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Influence of the light spectrum on the daily rhythms of stress and humoral innate immune markers in pikeperch *Sander lucioperca*

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ABSTRACT

This study investigated the daily variations of stress markers namely plasma cortisol and glucose and some humoral innate immune markers, including peroxidase, lysozyme and complement activities, of pikeperch (*Sander lucioperca*) and the effect of light spectrum on these variations. Fish were reared under a white or red light spectrum at a constant photoperiod (12D:12 L). Samples were collected at 22:00, 04:00, 10:00 and 16:00 at days 1 and 42 of the experiment. After 42 days, the use of a red light spectrum led to a significant increase in final bodyweight. Specific growth rate reached 2.1 ± 0.18 and 1.8 ± 0.17% d^{-1} under red and white spectra respectively. The profiles of plasma cortisol followed a cyclic activity with a surge during photophase at 10:00 without any effect of the light spectrum at day 42. Both lysozyme and peroxidase activities in blood followed a day-night variation with a peak at 4:00 corresponding to low cortisol values. No rhythmicity was detected for the complement activity but higher values were observed at 16:00 when cortisol values were lowest. Light spectra also influenced humoral immune markers with an increase in lysozyme activity and a decrease in peroxidase activity in a red light environment. The present results indicate a strong effect of the light environment, including the light-dark cycle and the light spectrum, on pikeperch physiology. Especially, some innate immune status seemed stimulated during the dark phase in relation to a decrease in the stress level markers. Such parallelism in the relationship between the immune status and stress markers may be affected positively or negatively by the light characteristics. Humoral immune markers were also modulated according to the light spectrum without no clear trend (stimulation or inhibition) for the immunocompetence status.

1. Introduction

Due to its fast growth, high quality flesh and high economical expectation, pikeperch *Sander lucioperca* is one of the most promising freshwater fish species for the diversification of European inland aquaculture (Wang et al., 2009; Dalsgaard et al., 2013; Overton et al., 2015). However, its culture is still limited by impairment in growth rate and high mortality rate during the young developmental stages. These failures may be related to inadequate rearing conditions inducing high stress level since the pikeperch aquaculture management has not been optimized yet. It has been shown that percid fish are more sensitive to aquaculture stressors than other species with a longer history of domestication (Jentoft et al., 2005). And since decreased welfare may lead to increased stress level and to disease outbreaks, it is essential to improve its management strategy. In previous studies (Luchiari et al., 2006, 2009; Baekelandt et al., 2018), light was defined as a determining factor affecting physiology and, by the way, culture of pikeperch. However, the effects of the light environment, including light-darkness cycle and light spectrum, on physiology and immunity of pikeperch, are poorly documented and would merit more attention.

The aquatic environment is critical for the maintenance of fish homeostasis. It is well established that a perturbation of the pathogen-host-environment balance favors disease outbreaks that can severely limit aquaculture success (Esteban et al., 2006). From environmental cues, photoperiod is one of the major factors regulating a wide range of biological processes. The light-darkness cycle is perceived by photoreceptors and integrated into a melatonin rhythmic signal. It has been described several times to play in almost all vertebrates, a central role in driving circadian rhythms, including locomotor activity, thermal preferences, rest, osmoregulation and metabolic activity, as well as annual processes such as growth and sexual maturation (Falcón et al., 2007, 2010). Few studies also support a circadian and circannual activity of the immune system (Esteban et al., 2006; Morgan et al., 2008). Esteban et al. (2006) pointed out a variation of some humoral immune markers in seabream and sea bass based on the light-darkness cycle. In addition to vary seasonally, immunity was shown to be influenced by

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artificial photoperiod (Leonardi and Klempau, 2003). While only little is known on this matter, a better understanding of the immune regulation by the light-darkness cycle would improve management strategies of cultured species. And as already used to control timing of broodstock spawning, smoltification and early maturation in several fish species (Falcón et al., 2010), photoperiod manipulation could be an interesting tool for improving their immunocompetence.

Environmental colors affect the vision of teleosts, influencing for example food intake, signals for hierarchical status, reproduction, growth and even survival (Downing, 2002; Politis et al., 2014; Karakatsouli et al., 2007; Luchiari and Pirhonen, 2008). The use of optimal light colors was described to decrease stress status and stress-induced cortisol response in several fish species (Volpato and Barreto, 2001; Karakatsouli et al., 2007; Eslamloo et al., 2015). Unnatural light spectra also negatively affect important aspects of larval development and performance (Villamizar et al., 2009; Blanco-Vives et al., 2010). In pikeperch, it was shown that the use of red light improved specific growth rate and feed efficiency, with no consideration on the stress status or the immune system (Luchiari et al., 2009). These species-dependent preferences are explained by their specific natural habitat characteristics in relation to the adaptations of their visual system (Karakatsouli et al., 2007; Migaud et al., 2007). In the case of pikeperch, natural habitats are typically eutrophic and only light above 600 nm, including red wavelengths, penetrates below 1 m (Luchiari et al., 2009). Considering current practices in pikeperch culture and previous data (Baekelandt et al., 2018), red and industrial white light spectra were chosen to better evaluate the potential effects of the light spectrum on humoral innate immune markers.

As a potent immunosuppressive agent in vertebrates with complex actions on immune cells, cortisol has to be taken into account when investigating the potential effects of the light environment on the immune system (Tort, 2011). This glucocorticoid is well known to be part of the stress response and to be a major stress indicator promoting the metabolic pathways that increase plasma glucose levels in response to energy expenditure (Laiz-Carrión et al., 2003; Milla et al., 2010; Oliveira et al., 2013).

Globally, involvement of light characteristics in the regulation of the immune system in fish is poorly documented. Therefore, as a first step in describing the immunomodulation by the light environment, we tested in pikeperch the 24-h profiles for rhythmicity in cortisol and glucose release and in humoral innate immune markers, including peroxidase, lysozyme and complement activities. Furthermore, the effects of two light spectra (red and white) on stress status and on the latter immune markers were assessed.

2. Materials and methods

2.1.1. Animals and rearing conditions

A stock of 960 pikeperch (*S. lucioperca*) juveniles from Asialor farm (Dieuze, France) were transferred to URBE facilities at the University of Namur, Belgium. Animals were randomly distributed in 24 indoor 100 l-tanks. They were acclimated for 20 days under constant white lightning conditions (industrial white spectrum, 10 lx, 12 h of night duration from 8 pm to 8 am) and 22 ± 0.5 °C water temperature until they reached 10 ± 1 g bodyweight. At day 1 of the experiment, the white spectrum was replaced by a red spectrum (610 nm) for half of the tanks. Intensity was maintained at 10 lx at water surface. Fish were reared under these conditions (red or white spectrum) with a 12 L:12 D daily cycle for 42 days. They were fed twice a day at 10:30 and 18:00 with a commercial pellet diet (44% proteins and 26% lipids; Coppens, Netherlands) at 2.0% biomass during all the experimental period. These rearing conditions were adapted according to a previous multifactorial experiment comparing major husbandry practices in pikeperch culture (Baekelandt et al., 2018).

The present protocol (16 276 KE) has been carried out in agreement with the local Ethics Committee for Animal Experiments.

2.1.2. Sampling procedures

Samplings occurred at 22:00, 4:00, 10:00 and 16:00 h (5 fish per time and per tank) at both days 1 and 42. To avoid repetitive stressful events on fish and potential artefacts on results, 6 tanks (3 per condition) were assigned at each time of sampling. Each treatment group had thus 3 replicates. Fish were starved one day before samplings. Five fish were removed randomly from each tank and anesthetized with MS-222 (150 mg l^{-1}) in a bucket covered with a tissue. As soon as they were anesthetized, fish head was covered with a tissue and blood was quickly collected by caudal vein puncture with heparinized syringes and centrifuged at 3000*g* during 10 min at 4 °C. The time between fish capture from their rearing tank and the blood sampling was optimized within 4 min. Plasma was aliquoted and stored at −80 °C until assayed.

2.2. Husbandry variables

Final individual weight (FIW) and Specific Growth Rate (SGR) were determined on day 42 for each experimental condition. SGR was estimated according to the formula: ((Ln(final individual weight) – Ln (initial individual weight)) *100 / duration of the experiment).

2.2.1. Cortisol and glucose assays

Cortisol was assayed in triplicate using a cortisol ELISA kit (DRG, EIA-1887), following the manufacturer's instructions (BioSource, Belgium). The intra-assay coefficient of variation was 3.6%, the assay dynamic range was between 0 and 800 ng ml^{-1} and the analytical sensitivity was 2.5 ng ml^{-1}. Plasma glucose, also assayed in triplicate, was determined calorimetrically based on a glucose oxidase/peroxidase method described by Trinder (1969).

2.2.2. Plasma lysozyme activity

Lysozyme activity was evaluated in plasma samples by the turbimetric method (Siwicki and Studnicka, 1987; Douxfils et al., 2012). Plasma samples (10 µl) were mixed with 10 µl of Na_2HPO_4 0.05 M pH 6.2 and 140 µl *Micrococcus lysodeikticus* (Sigma-Aldrich) solution (0.6 g l^{-1}). This assay was performed in triplicate. Absorbance was measured at 450 nm every 2 min during 20 min at room temperature. Lysozyme activity (units) is defined as the amount of enzyme decreasing the turbidity of 0.001 OD per min.

2.2.3. Peroxidase activity

The total peroxidase activity in plasma was assessed following the method described in Quade and Roth (1997). Briefly, 15 µl of plasma was diluted in 140 µl of HBSS without Ca + 2 or Mg + 2 and mixed with 50 µl of 20 mM 3,3′,5,5′-tetramethylbenzidine hydrochloride (Sigma) and 5 mM H2O2. The reaction was stopped after 2 min by adding 50 µl of 4 M sulphuric acid and absorbance was measured at 450 nm. The peroxidase activity was determined defining as one unit the peroxidase that produces an absorbance change of 1 OD.

2.2.4. Plasma alternative complement pathway

The alternative complement pathway (ACH50) was assayed by measuring the haemolytic activity in plasma samples using rabbit erythrocytes as targets (Sunyer and Tort, 1995). Briefly, 10 µl of rabbit red blood cells suspension suspended at 3% in veronal buffer (Biomerieux, Marcy-l'Etoile, France) were mixed with serial dilutions of plasma (from 40 to 800 times). Plates were then read at 405 nm after incubation at 28 °C for 120 min. The spontaneous hemolysis was obtained by adding veronal buffer to 10 µl of rabbit erythrocytes and total lysis was obtained by mixing 10 µl of rabbit erythrocytes to distilled water (total volume = 70 µl). ACH50 was calculated using 4-parameter logistic regression and corresponds to the lysis of 50% of the rabbit erythrocytes.