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Kartlegging av hvilke lyskvaliteter berggylt er i stand til å se i fiskens ulike livsstadier

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Kartlegging av hvilke lyskvaliteter berggylt er i stand til å se i fiskens ulike livsstadier

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HAVFORSKNINGSINSTITUTTET
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Spectral Sensitivity of Larval-Juvenile and Adult Ballan Wrasse with Suggestions for Lighting in Indoor Intensive Culture Environments

Submitted by Professor Ellis Loew

Part I Visual Pigments and Overall Spectral Sensitivity

Microspectrophotometry (MSP) was performed on cultured larval-juvenile and adult ballan wrasse, *Labrus bergylta*. This technique measures the spectral absorbance of the visual pigments present in individual retinal photoreceptor cells, the rods and cones, isolated from the eyes of fish under study. The visual pigments, composed of a molecule of vitamin A aldehyde, the chromophore, bound to a protein, the opsin, absorb the light that has entered the eye and transduce it into neuronal signals. Only photons of light absorbed by the visual pigments can stimulate vision. Thus, the absorbance spectrum of the visual pigment present in a photoreceptor establishes the maximum spectral sensitivity of that cell. A survey of the photoreceptor cell population in the retina will, therefore, define the fish's spectral sensitivity. The wavelength range over which a visual pigment absorbs light is determined by the amino acid sequence of the opsin (different opsin genes code for different opsins) and the vitamin A complexed with it (either vitamin A_1, or vitamin A_2). For the same opsin, substitution of vitamin A_2 for vitamin A_1 shifts the absorbance spectrum towards longer wavelengths. This is of relevance here because ballan wrasse, along with other wrasses that have been studied, use both opsin gene expression and chromophore mixing to determine the final spectral absorbance of individual photoreceptor cells.

The ultimate spectral sensitivity of the eye will be determined by the visual pigments and any pre- or post-retinal color filters. In the case of the ballan wrasse, there is an 'orange' pigment present in the retinal pigment epithelial (RPE) cells that surround the photoreceptor cells providing metabolic support. The 'orange' color arises due to absorption of short wavelength light which in and of itself suggests a lack of 'violet' sensitivity. While present in juveniles, this pigment is particularly dense in adults.

The retina of the ballan wrasse contains both rods and cones. Rods subserve dim-light, nocturnal (scotopic) vision while the cones are responsible for bright-light (photopic) and color vision. Color vision requires the presence of at least two spectral classes of cone differing in their spectral sensitivity and the neuronal circuitry necessary for the comparison of the signals from the different cone classes. While the presence of different cone spectral classes is suggestive of color vision capabilities, only behavioural testing can confirm its presence.

Figure 1 shows typical absorbance records from individual photoreceptors of ballan wrasse. The noise is due to the small size of the cells and the necessity of using very dim light to make the measurements (by their very nature visual pigments are 'destroyed' when they absorb light and must be continually regenerated if vision is to persist). Because all visual pigments have similar shapes, template curves can be matched to the noisy spectra to establish the wavelength of maximum light absorbance, the λ_{max}.

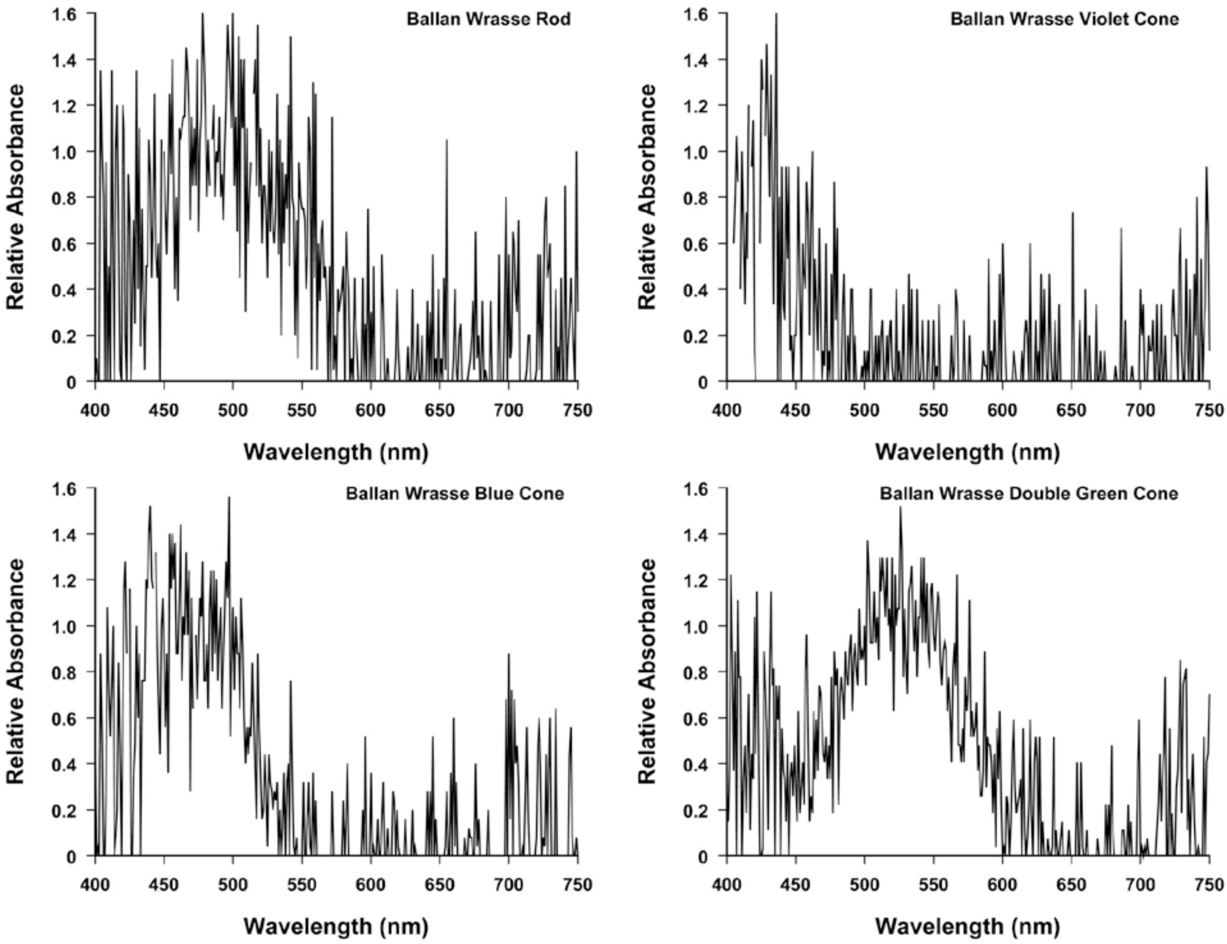

Figure 1. Representative absorbance spectra from the rods and cones of ballan wrasse measured using MSP.

Thus, visual pigments are classified according to the spectral region of the λ_{max}. For example, a λ_{max} at 535nm would make that cell a 'green' cell, etc.

While there is some variability in the λ_{max} of different cones classes in the retinas of different fish due to differential opsin expression and chromophore mixing, the majority of cones fall within three λ_{max} ranges – violet, blue and green. Of particular relevance here is the fact that the violet cone is only found in larval/juvenile wrasse, and not in the adults. The loss of short-wavelength-sensitive cones with aging is quite common in fish. This loss is usually correlated with a change in diet such as moving from planktivory to piscivory, or a change in the spectral quality of the environment due to a movement from near-surface to deeper waters. Figures 2 and 3 show the best-fit template curves for the suite of cone visual pigments for larval/juvenile (Figure 2) and adult (Figure 3) fish.

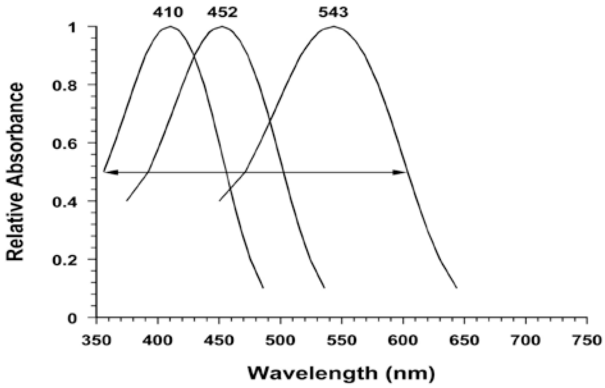

Figure 2. Visual pigments present in cones of larval/juvenile ballan wrasse. The number above each curve is the λ_{max} of the pigment. The horizontal line at the 0.5 relative absorbance level is an indicator of the potential spectral sensitivity range of the eye – that is, the eye of larval-juvenile wrasse will not be sensitive to light at wavelengths that fall below this line. Importantly, these fish have very little sensitivity to red light.

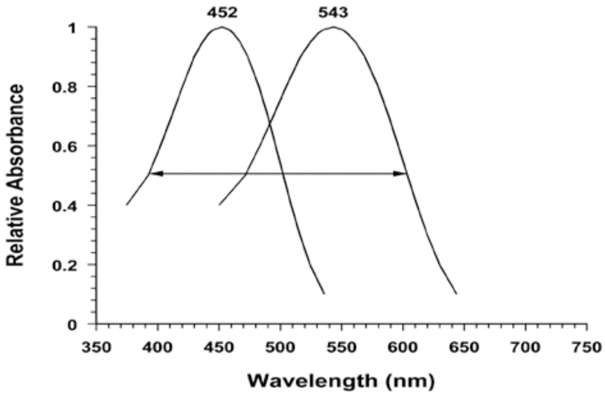

Figure 3. Visual pigments present in cones of adult ballan wrasse. The number above each curve is the λ_{max} of the pigment. The horizontal line at the 0.5 relative absorbance level is an indicator of the potential spectral sensitivity range of the eye – that is, the eye of adult wrasse will not be sensitive to light at wavelengths that fall below this line. Importantly, these fish have very little sensitivity to red light.

Tables 1 and 2 relates the presence of the individual cones classes in different size fish as indicated by standard length (S.L.).

Table 1. Average visual pigment λ_{max} from ballan wrasse. AVG = average λ_{max}; SD = standard deviation.

Cell	**Rod (r)**	**Violet (v)**	**Blue (b)**	**Green (g)**
avg	509	410	452	543
sd	4	5	3	6

Table 2. Photoreceptor cell classes found in ballan wrasse of various standard lengths (S.L).

S.L. (mm)	**6**	**48**	**68**	**72**	**74**	**82**	**120**	**221**	**225**
	R	R	R	R	R	R	R	R	R
	V	V	V	V	V	V	V	-	-
	B	B	B	B	B	B	B	B	B
	G	G	G	G	G	G	G	G	G

The importance of the different cone classes for specific visual tasks such as feeding, predator avoidance, etc. is unknown, but adequate stimulation of all classes would be the best strategy for determining the types of lighting to use in culture conditions. This practical aspect of the results is discussed further below.

Part II

When setting up lighting for an indoor intensive culture system it is important to know three things:

1) the relevant visual tasks necessary for growth and development (e.g. prey detection/ recognition, mate selection, etc.),

2) best solution for lighting, and

3) best physical specifications (e.g. diameter and depth of tank, temperature, oxygenation, etc.).

Of relevance to this report is the most suitable lighting and tank color given the observed spectral sensitivity of the fish. It is assumed here that feeding is the most relevant visual task.

The choices for lighting are natural sunlight, incandescent or gas discharge bulbs, fluorescent lamps (either traditional tubes or the newer compact types) or the newer light emitting diode (LED) sources. Lamps are usually specified by their energy output in Watts, their luminance (their brightness to the human eye), their color temperature measured in degrees Kelvin (the higher the color temperature, the 'bluer' the light), and it's color rendition index (CR) that is a measure of how well the lamp allows colors to be perceived and discriminated by the human eye.

Natural Sunlight. This would seem to be the obvious choice since all important behaviours should be supported by the lighting in which the behaviours evolved. When dealing with sunlight as the light source, one is actually dealing with two sources – the solar disk which is yellow with a color temperature of between 3000^o K and 4000^o K, and blue skylight with a much higher color temperature. There is going to be quite a bit of uncontrollable variability in using natural lighting since the solar disk changes position in the sky and atmospheric condition can affect both the solar disk and the quality of skylight. There is also an inability to independently control the light cycle under skylight. It may be necessary to maintain 'summer' lighting conditions during 'winter' months in order to maximize growth rate or other factors. There is also the question of how sunlight interacts with the properties of the water in which the fish naturally live. For example, natural waters may be coloured by chlorophyll or dissolved organic that act as light filters. The filtering effect is also affected by depth. These factors are hard to reproduce given the requirement for circulating water and tanks of reasonable size and depth. If the tanks must be kept indoors, there is the added problem of providing skylight that allows the full solar spectrum to be transmitted. This is particularly necessary where ultraviolet light is important. For these reasons, natural sunlight is not the common light source used for controlled culture situations.

Incandescent/gas discharge sources. The common tungsten-filament incandescent bulb is a good choice for many culture situations as it has a continuous spectrum (see Figure 5) and can be matched to the solar spectrum with simple filters. However, it has several drawbacks. First and foremost is that much of its emission falls in the far infrared and is radiated as heat. This carries with it an economic cost as this 'wasted' energy must be paid for. They also have a relatively short lifetime which carries with it replacement costs. Of more practical relevance here, many governments are mandating the total elimination of incandescent bulbs as a 'green' measure. While commercial entities will probably be exempt, the bulbs may be harder to get and could be more expensive. Even the more efficient quartz/halogen incandescent lamps may ultimately be banned. Gas discharge lamps such as xenon or mercury arc lamps or other high intensity discharge (HID) sources have the same limitations as incandescent sources although they may be more energy efficient and can be more easily 'tuned' as regards color temperature. In view of the above, it is best to abandon the use of incandescent or gas discharge sources if at all possible.

Fluorescent lamps. These are probably the most common sources currently in use for indoor or sheltered culture situations. They are extended sources which allows for more even illumination of larger tanks, are much more energy efficient than incandescent or gas discharge sources, have relatively long lifetimes and can be obtained in a number color temperatures (e.g. warm white, white, solar). However, these also have their problems. The output relies on the passage of electrical current through a gaseous mercury atmosphere within the tube that generates a lot of UV light. This light is absorbed by the phosphor painted on the inner surface of the bulb that is stimulated to release light covering the spectrum that is visible to HUMANS. The spectrum produced by excitation of mercury is not continuous like an incandescent lamp, but consists of a line spectrum with very narrow peaks. The phosphor output is much more continuous, but remnants of the line spectrum remain. Figure 4 shows the spectral output of two common fluorescent tubes having the same color temperature and CR index for humans. Note the obvious difference in the spectral output even though they appear the same to humans. In previous studies with yellowfin tuna in culture, it has been shown that survival and growth are different under the two different bulbs even though integrated intensity as measured with a radiometer are the same (personal observations). It is hard to predict how well a particular fluorescent tube will fare in a fish culture situation without knowing its spectral output and matching it to the spectral sensitivity of the fish. Even with that, significant trial and error would be required to determine which produces the best result. It should be noted that there is a continuing trend to move away from fluorescent sources altogether due to their contained mercury.

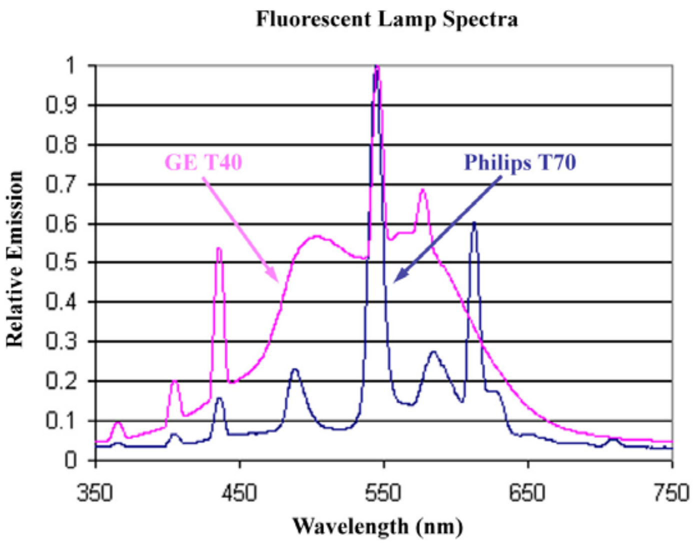

Figure 4. Spectral emission of two common types of fluorescent tube. The positions of the peaks are due to excitation of the mercury vapor. Note in particular the peaks at 435 nm and that around 550 nm, while the more continuous output between the peaks is a result of the phosphor mix.

LED sources. These are the lamps of the future. They are very efficient, they can be tailored to have a multitude of spectral outputs, they produce very little heat, they are dimmable over their full output range (unlike fluorescent or gas discharge bulbs), they have very long lifetimes and they are available in all common form factors including long fluorescent tube types. While they are currently the most expensive of artificial light sources, this will certainly change as the technology matures and the market grows. Are current LED sources suitable for fish culture? The answer is a definite yes! LED arrays are available consisting of numerous individual LEDs having different spectral outputs. For example, arrays to be used as grow-lights contain UV, blue and red LEDs in a matrix and provide the right spectral output for supporting plant growth. Large white arrays are also available for uniform area illumination. It is certainly feasible to place a number of such arrays containing LEDs of specified spectral output over tanks to replace current sources. However, it would be more convenient to simply use regular household bulb replacements if they are suitable since these are where all the R&D is being directed.

Figure 5 shows the spectral output of two common color variants of LED bulb, warm white (color temperature of 3000^{o} K) and white (5000^{o} K) along with that of a standard incandescent lamp (2800^{o} K).

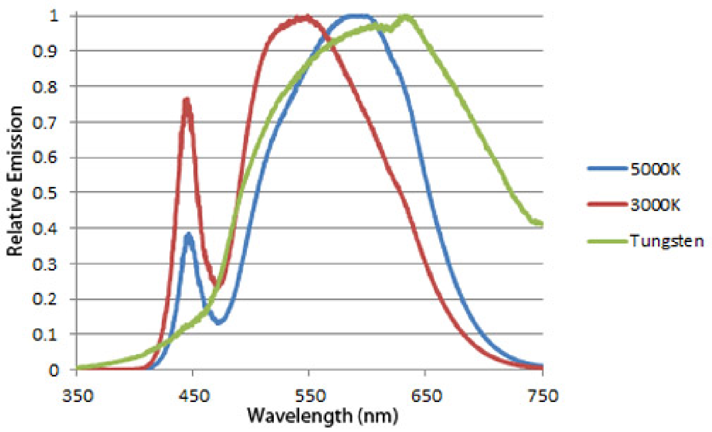

Figure 5. Spectral output of two commercial LED bulbs, warm white (3000^{o} K) and white (5000^{o} K) and a standard 60W incandescent bulb (2800^{o} K). To get the continuous spectrum, a mix of phosphors is layered over a high intensity blue led, the output of which is responsible for the output peak around 440 nm.

Comparing the spectral sensitivity of adult ballan wrasse (Figure 3) with the spectral emissions shown in Figure 5, it is clear that the warm white LED would provide suitable stimulation of both cone classes and would be an excellent replacement for current sources. For larvae and juveniles the ‘fit’ is not as good given the output of the LEDs in the violet region where the third visual pigment is located. However, I feel that there is enough overlap between the LED emission spectrum and the violet pigment absorbance to allow for adequate stimulation of this cone. Given this, I would suggest switching to the warm white LED lamps in a few tanks to test their effectiveness under ‘real life’ conditions.

As for tank color, black would seem to be the best choice since it would provide maximum possible contrast between ‘target’ and background when the light source is above the tank (the ‘darkfield’ situation). There is also evidence that black tanks increase survival of larval fish by decreasing death due to collision with tank walls (personal observation). The other common tank colors are ‘fiberglass green’ and blue. These choices are probably made in an effort to mimic the natural background environmental spacelight in waters where the fish live. Without more data like the spectral reflectance of the tanks, it is not possible to say which tank ‘color’ is better. I would continue with the current tanks and see how the combination of tank color and LED output affect survival.