter the light, within the operating range of the photoreceptor cell, the greater will be the contrast and therefore the visibility. Brightness is also associated with acuity and optical resolution. The object to be viewed must be of a size such that its image on the retina is of sufficient clarity to be recognized as regards shape and fine features. The brighter the light, the better the resolution. This also affects the range at which a fish can detect and recognize a parasite on the side of the fish.

I. Material and Methods.

The four species of cleaner fish used for this study were:

1. The Ballan wrasse, *Labrus bergylta*
2. The Corkwing wrasse, *Symphodus melops.*
3. The Goldsinny wrasse, *Ctenolabrus rupestris*
4. The Lumpsucker, *Cyclopterus lumpus.*

The Ballan wrasse and lumpsuckers were obtained from culture tanks at the Institute of Marine Research, Austevoll, Norway. The Corkwing and Goldsinny wrasses were wild-caught.

II. Visual Cells.

All three of the wrasses had the same complement of retinal photoreceptor cells – rods, both long and short single cones, and double cones. This is in agreement with the description of the adult Ballan wrasse retina by Engstrom (1963). The lumpsucker has a similar distribution of photoreceptor cell types. The rods are responsible for low-light, black & white vision. They are sensitive to motion, but yield a neural image that has poor spatial features (i.e. it is blurred). The cones are responsible for high acuity colour vision as well as bright-light motion detection. In order to have colour vision, a retina must contain at least two spectral classes of cone and the necessary wiring to specify a colour over a wide range of brightness (i.e. the colour perceived does not change appreciably with the brightness of the light).

III. Visual Pigments.

As has been found for other species of wrasse, there is variability in visual pigment absorbance maximum (λ_{max}) among the three studied here as well as for the lumpsucker. Because all visual pigments have essentially the same shape when plotted correctly and certain chemical conditions are taken into account, visual pigments can be fully characterized by their wavelength of maximum absorption after fitting of the spectrum to known template curves. This is the $\lambda_{max.}$ The wavelength of maximum absorbance of the non-fitted curve is the x_{max}. For identification purposes the visual pigments and cones are often labeled by the colour to humans of the λ_{max}. For example a blue cone (also called SWS) would have its λ_{max} value in the blue part of the visible spectrum. Estimation of λ_{max} was performed by template fitting using accepted methods and selection criteria (see Loew, 1994). It should be noted that many more cells than met the selection criteria were scanned with x_{max} found to fall within the λ_{max} ranges of the cell classes reported below.

A. Ballan wrasse. A rod with λ_{max} at 508 nm in the 'blue-green' was identified (Figure 1). The majority of the double cones had the same, 'green-yellow' pigment in both members, although a small number of doubles were found with 'green-yellow' in the principal (longer) member and 'blue' in the accessory (shorter) member (these are the LWS cones). The absorbance spectra for these cones is seen in Figure 1. These spectral positions are what is expected for fish adapted for shallow to mid-water visual tasks although the spectral position of the green-yellow cone at 542 nm would suggest adaptation for blueish-green waters as might.be found in coastal locations.

B. Corkwing wrasse. The pattern here is like that of the Ballan wrasse with a rod visual pigment at λ_{max} 505 nm, essentially identical to that of the Ballan (Figure 3). However, the spectral positions of the two cone pigments are shifted somewhat towards the red. The significance of this shift is uncertain, except that it would make this fish more adapted to greenish-yellow waters.

C. Goldsinny wrasse. Again, the visual pigment pattern here is like that for the other wrasses except for a significant spectral shift of ALL receptors towards the blue (Figure 5). This is what would be expected for fish adapted to deeper, blue waters.

D. Lumpsucker. The pattern here is like for the wrasses except for the LWS cone. The absorbance spectrum for these cells was NOT best fit by a single visual pigment template curve. Rather, it required a mixture of two visual pigment templates to best match the measured absorbance. This is seen in Figure 7. Among the individuals studied (N = 3), there was an approximately 10 nm variation in the measured absorbance of the LWS cones. This degree of variability was NOT seen for the SWS cone or the rod. This supports the idea of co-expression of two opsins in the LWS cones yielding the mixture of two A1-based visual pigments as opposed to a mixture of vitamin A1- and A2-based visual pigment using a single opsin. Regardless of the mixture ratio, the spectral range covered by the LWS cells as well as the spectral positions of the rod and SWS cones is what would be expected of a shallow to mid-water species.

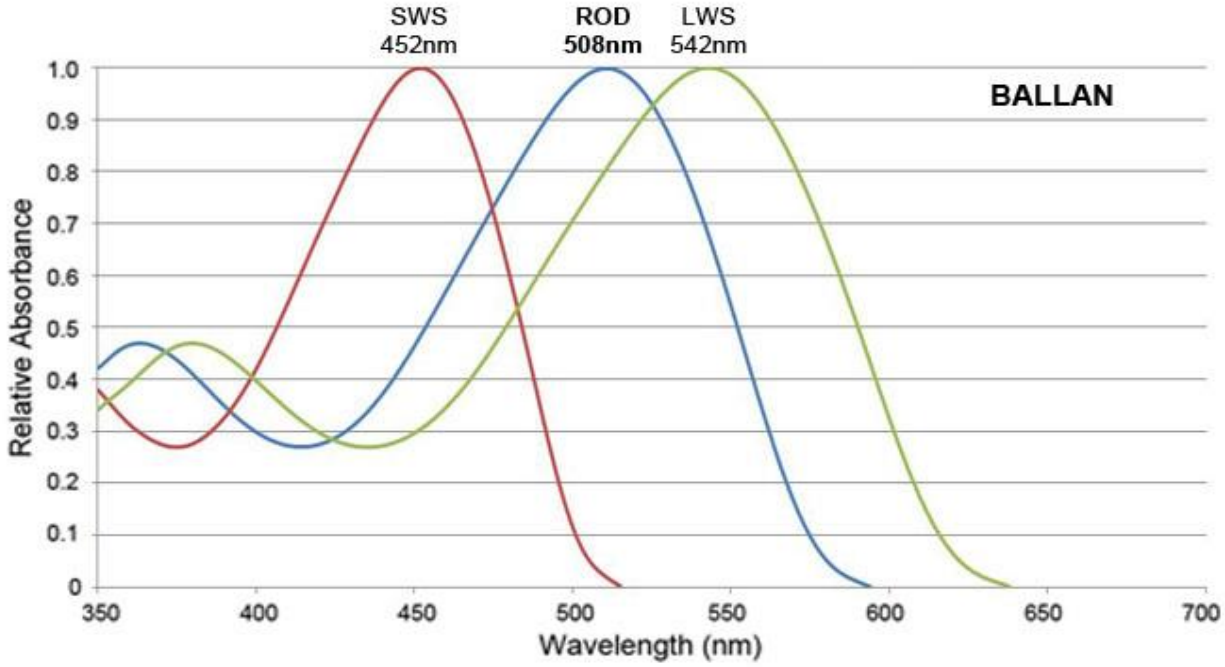

Figure 1. Absorbance spectra of the retinal photoreceptors of the juvenile Ballan wrasse. There is a single rod visual pigment with λ_{max} at 508nm (N = 19), and two cone visual pigments with λ_{max} at 452nm (N = 3) and 542nm (N = 6). All pigments are best fit by vitamin A1-based template curves. SWS = short wave sensitive. LWS = long wave sensitive.

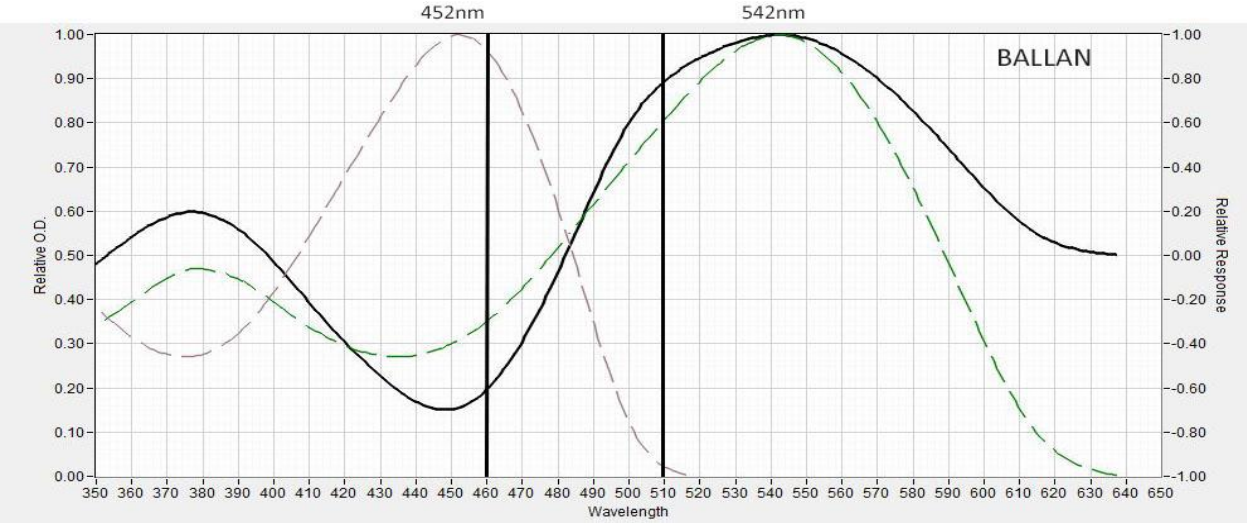

Figure 2. Opponent curve derived by subtracting the 452nm absorbance spectrum from the 542nm spectrum. The two vertical lines bracket the region where hue is uniquely encoded as horizontal cell membrane potential change.

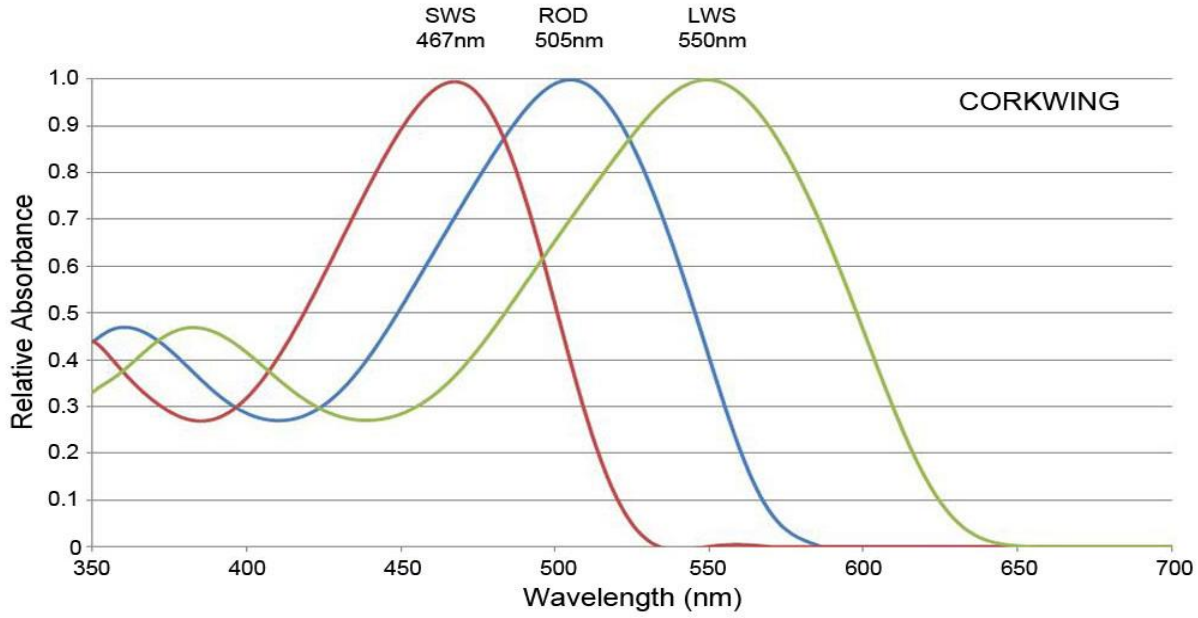

Figure 3. Absorbance spectra of the retinal photoreceptors of the adult Corkwing wrasse. There is a single rod visual pigment with λ_{max} at 505nm (N = 7), and two cone visual pigments with λ_{max} at 467nm (N = 2) and 550nm (N = 7). All pigments are best fit by vitamin A1-based template curves. SWS = short wave sensitive. LWS = long wave sensitive.

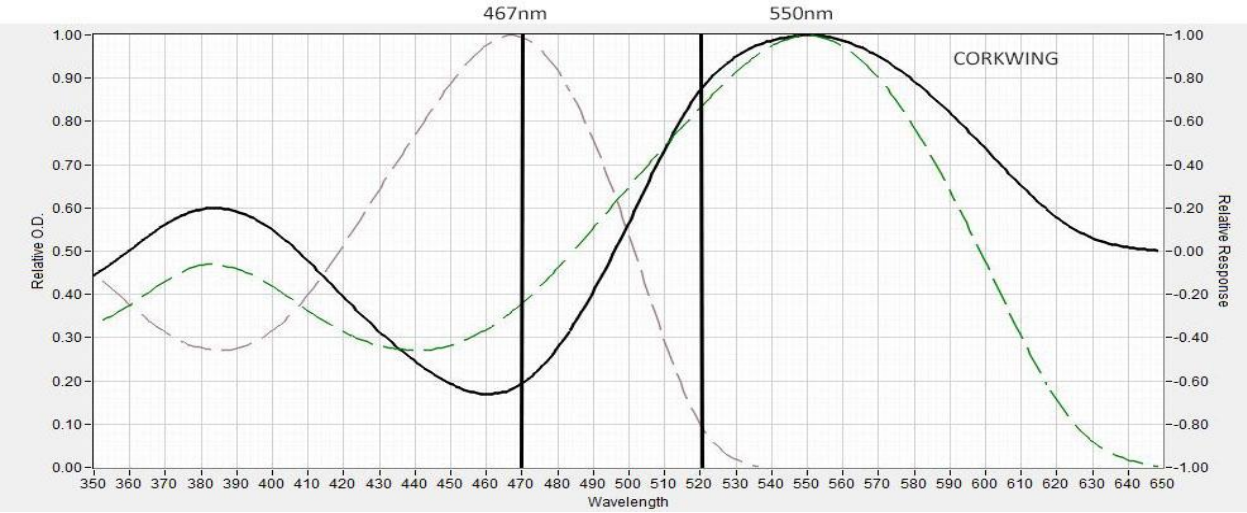

Figure 4. Opponent curve derived by subtracting the 467nm absorbance spectrum from the 550nm spectrum. The two vertical lines bracket the region where hue is uniquely encoded as horizontal cell membrane potential change.

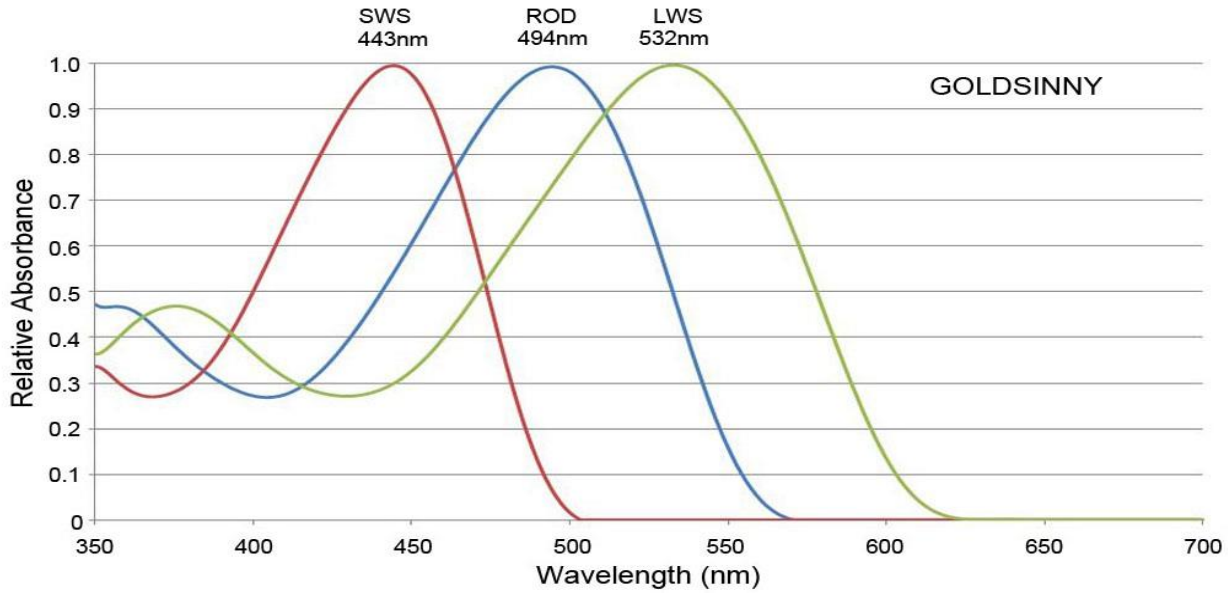

Figure 5. Absorbance spectra of the retinal photoreceptors of the adult Goldsinny wrasse. There is a single rod visual pigment with λ_{max} at 494nm (N = 4), and two cone visual pigments with λ_{max} at 443nm (N = 2) and 532nm (N = 5). All pigments are best fit by vitamin A1-based template curves. SWS = short wave sensitive. LWS = long wave sensitive.

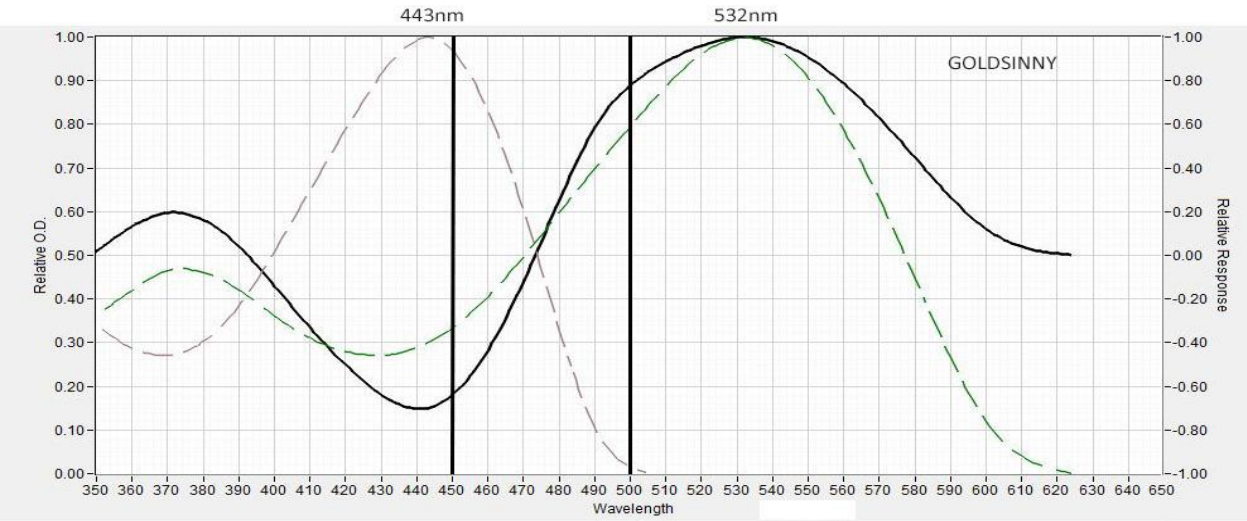

Figure 6. Opponent curve derived by subtracting the 443nm absorbance spectrum from the 532nm spectrum. The two vertical lines bracket the region where hue is uniquely encoded as horizontal cell membrane potential change.

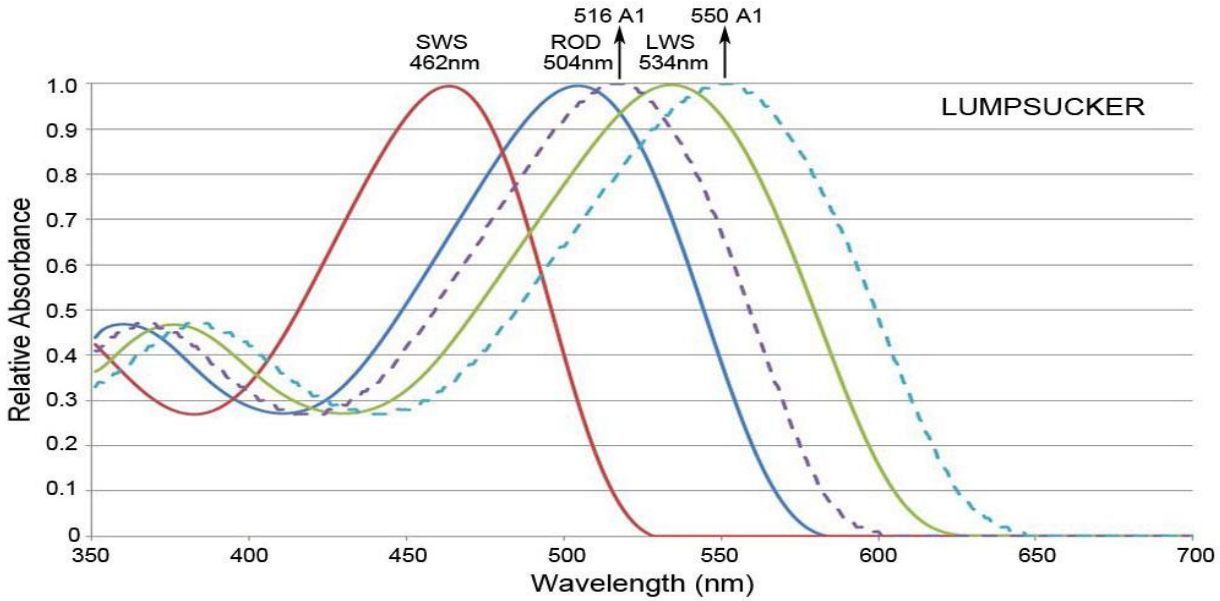

Figure 7. Absorbance spectra of the retinal photoreceptors of the juvenile Lumpsucker. There is a single rod visual pigment with λ_{max} at 504nm (N = 4), and an SWS visual pigment with λ_{max} at 462nm (N = 1). These two visual pigments are best fit by vitamin A1-based template curves. The curve with x_{max} at 534nm is best fit by a mixture of a 516nm A1-based pigment and a 550nm A1-based pigment (both indicated by the dashed curves). The suggestion here is that there is coexpression of two LWS opsins in these cones.

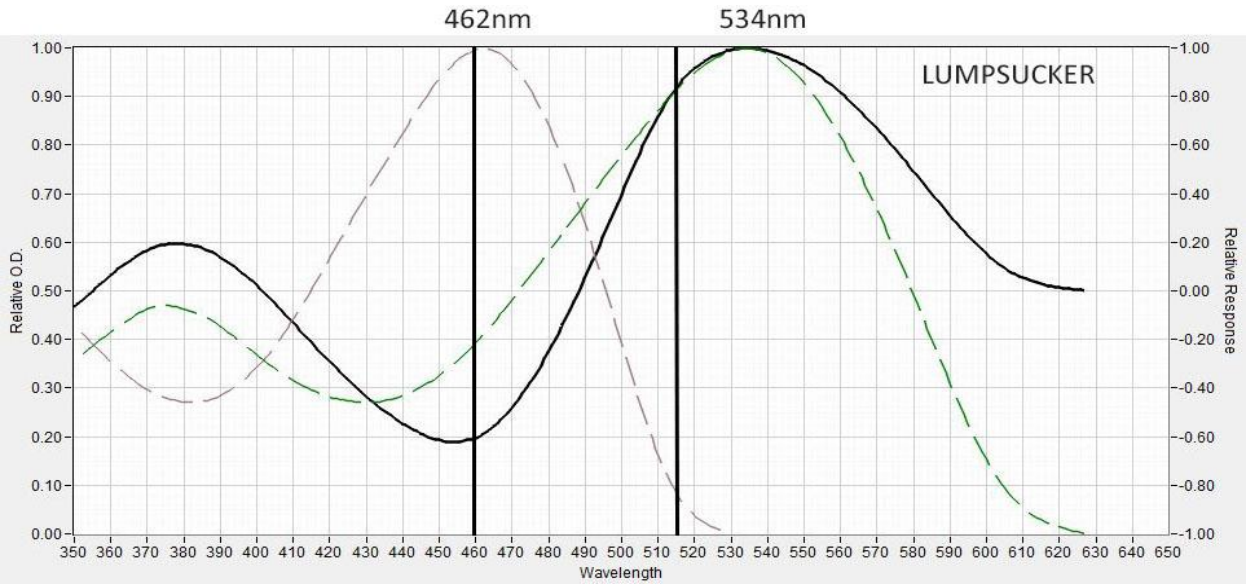

Figure 8. Opponent curve derived by subtracting the 462nm absorbance spectrum from the 534nm spectrum. The two vertical lines bracket the region where hue is uniquely encoded as horizontal cell membrane potential change.

IV. Contrast Potentials.

All vision proceeds from contrast between and among objects and their backgrounds. The two formulas used to calculate contrast are:

$$\text{Weber Contrast} = \frac{I - I_b}{I_b}$$

Where I is the luminance (brightness) of the target and I_b is the luminance of the background. This formula is used when dealing with small targets viewed against a large uniform background.

$$\text{Michelson Contrast} = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

Where I_{max} and I_{min} are the highest and lowest brightnesses. This formula is more appropriate for patterns viewed against an average background (the denominator).

Given that the visual task to be modeled here is the detection of small objects against a uniform tank side or the flank of a fish, the Weber formula is used. The goal is to use the information on visual pigment complement to predict conditions that would maximize the detectability (related to contrast) of targets under different conditions. To do this, a computer

model is employed with inputs of target spectral radiance and irradiant spectrum (a full description of this model can be found in Loew & Zhang, 2005).

For example, let's assume three cone pigments with λ_{maxes} at 405 nm, 455 nm and 545 nm. Sixteen targets (Figure 9) varying in spectral radiance are placed 10 cm in front of the eye in clear blue water with downwelling irradiance similar to that of sunlight. Using the above visual pigments an artificial color space is constructed with the 16 targets placed within its boundaries (Figure 10A). The rules for this kind model are that any of the circles that touch are not discriminable, and the greater the distance between circles, the greater will be the contrast and, therefore, the detectability. If I now replace the 545 nm λ_{max} visual pigment with a 624 nm λ_{max} one, the distances between many of the targets along a line from the center to the 'red' vertex is increased (Figure 10B). Thus, it would be easier to detect those targets with this set of pigments than with the first set. However, this change also results in reduced discrimination for some of the targets. Clearly there is a tradeoff when 'playing' with which visual pigments to express!

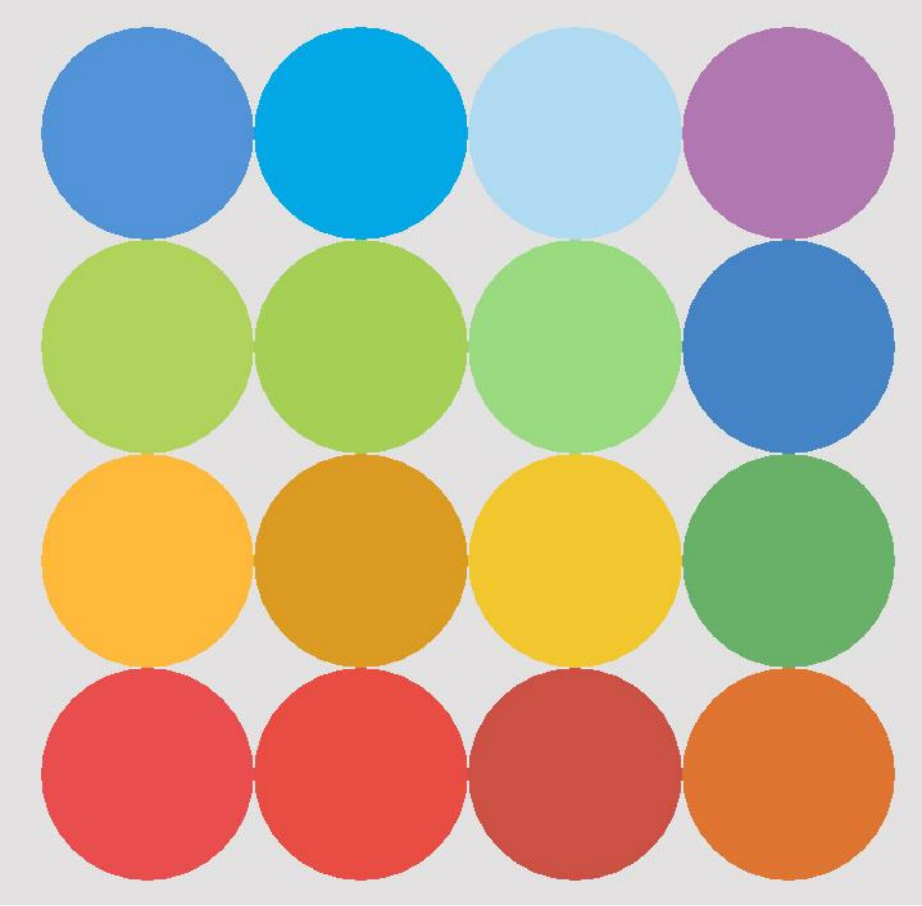

Figure 9. Appearance of the 16 color targets to the human eye.

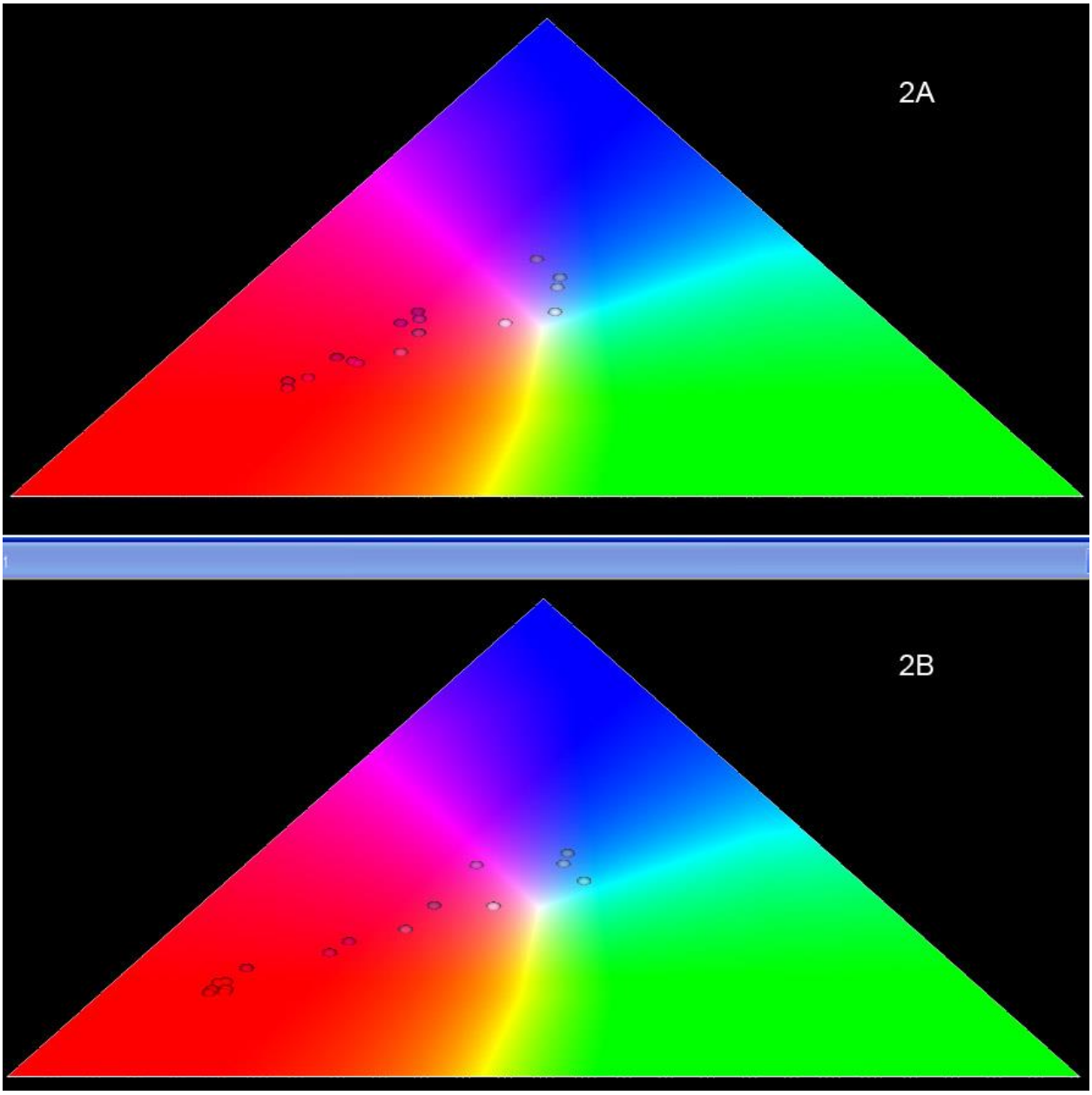

Figure 10AB. The figure 2A shows the 16 colored targets placed into a color space defined by visual pigments with λ_{maxes} at 405 nm, 455 nm and 545 nm. Figure 2B shows the same targets but placed into a space with the 545 nm pigment replaced by a 624 nm one.

Another way to model the data is to assume opponent processing as the physiological process for colour discrimination as seen for humans and a number of other vertebrate species. The assumption is that two different spectral classes of cone connect with a single, third cell (assumed to be a horizontal cell). The LWS cell stimulates the horizontal cell while the SWS

cell is inhibitory. The measured output of the horizontal cell would represent the sum of the stimulatory (+ or depolarizing) and inhibitory (- or hyperpolarizing) inputs. This yields a function that is relatively insensitive to brightness changes that would affect both receptors equally, but produces an output for colour over a certain spectral range. For modeling here, the absorbance spectrum for the SWS cone is subtracted from the absorbance spectrum of the LWS cone. The results of this for the four species are seen in Figures 2, 4, 6, and 8. The two vertical lines isolate that spectral region where maximum colour discrimination should be found. For the four species, this spectral extant covers about 50 nm, although the central wavelength for individual species is shifted. The greater the signal difference produced by two coloured stimuli, the easier will be a discrimination task. The steeper is the opponent function; the more colours can be discriminated over the range specified by the vertical lines.

V. Synthesis.

The goal of this study was to identify the spectral sensitivity, both dim- and bright-light, in order to be able to model contrast situations and make predictions as to what lighting should produce the best contrast between parasites and fish they parasitize.

1. Dim-light tasks. These would be mediated by the rods. Maximum sensitivity is in the blue-green (Figs. 1, 3, 5 and 7) meaning that increased lighting in this spectral region should increase low-light detection and, therefore, feeding. Note that the 'colour' would not be perceived as such by the fish, only its brightness. Putting aside potential feeding behaviour changes associated with short, winter days, adding illumination could increase the time over which the cleaner fish could detect and remove parasites based solely on brightness differences.

2. Bright-light, colour tasks. Brightness tasks at light levels that saturate the rods is almost always carried out by the LWS cones. These are typically the most numerous and widely distributed across the retina. Colour differences are not necessary, only brightness differences. Bright-light motion detection is also mediated by the LWS receptors. If it is this type of 'vision' being used by cleaner fish, the best colours to use for illumination would be in the green-yellow-orange range. Given that the parasites are essentially black or brown and are being viewed against a silvery-white or greenish fish flank, high level brightness differenes might be the most important clue used by the cleaner fish. If colour is to be used, there must be the addition of shortwave light. The best range would be between the vertical lines as seen in Figs. 2, 4, 6 and 8. A mixture of green-blue and yellow LED lamps would be best for this illumination and would eliminate the need for filters.

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