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RESEARCH

The crosstalk between photoperiod and early mild stress on juvenile oscar (*Astronotus ocellatus*) after acute stress

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Abstract Early mild stress (EMS) is like preparedness and might help fish deal with stress appropriately. This study investigated how EMS and photoperiod changes can impact growth, haematology, blood biochemistry, immunological response, antioxidant system, liver enzymes, and stress response of oscar (*Astronotus ocellatus*; 7.29 ± 0.96 g) before and after acute confinement stress (AC stress). Ten experimental treatments included five different photoperiods 8L16D (08:16 light to dark), 12L12D (12:12 light to dark), 16L8D (16:08 light to dark), 20L4D (20:04 light to dark), and 24L0D (24:00 light to dark), and these five photoperiod schedules were conducted in an EMS condition. After 9 weeks, no significant differences were found in growth parameters, survival rate, and body composition. At the end of the experiment and after AC stress, fish farmed in 24 light hours had the lowest haematocrit, white blood cells, total protein, blood performance, lysozyme, immunoglobulin M, complement C3, superoxide dismutase,

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and catalase. Fish that experienced EMS had significantly higher survival rates than those farmed in normal conditions (80.67% vs 61.33%). In conclusion, considering all measured parameters, 8-h light can be suggested as an optimum photoperiod for this fish species. Under 24L0D (no EMS) conditions, there were many negative effects apparent. In addition, a positive effect of EMS was evident in terms of survival after AC stress. AC stress decreased some health parameters under 24-h light treatment, while these results were not observed in EMS-exposed fish. Therefore, the EMS schedule can be a useful tool in preventing the negative effects of stress.

Keywords Antioxidant response · Blood performance · Blood biochemistry · Stress physiology · Continuous light

Introduction

Photoperiod has been known since the past century as an external factor that affects the growth, reproduction, and health of fish (Harrington Jr 1956). Therefore, manipulation of photoperiod has been a common practice in modern farming systems, like indoor systems and aquariums. Aquatic animal species responded to a change of photoperiod differently. Photoperiod manipulation has been successfully applied to improve the growth of several finfish species. For example, red sea bream (*Pagrus major*) (Biswas et al. 2006a, b), rainbow trout (*Oncorhynchus mykiss*) (Valenzuela et al. 2006), tilapia (*Oreochromis niloticus*) (Biswas et al. 2004), gibel carp (*Carassius auratus*) (Wei et al. 2019), coho salmon (*Oncorhynchus kisutch*) (Fang et al. 2019), zebrafish (*Danio rerio*) (Abdollahpour et al. 2020), and beluga (*Huso huso*) (Bani et al. 2009) grew well under 24-h continuous light. However, other fish species experienced stress, aggressiveness, and reduced growth with manipulation of photoperiod, including but not limited to African catfish (*Clarias gariepinus*) (Almazán-Rueda et al. 2005) with increased light, blunt snout bream (*Megalobrama amblycephala*) (Tian et al. 2019), and golden mahseer (*Tor putitora*) (Akhtar et al. 2020) with 8L:16D. The effect of photoperiod in parameters such as growth (Tian et al. 2019; Abdollahpour et al. 2020; Akhtar et al. 2020), metabolic rate (Hvas 2022), haematological parameters, liver and antioxidant enzymes (Biswas et al. 2004; Valenzuela et al. 2006; Kitagawa et al. 2015; Malinovskyi et al. 2022), maturation (Hansen et al. 1992), and immune system (Ángeles Esteban et al. 2006) has been widely investigated.

The market size of ornamental fish species is trending upward, and this sector continues to play a central role in the aquaculture industry. This market is predicted to expand at a compound annual growth rate of 8.5% from 2022 to 2030 and to reach more than USD 11 billion by 2030 (Ghosi Mobaraki et al. 2020). However, the increasing production trend requires more stock from hatcheries and improvements to productivity. Oscars are popular ornamental species of freshwater aquariums known for their large size and sociable personalities. Their intensive production, which is common in ornamental aquaculture, can potentially result in stress (Martos-Sitcha et al. 2020).

Stress causes cellular instability and imbalance in homeostasis (Adam et al. 2011) and any factor (both internal and external) that disturbs homeostasis can be named stress. Photoperiod can affect the stress response of fish in different ways. Melatonin is responsible for night and day patterns, and as a result, changes in this hormone drive many physiological alterations and have key roles in the redistribution of energy to maximise fitness and survival (Walton et al. 2011). The molecular and biological functions and related pathways to melatonin metabolism were reviewed elsewhere (Reiter 2003). Melatonin has direct interactions with the immune and antioxidant systems, and therefore, photoperiods, which have a direct impact on melatonin, can directly affect the growth, immune, and antioxidant systems of fish.

Early mild stress can occur positively in early-life stages, which means that mild chronic stress in early life can potentially shape positive phenotypes later in life. EMS is defined as a situation whereby animals do not die or suffer significantly from chronic stress, and they have a short-term response. In aquatic species, studies related to EMS, which can be categorised as eustress, are limited to our previous work and other investigations that lower hypothalamus catecholaminergic; brainstem serotonergic responses to stress and cortisol responsiveness were reported (Auperin and Geslin 2008; Madaro et al. 2015; Pederzoli & Mola 2016; Vindas et al. 2016). Further, higher brain catecholaminergic signalling and neuronal activity were reported in fish (Vindas et al. 2018). In our works, the interaction of EMS with fish meal replacement (Zare et al. 2023a), temperature (Esmaeili et al. 2024), and different levels of lipid (Esmaeili et al. 2022) and protein (Zare et al. 2024) was tested. The relationship between the onset of EMS and final acute confinement stress (AC stress) was also investigated (Zare et al. 2023b). The highest survival rate after final acute stress in oscar (*Astronotus ocellatus*) exposed to 2 weeks of EMS out of 10 weeks of the trial was observed (Esmaeili et al. 2022; Zare et al. 2023a, b). These results indicated that 2 weeks of EMS had the highest survival rate in oscar regardless of lipid or protein contents in diets. To the best of our knowledge, no study has investigated the effect of photoperiod on the physiology of oscar species. Further, no research tested how EMS can interact with photoperiod in animals and affect survival rate after an acute stress event. Therefore, in follow-up research with the same experimental conditions, 2 out of 10 weeks of scheduled EMS stress and five photoperiods were considered to see their effects on growth performance, haematology, blood biochemistry, immune response, antioxidant activities, stress response, and liver enzymes of juvenile oscar. In addition, a final AC stress event was carried out for the assessment of fish survival rate and biomarkers.

Materials and methods

Animal ethics, fish, and experimental conditions

A National Animal Care and Committee approved all experimental protocols (281–1385) (Safavi et al. 2019; Tazikeh et al. 2020; Ahmadi-Noorbakhsh et al. 2021). For this experiment, 621 oscars (initial weight, 7.29 ± 0.96 g) were obtained from the Abzian Center (Mahallat, Markazi, Iran). The acclimatisation steps to the trial condition were for 2 weeks and fish was fed with a commercial diet (500 and 150 g/kg crude protein and lipid, respectively). Twenty-two fish were randomly distributed into 30 rectangular glass tanks (100 L), with three replicate aquaria per experimental treatment. The fish were provided with their respective diets three times per day (09:00 h, 14:00 h, and 19:00 h) for 10 weeks to apparent satiety. For the 8L16D group, we fed them twice per day (09:00 h, 14:00 h) to apparent satiety.

Table 1 Experimental design for the effect of two-time scheduled stress (doing netting after the exchange water on Monday and Friday of the proposed week) and photoperiod and AC stress at the end of the experimental period

	8L16D	12L12D	16L8D	20L4D	24L0D	ES8L16D	ES12L12D	ES16L8D	ES20L4D	ES24L0D
Week 1	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress
Week 2	Without stress	Without stress	Without stress	Without stress	Without stress	EMS	EMS	EMS	EMS	EMS
Week 3	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress
Week 4	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress
Week 5	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress
Week 6	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress
Week 7	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress
Week 8	Without stress	Without stress	Without stress	Without stress	Without stress	EMS	EMS	EMS	EMS	EMS
Week 9	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress
Calculation of weight of all fishes, growth, sampling of blood, and other factors										
Week 10	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress	Without stress

EMS (early mild stress). Final stress (AC stress) and sampling for serum and haematology parameters

The ten experimental treatments were 8L16D (08:16 light to dark), 12L12D (12:12 light to dark), 16L8D (16:08 light to dark), 20L4D (20:04 light to dark), and 24L0D (24:00 light to dark), and fish in these five photoperiods were farmed with or without an EMS schedule (Table 1). To remove faeces and debris, approximately 25% of the water in each tank was exchanged daily with dechlorinated water during the experiment. The water quality parameters (dissolved oxygen (6.8 ± 0.8 mg/L), temperature (24.7 ± 0.9 °C), ammonia (0.47 ± 0.1 mg/L), nitrite (0.027 ± 0.005 mg/L), nitrate (22.7 ± 2.0 mg/L), pH (7.3 ± 0.9)) were measured regularly and kept in standard levels. Water quality was maintained by aeration with compressed air and mechanical filtration, which was detailed in our earlier experiments from this project (Esmaeili et al. 2022; Zare et al. 2023a).

Diet formulation and experimental design

An optimal isonitrogenous (450 crude protein/kg feed and 180 g/kg fish meal) and isolipidic (180 g/kg lipid) (Zare et al. 2024) diet strategy was used for this experiment. The same diet preparation and formulation processes were previously reported (Hosseinpour Aghaei et al. 2018; Esmaeili et al. 2022). During the 9-week feeding experiment, two scheduled EMS stress events were conducted in weeks 2 and 8, on both Monday and Friday of that week (Table 1). This involved dragging an aquarium net around the tank for 5 min after a water exchange without actively chasing or removing any fish. This stress, for the first time, was applied in a previous study (Esmaeili et al. 2022) and then others (Zare et al. 2023a, 2023b).

Sample collection and growth performance

Prior to final measurements (end of week 9), the fish were fasted for 24 h and then anaesthetised using clove oil stock solution (50 ppm) before removal from tanks (Esmaeili et al. 2017). Standard methods and relationships were used to calculate weight gain, specific growth rate, feed conversion ratio (FCR), daily feed intake, hepatosomatic index (HSI), viscerosomatic index, and condition factor at the end of the experiment (Zaretabar et al. 2021). Further, four fish were chosen at random from each tank, and after collecting blood, their liver and viscera were sampled and weighed. The dissected fish were returned to a bag (minus the blood) and kept frozen until further analysis. The remaining fish were returned to their respective tanks for a further 1-week period of feeding with no EMS event prior to the application of AC stress below.

Chemical analysis of diets and fish body composition

The proximate composition of the diets and whole body samples was measured by AOAC methods (AOAC 2000). Briefly, nitrogen (nitrogen × 6.25) was determined using the Kjeldahl method and an automatic Kjeldahl system (Kjeltec Analyser unit 2300, Sweden). The Soxhlet extraction method was used to examine crude lipids (Soxtec 2050 FOSS Model, Switzerland). Moisture was determined gravimetrically by oven drying samples at 105 °C oven for 12 h. The ash content was determined using a Nabertherm muffle furnace (Model K, Germany) at 550 °C for 4 h.

Blood collection and sample preparation

The serum from four fish from each tank was tested for haematology, immune response, blood biochemistry, antioxidants, and serum enzymes at the end of the experiment (week 9 and week 10). The blood samples were quickly collected (within 2 min) via venipuncture of the caudal vein with an 18-G needle connected to a sterile 5-mL syringe. Aside from sampling blood with anticoagulant (EDTA) for haematology analysis, we sampled blood in tubes without anticoagulant for serum. Following that, blood was refrigerated for 2 h before serum was collected after centrifuging at 3000 g at 4 °C for 2 min (Esmaeili et al. 2017) and then stored at − 20 °C until further analyses.

Haematology profile

Red blood cells (RBCs) and white blood cells (WBCs) were counted in a Neubauer haemocytometer and the Neubauer chamber, respectively, as described earlier (Kenari et al. 2013; Esmaeili et al. 2022). Further, the haemoglobin (Hb) and haematocrit (Ht) were determined by cyanmethaemoglobin and the microhaematocrit method (Řehulka et al. 2004; Esmaeili et al. 2022). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) (Wintrobe 1929), and blood performance (BP) (Esmaeili 2021) were calculated according to the below formulas:

$$\text{Mean corpuscular volume (MCV) (fl)} = (\text{Ht}/(\text{RBC } 10^6/\text{mm}^3)) \times 10,$$

$$\text{Mean corpuscular haemoglobin (MCH) (pg)} = \text{Hb}/\text{RBC}10^6/\text{mm}^3 \times 10,$$

$$\text{MCHC} = \text{Hb}/\text{Ht} \times 100,$$

$$\text{Blood performance} = \text{Ln Hb(g/dL)} + \text{Ln Ht(\%)} + \text{Ln RBC}(\times 10^5/\text{mm}^3) + \text{Ln WBC}(\times 10^3/\text{mm}^3) + \text{Ln total protein (g/L)}.$$

Blood biochemistry, antioxidant enzyme activities, serum enzymes, and cortisol

Plasma biochemical parameters, glucose, total protein (TP), albumin, globulin, high-density lipoproteins (HDL), low-density lipoproteins (LDL), cholesterol, triglycerides, lactate, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine aminotransferase (ALT), were analysed using commercial clinical investigation kits (Pars Azmoon Kit, Karaj, Iran). The antioxidant enzymes, including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and malondialdehyde (MDA), were measured using ELISA kits, according to the kit protocol (ZellBio, GmbH, Germany) which were used in earlier studies (Esmaeili et al. 2022; Zare et al. 2023a, 2023b). Cortisol levels in serum were measured using fluorescence immunoassay (FIA) and a commercial kit (iCHROMA, South Korea) and the protocol provided with the kit. Linearity and parallelism were tested using serial dilutions of samples (0, 25, 50, and 75%). An R^2 of more than 90% was considered to be the threshold to pass the limit for the linearity test. For the parallelism test, the coefficient of variation (% CV) between the slope of the standard curve and the samples was calculated (values of ≤ 20% passed the test). The recovery range of 80–120% CV was considered threshold. Intra-assay and intra-assay precision thresholds were 20% CV.

Nonspecific immune parameters

To determine serum lysozyme, gram-positive bacteria sensitive to the lysozyme enzyme method were used (*Micrococcus lysodeikticus*) (Clerton et al. 2001) as substrate. Alternative complement pathway hemolytic activity (ACH50) was determined by haemolysis of rabbit RBCs (RaABC) (Amar et al. 2000). Serum immunoglobulin M, complement C3 (C3), and complement C4 (C4) levels were measured by ELISA method using CUSABIO and MyBioSource kit companies (CUSABIO-CSB-E12045Fh and CUSABIO, CSB-E09727s) based on the protocol available in the kit package. The complete methods for measuring these parameters were described in our previous study (Hosseini et al. 2022). The qualitative and quantitative checks of data were done, as previously explained in the "Blood biochemistry, antioxidant enzyme activities, serum enzymes, and cortisol" section.

Acute confinement stress (AC stress)

After the 10 weeks of the photoperiod and EMS experiment, oscars were exposed to AC stress based on our previous studies to test fish's ability to tackle stressful situations (Asgari et al. 2020; Esmaeili et al. 2022). After collecting samples at the end of week nine, ten fish per tank were biomass adjusted

Table 2 Growth performance of oscar (*Astronotus ocellatus*) under different photoperiods and early mild stress treatments after 9 weeks of feeding

	8L16D	12L12D	16L8D	20L4D	24L0D	ES8L16D	ES12L12D	ES16L8D	ES20L4D	ES24L0D
Initial weight (g)	6.63 ± 0.93	7.53 ± 0.93	6.70 ± 1.18	6.87 ± 0.60	8.40 ± 1.08	7.47 ± 0.85	6.67 ± 0.94	7.67 ± 1.19	7.60 ± 0.90	7.40 ± 0.87
Weight gain (g)	52.13 ± 8.27	47.43 ± 7.29	50.33 ± 8.99	45.30 ± 7.97	47.13 ± 6.23	46.40 ± 7.91	48.40 ± 9.04	51.17 ± 10.37	50.30 ± 8.05	40.83 ± 5.22
SGR (%/day)	3.46 ± 0.47	3.16 ± 0.43	3.40 ± 0.52	3.21 ± 0.29	3.00 ± 0.34	3.13 ± 0.46	3.35 ± 0.42	3.23 ± 0.56	3.22 ± 0.44	2.98 ± 0.38
FCR	1.98 ± 0.29	2.06 ± 0.33	2.24 ± 0.50	1.86 ± 0.24	1.82 ± 0.34	2.10 ± 0.23	1.99 ± 0.31	2.08 ± 0.44	1.92 ± 0.36	2.03 ± 0.30
DFI (%/day)	4.99 ± 0.75	4.91 ± 0.51	5.54 ± 0.77	4.51 ± 0.54	4.21 ± 0.53	5.04 ± 0.72	4.95 ± 0.99	5.00 ± 0.87	4.67 ± 0.96	4.73 ± 0.96
HSI (%)	2.28 ± 0.40	2.47 ± 0.81	2.04 ± 0.23	2.12 ± 0.29	2.30 ± 0.29	2.10 ± 0.30	2.29 ± 0.39	2.37 ± 0.51	2.52 ± 0.16	2.27 ± 0.63
VSI (%)	5.97 ± 0.45	5.60 ± 0.75	6.14 ± 0.70	5.90 ± 0.40	5.53 ± 0.93	6.13 ± 0.57	6.41 ± 0.49	6.70 ± 0.46	6.37 ± 0.45	6.57 ± 0.78
Condition factor	2.39 ± 0.20	2.68 ± 0.46	2.78 ± 0.11	2.13 ± 0.59	2.70 ± 0.64	2.22 ± 0.23	2.10 ± 0.48	2.27 ± 0.71	2.94 ± 0.59	2.18 ± 0.10
Survival rate (%)#	90.91 ± 4.55	92.42 ± 2.62	90.91 ± 4.55	92.42 ± 2.62	92.42 ± 9.46	95.45 ± 4.55	93.94 ± 6.94	90.91 ± 4.55	95.45 ± 4.55	93.94 ± 2.62
Survival rate after AC (%)	56.67 ± 20.82	60.00 ± 10.00	66.67 ± 15.28	60.00 ± 17.32	63.33 ± 15.28	83.33 ± 5.77	80.00 ± 20.00	76.67 ± 5.77	86.67 ± 11.55	76.67 ± 20.82

Values are represented by means ± SDM of triplicate tanks. There was no significant difference in initial weight

WG = final weight − initial weight

SGR, specific growth rate = ((Ln W2 − Ln W1)/63 days)) × 100

FCR, feed conversion ratio = dry feed consumed (g)/WG (g)

DFI, daily feed intake (%body weight.day^{-1}) = 100 × feed consumed (g)/((initial weigh + final weight) × 0.5 × days)

HSI, hepatosomatic index = (liver weight (g)/body weight (g)) × 100

VSI, viscerosomatic index = (visceral weight (g)/body weight (g)) × 100

CF, condition factor = (W2 (g)/length3) × 100

#Survival rate (%) = (number of fish in each group remaining at the end of experiment/initial number of fish: 20) × 100

The ten treatments were 8L16D (08:16 light to dark), 12L12D (12:12 light to dark), 16L8D (16:08 light to dark), 20L4D (20:04 light to dark), 24L0D (24:00 light to dark), ES8L16D (08:16 light to dark and early stressed), ES12L12D (12:12 light to dark and early stressed), ES16L8D (16:08 light to dark and early stressed), ES20L4D (20:04 light to dark and early stressed), and ES24L0D (24:00 light to dark and early stressed) with three tanks per treatment. Then, the fish were fed as usual for 1 week to recover from stress, and we applied AC stress at the end of week 10. The acute stress was a succession of netting all of the fish in each tank followed by a 30-s air exposure before being transferred to a plastic mesh bucket at a density of 120 g/L in their original tank for 5 h. Aeration was maintained to prevent oxygen depletion and minimise any premature death as a result of the AC stress. Following the 5 h of confinement stress, blood sampling and serum extraction from fish that could survive were performed as previously described on three fish per tank (Esmaeili et al. 2022). The survival rate of fish after 48 h in various treatments is shown in Table 2. We needed to test the fish's ability and our hypothesis to determine whether fish that experienced EMS could tolerate AC stress at the end of the experiment better or not. To do so, we had to design an AC stress severe enough to kill some fish.

Statistical analysis

This study used a completely randomised design with nine treatments and three replications. After testing the normality and homogeneity of data, two-way ANOVA was used to investigate the "photoperiod effect" and the "EMS effect". When the *P* value of interaction was not significant, treatments were compared in different EMS groups and five photoperiod treatments in pooled data (Table 4). When the interaction was significant, the original data was unpacked, and treatments for each EMS group and photoperiod level were compared (figures). Further, data were compared before and after AC stress to see which parameter was changed with the AC stress in each treatment (Hosseini et al. 2022; Zare et al. 2024). In all analyses, a significant difference between treatments was defined as a difference of 5% or less. SPSS (version 21.0 for Windows) was used to analyse the data.

Results and discussion

It has been nearly half a century since the effect of photoperiod on fish growth, reproduction physiology, and health was investigated. As time passes, more fish species are farmed in controlled environmental conditions such as recirculation aquaculture systems and aquariums. It means that photoperiod manipulation to increase the growth of fish is possible and practical. Although many studies are available on different species, no studies have been conducted on the effect of photoperiod on oscar families. This study was a follow-up study from our EMS project. After four studies (Esmaeili et al. 2022; Zare et al. 2024, 2023a, 2023b), we understood that 2 out of 10-week stress events do not impair growth, stress response, and many blood parameters and even improved survival after AC stress. Further, the interaction of photoperiod and EMS and eventually their impacts on the final survival rate after acute AC stress was undiscovered. This study indicated that photoperiod does not affect fish growth, but consistent light schedule impaired fish health. This result shows that we can farm oscars even in the 8:16 (light to dark) regime without decreased growth. Unlike previously available studies (Esmaeili et al. 2022; Zare et al. 2023a, 2023b), EMS did not affect the growth and physiology of fish due to the probably stronger effect of photoperiod. As it is the first study on this topic, more studies are required to test the interaction of EMS and photoperiods on other species.

Growth performance and body composition

One of the most important consequences of photoperiod manipulation is its potential to affect the growth performance of fish. In the present research, the effect of photoperiod or EMS schedule on growth performance was not significant and showed that fish grew well on all five photoperiod schedules. Providing light for aquaculture systems can be costly, and even with 8 h of light, oscar can grow well. Similar to this output, other studies indicated fish growth was the same between 8:16 and 12:12 L:D, which has been considered the optimum for most fish species. While we fed fish twice per day in the 8:16 group, the growth was not affected, showing that fish were able to adjust their feeding with this photoperiod schedule. For example, in African catfish (Almazán-Rueda et al. 2005) and channel catfish (*Ictalurus punctatus*) (Stickney and Andrews 1971), these results were observed. Photoperiod similarly did not affect the growth performance of flounder (*Pleuronectes ferrugineus*) (Purchase et al. 2000), rainbow trout (Reddy and Leatherland 2003), turbot (*Psetta* *maxima*) (Imsland et al. 1995), and sunshine bass (*Morone chrysops* × *Morone saxatilis*) (Davis and Mcentire 2006). Other studies reported the positive effect of continued or increased light on the growth of fish such as gilthead sea bream (*Sparus aurata*) (Ginés et al. 2004), which was due to increased food conversion efficiency and suppressed sexual maturation. Since photoperiod can affect the level of plasma growth hormone and eventual growth, it is reasonable to hypothesise that levels of hepatic insulin-like growth factor I mRNA are indirectly regulated by photoperiod (Vera Cruz and Brown 2009). While there are possible mechanisms to improve growth by photoperiod, oscar was not sensitive to light in the present study, similar to several other fish species noted above. In fish species like catfishes, longer periods of light can be stressful as more time is needed to show dominant behaviour or escape from dominant individuals by subordinate ones (Boeuf and Falcon 2001). Oscars may have less dominant-subordinate and social hierarchies, and because of that, continued light did not negatively affect fish growth. More behavioural studies may be warranted to better understand the relationship between social hierarchies and photoperiod.

In the present study, DFI, HSI, feed intake, and FCR were not affected by photoperiod (Table 2), which is unlike other studies that showed, for example, improved FCR with decreased and increased light period (Purchase et al. 2000; El-Sayed and Kawanna 2004). However, similar results were observed in tilapia (Wang et al. 2023), barramundi (*Lates calcarifer*) (Barlow et al. 1995), and yellowtail flounder (Purchase et al. 2000). Lack of any change in these parameters is in line with growth data indicating that oscar had no issue with different photoperiod schedules.

The survival rates in the current study were insignificant among treatments and greater than 90% at the end of the 9-week experiment, indicating that all fish were grown in a suitable environment. However, the effect of EMS on survival after final AC stress was significant, and those subjected to the EMS schedule had a higher survival (80.67%) than those grown in normal conditions without EMS (61.33%) ($P < 0.05$). The higher survival rate in EMS groups is consistent with our previous studies (Esmaeili et al. 2022; Zare et al. 2023a, 2023b) and is further evidence that the fish experienced adaptation and that allowed them

Table 3 Carcass chemical composition (g/kg wet weight basis) of oscar (*Astronotus ocellatus*) under different photoperiods and early mild stress treatments

	8L16D	12L12D	16L8D	20L4D	24L0D	ES8L16D	ES12L12D	ES16L8D	ES20L4D	ES24L0D
Protein	174.8 ± 8.5	175.4 ± 11.9	182.3 ± 9.4	178.2 ± 9.5	170.5 ± 12.4	172.9 ± 3.1	176.1 ± 17.3	177.1 ± 12.4	173.4 ± 6.1	169.6 ± 4.1
Fat	58.7 ± 5.1	62.1 ± 4.9	56.3 ± 6.8	52.9 ± 11.2	61.0 ± 5.5	57.0 ± 4.3	60.4 ± 5.5	55.4 ± 9.3	59.9 ± 7.0	64.6 ± 5.1
Ash	29.6 ± 3.7	29.3 ± 3.5	26.6 ± 6.6	32.6 ± 2.5	31.7 ± 4.9	28.8 ± 2.0	32.0 ± 4.5	31.7 ± 2.1	30.5 ± 4.7	31.3 ± 3.1
Moisture	681.1 ± 22.2	675.4 ± 36.1	655.4 ± 50.5	666.7 ± 59.9	671.4 ± 45.1	689.3 ± 43.7	677.5 ± 57.8	696.2 ± 33.6	691.5 ± 47.0	682.3 ± 43.3

Values are represented by means ± SDM of triplicate tanks

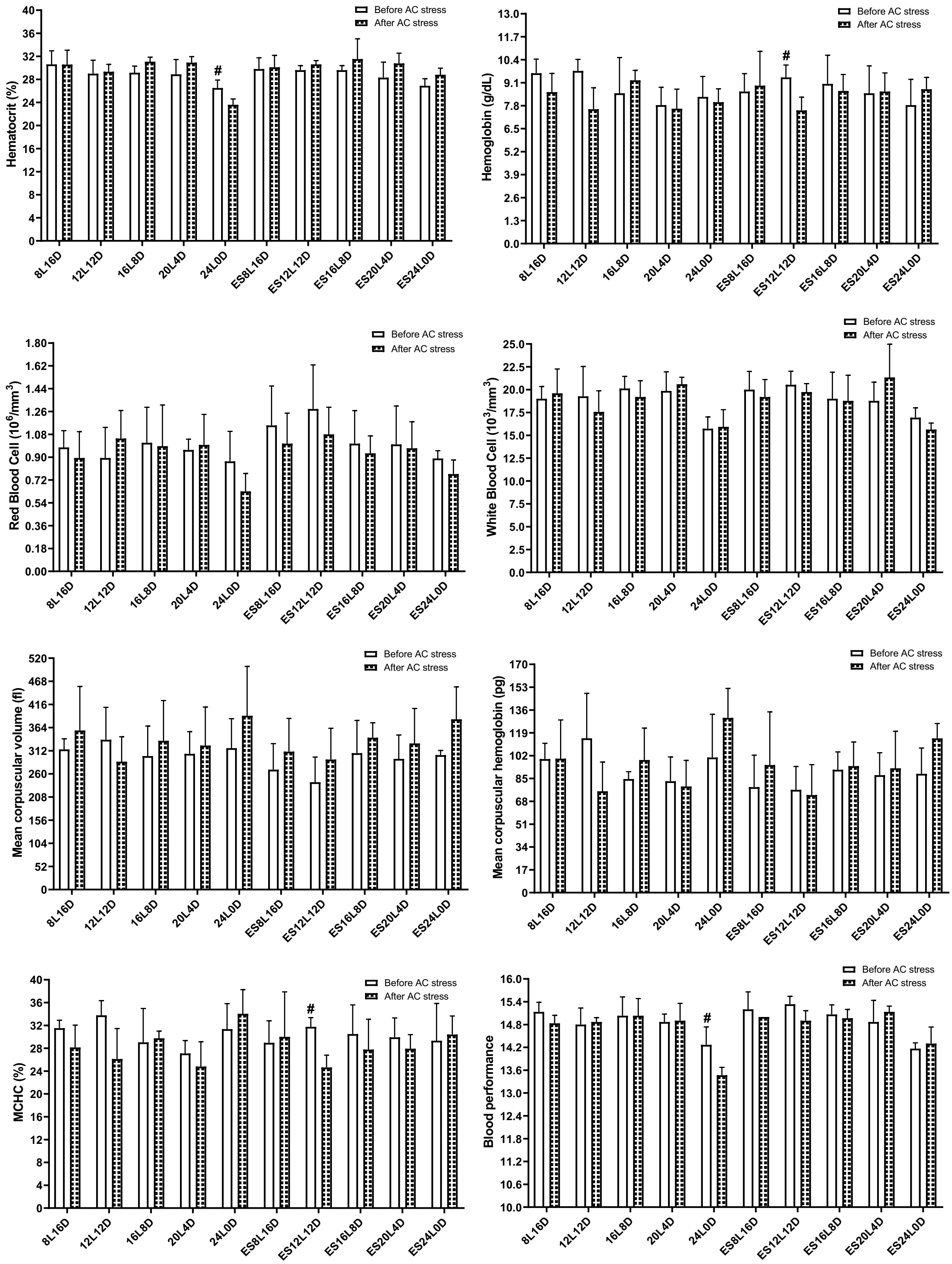
The ten treatments were 8L16D (08:16 light to dark), 12L12D (12:12 light to dark), 16L8D (16:08 light to dark), 20L4D (20:04 light to dark), 24L0D (24:00 light to dark), ES8L16D (08:16 light to dark and early stressed), ES12L12D (12:12 light to dark and early stressed), ES16L8D (16:08 light to dark and early stressed), ES20L4D (20:04 light to dark and early stressed), and ES24L0D (24:00 light to dark and early stressed)

Table 4 The results of two-way ANOVA analysis with SPSS for measured parameters. When the interaction was not significant, we compared the "early stress (EMS) effect" via independent sample *T*-test and "photoperiod effect" via Duncan's new multiple range test for the case these effects were significant ($P < 0.05$). The non-significant parameters were not reported. The letters a, b, and c indicate significant differences among treatments exposed to different stress numbers

	P value			Main effects (mean)						
	Photoperiod effect	Early stress effect	Interactions	Without early stress	With early stress	8L16D	12L12D	16L8D	20L4D	24L0D
Survival rate after AC	0.993	0.002	0.826	61.33	80.67					
Haematocrit	0.041	0.985	0.946			30.20a	29.30a	29.38a	28.58ab	26.70b
WBC	0.035	0.740	0.680			19.90a	19.57a	19.50a	19.31a	16.33b
Total protein	0.028	0.721	0.883			7.05a	6.12ab	7.32a	6.70a	5.02b
Blood performance	0.002	0.448	0.641			15.17a	15.07a	15.05a	14.87a	14.22b
Ht-stress#	0.001	0.067	0.078			30.33a	29.97a	31.30a	30.83a	26.20b
WBC-stress#	0.008	0.656	0.797			19.40a	18.65a	18.98a	20.97a	15.78b
BP-stress#	0.001	0.036	0.108			14.92a	14.88a	15.00a	15.02a	13.88b
Total protein-stress#	0.008	0.233	0.208			6.60a	6.77a	6.63a	6.72a	4.75b
LDL	0.152	0.046	0.213	28.74	32.76					
Lysozyme	0.003	0.219	0.059			46.75 a	43.95a	41.92a	43.62a	29.95b
Immunoglobulin M	0.027	0.032	0.202	22.96	18.83	24.88a	23.27a	21.43ab	19.65ab	15.25b
Complement C3	0.048	0.319	0.750			126.68a	125.38a	124.25a	131.48a	90.88b
Immunoglobulin M-stress#	0.006	0.641	0.215			17.92bc	22.73ab	27.33a	25.02a	15.95c
Lysozyme-stress#	0.001	0.543	0.119			35.28a	43.55a	37.00a	43.30a	24.72b
ACH50-stress#	0.045	0.331	0.284			196.47ab	191.87ab	182.15ab	225.38a	159.15b
SOD	0.001	0.002	0.052	58.29	46.99	60.27a	59.78a	48.97a	56.32a	37.88b
Catalase	0.005	0.001	0.668	35.53	48.54	46.38a	43.42a	46.47a	44.87a	29.03b
SOD-stress#	0.004	0.503	0.558			52.12a	50.92a	50.75a	48.62a	33.05b
Catalase-stress#	0.001	0.840	0.413			47.50a	42.33a	49.32a	48.73a	28.10b
GPx-stress#	0.022	0.043	0.440	164.77	189.58	171.83ab	197.62a	169.32ab	204.22a	142.88b
AST	0.045	0.208	0.956			181.88b	204.57ab	185.45b	195.23b	248.17a
MCH-stress#	0.034	0.761	0.896			97.39ab	74.05b	96.51ab	85.80b	122.51a

The interaction effect for ALT and ALT-stress was significant, which was unpacked and shown in figures

#The parameters measured after AC stress


Before AC stress
After AC stress
Hematocrit (%)
Hemoglobin (g/dL)
Red Blood Cell (10^6/mm^3)
White Blood Cell (10^3/mm^3)
Mean corpuscular volume (fl)
Mean corpuscular hemoglobin (pg)
MCHC (%)
Blood performance
8L16D
12L12D
16L8D
20L4D
24L0D
ES8L16D
ES12L12D
ES16L8D
ES20L4D
ES24L0D
#

◂**Fig. 1** Haematological parameters of oscar exposed to different photoperiods and early mild stress treatments during 10 weeks plus data related to after acute confinement stress (AC stress). Values were represented by means ± SDM of triplicate samples

to tolerate an acute stress condition better. This finding supports the notion that in a healthy population of high-performing fish, equal growth performance after an EMS or even AC stress does not necessarily translate to more robust fish. Nutritional status, cultural system, size of fish, and species are other variables that may invariably affect the results of growth performance. While both biotic (age, gender, and size) and abiotic factors (water quality, season, and geographical location) can affect the proximate body composition of aquatic species, the diet is most likely responsible for most of the changes (Shearer 1994). Unsurprisingly, as oscars in the present study were fed the same diets, there was no significant difference in protein, fat, ash, and moisture contents (Table 3). These results align with earlier studies investigating EMS (Esmaeili et al. 2022) and photoperiod (Biswas et al. 2006a, b; Biswas et al. 2008, 2016; Zolfaghari et al. 2011; Tian et al. 2019). However, other studies on marine species, such as gilthead sea bream, have reported changes to the proximate composition of fish in response to different photoperiod regimes (Ginés et al. 2004).

Haematology and blood biochemistry

Environmental factors such as photoperiod and stress drive alterations in haematology and biochemistry parameters, and these parameters can be indicators of the health status of fish. Our results indicated that some markers were affected by the photoperiod including haematocrit, WBC, and blood performance before and after AC stress ($P < 0.05$; Table 4). In detail, fish exposed to 24 h of light had lower haematocrit and WBC levels. It can be hypothesised that 24 h of light was a bit stressful for oscar, which eventually decreased their haematocrit and WBC. Decreasing these parameters with stress has been reported earlier (Gao et al. 2021; Shin et al. 2016). These results are consistent with our previous EMS works (Esmaeili et al. 2022). Other studies showed that photoperiod has significantly altered the haematology parameters. For example, in coho salmon (Fang et al. 2019) and piracanjubas (*Brycon orbygnianus*) (Machado et al. 2016), the same results were observed. In contrast, some other species, such as beluga (Bani et al. 2009), red sea bream (Biswas et al. 2006a, b; Biswas et al. 2006a, b; Biswas et al. 2010), and pacamã (*Lophiosilurus alexandri*) (Kitagawa et al. 2015), experienced no alterations in haematocrit under photoperiod treatments. The WBC is an important parameter showing the immune status of fish (Esmaeili 2021). A decrease in this parameter in the 24L0D group could be evidence of a suppressed immune system with continuous light. Photoperiods did not change the WBC in iridescent shark catfish (*Pangasionodon hypophthalmus*) (Windarti et al. 2021). However, this parameter was changed in other species such as pikeperch (*Sander lucioperca*) (Pourhosein Sarameh et al. 2013), Persian sturgeon (*Acipenser persicus*) (Falahatkar et al. 2012), rainbow trout (Valenzuela et al. 2022), tambaqui (*Colossoma macropomum*) (Pereira et al. 2021), and carp (*Cyprinus carpio*) (Ruchin 2006). The observed various outputs can highlight the fact that fish species respond differently to photoperiod regardless of trophic levels.

A blood performance (BP) marker has previously been used as an indicator of fish growth and health (Esmaeili 2021). In the current data, the photoperiod effect for BP before and after AC stress was significant, and accordingly, the 240LD group (14.22 and 13.88 for before and after AC stress, respectively) had a lower value than the others (Fig. 1). These results show that BP can be an indicator of immunity of fish as the immune parameters, WBC, and haematocrit were lower in continued light. To further validate, new investigations related to BP and photoperiod to compare with the current data are still required. Unlike the present data, in other studies from this project (Esmaeili et al. 2022; Zare et al. 2024, 2023a, 2023b) and also other studies (Esmaeili et al. 2017; Hosseini et al. 2021, 2022; Montazeri et al. 2021; Ravardshiri et al. 2021), the connection of growth and BP was observed. Earlier research found that a lower BP level can indicate a weaker fish in terms of its overall health status. Accordingly, when fish were fed too much soybean (Montazeri et al. 2021), total carbohydrate (Ravardshiri et al. 2021), and meat and bone meal (Esmaeili et al. 2017), the BP was lower when compared to the control group.

A blood biochemistry panel of markers is considered an indicator of fish metabolism as it is in other species and humans. Some blood biochemistry parameters like albumin and total protein are also markers of the immune system status. In the current study, total protein was the only parameter affected by photoperiod, with the 24L0D group having the lowest value before (5.02 g/dL) and after (4.75 g/dL) AC stress (Fig. 2). Proteins have key roles in almost all physiological metabolism, such as the transport of bilirubin, hormones, metals, vitamins, drugs, and lipid metabolism. Most importantly, almost all immune system components are protein-based, such as carrier proteins, enzymes, complements, and immunoglobulins. Some studies indicated a change in total protein with photoperiod. For example, in golden mahseer (Akhtar et al. 2020) and largemouth bass (*Micropterus salmoides*) (Malinovskyi et al. 2022), these results were observed. However, no change in this parameter was observed in Murray cod (*Maccullochella peelii*) (Di et al. 2023), Nile tilapia (Wang et al. 2023), and red sea bream (Biswas et al. 2006a, b).

Immune and stress response

The EMS can positively improve the immune and stress response and can act as a preparedness for the fish in terms of future stressful conditions. We observed the positive effect of EMS on the immune system and stress response of oscars in several prior studies (Esmaeili et al. 2022; Zare et al. 2023a, 2023b) but not in the present study where the effect of EMS was only significant for immunoglobulin M, being lower in the EMS group (Fig. 3). However, the effect of photoperiod before AC stress for lysozyme, immunoglobulin M, and complement C3 was significant. The oscar that was grown in the continued light and before AC stress had the lowest value of lysozyme (29.95 µ/mL), immunoglobulin M (15.25 mg/mL), and complement C3 (90.88 mg/dL). After the AC stress was applied, the fish followed the same trend, and the 24L0D group had the lowest values of immunoglobulin M (15.95 mg/mL), lysozyme (24.72 µ/mL) and ACH50 (159.15 µ/mL). The photoperiod and immune system are closely related to each other. It has been well-known that the immune system is affected by photoperiods and, in most cases, improved immunity in less light is observed. For example, increased spleen and thymus mass and numbers of lymphocytes and neutrophils are documented (Nelson et al. 1996). Melatonin influences the light–dark rhythm in most vertebrates, including fish, and eventually affects the humoral innate immune system (Ángeles Esteban et al. 2006). In the present study, the immune system was decreased in 24 h of light but with no change in stress response parameters like glucose and cortisol. This is potential evidence that it has not been due to suppression of immunity by the endocrine system. Chronic stress also prevents immune cells and signalling networks from communicating with each other properly (Bae and Shin 2019). It can be hypothesised that the relationship between immunity and photoperiod comes from melatonin. Unfortunately, we could not measure this parameter and more hormonal studies are required.

Lysozyme plays a key role in the nonspecific immune response of fish and is one of the most commonly measured parameters in aquaculture. In other studies, 24 h of light during culture decreased lysozyme, erythrocytes, leucocytes, lymphocytes, monocytes, and polymorphonuclear in rainbow trout compared with the 12:12 light to dark cycle (Burgos et al. 2004; Valenzuela et al. 2022). Similar to our data, decreased lysozyme was observed in gilthead seabream and European sea bass (*Dicentrarchus labrax*) (Ceballos-Francisco et al. 2020) as well. However, other studies reported no change in lysozyme with photoperiod in other species including golden mahseer (Akhtar et al. 2020), Atlantic halibut (*Hippoglossus hippoglossus*) (Bowden et al. 2004), European sea bass (Ángeles Esteban et al. 2006), and Nile tilapia (Atwood et al. 2003). Immunoglobulin M was another parameter that was declined in the 24L0D group. Decreased immunoglobulin with increased light in gilthead seabream and European sea bass (Ceballos-Francisco et al. 2020) and also other animals (Guo et al. 2010; Park et al. 2015) was observed, which is in line with the current study.

The 24L0D groups had the lowest values of some haematological parameters and total proteins as well that can be connected to the immune system results. Although it was not significant, the 24L0D groups also had numerically lower growth (around 32 g compared to others, around 37 g), which can potentially be connected to these blood chemistry results. However, the 24L0D groups had the same survival rate compared to other pre-AC stresses, while the survival rate was significantly lower in the 24L0D groups after AC stress. It is possible that the stress events were not

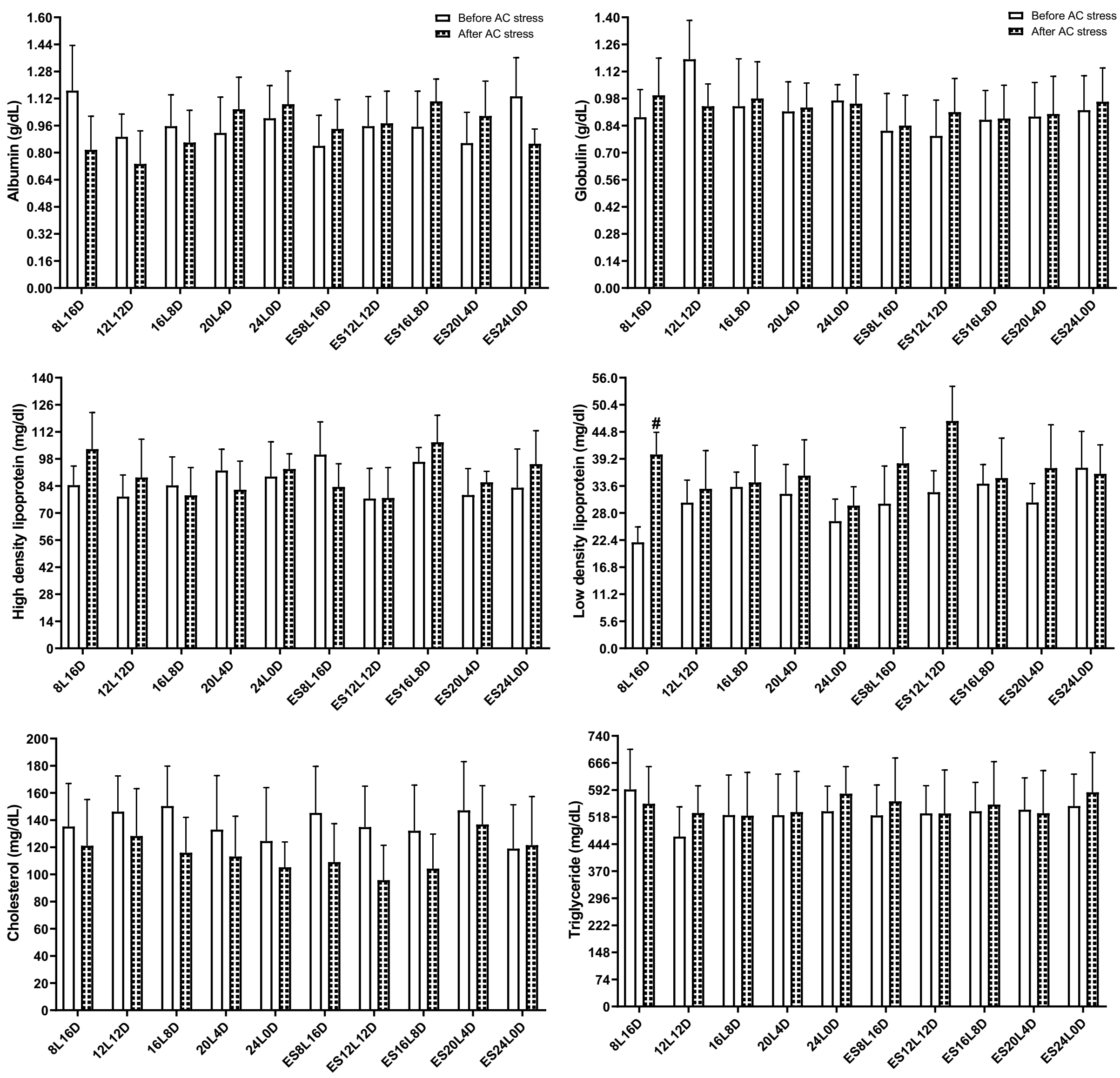


Fig. 2 Blood biochemistry parameters of oscar exposed to different photoperiods and early mild stress treatments during 10 weeks plus data related to after acute confinement stress (AC stress). Values were represented by means ± SDM of triplicate samples

sufficient to affect the growth and survival rate significantly after EMS stress, but the immune parameters were changed. Our previous studies observed that decreased immunity was responsible for decreased growth and survival rate after AC stress (Esmaeili et al. 2022; Zare et al. 2023a, 2023b). To the best of our knowledge, no study has measured fish in a vast panel of immunity parameters under photoperiod treatments in oscar. Further research is needed to determine how different fish species' immune systems react to EMS and photoperiod and how this eventually translates to growth performance and survival.

The present study indicated no significant differences in stress parameters' levels such as glucose, lactate, and cortisol (Fig. 4). It should be noted that after AC stress, these parameters were higher in most treatments but not significant. The result of this study is unlike our previous studies that showed AC stress increased stress parameters (Esmaeili et al. 2022; Zare et al. 2023a, 2023b). However, as

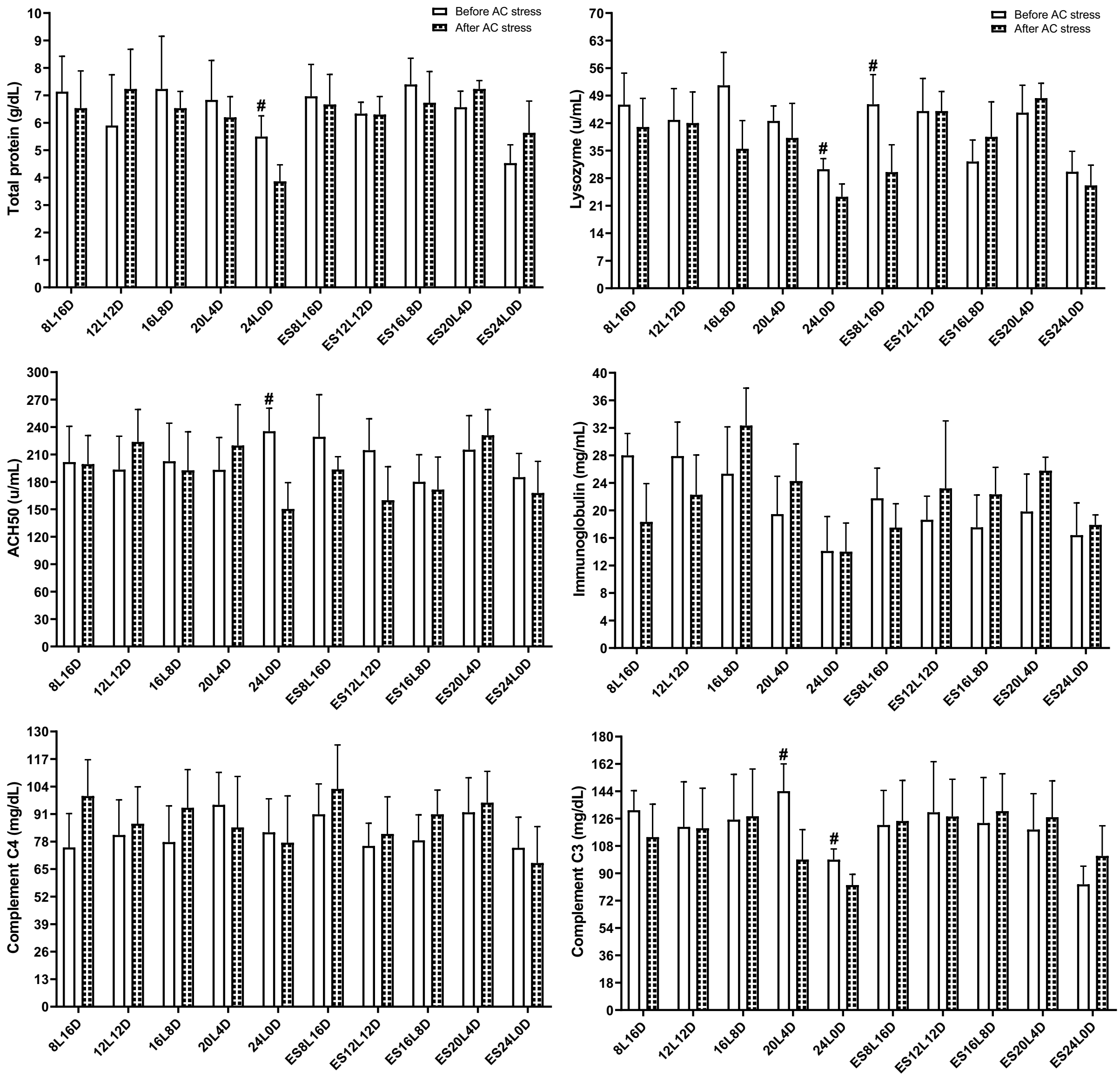


Fig. 3 Immune response parameters of oscar to different photoperiods and early mild stress treatments during 10 weeks plus data related to after acute confinement stress (AC stress). Values were represented by means ± SDM of triplicate samples. Hashtag (#) indicates the significant difference in each treatment between farmed fish with and without early stress according to the independent sample *T*-test ($P < 0.05$) (for example, 24L0D compared to ES24L0D). X, Y, and Z indicated significant differences across different photoperiod groups after AC stress (the interaction effect was significant for this parameter)

previously mentioned, the fish grown in the EMS schedule had a higher survival rate after AC stress. Aquaculture sustainability depends on growth performance and survival as key indicators, and reduced stress responsiveness can improve these parameters. The present study clearly demonstrates that glucose, lactate, and glucose cannot always be relied upon as the only indicators in fish studies to show stress responsiveness after acute stress. It has been reported that stress does not always cause an increase in cortisol, glucose, and lactate. The possible reasons can be impaired cortisol secretion by

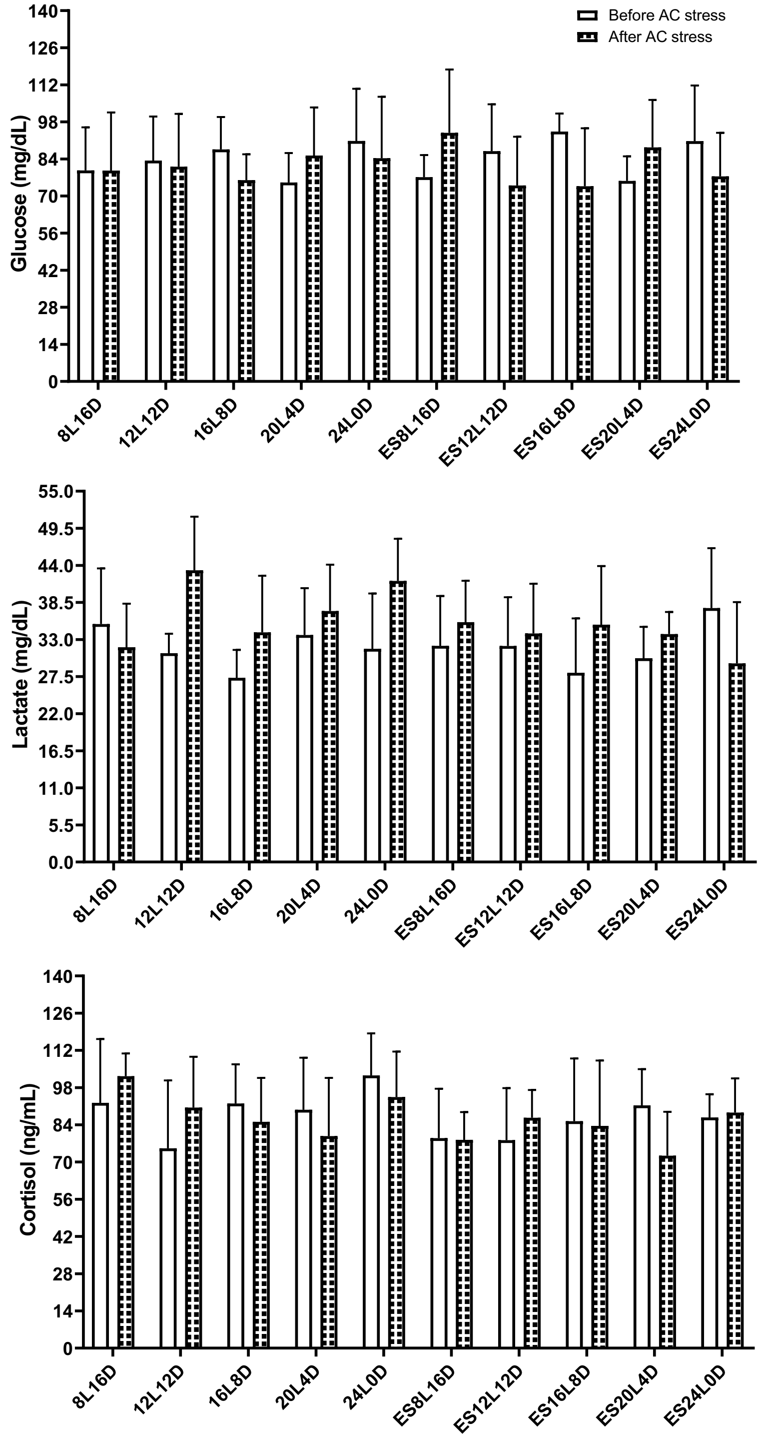


Fig. 4 Stress response parameters of oscar exposed to different photoperiods and early mild stress treatments during 10 weeks plus data related to after acute confinement stress (AC stress). Values were represented by means ± SDM of triplicate samples

interrenal exhaustion and environmental factors or the HPI axis is no longer being stimulated, as the fish "acclimate" through gradual loss of awareness of the stress factor (Ellis et al. 2012).

Antioxidant enzyme activities

Antioxidant enzymes such as SOD, CAT, GPx, and MDA are also indicators of health status and are

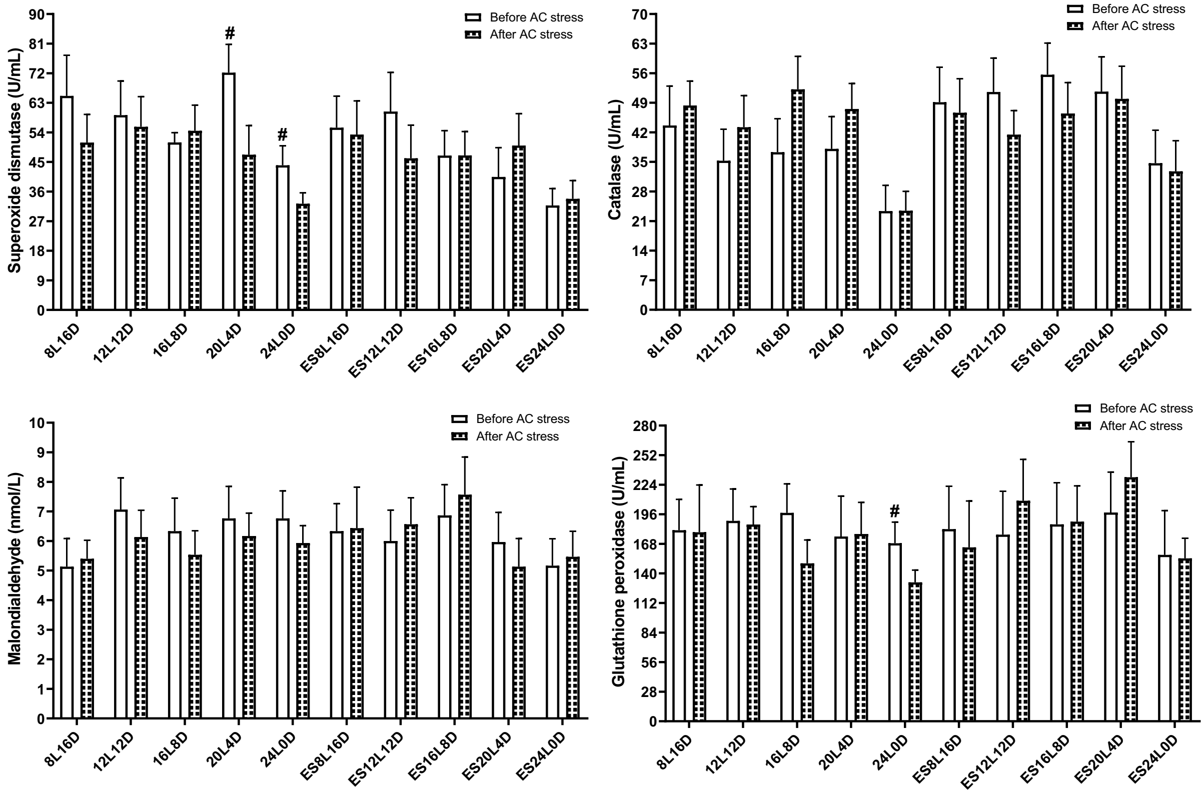


Fig. 5 Antioxidant system parameters of oscar exposed to different photoperiods and early mild stress treatments during 10 weeks plus data related to after acute confinement stress (AC stress). Values were represented by means ± SDM of triplicate samples. Hashtag (#) indicates the significant difference in each treatment between farmed fish with and without early stress according to the independent sample *T*-test ($P < 0.05$) (for example, 24L0D compared to ES24L0D). x, y, and z and also, a, b, and c indicated significant differences across different photoperiod groups with and without early stress, respectively (the interaction effect was significant for this parameter)

commonly employed in aquaculture studies. These enzymes protect cells from uncontrolled oxidative reactions that produce superoxide and H_2O_2 radicals (Hoseinifar et al. 2020). The current data indicated that before AC stress, SOD and catalase were affected by both EMS and photoperiod. SOD and catalase were higher and lower in the "without EMS" groups (Fig. 5). Like several other measured parameters explained in previous sections, 24L0D groups had the lowest values of SOD and catalase before (37.88 U/mL and 29.03 U/mL, respectively) and after AC stress (33.05 U/mL and 28.10 U/mL, respectively). After AC stress, GPx in the 24L0D and without EMS groups had the lowest level compared to other treatments. It can be seen that, generally, the 24L0D had a lower value of antioxidant system enzymes, which may indicate this group of fish was under oxidative stress. The link between growth and antioxidant activities was well-reviewed (Hoseinifar et al. 2020), and previously, we linked the lower survival rate to decreased cellular resistance to oxidative stress and impaired maintenance of the antioxidant-ROS balance (Esmaeili et al. 2022; Zare et al. 2023a, 2023b). In the present study, the photoperiod effect was more dominant and influential in changing antioxidant enzymes rather than EMS. Other studies similarly indicated that SOD, catalase, and GPx were changed with photoperiod in European sea bass (Li et al. 2021), blunt snout bream (Tian et al. 2019), golden hamster (Mukherjee and Haldar 2015), and gibel carp (Wei et al. 2019) where the lowest antioxidant status was observed in the groups with highest light exposure. However, no change was observed in black sea bass (*Centropristis striata*) (Ren et al. 2020). As we can see, most of the literature reported decreased antioxidant activities with higher or continued light. The possible reason for the lower antioxidant status in the 24L0D group of the present study can be related to a higher rate of metabolic oxygen consumption (Martínez-Álvarez et al. 2005). However, this group did not have a significantly lower growth or survival rate as a result of oxidative stress.

Liver enzymes

Liver or serological enzymes (LDH, ALP, AST, and ALT) can show the health status of fish, especially the liver, which is the central metabolic organ. Consistent with other markers examined in this study, the AST in the 24L0D group before AC stress was higher than in the other groups (Fig. 6). Other parameters did not change with EMS or photoperiod and can be a positive sign that oscars were in a healthy/normal condition. In previous studies, higher liver enzymes in oscar were evidence of stress, leading to decreased growth (Esmaeili et al. 2022). The interaction effect for ALT before and after AC stress was significant, as shown in Fig. 6. In the without EMS group, before and after AC stress, and in EMS treatment before AC stress, the 24L0D group had the highest value among the others. Other studies indicated that liver enzymes were changed with photoperiod in common carp, largemouth bass (Malinovskyi et al. 2022), Malaysian red tilapia (Malambugi et al. 2020), and tiger puffer (*Takifugu rubripes*) (Ma et al. 2021) and no change in Murray cod (Di et al. 2023). These results are matched with other parameters, such as lower

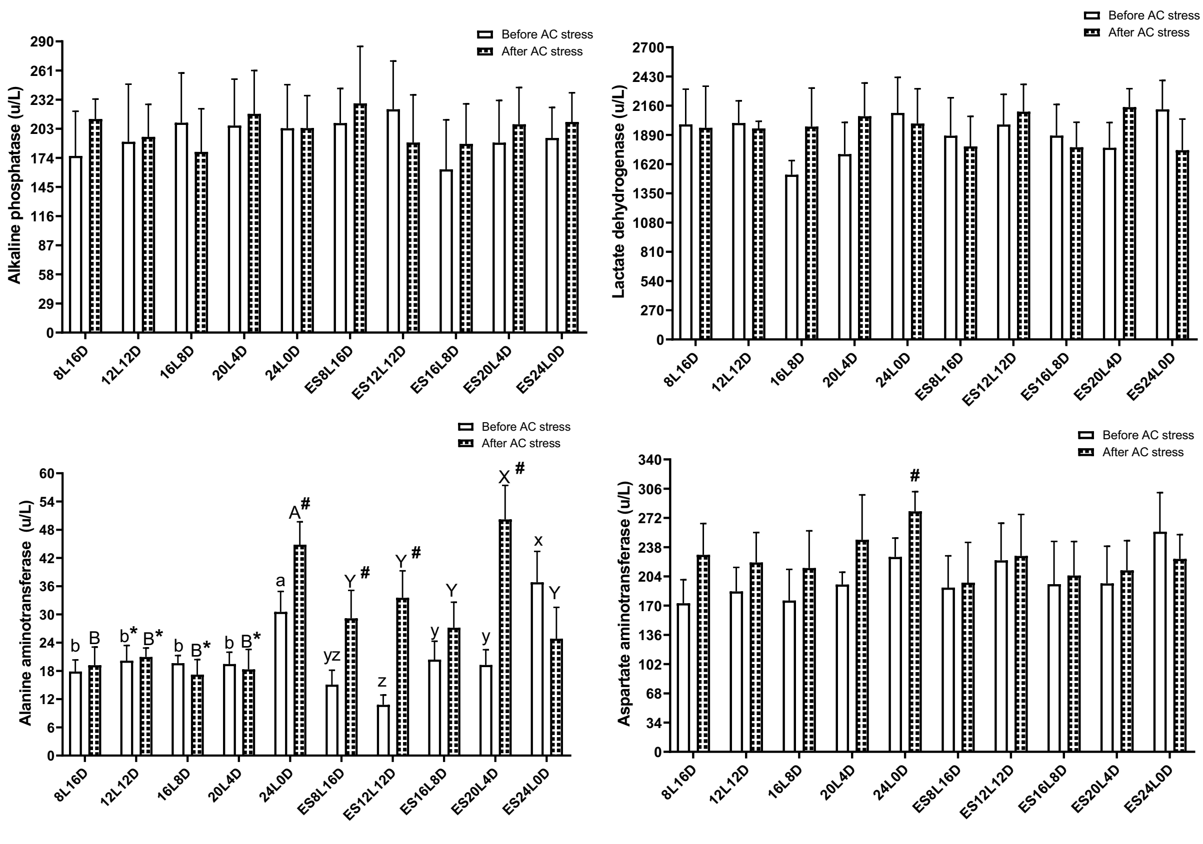


Fig. 6 Liver enzyme parameters of oscar to different photoperiods and early mild stress treatments during 10 weeks plus data related to after acute confinement stress (AC stress). Values were represented by means ± SDM of triplicate samples. Hashtag (#) indicates the significant difference in each treatment between farmed fish with and without early stress according to the independent sample *T*-test ($P < 0.05$) (for example, 20L4D compared to ES20L4D). Further, x, y, and z and also, a, b, and c indicated significant differences across different photoperiod groups with and without early stress, respectively (the interaction effect was significant for this parameter). In addition, X, Y, and Z indicated significant differences across different photoperiod groups after AC stress (patterned columns)

antioxidant enzymes and immune system parameters in the 24L0D group. It was earlier mentioned that a lower survival rate after AC stress and higher liver enzymes are further evidence that this treatment was not displaying normal physiological responses (Zare et al. 2023a). Aquaculture studies have reported elevated ALT and AST in response to different stresses in a wide range of fish species (Tejpal et al. 2009; Liu et al. 2016; Sun et al. 2019; Dawood et al. 2021; Hoseini et al. 2022). More research is necessary to demonstrate how EMS, photoperiod, and the liver's physiological status react with each other.

Conclusion

The result of the present research suggests that neither EMS nor photoperiod affected fish growth performance or survival rate after a 9-week experiment. Fish exposed to continued light (24L0D) showed negative responses to many markers examined, such as lower haematological parameters, immune system parameters, antioxidant enzyme activities, and higher liver enzymes. However, none of these alterations led to impaired fish growth performance. Survival rate was significantly changed after AC stress, with fish exposed to EMS regimes being more able to cope with stress. AC stress decreased some health parameters under 24-h light, while such results were not observed in the EMS-exposed fish. Therefore, the EMS schedule can be a useful tool in preventing the negative effects of acute stress. More programmed stresses and measurements of more parameters at the classical and molecular levels are needed, as well as a nutritional fortification, to illustrate the various mechanisms of EMS and its interaction with photoperiod in fish. EMS can directly influence the behaviour of fish; behavioural and learning mechanisms should be focused on in future studies.

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Author contribution Mahyar Zare: statistical analysis, investigation, resources, research; Mohammad Kazempour: student, research, lab works, investigation; Seyedeh Mahsa Hosseini: student, research, lab works, investigation; Sobhan R. Akhavan: validation, revision; Michael Salini: validation, revision; Artur Rombenso: validation, revision; Noah Esmaeili: ideas, supervision, validation, writing.

Data Availability Data available on request due to privacy/ethical restrictions (The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.)

Declarations

Ethical approval A National Animal Care and Committee approved all experimental protocols (281–1385) (Safavi et al. 2019; Tazikeh et al. 2020; Ahmadi-Noorbakhsh et al. 2021).

Competing interests The authors declare no competing interests.

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