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Differential light intensity and spectral sensitivities of Atlantic salmon, European sea bass and Atlantic cod pineal glands *ex vivo*

L.M. Vera, A. Davie, J.F. Taylor, H. Migaud *

Reproduction and Genetics Group, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK

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ABSTRACT

Photoperiod is perceived by pineal photoreceptors and transduced into rhythmic melatonin signals. These rhythms can be influenced by light intensity and spectral content. In this study we compared the light sensitivity of Atlantic salmon, European sea bass and Atlantic cod by testing *ex vivo* the effect of different intensities and narrow bandwidth lights on nocturnal melatonin suppression by isolated pineal glands in a flow-through culture system. Using combinations of neutral density and bandpass interference filters we tested a range of light intensities (ranging from 1.22×10^{13} to 3.85×10^{6} photons s^{-1} cm^{-2}) and three wavelengths of 80 nm width (472, 555 and 661 nm corresponding to blue, green and red, respectively). Results showed clear species specific light intensity and spectral sensitivities, with cod being from 100 to 1000 times more sensitive than sea bass and salmon. Regarding the influence of spectrum, red light was less efficient on suppressing melatonin than blue and green in salmon but results were not as clear in the two other species studied. Finally, the first evidence of relative photoreception in teleosts was obtained in cod suggesting that the definition of illuminance thresholds (day/night perception) would depend on the day intensity. Indeed, a single order of magnitude increase or decrease in day intensity was shown to elicit a significant shift in the intensity response curve of night-time melatonin suppression. Taken together, this study demonstrated species specific light intensity and spectral sensitivities within temperate teleosts.

1. Introduction

The pineal gland of teleost fish is an evagination of the diencephalon containing cone-like photoreceptors that are functional light sensors (Falcón, 1999). In most vertebrates, this gland is described as a photoneuroendocrine system composed of photoreceptor cells, self-sustained oscillators and neuroendocrine effectors (Ekström and Meissl, 1997; Korf et al., 1998) that synthesizes the indolamine melatonin in response to the ambient illumination. Highest melatonin levels are produced during the dark phase and circadian rhythms are self-sustained under continuous darkness (Falcon et al., 1989; Iigo et al., 1994; Masuda et al., 2003). However, within teleosts, salmonids appear to be exceptions to this generalized system as melatonin rhythms are not sustained under prolonged dark periods suggesting that they have lost the clock regulation of rhythmic melatonin release (Iigo et al., 2007). The photic regulation of the melatonin production is complex and involves photoreceptors in the eyes, pineal gland and possibly deep brain with divergent circadian organizations found within teleosts (Migaud et al., 2007). It is likely that the light sensitivity of these different systems will differ possibly due to the type of photoreceptors involved, their location but also the range of photic environments inhabited by teleosts. However, to date, very few comparative studies have been performed despite the significant implications for the control and management of fish physiology (i.e., reproduction, migration, feeding, locomotor activity...). This would certainly help to better characterize and understand local adaptations to specific environments.

Previous studies have shown that light sensitivity of the melatonin cascade greatly differ between teleost species. The lowest light intensity to suppress melatonin production *in vivo* in sea bass *Dicentrarchus labrax* was 6.0 $\mu W/cm^2$ (equivalent to 1.92×10^{13} photons s^{-1} cm^{-2}) (Bayarri et al., 2002) whereas in Senegal sole it was 5.3 $\mu W/cm^2$ (1.70×10^{13} photons s^{-1} cm^{-2}) (Oliveira et al., 2007) and in tench 3.3 $\mu W/cm^2$ (1.10×10^{13} photons s^{-1} cm^{-2}) (Vera et al., 2005) when 1 h light pulse was tested in the middle of the night. However, results largely differed in other *ex vivo* studies with theoretical *in vivo* threshold of light intensity (taking into account the light penetration through the skull), determined between 3.8×10^{-5} and 3.8×10^{-6} W/m^2 (equivalent to 1.22×10^{10} and 1.22×10^{9} photons s^{-1} cm^{-2}) in sea bass (Migaud et al., 2006). These discrepancies could be explained by experimental differences between both studies as light tested in the later was continuously ap-

* Corresponding author. Fax: +44 1786 472133.
E-mail address: herve.migaud@stir.ac.uk (H. Migaud).

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plied throughout the dark phase (12 h period) which might explain the increased photic sensitivity as compared to light pulses. This is known as dark adaptation and well documented in the mammalian visual system (Refinetti, 2001). Atlantic salmon, *Salmo salar* appeared to have much lower light sensitivity of the pineal gland than sea bass with a threshold of day and night melatonin levels found between 3.8×10^{-4} and 3.8×10^{-5} W/m^2 (equivalent to 1.22×10^{11} and 1.22×10^{10} photons s^{-1} cm^{-2} (Migaud et al., 2006). The photic sensitivity of the melatonin system will also depend on the spectral properties of the light. Several photopigments have been identified in the outer segment of the pineal photoreceptors in fish. In rainbow trout, *Oncorhynchus mykiss*, it was reported the existence of two populations of photoreceptors with two different action spectra peaking in the blue and in the green region of the visible spectrum (Marchiafava and Kusmic, 1992). In mesopelagic fish the pineal morphological organization is similar to that observed in shallow-water species with photopigments that have a λ_{max} between 485 and 503 nm whereas in the deep demersal eel, *Synaphobranchus kaupi*, the pineal photopigment has a λ_{max} at 515 nm (Bowmaker and Wagner, 2004). Several *in vivo* and *ex vivo* experiments have tested the efficiency of different wavelengths to reduce nocturnal melatonin and have shown that in sea bass the blue end of the visible spectrum (blue, 434–477 nm) was more effective than longer green and red wavelengths (Bayarri et al., 2002). In zebrafish, however, green light (512 nm) was shown to be the most efficient in suppressing melatonin production by pineal glands in culture (Ziv et al., 2007). While there are many indirect evidences of differential photic sensitivities in temperate fish species through assessment of the effects of artificial lighting regimes on growth (Boeuf and Le Bail, 1999; Ruchin, 2004), reproduction (Popek et al., 1992; Randall et al., 1995), behavior such as light attraction, feeding and locomotor activity (Ballagh et al., 2008; Giménez and Esteve, 2008; Kavaliers, 1981; Underwood, 1989) and embryo/larvae development and performances (Downing and Litvak, 2002; Monk et al., 2006), there is still a lack of clear demonstration of these at the pineal level, especially with regards to spectrum.

The objective of our study was therefore to (1) determine the light intensity threshold of melatonin production by Atlantic cod (*Gadus morhua*) pineal gland cultured *ex vivo* and compare results with previously published data on Atlantic salmon and European sea bass (Migaud et al., 2006), (2) compare spectral sensitivity of Atlantic salmon, European sea bass and Atlantic cod pineal glands *ex vivo* and (3) investigate relative pineal sensitivity in Atlantic cod.

2. Materials and methods

2.1. Animals

Atlantic salmon (body weight: 92.9 ± 4.0 g, total length: 20.7 ± 0.2 cm), European sea bass (body weight: 368.3 ± 15.6 g, total length: 30.8 ± 0.5 cm) and Atlantic cod (body weight: 256.6 ± 16.6 g, total length: 28.6 ± 0.7 cm) were obtained from the Machrihanish Environmental Research Laboratories of the Institute of Aquaculture (Scotland). Fish were reared and acclimated to a constant 12L: 12D artificial photoperiod (lights on at 08:00, lights off at 20:00) and a temperature of 14 ± 1 °C for a period of at least 2 weeks prior to the start of the experiments. Fish were killed by a lethal dose of 2-phenoxyethanol solution (1 mL L^{-1}, SIGMA, Ref. P 1126). All experiments were carried in accordance with the Animal (Scientific Procedures) Act 1986, UK.

2.2. Experiment 1: ex vivo pineal light intensity sensitivity in Atlantic cod

Fish were culled between 15:00 and 16:00 h and pineal glands dissected by opening the skull dorsally around the pineal window and extracting the intact gland. After removal, pineal glands ($n = 4$) were washed with culture medium and then placed individually in the culture chambers. The pineal culture system consisted of a continuous flow-through system regulated by a peristaltic pump at a flow rate of 1.5 mL of culture medium/hour and samples were collected every hour by an automatic fraction collector as previously described by Migaud et al. (2006). The culture media (Ref. RPMI 1640; Sigma), which was changed daily, was supplemented with HEPES sodium salt (Ref. H3784, 4.77 g/L; Sigma) to buffer the pH adjusted to 7.4 and penicillin–streptomycin (10 mg/L) and Fungizone (5 g/mL) to avoid bacterial and fungal development. Samples were removed from the culture system daily and stored at −70 °C prior to analysis. The pineal glands were subjected to a matching photoperiod regime *ex vivo* as they had previously been acclimated to *in vivo* with culture media samples being collected from 17:00 on the day of pineal removal. Each *ex vivo* trial started and ended by a 12L:12D cycle to which fish were acclimatized to, serving as controls for normal melatonin production by the pineal glands. On the second and third night of culture (subjective night, SN), pineal glands were subjected to one of a range of seven different intensities from 3.85×10^6 to 3.85×10^{12} photons s^{-1} cm^{-2} (Table 1). Illumination was supplied by dichroic halogen bulbs with an emission spectrum equivalent

Table 1
Light intensity treatments expressed in Lux, W/m^2 and photons flux (photons/s/cm^2). Transmittance, light intensity ratio between day and night and nominal density filters used are also described. Ambient day lighting was recreated by the use of a solux bulb (4700 K CRI 99, 10° spread) which deliver a similar spectrum than in ambient natural conditions. Light intensity provided by one solux bulb was 22000 lux, 116 W/m^2 equivalent to 3.72×10^{16} photons/sec/cm^2. Transmittance (%) related to the max bulb intensity. C, S, SB and X refer to treatments applied in cod, salmon, sea bass and the three studied species, respectively.

Periods	Transmittance (%)	Light intensity ratio at subjective night/day	Nominal density of neutral density filters used	Lux	W/m^2	Photons/s/cm^2	Experiment 1	Experiment 1	Experiment 1
Daylight	50	n/a	0.3	11,026	58	1.92×10^{16}			X
	5 (Ref.)	LL 100	1.3	1102	5.8	1.92×10^{15}	X	X	
	0.5	n/a	2.3	110	0.58	1.92×10^{14}			X
Light treatment	0.032	0.6	2.5	7	3.8×10^{-2}	1.22×10^{13}		S	
during	0.01	0.2	4	2.2	1.2×10^{-2}	3.85×10^{12}	X		
subjective	0.0032	0.06	3.5	0.7	3.8×10^{-3}	1.22×10^{12}		S, SB	
night	0.00032	0.006	5.5	0.07	3.8×10^{-4}	1.22×10^{11}	X	S, SB	X
	0.000032	0.0006	6.5	0.007	3.8×10^{-5}	1.22×10^{10}	X	C, SB	
	0.00001	0.00019	7	0.0022	1.2×10^{-5}	3.85×10^{9}	X		
	0.0000031	0.00006	7.5	0.0007	3.8×10^{-6}	1.22×10^{9}	X	C	X
	0.00000031	0.000006	8.5	7×10^{-5}	3.8×10^{-7}	1.22×10^{8}	X	C	
	0.0000001	0.0000019	9.0	2×10^{-5}	1.2×10^{-7}	3.85×10^{7}	X		X
	0.00000001	0.00000019	10.0	2×10^{-6}	1.2×10^{-8}	3.85×10^{6}	X		