THE INFLUENCE OF LIGHT INTENSITY ON HEMATOLOGICAL INDICATORS OF JUVENILE CARP REARED IN A RECIRCULATING SYSTEM

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Abstract

This paper aims to analyze the effects of light intensity on the main hematological indicators of carp, reared under a recirculating system condition. Two light intensity levels were tested, each in duplicate: 280 lx and 90 lx. In both experimental lighting variants, the hemoglobin concentration showed an upward trend at the end of the experimental period. The analysis of hematocrit values showed a similar trend to that observed for hemoglobin concentration. Regarding the dynamics of erythrocytes, a significant decrease in their number was observed under white light exposure ($p<0.05$), and a slight increase was recorded in the blue light variant. The values for MCV and MCH showed a statistically significant increase ($p<0.05$) in both lighting variants at the end of the experiment. The MCHC decreased significantly in the white light variant, while in the blue light variant, there was a significant increase ($p<0.05$). The results obtained indicate that hematological indicators suggest that juvenile carp were able to adapt due to judicious feed management and, to a lesser extent, due to light intensity.

Key words*: carp, light intensity, hematological parameters*

INTRODUCTION

The specialized literature provides comprehensive information on the physiology of the visual system in fish, supporting the hypothesis that it is sufficiently evolved to respond to a wide range of wavelengths and light intensities. The influence of light on the growth dynamics of a cultured species is evaluated in various studies in the literature through the prism of its main characteristics, namely: color, intensity, and photoperiodicity. Numerous experiments have demonstrated that the physiological state and, implicitly, the growth of fish can be influenced by the light spectrum and its intensity [1, 2, 3, 4] and photoperiodicity [5, 6, 7, 8, 9]. The two parameters of light, namely spectral structure and intensity, can be easily manipulated from a technical point of view and at low cost in recirculating systems [10].

The specialized literature mentions, in the case of carp juveniles, a series of experiments in which the influence of certain parameters of the light regime on growth dynamics was tested in a differentiated manner. One of the experiments aimed to evaluate the influence of light colour on growth performance, concluding that red light leads to better results than blue light [4]. Another experiment on the influence of light regime on carp fry growth refers to light intensity,

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establishing that the optimal range for this parameter is 150-300 lx [4].

Light intensity influences both positively and negatively the development, growth, and other physiological processes of fish and amphibians. The quality of the response to light intensity can be influenced by the developmental stage of the larvae, age, feeding status, season, time of day, and physiological state of the fish [11].

Previous research has shown that for two of the most economically valuable species in our country, *Cyprinus carpio* and *Oncorhynchus mykiss*, red light can stimulate growth more effectively than white light [4, 10]. In other species, such as *Sparus aurata*, blue light has also been found to have a positive effect on growth performance.

The complexity of the issues related to the modification of the physiology of different cultured species in relation to light regimes, a tool for improving fish growth performance, requires further research in this field to better understand the effect of light [1].

Light has a significant impact not only on growth performance but also on the physiological and biochemical processes of fish species, with blood being a direct indicator of metabolic changes, accurately reflecting the changes taking place in their bodies [14, 15]. Blood hematological indicators (Hb, Ht, erythrocytes, and leukocytes) are good indicators of the physiological response to external stimuli, and all changes that occur are reflected in their morphology and distribution in the blood [16]. Previous studies on fish hematology have shown that interpreting blood indicators is quite difficult because blood changes are caused by both internal and external factors [17]. In this context, the objective of the experiment is to assess the health status of carp juveniles reared in an intensive recirculating aquaculture system, depending on the light regime (color and intensity), by analyzing the main hematological indicators.

MATERIAL AND METHOD

Based on the hypothesis that light influences the health of carp juveniles in a recirculating industrial aquaculture system, two experimental variants were designed and implemented:

a) Variant V1 (Light intensity - 280 lx):

- Growth unit B1, initial stocking density- 70.54 kg/m^3, number of fish - 251, average body weight - 141 g/fish;
- Growth unit B2, initial stocking density - 70.65 kg/m^3, number of fish - 244, average body weight - 145 g/fish.

b) Variant V2 (Light intensity - 90 lx):

- Growth unit B3, initial stocking density - 70.77 kg/m^3, number of fish - 253, Average body weight - 140 g/fish;
- Growth unit B4, initial storage density - 71.42 kg/m^3, number of fish - 258, average body weight - 138 g/fish.

The synoptic diagram of the experimental design, developed to assess the influence of light on the technological plasticity of carp juveniles raised in a recirculating system, is shown in Figure 1 [18]. In order to maintain a stable aquatic environment, a key concern in organizing the experiment was to ensure that water quality parameters were maintained within technologically optimal limits through rigorous control of the operation of all treatment stages in the recirculating system configuration. The following water quality parameters were monitored daily: temperature and dissolved oxygen at the four rearing units; pH, ammonium, and nitrates at the water inlet point to the rearing units.

The experimental variants differ mainly in the color of the light used in the growing units and, secondarily, in its intensity. Thus, the first variant corresponds to two growing units covered with caps/filters made of white-colored polycarbonate plates, and the second variant corresponds to two growing units covered with caps/filters made of blue-colored polycarbonate plates.

The filtering of light through these covers naturally led to a differentiation in the

spectral structure of the light radiation. The technological space in which the experiment took place, isolated from natural light, was permanently illuminated, both day and night, with a set of metal vapor fluorescent lamps, with a power of 36 watts/lamp. The light intensity measured in the technological space was kept constant throughout the experiment at around 400 lx.

The light intensity was measured at the water surface using a TESTO 545 lux meter, recording an average value of 280 lx in the white light version and 90 lx in the blue light version. These values represent the average light intensities determined at points considered representative within the breeding units, namely: the center of the tank, the point located halfway between the center and the side wall, and the area adjacent to the tank wall.

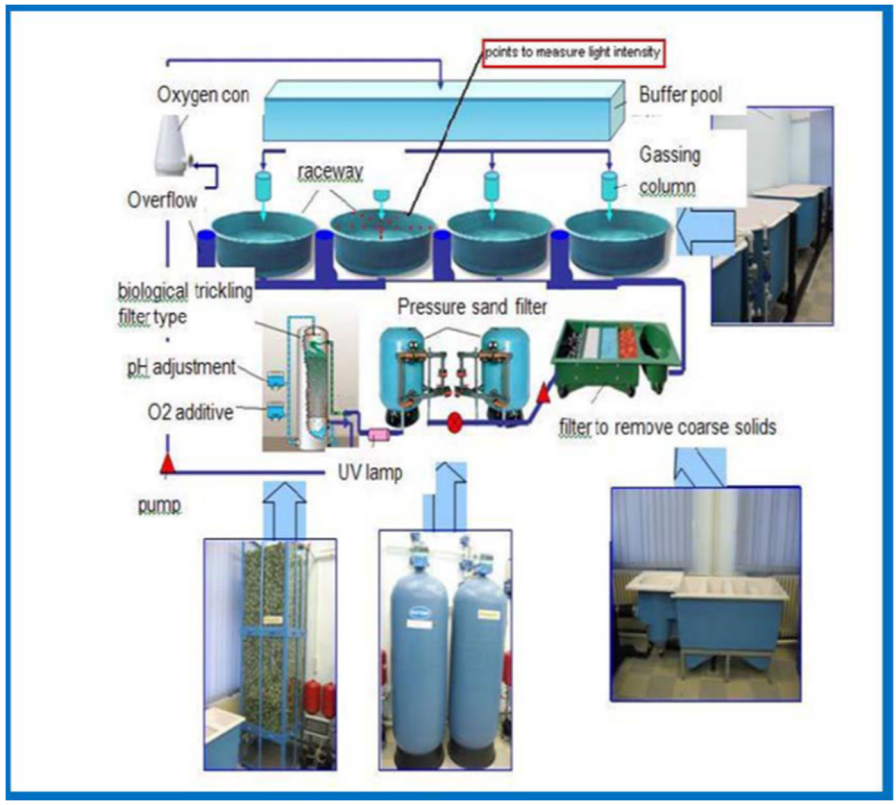

Figure 1. Pilot recirculation system configuration (Cristea V., 2008)

The biological material used consisted of 16-month-old carp juveniles obtained from the experimental laboratory of the Institute of Research and Development for Aquatic Ecology, Fishing, and Aquaculture in Galati. The fish were fed the same type and quantity of feed in all four breeding units, with a protein content of 35%. The feeding intensity in both experimental variants, expressed as the ratio between the amount of feed administered daily and the amount of biomass in the rearing units, was 1.3% BW (kg feed/kg biomass*day). The daily ration was distributed automatically, in a continuous/continuous flow regime, between 9 a.m. and 9 p.m. Before

introduction into the breeding units, the health status of the carp juveniles used in the experiment was assessed. For this purpose, five carp were clinically and anatomo-pathologically investigated to identify and eliminate any pathogens.

In order to characterize the hematological profile of the culture biomass, at the start of the experiment, 10 carp from each tank were marked with coded chips, each fish corresponding to a specific code. At the beginning and end of the experiment.

The following methods were used to determine the main hematological indicators:

- Hemoglobin was determined using the colorimetric method with Drabkin reagent. Blood samples were analyzed using the SPECORD 210 Analytikjena spectrophotometer at a wavelength of 540 nm.
- Hematocrit was determined using the microhematocrit method, which involves centrifuging blood in heparinized microhematocrit capillary tubes. Centrifugation is performed using the HETTICH HAEMATOCKIT 210 device at a speed of 12,000 rpm for 5 minutes.
- The number of red blood cells was determined using the classical method, using Vulpian's solution as the diluent, a Neubauer counting chamber, and a Potain pipette for erythrocytes.

Based on the results obtained from the determination of hemoglobin, hematocrit, and red blood cell count, the derived erythrocyte constants, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated, which are significant indicators in characterizing the physiological state of fish.

The experimental data were statistically processed using the following statistical test: the parametric t-Student test (comparisons between means, significance $p < 0.05$).

RESULTS

The water quality parameters monitored daily, including temperature and dissolved oxygen concentration in the four breeding units, as well as pH values, ammonium, and nitrate concentrations at the water inlet point in those units, were within the optimal range for the species studied. Following the analysis and statistical processing of blood samples collected from carp juveniles in the two experimental variants, erythrocyte reactions were characterized based on indicators specific to these blood cells, determined both at the beginning and at the end of the experimental period. The results obtained are presented in Table 1, as well as in the figures below, which illustrate the evolution of the parameters analyzed during the experimental period. In order to objectively assess the health status of carp juveniles exposed to different light intensity regimes, the values obtained for the main hematological indicators were compared with the reference ranges available in the literature (Table 2).

DISCUSSIONS

According to data in the literature, normal hemoglobin levels vary significantly depending on the age of the fish, with a general tendency to increase with age [23]. Studies also indicate a decrease in hematocrit values under stress conditions [24], suggesting that the physiological response of fish to stress involves exceeding the capacity of the spleen, with the release of an increased number of erythrocytes into the peripheral circulation.

Maintaining internal homeostatic balance is essential for the normal functioning of the body, and in the event of a disturbance, regardless of its nature, the body will attempt to establish a new balance. Any disturbance in the environment can be considered a potential source of stress, which will require the body to make a series of adaptive responses to cope with the physiological changes triggered by external changes [25].

Table 1. Hematological parameters of carp from the two variants

Experimental variants		Hematological parameter (Med. ±StDev)					
		Ht %	Hb (g/dl)	Red blood cells (mil/µl)	MCV (µm3)	MHC (pg)	MCHC(g/dl)
Variant 1	B1 initial	25.2±4.1	9.36±1.13	2.004±0.14	126.12±20.57	46.86±6.04	37.78±5.72
	B1 final	30.4±5.3	9.82±1.55	1.726±0.26	175.67±9.52	57.04±4.92	32.50±2.56
	B2 initial	24.2±4.4	8.06±0.14	1.836±0.21	131.98±19.49	44.54±5.58	34.46±6.49
	B2 final	28.0±2.8	8.74±1.63	1.467±0.11	190.83±12.31	59.29±8.24	31.02±3.5
Variant 2	B3 initial	24.0±3.4	6.66±0.55	1.563±0.22	156.95±32.1	43.01±3.45	28.27±4.48
	B3 final	28.4±3.4	8.00±1.72	1.546±0.07	183.66±19.62	51.86±11.5	28.15±5.61
	B4 initial	26.8±2.8	5.22±1.05	1.645±0.09	163.60±21.36	31.58±5.46	19.88±4.87
	B4 final	29.6±1.0	8.88±1.38	1.703±0.10	174.45±12.65	52.45±9.77	30.09±5.09

Table 2. Normal values of the main hematological indicators in carp

Bibliographical references	Red blood cells (10^6/µl)	Hb (g/dl)	Ht (%)	MCV (µm3)	MHC (pg)	MCHC (g/dl)
Ghittino, 1983 [19]	1.10-2.20	6.5-10.6	32.-43.8	152-364	50-63	15-25
Bastami K.D, et al, 2009, [20]	1.397	8.73	31.5	225	59-64	27.4-28.8
Sieroslawska A. et al., 2012, [21]	1.5	6.439	29	198.62	43.07	21.788
Kumar V.et al., 2010, [22]	1.58	5	29.75	189.3	31.8	17.14

Erythrocyte reactions describe the variation in hematological indicators (hemoglobin, hematocrit, erythrocytes, and erythrocyte constants) recorded at the start and end of the experiment. Their analysis leads to a series of qualitative and quantitative assessments presented below.

Concerning hemoglobin (Hb) concentration, there are more or less significant differences in both experimental variants between the initial and final values. There are also notable variations in the dynamics of hemoglobin concentration between the two experimental variants (Figure 2). The qualitative assessments of hemoglobin concentration are supported, from a quantitative point of view, by the following data:

- the amount of hemoglobin in the variant using white light and an intensity of 280 lx increases, but not statistically significantly ($p>0.05$; $p=0.19$), from 8.71 g/dl (initial value) to 9.28 g/dl (final value);
- in the second experimental variant, in which blue light and an intensity of 90 lx were used, the increase in hemoglobin at the end of the experiment was much more pronounced and statistically significant ($p<0.05$; $p=0.0006$), in the sense that from an initial average value of 5.94 g/dl, located at the lower limit of the normal range, the final average value was 8.44 g/dl, which is close to the upper limit of the normal range;
- the amount of hemoglobin recorded at the end of the experiment in the two variants is different, higher in the case of white light, but the differences in concentration are not statistically significant ($p>0.05$; $p=0.14$). The hemoglobin values obtained at the end of the experiment for the two experimental variants fall within the optimal range for the species studied and are comparable to those in the specialized literature [26, 27].

For the *Carassius gibelio* species, better hemoglobin values were obtained for the variant using dark blue light compared to red, green, light blue, and violet light. For the Siberian sturgeon species, an increase in hemoglobin values can be observed in the case of green, dark blue, and light blue light [15].

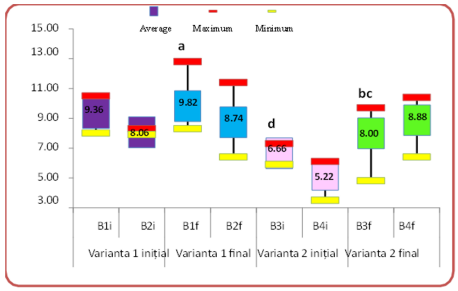

Fig. 2. Hemoglobin variation of carp in the two variants

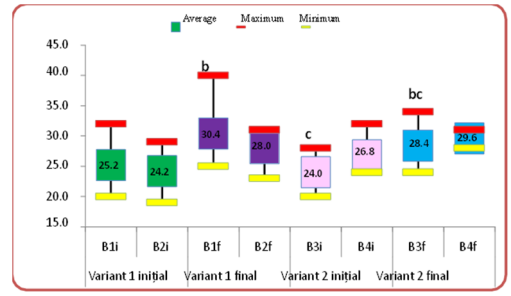

Fig. 3. Variation of hematocrit in carp during the experiment

a - statistically insignificant differences between the initial and final moments, p>0.05;
b - statistically significant differences between the initial and final moments, p<0.05;
c - statistically insignificant differences between the two variants, p>0.05;
d - statistically significant differences between the two variants, p<0.05.

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Quantitative observations regarding hematocrit (Ht) dynamics in the two experimental variants are presented in Figure 4. The following variations were observed for this indicator:

•in the variant in which white light was used, there was a statistically significant increase ($p<0.05$; $p=0.02$) at the end of the experiment, from 24.7% to 29.2%;

•in the variant using blue light, the hematocrit also showed a significant increase ($p<0.05$; $p=0.01$), from 25% at the start of the experiment to 29% at the end of the experiment;

•the hematocrit dynamics in the two experimental variants are similar, with no statistically significant differences between the two variants at the end of the experiment ($p>0.05$; $p=0.45$).

Some comments can be made regarding the phenomenon of hematocrit increase recorded in both light regimes. Thus, a possible cause that may explain the increase in hematocrit, a cause cited/confirmed by the literature [28] consists in the increase in the number of erythrocytes as a result of the intensification of erythropoiesis in response to the potentially stressful action of light; this situation is not found in our experiment, as the number of erythrocytes recorded at the start and end of the experiment was virtually constant.

A second possible cause that may explain the increase in hematocrit while maintaining a constant number of erythrocytes, a situation that we consider plausible/feasible in the case of the experiment performed, would be an increase in the diameter/volume of erythrocytes as a result of an increase in hemoglobin content, a statement supported by the data presented in Table 1.

The evolution of the erythrocyte count in the two variants regarding the light regime is presented graphically in Figure 4. The first finding that emerges from the analysis of the dynamics of this indicator is that in the case of white light, the number of erythrocytes shows a significant decrease, the difference between the initial and final values of this indicator being 16.66%, which is considered statistically significant ($p<0.05$; $p=0.02$), while in the case of blue light there is a slight increase in the number of erythrocytes (1.2%), but the difference between the two moments of evaluation is not statistically significant ($p>0.05$). Another remark regarding the evolution of erythrocytes in this experiment is that, as a consequence of the different lighting

regimes, starting from different values of the number of erythrocytes in the two variants, the values were finally found to be significantly equal ($p>0.05$; $p=0.37$) in both light regimes.

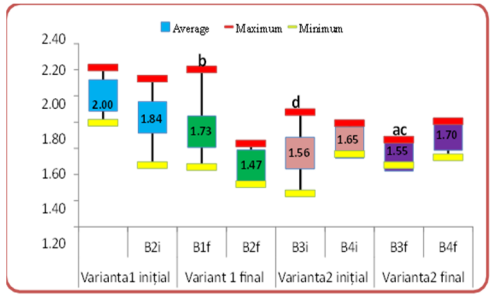

Fig. 4. Variation in the number of erythrocytes

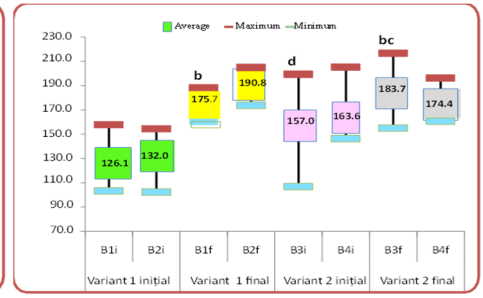

Fig. 5. Evolution of Mean Corpuscular Volume (MCV)

The results obtained in this experiment, in terms of hematocrit values, hemoglobin concentration, and erythrocyte count, are similar to those reported for the species *Polyodon spathula* (Walbaum, 1792), indicating that there are no statistically significant differences between the experimental variant exposed to white light and the one exposed to green light [29].

The values obtained for hemoglobin, hematocrit, and red blood cell count are used in formulas to calculate and characterize derived red blood cell constants. The erythrocyte constants of blood (Table 1) reflect the degree of normality of erythrocytes, their average hemoglobin content, providing useful information on the functional integrity of respiratory function [30]. Interpretation of the data in Table 1 regarding the VEM, HEM, and CHEM indicators leads to a series of relevant conclusions, including:

- Between the two moments of the experiment, the beginning and the end, a statistically significant increase ($p<0.05$) in the mean erythrocyte volume (MVC) both in the variant where white light was used, from a value below the normal range, and in the variant where blue light was used, from a value at the lower limit of the normal range (Figure 5). However, when comparing the mean corpuscular volume (MCV) values from the two experimental variants at the end of the experiment, no statistically significant differences ($p>0.05$) were observed; these values fell within the normal range for carp, according to the data in the literature.
- Mean corpuscular hemoglobin (MCH) (Figure 6) shows the same upward trend in both variants at the end of the experiment as mean erythrocyte volume (VEM). The initial values of mean corpuscular hemoglobin in both experimental variants are outside the normal range for the species studied, which suggests that the carp juveniles were slightly anemic at the start of the experiment. By applying appropriate feed management, it can be observed that the values of this parameter increase, falling within the normal range for the species at the end of the experiment. The increase in mean erythrocyte hemoglobin at the end of the experimental period is statistically significant ($p<0.05$), both in the variant where white light was used and in the variant where blue light was used. At the end of the experiment, the mean erythrocyte hemoglobin was significantly equal in both experimental variants, and statistical processing of the data did not reveal any statistically significant differences ($p>0.05$).
- At the end of the experiment, mean corpuscular hemoglobin concentration (MCHC) showed a significant decrease ($p<0.05$; $p=0.04$) from 36.12 g/dl to 31.76 g/dl in the variant where white light was

used and a significant increase ($p<0.05$, $p=0.04$) from 24.07 g/dl to 29.12 g/dl in the variant using blue light. Statistical comparison of the mean erythrocyte hemoglobin concentration values in the two experimental variants shows that these values are sensibly equal and do not present statistically significant differences ($p>0.05$; $p=0.11$) at the end of the experiment; the variation in mean erythrocyte hemoglobin concentration is shown graphically in Figure 7.

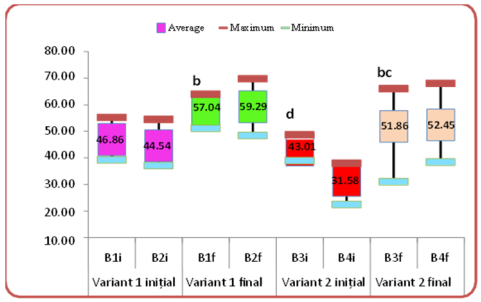

Fig. 6. Mean corpuscular hemoglobin (MCH)

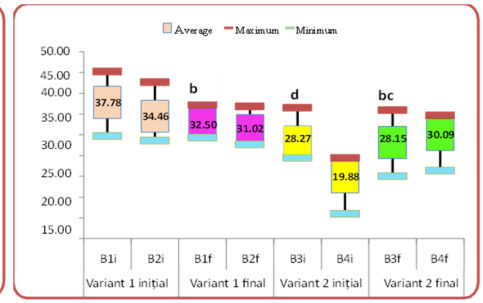

Fig. 7. Mean corpuscular hemoglobin concentration (MCHC)

CONCLUSIONS

The main scientific objective of the experiment, formulated in accordance with the working hypothesis, was to assess the health status of carp juveniles raised in an intensive recirculating aquaculture system.

The infrastructure in which the experiment was organized ensured, thanks to advanced water quality control techniques, normal conditions for the development of crop biomass in the two experimental variants, which lends scientific credibility to the results obtained.

The main conclusion that emerges from the analysis of the values of the most important hematological indices, hemoglobin and hematocrit, is that both indicators showed a significant increase during the experiment. Thus, while at the beginning of the study the hematocrit values were below the limits considered normal for the species analyzed, according to data from the specialized literature, at the end of the experiment, they fell within the reference range specific to the species. It is known from the literature that a lower hematocrit and leukocyte count suggest the onset of mild anemia. The low hematocrit value, approximately 25% in the present experiment, indicates that the carp juveniles in both experimental variants had mild anemia at the start of the experiment.

The higher hematocrit values at the end of the experimental period show an improvement in the health of the culture biomass, primarily due to judicious feed management (feeding intensity and nutritional value of the feed administered) and less likely due to the light regime (color and intensity). Analysis of the evolution of the erythrocyte count in the two experimental variants highlights one main conclusion: as a result of different lighting regimes and distinct initial values of this indicator, opposite trends were observed – an upward trend under blue light and a downward trend under white light. Ultimately, these trends led to approximately equal values for the number of erythrocytes in both lighting variants.

Although differences were observed in the evolution of erythrocytes and other hematological indices between the two lighting variants (white and blue), the impact of the lighting regime on health remains inconclusive, requiring further research. The complexity of the interaction between light intensity and the

appropriateness of using light intensity in breeding units as the main technological management tool requires further studies to clarify the potential of the lighting regime as a technological optimization tool in intensive aquaculture.

From a practical perspective, lighting in aquaculture must be carefully designed, adapted to each stage of the organisms' life cycle, and correlated with the specific preferences of each species. Light must create optimal conditions for the welfare of aquatic organisms, thus contributing to maintaining the quality of the farming facilities and achieving high productivity.

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