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# Optical properties of the pineal window of Atlantic salmon (*Salmo salar* L.)

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**Abstract**

The spectral composition and intensity of light penetrating different parts of fresh preparations of the upper part of the skull of Atlantic salmon (*Salmo salar*) (fork length 25–30 cm) was investigated. All measurements were made in an aqueous medium, by moving the tip of an optical fibre in a three dimensional lattice below preparations that were illuminated by a parallel light source from above. The intensity of the transmitted light showed a well-defined maximum just below the pineal groove. Light that penetrated the skull from a source vertically above was refracted to produce a focusing point in the approximate position of the pineal body. Light projected from angles of 45° relative to the vertical position was only slightly (25%) attenuated, thus indicating a wide acceptance angle.

There was an almost uniform transmission of light (of 3%) between 500 and 700 nm. The transmittance of UV light (350 nm) was about 10% of that of green light. These differences are small when compared with the intensity range reported for the pineal light receptors. The transmission properties are discussed in relation to the known diurnal changes in the spectral composition of natural light.

## Introduction

Many processes in and between animals are triggered or mediated by the photoperiodicity of their natural habitats. Among the salmonid fishes, the seasonal changes in the photoperiod usually serve to synchronize such processes as sexual maturation, spawning, growth, smoltification and migration (*e.g.*, Davies *et al.* 1986). There is at present a considerable interest in manipulation of the photoperiod in order to accelerate or delay these processes.

The photosensitive pineal organ of teleosts has been reported to mediate the effects of the photoperiod on both the circadian and the seasonal rhythms in fish (*e.g.*, de Vlaming and Olcese 1981). In spite of the considerable research made on the innervation (*e.g.*, Ekström and van Veen 1983), histology (Ekström and Korf 1985) biochemistry (Dye *et al.* 1986) and electrophysiology (Meissl and Dodth 1981) of the pineal organ, little information exists on the optical transmission properties of skull tissues surrounding the pineal organ. Several vertebrates possess specialised areas on the head, through which light enters the brain cavity and strikes the pineal complex (Gruber *et al.* 1975). Weber and Smith (1980) have suggested a pineal mechanism by which photoperiodic effects may be mediated. They found that the activity of the enzymes involved in melatonin synthesis in rainbow trout (steelhead) decreased with increasing photoperiod, and suggested that melatonin production in salmonids may therefore decrease during the spring.

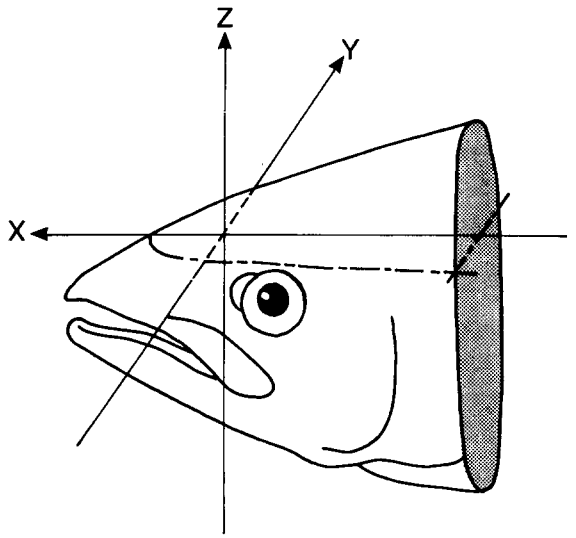


*Fig. 1.* The main axes used in the experiments. The origo was defined as the point of maximum intensity below the skull preparation. This point was located slightly below the pineal groove. The preparation was illuminated along the z-axis (vertical), and in some cases (the tilting experiments) at 45° to this axis, either frontally (x) or laterally (y) in both directions. The dotted line indicates where the horizontal cut was made.

The aim of the present work was to study the optical properties of the skull and of the pineal window of Atlantic salmon (*Salmo salar* L.) in relation to the transmission and conus of acceptance of light. Any threshold values for the photosensory pineal organ must be evaluated in relation to our knowledge of the filtering, focusing and extinction properties of the tissues above the pineal organ area.

## Materials and methods

Seawater-adapted Atlantic salmon (fork length 25.0–30.0 cm) were used in this study. Skull preparations were made, 5 in all, by cutting across horizontally just above the upper margin of the eye cavity (see Fig. 1). The preparation was thereafter transferred to a heparinized Ringer's solution (at 0°C). The ventral side of the preparation was cleaned, any remaining brain tissue, including the pineal body, removed. The preparation was then sewed on to a ring shaped holder, which could be rotated both horizontally and vertically.

The holder with the attached skull preparation was positioned in a small rectangular container equipped with a double window of thin glass (0.3 mm) facing towards the light source. This container had adjacent compartments that contained melting ice in order to maintain the temperature of the Ringer's solution at 0°C. The preparation was mounted with its dorsal side facing the light source.

A uniform light beam (diameter 2 cm) of near parallel light rays was provided by a 100W, 12V halogen lamp equipped with quartz lenses. The light intensity was adjusted with series of quartz neutral-density filters. The light that was transmitted through the preparation was collected by a plastic optical fibre (HFBR-3579), with a core diameter (aperture) of 1 mm pointed towards the light source. The position of the tip of the fibre was controlled by a micromanipulator along three separate dimensions, the x-, y-, z-axes (see Fig. 1). The angle of illumination was determined in relation to the approximate upright position of a fish swimming in the horizontal plane. The site of maximum transparency (the origo of the coordinate system) was determined for each preparation. The distal end of the optical fibre was connected to a monochromator system (Ferrand Optical Co., half width 4 nm) with a motor drive. The light intensity was measured by a photo-multiplier (Hamamatsu R928) connected to the monochromator, and recorded by a pen recorder.

The measuring programme consisted of three different series of experiments. The first was performed by illuminating the preparations along the z-axis. Transmission at 600 nm was recorded close to the tissue at intervals of 1.0 mm along the longitudinal (x-axis) and transverse (y-axis) axes through the origo. In the second, the refraction of the transmitted light was investigated by recording the light intensity as the optical fibre was moved away from the tissue along the axis of illumination (z-axis). Finally, the spectral characteristics in the origo of the transmitted light (from 350 to 700 nm) were recorded whist the preparation was being illuminated either along the z-axis, or at a direction of 45° to the z-axis, in either the anterior or both lateral directions, respectively.

The transmission properties of the Ringer's solu-

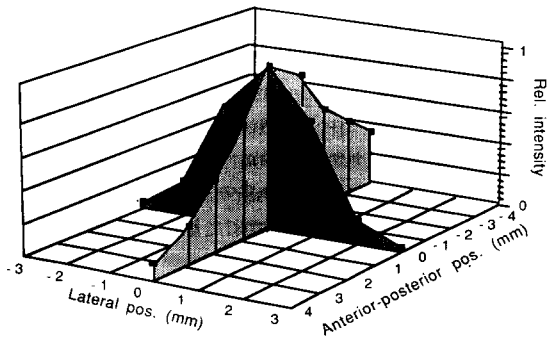


*Fig. 2.* The relative intensity distribution of light in the vertical (x) and lateral (y) directions from the pineal window (origo). Light intensity was measured at intervals of 1.0 mm. All values are given relative to the maximum intensity value.

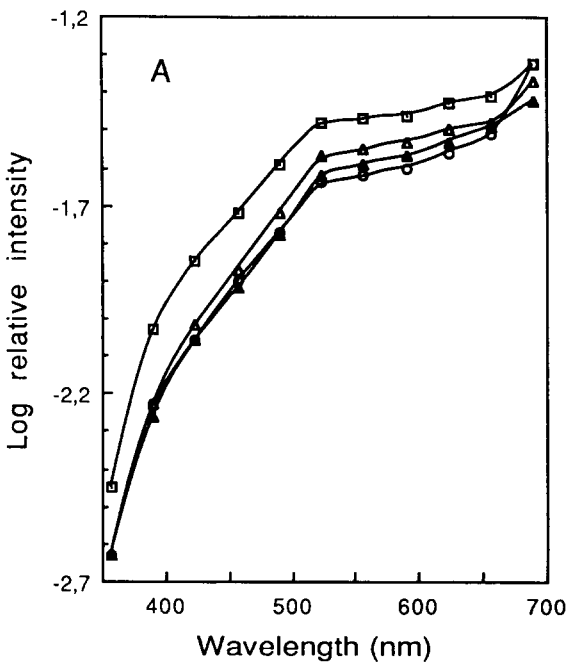


*Fig. 3a.* The relative spectral composition (log-values) of transmitted light. The various spectra represent different illumination angles. The light transmission value at each wavelength is expressed as a fraction of the corresponding value above the skull preparation. Illumination direction: vertical (□), 45° frontal (△), 45° lateral (▲, ○).

tion, as well as the position of origo, were controlled both before and after each recording session. The results have been adjusted to allow for the absorption by the Ringer's solution.

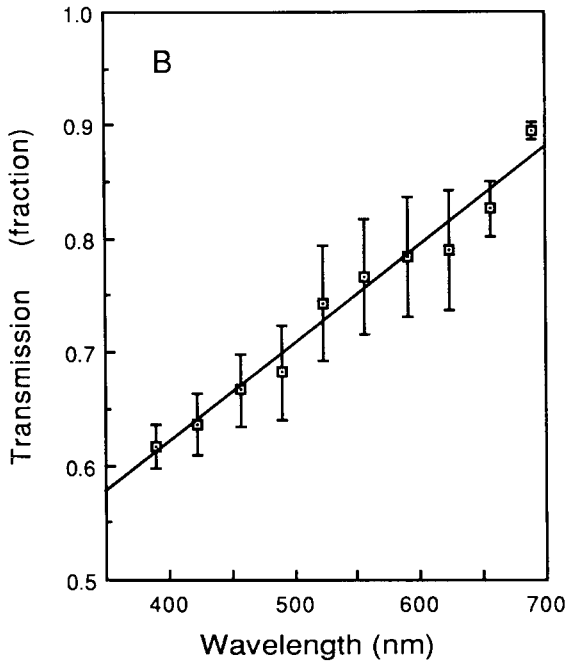


*Fig. 3b.* The spectral attenuation caused by tilting the illumination angle at 45° to the vertical position. Each point represents the mean of all values (SD indicated) obtained when the skull preparation was illuminated from the frontal and the two lateral directions. The light transmission value at each wavelength is expressed as a fraction of the corresponding value when the preparation was illuminated vertically.

## Results

In all the experiments, the point of maximum transparency was located at the position of the pineal groove. The intensity of transmitted light decreased substantially, (Fig. 2) in both the longitudinal and lateral directions, with the distance away from this origo. However, in the posterior (x-axis) direction, corresponding to longitudinal positions, the optical transmittance was quite high compared to that in the other direction.

The mean extinction coefficient in the spectral range 525–650 nm was about 1.5 (in log units), corresponding to a transmittance of about 3% (Fig. 3a). Below 500 nm towards the UV-region, the extinction increased to about 2.5.

Tilting the axis of illumination at 45° from the vertical direction, both frontally and laterally, resulted in a mean reduction in transmittance of

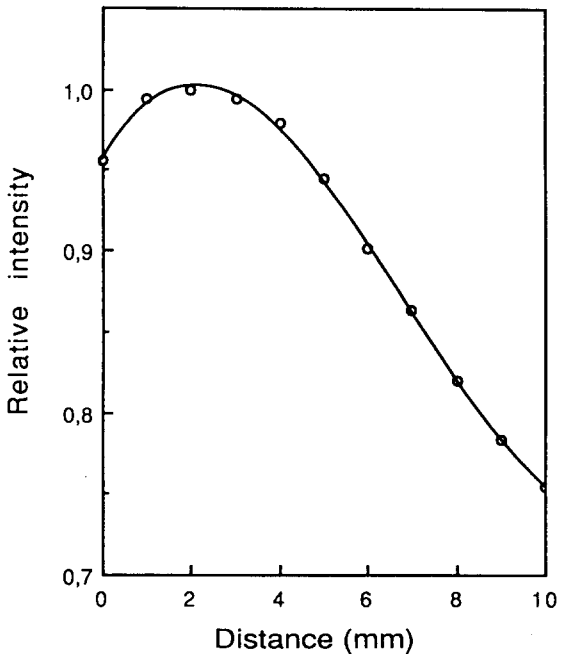


*Fig. 4.* The relative intensity distribution of light along the illumination axis below the pineal groove. Maximum intensity was recorded about 2.0 mm below the inner margin of the skull.

about 25%. The fraction of the light transmitted, relative to that from illumination with the preparation held upright was almost linearly dependent on wavelength and varied from about 0.6 in the UV-region to about 0.9 at 700 nm (Fig. 3b).

The intensity of transmitted light, as a function of the distance beneath the pineal groove (z-axis), increased initially (Fig. 4). At greater distanced the expected decrease was recorded, followed by a further decrease at even greater distances away. This can only be due to light refraction within the partial transparent skull producing a light focus about 2 mm below the inner surface of the bone in the pineal groove.

## Discussion

The use of a flexible optical fibre, which can be considered as an adjustable light collector with a small aperture, enabled measurement of the intensity of transmitted light in a three dimensional lattice below the skull roof to be carried out in an aqueous medium. This technique is different from that used by previous investigators.

A pronounced feature of fish skulls is the thinness of the parietal bone immediately above the pineal vesicle. It gives the impression of forming a window-like area, the pineal window. In nature, the skin and other tissues covering the pineal area are semi-transparent (Jafri and Ensor 1983). The existence of a window-like area is also borne out by the results of our measurements. The window has no clearcut borders, since the light transmission value in the region behind the origo was still high.

The optical density of the pineal window region (400–700 nm) of the Atlantic salmon is within the range of 1–2 log units, *i.e.*, similar to the previously recorded values for other fish species (Morita 1966; Hartwig and van Veen 1979; Meissl and Dodt 1981; Tamotsu and Morita 1986), but according to our data the transmission is somewhat more uniform in the investigated spectral range. Such results were reported on the teleost *Plecoglossus altivelis* by Hanyu *et al.* 1978. We have not been able, however, to find other comparable, reported measurements from the UV-spectral area.

We found no significant declines in transmission values that could be attributable to the absorption characteristics of hemoglobin. This may have been due to the use of a heparinized Ringer's solution, which may have caused a decrease in the hemoglobin content of the tissues. The linear decrease in the intensity of the transmitted light towards the UV region (Fig. 3b) with change in the illumination angle is probably due to the increase in scattering from the greater length of the ray path through the tissues.

The most commonly described electrophysiological response of the pineal body of fish is the acromatic (luminance) response, which varies linearly with the logarithmic expression of the light intensity values over a range of at least 5 log units (*e.g.*, Morita 1966; Falcón and Meissl 1981). The absolute threshold of this response for exposed pineal bodies in fish is about 7–10 log units below the intensity of bright sunlight (Meissl and Dodt 1981).

The pineal body also produces a chromatic response: Illumination by short wavelengths lead to inhibition, whereas light of long wavelengths causes

an excitatory response (Dodt and Heerd 1962). The response of the pineal body, therefore, depends on the spectral composition of the ambient light. The inhibitory component is strongest in fish and amphibians at 355 nm, whereas the excitatory component reaches its maximum in the green-red region (Meissl and Dodt 1981). The spectral range of this sensory apparatus thus extends into the UV-region, beyond that of visible light. It should be noted that the diurnal shifts in the spectral composition of natural light, when the UV-region and the green-red spectral regions are compared, are much larger than those obtained when comparing other parts of the visible spectrum (Nordtug and Melø 1988). Because of such a combined exitatory-inhibitory input from the receptors, these animals possess a sensory apparatus that is well suited for the detection of cyclic variations in the spectral composition of light. These variations are, at least at high latitudes, probably the most precise environmental parameter associated with the rotation of the earth (Krüll 1976). Our results indicate that the difference between the attenuation of UV-light (355 nm) and of green-orange light (600 nm) by the tissues covering the pineal body is about 1 log unit. In natural daylight the photon content of UV-light is lower than that of green-orange light. The difference between these spectral components at the position of the pineal body is therefore expected to be more than 1 logarithmic unit. The sensitivity inhibitory (UV; 360 nm) component in the toad *Xenopus laevis* was found to be 2.2 log units higher than that of the exitatory (green; 520 nm) response (Korf *et al.* 1981). A similar difference in sensitivity between corresponding components in the Atlantic salmon would compensate for both the increased extinction and the smaller ambient intensity of UV relative to green light at the surface. The possibility that the chromatic system is used for the detection of cyclic variations in the spectral composition of light, especially during twilight, was suggested earlier (Korf *et al.* 1981).

By the recording technique used, we were able to carry out all measurements in an aqueous medium (Ringer's solution) that had a refractive index close to that of the body fluids of the fish, thus permitting simple measurements of the spatial transmission properties of the skull to be made. The results of the experiments in which the angle of the incident light was changed show that the cone of acceptance of light reaching the pineal area is a wide one. This may be partly due to the focusing properties of the skull roof, as is apparent from the spatial distribution of light along the axis below the pineal window. In the live animal the position of the focal point may lie closer to the inner margin of the skull. The inner surface of the pineal groove is highly concave. Light that passed from the bone into the less dense Ringer's solution will have undergone some divergence, whereby the focal point will have been displaced away from the bone. In live animals the optical density of the underlying tissue (pineal body) can be expected to be greater than that of the Ringers solution, thus causing a further reduction in the focal length. Light rays striking the fish head from above, will thus converge at a point that corresponds to the position of the pineal body. We are not able to decide whether the focusing properties are due to specialized structures of increasing optical density, or to other particular structural features of the tissues situated above the pineal body. Ray tracing experiments on the skull roof, at present in progress, may clarify this point.

Both the wide conus of acceptance and the above-mentioned focusing properties of the tissues increase the light gathering capacity of the pineal window. However, the transmission value is still only about 3% of the incident light, due to the fairly heavy pigmentation of the skin covering the skull. The easiest way to achieve a further increase in light transmittance would be to reduce this pigmentation. This, though, would disrupt the natural pigmentation pattern, which might be disadvantageous to the protective camouflage. Nevertheless, both the wide acceptance angle and the focusing properties may compensate for some of the attenuation due to the heavy skin pigmentation.

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