Science of the Total Environment 511 (2015) 516–522

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Spotlight on fish: Light pollution affects circadian rhythms of European perch but does not cause stress

Anika Brüning [a],*, Franz Hölker [a], Steffen Franke [b], Torsten Preuer [a], Werner Kloas [a]

[a] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany
[b] Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany

## HIGHLIGHTS

- We studied the impact of light at night on melatonin and cortisol rhythms in perch
- Sampling was non-invasive and hormones were extracted out of the holding water
- Melatonin rhythm was significantly impaired by artificial light at night
- Cortisol was not affected by artificial light at night

## ARTICLE INFO

*Article history:*
Received 16 September 2014
Received in revised form 22 December 2014
Accepted 28 December 2014
Available online xxxx

Editor: Mark Hanson

*Keywords:*
*Perca fluviatilis*
Light pollution
Light intensity
Non-invasive hormone measurement
Fish

## ABSTRACT

Flora and fauna evolved under natural day and night cycles. However, natural light is now enhanced by artificial light at night, particularly in urban areas. This alteration of natural light environments during the night is hypothesised to alter biological rhythms in fish, by effecting night-time production of the hormone melatonin. Artificial light at night is also expected to increase the stress level of fish, resulting in higher cortisol production. In laboratory experiments, European perch (*Perca fluviatilis*) were exposed to four different light intensities during the night, 0 lx (control), 1 lx (potential light level in urban waters), 10 lx (typical street lighting at night) and 100 lx. Melatonin and cortisol concentrations were measured from water samples every 3 h during a 24 hour period. This study revealed that the nocturnal increase in melatonin production was inhibited even at the lowest light level of 1 lx. However, cortisol levels did not differ between control and treatment illumination levels. We conclude that artificial light at night at very low intensities may disturb biological rhythms in fish since nocturnal light levels around 1 lx are already found in urban waters. However, enhanced stress induction could not be demonstrated.

## 1. Introduction

Recent decades have seen a profound transformation of nightscapes, with an increasing proportion of the Earth's surface being illuminated at night. Global light emissions increased at a rate of around 3–6% per year in recent decades (Hölker et al., 2010a). This substantial transformation demonstrates a pressing need to understand the effects of artificial light at night on biological processes. In particular, information regarding the ecological impact of light pollution on animal populations and whole ecosystems is crucial. Possible consequences of artificial light at night on many behavioural and physiological processes in various classes of animals have been reviewed recently (Gaston et al., 2013; Hölker et al., 2010b; Navara and Nelson, 2007). Most of these processes are coupled to circadian or seasonal rhythms (Falcón et al., 2003) which may be disrupted by light pollution.

In fish, seasonal rhythms include reproduction, growth and development, and migration, while patterns like locomotor activity, food intake, shoaling or diel vertical migration are mainly driven by circadian rhythms (Boeuf and Le Bail, 1999; Downing and Litvak, 2002; Duston and Bromage, 1986; Lowe, 1952; Mehner, 2012; Ryer and Olla, 1998; Vowles et al., 2014). The most important mechanism of the circadian system in vertebrates is the light-dependent production of melatonin (production at night, suppression during the day) by the pineal organ. The pineal organ of fish is light-sensitive and directly processes photoperiodic information, resulting in a circadian melatonin rhythm that provides periodic information for cells and organs, such as time of the day and season (Ekstrzm and Meissl, 1997; Falcón and Collin, 1989; Kulczykowska et al., 2010; Underwood and Goldman, 1987).

---

* Corresponding author.
*E-mail addresses:* bruening@igb-berlin.de (A. Brüning), hoelker@igb-berlin.de (F. Hölker), steffen.franke@inp-greifswald.de (S. Franke), preuer@igb-berlin.de (T. Preuer), werner.kloas@igb-berlin.de (W. Kloas).

http://dx.doi.org/10.1016/j.scitotenv.2014.12.094
0048-9697/

The influence of artificial light at night on fish has been an area of interest for researchers with respect to aquaculture, e.g., how to control growth (Biswas et al., 2005; Boeuf and Le Bail, 1999; Kissil et al., 2001), development (Porter et al., 1998; Thrush et al., 1994) and reproduction (Kissil et al., 2001). However, while photoperiod manipulation has proven beneficial for aquaculture, artificial light can have detrimental effects in nature.

Most studies that investigated the influence of artificial light at night on the melatonin rhythm used high light intensities above 100 lx and only a few studies using low light intensities (Bayarri et al., 2002; Migaud et al., 2006a; Takemura et al., 2006), which may occur in light polluted urban areas. However, none of these studies addressed the possible effect of artificial light at night on the physiology of fish and very little is known about dose–response relationships for a range of biological impacts (Gaston et al., in press).

Cortisol is the most measured indicator for stress in fish. Moreover, two of the major actions of cortisol in fish are hydromineral balance (e.g., seawater adaption in migratory fish) and energy metabolism (carbohydrate, protein, lipid metabolism).

In most fish species, blood levels of cortisol also exhibit a circadian rhythm. However this rhythm is species specific, subjected to seasonal influences and affected by other environmental cues (Wendelaar Bonga, 1997). In goldfish (*Carassius auratus*) for instance, the schedule is linked to the photoperiod and peak titres occur around light onset, minimum titres at light offset (Noeske and Spieler, 1983). In humans this pattern is called the cortisol awakening response (Kirschbaum et al., 2000) and was also found in Nile tilapia (*Oreochromis mossambicus*) (Binuramesh and Michael, 2011). However, in goldfish the feeding schedule can override photoperiod to trigger circadian serum-cortisol variations (Spieler and Noeske, 1984). In some salmonids, cortisol peaks were found during the dark phase of the photoperiod but also connected to feeding time (Laidley and Leatherland, 1988; Pickering and Pottinger, 1983), whereas in sticklebacks (*Gasterosteus aculeatus*) no circadian rhythm could be identified (Audet et al., 1986).

Previous studies regarding the effect of light at night on cortisol levels revealed no obvious impact. But most studies were working with prolonged or continuous photoperiods and relatively high light intensities. Biswas et al. (2006, 2008) for example tested a constant illumination of 1500 lx on red sea bream (*Pagrus major*) and striped knifejaw (*Oplegnathus fasciatus*), but cortisol concentrations showed no significant differences to normal photoperiods. Bluefin tuna (*Thunnus orientalis*) showed no changes of circadian cortisol levels when subjected to 5, 15 or 150 lx compared to a 0 lx control (Honryo et al., 2013). However, the invasive sampling procedures (blood or whole body sampling) in the abovementioned examples may have introduced a sampling artefact that obscured potential differences in the stress response between treatments. The dynamics of the stress response to handling stress associated with surgical sampling methods cannot be generalized and seems to be highly species specific ranging from a few minutes (Ramsay et al., 2009) to one or several hours (Wendelaar Bonga, 1997). However, with a non-invasive sampling method, existing differences in cortisol rhythms in response to artificial light at night might be uncovered.

Our study presents data on the influence of artificial light at night on circadian rhythm and stress response of European perch (*Perca fluviatilis*). Perch belong to the most dominant fish species in Central European aquatic systems and inhabit a wide range of habitats, including all types of lakes and most streams (Kottelat and Freyhof, 2007). Perch are diurnal feeders and become inactive during the night, especially in the presence of nocturnal predators (Hölker et al., 2007).

We measured melatonin concentrations to assess the influence of light on the biological rhythms of perch. Cortisol was measured to evaluate the stress response to light pollution. In contrast to earlier studies, we based our results exclusively on non-invasive measurements, by extracting the hormones from water samples taken without disturbing the experimental animals and determined their concentrations. Ellis et al. (2004) proved a correlation between concentrations of cortisol in water and serum in rainbow trout (*Oncorhynchus mykiss*) with a phase delay of about 2 h due to excretion route and accumulation in the water. The work of Ellis et al. (2005) indicated a similar correlation for melatonin.

We hypothesised that artificial light at night has a clear effect on the natural circadian rhythm of melatonin production. We expected that with increasing light intensity, the nocturnal melatonin production is suppressed. Furthermore, we hypothesised a stress response caused by the presence of artificial light at night. Using non-invasive methods we expected to identify an increase in cortisol concentrations corresponding to increasing light intensity, especially during the dark phase of the photoperiod.

## 2. Methods

### 2.1. Experimental fish

European perch were taken from an existing population at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany. They were originally obtained from the nearby Lake Müggelsee that has a periurban surrounding including forests and housing. Prior to the experiment they were held in 600 L indoor tanks in a flow-through system with ground water, aeration and natural photoperiod and fed daily with frozen red bloodworms. Body mass of the fish at the time of the experiment was 14.2 ± 3.9 g (mean ± SD).

### 2.2. Experimental setup

Experiments were conducted in a climate chamber in 12 aquaria (80 cm length, 30 cm height, 35 cm width). The temperature of the climate chamber was adjusted to maintain a water temperature of 16 °C. The aquaria were taped with black foil from all sides to make them lightproof.

The lighting system in the cover of the aquaria provided a daytime illumination of up to 7000 lx. Additionally, a three hour dusk and three hour dawn period was programmed to resemble natural twilight conditions. The night-time illumination was adjusted to four different light intensities: 0 lx (control), 1 lx (potential light level in urban waters), 10 lx (nocturnal street illumination) and 100 lx (for details see (Franke et al., 2013)). Each treatment group had three replicates and the experiment was repeated once with a different set of fish to obtain a sufficient replicate number ($n = 6$) for statistical analysis. Thus, in total $4 \times 6 = 24$ experimental units were used.

### 2.3. Experiment

Each aquarium contained 84 L water and was stocked with 30 perch. The flow-through was adjusted to approximately 10 L per hour with aerated tap water. During acclimatisation the photoperiod was 14 hour light/10 hour darkness with complete daylight from 9:30 am to 5:30 pm and a dusk and dawn starting at 6:30 pm and 5:30 am, respectively. The night was kept dark in all aquaria and the fish were fed with frozen red bloodworms twice a day. Acclimatisation conditions were applied for 14 days.

After acclimatisation time, the night-time illumination was activated. Water flow was reduced to 4.1 ± 0.2 L/h. When applying the non-invasive method in a flow through system the water exchange rate has to be lowered depending on tank volume and fish biomass to allow the accumulation of the hormones in order to meet the sensitivity range of the applied test.