Received: 27 September 2021 | Revised: 22 December 2021 | Accepted: 18 January 2022

DOI: 10.1002/jez.2584

RESEARCH ARTICLE

JEZ-A ECOLOGICAL AND INTEGRATIVE PHYSIOLOGY WILEY

Combined blue light and daily thermocycles enhance zebrafish growth and development

Gonzalo de Alba | Sherezade Carrillo | Francisco Javier Sánchez-Vázquez | José Fernando López-Olmeda

Department of Physiology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia, Spain

Correspondence
Gonzalo de Alba, Department of Physiology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia, Spain.
Email: gonzalode.alba@um.es

Funding information
Secretaría de Estado de Investigación, Desarrollo e Innovación,
Grant/Award Numbers: AGL2017-82582-C3-3-R, RED2018-102487-T, RTI2018-100678-A-I00, RYC-2016-20959, Seneca Foundation, 19899/GERM/15

Abstract

In the wild, the light/temperature environment cyclically oscillates insofar as the temperature rises after dawn and drops after dusk. In the underwater photo-environment, light is filtered through the water column so that blue photons reach greater depths. This paper investigates the combined effects of both factors with two temperature regimes (constant temperature = 26°C, CTE vs. daily thermocycle = 28°C day:24°C night, TC) and three light wavelengths (white-W, blue-B, red-R) on *Danio rerio* embryos and larvae from fertilization to 30 days post-fertilization (dpf). It studied hatching rate, larval survival, growth, and food intake (gut content). It analyzed the expression of the genes involved in stress (*crh*), somatic growth (*gh*, *ifg1a*, *igf2a*), and food intake control (*npy*, *agrp*, *ghrelin*, *orexin*, *mch1*, *mch2*, *grp*, *cck8)* at 10 and 30 dpf. The results revealed that the lowest hatching rate was in R regardless of the temperature regime. The highest growth rate was for the larvae reared with B + TC, which was consistent with the highest expression values of the growth factors. The highest feeding and expression levels of the genes involved in food intake were for the larvae in B (regardless of the temperature regime) and W + TC. Conversely, the R + CTE combination obtained the worst growth and feeding results. These findings indicate that the best larval performance can be achieved with combinations of blue wavelengths and cyclic temperature regimes that come closer to those in the natural environment. These results should be considered when optimizing rearing protocols to improve the growth and welfare of the fish larvae.

KEYWORDS
daily thermocycle, larvae development, light spectrum, Zebrafish

1 | INTRODUCTION

The Earth is submitted to environmental cyclic variations caused by predictable geophysical cycles, such as the Earth's axial rotation. Thus, daily temperature and light cycles became a selective pressure and favored the appearance of biological clocks, which animals use to keep track of time and to anticipate periodic events to optimize physiological processes and survival success (Aschoff, 1981).

Although not all species are subjected to them (e.g., deep-sea fish living below the thermocline or fish living in caves), light and temperature in most aquatic environments oscillate daily in a linked dynamic way: water temperature rises after sunrise and drops after

sunset (i.e., daily thermocycle). Daily thermocycles can synchronize the daily rhythms of most organisms, ranging from unicellular algae to vertebrates (Rensing & Ruoff, 2002). In zebrafish (*Danio rerio*), the thermophase (high temperature) produces a similar physiological response to the light phase, while the cryophase (low temperature) is associated with nocturnal responses (López-Olmeda & Sánchez-Vázquez, 2011). Indeed, daily temperature cycles affect a wide range of fish larval activities, including survival, development, sex differentiation, behavioral rhythms, and the molecular clock as it has been described in Senegalese Sole (*Solea senegalensis*) and zebrafish (Blanco-Vives et al., 2010, 2011; Lahiri et al., 2005; López-Olmeda et al., 2006; Villamizar et al., 2012). In addition to water temperature, photoperiod, the light spectrum, and intensity strongly influence fish physiology in all life stages from fertilization to sexual maturation (Ikegami et al., 2014; Mangor-Jensen & Waiwood, 1995; Konstantinov et al., 2003; Villamizar et al., 2011, 2014). The water column acts as a potent chromatic filter by modifying the spectral profile of sunlight so that the wavelengths below violet ($\lambda < 390$ nm) and beyond red ($\lambda > 600$ nm) are selectively absorbed. Therefore, blue wavelengths reach greater depths (McFarland, 1986). Phototransduction in zebrafish is mediated mainly by the pineal gland and the retina (Falcón et al., 2003; Vatine et al., 2011). In the teleost retina, light is absorbed by photopigments of photoreceptor cells (rod and cones), which are involved mainly in brightness/intensity detection, and also in visual acuity and color discrimination, respectively (Kusmic & Gualtieri, 2000). Furthermore, nonvisual photoreceptors are present in every zebrafish cell (Whitmore et al., 2000). A wide variety of these nonvisual photoreceptors, which cover sensitivity to the entire visible light spectrum, has been described in zebrafish (Hankins et al., 2014).

The influence of light spectrum and diel thermocycles during early fish development has been separately investigated and shows different physiological responses depending on fish species as it has been reviewed in some aquacultured teleost species (Villamizar et al., 2011). The light wavelength and intensity to which fish embryos and larvae are exposed can have profound effects on fish survival, malformations, growth, and performance (for a review see Ruchin, 2020, 2021; Villamizar et al., 2011). Diel thermocycles can influence the metabolic rate, food intake control, and growth efficiency in some wild fish species such as yellow perch (*Perca flavescens*) and green sturgeon (*Acipenser medirostris*) (Coulter et al., 2016; Konstantinov et al., 2005; Rodgers et al., 2018). On the other hand, other authors suggested that daily thermocycles may impact negatively or not significantly on fish metabolism and growth (Morash et al., 2018). Given the scarcity of studies the metabolic effects of thermocycles in fish, there is a need for more research on this topic.

Physiologically, the light wavelength and temperature can both stimulate and suppress fish nutrition influencing the development, growth, and other physiological processes of fish (Ruchin, 2021). Biochemically, fish growth development can be explained by changes in the neuropeptide expression involved in the endocrine regulation of survival, somatic growth, food intake control, and digestion process (Bertucci et al., 2019; Rønnestad et al., 2017). Regarding survival, corticotropin-releasing hormone (Crh) is the main stress response-regulating factor in fish and is also related to anxiogenic, anxiolytic, and appetite-inhibiting roles (Matsuda, 2013; Volkoff et al., 2005). The most important neuropeptides involved in somatic growth regulation are the growth hormone (Gh) and insulin-like growth factors (Igf) as a decrease in the previous factors can inhibit growth and other physiological functions in fish (i.e., food intake) (Bertucci et al., 2019; Canosa & Bertucci, 2020; Reinecke et al., 2005; Saera-Vila et al., 2009). Other hormones are considerably implicated in the endocrine control of food intake and digestion. These factors act as appetite-stimulating factors (orexigenic peptides): neuropeptide Y (Npy), agouti-related peptide (Agrp), ghrelin, orexin, melanin-concentrating hormones 1 and 2 (Mch1 and Mch2); appetite-inhibiting factors (anorexigenic peptides): gastrin-releasing peptide (Grp) and cholecystokinin-8 (Cck8) (Bertucci et al., 2019; Koven & Schulte, 2012; Matsuda, Azuma, et al., 2012; Matsuda, Sakashita, et al., 2012; Rønnestad et al., 2017; Takahashi et al., 2016).

Zebrafish is a widespread fish model used by biomedical and aquaculture research (Ribas & Piferrer, 2014). It is a eurythermal species that live in environments subjected to light and thermal fluctuations. For instance, in one of its natural environments, the River Ganges, both solar radiation and air thermal variation cause daily water temperature changes that can oscillate several degrees (between 0.1°C and 5.6°C) in the daytime (Payne et al., 1996). In zebrafish larvae, the effects of the light spectrum (from violet to red) and temperature regimes (constant vs. cycling) have been separately studied (Sánchez-Vázquez & López-Olmeda, 2018; Villamizar et al., 2012, 2014; Wang & Xia, 2019). In zebrafish, blue light elicited the best larval performance in terms of hatching, growth, survival, and occurrence of malformations while red lights presented the worst results, causing the death of all larvae during development (Villamizar et al., 2014). In addition, beneficial effects on early larval development were found in zebrafish reared under daily thermocycles compared to constant temperatures (Villamizar et al., 2012). Although these studies showed separately the profound effects of light spectrum and temperature regimes on zebrafish development, no study to date has evaluated the effect of the combination of both factors. Moreover, these previous studies did not delve into the underlying physiological mechanisms that generate such effects, neither the ones that cause the improvement observed under blue light or thermocycles nor the ones that cause the lethal effects of red light applied to zebrafish during development.

Therefore, this study aimed to investigate the combined effect of daily thermocycles and light spectrum during early development in zebrafish, especially on understanding which mechanisms drive the enhancement under blue light or thermocycles and the deleterious effects of red wavelengths. For this purpose, the following developmental markers and physiological parameters were evaluated: hatching rate, survival, larval growth, food intake, and the messenger RNA (mRNA) expression of genes related to stress, growth, and food intake control (Figure 1).

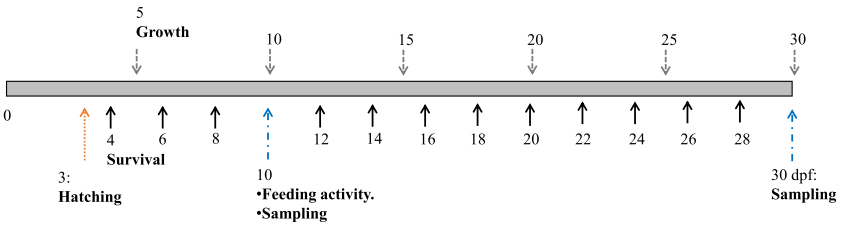

FIGURE 1 Schematic representation of the experimental design indicating the variables analyzed from Day 0 to 30 post fertilization (dpf). The orange dotted arrow indicates the hatching rate measurement at 3 dpf. The continuous black arrows denote the mortality measurement, taken every 2 days from 4 dpf. The grey continuous arrows represent the growth measurement, taken every 5 days from 5 dpf. The blue dotted arrows indicate the sampling points for analyzing gene expression. In addition at 10 dpf, food intake was measured

2 | MATERIALS AND METHODS

2.1 | Animals and housing

The present research was conducted at the facilities of the Department of Physiology of the University of Murcia (Spain). Fish were reared following Spanish legislation on Animal Welfare and Laboratory Practices. Experimental protocols were performed following the Guidelines of the European Union (2010/63/UE) and Spanish legislation (RD 53/2013 and Law 32/2007) for the use of laboratory animals. They were approved by the National Committee and the Committee of the University of Murcia on Ethics and Animal Welfare.

Zebrafish adults and larvae were raised at the Fish Chronobiology laboratory in the Faculty of Biology at the University of Murcia. Broodstocks were obtained from a local supplier (Alimar Pets; Murcia) and acclimatized to laboratory conditions for 2 months according to standard methods (Nusslein-Volhard & Dahm, 2002). Six groups of sexually mature zebrafish were used, with a 1:2 (female:male) sex ratio per group. The broodstock groups were set in the afternoon and kept overnight, and fertilized eggs were collected within the first 2 h after lights on. Then all the fertilized eggs were pooled and sorted into groups of 30 eggs in floating Petri dishes (85 × 10 mm) with embryo medium (E3 medium, Westerfield, 2000).

2.2 | Experimental design

The complete experimental procedure was repeated three times using different broodstock groups, which means that the presented results came from three independent experiments. In all the experiments, 1728 (N) embryos and larvae were employed. In a single independent experiment, 576 fertilized embryos were distributed into six different experimental treatments using a given combination of three different wavelengths (white, W, blue, B, and red, R) and two distinct temperature regimes (constant temperature vs. daily thermocycles) (Figure S1). For each experimental group, four Petri dishes ($n = 96$ embryos, 24 embryos per Petri dish) were utilized. Spectral analysis of lights was performed with a spectroradiometer (FieldSpec® Hand-Held spectroradiometer UV/VNIR; ASD Colorado) and a lux meter (MX Elektronik). This gave the spectral composition of each LED light expressed as the percentage of irradiance (white $\lambda_{peaks} = 466$ nm and 668 nm; blue $\lambda_{peak} = 472$ nm, and red $\lambda_{peak} = 665$ nm) (Figure S1a). The photoperiod was set at 12 L:12D h (light:dark cycle) (lights on at 09:00 h) in all the groups. The two tested temperature regimes were: constant temperature (CTE) of 26°C (26.1 ± 0.1°C) or daily thermocycle (TC) with a thermophase of 28°C (27.9 ± 0.1°C) occurring in the daytime and the cryophase of 24°C (24.1 ± 0.1°C) occurring at nighttime (Figure S1b). Water temperature was modified by water heaters (Askoll) and cooler units (Aqua Medic Titan 1500 GmbH) controlled by an electronic timer (Bachmann GmbH & Co.). Water temperature was recorded continuously throughout the experiment by underwater data recorders (HOBO PENDANT; Onset Computer Corporation). Zebrafish embryos and larvae were reared under these conditions from 0 to 30 days post-fertilization (dpf). At 5 dpf, larvae were transferred from the Petri dish to 2.5-liter nursery net cages (SERA GmbH; Heinsberg). Larvae mouth opening occurred 2 days after hatching (5 dpf). From that time, they were fed powder feed (Vipan Baby; SERA) to satiety twice a day at 11:00 h and 16:00 h (Westerfield, 2000).

To evaluate the performance of both the embryo and larvae reared under the six different experimental conditions, the following variables were analyzed: hatching rate, survival, growth, feeding activity. Whole larvae samples were collected before the first feeding point at 10 and 30 dpf for the mRNA expression analysis of the genes involved in the stress response, growth, and food intake control. For the gene expression studies, larvae were immediately euthanized by anesthetic overdose and death was confirmed under the microscope according to the guidelines for fish euthanasia (RD 1386/2018). Then larvae were pooled for each replicate to obtain two replicates per group/experiment, which gave six replicates ($n = 6$) during the three independent experiments. The number of larvae used in each pool replicates differed depending on the stage: 12 larvae/pool at 10 dpf and 3 larvae/pool at 30 dpf. Larvae were stored in 1.5 ml sterile tubes and immediately frozen at −80°C until the gene expression analysis.

2.3 | Hatching and survival rates

The hatching rate was calculated as the percentage of embryos hatched at 3 dpf from the total number of embryos. Survival was measured every 2 days from 4 to 30 dpf (Figure 1) and was calculated as the percentage of live larvae from the total of larvae at 4 dpf. To measure larvae

mortality, a binocular microscope (Leica EZ4 HD; Leica Microsystems GmbH) was used to observe the cessation of heartbeat and blood circulation, which was set as an endpoint (Villamizar et al., 2014).

2.4 | Growth rate and food intake (gut content)

The growth rate was assessed by longitudinally measuring the total lengths (TL) of 10 larvae per experimental group every 5 days from 5 to 30 dpf (Figure 1). Larval measurements were taken from living animals by a digital camera mounted on a binocular microscope and using the "ImageJ" image processing software (v. 1.8.0_112, Wayne Rasband; National Institute of Mental Health) (Abràmoff et al., 2004). At 10 dpf, the food intake of six larvae per experimental group was determined by calculating the proportion of larvae's digestive tube (DT) filled with food 1 h after being fed in relation to its TL (Villamizar et al., 2009).

2.5 | Gene expression analysis: Real-time reverse-transcription polymerase chain reaction (RT-PCR) analysis

Larvae pool samples were individually homogenized in TRIzol reagent (Ambion; Thermo Fisher Scientific) using a tissue homogenizer for mechanical homogenization (TissueLyser LT; Qiagen). RNA was extracted according to the manufacturer's instructions. The RNA pellet from the larvae samples of 10 and 30 dpf was dissolved in 15 and 50 µl of sterile DEPC water (Invitrogen), respectively. The RNA concentration was determined by spectrometry (Nanodrop ND-1000; Thermo Fisher Scientific). Then RNA (1 µg) was first treated with 1U of DNase I (Thermo Fisher Scientific), followed by retrotranscription with a commercial kit (QSCRIPT cDNA Synthesis Kit; Quantabio). All the complementary DNA (cDNA) samples were diluted (1:10) in nuclease-free water (Thermo Fisher Scientific) and stored at −20°C for subsequent analyses. The quantitative PCR (qPCR) reactions were performed using Perfecta® SYBR® Green Fastmix (Quantabio). All the samples were run in duplicate and qPCR reactions were performed in a final volume of 20 µl. The qPCR analyses were run in a light thermocycler (7500 RT-PCR system; Applied Biosystems) following this protocol: 15 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Melting curves were run after amplification to ensure that only one DNA species was amplified. Twelve genes were selected and divided according to their physiological functions in embryo and larvae performance: stress and survival (*crh*), growth (*gh*, *igf1a*, *igf2a*), and food intake control (*npy*, *agrp*, *ghrelin*, *orexin*, *mch1*, *mch2*, *grp*, *cck8*). All the primer sequences (Table 1) were designed with the Primer 3 plus software (Untergasser et al., 2012). The relative amplification efficiencies of all the genes were analyzed by cDNA dilution curves. The primer concentrations were determined by a primer dilution curve. The primers of *crh*, *igf2a*, *agrp*, *ghrelin*, *orexin*, *mch1*, *grp*, and *cck8* were added at a final concentration of 200 nM. The primers of *gh*, *igf1a*, *npy*, and *mch2* were added at a final concentration of 400 nM. The relative expression of all the genes was calculated by the $2^{-\Delta\Delta C_t}$ method, and using zebrafish *ef1a* as the housekeeping gene after assessing that its coefficient of variation (CV) was lower than 5% between the experimental groups.

2.6 | Statistical analysis

All the results are expressed as the mean ± SEM. The SPSS software (v. 19.0; IBM Analytics) performed the statistical analysis. The normality of data distribution was checked by the Kolmogorov–Smirnov test and homogeneity of variance by Levene's test. The data from each gene and day were subjected to a one-way analysis of variance (ANOVA) to check for statistically significant differences between the six experimental groups, and to a two-way ANOVA to analyze the effects of light wavelength (W, B, R), temperature regimes (TC, CTE) and their interaction. The one-way ANOVA was followed by Duncan's post hoc test to determine statistically significant differences between the experimental groups. The significance threshold was set at $p = 0.05$ in all statistical tests.

3 | RESULTS

3.1 | Effects of light wavelength and daily thermocycles on hatching, survival, and growth

At 3 dpf, all the larvae had either hatched or died during embryonic development, and the hatching rate was calculated on this day. The analysis detected statistically significant differences between lighting conditions ($p = 0.044$), but not between temperature regimes ($p = 0.809$) (two-way ANOVA, Table S1, Figure 2). The embryos left in B and W light obtained higher hatching rates (70.4 ± 5.1% and 69.2 ± 3.5%, respectively) than those reared in R light (59.7 ± 5.9%) (two-way ANOVA, $p < 0.05$) (Figure 2).

From 4 to 30 dpf, survival was measured every 2 days in all the groups (Figure 1). Zebrafish larvae survival presented striking differences, which depended mainly on the light wavelength, although an effect of temperature regime was also detected (Figure 3) (one- and two-way ANOVA, $p < 0.05$, Table S1). The highest survival rates were for the larvae reared in either W or B, with a final survival (at 30 dpf) of 78.9 ± 4.8% and 75.5 ± 3.2%, respectively (Figure 3). In contrast, R obtained 100% mortality, although some differences were observed depending on the temperature regime (Figure 3) (two-way ANOVA, $p < 0.001$). In R + CTE, survival was significantly lower right from the start (6 dpf), while the survival of the larvae reared in R + TC was significantly lower as of 14 dpf. A significant effect of temperature regime was detected from 6 to 14 dpf, with the groups reared in TC surviving better than in CTE (two-way ANOVA, $p < 0.01$). No significant differences in survival rates were observed between the larvae reared in W and B regardless of the temperature regime (two-way ANOVA, $p > 0.05$, Table S1).

The larval length was measured every 5 days from 5 to 30 dpf to evaluate the effects of the different experimental conditions on growth. Statistically significant differences in length were observed from 10 dpf onward (Figure 4) (one-way ANOVA, $p < 0.05$; Table S1). At 10 dpf,

TABLE 1 The primer sequences used for the quantitative PCR analyses

Gene	F/R	Sequence (5′–3′)	GenBank Accession number
ef1a	F	CTGGAGGCCAGCTCAAACAT	NM_131263.1
	R	ATCAAGAAGAGTAGTACCGCTAGCATTAC	
crh	F	GCCGCGCAAAGTTCAAAA	BC085458.1
	R	GCGAGGAGAATCTGTGCGTAA	
gh	F	AAGATCAGTGTTCAAAGGGTTCACT	NM_001020492.2
	R	TTAAGGCAAGAATCTATCAGACAGACA	
igf1a	F	CAGGCAAATCTCCACGATCTC	AH010825.2
	R	CTTTGGTGTCCTGGAATATCTC	
igf2a	F	GTGAAGTCGGAGCGAGATTGTT	NM_131433.1
	R	GAGCCTGTGACACTG GGAAGA	
npy	F	GACTCTCACAGAAGG GTATCC	BC162071.1
	R	GGTTGATGTAGTGTCTTAGTGCTG	
agrp	F	TCGCACAGAGAATCCACAGAG	NM_001328012.1
	R	TAAAACCGCAGCCAATGGTG	
ghrelin	F	CAGCTTCCTCAGTCCGACTCA	EU908735.1
	R	TTCTCTTCTGCCCACTCTTGGT	
orexin	F	GTCGCCAGACATTTAGTGCATC	NM_001077392.2
	R	TTCGCCCACTTTACGTTTGC	
mch1	F	CAAACCGCTAAAGCAAACGC	NM_001162488.1
	R	AAAGTGCAACGGTGATGAGG	
mch2	F	GCTGGCAAGCTTGAAAATGG	FJ204828.1
	R	TTGCAAGATCAAGGGATGGC	
grp	F	GACAACACAGAGGTCAACGCTTT	NM_001161350
	R	ACTGGCGTCCCTTTTCGAT	
cck8	F	CAAAGGCTCATACCGCAGAAG	XM_001346104
	R	TCTGTGAGATGCACCCATGGT	

Abbreviation: PCR, polymerase chain reaction.

statistically significant differences due to the lighting conditions, but regardless of temperature, were noted (two-way ANOVA, $p < 0.05$; Table S1). From 15 to 30 dpf, significant differences in growth were due to not only lighting but also to the temperature conditions (two-way ANOVA, $p < 0.05$; Table S1). In general, the larvae that obtained the highest growth rates were those reared in B + TC (Figure 4). This effect was evidenced from 20 dpf onward, when the larvae from B + TC were longer (9.2 ± 0.1 mm) than those in the other groups (6.9–7.3 mm) (one-way ANOVA, $p < 0.05$). When examining the lighting conditions, the growth rate for the fish maintained in B was higher than for those reared in W from 10 to 30 dpf, and then those larvae reared in R, which were shorter than those in B and W (Figure 4) (two-way ANOVA, $p < 0.01$). Moreover from 15 dpf to the end of the experiment (30 dpf), the larvae reared in TC had a higher growth rate than those reared in CTE (Figure 2) (two-way ANOVA, $p < 0.01$).

3.2 | Effects of light wavelength and thermocycles on food intake (gut content)

To test whether the survival and growth results were related to food intake, we analyzed the presence of food in the digestive tract of the 10 dpf larvae 1 h after mealtime. Statistically significant differences were observed in the experimental groups (Figure 5) (one-way ANOVA, $p < 0.001$; Table S1). The highest food intake levels were for those in B + TC (67.0 ± 1.1%), W + TC (54.3 ± 1.8%), and B + CTE (53.1 ± 2.2%), whereas the lowest food intakes were found in the larvae in R (0.8 ± 0.2 and 8.4 ± 1.0% in R + CTE and R + TC, respectively). In general, the larvae reared in B and W presented higher food intake than those reared in R light (Figure 5) (two-way ANOVA, $p < 0.001$). On the effects of temperature regime, the larvae reared in TC had a higher food intake compared to those in CTE (two-way ANOVA, $p < 0.001$).

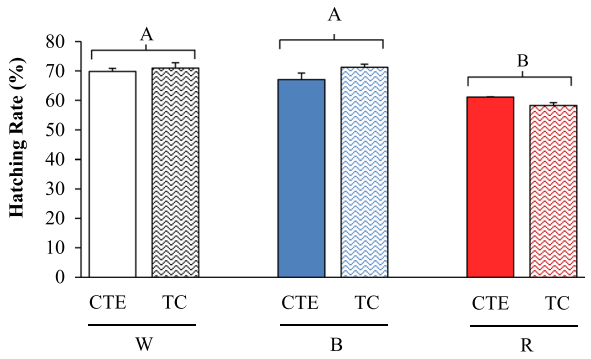

FIGURE 2 The hatching rates (%) of the zebrafish exposed to the combination of three different light wavelengths (white, W; blue, B; red, R) and two temperature regimes (a thermocycle of 28:24°C thermophase:cryophase, TC; and a constant temperature of 26.1 ± 0.1°C, CTE). Data ($n = 6$) are expressed as mean ± SEM of the percentage of hatched eggs, calculated at 3 days post fertilization (dpf). Different upper case letters indicate significant differences between light treatments (two-way ANOVA, $p < 0.05$). ANOVA, analysis of variance

3.3 | Effects of light wavelength and thermocycles on gene expression

As we observed marked differences in survival, growth, and food intake, especially between larvae reared in R compared with the rest of groups, we went on to elucidate the underlying molecular mechanisms that could lead to these effects. For this purpose, we analyzed the mRNA expression of the multiple factors involved in stress, growth, and food intake control in the 10 and 30 dpf larvae of all the experimental groups (Figure 1).

3.3.1 | Genes involved in stress response

One of the processes that can be affected by light and temperature conditions is the stress response mediated by Crh, which is related to survival. The expression of *crh* at 10 dpf showed statistically significant intergroup differences (Figure 6, left panel) (one-way ANOVA, $p < 0.05$). The larvae reared in R + CTE presented a higher *crh* expression than the

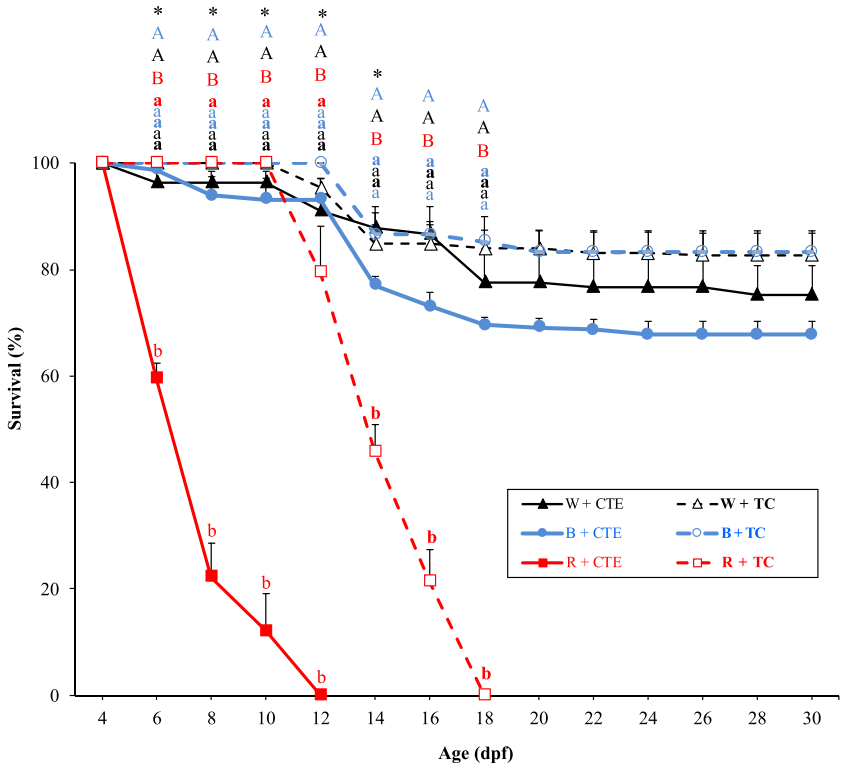

FIGURE 3 Effect of three different light wavelengths (white, W; blue, B; and red, R) and two temperature regimes (a thermocycle of 28:24°C thermophase:cryophase, TC; and constant temperature of 26.1 ± 0.1°C, CTE) on zebrafish larval survival from 4 to 30 days post-fertilization (dpf). Triangles, circles, and squares indicate the groups in the W, B, and R light spectra, respectively. Dashed and continuous lines denote the groups kept at the TC and CTE temperatures, respectively. Different lower case letters indicate significant differences between the experimental groups within the same dpf (one-way ANOVA, $p < 0.05$). Different upper case letters and asterisks indicate significant differences between light treatments and rearing temperature regimes, respectively, at the same dpf (two-way ANOVA, $p < 0.05$). Data ($n = 4$) are represented as mean ± SEM. ANOVA, analysis of variance

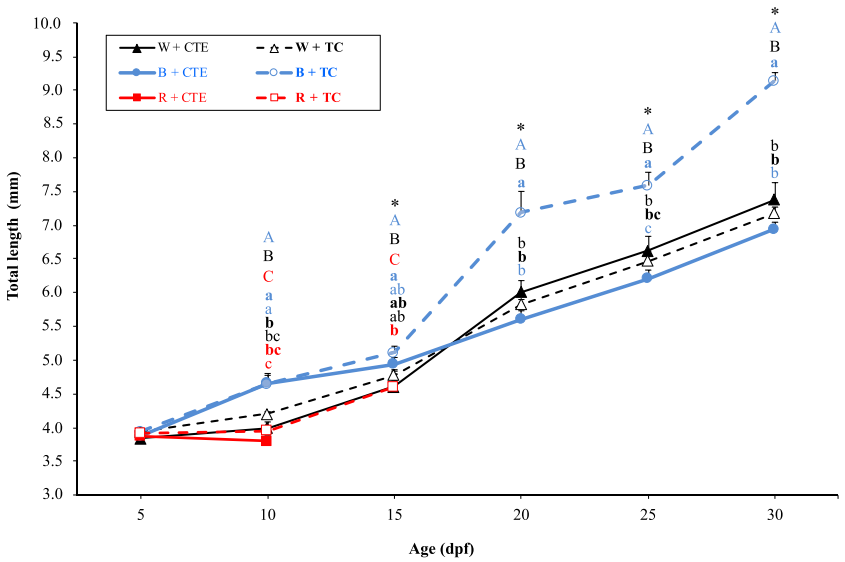

FIGURE 4 Effect of three different light wavelengths (white, W; blue, B; and red, R) and two temperature regimes (a thermocycle of 28:24°C thermophase:cryophase, TC; and constant temperature of 26.1 ± 0.1°C, CTE) on the total length (mm) of zebrafish larvae from 5 to 30 days post-fertilization (dpf). Triangles, circles, and squares indicate the groups in the W, B, and R light spectra, respectively. Dashed and continuous lines denote the groups kept at the TC and CTE temperatures, respectively. Different lower case letters represent significant differences between the experimental groups on the same dpf (one-way ANOVA, $p < 0.05$). Different upper case letters and asterisks indicate significant differences between the light treatments and rearing temperature regimes, respectively, on the same dpf (two-way ANOVA, $p < 0.05$). Data ($n = 10$) are represented as mean ± SEM. ANOVA, analysis of variance

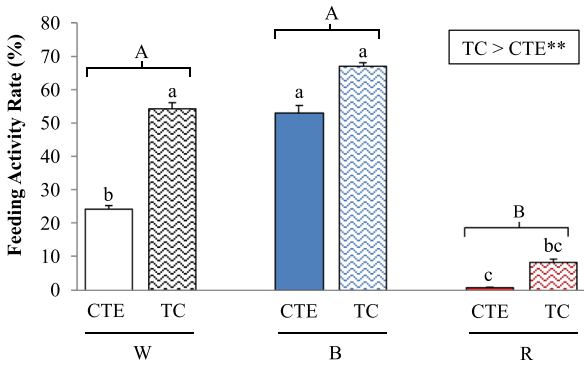

FIGURE 5 Effect of three different light wavelengths (white, W; blue, B; red, R) and two temperature regimes (a thermocycle of 28:24°C thermophase:cryophase, TC; and constant temperature of 26.1 ± 0.1°C, CTE) on the food intake (gut content) in the zebrafish larvae at 10 days post-fertilization (dpf). Different lower case letters indicate significant differences between the experimental groups on the same dpf (one-way ANOVA, $p < 0.05$). Different upper case letters and asterisks denote significant differences between the light treatments and rearing temperature regimes, respectively, on the same dpf (two-way ANOVA, $p < 0.05$). Data ($n = 10$) are represented as mean ± SEM. ANOVA, analysis of variance

other groups. Significant differences depending on temperature regime were also detected (two-way ANOVA, $p = 0.023$; Table S1), with the larvae reared in CTE displaying a higher *crh* expression than those in TC (Figure 6, left panel). No significant differences in *crh* expression were found at 30 dpf (Figure 6, right panel) (Table S1).

3.3.2 | Genes involved in growth

We also analyzed the expression of the main endocrine factors involved in somatic growth regulation: *gh*, *igf1a*, and *igf2a*. Statistically, significant differences were observed in groups for *gh* expression at both 10 and 30 dpf (Figure 7) (one-way ANOVA, $p < 0.05$; Table S1). In both developmental stages, the highest *gh* expression was detected in the larvae reared in B + TC. A significant effect of temperature regime was observed at both 10 and 30 dpf, with a higher *gh* expression in TC than in CTE (Figure 7a) (two-way ANOVA, $p < 0.05$; Table S1). At 30 dpf, *gh* expression also differed between light treatments, with B inducing a higher expression than W (Figure 7a, right panel) (two-way ANOVA, $p < 0.05$).

The highest expression for *igf1a* at 10 dpf was observed in the larvae reared in B + TC and the lowest for the larvae left in R + CTE (Figure 7b, left panel) (one-way ANOVA, $p < 0.05$; Table S1). The light wavelength also influenced *igf1a* expression, with higher values for the larvae reared in W and B than in R (two-way ANOVA, $p < 0.05$). No statistically significant differences in *igf1a* appeared at 30 dpf (Figure 7b, right panel) (one- and two-way ANOVAs, $p > 0.05$).

Finally, *igf2a* expression at 10 dpf was significantly higher in all the groups reared in W and B than in the groups reared in R (Figure 7c, left panel) (one- and two-way ANOVAs, $p < 0.05$). For the 30 dpf larvae, *igf2a* was higher in the larvae in W + TC than in the W + CTE and B + CTE larvae (Figure 7c, right panel) (one-way ANOVA, $p < 0.05$). The statistical analyses also revealed a significant effect of temperature regime because the larvae in TC showed higher *igf2a* expression than those in CTE (two-way ANOVA, $p < 0.05$).

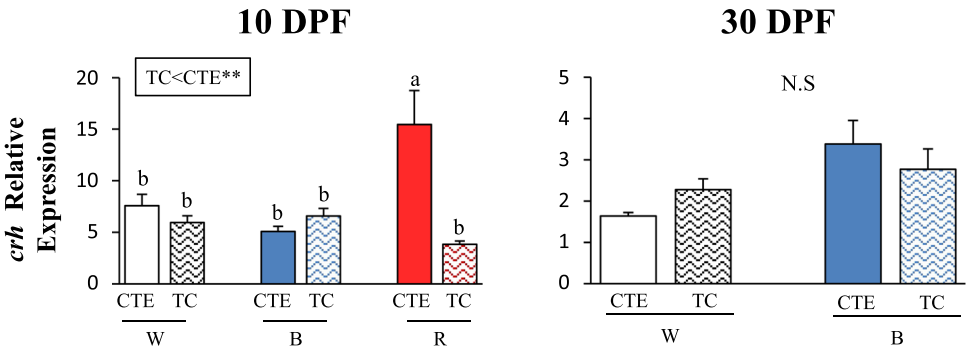

FIGURE 6 Relative mRNA expression of *crh* of the 10 (left panel) and 30 (right panel) days post fertilization (dpf) zebrafish larvae reared in three different light wavelengths (white, W; blue, B; red, R) and in two temperature regimes (a thermocycle of 28:24°C thermophase:cryophase, TC; and constant temperature of 26.1 ± 0.1°C, CTE). Different lower case letters indicate significant differences between the experimental groups on the same dpf (one-way ANOVA, $p < 0.05$). Different upper case letters and asterisks denote significant differences between the light treatments and rearing temperature regimes, respectively, on the same dpf (two-way ANOVA, $p < 0.05$). Data ($n = 6$) are represented as mean ± SEM. ANOVA, analysis of variance; mRNA, messenger RNA

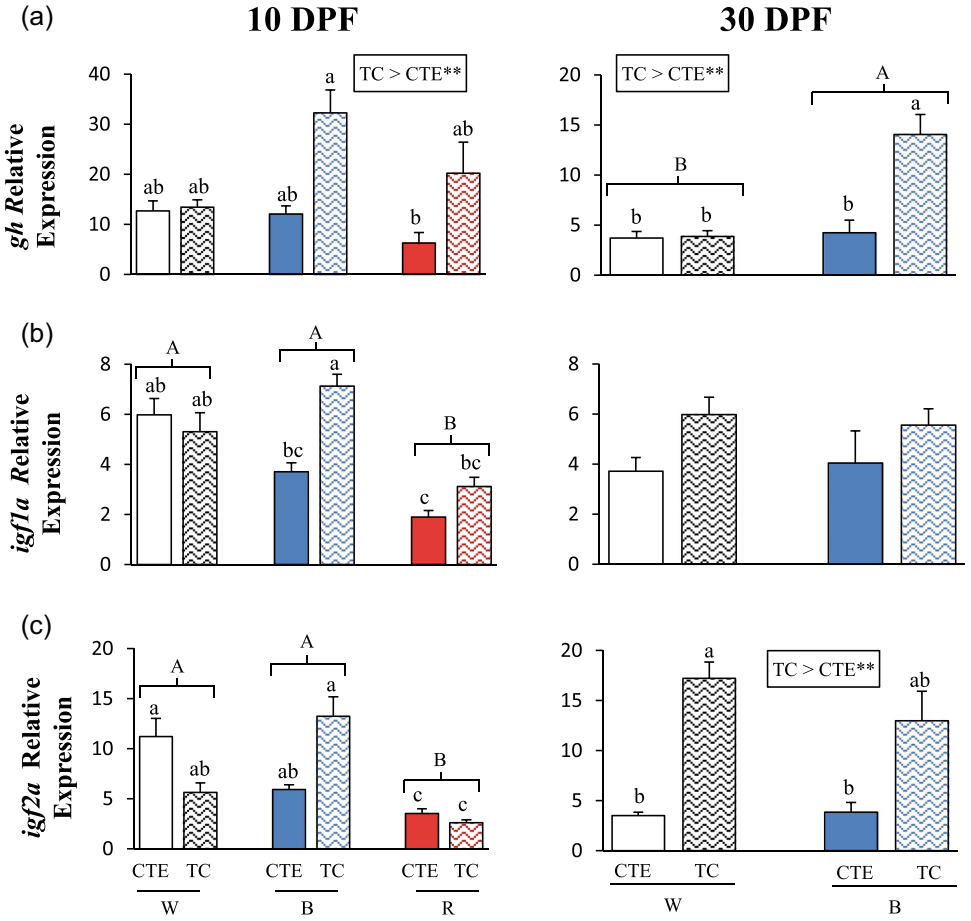

FIGURE 7 Relative mRNA expression of *gh* (a), *igf1a* (b), and *igf2a* (c) of the 10 (left panels) and 30 (right panels) days post fertilization (dpf) zebrafish larvae reared in three different light wavelengths (white, W; blue, B; red, R) and in two temperature regimes (a thermocycle of 28:24°C thermophase:cryophase, TC; and constant temperature of 26.1 ± 0.1°C, CTE). Different lower case letters indicate significant differences between the experimental groups on the same dpf (one-way ANOVA, $p < 0.05$). Different upper case letters and asterisks denote significant differences between the light treatments and rearing temperature regimes, respectively, on the same dpf (two-way ANOVA, $p < 0.05$). Data ($n = 6$) are represented as mean ± SEM. ANOVA, analysis of variance; mRNA, messenger RNA

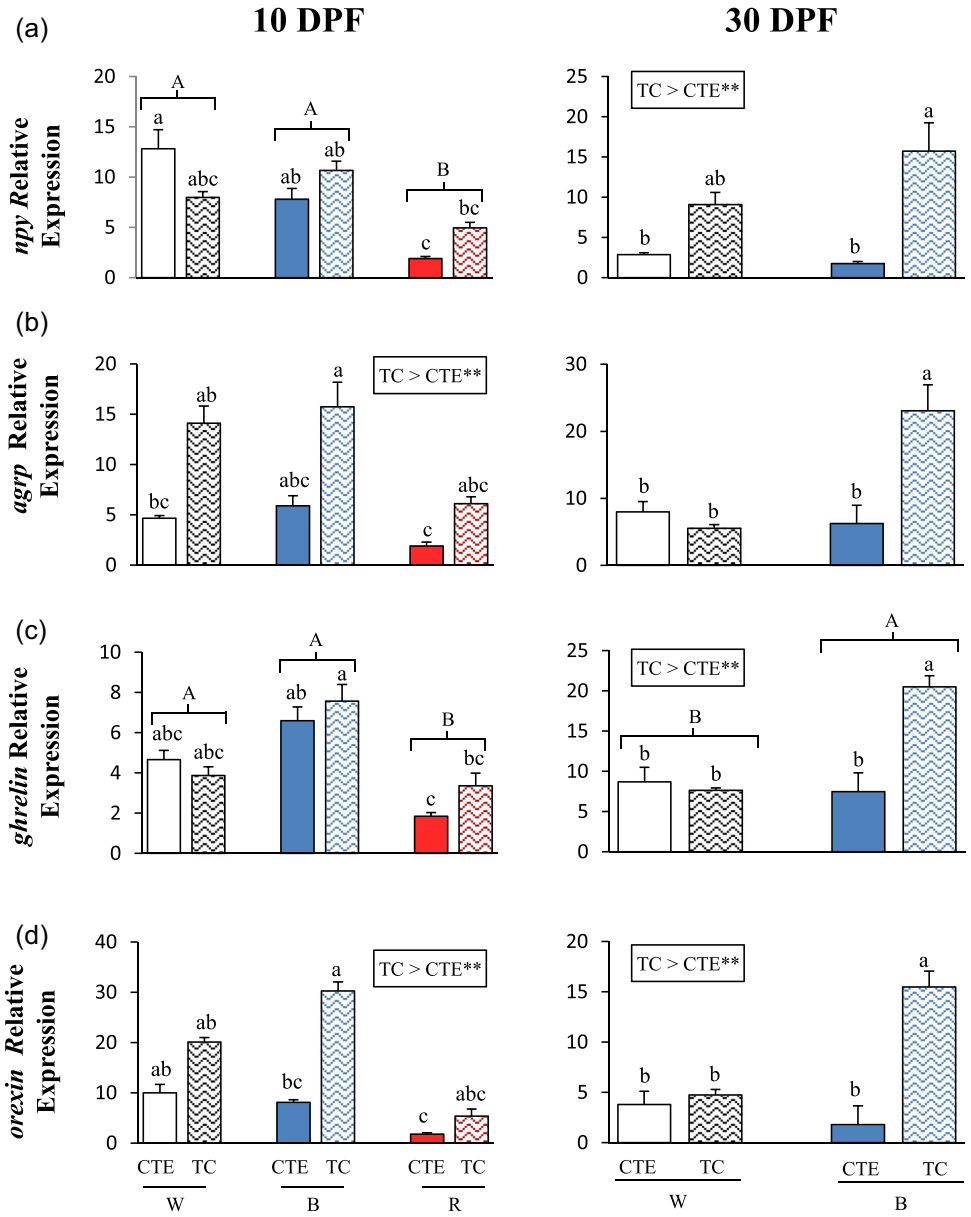

FIGURE 8 Relative mRNA expression of *npy* (a), *agrp* (b), *ghrelin* (c), *orexin* (d), *mch1* (e), *mch2* (f), *grp* (g), and *cck8* (h) of the 10 (left panels) and 30 (right panels) days post fertilization (dpf) zebrafish larvae reared in three different light wavelengths (white, W; blue, B; red, R) and in two temperature regimes (a thermocycle of 28:24°C thermophase:cryophase, TC; and constant temperature of 26.1 ± 0.1°C, CTE). Different lower case letters indicate significant differences between the experimental groups on the same dpf (one-way ANOVA, $p < 0.05$). Different upper case letters and asterisks denote significant differences between the light treatments and rearing temperature regimes, respectively, on the same dpf (two-way ANOVA, $p < 0.05$). Data ($n = 6$) are represented as mean ± SEM. ANOVA, analysis of variance; mRNA, messenger RNA

3.3.3 | Genes involved in food intake control

As the different treatments affected food intake, we evaluated the mRNA expression of several hormones involved in the endocrine control of food intake and digestion. These factors intervene as either appetite-stimulating factors (orexigenic peptides: *npy*, *agrp*, *ghrelin*, *orexin*, *mch1*, and *mch2*) or appetite-inhibiting factors (anorexigenic peptides: *grp* and *cck8*).

At 10 dpf, significant intergroup differences were observed in all the genes (Figure 8, left panels) (one-way ANOVA, $p < 0.05$; Table S1), except for *mch1* (one-way ANOVA, $p > 0.05$). The highest expression levels were generally detected in the larvae reared in B + TC, whereas the lowest expression was detected in the group reared in R + CTE (Figure 8, left panels). The expression of *ghrelin*, *mch2*, and *grp* was also higher in B + TC than in R + TC (Figure 8c,f,g). The expression of *orexin*, *mch2*, and *grp* was higher in B + TC than in

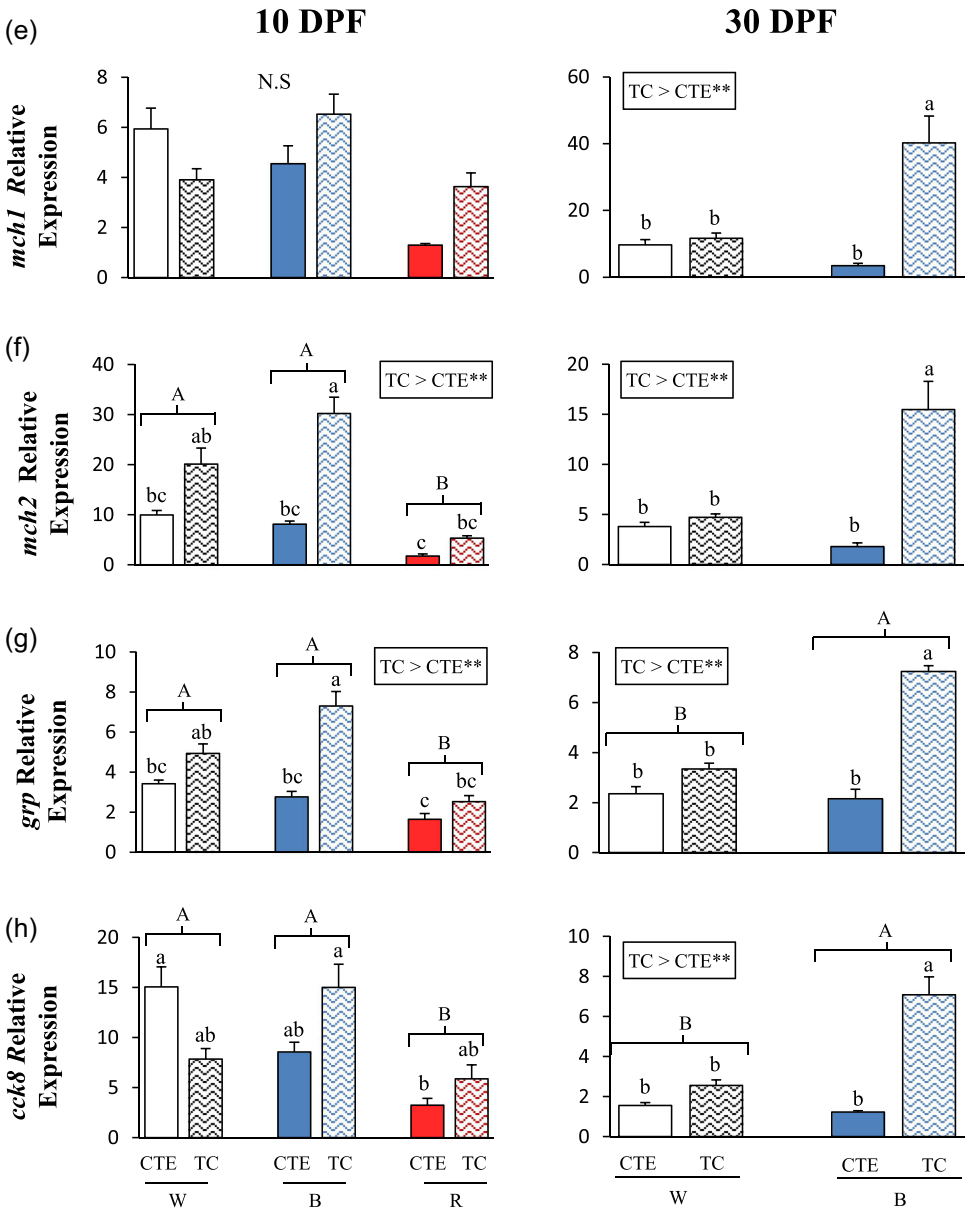

FIGURE 8 Continued

B + CTE (Figure 8d,f,g). The two-way ANOVA revealed significant differences depending on lighting conditions in the expression of *npy*, *ghrelin*, *mch2*, *grp*, and *cck8* (Figure 8a,c,f,g,h) (two-way ANOVA, $p < 0.05$; Table S1). In these genes, the expression levels of the larvae reared in W or B lights were higher than the larvae in R. Significant differences were also reported according to the temperature regime in *agrp*, *orexin*, *mch2*, and *grp* (Figure 8b,d,f,g) (two-way ANOVA, $p < 0.05$). In these genes, the larvae in TC presented higher expression levels than those in CTE.

At 30 dpf, the expression of the analyzed genes involved in food intake control followed similar patterns. All the genes showed significant intergroup differences (Figure 8, right panels) (one-way ANOVA, $p < 0.05$; Table S1). The B + TC-reared larvae showed a higher expression than the other groups (B + CTE, W + TC, and W + CTE) in all the analyzed genes, except for *npy*, for which differences were only found between B + TC versus W + CTE and B + CTE (Figure 8a). The two-way ANOVA also revealed a significant effect of lighting conditions on *ghrelin*, *grp*, and *cck8* (Figure 8c,g,h) ($p < 0.05$; Table S1). The expression levels of these genes were higher in the larvae in B light than in W. All the genes except for *agrp* showed a significant effect of temperature regime, with the groups in TC displaying higher expression levels than in CTE (Figure 8, right panels) (two-way ANOVA, $p < 0.05$).

4 | DISCUSSION

The present research findings revealed that the combined effects of the light spectrum and daily temperature conditions (cycling vs. constant) considerably influenced zebrafish development from very

early stages. The most favorable combination was blue light with daily thermocycles (B + TC), which positively affected hatching and survival, and stimulated growth and food intake. On the contrary, the combination of red light and constant temperature (R + CTE) obtained the worst results. These profound effects were consistent with the results obtained in the gene expression analysis performed under the different environmental conditions.

The effect of different light intensities and spectrums has been reviewed in fish (Villamizar et al., 2011). In zebrafish, the use of short wavelengths enhanced early larval development whereas long wavelengths caused the worst results (Villamizar et al., 2014). In addition to light wavelength, the use of thermocycles versus constant temperatures showed similar beneficial effects in zebrafish larval performance (Villamizar et al., 2012). However, the combined effects of light spectrum and daily thermocycles on fish larval development, as well as on the underlying molecular mechanisms that lead to these effects, had not been studied to date in zebrafish. Our results showed that the zebrafish larvae reared in lights W and B had a higher hatching rate than those kept in light R. This finding suggests that zebrafish embryos can detect light wavelength from very early development stages. In agreement with our findings, other studies have revealed that zebrafish hatching is affected by light spectrum, and ranges from 90.1% in violet light to 79.4% in red light (Villamizar et al., 2014). However, in other species, such as turbot (*Scophtalmus maximus*), the use of different light spectra led to no substantial differences in hatching rates (Wu et al., 2019). About the temperature regime effect, the lack of differences in the hatching rate between CTE and TC herein observed agrees with other studies performed in zebrafish and Nile tilapia (*Oreochromis niloticus*) (Santo et al., 2020; Villamizar et al., 2012).

The present paper revealed that zebrafish larvae showed a higher survival rate for the B and W light spectra than for R. Indeed, all the larvae reared in light R died while developing. This implies that light wavelength is one of the main factors for survival rates. The light spectrum effect during early fish development seems species-specific. In Senegalese sole and Russian Sturgeon (*Acipenser baerii*), short wavelengths increase survival as in zebrafish (Blanco-Vives et al., 2010; Ruchin, 2016; Villamizar et al., 2014). In contrast, European eel (*Anguilla anguilla)* survival is enhanced by long wavelengths (Politis et al., 2014). Regarding the temperature regime, the effects of daily thermocycles on fish survival have been poorly studied to date. In the present research work, daily thermocycles were relevant on the first days of larval development. Thermocycles enhanced the survival of the larvae reared in R light because those reared with daily thermocycles died later than those maintained at a constant temperature. However, thermocycles were not enough to counteract the deleterious effects of red light. In Senegalese sole and brook trout (*Salvelinus fontinalis*), beneficial effects of thermocycles in early developmental stages have been described: they increased survival rate and lowered the incidence of malformations (Blanco-Vives et al., 2010; Pisano et al., 2019).

As we observed marked differences in survival, growth, and feeding between experimental groups, we went on to elucidate the underlying molecular mechanisms that could lead to these effects. The present study analyzed the expression of the genes involved in processes like survival, growth, and food intake control to investigate whether the differences in biometric parameters can be explained by the differences in the expression of these factors. Of them, *crh* expression was evaluated to explain survival differences. Higher *crh* expression levels are related to the stress response in zebrafish and can limit important physiological processes like food intake, survival, and larval development (Alderman & Bernier, 2009; Ruchin, 2020; 2021). In our experiments, the larvae reared in R and CTE obtained the highest *crh* expression levels, which coincides with previous studies performed in zebrafish and European sea bass (Villamizar et al., 2014; Yan et al., 2019). However, the use of thermocycles lowered both the *crh* expression levels and survival rate differences with the remaining groups. This scenario suggests that zebrafish larvae under red light and constant temperature may be under a stressed state, which could be partly responsible for the deleterious effects of these environmental conditions. Daily thermocycles may improve zebrafish larval survival by minimizing such stressing effects.

The B light and TC temperature combination led to the highest growth rate. In agreement with our results, the use of daily thermocycles has also shown better early development for zebrafish (Villamizar et al., 2012), Nile tilapia (Espirito Santo et al., 2020), Senegalese sole (Blanco-Vives et al., 2010), green sturgeon (Rodgers et al., 2018), and perch (Coulter et al., 2016). Nevertheless, the effect of daily thermocycles on the growth rate depends on the fish species. Nonsignificant effects have been found for juvenile Nile tilapia (Azaza et al., 2010) and Chinese bream (*Parabramis pekinensis*) (Peng et al., 2014), while a lower growth rate has been reported for rainbow trout (*Oncorhynchus mykiss*) (Flodmark et al., 2004), Atlantic Salmon (*Salmo salar)* (Imholt et al., 2011; Morissette et al., 2021), and brook trout (Chadwick & McCormick, 2017). Not only can daily thermocycles have major effects on growth, but so can the light spectrum. In general, higher growth rates with shorter than longer wavelengths have been reported in zebrafish (Villamizar et al., 2014), European sea bass (*Dicentrarchus labrax*) (Villamizar et al., 2009), Senegalese sole (Blanco-Vives et al., 2010), Atlantic cod (*Gadus morhua*) (Migaud et al., 2009; Sierra-Flores et al., 2016), gilthead seabream (*Sparus aurata*) (Karakatsouli et al., 2007), turbot larvae (Sierra-Flores et al., 2016), goldfish (*Carassius carassius*), Chinese sleeper (*Perccottus glenii*), and guppy (*Poecilia reticulata*) juveniles (Ruchin, 2004, 2016, 2020). However, other species like rainbow trout, perch, and common carp (*Cyprinus carpio*) have presented higher growth, weight gain, and feed efficiency rates with red light (Head & Malison, 2000; Karakatsouli et al., 2008, 2010).

Very little information is available about how the combination of different light spectra and thermal regimes can act on fish growth. In the present study, short wavelengths stimulated the mRNA expression levels of somatic growth factors (*gh*, *igf1a*, and *igf2a*), which correlated with a higher growth rate, while long wavelengths reduced the expression of growth factors and negatively influenced the growth rate, and probably other physiological functions (Canosa & Bertucci, 2020; Reinecke et al., 2005; Saera-Vila et al., 2009).

The stimulatory effect of short wavelength on growth factors has also been recently described in yellowtail clownfish (*Amphiprion clarkia*) (Shin et al., 2012), goldfish (*Carassius auratus*) (Shin & Choi, 2014), barfin flounder (*Verasper moseri*) (Takahashi et al., 2004, 2016; Yamanome et al., 2009), and zebrafish (Villamizar et al., 2014). In other studies performed in rainbow trout and European sea bass, the somatic growth factors were stimulated in red light (Karakatsouli et al., 2007, 2008; Yan et al., 2019). Apart from the light wavelength, water temperature is also a determining factor that directly affects embryonic and fish larval growth. This environmental cue can modify the plasma levels and mRNA expression of GH and IGFs (Gabillard et al., 2005). In most fish species, higher temperatures increase the expression of GH and IGF1, which fluctuates in parallel to the growth rate. However, cold temperatures decrease the expression profiles of growth factors (for a review, see Deane & Woo, 2009). In the natural environment, seasonal water temperature changes affect the activities of metabolic enzymes and expression profiles by increasing the growth rate and the mRNA expression at the GH level, and in warmer months compared to colder months (for a review, see Deane & Woo, 2009). In addition to seasonal water temperature changes, many fish in nature undergo daily temperature fluctuations. The results of the present research work suggest that thermocycles can enhance the growth rate by upregulating the expression of several growth factors, but more studies are necessary to test this hypothesis.

The findings observed in the growth and survival rates can be partly explained by the differences in the food intake levels between the combinations of light wavelengths and temperature regimes. In several fish species, light wavelength influences food consumption and the efficiency of its utilization for fish growth (for a review see Ruchin 2020). The zebrafish larvae reared in the W and B light wavelengths presented a higher food intake than those in R light in correlation to survival and growth parameters. Similar findings have been found in haddock larvae with a higher feeding rate in blue light than either full-spectrum (white) or green light (Downing & Litvak, 2001; Downing, 2002). This more efficient adaptive response of zebrafish to shorter wavelengths can be mediated by visual and nonvisual photopigments (Villamizar et al., 2014). With blue wavelengths, the retina of zebrafish larvae presents a large amount of UV and blue cones of zebrafish larvae retina (Allison et al., 2010). Thus the marked presence of these visual pigments may have helped the zebrafish reared in blue light to acquire greater visual acuity for capturing food and enhancing the feeding rate (Ruchin 2020).

Temperature also played an important role in food intake because, for instance, the larvae reared in W + TC obtained higher food intake than those in W + CTE. These results agree with previous research works performed in Nile tilapia, in which the larvae reared in TC presented higher growth rates than those reared in CTE. These results suggest that thermocycles can enhance food intake and digestion process synchronization by improving feeding efficacy and, consequently, growth rates (Espirito Santo et al., 2020). Both short wavelengths and thermocycles directly affect the intake control system. For this reason, we analyzed the mRNA expression of orexigenic (*npy*, *agrp*, *ghrelin*, *orexin*, *mch1*, *mch2*) and anorexigenic (*grp* and *cck*) neuropeptides. Npy acts as a potent orexigenic neuropeptide involved in feeding regulation and Gh stimulation in fish (Delgado et al., 2017; Matsuda, Azuma, et al., 2012; Yokobori et al., 2012). Gh can act in AgRP production, a stimulator of appetite and feeding. Ghrelin is a peptide that exerts a synergic effect with orexin, and both stimulate Gh secretion and appetite via Npy/AgRP (Kaiya et al., 2008; Matsuda, Sakashita, et al., 2012; Yokobori et al., 2012). Recently, Mch has been considered to be a potent orexigenic regulator of fish appetite because it participates in the transduction of photic conditions by modulating feeding behavior (Takahashi et al., 2004, 2014). Cck and Grp act as satiety (anorexigenic) signals in fish and participate in the secretion of pancreatic enzymes (Koven & Schulte, 2012; Volkoff et al., 2005). In the present study, the different light and temperature treatments induced differences in the expression of most of the analyzed genes. Regarding lighting conditions, all the genes involved in food intake control and digestion, except for *agrp*, *orexin*, and *mch1*, presented higher expression levels in B and W than in R. Similar stimulatory effects of short wavelengths on expressions *mch1* and *mch2* in association with *npy* and *orexin* have been described in spotted halibut (*Verasper variegatus*) (Shimizu et al., 2019), barfin flounder (Takahashi et al., 2004, 2016, 2018; Yamanome et al., 2009), yellowtail clownfish (Shin et al., 2012) and goldfish (Shin & Choi, 2014). In these studies, the stimulatory effects of short wavelengths on the food intake system correlated with enhanced food intake and subsequently higher levels of somatic growth factors and growth rate, which also occurred in our study. Regarding temperature, the effect of diel thermal cycles on the endocrine control of feeding and appetite has been less investigated than light. Konstantinov et al. (2005) showed that young sturgeons reared under daily and seasonal water changes presented higher feeding and food energy to growth than those kept at a constant temperature. Here we observed that TC increased the expression of several of the peptides involved in food intake control (*agrp*, *orexin*, *mch2*) and digestion (*grp*) in very early development stages, such as 10 dpf. Moreover, the stimulatory effects of thermocycles were more pronounced at 30 dpf as the expression of all the genes was higher in TC than in CTE (except for *agrp*). One hypothesis to explain this effect is that circadian clocks can be entrained by temperature cycles (Lahiri et al., 2005). It has been suggested in Nile tilapia that thermocycles induce a better synchronization of the rhythms of food intake factors to, thus, improve the timing of digestion processes, metabolism efficiency, and, consequently, growth (do Espirito Santo et al., 2020). However, more research is necessary to test this hypothesis.

5 | CONCLUSION

As far as we know, the present research is the first to show that the combination of light wavelengths and different temperature regimes considerably affects fish larval development by generating changes in both biometric parameters (hatching, survival, growth, food intake) and the mRNA expression of the genes related to them. The combined effects of both shorter wavelengths and thermocycles, which

come closer to the natural underwater environment, positively influenced most of the analyzed parameters. In addition, the present study identified several physiological factors that are affected by long wavelengths and whose changes may be responsible for the deadly effects observed when fish are reared under red light.

From a practical point of view, our findings suggest that using similar environmental conditions that fish experience in their aquatic environment may have a beneficial impact on both their development and survival. Therefore, these findings should be considered to optimize the growth and welfare of the fish reared in a laboratory and fish farming.

ACKNOWLEDGMENTS

This study was funded by Project "BLUESOLE" (AGL2017-82582-C3-3-R), granted by the Spanish Ministry of Economic Affairs and Competitiveness (MINECO) to FJSV; by project "CHRONOLIPOFISH" (RTI2018-100678-A-I00), granted by the Spanish Ministry of Science to JFLO; the thematic network in fish chronobiology (CRONOFISH), granted by the Spanish MINECO (RED2018-102487-T); and Project CHRONOHEALTH (Seneca Foundation, 19899/GERM/15). JFLO was also funded by a "*Ramón y Cajal*" research fellowship granted by the Spanish MINECO (RYC-2016-20959).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Gonzalo de Alba, Sherezade Carrillo, José Fernando López-Olmeda, and Francisco Javier Sánchez-Vázquez conceived and designed the experiments, and wrote the manuscript; Gonzalo de Alba and Sherezade Carrillo performed the experiments; Gonzalo de Alba, Sherezade Carrillo, and José Fernando López-Olmeda analyzed the data; Francisco Javier Sánchez-Vázquez and José Fernando López-Olmeda provided funding.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with Image *J. Biophotonics international*, *11*(7), 36–42.

Alderman, S. L., & Bernier, N. J. (2009). Ontogeny of the corticotropin-releasing factor system in zebrafish. *General and Comparative Endocrinology*, *164*(1), 61–69.

Allison, W. T., Barthel, L. K., Skebo, K. M., Takechi, M., Kawamura, S., & Raymond, P. A. (2010). Ontogeny of cone photoreceptor mosaics in zebrafish. *Journal of Comparative Neurology*, *518*(20), 4182–4195.

Aschoff, J. (1981). *Handbook of behavioral neurobiology. Biological rhythms* (Vol. 4). Plenum Press.

Azaza, M. S., Legendre, M., Kraiem, M. M., & Baras, E. (2010). Size-dependent effects of daily thermal fluctuations on the growth and size heterogeneity of Nile tilapia *Oreochromis niloticus*. *Journal of Fish Biology*, *76*(3), 669–683.

Bertucci, J. I., Blanco, A. M., Sundarrajan, L., Rajeswari, J. J., Velasco, C., & Unniappan, S. (2019). Nutrient regulation of endocrine factors influencing feeding and growth in fish. *Frontiers in Endocrinology*, *10*, 83.

Blanco-Vives, B., Villamizar, N., Ramos, J., Bayarri, M. J., Chereguini, O., & Sánchez-Vázquez, F. J. (2010). Effect of daily thermo-and photocycles of different light spectrum on the development of Senegal sole (*Solea senegalensis*) larvae. *Aquaculture*, *306*(1–4), 137–145.

Blanco-Vives, B., Vera, L. M., Ramos, J., Bayarri, M. J., Mañanós, E., & Sánchez-Vázquez, F. J. (2011). Exposure of larvae to daily thermocycles affects gonad development, sex ratio, and sexual steroids in *Solea senegalensis*, Kaup. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *315*(3), 162–169.

Canosa, L. F., & Bertucci, J. I. (2020). Nutrient regulation of somatic growth in teleost fish. The interaction between somatic growth, feeding and metabolism. *Molecular and Cellular Endocrinology*, *518*, 111029.

Chadwick, J. G., & McCormick, S. D. (2017). Upper thermal limits of growth in brook trout and their relationship to stress physiology. *Journal of Experimental Biology*, *220*(21), 3976–3987.

Coulter, D. P., Sepúlveda, M. S., Troy, C. D., & Höök, T. O. (2016). Species-specific effects of sub-daily temperature fluctuations on consumption, growth and stress responses in two physiologically similar fish species. *Ecology of Freshwater Fish*, *25*(3), 465–475.

Deane, E. E., & Woo, N. Y. (2009). Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: A review. *Reviews in Fish Biology and Fisheries*, *19*(1), 97–120.

Delgado, M. J., Cerdá-Reverter, J. M., & Soengas, J. L. (2017). Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: Involvement in the control of food intake. *Frontiers in Neuroscience*, *11*(1), 354.

Downing, G. (2002). Impact of spectral composition on larval haddock, *Melanogrammus aeglefinus L.*, growth and survival. *Aquaculture Research*, *33*(4), 251–259.

Downing, G., & Litvak, M. K. (2001). The effect of light intensity and spectrum on the incidence of first feeding by larval haddock. *Journal of Fish Biology*, *59*(6), 1566–1578.

Falcón, J., Gothilf, Y., Coon, S. L., Boeuf, G., & Klein, D. C. (2003). Genetic, temporal and developmental differences between melatonin rhythm generating systems in the teleost fish pineal organ and retina. *Journal of Neuroendocrinology*, *15*(4), 378–382.

Flodmark, L. E. W., Vøllestad, L. A., & Forseth, T. (2004). Performance of juvenile brown trout exposed to fluctuating water level and temperature. *Journal of Fish Biology*, *65*(2), 460–470.

Gabillard, J. C., Weil, C., Rescan, P. Y., Navarro, I., Gutiérrez, J., & Le Bail, P. Y. (2005). Does the GH/IGF system mediate the effect of water temperature on fish growth? A review. *Cybium*, *29*(2), 107–117.

Hankins, M. W., Davies, W. I., & Foster, R. G. (2014). The evolution of non-visual photopigments in the central nervous system of vertebrates, In D. M. Hunt (ed.), *Evolution of visual and non-visual pigments* (pp. 65–103). Springer.

Head, A. B., & Malison, J. A. (2000). Effects of lighting spectrum and disturbance level on the growth and stress responses of yellow perch *Perca flavescens*. *Journal of the World Aquaculture Society*, *31*(1), 73–80.

Ikegami, T., Takeuchi, Y., Hur, S. P., & Takemura, A. (2014). Impacts of moonlight on fish reproduction. *Marine Genomics*, *14*, 59–66.

Imholt, C., Malcolm, I. A., Bacon, P. J., Gibbins, C. N., Soulsby, C., Miles, M., & Fryer, R. J. (2011). Does diurnal temperature variability affect growth in juvenile Atlantic salmon *Salmo salar*? *Journal of Fish Biology*, *78*(2), 436–448.

Kaiya, H., Miyazato, M., Kangawa, K., Peter, R. E., & Unniappan, S. (2008). Ghrelin: A multifunctional hormone in non-mammalian vertebrates.

Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, *149*(2), 109–128.

Karakatsouli, N., Papoutsoglou, E. S., Sotiropoulos, N., Mourtikas, D., Stigen-Martinsen, T., & Papoutsoglou, S. E. (2010). Effects of light spectrum, rearing density and light intensity on growth performance of scaled and mirror common carp *Cyprinus carpio* reared under recirculating system conditions. *Aquacultural Engineering*, *42*(3), 121–127.

Karakatsouli, N., Papoutsoglou, S. E., Panopoulos, G., Papoutsoglou, E. S., Chadio, S., & Kalogiannis, D. (2008). Effects of light spectrum on growth and stress response of rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions. *Aquacultural Engineering*, *38*(1), 36–42.

Karakatsouli, N., Papoutsoglou, S. E., Pizzonia, G., Tsatsos, G., Tsopelakos, A., Chadio, S., & Papadopoulou-Daifoti, Z. (2007). Effects of light spectrum on growth and physiological status of gilthead seabream *Sparus aurata* and rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions. *Aquacultural Engineering*, *36*(3), 302–309.

Konstantinov, A. S., Pushkar, V. Y., Zdanovich, V. V., Aver'yanova, O. V., & Rechinskiy, V. V. (2005). Energy budget of some young sturgeons under optimal static and astatic thermal conditions. *Hydrobiological Journal*, *41*, 34–44.

Konstantinov, A. S., Vechkanov, V. S., Kuznetsov, V. A., & Ruchin, A. B. (2003). Effect of fluctuation of intensity and spectral structure of light on the growth and energetics of young fish. *Hydrobiological Journal*, *39*, 95–106.

Koven, W., & Schulte, P. (2012). The effect of fasting and refeeding on mRNA expression of PepT1 and gastrointestinal hormones regulating digestion and food intake in zebrafish (*Danio rerio*). *Fish Physiology and Biochemistry*, *38*(6), 1565–1575.

Kusmic, C., & Gualtieri, P. (2000). Morphology and spectral sensitivities of retinal and extraretinal photoreceptors in freshwater teleosts. *Micron*, *31*(2), 183–200.

Lahiri, K., Vallone, D., Gondi, S. B., Santoriello, C., Dickmeis, T., & Foulkes, N. S. (2005). Temperature regulates transcription in the zebrafish circadian clock. *PLOS Biology*, *3*(11), e351.

López-Olmeda, J. F., & Sánchez-Vázquez, F. J. (2011). Thermal biology of zebrafish (*Danio rerio*). *Journal of Thermal Biology*, *36*(2), 91–104.

López-Olmeda, J. F., Madrid, J. A., & Sánchez-Vázquez, F. J. (2006). Light and temperature cycles as zeitgebers of zebrafish (*Danio rerio*) circadian activity rhythms. *Chronobiology International*, *23*(3), 537–550.

Mangor-Jensen, A., & Waiwood, K. G. (1995). The effect of light exposure on buoyancy of halibut eggs. *Journal of Fish Biology*, *47*(1), 18–25.

Matsuda, K. (2013). Regulation of feeding behavior and psychomotor activity by corticotropin-releasing hormone (CRH) in fish. *Frontiers in Neuroscience*, *7*, 91.

Matsuda, K., Azuma, M., & Kang, K. S. (2012). Orexin system in teleost fish. *Vitamins & Hormones*, *89*, 341–361.

Matsuda, K., Sakashita, A., Yokobori, E., & Azuma, M. (2012). Neuroendocrine control of feeding behavior and psychomotor activity by neuropeptideY in fish. *Neuropeptides*, *46*(6), 275–283.

McFarland, W. N. (1986). Light in the sea—Correlations with behaviors of fishes and invertebrates. *American Zoologist*, *26*(2), 389–401.

Migaud, H., Davie, A., Carboni, S., Murray, J., & Lysaa, P. A. (2009). Treasurer effects of light on Atlantic cod (*Gadus morhua*) larvae performances: Focus on spectrum. *Fish and Shellfish Larviculture Symposium*, *1*(4), 265–269.

Morash, A. J., Neufeld, C., MacCormack, T. J., & Currie, S. (2018). The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. *Journal of Experimental Biology*, *221*(14), 1–14.

Morissette, J., Swart, S., MacCormack, T. J., Currie, S., & Morash, A. J. (2021). Thermal variation near the thermal optimum does not affect the growth, metabolism or swimming performance in wild Atlantic Salmon *Salmo salar*. *Journal of Fish Biology*, *98*(6), 1585–1589.

Nusslein-Volhard, C., & Dahm, R. (2000). *Zebrafish* (Vol. 8, pp. 1–31). Oxford University Press.

Payne, A., Temple, S., & Singh, H. R. (1996). River and floodplain fisheries in the Ganges Basin. *Final Technical Report*, *5485*(1), 1–15.

Peng, J., Cao, Z. D., & Fu, S. J. (2014). The effects of constant and diel-fluctuating temperature acclimation on the thermal tolerance, swimming capacity, specific dynamic action and growth performance of juvenile Chinese bream. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *176*, 32–40.

Pisano, O. M., Kuparinen, A., & Hutchings, J. A. (2019). Cyclical and stochastic thermal variability affects survival and growth in brook trout. *Journal of Thermal Biology*, *84*, 221–227.

Politis, S. N., Butts, I. A., & Tomkiewicz, J. (2014). Light impacts embryonic and early larval development of the European eel, *Anguilla anguilla*. *Journal of Experimental Marine Biology and Ecology*, *461*, 407–415.

Reinecke, M., Björnsson, B. T., Dickhoff, W. W., McCormick, S. D., Navarro, I., Power, D. M., & Gutiérrez, J. (2005). Growth hormone and insulin-like growth factors in fish: Where we are and where to go. *General and Comparative Endocrinology*, *142*(1-2), 20–24.

Rensing, L., & Ruoff, P. (2002). Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. *Chronobiology International*, *19*(5), 807–864.

Ribas, L., & Piferrer, F. (2014). The zebrafish (*Danio rerio*) as a model organism, with emphasis on applications for finfish aquaculture research. *Reviews in Aquaculture*, *6*, 209–240.

Rodgers, E. M., Cocherell, D. E., Nguyen, T. X., Todgham, A. E., & Fangue, N. A. (2018). Plastic responses to diel thermal variation in juvenile green sturgeon, *Acipenser medirostris*. *Journal of Thermal Biology*, *76*, 147–155.

Ruchin, A. B. (2004). Influence of colored light on growth rate of juveniles of fish. *Fish Physiology and Biochemistry*, *30*(2), 175–178.

Ruchin, A. B. (2016). Effect of light on the development of the hard roe of *Acipenser baerii* Brandt. 1869. *Indian Journal of Science and Technology*, *9*, 89110.

Ruchin, A. B. (2020). Environmental colour impact on the life of lower aquatic vertebrates: Development, growth, physiological and biochemical processes. *Reviews in Aquaculture*, *12*(1), 310–327.

Ruchin, A. B. (2021). Effect of illumination on fish and amphibian: Development, growth, physiological and biochemical processes. *Reviews in Aquaculture*, *13*(1), 567–600.

Rønnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jönsson, E., & Volkoff, H. (2017). Appetite-controlling endocrine systems in teleosts. *Frontiers in Endocrinology*, *8*, 73.

Saera-Vila, A., Calduch-Giner, J. A., Prunet, P., & Pérez-Sánchez, J. (2009). Dynamics of liver GH/IGF axis and selected stress markers in juvenile gilthead sea bream (*Sparus aurata*) exposed to acute confinement: differential stress response of growth hormone receptors. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *154*(2), 197–203.

Sánchez-Vázquez, F. J., & López-Olmeda, J. F. (2018). Environmental cycles and biological rhythms during early development. In M. Yúfera (Ed.), *Emerging issues in fish larvae research* (pp. 37–50). Springer.

Santo, A. H. E., de Alba, G., da Silva Reis, Y., Costa, L. S., Sánchez-Vázquez, F. J., Luz, R. K., & López-Olmeda, J. F. (2020). Effects of temperature regime on growth and daily rhythms of digestive factors in Nile tilapia (*Oreochromis niloticus*) larvae. *Aquaculture*, *528*(1), 735545.

Shimizu, D., Kasagi, S., Takeuchi, R., Maeda, T., Furufuji, S., Mizusawa, K., & Takahashi, A. (2019). Effects of green light on the growth of spotted halibut, *Verasper variegatus*, and Japanese flounder, *Paralichthys olivaceus*, and on the endocrine system of spotted halibut at different water temperatures. *General and Comparative Endocrinology*, *271*, 82–90.

Shin, H. S., & Choi, C. Y. (2014). The stimulatory effect of LED light spectra on genes related to photoreceptors and skin pigmentation in goldfish (*Carassius auratus*). *Fish Physiology and Biochemistry*, *40*(4), 1229–1238.

Shin, H. S., Lee, J., & Choi, C. Y. (2012). Effects of LED light spectra on the growth of the yellowtail clownfish Amphiprion clarkii. *Fisheries Science*, *78*(3), 549–556.

Sierra-Flores, R., Davie, A., Grant, B., Carboni, S., Atack, T., & Migaud, H. (2016). Effects of light spectrum and tank background colour on Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) larvae performances. *Aquaculture*, *450*, 6–13.

Takahashi, A., Kasagi, S., Murakami, N., Furufuji, S., Kikuchi, S., Mizusawa, K., & Andoh, T. (2016). Chronic effects of light irradiated from LED on the growth performance and endocrine properties of barfin flounder *Verasper moseri*. *General and Comparative Endocrinology*, *232*, 101–108.

Takahashi, A., Kasagi, S., Murakami, N., Furufuji, S., Kikuchi, S., Mizusawa, K., & Andoh, T. (2018). Effects of different green light intensities on the growth performance and endocrine properties of barfin flounder *Verasper moseri*. *General and Comparative Endocrinology*, *257*, 203–210.

Takahashi, A., Mizusawa, K., & Amano, M. (2014). Multifunctional roles of melanocyte-stimulating hormone and melanin-concentrating hormone in fish: Evolution from classical body color change. *Aqua-BioScience Monographs*, *7*(1), 1–46.

Takahashi, A., Tsuchiya, K., Yamanome, T., Amano, M., Yasuda, A., Yamamori, K., & Kawauchi, H. (2004). Possible involvement of melanin-concentrating hormone in food intake in a teleost fish, B*arfin flounder*. *Peptides*, *25*(10), 1613–1622.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115.

Vatine, G., Vallone, D., Gothilf, Y., & Foulkes, N. S. (2011). It's time to swim! Zebrafish and the circadian clock. *FEBS Letters*, *585*(10), 1485–1494.

Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S., & Sanchez-Vazquez, F. J. (2011). Effects of light during early larval development of some aquacultured teleosts: A review. *Aquaculture*, *315*(1-2), 86–94.

Villamizar, N., García-Alcazar, A., & Sánchez-Vázquez, F. J. (2009). Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture*, *292*(1-2), 80–86.

Villamizar, N., Ribas, L., Piferrer, F., Vera, L. M., & Sánchez-Vázquez, F. J. (2012). Impact of daily thermocycles on hatching rhythms, larval performance and sex differentiation of zebrafish. *PLOS One*, *7*(12), e52153.

Villamizar, N., Vera, L. M., Foulkes, N. S., & Sánchez-Vázquez, F. J. (2014). Effect of lighting conditions on zebrafish growth and development. *Zebrafish*, *11*(2), 173–181.

Volkoff, H., Canosa, L. F., Unniappan, S., Cerda-Reverter, J. M., Bernier, N. J., Kelly, S. P., & Peter, R. E. (2005). Neuropeptides and the control of food intake in fish. *General and Comparative Endocrinology*, *142*(1-2), 3–19.

Wang, G. Q., & Xia, J. G. (2019). Effects of constant and diel-fluctuating temperature on thermal tolerance of zebrafish at different life-history stages. *Chinese Journal of Ecology*, *38*(1), 2133–2137.

Westerfield, M. (2000). *The zebrafish book. A guide for the laboratory use of zebrafish* (Danio rerio) (Vol. 1, pp. 1–10). Oregon Press.

Whitmore, D., Foulkes, N. S., & Sassone-Corsi, P. (2000). Light acts directly on organs and cells in culture to set the vertebrate circadian clock. *Nature*, *404*(6773), 87–91.

Wu, L., Han, M., Song, Z., Xu, S., Li, J., Li, X., & Li, X. (2019). Effects of different light spectra on embryo development and the performance of newly hatched turbot (*Scophthalmus maximus)* larvae. *Fish & Shellfish Immunology*, *90*, 328–337.

Yamanome, T., Mizusawa, K., Hasegawa, E. I., & Takahashi, A. (2009). Green light stimulates somatic growth in the barfin flounder *Verasper moseri*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *311*(2), 73–79.

Yan, H., Liu, Q., Cui, X., Shen, X., Hu, P., Liu, W., & Liu, Y. (2019). Growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae cultured under different light spectra and intensities. *Aquaculture Research*, *50*(8), 2066–2080.

Yokobori, E., Azuma, M., Nishiguchi, R., Kang, K. S., Kamijo, M., Uchiyama, M., & Matsuda, K. (2012). Neuropeptide Y stimulates food intake in the zebrafish, *Danio rerio*. *Journal of Neuroendocrinology*, *24*(5), 766–773.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: de Alba, G., Carrillo, S., Sánchez-Vázquez, F. J., & López-Olmeda, J. F. (2022). Combined blue light and daily thermocycles enhance zebrafish growth and development. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 337, 501–515. https://doi.org/10.1002/jez.2584