CONTINUOUS HIGH LIGHT INTENSITY CAN INDUCE RETINAL DEGENERATION IN ATLANTIC SALMON, ATLANTIC COD AND EUROPEAN SEA BASS

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ABSTRACT

Retinal photodamage has previously been studied in teleost fish but very few have been performed on aquaculture species. To study retinal damage, Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*) and European sea bass (*Dicentrarchus labrax*) were previously acclimated to a control 12L:12D photoperiod with standard experimental low light intensity (0.1 W/m^2, equivalent to 3.2 x 10^{13} photons /s /cm^2) for at least 4 weeks and then kept under constant darkness (DD) for 3 days. Thereafter, fish were exposed to continuous high intensity light (51-380 W/m^2, equivalent to 1.63 x 10^{16} – 1.22 x 10^{17} photons /s /cm^2) for 3, 7, 15 or 25 days before returning to a control 12L:12D photoperiod (same intensity than during acclimation period) to study retinal regeneration over a period of 30 days. Retinal damage was exclusively assessed through the analysis of morphometric parameters. Results showed the presence of light-induced damage in the three species examined, as well as recovery once the control photocycle was restored. Cod was the most light-sensitive species as demonstrated by early signs of retinal damage (from three days of exposure) and reduced photoreceptor layer thickness (PRos/is) (43.1% relative to basal value in comparison to 51.6% and 73.3% respectively in salmon and sea bass). However, once the light-dark cycle was resumed the retina recovered in the three species studied (after 15 days in cod and 30 days in salmon and sea bass). Exposure to continuous high intensity light also resulted in significantly increased plasma cortisol levels in cod at LL15 (13.4 ± 2.0 ng/ml) and sea bass at LL3 (120.6 ± 12.2 ng/ml) and LD15 (54.2 ± 7.1 ng/ml). These results have important welfare implications with regards to the use of artificial light in culture and should be considered when designing lighting protocols in the aquaculture industry.

1. INTRODUCTION

Most adult fish possess a well-developed eye, effective for prey location and predator detection (Browman et al., 1990; Hawryshyn, 1997). The retina is a projection of the brain originating from the optic vesicles of the diencephalon and its morphology is not so much determined phylogenetically but rather by the functional requirements imposed upon the visual system by ecological and ethological factors (Ali and Anctil, 1976; Douglas and Djamgoz, 1990). It consists of several neuron layers (Wagner, 1990) from the sclera side to the vitreous side, these being 1) the visual cell layer, composed of two main types of photoreceptors (rods and cones) that have an outer segment (PRos) with lamellar disks containing the visual pigment (Kusmic and Gualtieri, 2000); 2) the outer nuclear layer (ONL) composed of photoreceptor cell bodies; 3) the inner nuclear layer (INL) composed of cell bodies of bipolar cells, Müller glia and other processing neurons, and 4) the ganglion cell layer (GCL) composed of ganglion cells (Wagner, 1990; Cameron et al., 1995; Kusmic and Gualtieri, 2000; Ferguson et al., 2006).

The eye in teleost species is similar to the human eye (Wagner et al, 1998; Kusmic and Gualtieri, 2000) although there are two main differences, the absence of eyelids (palpebra) and a fixed pupil aperture (Ferguson et al., 2006). This makes the fish retina more vulnerable to potential light induced damage as they are constantly exposed to the surrounding light as opposed to higher vertebrates. Fish have therefore developed alternative, efficient protective mechanisms to cope with this impinging light intensity such as photoreceptor mobility and migration of melanin granules,.

Light is essential for vision but it also generates potentially damaging reactive oxygen species within the eye. The visual pigments (mainly rhodopsin) are thought to be one of the main factors responsible for photochemical retinal damage. The reaction of light with photopigments results in a cascade of chemical events that could lead to

impairment of cellular function and cytotoxicity (Boulton et al., 2001). Unlike mammals, one key feature in teleosts is that the retina grows throughout the life cycle of the animal (Johns and Easter, 1977; Fernald, 1985). There are two different populations of self-renewing stem cells: the first is located in the INL and their mitotic progeny migrate radially outward into the ONL to replenish the population of rod precursors (Julian et al., 1998). The other population is located in the circumferential germinal zone (CGZ) at the retinal margin and produces the remaining retinal cell types (Hitchcock et al., 2004). Moreover, recent studies have shown that in zebrafish (*Danio rerio*), Müller glia function as multipotent retinal stem cells that generate retinal neurons by homeostatic and regenerative developmental mechanisms (Bernardos et al., 2007). Retinal cell regeneration can be stimulated by destruction or significant damage to the photoreceptor cells (Braisted and Raymond, 1992; Braisted et al., 1994). Morris et al. (2008) showed that rod death in zebrafish caused an increase of rod progenitors in the ONL but not in the INL. However, cones can also be generated from INL stem cells in response to photoreceptor damage. Furthermore, it has been suggested that ONL cell death is also able to initiate cell regeneration in the teleost retina (Negishi et al., 1987; Raymond et al., 1988a; Braisted and Raymond, 1992; Otteson and Hitchcock, 2003), although in the chicken, toxin-induced cell death in the inner retina can induce regeneration without damage to the ONL (Fischer and Reh, 2001).

To date, very few studies have been performed on light induced retinal damage and regeneration in fish, with data only available in a few albino teleost species such as zebrafish (Vihtelic and Hyde, 2000; Vihtelic et al., 2006) and rainbow trout, *Oncorhynchus mykiss* (Allison et al., 2006). Furthermore, mechanisms underlying cell damage and regeneration processes are not well described in fish although these would be relevant to the whole vertebrate class. Continuous light (LL) regimes however are

routinely used within the aquaculture industry throughout the production cycle of cod (*Gadus morhua*), salmon (*Salmo salar*) and sea bass (*Dicentrarchus labrax*) to suppress early maturation during ongrowing (Bromage et al., 2001; Begtashi et al., 2004), optimize larvae performances (Carrillo et al., 1995; Monk et al., 2006), manipulate smoltification (Stefansson et al., 2007) and enhance growth (Taylor et al., 2006). This emphasises the need to further understand species light sensitivities and potential welfare and stress implications behind the use of increasingly powerful lighting systems (Barton and Iwama, 1991; Migaud et al., 2007). Not only potential light-induced retinal damage should be better characterized, but also the stress response through the activation of the hypothalamic-pituitary-interrenal (HPI) axis and subsequent release of cortisol during exposure to adverse rearing conditions (Mommsen at al., 1999). The aims of our investigation were 1) to determine whether constant high intensity light can result in retinal damage in normally pigmented Atlantic cod, salmon and European sea bass and 2) to assess retinal recovery once normal light conditions are restored.

2. MATERIALS AND METHODS

2.1. Animals

Atlantic cod (body weight: 55.1 ± 3.5 g, total length: 17.7 ± 0.4 cm, 8-10 month post hatch), post smolt Atlantic salmon (body weight: 75.8 ± 6.4 g, total length: 20.1 ± 0.4 cm, 12 month post hatch) and European sea bass (body weight: 60.4 ± 4.2 g, total length: 17.4 ± 0.4 cm, 10-11 month post hatch) were obtained from the Machrihanish Marine Environmental Research Laboratories, Institute of Aquaculture (Scotland). During the acclimation phase and the experimental trials, seawater temperature was maintained at 14 ± 1ºC and salinity at 35 ppt for all species. Fish were fed once a day to satiation with commercial diets (Biomar Ltd., UK) and water quality

was monitored daily. Fish were euthanized prior to sampling and all experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986, UK. All trials were carried out from August 2007 to March 2008.

2.2. Experimental design

Fish were reared and acclimated to a control LD cycle (12L:12D, 0.1 W/m^2, equivalent to 3.21 x 10^{13} photons/s/cm^2) for at least four weeks prior to the start of the experiments. All fish/species were stocked in isolated tanks within a flow through system. Prior to the light treatment exposure, fish were kept for 3 days under constant darkness (DD) to increase retinal sensitivity. Blood and eye samples were taken at the end of the SNP and DD phases, respectively, as light and dark controls.

The light treatment consisted in exposing each species to constant (LL) high intensity light for 3, 7 and 15 days (LL3, LL7 and LL15, respectively). Sea bass were also exposed to this regime for an additional 10 days (25 days, LL25), as no damage was observed at LL15. Trials were performed consecutively for each species. For each trial, 12-16 fish (maximum biomass at the start of the experiment between 16 and 20 kg/m^3) were placed in a flow through rectangular 54 L glass tank (60x30x30cm) in a temperature/photoperiod controlled room surrounded by 10 tungsten halogen floodlights (Armley 500 W, UK) placed 30 cm from the glass wall. Light intensity was measured using a single sensor light channel watts meter (Skye Instruments Ltd., UK) calibrated to National Physics Laboratory (UK) standards. Intensities recorded in the fish tank ranged from 51 W/m^2 (equivalent to 1.63 x 10^{16} photons/s/cm^2) in the centre to 380 W/m^2 (equivalent to 1.22 x 10^{17} photons/s/cm^2) at the edge. Based on the results obtained in the first series of trials, retinal regeneration was monitored in fish from the same stocks (all three species) exposed to LL15 and then control LD (same as during

acclimation) for 30 days, with samples taken at 15 and 30 days post LD onset (LD15 and LD30, respectively). Blood samples and both eyes were extracted from fish (n=4) previously euthanized (lethal dose of 2-phenoxyethanol solution, 1 $mL.L^{-1}$, SIGMA, Ref. P 1126) after four weeks under LD, 3 days under DD and 3, 7, 15 and 25 (only for sea bass) days under LL as well as 15 and 30 days when returned to control LD conditions. Each sampling took place at the same time of the day (09:00 am) to avoid potential circadian morphological changes as already shown in the vertebrate retina (Ruang et al., 2006).

2.3. Histology and retinal morphometric analyses

Once the eye was removed, a small incision was made in the sclera 90º to the right of the choroid fissure to allow fixative penetration. Eyes were fixed overnight in Bouin's fixative, washed and then transferred into 70% ethanol until further processing. Eyes embedded in paraffin wax were sectioned in the dorsal-ventral plane to include the optic nerve and sections were stained with haematoxylin and eosin (H&E).

Retinal measurements were made on the histological sections using image analysis software (Image Pro Plus, v. 4.5, Media Cybernetics, Inc. USA). Analysis consisted in measuring the following parameters: the thickness of the photoreceptor layer (PRos/is) excluding the outer nuclear layer (ONL) (i.e. distance from the outer limit of the retinal pigment epithelium (RPE) to the nearest membrane of the ONL), the thickness of the ONL, the thickness of the melanin granules within the RPE and the number of ONL nuclei/100 µm (Fig. 1). Ten measurements for each of these parameters were made from each retina (n=4/sampling/species/treatment) in the central, dorsal and ventral regions.

2.4. Cortisol analysis

Blood samples were collected within 5 min of netting, stored on ice and then centrifuged at 2500 RPM for 15 min to extract the plasma. Plasma cortisol levels were determined by direct radioimmunoassay (North et al., 2006). The tritiated label was supplied by Amersham Biotech (UK) and a sheep anti-cortisol antibody from Diagnostic Scotland (UK). Intra- and inter-coefficients of variation were 2 and 11% respectively (n=4), with a minimum sensitivity of 12 pg ml^{-1}.

In order to validate the method in cod and sea bass, the existence of parallelism between plasma serial dilutions and the standard curve was tested. Cortisol data from salmon are not presented in this paper due to technical problems during sample processing.

2.5. Statistical analysis

All statistical tests were carried out using SPSS v16.0 (SPSS Inc., USA). Initial data were tested for normality using the Kolmogorov-Smirnov test and for homogeneity of variances by Levene's test, and if necessary data was log-transformed. A General Linear Model (GLM) multivariate analysis was performed to provide regression analysis and analysis of variance for the different retinal parameters measured. For this, the following fixed factors were considered: light treatment, retinal region and species. Statistical differences between sampling times within each species were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test. In all analyses, a $P < 0.05$ was taken as the statistically significant threshold. Data are expressed as mean ± S.E.M (n=4).

3. RESULTS

The morphometry of retinas from fish exposed to the control LD regime showed species differences. ONL nuclei number and thickness showed inter-species differences (sea bass > cod > salmon). Furthermore, the number of nuclei in the central region of the sea bass retina was significantly higher than in the dorsal and ventral regions, whereas the ONL thickness differed among retinal regions in cod (ventral > central > dorsal) (Table I). However, the photoreceptor outer segment length did not differ significantly between species or between retinal regions ranging from 110 to 139 μm.

When cod, salmon and sea bass were exposed to high light intensity, several morphological changes were observed in the central region of the retina in comparison to the control LD (Figs. 2A, 3A, 4A, respectively) and the control DD (Figs. 2B, 3C, 4C respectively). Statistical analysis showed that the effect of light treatments differed between species, indicating different degrees of sensitivity (cod > salmon > sea bass). Light treatment effect on the ONL thickness and nuclei was dependent on the retinal region. However, no regional differences were found in the PRos/is thickness.

Fish exposed to high light intensity (LL15 in cod and salmon and LL25 in sea bass, Fig 2C, 3C and 4C) appeared to have retinas with higher melanin density, forming granules around the photoreceptor cells, than fish in the control LD prior exposure to LL (Fig 2A, 3A, 4A) or when returned to LD for 30 days following LL exposure (LD30)(Fig 2B, 3B and 4B). Photoreceptor necrosis was also observed characterised by the lack of outer and inner segments and clear disorganization within the ONL (Fig. 2C, 3C, 4C). Importantly, species differences were observed with changes appearing earlier in cod (from 3 days of exposure). After 15 days of LL exposure, cod retina (central region) was significantly damaged. Retinas then recovered in all species when exposed to LD with no sign of damage after 30 days of exposure (Figs. 2D, 3D, 4D). The time course of retinal recovery also differed between species with a return to normal retinal

morphology in most fish within 15 days of LD exposure. However, in sea bass, the recovery process was slower with retinas from LD15 fish still displaying clear signs of degeneration (not shown).

Measurements of melanin thickness showed the same tendencies in Atlantic cod and salmon in the three retinal regions examined: highest values were recorded from the LD acclimated fish (80-100 μm) and lowest in fish kept under DD for three days (20-40 μm). During the LL phase, melanin thickness significantly decreased from 3 to 15 days of exposure in comparison to LD. However, when the LD cycle was restored the thickness of the melanin layer increased again (Fig. 5A, B) although it still remained significantly lower than during control LD, except in the ventral region of the salmon retinas. However, in sea bass, values significantly increased in the central region of the retina after 25 days of exposure to continuous high light intensity as compared to DD. Melanin thickness returned to normal values (as in control LD fish) in fish returned to LD conditions for 30 days (Fig. 5C). Species differences were observed with significantly lower values shown in sea bass under control LD (circa 70 μm).

The number of ONL nuclei under control LD showed high variability between species (cod: 250-310, salmon: 100-120 and sea bass: 320-420/100 μm, Fig. 6). When fish were exposed to LL the number of ONL nuclei decreased gradually especially in the central region of cod, salmon and sea bass retinas. In cod, the values significantly dropped from 286.5 ± 18.5 nuclei/100 μm in LD fish to 53.3 ± 6.8 nuclei/100 μm in LL15 fish (LD>LL3>LL7=LL15) (Fig. 6A). When the LD cycle was restored for 30 days, the number of nuclei significantly increased again in the central region of the retina to reach similar values than under LD (227.1 ± 9.9 nuclei/100 μm). However, in the dorsal and ventral regions of the retina only LD values significantly differed from those obtained under all other light conditions except for ventral DD (Fig. 6A). In

salmon the difference between LD and LL15 was also significantly different in the central region of the retina (124.0 ± 6.9 *versus* 70.9 ± 11.2 nuclei/100 µm, respectively) although less pronounced than for cod. Thereafter, ONL nuclei recovery was only observed in fish returned to LD for 30 days (93.0 ± 11.3 nuclei/100 µm)(Fig. 6B). No significant differences were observed in both the dorsal and ventral areas of the salmon retinas. In sea bass, fish had to be exposed for 25 days to LL before a significant decrease in the number of ONL nuclei (30% of SNP value) could be observed in the central region of the retina. No significant differences were observed in both the dorsal and ventral areas of the sea bass retinas. Recovery was only observed 30 days after the return to LD with however significantly lower values than under LD remaining (Fig. 6C).

Measurements of the ONL thickness in the central retina were correlated to the data on ONL nuclei number. No differences were observed at the dorsal or ventral regions of the retina (data not shown) in all species studied. However, in the central region of the retinas from all three species, the thickness of the ONL decreased gradually when fish were exposed to high light intensity (Fig. 7). Large inter-species differences were found, since after 15 days of exposure to LL, the percentage of the ONL thickness relative to the control LD values were 18.3, 82.5 and 82.1% for cod, salmon and sea bass respectively. Furthermore, in cod, the significant decrease of the ONL thickness, in comparison to LD, started earlier (from LL3, approximately 50% thinner than in the LD group) than in salmon (from LL15, only 12% thinner) and sea bass (from LL7, only 14% thinner) (Fig. 7). Finally, fish showed recovery of the ONL thickness when returned to LD for 30 days although both cod and sea bass values were still significantly lower than in LD fish at the start of the experiment.

The thickness of the photoreceptor layer as described in the Materials and Methods (PRos/is corresponding to thickness of both RPE and photoreceptor inner segment) in the central retina also varied between sampling points in the three species (Fig. 8). The lowest values were observed in fish exposed to LL15 (cod and salmon) and LL25 (sea bass). Recovery was observed in all species when returned to LD for 30 days. Percentages of PRos/is thickness at the end of the LL phase in relation to the LD values were 43.1, 51.6 and 73.3% in cod, salmon and sea bass, respectively. Changes in the PRos/is thickness were also observed in the dorsal and ventral regions of the retina in the three species, although in cod, photoreceptor regeneration at LD15 and LD30 was less noticeable in these regions than in the central retina: 114.8 ± 4.6µm at LD30 in central region *versus* 73.5 ± 1.1 and 82.6 ± 1.8µm in dorsal and ventral regions, corresponding to 89.8 %, 66.7% and 69.0 % of LD values respectively (Fig. 8).

Cortisol levels showed significant differences between experimental groups in cod and sea bass. In cod, levels gradually increased during LL exposure and peaked at LL15 (13.4 ± 2.0 ng/ml) and decreased when the LD cycle was restored to 1.9 ± 0.4 ng/ml at LD30 (Fig. 9A). In sea bass, two cortisol peaks were observed at LL3 (120.6 ± 12.2 ng/ml) and LD15 (54.2 ± 7.1 ng/ml) (Fig. 9B).

4. DISCUSSION

The present study showed that the application of continuous high levels of illumination resulted in the induction of retinal damage in Atlantic cod, Atlantic salmon and European sea bass. Damage was characterized by a reduction in morphometric parameters as well as other pathological changes (e.g. melanin aggregation and cellular disorganization). Inter-species and retinal regional differences were observed, with cod being the most sensitive species and the retinal damage more acute in the central region.

In contrast, sea bass appeared far less sensitive to the light treatment. These results could reflect adaptations to different photic habitats since sea bass is a coastal diurnal Mediterranean fish species, therefore exposed to high light intensities in their natural habitat (up to 100,000 lux, equivalent to approximately 146 W/m^2) (Jerlov, 1968; McFarland, 1986). However, when fish were returned to control photic conditions characterised by a light/dark cycle (LD) and low day light intensity, these morphological changes were reversed, supporting previous findings about retinal regeneration in fish. The control retinas of cod, salmon and sea bass also presented morphological differences. Under LD, the number of ONL nuclei/100µm and thickness varied between species (sea bass > cod > salmon). Previous studies have shown that the number of visual cells is higher in nocturnal species vs. diurnal (Fishelson et al., 2004). Furthermore, photoreceptor density in retinas from mid- and deep-sea fish is also higher than in fish inhabiting shallow waters, and very often a pure-rod retina has been observed in those species (Wagner et al., 1998; Ferguson, 2006). Salmon and sea bass have been described as dual species (showing both diurnal and nocturnal activity) (Richardson and McCleave, 1974; Sánchez-Vázquez et al., 1995) and cod is able to perform diel vertical migrations in the water column depending on prey availability (Strand and Huse, 2007). Therefore, these species could have adapted to different photic environments although the link between retinal structure and spatial or temporal habitat remains unclear.

Species that are exposed to large variations in light intensity (from bright light at day to dim light at night) have developed mechanisms to protect their retinal photoreceptors against potential light induced damage, e.g. melanosomes within the retinal pigment epithelium (RPE) can move in an apical direction enshrouding the photoreceptors (Walls, 1942; Allen and Hallows, 1997; Kusmic and Gualtieri, 2000). In

the current study, no such melanin migration pattern was observed as melanin thickness decreased when cod and salmon were exposed to the bright light treatment. However, after close examination of the retinal histological sections, increased melanin density in the retinas of fish exposed to the bright LL treatment could be observed in comparison to fish under the control LD group. Unfortunately, this could not be quantified in the present study.

The present study focused on normally pigmented individuals of commercially important temperate species for aquaculture. Most previous studies on light induced retinal damage have been performed in albino fish. The first study reporting light-induced damage in fish showed the existence of retinal disintegration in golden shiners, *Notemigonus crysoleucas*, after exposure to LL for 14 days (Penn, 1985). Later, Bassi and Powers (1990) showed that light exposure (340 lux) for 7 days resulted in a 60% reduction of the rod outer segment (ROS) in goldfish, *Carassius auratus*, retinas. In salmon, no signs of morphological retinal damage were found when fish were exposed to white or blue LED (light emitting diode) light (intensities ranging from 0.199 and 2.7 W/m^2, equivalent to 6.38×10^{13} and 8.66×10^{14} photons/s/cm^2). However, acute stress responses characterized by short term increase in plasma cortisol and glucose concentrations were observed (Migaud et al., 2007). In the present study, salmon were reared under a much higher light intensity (51-380 W/m^2) which resulted in a reduction of the ONL nuclei and PRos/is thickness after 7 days of exposure in some individuals and was generalised after 15 days of exposure.

Large variations between retinal regions within a given species were observed. In the central retinal region of the three species studied, the number of ONL nuclei progressively reduced during the LL phase and then regenerated when the LD cycle was resumed, especially in cod. However, no significant differences between sampling

points were found in the dorsal or ventral regions of salmon and sea bass whereas in cod only the LD values differed from the other groups. In contrast, the PRos/is showed a decrease in thickness under high light intensity in all retinal regions for all species tested. These results differ from previous studies performed in other teleosts such as the golden shiners (Penn, 1985), *albino* zebrafish (Vihtelic et al., 2006), goldfish (Wheeler, 1978) and in mammals such as rats (Gordon et al., 2002) in which light-induced retinal damage differed between retinal regions. Some authors have suggested that these regional differences could be due to physical and biochemical adaptations of the fish retina to the underwater photic environment, characterised by downwelling light (Migaud et al., 2007; Vihtelic et al., 2006). Light, in the current study, was applied horizontally to mimic the effects of submerged artificial light units in a cage system and it is therefore unlikely that such differences could be explained by different levels of illumination of retinal regions. The different sensitivities between retinal regions may be the result of light transmission through the lens or gene expression differences (Vihtelic et al., 2006). Furthermore, fish retina have a range of variable features including cell density (ganglion cells and photoreceptors) (Collin and Collin, 1999) that could also explain region specific light sensitivities. A better comparative physical and biochemical characterization of cod, salmon and sea bass retinas is clearly required. On the other hand, the fact that changes in the photoreceptor layer outer segments were only found in dorsal and ventral regions with no corresponding changes in the ONL may confirm the theory that visual pigments act as photosensitisers, leading to retinal damage (Noell et al., 1966; Sperling et al., 1980; Boulton et al., 2001).

When fish were returned to an LD cycle following the exposure to LL, retinal regeneration took place in the three temperate teleosts studied although the time course of regeneration was species specific. Indeed, in cod, regeneration/recovery appeared to

be quicker (ONL nuclei and thickness values at LD15 were significantly higher than at LL15) than in salmon and sea bass (only at LD30). However, it is not clear whether retinal regeneration is a continuous process or not and whether damage/recovery at one given time depends on the relative speed of disintegration *vs.* regeneration. Allison et al. (2006) showed that in *albino* rainbow trout the light-induced rod death occurred without a decrease in the number of rod nuclei, suggesting that proliferation had to be replacing cells, although this proliferative process was insufficient to replace all rods in the central region. Previous studies performed in the *albino* zebrafish have shown that the retina was capable of self-regeneration following light-induced retinal damage resulting from 3 days of LL exposure. This was associated with high levels of cell proliferation within the INL, assessed by PCNA immunohistochemistry and BrdU labelling (Vihtelic and Hyde, 2000). Moreover, functional retinal regeneration has been reported in zebrafish within 98 days of retinal destruction by ouabain (Sherpa et al., 2008) and in goldfish after removal of up to 95% of neural retina (Mesinger and Powers, 2007). Unfortunately, in the present study apoptosis and proliferation analyses of the retina are not available. Further research has showed that α1tubulin (α1T), a neuron-specific microtubule protein, is involved in retinal regeneration. Thus, in injured zebrafish retina, α1T-expressing Müller glia dedifferentiate and mediate regeneration (Fausett and Goldman, 2006). This process appears to be mediated by the achaete-scute-complex-like 1a (ascl1a) which convert quiescent Müller glia into actively dividing retinal progenitors (Fausett et al., 2008). Molecular techniques have proven to be very useful to confirm and better understand retinal damage and regeneration processes in fish. The current study only focused on the comparative histological assessment of retinal morphological changes (degeneration and regeneration) induced by light in temperate

teleosts. However, the next step should involve the biochemical and molecular characterisation of these processes.

The exposure of fish to LL also clearly resulted in plasma cortisol increases in both cod and sea bass which could indicate light induced stress responses. Furthermore, species differences were observed. In cod, cortisol secretion in response to the light treatment was in accordance with previous data obtained in juvenile rainbow trout exposed to LL for 60 days, as in both studies plasma cortisol levels gradually increased during the light treatment and decreased when fish returned to control LD conditions. This is typical of a chronic stress response (Leonardi and Kempau, 2003). However, in sea bass results support the generally accepted idea proposing that cortisol release declines after the acute response, even if the stressful stimulus is maintained and therefore, plasma cortisol levels would not be very informative to detect chronic stress conditions (Mormède et al., 2007). Cortisol levels found in sea bass are in accordance with previous studies reporting cortisol basal levels around 20 ng/ml and 90 ng/ml following exposure to a stressor (Di Marco et al., 2008). Unfortunately, cortisol data in salmon was not available in the current study.

In summary, this study demonstrated light-induced retinal damage for the first time in three of the most commercially important aquaculture species in Europe. Large species specific differences in light sensitivities have also been reported. These results may have direct implications for the use of artificial lighting regimes in the culture of these species although further studies are needed to determine critical thresholds of exposure. Further investigations are also clearly needed to improve the understanding in underlying mechanisms regulating the degeneration and regeneration of the fish retina.

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FIGURE LEGENDS

Figure 1. Histological section of a retina from Atlantic salmon sampled during day time under the LD regime. The slide illustrates measurements taken in our study: RPE, thickness of the retinal pigment epithelium; PRos/is, thickness of the photoreceptor layer (including RPE, PRos and PRis); ONL, thickness of the outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer. A 100-µm scale bar is shown at the bottom.

Figure 2. Histological sections of Atlantic cod retina in the central region sampled at the end of the LD (A) and DD (B) phases, after 15 days of exposure to constant high light intensity (C) and 30 days after return to LD (D). (C) The asterisk points the aggregated melanin granules and arrows the remaining photoreceptors outer segments. The brace shows the ONL thickness reduction. The 100-µm scale bar shown in (A) is the same for all panels. M, melanin granules; PRis, photoreceptor layer inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer.

Figure 3. Histological sections of Atlantic salmon retina in the central region sampled at the end of the LD (A) and DD (B) phases, after 15 days of exposure to constant high light intensity (C) and 30 days after return to LD (D). (C) The asterisk points the aggregated melanin granules, arrows the photoreceptor layer reduction and arrowheads the cellular disorganization and loss of density within the ONL. The 100-µm scale bar shown in (A) is the same for all panels. M, melanin granules; PRis, photoreceptors layer inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer.

Figure 4. Histological sections of European sea bass retina in the central region sampled at the end of the LD (A) and DD (B) phases, after 25 days of exposure to

constant high light intensity (C) and 30 days after return to LD (D). (C) The asterisk points the aggregated melanin granules and arrowheads the remaining photoreceptor outer segments. The brace shows the ONL thickness reduction. The 100-µm scale bar shown in (A) is the same for all panels. M, melanin granules; PRis, photoreceptor layer inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer.

Figure 5. RPE (µm) in Atlantic cod (A), Atlantic salmon (B) and European sea bass (C) measured in dorsal (black bars), central (grey bars) and ventral (white bars) retinal regions at the end of the LD and DD phases, after exposure to constant high light intensity for 3, 7, 15 and 25 days (only in sea bass) and 15 and 30 days after fish returned to LD. Data are expressed as mean + SEM (n=4 fish/time/treatment/species with 10 measures/fish). Superscripts denote significant differences between sampling points for a given retinal region.

Figure 6. Nuclei density of the outer nuclear layer (number/100 µm sections) in Atlantic cod (A), Atlantic salmon (B) and European sea bass (C) measured in dorsal (black bars), central (grey bars) and ventral (white bars) retinal regions at the end of the LD and DD phases, after exposure to constant high light intensity for 3, 7, 15 and 25 days (only in sea bass) and 15 and 30 days after fish returned to LD. Data are expressed as mean + SEM (n=4 fish/time/treatment/species with 10 measures/fish). Superscripts denote significant differences between sampling points for a given retinal region.

Figure 7. Outer nuclear layer thickness (µm) in Atlantic cod (black bars), Atlantic salmon (grey bars) and European sea bass (white bars) measured in the central retinal region at the end of the LD and DD phases, after exposure to constant high light intensity for 3, 7, 15 and 25 days (only in sea bass) and 15 and 30 days after fish returned to LD. Experimental treatments are indicated under the X-axis. Data are

expressed as mean + SEM (n=4 fish/time/treatment/species with 10 measures/fish). Superscripts denote significant differences between sampling points for a given species.

Figure 8. Thickness of the photoreceptor layer (μm) in Atlantic cod (A), Atlantic salmon (B) and European sea bass (C) measured in dorsal (black bars), central (grey bars) and ventral (white bars) retinal regions at the end of the LD and DD phases, after exposure to constant high light intensity for 3, 7, 15 and 25 days (only in sea bass) and 15 and 30 days after fish returned to LD. Experimental treatments are indicated under the X-axis. Data are expressed as mean + SEM (n=4 fish/time/treatment/species with 10 measures/fish). Superscripts denote significant differences between sampling points.

Figure 9. Plasma cortisol levels in Atlantic cod (A) and European sea bass (B), sampled at the end of the LD and DD phases, after 3, 7 and 15 days of exposure to constant high light intensity and 15 and 30 days after fish returned to LD. Data expressed as mean + SEM (n=4 fish/time/treatment/species). Superscripts denote significant differences between sampling points.

Table I: Retinal morphometry of control retinas of Atlantic cod, Atlantic salmon and European sea bass. Values are expressed as mean ± SE (n=4). The superscripted lower case letters indicate significant differences between retinal regions for a given species (ANOVA, $P< 0.05$) and superscripted capital letters indicate significant differences between species for a given retinal region. PRos/is: photoreceptor layer; ONL: outer nuclear layer.

	PRos/is length (µm)			**ONL nuclei/100 µm)**			**ONL thickness (µm)**		
	Dorsal	Central	Ventral	Dorsal	Central	Ventral	Dorsal	Central	Ventral
Atlantic cod	110 ± 2	128 ± 1	120 ±4	261 ± 7^{B}	287 ± 19^{B}	312 ± 2^{B}	$33 \pm 1^{a;\,B}$	$37 \pm 1^{b;\,A}$	$40 \pm 1^{c;\,AB}$
Atlantic salmon	123 ± 5	139 ±3	123 ± 6	101 ± 6^{A}	124 ± 7^{A}	103 ± 16^{A}	28 ± 1^{A}	29 ± 1^{A}	28 ± 1^{A}
European sea bass	112 ±2	132 ± 2	111 ± 2	$327 \pm 22^{a;\,B}$	$410 \pm 30^{b;\,B}$	$350 \pm 19^{ab;\,B}$	41 ± 1^{C}	47 ± 1^{B}	44 ± 1^{B}

Figure 1

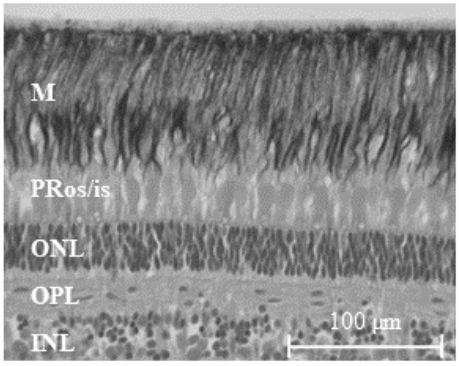
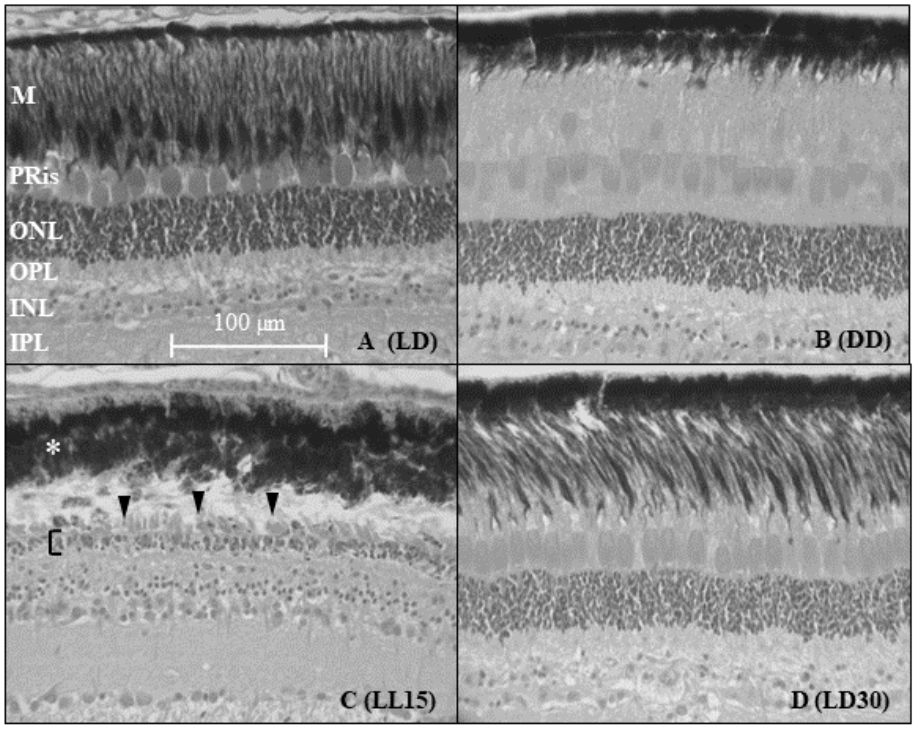


Figure 2



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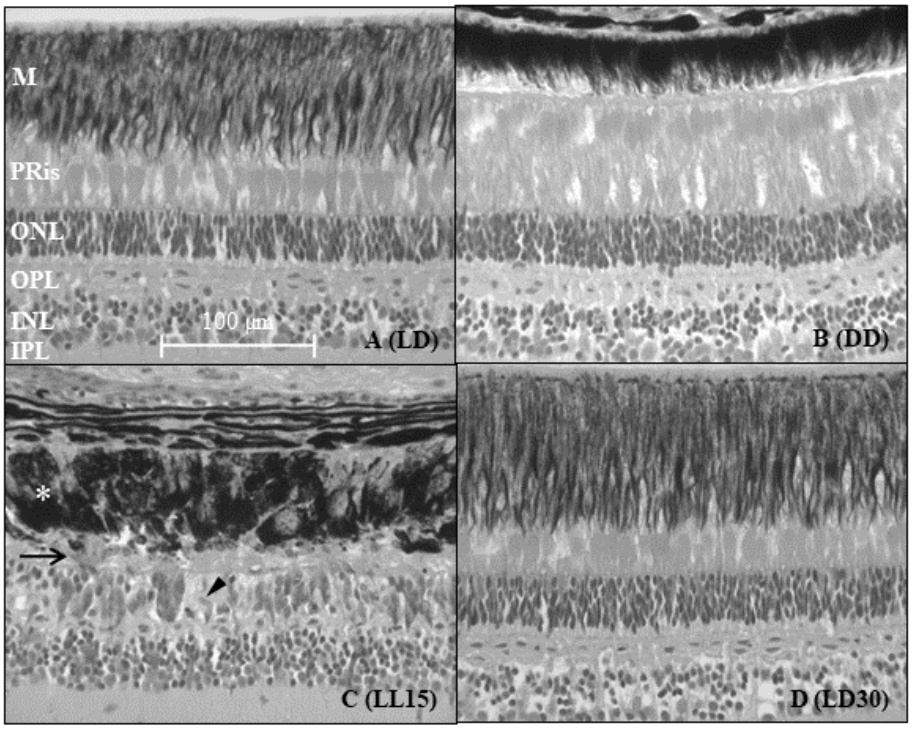


Figure 4

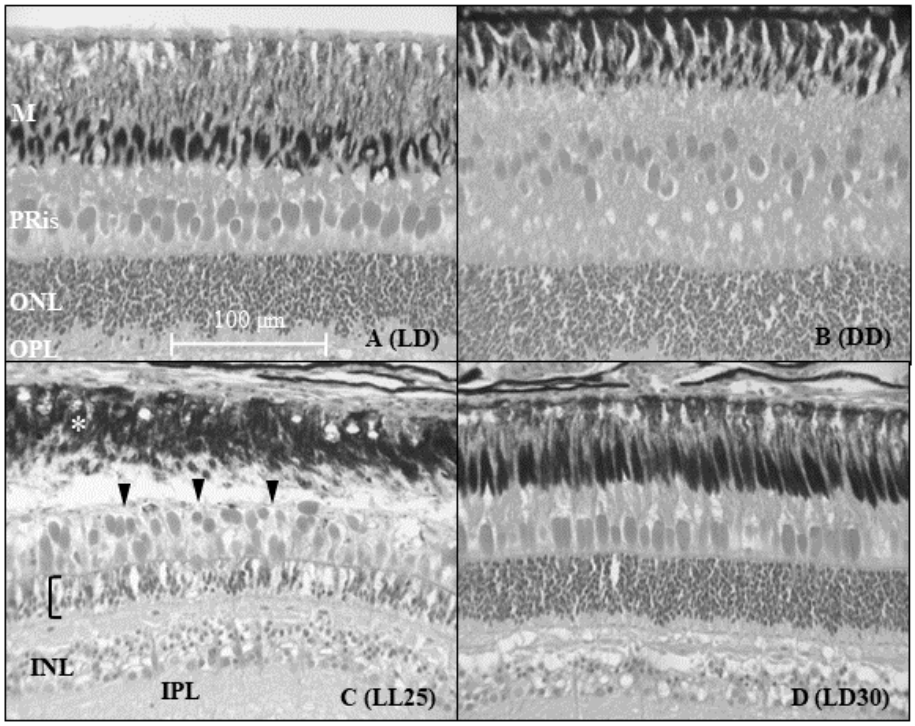


Figure 5

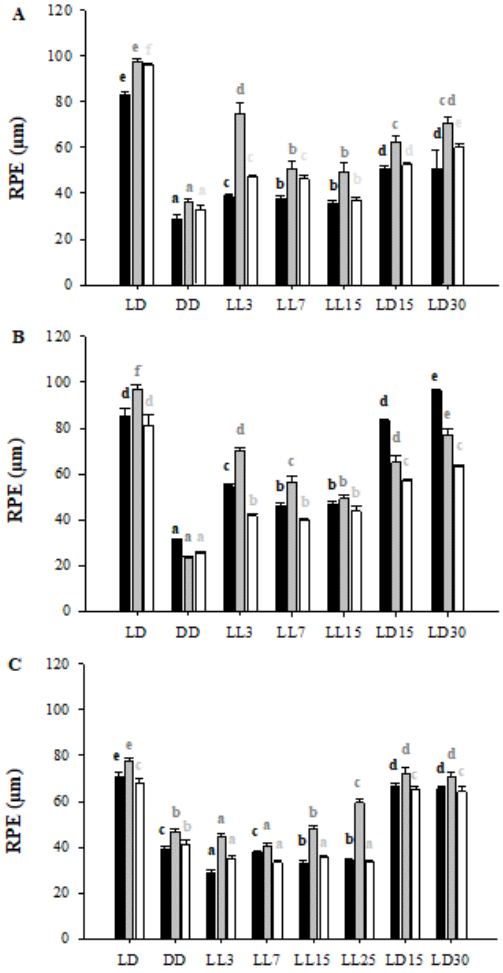


Figure 6

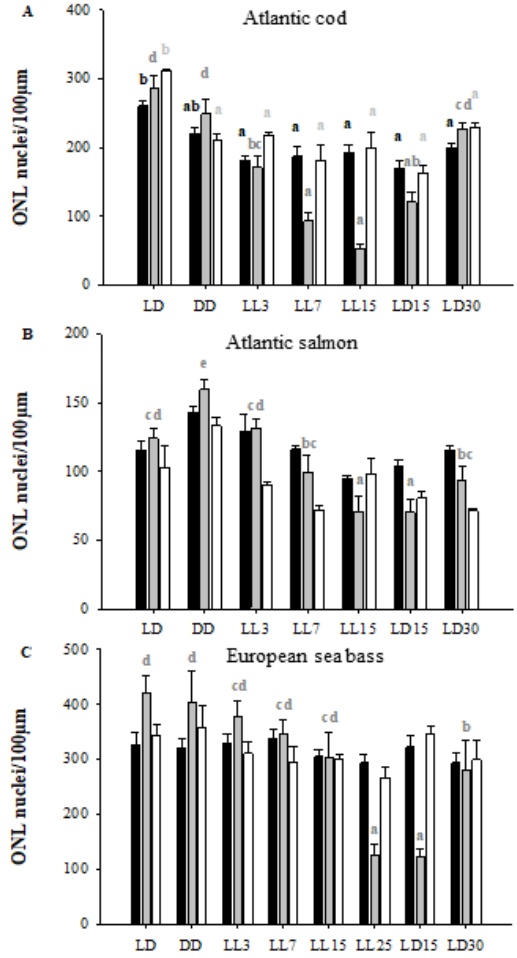

A
Atlantic cod
ONL nuclei/100µm
400
300
200
100
0
LD
DD
LL3
LL7
LL15
LD15
LD30
B
Atlantic salmon
ONL nuclei/100µm
200
150
100
50
0
LD
DD
LL3
LL7
LL15
LD15
LD30
C
European sea bass
ONL nuclei/100µm
500
400
300
200
100
0
LD
DD
LL3
LL7
LL15
LL25
LD15
LD30

Figure 7

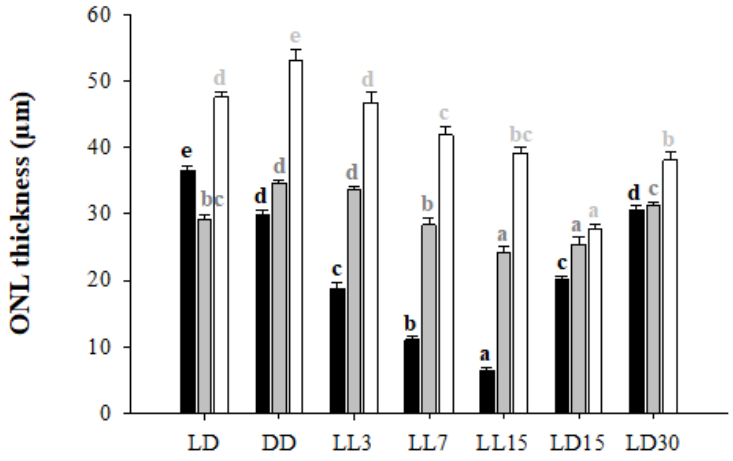


Figure 8

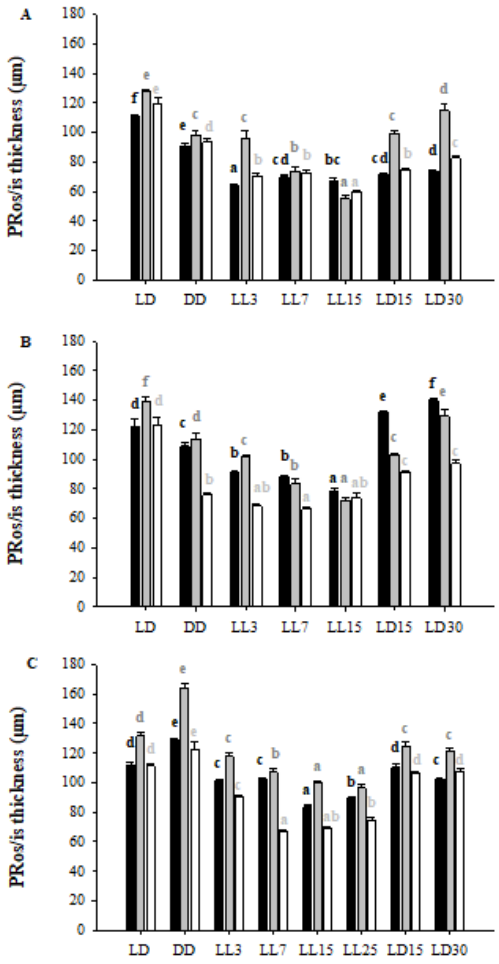
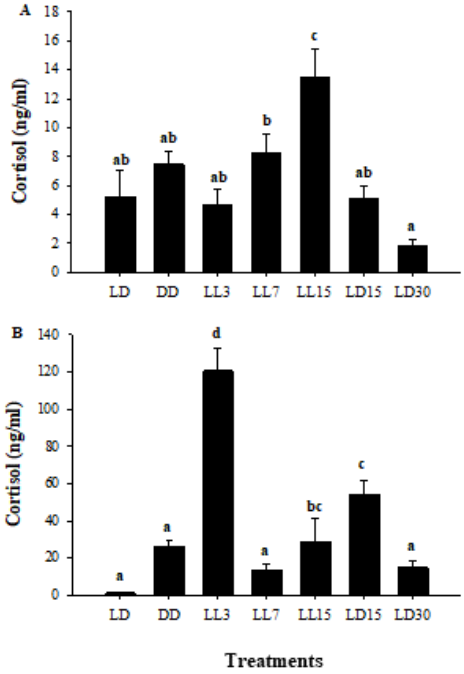

A
B
C
PRos/is thickness (µm)
0
20
40
60
80
100
120
140
160
180
LD
DD
LL3
LL7
LL15
LL25
LD15
LD30

Figure 9


A
Cortisol (ng/ml)
18
16
14
12
10
8
6
4
2
0
ab
ab
ab
b
c
ab
a
LD
DD
LL3
LL7
LL15
LD15
LD30
B
Cortisol (ng/ml)
140
120
100
80
60
40
20
0
a
a
d
a
bc
c
a
LD
DD
LL3
LL7
LL15
LD15
LD30
Treatments