The potential of alternative lighting-systems to suppress pre-harvest sexual maturation of 1+ Atlantic salmon (Salmo salar) post-smolts reared in commercial sea-cages

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CHAPTER 5

PAPER VI

RESEARCH ARTICLE

THE POTENTIAL OF ALTERNATIVE LIGHTING-SYSTEMS TO SUPPRESS PRE-HARVEST SEXUAL MATURATION OF 1+ ATLANTIC SALMON (*SALMO SALAR*) POST-SMOLTS REARED IN COMMERCIAL SEA CAGES.

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Contributions: The present manuscript was compiled and written in full by the author of this thesis. Sampling, lab and statistical analyses have been carried out by the candidate except for trial 1 and the melatonin analyses done by Dr. Matthew Sprague. Supervisors (Drs Herve Migaud and John Taylor) provided assistance with the design of the trials, sampling and proofreading of the manuscript.

Keywords: *Salmo salar*, lighting-technology, light-intensity, sexual maturation, melatonin, growth.

Abstract

The aim of this study was to compare the efficiency of new candidate lighting-technologies (50W 'blue' light-emitting-diode (B, λ_{max} = 465 nm); 232 W 'green' hot cathode, (G, λ_{max} = 546 nm); 400 W 'red' tungsten-halogen, (R, λ_{max} = 667 to 740 nm)) against a standard 400 W 'white' metal-halide used as control technology (C, broad spectrum) at suppressing sexual maturation of 1+ Atlantic salmon (*Salmo salar*) in sea-cages. A total of seven experimental set-ups were tested on a commercial-scale in three trials using a standardised photoperiod regime in the form of continuous artificial-light (LL) applied from winter to summer solstice during the second year at sea. The experimental stocks were raised under an ambient thermal regime that was similar across all trials.

Technical performances (spectral output, light-attenuation and irradiance distance) of the individual light-units were measured and light-perception was assessed by quantifying plasma melatonin levels. Body-size parameters (BW, FL, K) were measured at the switch-on and turn-off of the photoperiod regimes. Maturation rates were estimated at the end of the light-treatments and at harvest. The B-unit provided the shortest effective irradiance distance (distance from the light-bulb to the minimum irradiance suppressing plasma melatonin to basal day-time level = 0.016 W m^{-2}) but the longest relative to its energy consumption; while the G- and R-units did not offer a comparative advantage over the C-unit in that regard (B>C>G>R). Nocturnal plasma-melatonin and maturation rate decreased proportionally to the light-intensity provided using a range of technologies emitting distinct spectral profiles. Light-intensity rather than light-spectral composition appeared to be the prime parameter negatively affecting sexual maturation. Maximal suppression of maturation was observed in treatments depressing nocturnal plasma melatonin to a 1.2-fold but not to a 1.7-fold increase

compared to daytime levels, confirming that a threshold level of light-irradiance is necessary to obtain the desired effect. Results suggest that this can be achieved under standard commercial practices by applying, over the photoperiod regime presently used, continuous artificial-illumination with an (electrical) energy consumption of 0.28 Wh m^{-3} generating a mean-irradiance of 0.012 W m^{-2} and providing a minimum volume of effective irradiance equivalent to 12% of the rearing-environment. Such a low volume of biologically effective irradiance was likely sufficient due to the strong photic attraction already reported in Atlantic salmon. Maximal suppression of pre-harvest sexual maturation can be achieved in the Atlantic salmon on-growing industry using alternative light-technologies. Present data provides methods and threshold values favouring the implementation of photoperiod-manipulation to suppress pre-harvest maturation at the most advantageous scale and cost.

1. Introduction

In Atlantic salmon (*Salmo salar*) sexual maturation is concomitant with an altered feeding pattern (Kadri et al., 1996, 1997, Leclercq et al., 2010a), an increased pathogen susceptibility (St-Hilaire et al., 1998; Currie and Woo, 2007) and a deterioration of flesh and skin colour quality (Aksnes et al., 1986, Leclercq et al., 2010b). These detrimental effects can compromise the performance of the cohabiting immature cohort and reduce the volume of commercially valuable biomass. The control of pre-harvest maturation is therefore a priority in the on-growing salmon industry. This is successfully achieved on a commercial-scale by applying continuous artificial-light (LL) from the winter to the summer solstice during the second year at sea. The onset of LL in January has been shown to be the most effective at inhibiting gonadal development in fish below pre-determined developmental thresholds (Thorpe, 1994;

Taranger et al., 1998, 1999; Endal et al., 2000; Bromage et al., 2001; Leclercq et al., 2010c). This 6-month LL-window is routinely applied by the industry using powerful, wide-spectrum lighting-systems (metal-halide) which have a high-running-cost and potential welfare impacts (Migaud et al., 2007a). The industry would therefore greatly benefit from the implementation of optimized lighting-strategies that reduce operational costs and maximise the targeted biological effects, i.e. reduce maturation rate but also increase growth rates.

The annual photoperiod is widely acknowledged as the key environmental "zeitgeber" synchronizing the endogenous reproductive cycle of salmonids to the annual calendar-time (Bromage et al., 2001; Migaud et al., 2010; Taranger et al., 2010). In comparison, temperature has a minor role in the proximate control of salmonid reproductive cycles and acts as an ultimate cue synchronising, in particular, final gamete maturation and spawning (Bromage et al., 2001; Taranger et al., 2010; Pankhurst and King, 2010). The intensity (quantity) and spectral composition (quality) of incident light are key properties affecting the physiological response of teleosts with, among others, effects on growth, reproduction, behaviour and stress documented (Oppedal et al., 1997, 1999; Boeuf and Le Bail, 1999; Marchesan et al., 2005; Karakatsouli et al., 2007; Migaud et al., 2010). The effects of light-intensity have been well studied over recent years and findings clearly suggest that exposure to threshold intensity levels is required to manipulate physiological functions in various teleosts (Oppedal et al., 1997; Porter et al., 1999; Taylor et al., 2005, 2006; Migaud et al., 2006, 2010). In Atlantic salmon, exposure to LL-regimes was shown to inhibit sexual maturation and enhance growth compared to natural photoperiod (NP) and at increased rates with higher light intensities (Wallace et al., 1988; Stefansson et al., 1993; Oppedal et al., 1997; 1999). However, recent findings showed that excessively high light

intensities could induce an acute transient stress response (Wallace et al., 1988; Migaud et al., 2007a) and even retinal damage (Vera et al., 2009).

Various endocrine studies both *in-vitro* and *in-vivo* have demonstrated that synthesis of the hormone melatonin, released by the light-sensitive pineal gland, accurately reflects the prevalent photoperiod in teleosts (reviewed by Falcón et al., 2010). As such melatonin is regarded as the key time-keeping hormone that can be used as a reliable indicator of light perception as its production varies inversely with the level of light-irradiance on the pineal organ (Randall et al., 1995; Yáñez and Meissl, 1996; Falcón et al., 2010; Migaud et al., 2010). More specifically, there appear to be species-specific light-irradiance thresholds above which the circadian melatonin rhythm is suppressed to basal levels such that nocturnal artificial-light is perceived as daylight (Migaud et al., 2006). This threshold would be in the region of 0.016 W m^{-2} in Atlantic salmon (Migaud et al., 2006; Vera et al., 2010).

If many studies have focused on the effects of light-intensity, only a few have looked at the effects of light spectral composition on fish physiology. The teleost pineal gland also exhibits a spectral sensitivity to the incident light which appears to be adapted to the species natural habitat (Karakatsouli et al., 2007; Vera et al., 2010). In European sea bass (*Dicentrarchus labrax*), shorter wavelengths (blue light λ 450 nm) were found to be the most effective at suppressing circulating melatonin levels although longer wavelengths (red light λ 700 nm) were also potent if applied above intensity thresholds (Bayarri et al., 2002; Vera et al., 2010). Similarly, in Atlantic salmon, *in-vitro* studies showed that red light (λ 650 nm) was less efficient at suppressing melatonin than blue (λ 450 nm) and green (λ 550 nm) light although data on spectral sensitivity remain scarce in this species (Migaud et al., 2010; Vera et al., 2010). In rainbow trout (*Oncorhynchus mykiss*) and gilthead seabream (*Sparus aurata*), blue and

red wavelengths appeared to decrease growth performance and increase stress factors in comparison to full visible spectrum from white fluorescent lamps (Karakatsouli et al., 2007; 2008).

To date, present knowledge on spectral-sensitivity and threshold light-intensity have not been applied to Atlantic salmon reared in commercial sea-cages. Narrow band-width lighting-systems offer the potential for tailoring the spectral output to the sensitivity of the species thereby optimizing the use of energy into generating the most suitable wavelengths (Loew and McFarland, 1990; Migaud et al., 2006). In addition to energy-savings, the biological potency of an increased range of lighting-technologies would allow selection of the most appropriate based on a variety of technical, practical and health and safety considerations. The aim of this study was to compare the efficiency of alternative lighting-technologies and assess their biological impact on 1+ Atlantic salmon sexual maturation in comparison to an industry standard lighting-system. Comparisons are made at the methodological, technical and economical levels with the view to assist selection of new candidate lighting-systems for use in the Atlantic salmon industry.

2. Materials and Methods

Four submerged lighting-technologies generating distinct spectral outputs and intensities were assessed in a serie of trials. Each trial used mixed-sex 1+ post-smolt Atlantic salmon of the same strain (AquaGen AS, Trondheim, Norway) stocked in commercial sea-cages (Marine Harvest Ltd., UK) between February and April and exposed to an LL-regime from January to June during their second year at sea. The square-cages were set-up in rows of two (1.5 m to 2 m distance between cages) with, at

most, one cage-side adjacent to another experimental cage-side in order to prevent possible light pollution between cages.

2.1. Fish stock and rearing conditions

Trial 1

The first trial was conducted at a commercial salmon farm (56.41^{o}N, 5.10^{o}W, Loch Leven; Marine Harvest Ltd., UK) using 1+ Atlantic salmon stocked at sea in March 2004. All fish were held under ambient conditions prior to the commencement of the trial. On the 15th January 2005, one sea winter (1-SW) fish with a mean live body-weight (BW) of 1850 ± 260 g were distributed in six seawater cages (4000 m^{3}; 20 x 20 x 10 m; n = 15,500-17,500 fish/pen). Duplicate unlit cages, separated from the other experimental cages by unlit (non-experimental) cages to prevent artificial-light pollution, were maintained under ambient light-conditions as natural photoperiod controls (NP; sunlight has a continuous spectrum of all visible wavelengths) and four others were subjected to continuous artificial-light (LL) from the 22nd January 2005. In those, two different light-technologies were tested: wide-spectrum 'white' metal-halide lamp as control light-technology (C, 400 W unit^{-1}, 3700°K, Pisces 400, BGB Engineering, Grantham, UK; same technology as used in trial 2 and 3) and a narrow bandwidth 'blue' light-emitting-diode (LED) system (B, 50 W unit^{-1}, Akvasmart UK Ltd., Inverness, UK). For each technology, two and six units per cage were installed providing a total of four LL-treatments (Metal-halide: 2C, 6C; 'Blue' LED: 2B and 6B). No replicated design could be performed in this trial except for control natural photoperiod pens (NP). Positioning of the light-units was standardised between technologies and selected in an attempt to maximize light distribution within the rearing volume: units were submerged at 4.5 m depth in cages receiving two lamps (2C and 2B;

Fig. 1a) while, in cages equipped with six units (6C and 6B), they were submerged at 3 m and 6 m depth in two inverted triangular formations (Fig. 1a). All pens were returned to NP on 5th June 2005 which was immediately followed by a three-way size grading after which treatments could not be kept discrete due to commercial requirements. Ambient water temperature ranged from 7.5°C to 13°C during the period of light-manipulation and fish were fed a commercial diet (MHS Atlantic, Skretting, UK) according to standard commercial feeding protocols.

Trial 2

The second trial was conducted at a nearby commercial salmon farm (56.41°N, 5.42°W, 35 km from trial 1 sea-site, Loch Sunart; Marine Harvest Ltd., UK) using 1+ fish stocked at sea in March-April 2007. All fish were held under ambient conditions prior to the commencement of the trial. On the 3rd January 2008, four 6912 m^3 cages (24 x 24 x 12 m) holding 1-SW fish (n = 23,000 to 26,500 fish/pen; BW = 1631 ± 27 g) were exposed to LL using 2 different lighting-technologies in duplicate design. Two cages received 4 metal-halide units as control light-technology (4Ca; 400 W $unit^{-1}$; same technology as used in trial 1 and 3) and the other two 4 narrow bandwidth 'green' hot cathode lamps (4G, 232 W $unit^{-1}$, Intravision Aqua AS, Snarøya, Norway). In all treatments, light-units were positioned in a 12 x 12 m square formation and submerged to depths of 4 and 8 m (2 units/depth; Fig. 1b). The submersion depth of the G units, which were 1.80m long, refers to the middle point of the bulb. All pens were returned to NP on 18th June 2008 and experimental groups were kept discrete until harvest in October 2008. Ambient water temperature ranged from 7.1°C to 12.1°C during the period of light-manipulation and fish were fed a commercial diet (Biomar, Grangemouth, UK) according to standard commercial feeding protocols.

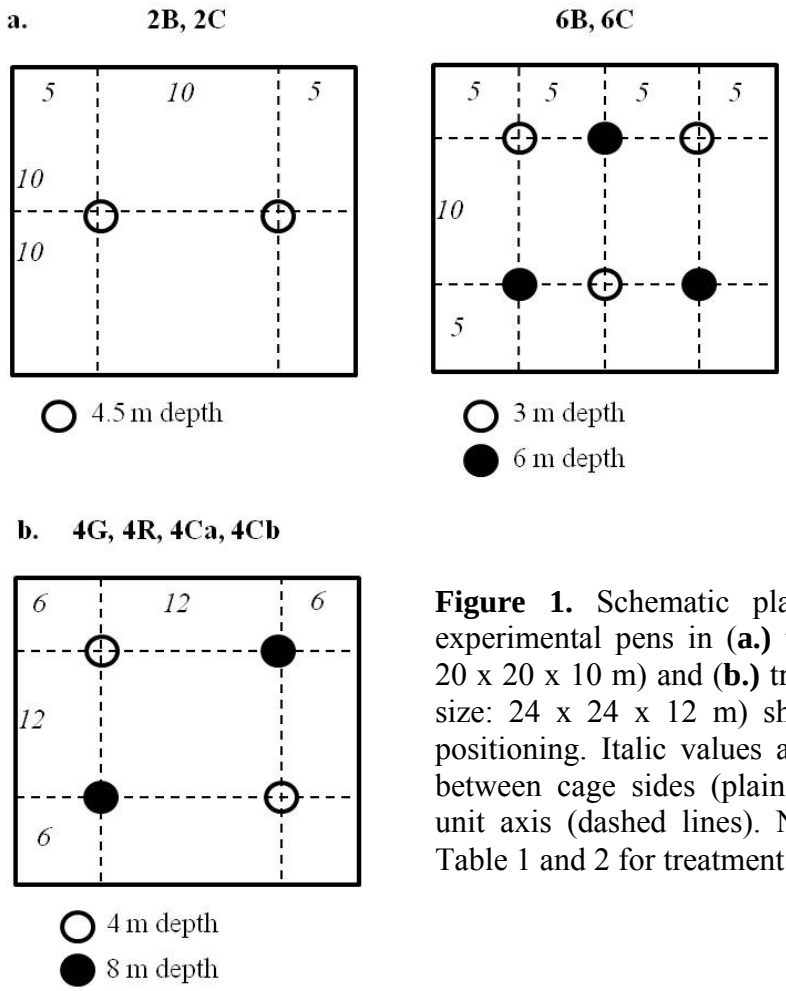


Figure 1. Schematic plan view of the experimental pens in (**a.)** trial 1 (pen-size: 20 x 20 x 10 m) and (**b.)** trial 2 and 3 (pen-size: 24 x 24 x 12 m) showing light-unit positioning. Italic values are distances (m) between cage sides (plain line) and light-unit axis (dashed lines). Not at scale, see Table 1 and 2 for treatment description.

Trial 3

The third trial was conducted at the same site as trial 1 using 1+ fish stocked at sea in February 2008. All fish were held under ambient conditions prior to the commencement of the trial. On the 19th January 2009, four 6912 m^3 cages (24 x 24 x 12 m) holding 1-SW fish (n = 29,000 to 35,000 fish/pen) with a BW of 2293 ± 95 g were exposed to LL using 2 different lighting-technologies in duplicate design. Two cages received 4 metal-halide units as control light-technology (4Cb; 400 W $unit^{-1}$; same technology as used in trial 1 and 2) and the other two cages received 4 'red' tungsten-halogen lamps (4R, 400 W $unit^{-1}$, Atlantis-light, Inishowen Engineering, Shandrum, Ireland). All units were submerged to depths of 4 and 8 m as in trial 2. All pens were returned to NP on 5th June 2009 and experimental groups were kept discrete until

harvest in September 2009. Ambient water temperature ranged from 6.6°C to 13.9°C over the period of light-manipulation and fish were fed a commercial diet (Biomar, Grangemouth, UK) according to standard commercial feeding protocols.

2.2 Light-output and light-perception

Characteristics of the light emitted by the different technologies (B, G, R, C) were measured on the same night and sea-site (May 2010; Loch Leven) on individual light-units submerged at 3 m depth. The spectral composition (over the visible spectrum: λ 400-740 nm) was determined using a portable spectroradiometer (StellarNet Inc. EPP2000c; Tampa, FL, USA; calibrated to National Physics Laboratory UK standards; Migaud et al., 2007b) directly pointing at the light-source and positioned 0.5 m from each unit. Horizontal profiles of light-attenuation were determined by measuring light-irradiance over the visible spectrum (λ 400-740 nm; W m^{-2}) using a single channel light-energy sensor pointing directly at the light-source and connected to a hand-held digital meter (Skye Instruments Ltd., Powys, UK). Measurements were made from the light-source (0 m) and then at 0.5 m horizontal increments until the detection limit of the light-energy sensor was reached (<0.0001 W m^{-2}). The equation of the regression curve was used to calculate the attenuation coefficients at 1m intervals within 1 to 10 m of the light-source (values were then averaged). This equation was also applied to determine the greatest distance from the light-source to an irradiance effective at suppressing plasma melatonin to day-time levels in salmonids (≥0.016 W m^{-2}; previously determined by Migaud et al., 2006). This distance was then used as the radius of a "theoretical sphere" to calculate the volume of effective irradiance emitted by point-source bulbs (B, R, C) while for light-unit G, the volume of a cylinder holding the same radius and the height of the bulb-length (1.80 m) was added. The effective irradiance

volume of individual lamps was finally used to calculate the percentage of the rearing volume theoretically subjected to an effective irradiance which assumes optimal positioning of the light-units. Using the same apparatus but with the light-energy sensor pointing upwards, vertical down-welling light-irradiance was measured in half of each treatment cage in May 2005, May 2008 and May 2009 in trial 1, 2 and 3 respectively. To do so, light-irradiance was measured in a grid format (starting in a cage corner) at 2 m horizontal increments in both horizontal directions and at 2 m (trial 1) or 1 m (trial 2 and 3) depth increments then averaged at each depth. Light-properties within the treatment pens were not measured during day-time in this study. The vertical intensity profile of artificial-illumination using C light-units in cages was previously quantified and found to be approximately 99.8% lower than natural day-light (under light-cloud conditions) (Leclercq et al., unpublished). This suggests that natural sunlight would prevail over the continuous artificial-illumination such that day-time light-conditions were consistent across all treatments. In addition, variations in ambient day-light conditions (e.g. from cloud-cover and surface-reflection) are unlikely to alter the perception of daylight as the natural photophase.

Light perception was assessed in trial 1 during both day- and night-time by measuring plasma melatonin levels at mid-day and mid-night (n = 20 fish/pen) on the date of light-irradiance assessment (May 2005). Blood was withdrawn (under dim red light at night) from the caudal vein of culled fish (MS-222 bath, 150 ppm for 2 to 3 min; Alpharma, Fordingbridge, England; followed by cranial percussion), centrifuged (1200 *g*; 15 min; 4°C) and plasma stored at -70^{o}C until analysis using a commercially available ELISA kit (IBL, Hamburg, Germany) previously validated in salmon (Migaud et al., 2007a, 2007b). The minimum sensitivity of the kits was 3.0 pg.ml^{-1} and the inter- and intra-assay coefficients of variation 3.8 % and 10.7 % respectively.

2.3 Body-size and maturational status

Body size parameters were assessed in each trial at both the onset and termination of LL-treatments in January and late May-June respectively. Fish were randomly sampled, anaesthetized (MS-222 bath, 30 ppm for 2 to 3 min) and individually measured for BW (± 5g) and fork length (FL, ± 1mm) (trial 1: 225 fish/pen on the 31st January and 25th May 2005, replicated photoperiod control only; trial 2: 60 fish/pen on the 10th January and 20th June 2008, replicated treatments; trial 3: 120 fish/pen on the 20th January by batch sample-weight and 20 fish/pen on the 11th June 2009, replicated treatments). Fulton condition factor (K) was calculated as K = (BW x 100) / FL^3. The relative weight gain (RWG; %) over the period was calculated as RWG = [(BW_f / BW_i) x 100]; where BW_f and BW_i are the mean final and initial BW respectively. The specific growth rate (SGR; % day^{-1}) was calculated using SGR = [exp(g) - 1] × 100; where g = ($LnBW_f$ - $LnBW_i$) / ($t_f - t_i$), BW_f and BW_i are the same parameters as for RWG calculation and ($t_f - t_i$) is expressed in days.

Sexual maturation was assessed on the day of body-size assessment in late May-June when recruitment into sexual maturation is determined in Atlantic salmon populations of the Northern hemisphere (Taranger et al., 1999; Leclercq et al., 2010a). Fish were sacrificed, sexed and gonad-weight measured (GW; ± 0.001g) to calculate the gonadosomatic index (GSI) as GSI = (GW / BW) x 100 (n = 25, 30 and 20 fish/pen in trial 1, 2 and 3 respectively). Ovary samples were preserved in 10% buffered formalin for histological analysis and classified according to their leading oocyte stage using the primary yolk stage (the first stage of exogenous vitellogenesis) as an indicator of commitment toward maturation (Taranger et al., 1999). Males were classified as immature or sexually recruited based on the bimodal GSI frequency distribution in the

population with a threshold value of GSI = 0.2% (Kadri et al., 1997; Taranger et al., 1998; Leclercq et al., 2010a). In addition, blood was withdrawn from randomly selected fish, centrifuged (1200 *g*; 15 min; 4°C) and plasma stored at -70°C for analysis of testosterone (T) level. Plasma T was analysed using an indirect competitive radioimmunoassay method (modified from Duston and Bromage, 1987) with levels above 3 ng ml^{-1} indicating recruitment into maturation (Taranger et al., 1998). Minimum sensitivity was 1.9 $pgml^{-1}$, with an intra-assay coefficient of variation of 4.4% and inter-assay coefficient of variation of 9.8% (n=15). In trial 1, plasma was sampled during a three-way body-size grading performed in June four days after gonad sampling (n = 50, 25 and 25 fish/grade/pen in the large, medium and small grade respectively) to assess the efficacy of top-crop harvest at selectively harvesting a high proportion of maturing fish. In trial 2 and 3, plasma was sampled on the day of gonad sampling (n = 60 or 20 fish/pen respectively) and maturation rate was further estimated at harvest using nuptial skin colouration as a reliable indicator (n > 1000 observations/pen in October 2008 and September 2009 respectively; Leclercq et al., 2010a and b).

2.4 Statistical analysis

Linear regressions were performed using GraphPad Instat between the energy consumption of the experimental set-ups and the mean light-irradiance in the sea-cage, between both those parameters and the maturation rate observed and between the cost of electricity and the value of the biomass sexually inhibited. Linear regressions always conformed to a linear model with slopes significantly different to 0. Analyses of variance in body-size and maturation parameters were performed using Minitab v.15 statistical software package. Data sets were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Bartlett's test, examination of residual plots), log or square-root transformed when required and proportions arc-sin transformed. Replicate

data (NP photoperiod control in trial 1; all treatments in trial 2 and 3) were pooled when no significant differences occurred. Differences in BW, FL, K, GSI and plasma-T between treatments within each trial were determined by one-way analysis of variance (ANOVA). In trial 3, two-way analysis of variance was used to test the effect of size-grade and light-treatment on plasma-T levels. Analysis of plasma-T variance systematically included BW as covariate (ANCOVA) which always had a significant effect except in trial 3 ($p = 0.083$). Where statistical differences were found, *post-hoc* multiple comparisons were applied (Tukey's test; Zar, 1999). A statistical significance of $p < 0.05$ was applied to all statistical tests. All data are presented as mean ± S.E.M.

3. Results

3.1. Light-output

Light emitted by the four submerged technologies tested in the marine environment displayed distinct spectral profiles over the visible spectrum (Fig. 2).

The 50 W 'blue' LED unit (B) generated a single peak at λ 465 nm, corresponding to the visible blue wavelengths, and the 232 W 'green' hot cathode (G) a main peak at λ 546 nm within the green wavelengths. Both B and G light-units can be considered as narrow bandwidth lighting-systems. In comparison, the spectral composition of the 400 W 'red' tungsten-halogen lamp (R) progressively increased from the blue to the red end of the visible spectrum: Normalized intensity level reached 50% at λ 586 nm (orange) and 80% at λ 667 nm within the visible red. Finally, the 400 W 'white' metal-halide control technology (C) generated a number of peaks over 30% of normalized intensity: at λ 475 nm (blue/cyan; 31%), λ 511 nm (green; 51%) and λ 571 nm (visible yellow; 54%) while the main peak was at λ 593 nm (yellow/orange; 100%).

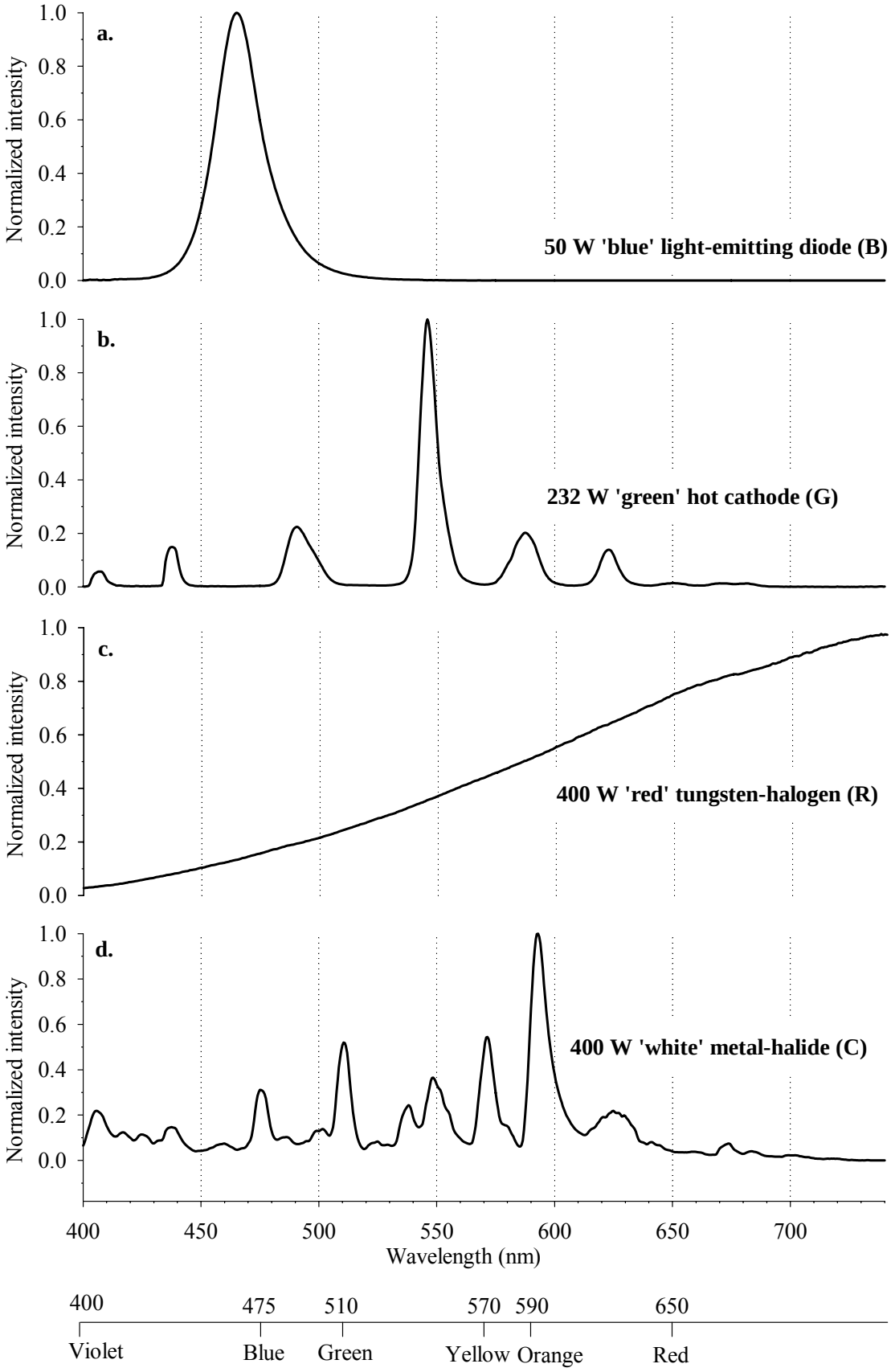


Figure 2. Spectral composition of the light emitted by the different lighting-technologies.

Horizontal profiles of visible light-attenuation were also different between lighting-technologies (Fig. 3). Irradiance (W m^{-2}) measured at 0.5 m from the source was lowest for G, followed by B, R and highest for C. The mean light-attenuation coefficient, directly related to the power of the regression curve, was higher for B (52.2±4.5% m^{-1}) and lowest for G (40.9±4.3 m^{-1}; Table 1). The distance from the light-source to an irradiance of 0.016 W m^{-2}, previously determined as the minimum light-intensity threshold suppressing plasma melatonin to day-time level (effective irradiance distance; Migaud et al., 2006) was longest for the C-unit (6.3 m; 100%) and comparatively reduced for all alternative technologies tested: R (4.6 m; 72.6%), G (3.3 m; 52.5%) and B (2.4 m, 37.8%; Table 1). The different systems also showed variation in their efficiency at converting energy-input into light-output. This is highlighted by the ratio of effective distance relative to the lamp energy use (m Wh^{-1}; B > C > G > R; data not shown) which was highest for B despite the higher attenuation of B-light in the aquatic environment. The volumes of effective irradiance emitted by the alternative light-units were always below 40% that of the C-unit (C: 1065 m^3, 100% > R: 407 m^3, 38.2% > G: 216 m^3, 20.3% > B: 58 m^3, 5.4%; Table 1). Adjusted to the number of lamps deployed in the experimental sea-cages, the theoretical volumes of effective irradiance varied between treatments (Table 2). In trial 1, it covered 100% and 53% of the rearing volume in 6C and 2C respectively but less than 10% in both B-treatments.

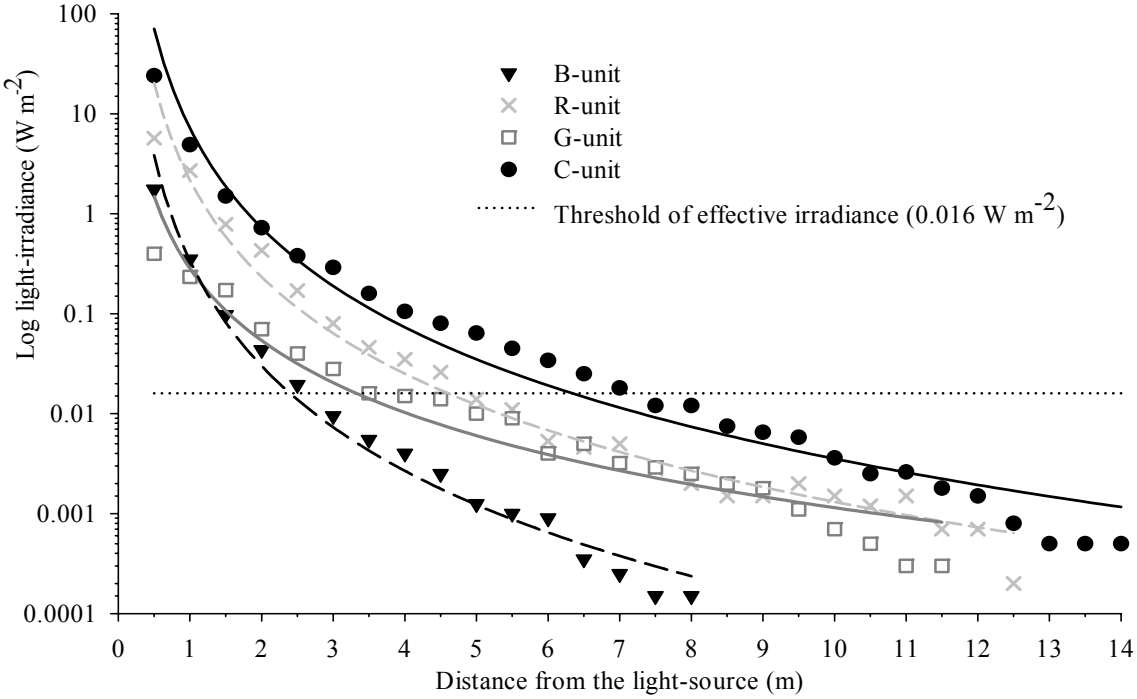


Figure 3. Horizontal profile of light-attenuation as generated by the different lighting-technologies assessed in the same environment over a single nocturnal sampling (See Table 1 for light-unit description). Equations of the respective light-attenuation curves were calculated by least-square regression (B: $y = 0.34x^{-3.50}$, $r^2 = 0.980$; G: $y = 0.28x^{-2.39}$, $r^2 = 0.927$; R: $y = 2.18x^{-3.22}$, $r^2 = 0.973$; C: $y = 7.14x^{-3.31}$, $r^2 = 0.965$ where x = distance from the light-source (m) and y = light-irradiance (W m^{-2}).

Table 1: Parameters of light-irradiance generated by the different lighting-technologies (B: 50 W 'blue' light-emitting diode; G: 232 W 'green' hot cathode; R: 400 W 'red' tungsten halogen; C: 400 W 'white' metal-halide).

Light[1]	Mean attenuation coefficient[2] (% m^{-1})	Greatest distance (m) from the source to an irradiance[2]:		Volume (m^3) of irradiance[5]:	
		Effective[3]	Detectable[4]	Effective	Detectable
B (58 Wh)	52.2±4.5	2.40	8.0	58	2145
G (232 Wh)	40.9±4.3	3.32	11.5	216	7118
R (398 Wh)	49.7±4.5	4.60	12.5	407	8181
C (460 Wh)	50.5±4.5	6.34	14	1065	11494

[1] Electrical energy consumption of light-units (Wh) provided by ligh-manufacturers. [2] The regression curves (Fig. 3) were used to calculate the mean attenuation coefficients within 1 to 10 m of the light-source and [3] determine the greatest distance from the light-source to a theoretical irradiance threshold shown to suppress plasma melatonin to basal day-time levels (Effective irradiance ≥ 0.016 W m^{-2}; Migaud et al., 2006). [4] The minimum irradiance instrumentally detectable was 0.0001 W m^{-2}. [5] Those distances were used to determine the corresponding volumes of irradiance.

Table 2: Theoretical volume and proportion of the experimental cages subjected to a biologically effective irradiance level (≥ 0.016 W m^{-2}). Fish were reared under natural photoperiod and under 2 or 6 B or C light-units (trial 1: NP, 2B, 6B, 2C and 6C respectively) or under 4 G and 4 C light-units (trial 2: 4G and 4Ca) or under 4 R and 4 C light-units (trial 3: 4R and 4Cb). See Table 1 for description of light-units.

Treatment	Effective irradiance in the rearing-volume	
	Volume[1] (m^3)	Proportion (%)
• **Trial 1** *(Cages volume = 4000 m^3)*		
NP	0	0
2B	115	2.9
6B	346	8.7
2C	2130	53.3
6C	6390	100
• **Trials 2 and 3** *(Cages volume = 6912 m^3)*		
4G	865	12.5
4R	1630	23.6
4Ca, b	4260	61.6

[1] The volume of effective irradiance in the rearing-volume was calculated by multiplying the volume of effective irradiance achieved by individual light-units (Table 1) by the number of units installed in the cage which does not take into account overlapping of light.

Variations between treatments also occurred but to a lesser extent in trial 2 and 3 with a volume of effective irradiance covering 62% of the rearing volume in 4Ca and 4Cb compared to 23.6% and 12.5% for 4R and 4G respectively. Measured on-site, vertical profiles of nocturnal down-welling light-irradiance were also lowest for 2B and 6B (Fig. 4a). Their maximum mean irradiance was 0.0004 W m^{-2} and 0.0008 W m^{-2} at 6 m and 8 m depth respectively compared to 0.0528 W m^{-2} and 0.0933 W m^{-2} at 6 m and 4 m depth for 2C and 6C respectively (trial 1). In trial 2 and 3, experimental set-ups using alternative light-units showed higher vertical irradiance profiles (Fig. 4b). Maximum mean irradiance was 0.0168 W m^{-2} at 8 m depth for 4G and 0.0063 W m^{-2} at 5 m depth for 4R as compared to 0.0284 W m^{-2} at 9 m depth for 4Ca.

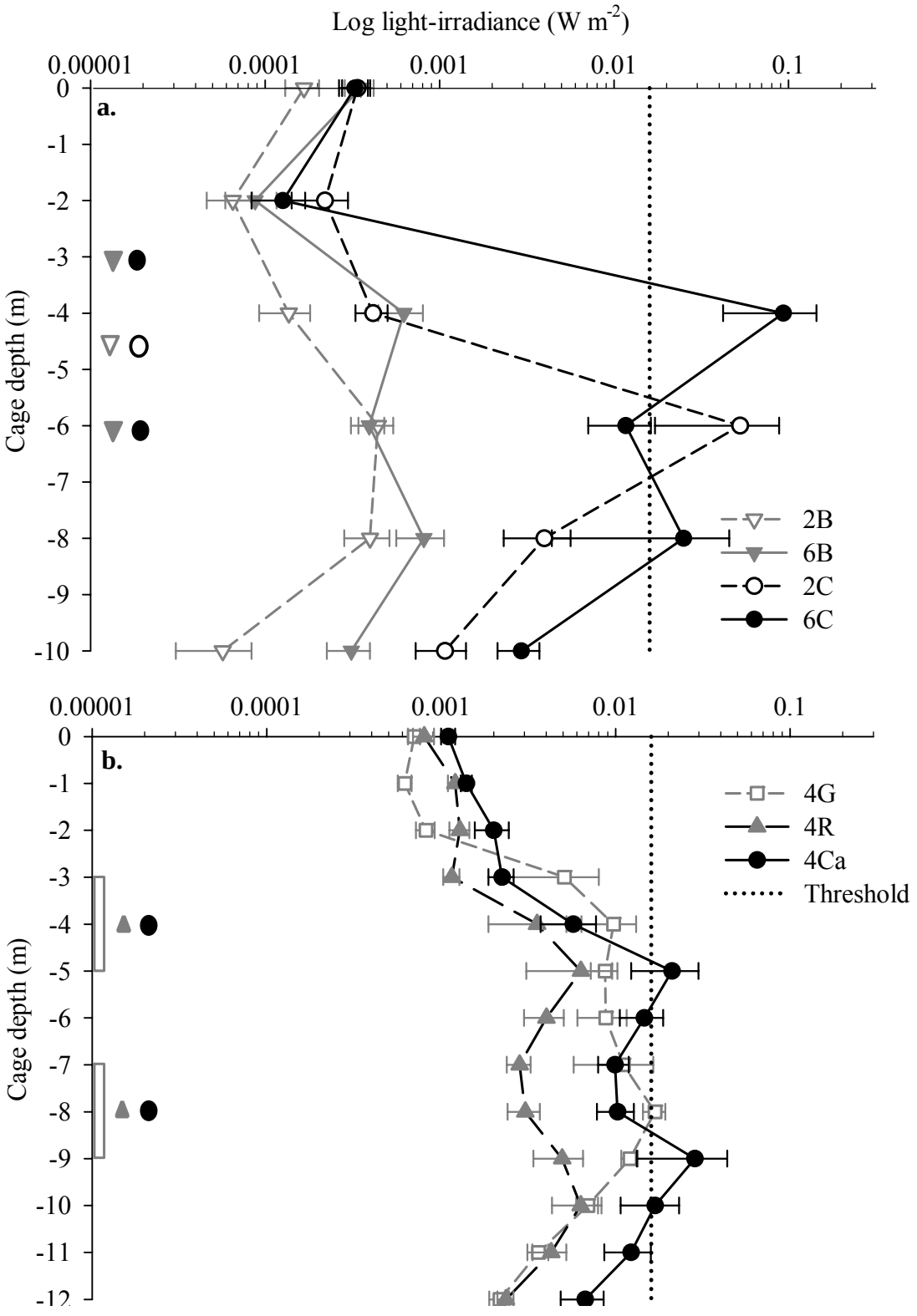


Figure 4. Vertical profile of nocturnal down-welling light-irradiance (W m^{-2}; mean ± SEM) within the experimental cages in (**a.**) trial 1 and (**b.**) trial 2 and 3 (See Table 1 and 2 for treatment description). The dashed line represents the minimum irradiance suppressing circulating plasma melatonin to basal day-time levels (0.016 W m^{-2}; Migaud et al., 2006).

3.2. Light perception: plasma melatonin levels

Day-time plasma melatonin level did not differ between treatments (data not shown) and were pooled for comparison with night-time levels (Fig. 5). At night, fish reared under LL always displayed significantly lower plasma melatonin levels than the unlit NP group which had the highest level. However, nocturnal plasma melatonin levels remained significantly higher than during the day in both B-treatments. Treatment 2B was the least effective at reducing plasma melatonin level followed by the significantly more potent 6B. Nocturnal illumination was most effective under 2C and 6C, both of which suppressed plasma melatonin to day-time levels. No such analysis could be performed in trial 2 and 3 due to the impossibility of crowding the stock within the 24 x 24 x 12m pen-systems at night.

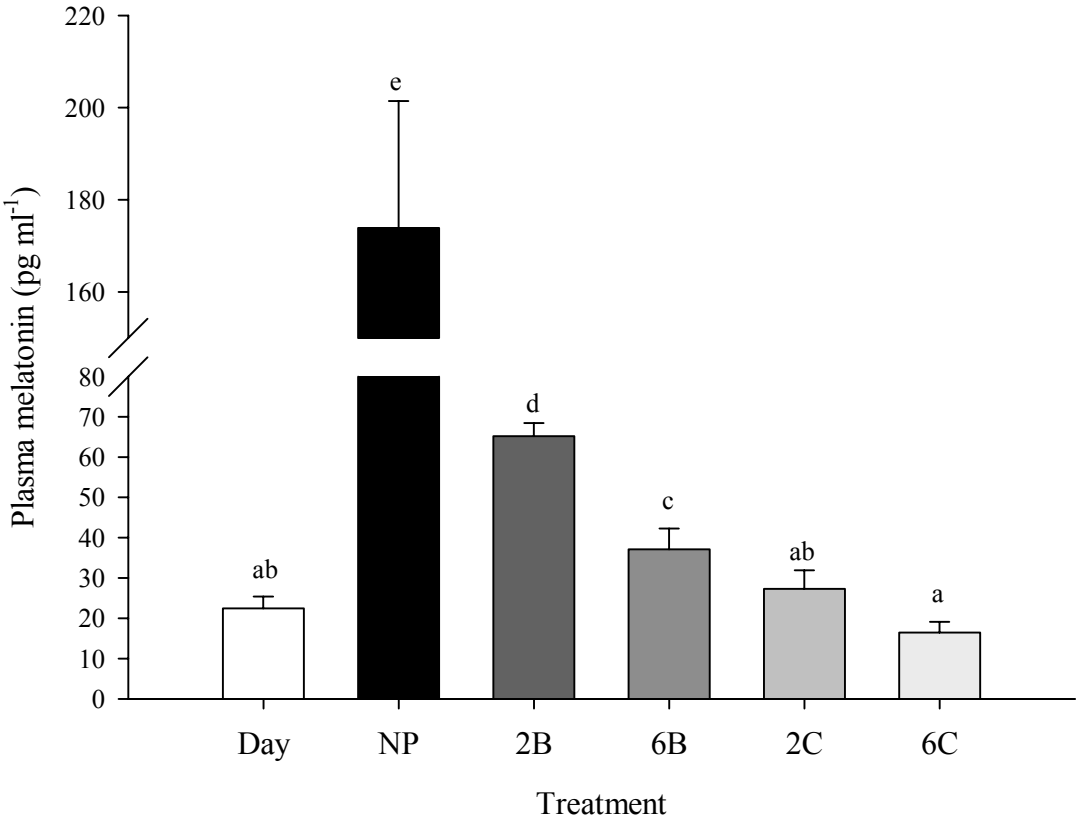


Figure 5. Nocturnal plasma melatonin levels (pg ml^{-1}) in fish exposed to different artificial-light treatments as compared to day-time levels (trial 1). Values are given as mean±SE with n = 20 fish/pen, NP in duplicate). Diurnal levels were not significantly different between treatments and therefore pooled. Different letters represent statistical differences (ANOVA, $p < 0.05$; see Table 1 and 2 for treatment description).

3.3 Body-growth

Before light application in January, there was no significant difference in body-size parameters between treatments within trial 2 and 3. However within trial 1, BW, FL and K were significantly higher in NP and lower in 6B (Table 3) and body-size parameters also varied between trials. The present experiment does not allow an accurate comparative assessment of the effect of light treatments on growth but data are briefly presented. Overall, body-growth parameters (RWG and SGR) appeared reduced when using alternative technologies (2B, 6B, 4G and 4R) compared to the experimental set-ups using the C-technology. Body-growth was lowest under the 2B treatment showing a RWG and SGR reduced by 38% and 32% respectively compared to C-treatments (trial 1). Both parameters were also lower in 4G compared to 4Ca (trial 2: RWG= - 4.0%; SGR = - 3.9%) and in 4R compared to 4Cb (trial 3: RWG = - 12.0%; SGR = - 9.5%).

Table 3: Body-size parameters between the onset (January) and the offset (June) of photoperiod manipulation using different lighting-technologies (See Table 1 and 2 for treatment description). Values are given as mean ± SEM with n = 250 fish/pen in trial 1 (NP in duplicate), n = 60 fish/pen in trial 2 (duplicate treatments) and n = 20 fish/pen in trial 3 (duplicate treatments). Different superscripts indicate significant differences between treatments within each trial and time point (ANOVA, $p < 0.05$). Differences between replicates are shown by italic values (as measured only for K in 4Cb treatment).

		TRIAL 1					**TRIAL 2**		**TRIAL 3**	
		NP	**2B**	**6B**	**2C**	**6C**	**4G**	**4Ca**	**4R**	**4Cb**
•	**January**									
BW	(g)	2233±25^{a}	1966±38^{b}	1560±29^{d}	1726±32^{c}	1759±22^{c}	1647±41	1616±43	2249±147	2350±92
FL	(mm)	559±2^{a}	544±3^{b}	510±3^{d}	526±3^{c}	524±2^{c}	517±3	510±4		
K		1.25±0.01^{a}	1.18±0.01^{b}	1.14±0.01^{c}	1.15±0.01^{c}	1.19±0.00^{b}	1.19±0.01	1.22±0.01		
•	**June**									
BW	(g)	3630±58^{a}	2828±62^{b}	2541±54^{c}	2938±54^{b}	3006±55^{b}	3364±73	3373±71	3710±157^{b}	4086±91^{a}
FL	(mm)	680±3^{a}	630±4^{c}	619±4^{c}	644±3^{b}	647±3^{b}	650±4	642±4	687±7^{b}	714±5^{a}
K		1.11±0.01^{a}	1.09±0.01^{ab}	1.04±0.01^{c}	1.07±0.01^{bc}	1.09±0.01^{ab}	1.21±0.01^{b}	1.26±0.01^{a}	1.13±0.04^{ab}	*1.19±0.03^{a}* *1.05±0.03^{b}*
•	**Body-growth during the window of light manipulation**									
RWG	(%)	62.6	43.8	62.9	70.2	70.9	104.3	108.7	65.0	73.9
SGR	(% day^{-1})	0.43	0.32	0.43	0.47	0.47	0.51	0.53	0.38	0.42

BW: live body-weight; FL: fork-length and K: Fulton condition-factor; RWG: relative weight-gain; SGR: Specific growth-rate.

3.4 Sexual maturation

Following LL-application and for both genders, no significant differences in mean-GSI between treatments were observed (Table 4 with number of fish sampled). Based on the bimodal-GSI distribution in males (GSI > 0.2%), the occurrence of exogenous vitellogenesis in females and plasma-T levels in both genders (> 3 ng ml^{-1}), no randomly sampled fish were deemed to be maturing in June in trial 2 and 3. This was confirmed at harvest (September-October) by observation of skin colouration in a large random sample of the population (n > 1000). Maturation rates were estimated below 2% in all treatments except in one replicate of treatment 4Cb (trial 3) where it reached 8.8%. In comparison, all indicators of sexual maturation showed a large variability between treatments in trial 1. None of the males displayed a GSI above 0.2% in the 2C and 6C treatments (trial 1) while around 12% were found in 2B, 6B and NP treatments. Similarly, none of the females sampled were undergoing exogenous vitellogenesis in 6C (trial 1), as observed in all treatments tested in trial 2 and 3, while it reached 12.5%, 30% and 40% in 2C, 6B-NP and 2B respectively (Table 4; Fig. 6). Although the low number of fish sampled for gonad analysis is acknowledged, plasma-T analysis overall confirmed the effect of light treatment determined from histological analysis and, in particular, the low maturation rates in trial 2 and 3 and in 2C and 6C treatments from trial 1 (Table 4). This parameter further highlighted the effect of body-size grading in June on segregating the maturing cohort within the leading body-weight cohort. As shown by the statistical differences in mean plasma-T, maturing fish (plasma-T > 3 ng ml^{-1}) were mainly present in the large-grade where they accounted for 45% of the fish sampled in NP compared to 30% and 26% in respectively 2B and 6B treatments. In comparison, maturation rate were always low in the medium and small size-grades (<4%).

Table 4: Indicators of sexual development at the offset of photoperiod manipulation (June) in the different experimental groups (See Table 1 and 2 for treatment description; Trial 1: duplicate photoperiod control (NP) only, Trial 2 and 3: duplicate treatments). Values are given as mean ± SEM with number of fish assessed given in the table. Different superscripts indicate significant differences between treatments. Different letters represent significant differences between body-size grades within experimental groups (ANOVA for GSI; ANCOVA for plasma-T using BW as covariate; $p < 0.05$). Italic values correspond to maturation rates determined at harvest based on nuptial skin colouration (Rep1: replicate 1 and Rep2: replicate 2; measured in October for trial 2 and September for trial 3; n > 1000 observations/pen).

	TRIAL 1					**TRIAL 2**		**TRIAL 3**	
	NP	**2B**	**6B**	**2C**	**6C**	**4G**	**4Ca**	**4R**	**4Cb**
•	**Male mean GSI (%) and, in brackets, number of males assessed/treatment and proportion of males with a GSI above 0.2%**								
	0.105±0.015 (23; 13.0%)	0.093±0.037 (11; 12.5%)	0.075±0.021 (12; 11.1%)	0.057±0.007 (14; 0.0%)	0.077±0.014 (13; 0.0%)	0.059±0.004 (33; 0.0%)	0.058±0.003 (31; 0.0%)	0.082±0.004 (19; 0.0%)	0.093±0.004 (22; 0.0%)
•	**Female mean GSI (%) and, in brackets, number of females assessed/treatment and proportion of ovaries undergoing exogenous vitellogenesis**								
	0.274±0.031 (27; 30.8%)	0.297±0.042 (14; 40.0%)	0.247±0.035 (13; 30.0%)	0.296±0.062 (11; 12.5%)	0.200±0.019 (12; 0.0%)	0.216±0.011 (27; 0.0%)	0.197±0.008 (29; 0.0%)	0.223±0.007 (21; 0.0%)	0.244±0.010 (18; 0.0%)
•	**Mean plasma T level (ng ml^{-1}) and, in brackets, number of fish assessed/treatment/grade and proportion of fish with plasma-T levels above 3 ng ml^{-1}**								
	Graded population					***Ungraded population***			
Large	3.24±0.29^{a}x (100; 44.6%)	2.56±0.38^{a}x (50; 30.0%)	1.86±0.23^{a} (50; 26.0%)	0.76±0.16^{b}xy (50; 4.0%)	1.68±0.17^{a}x (50; 4.0%)				
Med.	0.90±0.06^{a}y (50; 0.0%)	0.27±0.04^{a}y (25; 0.0%)	1.40±0.13^{b} (25; 4.0%)	0.24±0.08^{a}x (25; 0.0%)	0.58±0.19^{a}y (25; 4.0%)	0.64±0.02^{b} (120; 0.0%)	0.60±0.01^{a} (120; 0.0%)	0.70±0.01 (40; 0.0%)	0.77±0.02 (40; 0.0%)
						Rep 1: 0.62%	*0.38%*	*1.79%*	*1.61%*
						Rep 2: 1.52%	*0.91%*	*0.00%*	*8.82%*
Small	1.10±0.09^{bc}y (50; 2.0%)	0.26±0.04^{ab}y (25; 0.0%)	0.97±0.10^{c} (25; 0.0%)	1.01±0.23^{bc}y (25; 4.0%)	0.14±0.08^{a}y (25; 0.0%)				

GSI: Gonadosomatic index; T: Testosterone; Med.: Medium

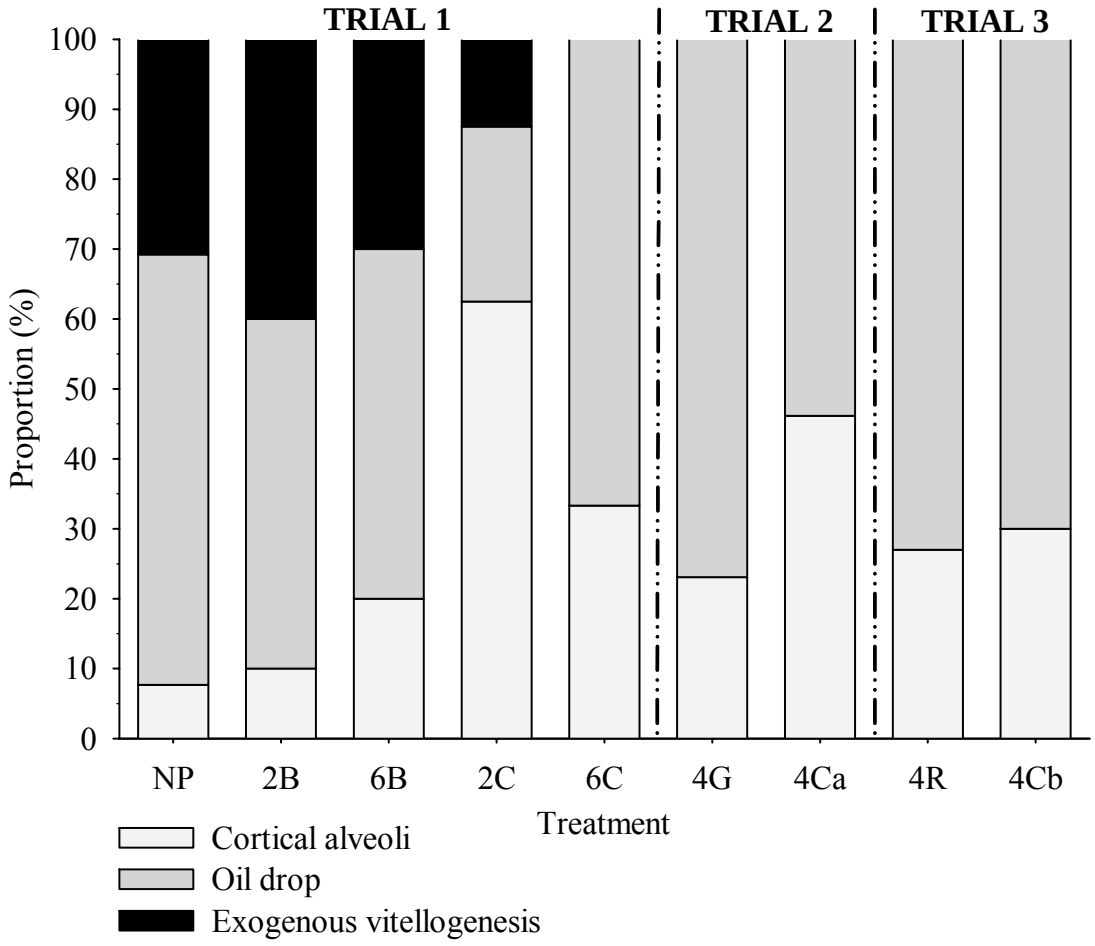


Figure 6. Proportion of females at the different oocyte leading stages in late June corresponding to the offset of continuous artificial-light (classified according to Taranger et al., 1999; see Table 1 and 2 for treatment description).

3.5. Cost-benefit analysis

Energy consumption, light-irradiance and inhibition of sexual maturation are presented jointly in Table 5. Experimental cages equipped with alternative lighting-systems always used less electrical energy relative to the rearing volume (Wh m^{-3}) than the C-treatments. In trial 1, electrical consumption of 2B, 6B and 2C were 4.2%, 12.6% and 33.3% respectively of the 6C set-up. In trial 2 and 3, energy consumption of 4G and 4R were 50.4% and 86.5% of 4Ca and 4Cb respectively.

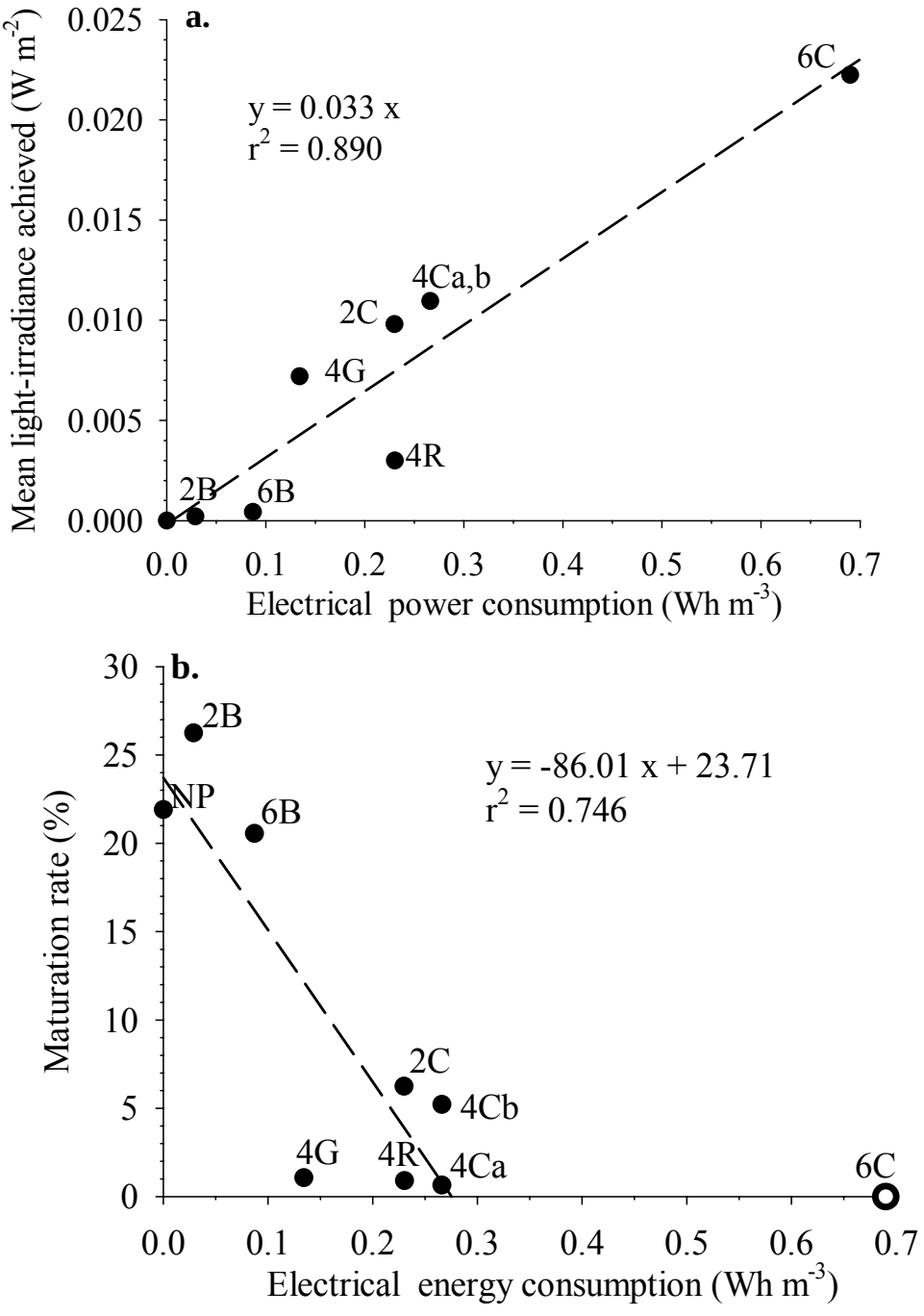
Table 5: (**a.)** Energy consumption, mean irradiance and maturation rate observed in the different experimental groups. (**b.)** Inhibition of maturation by light manipulation. **c.** Running cost-benefit analysis of the different lighting-systems tested (See Table 1 and 2 for treatment description).

			TRIAL 1				TRIAL 2		TRIAL 3	
		NP	**2B**	**6B**	**2C**	**6C**	**4G**	**4Ca**	**4R**	**4Cb**
a. Lighting-systems and maturation rate observed *(Cage volume = 4000 m^3 in trial 1and 6913 m^3 in trial 2 and 3)*										
Electrical consumption[1]	(Wh light-unit^{-1})		58	58	460	460	232	460	398	460
	(Wh pen^{-1})	0	116	348	920	2760	928	1840	1592	1840
	(Wh m^{-3})	0	0.029	0.087	0.230	0.690	0.134	0.266	0.230	0.266
Mean irradiance	(W m^{-2})	0	0.0002	0.0004	0.010	0.022	0.007	0.011	0.003	0.011
Total population[2]	(n)	14500	14500	14500	14500	14500	25000	25000	25000	25000
Maturation rate [3]	(%)	21.9	26.25	20.55	6.25	0	1.07	0.65	0.9	5.2
b. Inhibition of sexual maturation by light manipulation (as compared to NP)										
Population sexually inhibited	(n)	0	-631	196	2269	3176	5208	5313	5250	4170
Biomass sexually inhibited[4]	(kg)	0	-2838	881	10212	14290	23434	23906	23625	18765
	(kg m^{-3})	0	-0.71	0.22	2.55	3.57	3.39	3.46	3.42	2.71
	(kg kWh^{-1} m^{-3})	0	-97.9	10.1	44.4	20.7	174.6	89.8	102.6	70.5
c. Running cost-benefit analysis										
Total cost of electricity[5]	(£)	0	63	188	497	1490	501	994	860	994
	(£ m^{-3})	0	0.02	0.05	0.12	0.37	0.07	0.14	0.12	0.14
Value of biomass inhibited[6]	(£)	0	-8444	2621	30380	42512	69715	71121	70284	55826
	(£ m^{-3})	0	-2.11	0.66	7.59	10.63	10.08	10.29	10.17	8.08
Net saving (on electrical cost)[7]	(£)	0	-8507	2433	29883	41022	69214	70127	69425	54832
	(£ m^{-3})	0	-2.13	0.61	7.47	10.26	10.01	10.14	10.04	7.93
Economic return[8]		0	-134.8	13.9	61.2	28.5	139.1	71.6	81.8	56.2

[1]Provided by light manufacturers. [2]Standardized to a constant value and density approximating experimental data for validity of the comparison. [3]Trial 1: Based on the proportions of male with a GSI above 0.2% and of female undergoing exogenous vitellogenesis (Table 4) that were averaged (a strictly balanced [1:1] sex-ratio was used); trial 2 and 3: using the proportion of fish exhibiting nuptial display at harvest (Table 4). [4]Estimated mean live-body weight at harvest: 4.5 kg. [5]Cost of electricity = £0.15 k Wh^{-1} with usage of artificial-light standardized to 5 months (3600 h) in all treatments. [6]Gutted salmon market price = £3.5 kg^{-1} with a whole:gutted salmon ratio of 0.85. [7]Calculated as: value of inhibited biomass - cost of electricity. [8] Calculated as : value of inhibited biomass / cost of electricity. [7,8] Value of mature fish ("rebate") not considered.

Among all experimental groups, there was a significant positive linear correlation between the (electrical) energy consumption relative to the rearing volume (Wh m^{-3}) and the mean-irradiance achieved in the sea-cage (W m^{-2}; Fig. 7a). Of note, 4R generated the lowest mean light-irradiance relative to its energy use. Significant and negative linear correlations were measured between both the relative energy consumption or the mean-irradiance and the pen maturation rate (Fig. 7b, 7c). The 6C set-up had the highest energy use and mean-irradiance while inducing a total inhibition of maturation. The illumination provided by 6C was arguably far above the level required for maximum biological efficiency. This treatment was therefore not included in the analyses of regression between technical and biological parameters (Fig. 7b, 7c, 7d) in order to maintain the relevance of the relationships. Both regressions show that compared to 4G and 4R, all C treatments were less efficient at inhibiting sexual maturation relative to their energy use and irradiance achieved. Treatment 4G was the most effective at inhibiting sexual maturation relative to its energy use (Fig. 7b) while 4R had the lowest maturation rate relative to the irradiance emitted in the sea-cage (Fig. 7c). With virtually no effect on maturation rate, both B treatments had the highest maturation rate and lowest electrical consumption (Fig. 7b, 7c). The quantity of sexually inhibited biomass per unit of energy used and rearing volume (kg kWh^{-1} m^{-3}; Table 5b) further highlighted the relative efficiency of the different systems. It ranged from a gain of 175 kg kWh^{-1} m^{-3} and 103 kg kWh^{-1} m^{-3} in 4G and 4R respectively to 10 kg kWh^{-1} m^{-3} in 6B to a loss of 98 kg kWh^{-1} m^{-3} in 2B. Based on our dataset, a threshold of 0.28 Wh m^{-3} generating a mean irradiance of 0.0114 W m^{-2} is required to achieve a complete inhibition of sexual maturation (Fig. 7b, 7c). Electrical consumption and inhibition of sexual maturation were further translated into economic terms using standardized population size (and density) and duration of light exposure (Table 5c). Readily

apparent was the low electrical-cost relative to the value of the biomass sexually inhibited by photoperiod-manipulation. Among all LL-treatments, the average electrical-cost was £0.13 ± 0.04 m^{-3} against a value of inhibited biomass of £5.92 ± 1.27 m^{-3} such that the average net saving on the electrical-cost was £5.79 ± 1.25 m^{-3}. Lighting-strategies 4G and 4R had the highest economic return (value of biomass inhibited per unit of electrical running-cost; 4G: 139.1 > 4R > 4Ca > 2C > 4Cb > 6C > 6B, 13.9 > 2B, 0; Table 5c).



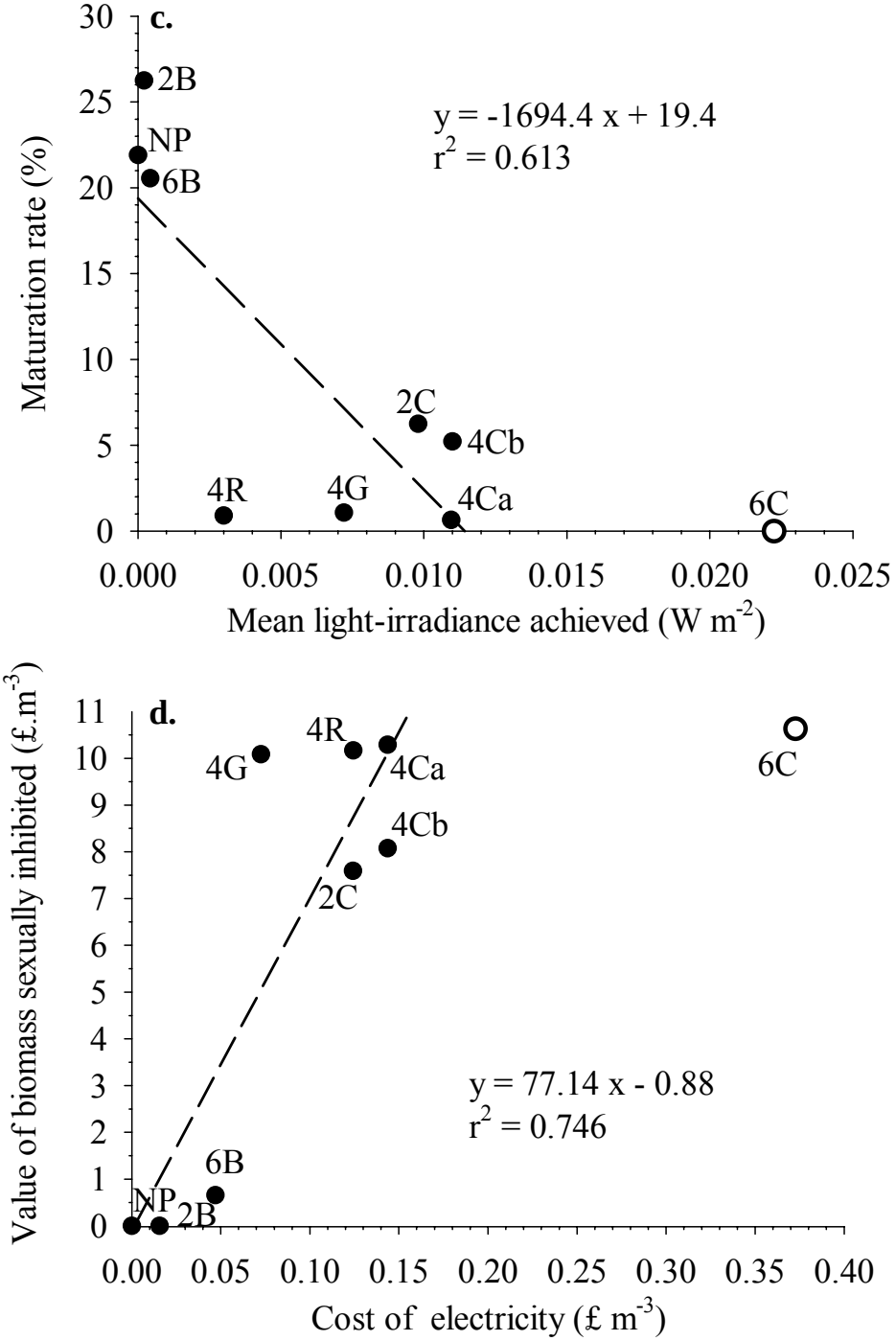


Figure 7. Linear relationship between (**a.)** maturation rate and relative consumption of electrical energy, (**b.)** maturation rate and mean light-irradiance in the rearing volume, (**c.)** mean light-irradiance in the rearing volume and relative consumption of electrical energy and (**d.)** relative value of biomass sexually inhibited and running cost of electricity related to light-manipulation (See Table 1 and 2 for treatment description and table 5 for data calculations). Dashed lines are the linear regression given with their respective equation and regression coefficient. All regression conformed to a linear model (a: $r^2 = 0.895$; $p < 0.001$; b: $r^2 = 0.778$; $p < 0.005$; c: $r^2 = 0.605$; $p < 0.05$ and d: $r^2 = 0.778$; $p < 0.005$). Note: Treatment 6C was not included in the regression analysis of datasets a. b. and c. as the light-power installed (W) and energy used (Wh) was excessively high.

4. Discussion

4.1. Comparison of light-outputs between technologies and set-ups

It is established knowledge that the red-end of the visible spectrum is the most attenuated in seawater followed by violet-blue light, which is further absorbed by fine particles such as silt, while blue-green light travels the greatest distance (Lobban and Harrison, 1994; Denny, 2008). In this study, the attenuation coefficient (% m^{-1}) of the narrow bandwidth green-spectrum (λ 546 nm) G-unit was nearly 30% lower than that of the wide-spectrum C-unit. This might however also reflect a more diffuse illumination due to the length of the G-unit bulb (1.80 m long). This is further supported by the fact that light-attenuation was similar for the R-and C-units and highest for the B-unit.

The level of irradiance emitted will also vary with the energetic consumption of the unit and its efficiency at converting energy-input into light-output. Strong linear correlations were measured between the electrical consumption and the biologically effective irradiance distance of individual lamps (r^2 = 0.873, data not shown) or the mean-irradiance measured in the rearing-volume (r^2 = 0.890, Fig. 7a.). Energy consumption of the lighting-system can therefore be considered as a key factor affecting irradiance level. However, light-output relative to energy consumption also varied between technologies and was higher for the B-unit suggesting that LED technology could ultimately achieve the same effective distance that the C-unit with a lower energy input. In contrast, the G- and R-unit did not offer a comparative advantage over the C-unit in terms of effective irradiance distance per unit of energy used.

Light-unit energy consumption and effective irradiance distance are simple and practical indicators of the illumination that can be achieved. The latter can be further converted into a theoretical volume of effective irradiance which is more relevant to the three-dimensional aquatic environment. In this study, the B-, G- and R-units achieved

5.4%, 20.3% and 38.2% of the C-unit effective irradiance volume respectively such that 18.4 B-, 4.9 G- and 2.6 R- units would be theoretically needed to equal 1 C-unit. Slight-variations in the effective irradiance distance are responsible for substantial variations in the corresponding volume as the volume of a sphere is proportional to the cube of its radius. This underpins the technical advantage of increasing the power, hence effective distance of a unit instead of multiplying their number.

Discrepancies in theoretical volume of effective irradiance also occurred within the experimental set-ups and this was reflected in the vertical profiles of nocturnal down-welling irradiance. Irradiance profiles measured in the rearing pen are dependent on light-unit positioning and hence represent the true illumination perceived by the stock. This is particularly true as they are based on down-welling light-irradiance which was shown, as opposed to up-welling irradiance, to suppress circulating plasma melatonin in seabass (Bayarri et al., 2002). This is expected to remain true in most teleost species due to the anatomic localization of the pineal gland on the dorsal surface of the teleost brain. Of note, these profiles show an apparent poorer performance of the 4R compared to the 4G set-up despite the longer effective distance of the former light-unit. This is likely to be due to the design of this early prototype incorporating a detrimental bottom cap acting as a barrier to down-welling light passage. Conversely, the length of the G-unit bulb favoured a more homogenous light distribution as evident from the more consistent irradiance between different depths.

4.2. Lighting-systems and suppression of maturation

In In Atlantic salmon, a significant GSI rise occurs at an advanced stage of gonadogenesis from July onward but recruitment into maturation for completion in the fall is already determined in spring/summer (Aksnes et al., 1986; Leclercq et al., 2010b). This study used three recognized indicators of maturation in June: plasma-T

levels above 3 ng ml^{-1} (Taranger et al., 1998; Oppedal et al., 1999), the upper GSI mode in the male cohort (Kadri et al., 1997; Leclercq et al., 2010a) and the occurrence of exogenous vitellogenesis in female gonads (Taranger et al., 1998), further confirmed using nuptial colouration in autumn (Leclercq et al., 2010b). All LL-treatments in trial 2 and 3 (4G, 4R, 4Ca,b) and 6C treatment (trial 1) were highly effective at suppressing sexual maturation. This concurs with previous studies, performed under varying conditions and latitudes using different fish stocks, demonstrating the potency of the photoperiod regime used in these trials at reducing Atlantic salmon pre-harvest sexual maturation (Hansen et al., 1992; Porter et al., 1999; Taranger et al., 1998, 1999; Endal et al., 2000; Leclercq et al., 2010c). In particular, similarly low maturation rates as those shown in trials 2 and 3 were previously reported (Oppedal et al., 1997, 2006). One exception is the 8.8% maturation rate observed in one 4Cb replicate (trial 3) despite being the established technology (metal-halide) used in the salmon farming industry. This replicate also exhibited a significantly higher K in June which, at this calendar-time, can be viewed as a consequence of the anabolic effect of sexual maturation (Kadri et al., 1996, 1997, Leclercq et al., 2010a).

4.3. Light-intensity threshold and suppression of maturation

The hormone melatonin, released by the light sensitive pineal-gland, accurately reflects the prevalent photoperiod and is a reliable indicator of light perception in teleosts (Falcón et al., 2010). Of note, plasma melatonin levels hence light-perception were not significantly different between treatments during day-time (trial 1). This confirms the idea that day-time natural illumination (sunlight) prevails and conceals the artificial-illumination regardless of variations in the lighting-technologies and in environmental conditions. During night-time, all LL-treatments in trial 1 caused a

reduction in plasma-melatonin levels below that measured under NP treatment (natural darkness). The suppression of nocturnal plasma melatonin further increased with mean-irradiance measured in the sea-cage (6C>2C>6B>2B>NP). This concurs with previous *in-vitro* and tank-based studies in Atlantic salmon and European sea bass (Yáñez and Meissl, 1996; Porter et al., 2001; Bayarri et al., 2002; Migaud et al., 2006) and was not previously reported in commercial sea-cages using different lighting-technologies, spectra and set-ups. It is acknowledged that, in the present study, discrimination between the effect of light-quantity and quality was not possible as the lighting-strategies tested emitted different intensities and spectra. However, and in line with our findings on melatonin, a significant negative linear correlation was found between mean-irradiance in the rearing volume (achieved from different spectra) and maturation rate. Our data suggest that light-intensity is the main light-property affecting biological potency and that the different light-spectrum tested can achieve the desired effects at similar intensities. Artificial-light must therefore be provided at sufficient intensity in order to mask the circadian amplitude of light-intensity to a threshold value below which it is perceived as continuous and affects reproductive events (Oppedal et al., 1997, 1999; Porter et al., 1999; Kissil, et al., 2001). In mammals it is similarly assumed that a 2-fold increase in basal day-time plasma melatonin induces a physiological response (Reiter, 1988). In this study, the increase in nocturnal plasma melatonin was 2.9-fold and 1.7-fold daytime levels in 2B and 6B respectively where maturation rates were virtually unaltered compared to NP. The levels of irradiance measured in those treatments may have therefore been too low to influence the circannual entrainment of reproduction. In contrast, 2C-treatment suppressed the nocturnal rise of plasma melatonin to a non significant 1.2-fold increase above day-time levels and maturation rate was indeed significantly inhibited. Present data show that the threshold ratio of

nocturnal:diurnal plasma melatonin levels effectively suppressing sexual maturation in 1+ Atlantic salmon was below 1.65 (2B) and around 1.22 (2C), as compared to a 7.7-fold rise under natural darkness. This confirms that light-intensity *per se* plays an important role in altering the physiological response related to sexual maturation and that plasma melatonin is a reliable indicator of light perception at the population level in a commercial sea-cage environment. However, further *in vivo* studies using experimental tank-based systems are required to distinguish the effects of light-intensity and spectrum on salmonid performance.

Another important factor affecting light perception and the potency of LL-regime is the strong photic attraction of Atlantic salmon which position themselves at the depth of the submerged light-units during night-time (Juell et al., 2003; Juell and Fosseidengen, 2004). Although not assessed in this study, this is likely to explain the statistically similar plasma melatonin levels and the low maturation rates observed in 2C and 6C groups which highlight their similar potency despite variations in energy use and irradiance levels (effective volume and down-welling profiles). Across all trials, all treatments except 2B and 6B were also similarly effective at suppressing sexual maturation. From the present dataset, the threshold volume of effective irradiance to provide would be around 12% (4G) of the sea-cage. The deployment of 0.28 Wh m^{-3} of light-energy consumption generating a mean-irradiance of 0.012 W m^{-2} in the rearing volume (Fig. 7b, c) can further be considered as a safe threshold to suppress sexual maturation of 1+ Atlantic salmon to basal levels. Although not directly comparable, it is interesting to note the proximity of this mean-irradiance threshold determined under commercial conditions with the minimum level of irradiance effectively suppressing plasma melatonin to day-time level previously determined under laboratory conditions (0.016 W m^{-2}; Migaud et al., 2006).

4.4. Lighting-system and growth

The present data do not allow accurate characterisation of the effect of light-intensity and light-spectrum on Atlantic salmon growth. A growth enhancement (SGR and RWG) was nonetheless apparent in populations exposed to greater irradiance levels (trial 1: 6C>2C>6B>2B; trial 2: 4Ca>4G, trial 3: 4Cb>4R). This supports previous reports in salmonids where growth and appetite were shown to be stimulated proportionally to the intensity of LL provided (Oppedal et al., 1997, 2003, 2006; Endal et al., 2000; Taylor et al., 2005, 2006). However, this was not the case in trial 2 where similar growth parameters were measured in 4Ca and 4G groups despite a reduced irradiance from the latter treatment. Not withstanding that present growth data should be interpreted with caution, this suggests a spectrum-specific stimulation of growth or a higher sensitivity of Atlantic salmon to blue-green wavelengths. Such spectral sensitivity was previously reported (Vera et al., 2010) and discussed as adaptative to the previously experienced photic environment (Lythgoe, 1980; Shand et al., 2008). The present findings, showing that different light-technologies effectively suppress sexual maturation, warrant further testing of the effect of light-property on Atlantic salmon growth. Together, this would allow identifying the most appropriate light-technologies to be used by the Atlantic salmon industry.

4.5. Cost-benefit analysis

Our preliminary cost-benefit analysis primarily highlighted the strong benefit of photoperiod manipulation inherent to the low running-cost of electricity in comparison to value of the biomass that would otherwise sexually mature. For example, the running-cost of electricity in treatment 4Ca and 4Cb (trial 2 and 3) were equal to the

value of 0.3% of the stocked biomass and the net savings on electrical cost (based on a 21.9% maturation rate observed in NP treatment) were always over £55,000 in trial 2 and 3. Although this analysis did not include other costs (capital, bulb and maintenance cost), this demonstrates that a complete suppression of maturation must be achieved through photoperiod manipulation to optimize the sustainability of the industry. With regards to the financial assessment, this also means that minor variations in maturation rate had a strong effect on the net saving on electrical cost. The economic return on light-manipulation (the value of biomass inhibited per unit of energy expenditure) is less sensitive hence more appropriate when comparing different treatments. In trial 2 and 3, this indicator was higher for 4G and 4R as these treatments achieved a similarly high inhibition of maturation with a lower energy use in comparison to 4Ca and 4Cb. Light-manipulation strategies 4G and 4R were the most cost-effective by providing the optimal level of light-intensity with biologically potent spectrums, despite the lower technical performances of units G and R (i.e. effective irradiance distance per unit of energy used). In contrast, both B-treatments showed poor economical performance due to a low suppression of sexual maturation which is likely to be due to the undersized light-power installed and irradiance achieved. However, B-units had the highest effective irradiance distance relative to energy use suggesting that they could offer the highest financial return if applied at higher intensities (e.g. >50W). Conversely, the 6C treatment successfully suppressed sexual maturation but was oversized hence its poorer economic performances.

This study demonstrated that pre-harvest sexual maturation can be efficiently suppressed using alternative lighting-technologies and with the best financial return by providing a properly scaled level of light-intensity. The optimal light-intensity is also likely to vary, to some extent, with the light-spectrum provided which requires further

experimental assessment. The possibility to choose from a wider range of lighting-technologies is of considerable advantage in itself as selecting an aquacultural lighting-technology is a trade-off between technical (e.g. consistency of output and reliability), practical (e.g. handling and maintenance), health and safety (e.g. voltage) and economic (e.g. capital and bulb cost, life-span) parameters. Extrapolation of the present results to other commercial environment must however proceed with caution due to variations in the genetic origins of the fish, environmental and husbandry conditions. The present study warrants further testing of the effect of light-property on Atlantic salmon growth and is also expected to facilitate the assessment and deployment of effective lighting-strategies in other aquacultural systems and species.

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