

The effect of metal halide and novel green cathode lights on the stress response, innate immunity, eye structure and feeding activity of Atlantic cod, *Gadus morhua* L.

Mairi Cowan, Andrew Davie & Hervé Migaud

Reproduction and Genetics Group, Institute of Aquaculture, University of Stirling, Stirling, UK

Correspondence: Dr H Migaud, Reproduction and Genetics Group, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK. E-mail: herve.migaud@stir.ac.uk

Abstract

High-intensity constant lighting is routinely used for photoperiod manipulation in the aquaculture industry in order to prevent early maturation. The potential welfare impacts of this technology, however, have not been extensively studied to date, and with the implementation of more efficient narrow bandwidth lighting technologies (cathode, light-emitting diodes), definitions of species-specific sensitivities are becoming essential. The objective of this study was to investigate the impact of traditional metal halide (MH) and novel green cathode lighting on the welfare (stress response, innate immunity, retina structure, feeding activity) and light perception of Atlantic cod. The results indicated that although acute responses to light were observed, there were no clear significant long-term effects of any of the lighting treatments on the stress levels (plasma cortisol, glucose), innate immune function (lysozyme activity), retina structure and population feeding activity (acute decline under all light treatments, most pronounced in fish exposed to higher illumination, but normal feeding activity was resumed within 8 days following light onset). Regarding light perception, interestingly, even when subjected to high-intensity constant lighting (MH mean tank intensity: 16.6 W m^{-2}), cod still demonstrated a day–night rhythm in melatonin release, which suggests perception of the overlying ambient photoperiod.

Keywords: *Gadus morhua* L., artificial light, green cathode, stress, lysozyme, retina, feeding activity

Introduction

Sexual maturation is a major welfare concern and economic burden during the on-growing of marine finfish as energy is directed into gonadal development, resulting in a loss in growth and product quality. Furthermore, during final maturation, there is an increased sensitivity to disease, a reduction in feeding activity and concerns exist over potential genetic interaction with native stocks through broadcast spawning or spawning interaction by escapees (Bromage, Porter & Randall 2001). Photoperiod manipulation is an efficient tool used to suppress early maturation in a number of commercially important marine teleosts, especially temperate species such as Atlantic cod, *Gadus morhua* L. (Hansen, Karlsen, Taranger, Hemre, Holm & Kjesbu 2001; Davie, Porter & Bromage 2003; Davie, Porter, Bromage & Migaud 2007), Atlantic salmon, *Salmo salar* L. (Endal, Taranger, Stefansson & Hansen 2000) and European sea bass, *Dicentrarchus labrax* L. (Bayarri, Rodriguez, Zanuy, Madrid, Sanchez-Vazquez & Carrillo 2003; Felip, Zanuy, Muriach, Cerda-Reverter & Carrillo 2008), in which seasonal changes in day–length act as the principal regulator of puberty onset (Bromage *et al.* 2001). It is believed that the indoleamine melatonin acts as the key light perception hormone and is released by the photosensitive pineal gland (Bromage *et al.* 2001; Falcon, Migaud, Munoz-Cueto & Carrillo 2009), with high levels of melatonin produced during darkness and lower levels produced during daylight (Porter, Duncan, Mitchell & Bromage 1999; Porter, Stefansson, Nyhammer, Karlsen, Norberg & Bromage

2000; Bromage *et al.* 2001; Bayarri, Madrid & Sanchez-Vazquez 2002), thus providing an entraining endocrine message. As such, plasma melatonin measurements are routinely used to assess an individual fish's perception of lighting systems (Porter *et al.* 2000; Migaud, Taylor, Taranger, Davie, Cerda-Reverter, Carrillo, Hansen & Bromage 2006). At present, photoperiod is manipulated in commercial, open cage systems through the use of metal halide (MH) light units. These systems, however, are not specifically designed for aquaculture and thus new, more cost-effective technologies [e.g. cathode lighting (CL) and light-emitting diodes (LED)] that allow the refinement of spectral content and reduce the energy requirement are now being used to develop species- and environment-specific lighting systems. *In vitro* and *in vivo* experiments in a number of species including sea bass and zebrafish have demonstrated the effectiveness of shorter wavelengths (blue-green) in reducing melatonin levels, in comparison with longer wavelengths (red) (Bayarri *et al.* 2002; Ziv, Toviv, Strasser & Gothilf 2007). These shorter wavelengths are also known to penetrate seawater more efficiently (Lalli & Parsons 1993). Currently, however, there is almost no published scientific information available regarding the technical performance of such systems in the marine environment. Likewise, there is limited information regarding the potential 'welfare' impact of these artificial lighting technologies on fish.

It is well known that aquaculture practices including stocking density, diet, feeding technique and management procedures may act as stressors in aquaculture and have strong effects on the health and performance of the fish (Pickering 1993; Wendelaar Bonga 1997; Schreck, Contreras-Sanchez & Fitzpatrick 2001). It is essential, therefore, that work is conducted on the effects of an abrupt change in lighting conditions and continuous (LL) high-intensity light on fish to avoid or mediate detrimental implications (Ashley 2007; Bowden 2008). There are a number of possible physiological and behavioural processes that artificial illumination could influence including the stress response, the immune system, eye damage and feeding activity. To date, only Migaud, Cowan, Taylor and Ferguson (2008) have directly considered the welfare impact of artificial blue LED lighting on Atlantic salmon. While the authors reported no chronic effects in this case, it is important to consider that species-specific sensitivities to light do exist (Migaud *et al.* 2006). Recent *in vitro* pineal studies have revealed that cod, in comparison with salmon and sea bass, have a much higher sensitivity to light (Vera, Davie, Taylor &

Migaud 2010). In addition, the cod retina has also been demonstrated recently to be more sensitive to light-induced damage than salmon and sea bass retina (Vera & Migaud 2009). Importantly, in cod on-growing, an increasing number of light units of escalating power and efficiency are being used in commercial cages as photoperiodic regimes used to date (MH systems) have failed to fully suppress early maturation and at best caused only a 4-month delay (Taranger, Aardal, Hansen & Kjesbu 2006). However, no documented studies have so far been performed on the welfare of cod regarding the effects of these increasingly high-intensity constant regimes.

The objective of our study was thus to investigate the potential welfare impact of two different types of artificial lighting (CL and traditional MH) currently being used to suppress maturation in commercial cod aquaculture through analysis of the stress response, innate immunity, retinal structure and feeding activity and also to determine cod light perception of these systems.

Materials and methods

Fish stock and initial rearing conditions

The trial was conducted at the Machrihanish Marine Environmental Research Laboratory (MERL, 55:44°N, 5:44°W) between 6th June and 16th August 2007. Groups of 50 mixed-sex juvenile Atlantic cod produced by MERL (mean wet weight \pm SEM = 142 \pm 3 g), previously reared in tanks under simulated natural photoperiod and ambient temperature regimes, were randomly stocked into 10 white 2 m diameter, covered tanks (volume 1.6 m³, 0.5 m deep, approximate initial stocking density: 4.4 kg m⁻³). Within each population, 20 individuals were selected at random and implanted with a passive integrated transponder tag (Avid Plc, Uckfield, UK). All tanks were supplied with fresh seawater, filtered to 60 μ m, at a flow rate of approximately 50 L min⁻¹ and drained to waste. The water temperature during the trial was 14 \pm 1 °C.

Experimental conditions

Fish were initially maintained on a 6-week acclimation period under a control simulated natural photoperiod regime (SNP, experimental light units were fitted in tanks but remained off). This control lighting was provided by two 9 W fluorescent bulbs (S G23 energy saver, Osram Dulux, St Helens, UK) that were

located on the underside of tank lids. Their operation was regulated by digital timers, which were adjusted weekly to match the ambient photoperiod throughout the trial. The intensity measured at the water surface was 0.32 W m^{-2} when illuminated. Intensity measurements (W m^{-2}) were performed using a single channel light sensor set to a wavelength range of 400–740 nm (Skye Instruments, UK) and calibrated to National Physics Laboratory (UK) standards. Spectral content was recorded using a portable spectroradiometer (Model EPP 2000c, Stellarnet, Tampa, FL, USA).

Following acclimation, fish were randomly assigned to one of five light treatments (duplicated) for 4 weeks. Control lighting was provided by fluorescent bulbs (as during acclimation) and experimental lighting was provided by a green cathode (CL, 40 W, Intra-vision Aqua, Oslo, Norway) or MH (400 W, BGB Engineering, Grantham, UK) units. Experimental treatments were designed to mimic the intensities that fish would be exposed to if they were to remain in close proximity to the lighting systems in a cage environment ($\leq 1.5 \text{ m}$) and were set to a continuous light (LL) or a simulated natural photoperiod (SNP) regime; daylength for SNP treatments ranged from 16 h at the start of the test period (19th July) to 15 h at the end (16th August). The treatments were as follows: (1) control (SNP), (2) low CL (1 U, LL), (3) high CL (4 U, LL), (4) MH-LL (1 U) and (5) MH-SNP (1 U). An SNP MH treatment was included in the trial in order to determine whether there was an effect of darkness following the highest intensity day-time lighting (Table 1). Regarding wavelength, the green cathode units emitted a clear prominent green peak (546 nm), whereas MH units emitted a broader range of wavelength throughout the visible spectrum (Fig. 1).

Fish were fed to satiation on commercial cod feed (Start/Pearl diet, Biomar, Grangemouth, UK) according to the manufacturer's guidelines via clockwork belt-feeders throughout the ambient daylight period.

Table 1 Mean light intensities recorded in tanks (W m^{-2})

Treatment	Photoperiod	Light intensity
Control	SNP	0.08 ± 0.03
Low CL	LL	0.47 ± 0.18
High CL	LL	0.82 ± 0.15
MH	(LL+SNP)	16.58 ± 8.77

Data presented as mean \pm SEM ($n = 2$).

MH, metal halide; CL, cathode lighting; SNP, simulated natural photoperiod.

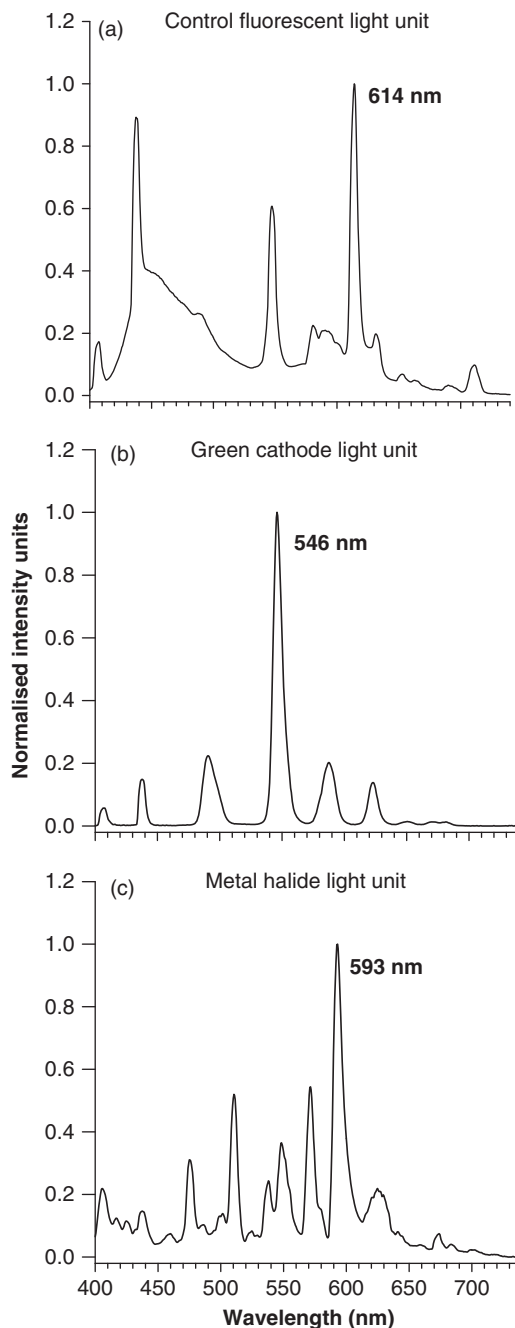


Figure 1 Normalized spectral profiles for (a) control fluorescent light, (b) cathode light and (c) metal halide light units.

In order to obtain data on population feed intake, tanks were also hand-fed to satiation four times (09:30, 12:00, 14:30, 17:00 hours) throughout the daylight period over 5 days before light onset (baseline feeding activity) and 11 days after light onset.

Sampling procedure

Five untagged fish per tank were sacrificed at six time-points during the experiment: pre-light exposure (3 and 2 weeks before exposure to the light treatments, during acclimation) and post-light exposure (3 h, 1, 2 and 4 weeks). At each time-point, fish were culled by lethal anaesthesia (MS222, 80 ppm, Pharmaq, Fordingbridge, UK). Immediately after death, a heparinized syringe was used to withdraw blood from the caudal vein for cortisol and glucose analyses: fish were then measured for whole body weight (± 0.1 g) and total length (± 1 mm) and then a sample of the head kidney was removed and frozen at -70°C for lysozyme analysis and both eyes were removed and fixed in bouin's fixative (Bios Europe, Lancashire, UK). Blood was sampled within 5 min of netting, stored on ice, centrifuged at 1200 *g* for 15 min and the resulting plasma was aliquoted and stored at -70°C until analysis. At the end of the trial, five fish were sacrificed during the night and five during the day from all tanks: 2 mL of blood was withdrawn and the plasma melatonin content was analysed.

Plasma analysis

Plasma cortisol levels were determined using a radioimmunoassay according to North, Turnbull, Ellis, Porter, Migaud, Bron and Bromage (2006) and validated in Atlantic cod by comparing serial dilutions of pooled cod plasma to check whether it was immunologically comparable to purified standards (data not presented). The tritiated label (TRK407) was supplied by Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, UK) and a sheep anti-cortisol antibody from Diagnostic Scotland (Carlisle, UK). Intra- and inter-coefficients of variation were 6.85% and 21.33%, respectively ($n = 4$), with a minimum sensitivity of 0.38 ng mL^{-1} .

Glucose concentration was analysed colourimetrically using InfinityTM Glucose Oxidase diagnostic kits (Alphalabs, Hampshire, UK).

Melatonin was analysed using a radioimmunoassay according to Porter *et al.* (2000).

Lysozyme analysis

Lysozyme activity was analysed using a modified version of the lysoplate method as described by Osserman and Lawlor (1966). The method is based on lysis of the bacterium *Micrococcus lysodeikticus* in 1% agarose prepared in 0.05 M sodium phosphate buffer

pH = 6.2. *Micrococcus lysodeikticus* is a Gram-positive cocci particularly susceptible to the lytic action of lysozyme. The diameter of the lysed zone was visualized by the lack of colour in contrast to the white unlysed area. The mean diameter ($n = 2$) of each zone was measured (± 0.5 mm) using a ruler.

Eye histology

Once the eye was removed, a small incision was made in the sclera 90° to the right of the choroid fissure to allow fixative penetration. Eyes were fixed overnight in Bouin's fixative (< 24 h) and then washed and transferred twice into fresh 70% ethanol, where they remained until processing. Eyes were oriented using the location of the ventral choroid fissure and trimmed in a dorsal–ventral plane to include the optic nerve. Subsequent processing to paraffin wax was routine and sections were stained using haematoxylin and eosin.

Retinal measurements were conducted using image analysis software (IMAGE PRO PLUS, v. 4.5, Media Cybernetics, Silver Spring, MD, USA) and taken at the central region of the retina ventral to the optic nerve. Two parameters were measured: (1) the thickness of the outer nuclear layer (ONL) ($n = 5$ measurements fish⁻¹) and (2) the number of ONL nuclei in a $50 \mu\text{m}$ band ($n = 2$ counts fish⁻¹) (Fig. 2). Measurements were conducted on the retina from the 2- and 4-week light exposure time-points.

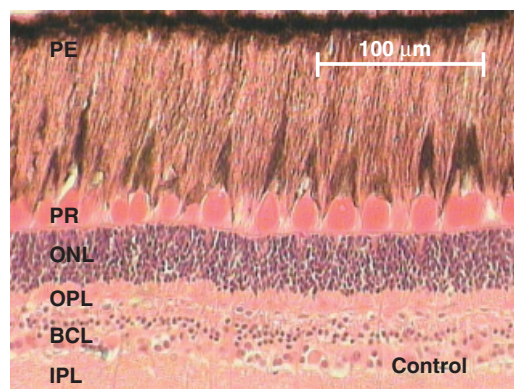


Figure 2 Histological section of Atlantic cod retina kept for 2 weeks under control conditions. Section illustrates different retinal layers: PE, pigment epithelium; PR, photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; BCL, bipolar cell layer; IPL, inner plexiform layer.

Population feed intake

Population feed intake (% body weight day⁻¹) was determined by hand-feeding tanks to satiation and dividing the total consumption by the number of fish.

Specific growth rate (SGR)

The SGR for all tagged individuals over the 4-week test period was calculated as follows:

$$\text{SGR} = \left(\text{Exp} \left(\frac{\text{Ln}(\text{weight end}) - \text{Ln}(\text{weight start})}{\text{Number of days}} \right) - 1 \right) \times 100$$

Statistical analysis

Statistical analysis was performed using MINITAB[®] version 15.0 (Minitab, Coventry, UK). All data sets were tested for normality using the Kolmogorov–Smirnov test and homogeneity of variances using Bartlett's test, and if necessary, were log transformed. All data expressed as a percentage were arcsine transformed before analysis. The effect of light treatment over time on all dependent variables was compared by analysis of variance manipulated using a General Linear Model that included a comparison of treatment replicates ($n = 2$) nested within the fixed treatment effect. When no significant replicate difference was found, the model only analysed treatment differences; however, for the SGR data, only where a replicate difference was present, analysis was performed between replicates independent of treatment. In all cases, a significance level of $P < 0.05$ was set, with significant interactions being analysed using the Tukey *post hoc* test.

Results

Cortisol, glucose and lysozyme

There were no significant differences between the light treatments in plasma cortisol (Fig. 3a) and glucose (Fig. 3b) levels. Although a significant elevation in cortisol (low CL at 1 week) was observed from baseline, this deviation was transitory, with a revert to baseline levels 2 weeks after light onset. There were no significant differences in lysozyme activity between treatments or timepoints; the mean activity (measured by clearance zone) ranged from 3.95 ± 0.25 to 5.45 ± 0.02 (mean \pm SD, $n = 2, 5$ fish-replicate⁻¹, data not shown).

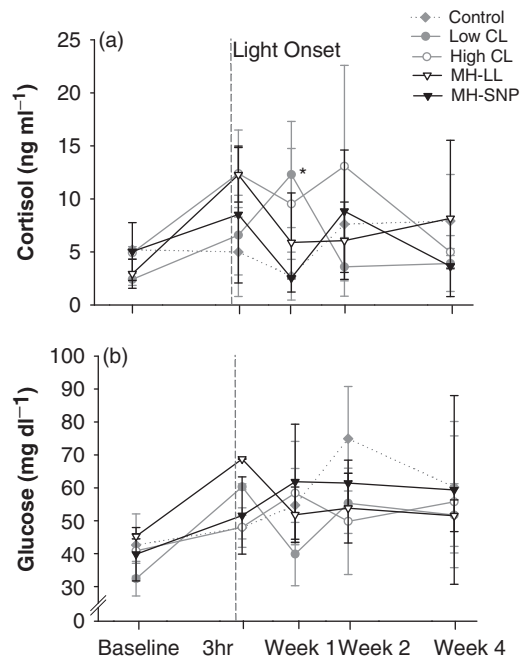


Figure 3 Plasma (a) cortisol and (b) glucose levels in Atlantic cod kept under control conditions, green cathode light (low CL and high CL) and metal halide (MH-LL and MH-SNP) light. Data presented as mean \pm SD ($n = 2, 5$ fish replicate⁻¹). Significant differences between baseline and post-light onset values are indicated by *.

Eye histology

No differences in ONL thickness or ONL nuclei number were observed between fish under different treatments after 2 or 4 weeks of light exposure (Table 2). ONL thickness ranged from 29.40 ± 3.75 to 37.09 ± 0.06 (mean \pm SD, $n = 2, 5$ fish replicate⁻¹) and the number of ONL nuclei ranged from 98 ± 19.94 to 126 ± 1.48 (mean \pm SD, $n = 2, 5$ fish replicate⁻¹).

Feeding intake

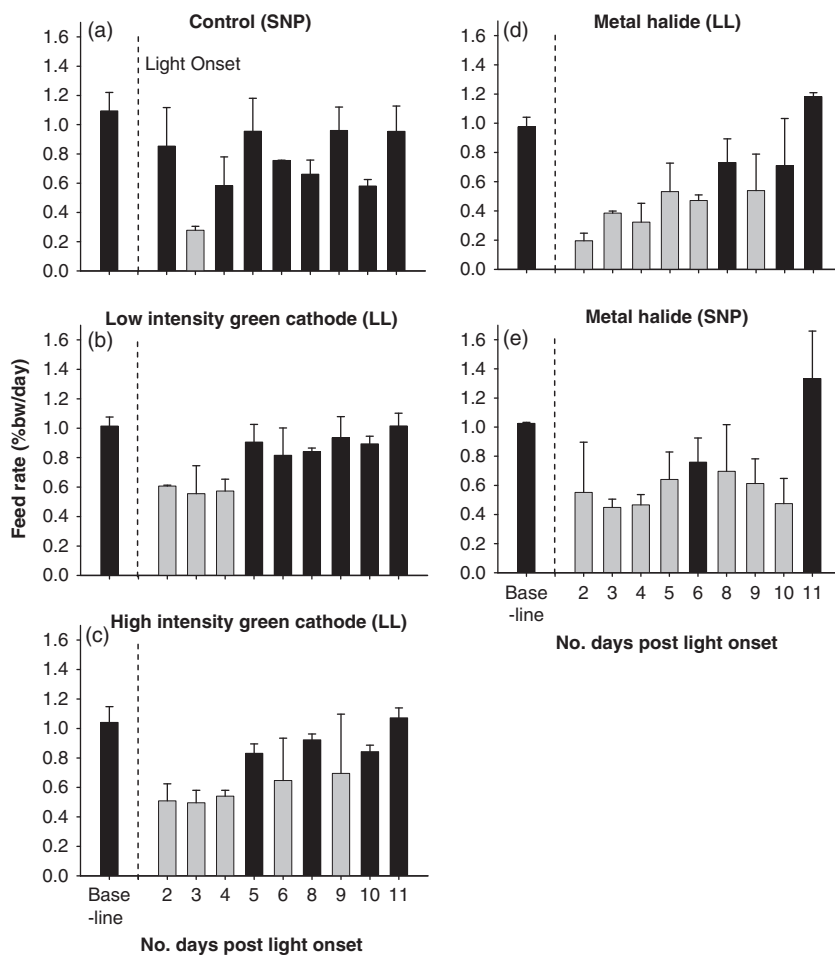
Population feed intake analyses indicated no long-term effects on the feeding activity of cod in any of the light treatments (Fig. 4). There was, however, a significant reduction in feed intake following light onset in all experimental treatments, with this being most pronounced in fish exposed to MH lighting. Before light onset, cod were feeding at $\geq 0.98\%$ body weight per day; however, following light onset, in the MH-LL treatment, this declined to 0.2%. By days 8 and 10 onwards, however, feeding rates were no different from the baseline levels. For the two CL

Table 2 Retinal morphometric measurements (central region) performed in Atlantic cod kept under control conditions, green cathode light (low CL and high CL) and metal halide light (MH-SNP and MH-LL) for 2 and 4 weeks following light onset

Parameter	Time	Treatment				
		Control	Low CL	High CL	MH-LL	MH-SNP
ONL Thickness	2 weeks	37.09 ± 0.06	34.12 ± 0.61	33.71 ± 0.58	30.95 ± 0.43	29.40 ± 3.75
	4 weeks	36.77 ± 1.47	36.46 ± 1.70	31.80 ± 2.09	34.14 ± 2.53	32.89 ± 0.42
ONL Nuclei	2 weeks	116 ± 15.95	111 ± 11.24	115 ± 0.00	102 ± 11.83	103 ± 18.21
	4 weeks	126 ± 1.48	107.58 ± 7.53	104 ± 4.79	106 ± 4.97	98 ± 19.94

Parameters measured include the thickness (μm) of the outer nuclear layer (ONL) and the number of ONL nuclei/ $50 \mu\text{m}^{-1}$. Data is presented as the mean \pm SD ($n = 2$, 5 fish replicate $^{-1}$).

MH, metal halide; CL, cathode lighting; SNP, simulated natural photoperiod.

**Figure 4** Population feeding behaviour in Atlantic cod kept under different lighting treatments. Data presented as mean feed rate (% body weight day $^{-1}$) per tank ($n = 2$) \pm SD. Dark bars indicate baseline feeding levels; light bars indicate a significant reduction from baseline levels. (a) Control (SNP); (b) Low-intensity green cathode (LL); (c) High-intensity green cathode (LL); (d) Metal halide (LL); (e) Metal halide (SNP).

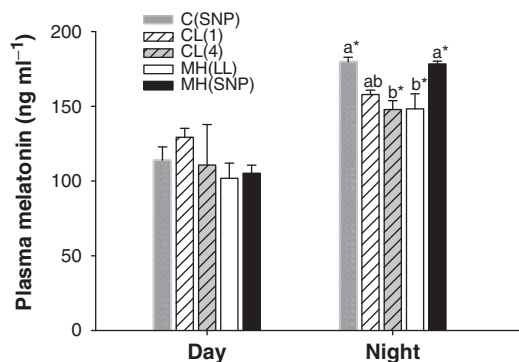


Figure 5 Plasma melatonin levels sampled at day and night in Atlantic cod under control conditions, green cathode light (low CL and high CL) and metal halide (MH-LL and MH-SNP) light. Data presented as mean ± SD ($n = 2, 5$ fish replicate⁻¹). Significant differences between day-time and night-time values are indicated by '*'. Significant differences between light treatments at a given time-point are indicated by different superscripts.

treatments, feeding intake declined to 0.5% (high CL) and 0.6% (low CL), with fish feeding normally once more after just 5 days. For control fish, there was no decline immediately following light onset; however, feed intake was significantly reduced on day 3.

Melatonin

While no significant differences between treatments were observed in melatonin levels during the day-time, there was a significant night-time elevation in plasma melatonin levels in fish under all treatments, except low CL (Fig. 5). Day-time and night-time low CL levels of melatonin did not differ significantly from other treatments at those time-points, however.

Survival and SGR

The survival rate over the trial period was 100%. No significant differences were found between treatments for SGR (Table 3); the mean SGR ranged between -0.05 ± 0.1 (MH-LL) and $0.58 \pm 0.14\%$ day⁻¹ (control).

Discussion

Photoperiod manipulation is used extensively throughout the aquaculture industry to induce out-of-season spawning, to control the timing and completion of

Table 3 Specific growth rate (SGR; % body weight day⁻¹) of Atlantic cod kept under different lighting treatments for 4 weeks

Treatment	SGR	
	Replicate 1	Replicate 2
Control	0.33 ± 0.06	0.58 ± 0.14
Low CL	0.38 ± 0.06	0.16 ± 0.12
High CL	0.30 ± 0.11	0.21 ± 0.10
MH-LL	-0.05 ± 0.10	0.27 ± 0.09
MH-SNP*	0.37 ± 0.12	0.16 ± 0.06

Data presented as treatment replicate mean ± SE ($n = 20$).

*Treatments exhibiting a significant replicate difference.

MH, metal halide; CL, cathode lighting; SNP, simulated natural photoperiod.

smoltification in salmonids and suppress early maturation (Endal *et al.* 2000; Bromage *et al.* 2001; Norberg, Brown, Halldorsson, Stensland & Bjornsson 2004; Davie *et al.* 2007). However, while considered to be less invasive than other techniques used to control puberty such as hormonal sex reversal for monosex production (Piferrer 2001; Hendry, Martin Robichaud & Benfey 2003; Taranger, Carrillo, Schulz, Fontaine, Zanuy, Felip, Weltzien, Dufour, Karlsen, Norberg Andersson & Hansen 2009) or chromosome manipulation for sterility induction (Benfey 2001; Tiwary, Kirubakaran & Ray 2004; Maxime 2008), studies investigating the potential 'welfare' impacts of such technology on fish are lacking. This is especially important in Atlantic cod that are being exposed to increasingly higher light intensities during on-growing in open cage systems owing to the relatively unsuccessful outcomes of photoperiodic manipulations (Taranger *et al.* 2006) in comparison with tank based studies where 100% suppression has been demonstrated (Davie *et al.* 2007).

Lighting treatments tested in the current study were designed to recreate the light intensities within a 1.5 m distance from a light source in a cage environment. Metal halide lights delivered a much brighter light intensity (20 ×) across a broad range of wavelengths in comparison with the CL technology. Surprisingly, no significant differences between treatments were observed in the stress response (cortisol and glucose) following light onset; however, cortisol did increase significantly with respect to the basal levels in the CL treatment (at 1 week). Although treatment differences were not apparent, it must be recognized that the large variability observed between individuals in conjunction with the limited

sampling size may have prevented the detection of further differences. When the current levels of cortisol are compared with other studies, however, it could be concluded that their range is not indicative of stress. The maximum mean cortisol value recorded was 12.3 ng mL^{-1} (low CL treatment), which, when compared with studies by King and Berlinsky (2006), King, Buckley and Berlinsky (2006) and Perez-Casanova, Rise, Dixon, Afonso, Hall, Johnson and Gamperl (2008), is far below the level representative of stress in cod. Although there are no studies specifically addressing the effect of light on stress levels of cod of a similar size, studies of stressors such as netting, transport and grading on smaller cod ($\sim 40 \text{ g}$) have been found to elicit a peak in the plasma cortisol concentration of over 60 ng mL^{-1} after 30–60 min, with a revert to basal levels after 24 h (King & Berlinsky 2006). Also, an increase in temperature of up to $16 \text{ }^\circ\text{C}$ resulted in cortisol levels of over 50 ng mL^{-1} (Perez-Casanova, Afonso, Johnson, Currie & Gamperl 2008). These results are similar to the cortisol levels reported in other teleosts subjected to similar stressors (Barton & Iwama 1991). Migaud *et al.* (2008) found that cortisol levels in Atlantic salmon following light onset reached a peak value $> 100 \text{ ng mL}^{-1}$. In haddock, *Melanogrammus aeglefinus* L., a 30-s net stressor resulted in a peak of 86 ng mL^{-1} plasma cortisol after 6 h (King *et al.* 2006). This said, however, in our study, it must be considered that as measurements were performed at 3 h following light onset, a temporary elevation within this window could have been missed (King *et al.* 2006). Glucose levels also showed large variability. According to Perez-Casanova, Rise *et al.* (2008), the maximum mean value recorded in our present study (74.91 mg dL^{-1} , control) was within the basal range ($60\text{--}100 \text{ mg dL}^{-1}$) for cod maintained under their control conditions. The relevance of glucose as a reliable indicator of stress in gadoids has been questioned, however (Perez-Casanova, Afonso *et al.* 2008).

Light treatments tested in this current study also appeared to have no significant effects on the innate immune response, studied through lysozyme activity. In fish, lysozyme activity is usually measured using the turbidity assay adapted from Lygren, Sveier, Hjeltnes and Waagbo (1999); however, due to the difficulties encountered with this methodology when used with cod, an agar plate (lyso-plate) method was developed and refined from Osserman and Lawlor (1966). Very few studies have been performed so far specifically looking at the immune response in this species, and it is therefore difficult to interpret the re-

sults when no baseline levels have been published (Bowden 2008). Regarding the literature relating to the effects of stressors on lysozyme activity, the results are very variable. For example, Migaud *et al.* (2008) demonstrated that a constant high light intensity had no effect on lysozyme activity in Atlantic salmon. In contrast, however, Demers and Bayne (1997) found that an elevation in plasma lysozyme was the typical immediate response of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to acute handling stress. Also, Taylor, Needham, North, Morgan, Thompson and Migaud (2007) demonstrated elevated plasma lysozyme activity in rainbow trout following seawater transfer. Clearly, the type and duration of an environmental change/stressor and the fish species involved will determine whether there is a consequent change in lysozyme activity.

In terms of retinal morphology, there were no significant differences in ONL thickness or ONL nuclei in any of the treatments; a reduction in ONL thickness or number of nuclei could be considered to be a sign of retinal damage (Allen & Hallows 1997; Vihtelic & Hyde 2000; Dawson, Nakanishi-Ueda, Armstrong, Reitze, Samuelson, Hope, Fukuda, Matsuishi, Ozawa, Ueda & Koide 2001). However, this was not apparent in these fish following light exposure.

Regarding feeding activity, acute effects of the light treatments on population feeding response were characterized by a transient reduction in feeding, in all treatments, although normal feeding resumed within a few days (approximately 8 days in fish exposed to MH light). Interestingly, the time needed to return to normal feeding behaviour appeared to be related to the light intensity of the treatments taking 5, 6 and 8 days under the CL treatments ($0.5, 0.8 \text{ W m}^{-2}$), MH-LL (16.6 W m^{-2}) and MH-SNP treatments respectively. It must be noted that although feeding activity remained steady immediately following light onset in control fish (SNP treatment), a reduction at day 3 was observed that cannot be explained and might simply reflect natural patterns of variation in feed intake (Kadri, Metcalfe, Huntingford & Thorpe 1996; Lokkeborg 1998).

Interestingly, with respect to the perception of the light by the cod populations, under the MH and high CL treatments, a day/night rhythm of plasma melatonin levels was still maintained, probably resulting from increased light intensities at day due to ambient light pollution entering the tanks through the feeding hatch. These results confirm previously obtained *in vitro* pineal results on the effects of day/night ratio on melatonin production (Vera *et al.* 2010). The poten-

tial entrainment of melatonin rhythm by internal clocks was ruled out as when Atlantic cod were subjected to constant lighting in fully light-proofed tanks on the same site: melatonin levels remained constant (Davie 2005).

As a whole, the results from this study indicate that the light treatments tested, which mimicked cod light exposure at night time in an open cage system when maintained within 1.5 m of the light unit, did not have any clear chronic effects on the stress response, immune function, retinal structure and feeding activity of cod. These results have relevant implications for cod culture where increasing light intensities are being used in an attempt to make the response to photoperiod management more consistent. Further studies should be carried out to determine whether there are light intensity thresholds above which the welfare of fish could be compromised as well as testing the effects of various spectral profiles.

Acknowledgments

The authors would like to thank the staff at Machrihanish Marine Environmental Research Laboratory for their help with fish husbandry and Dr Alison Morgan for her advice regarding analysis of lysozyme activity. This project was funded by the Scottish Aquaculture Research Forum (SARF) and CL systems were provided by Intravision Aqua (Norway).

References

- Allen D.M. & Hallows T.E. (1997) Solar pruning of retinal rods in albino rainbow trout. *Visual Neuroscience* **14**, 589–600.
- Ashley P.J. (2007) Fish welfare: current issues in aquaculture. *Applied Animal Behaviour Science* **104**, 199–235.
- Barton B.A. & Iwama G.K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* **1**, 3–26.
- Bayarri M.J., Madrid J.A. & Sanchez-Vazquez F.J. (2002) Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin. *Journal of Pineal Research* **32**, 34–40.
- Bayarri M.J., Rodriguez L., Zanuy S., Madrid J.A., Sanchez-Vazquez F.J. & Carrillo M. (2003) Effect of photoperiod manipulation on daily rhythms of melatonin and reproductive hormones in caged European sea bass (*Dicentrarchus labrax*). *Fish Physiology and Biochemistry* **28**, 37–38.
- Benfey T.J. (2001) Use of sterile triploid Atlantic salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada. *ICES Journal of Marine Science* **58**, 525–529.
- Bowden T.J. (2008) Modulation of the immune system of fish by their environment. *Fish and Shellfish Immunology* **25**, 373–383.
- Bromage N., Porter M. & Randall C. (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* **197**, 63–98.
- Davie A. (2005) *Effects of photoperiod manipulation on growth and reproduction in Atlantic cod (Gadus morhua L.)*. PhD Thesis, University of Stirling, Stirling, UK.
- Davie A., Porter M.J.R. & Bromage N.R. (2003) Photoperiod manipulation of maturation and growth of Atlantic cod (*Gadus morhua*). *Fish Physiology and Biochemistry* **28**, 399–401.
- Davie A., Porter M.J., Bromage N.R. & Migaud H. (2007) The role of seasonally altering photoperiod in regulating physiology in Atlantic cod (*Gadus morhua*). Part I. Sexual maturation. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 84–97.
- Dawson W., Nakanishi-Ueda T., Armstrong D., Reitze D., Samuelson D., Hope M., Fukuda S., Matsuishi M., Ozawa T., Ueda T. & Koide R. (2001) Local fundus response to blue LED and laser and infrared LED and laser sources. *Experimental Eye Research* **73**, 137–147.
- Demers N.E. & Bayne C.J. (1997) The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. *Developmental and Comparative Immunology* **21**, 363–373.
- Endal H.P., Taranger G.L., Stefansson S.O. & Hansen T. (2000) Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. *Aquaculture* **191**, 337–349.
- Falcon J., Migaud H., Munoz-Cueto J.A. & Carrillo M. (2009) Current knowledge on the melatonin system in fish. *General and Comparative Endocrinology* **165**, 469–482.
- Felip A., Zanuy S., Muriach B., Cerda-Reverter J.M. & Carrillo M. (2008) Reduction of sexual maturation in male *Dicentrarchus labrax* by continuous light both before and during gametogenesis. *Aquaculture* **275**, 347–355.
- Hansen T., Karlsen O., Taranger G.L., Hemre G.I., Holm J.C. & Kjesbu O.S. (2001) Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture* **203**, 51–67.
- Hendry C.I., Martin Robichaud D.J. & Benfey T.J. (2003) Hormonal sex reversal of Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture* **219**, 769–781.
- Kadri S., Metcalfe N.B., Huntingford F.A. & Thorpe J.E. (1996) Daily feeding rhythms in Atlantic salmon II: size-related variation in feeding patterns of post-smolts under constant environmental conditions. *Journal of Fish Biology* **50**, 273–279.
- King V.W. & Berlinsky D.L. (2006) Whole-body corticosteroid and plasma cortisol concentrations in larval and juvenile

- Atlantic cod *Gadus morhua* L. following acute stress. *Aquaculture Research* **37**, 1282–1289.
- King W.W., Buckley L.J. & Berlinsky D.L. (2006) Effect of acclimation temperature on the acute stress response in juvenile Atlantic cod, *Gadus morhua* L., and haddock, *Melanogrammus aeglefinus* L. *Aquaculture Research* **37**, 1685–1693.
- Lalli C.M. & Parsons T.R. (1993) *Biological Oceanography: An Introduction* (pp. 22–24). Butterworth-Heinemann, Oxford, UK.
- Lokkeborg S.V.E.I. (1998) Feeding behaviour of cod, *Gadus morhua*: activity rhythm and chemically mediated food search. *Animal Behaviour* **56**, 371–378.
- Lygren B., Sveier H., Hjeltnes B. & Waagbo R. (1999) Examination of the immunomodulatory properties and the effect on disease resistance of dietary bovine lactoferrin and vitamin C fed to Atlantic salmon (*Salmo salar*) for a short-term period. *Fish and Shellfish Immunology* **9**, 95–107.
- Maxime V. (2008) The physiology of triploid fish: current knowledge and comparisons with diploid fish. *Fish and Fisheries* **9**, 67–78.
- Migaud H., Taylor J.F., Taranger G.L., Davie A., Cerda-Reverter J.M., Carrillo M., Hansen T. & Bromage N.R. (2006) A comparative ex vivo and in vivo study of day and night perception in teleosts species using the melatonin rhythm. *Journal of Pineal Research* **41**, 42–52.
- Migaud H., Cowan M., Taylor J. & Ferguson H.W. (2008) The effect of spectral composition and light intensity on melatonin, stress and retinal damage in post-smolt Atlantic salmon, *Salmo salar*. *Aquaculture* **270**, 390–404.
- Norberg B., Brown C.L., Halldorsson O., Stensland K. & Bjornsson B.T. (2004) Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). *Aquaculture* **229**, 451–467.
- North B., Turnbull J.F., Ellis T., Porter M.J., Migaud H., Bron J. & Bromage N.R. (2006) The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **255**, 466–479.
- Osserman E.F. & Lawlor D.P. (1966) Serum and urinary lysozyme (Muramidase) in monocytic and monomyelocytic leukemia. *Journal of Experimental Medicine* **124**, 921–952.
- Perez-Casanova J.C., Afonso L.O.B., Johnson S.C., Currie S. & Gamperl A.K. (2008) The stress and metabolic responses of juvenile Atlantic cod *Gadus morhua* L. to an acute thermal challenge. *Journal of Fish Biology* **72**, 899–916.
- Perez-Casanova J.C., Rise M.L., Dixon B., Afonso L.O.B., Hall J.R., Johnson S.C. & Gamperl A.K. (2008) The immune and stress responses of Atlantic cod to long-term increases in water temperature. *Fish and Shellfish Immunology* **24**, 600–609.
- Pickering A.D. (1993) Growth and stress in fish production. *Aquaculture* **111**, 51–63.
- Piferrer F. (2001) Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture* **197**, 229–281.
- Porter M.J.R., Duncan N.J., Mitchell D. & Bromage N.R. (1999) The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilising. *Aquaculture* **176**, 237–244.
- Porter M.J.R., Stefansson S.O., Nyhammer G., Karlsen O., Norberg B. & Bromage N.R. (2000) Environmental influences on melatonin secretion in Atlantic cod (*Gadus morhua* L.) and their relevance to commercial culture. *Fish Physiology and Biochemistry* **23**, 191–200.
- Schreck C.B., Contreras-Sanchez W. & Fitzpatrick M.S. (2001) Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* **197**, 3–24.
- Taranger G.L., Aardal L., Hansen T. & Kjesbu O.S. (2006) Continuous light delays sexual maturation and increases growth of Atlantic cod (*Gadus morhua* L.) in sea cages. *ICES Journal of Marine Science* **63**, 365–375.
- Taranger G.L., Carrillo M., Schulz D.W., Fontaine P., Zanuy S., Felip A., Weltzien F.A., Dufour S., Karlsen O., Norberg B., Andersson E. & Hansen T. (2009) Control of puberty in farmed fish. *General and Comparative Endocrinology* **165**, 469–482.
- Taylor J.F., Needham M.P., North B.P., Morgan A., Thompson K. & Migaud H. (2007) The influence of ploidy on saltwater adaptation, acute stress response and immune function following seawater transfer in non-smolting rainbow trout. *General and Comparative Endocrinology* **152**, 314–325.
- Tiwary B.K., Kirubakaran R. & Ray A.K. (2004) The biology of triploid fish. *Reviews in Fish Biology and Fisheries* **14**, 391–402.
- Vera L.M. & Migaud H. (2009) Continuous high light intensity can induce retinal degeneration in Atlantic salmon, Atlantic cod and European sea bass. *Aquaculture* **296**, 150–158.
- Vera L.M., Davie A., Taylor J.F. & Migaud H. (2010) Differential light intensity and spectral sensitivities of Atlantic salmon, European sea bass and Atlantic cod pineal glands ex vivo. *General and Comparative Endocrinology* **165**, 25–33.
- Vihtelic T.S. & Hyde D.R. (2000) Light induced rod and cone cell death and regeneration in adult Zebrafish (*Danio rerio*) retina. *Journal of Neurobiology* **44**, 289–307.
- Wendelaar Bonga S.E. (1997) The stress response in fish. *Physiological Reviews* **77**, 591–625.
- Ziv L., Tovim A., Strasser D. & Gothilf Y. (2007) Spectral sensitivity of melatonin suppression in the zebrafish pineal gland. *Experimental Eye Research* **84**, 92–99.