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SURVIVAL OF EMBRYOS AND LARVAE OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM, 1792) UNDER INFLUENCE OF OPTICAL RADIATION AT VARIOUS TEMPERATURE REGIMES

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In this article the authors showed result of the study survival of embryos and larvae of the rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) under influence of optical radiation at various temperature regimes. The optical emission of the red region of the spectrum has a significant effect on the individual lifetime of embryos and larvae of rainbow trout in vitro in the absence of feeding. The best results were obtained at a temperature of 8 ° C. As studies have shown, the temperature regime of growing aquaculture objects, even in the ranges of optimal values, is able to exert an effect on the magnitude of the stimulating effect of optical radiation. The obtained results create prospects for more effective use of low-intensity optical radiation in aquaculture technology of valuable fish species.

Key words: trout, aquaculture, optical radiation, temperature regimes.

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INTRODUCTION

Reproduction of valuable fish species is a complex technological process, including work with breeders, obtaining stocking material, and the formation of a repair and broodstock. In this technological chain, the weakest and most vulnerable link is the production of stocking material due to the high sensitivity of embryos to industrial growing conditions. Aquaculture of fish-breeding industrial complexes working on the recirculating aquaculture system (RAS) is actively developing in Belarus. Thus, only in recent years 13 projects have been implemented in the country aimed at creating an RAS for the cultivation of sturgeon, salmon, clarias, and acne fishes (Kostousov & Barulin 2013, Barulin 2015). The RAS can increase the level of intensification of reproduction technology in aquaculture.

With the aim of solving the problem of developing a new effective technology for growing a viable stocking of valuable species in hatchery industrial complexes, as a result of long-term fundamental and applied research, we have scientifically substantiated approaches that ensure the realization of the stimulating effect of low-intensity optical radiation on fish-biological and economically useful sturgeon stocking material and salmonids due to the effect of optical radiation on embryos (fertilized roe) and fish sperm. It is shown that the magnitude of the effect depends strongly on the polarization of the radiation and is practically independent of the degree of its monochromaticity, which indicates that it is possible to use in the technology of artificial reproduction and cultivation of valuable species of fish effects on eggs by radiation of both semiconductor lasers and LED sources after preliminary polarization of latest radiation. The conducted researches served as the basis for creating a series of laser-optical devices based on semiconductor lasers and LEDs for irradiating roe of valuable species of fish incubated in a stationary position and in Weiss apparatuses (Plavskii & Barulin 2008 a, 2008 b, 2008 c, 2010).

Development of technology for trout and sturgeon breeding is relevant for Belarus. In the technological chain of growing commercial fish, the most important is the stage of obtaining fish stocking material. Industrial methods of cultivation, intensification of production and artificial conditions are the strongest stress factors for embryonic development, leading to a decrease in the basic physiological parameters, survival and vitality throughout the life of the fish including the appearance of morphological abnormalities. Therefore, during the period of embryonic development in industrial aquaculture, it is necessary to carry out correction of development using various factors of influence on the body. One of such factors is low-intensity optical radiation which is successfully used in medicine for treatment, correction and therapy in various directions. As our long-term studies have shown, laser radiations as well as the emission of super-bright LEDs have a stimulating effect on sturgeon fish and their sexual products (roe and sperm) as well as on the development of gullet crustaceans. However, our previous studies were based on the effect of optical radiation on bio objects within the same temperature. The question of the most favorable temperature regimes in which the maximum effect of optical radiation on aquaculture objects is manifested remains open (Plavskii & Barulin 2008 a, 2008 b, 2008 c, 2010).

The aim of research is study the effect of optical radiation on the empirical and postembryonic development of rainbow trout *in vitro* under different temperature regimes.

MATERIAL AND METHODS

The research was carried out at the Department of Ichthyology and Pisciculture and "fish farm industrial complex" of Belarusian State Agricultural Academy. The object of research were unisex embryos (fertilized eggs at the eye) of female rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792), which during the study passed to the stage of a free embryo, and then to the stage of exogenous nutrition.

Fertilized eggs at the ocellus stage was purchased in the fish hatchery Viviers de Sarrance (France), which was transported in a thermoplastic ice pack on an airplane. Then the eggs was adapted in the incubator of a fish-breeding industrial complex, operating on the principle of RAS included pre-incubation trays, mechanical and biological purification systems, as well as oxygenation and disinfection of water by UV irradiation. After a two-day adaptation of the embryos (fertilized eggs in the ocellus stage) in the RAS of the incubation department, experimental and control groups were formed. The groups were placed in separate Petri dishes and transferred to the refrigerator, where their further two-day adaptation took place. Further embryos underwent optical radiation (test groups) or were not exposed but were in identical conditions (control group). As a source of optical radiation, a semiconductor laser (LD) of the phototherapeutic device "Lotos" (red spectral region $\lambda = 650$ nm) was developed in

Table 1. Characteristics of the probit (logit) models of the each ten-day average survival rate of embryos and larvae of rainbow trout at a temperature of 8 - 12 ° C, depending on the type of optical radiation

Groups	Slope coefficient	Linear probit (logit) equation	LD50
Temperature 12 ºC			
Control	2.15	probit(P) = - 7.8 + 2.15 ln(D)	38.83
LED	2.83	probit(P) = -10.81+ 2.83 ln(D)	45.29
LD	3.26	probit(P) = -12.40+ 3.26 ln(D)	44.48
Temperature 11 ºC			
Control	2.37	logitP) = -9.11 + 2.37 ln(D)	46.29
LED	3.17	logit(P) = -12.41 + 3.17 ln(D)	49.91
LD	4.77	logit(P) = -18.36 + 4.77 ln(D)	46.74
Temperature 10 ºC			
Control	1.83	logit(P) = -7.01 + 1.83 ln(D)	45.41
LED	3.82	logit(P) = -15.73 + 3.82 ln(D)	61.13
LD	3.13	logit(P) = -13.02 + 3.13 ln(D)	64.24
Temperature 9 ºC			
Control	1.74	logit(P) = -7.35+ 1.74 ln(D)	67.15
LED	5.64	logit(P) = -24.02 + 5.64 ln(D)	70.44
LD	4.98	logit(P) = -21.56 + 4.98 ln(D)	75.68
Temperature 8 ºC			
Control	3.04	logit(P) = -12.17 + 3.04 ln(D)	54.16
LED	5.11	logit(P) = -21.71+ 5.11 ln(D)	69.74
LD	4.78	logit(P) = -20.14+ 4.78 ln(D)	67.16

the design department «Lyuzar» and Institute of Physics National Academy of Sciences of Belarus, as well as a matrix of LED sources of the optical device «Stronga» (red spectral region λ = 630 ± 10 nm) developed at the Belarusian State Agricultural Academy and Institute of Physics of the National Academy of Sciences of Belarus. The effect of irradiation on the embryos was carried out for 5 days for 20 minutes at a power density of 3.0 mW / cm^2. After exposure to optical radiation, the embryos were immediately returned to the refrigerator on the appropriate shelf.

To study the effect of optical radiation on the survival of embryos and larvae of rainbow trout at different temperatures in the absence of food, five so-called "Temperature" study groups, including control and experimental (LD and LED) groups in triplicate for each temperature: 8, 9, 10, 11, 12 ºC were formed. Temperature control in the study groups was carried out by placing them in a refrigerator on the appropriate shelf in height. In the groups studied, water was replaced daily. The source of water was an artesian well. The water was previously subjected to de-ironing, disinfection (UV-irradiation) and temperature equalization.

Control of survival was performed by daily recording the number of live and dead larvae in the study groups. The dead larvae were disposed off after registration. Based on the obtained data on the number of live and dead larvae the mean survival rate for the period of the experiment, the each ten-day dynamics of the average survival rate during the experiment, and the individual lifetime were calculated.

R soft (R Core Team 2016), including packages R Commander (Fox 2005), PMCMR (Pohlert 2014), MASS (Venables & Ripley 2002), etc.,

Table 2. Deviance - analysis of probit (logit) models of the ten-day average survival rate of embryos and larvae of rainbow trout at a temperature of 8 - 12 ºC

Model No.	Remainder of Df.	Remainder of the deviance	Df	Deviance	p - criterion
Temperature 12 ºC					
1	22	10.02	-	-	-
2	18	7.49	4	2.52	0.63
Temperature 11 ºC					
1	22	24.92	-	-	
2	18	19.25	4	5.66	0.22
Temperature 10 ºC					
1	22	24.15	-	-	
2	18	12.92	4	11.22	**0.02**
Temperature 9 ºC					
1	25	34.59	-	-	
2	21	17.87	4	16.71	**0.002**
Temperature 8 ºC					
1	28	31.25	-	-	
2	24	21.12	4	10.13	**0.03**

were used for statistical processing of results. Statistical significance of the differences was estimated by Tukey test provided that the data distribution is normal (assessed by the Shapiro-Wilk test) and the homogeneity of the group variances (evaluated by Levene's test). If non-observance of these conditions, a non-parametric Newman-Keils test was used. To evaluate the qualitative characteristics, the $\chi 2$ criterion («chi-square») was used. To construct a generalized linear model, we used the function glm () in the statistical environment R, which had the form: glm (formula, data = data. frame, family = family. generator). To compare the quality of the models built, the deviance was used, which directly results from the MLE, Maximum Likelihood Estimation. To test for differences in the replicates of the experiment, a logistic criterion and the Gehan -Wilcoxon test in the Peto modification were used.

RESULTS AND DISCUSSION

1. Influence of optical radiation on the each ten-day survival of embryos and larvae of rainbow trout as a function of temperature

To assess the effect of optical radiation on the each ten-day dynamics of the average survival rate of embryos and larvae of rainbow trout during the experiment, the generalized linear model GLM (Generalized Linear Model). To select the type of the GLM-model function, we compared two possible models and estimated them by the AIC-criterion (Akaike information criterion). The best model corresponded to its minimum. To analyze the effect of the type of optical radiation on each ten-day survival at a temperature of 8 - 12 ° C, we constructed a probit - or logit model (depending on the result of AIC-criterion) for each group under study. In doing so, we obtained the slopes of the individual regression for each study group (Table 1), as well as the linear probit (logit) model equations and the values of semi-lethal doses (LD50).

The slope coefficient (Fig.1) characterizes the rate of development of the effect with an increase in the duration of fasting (lack of food), and the half-lethal dose characterizes the average duration of fasting, in which 50% of the embryos and larvae of rainbow trout are killed.

To compare the quality of the constructed models, the deviance statistics which follows directly from the estimates of the maximum likelihood method were used (Table 2).

A statistical comparison for the deviation of the obtained probit (logit) model (model No. 2) from the deviation of the null model without predictors (model No. 1) using the anova () function in the statistical medium R shows (Table 2) that the inclusion of the optical emission factor in the approximated dependent dose-effect is not highly significant in terms of reducing the model error by the chi-square test for temperature regime 12 and 11 ºC and is highly significant for temperature regime 10, 9 and 8 ºC.

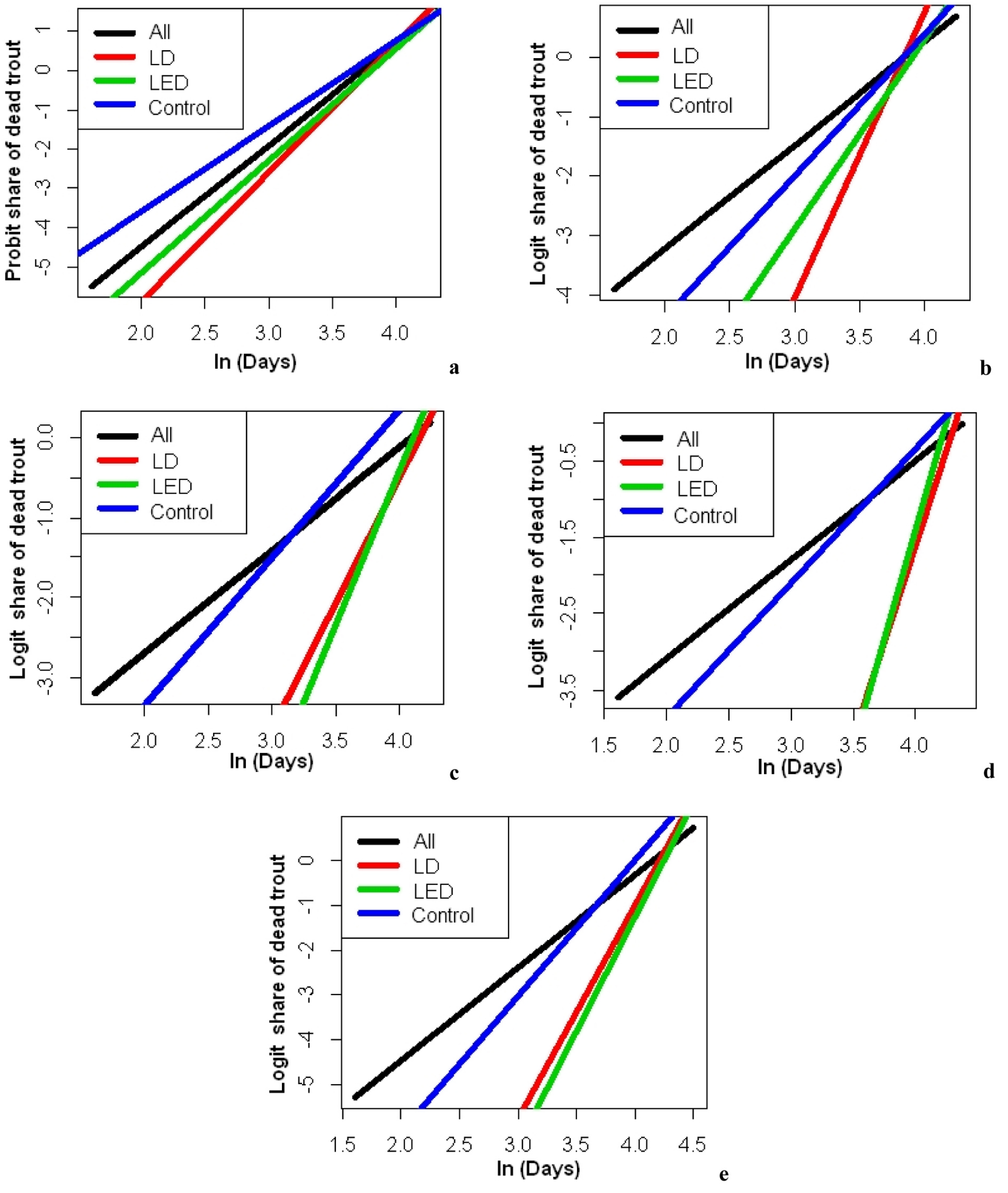

Fig. 1. Linear dependencies of the probit (logit) effect of the death of rainbow trout larvae *in vitro* from the logarithm of days of fasting for various types of optical radiation at a temperature of 12 (a), 11 (b), 10 (c), 9 (d), 8(e) ° C.

Table 3. Influence of laser (LD) and Light Emitting Diode (LED) optical radiation of the red spectrum on the duration of individual lifetime of embryos and larvae of rainbow trout *in vitro* in the absence of feeding at a temperature of 8-12 ºC

Groups	Mean, days	SD	SE (mean)	CV	N	Shapiro-Wilk test	Levene's test	Tukey Test
Temperature 12 ºC								
LD	46.0	20.1	7.1	0.4	8.0			p>0.05
LED	46.3	9.9	4.0	0.2	6.0	p>0.05	p>0.05	p>0.05
Control	43.0	8.71	3.29	0.2	7.0			-
Temperature 11 ºC								
LD	54.8	4.1	1.7	0.1	6.0			p>0.05
LED	51.1	11.0	3.7	0.2	9.0	p<0.05		p>0.05
Control	46.5	13.3	4.7	0.3	8.0			-
Temperature 10 ºC								
LD	62.7	11.4	4.7	0.2	6.0			**p<0.05**
LED	62.6	9.9	3.3	0.2	9.0	p<0.05		**p<0.05**
Control	51.7	13.0	4.6	0.2	8.0			-
Temperature 9 ºC								
LD	72.1	6.4	2.4	0.1	7.0			**p<0.05**
LED	70.3	5.4	1.8	0.1	9.0	p<0.05		**p<0.05**
Control	56.7	16.7	5.6	0.3	9.0			-
Temperature 8 ºC								
LD	81.0	2.8	1.3	0.0	5.0			**p<0.05**
LED	78.9	1.1	0.4	0.0	7.0	p<0.05		**p<0.05**
Control	63.9	17.9	6.3	0.3	8.0			-

2. Influence of optical radiation on the individual lifetime of embryos and larvae of rainbow trout as a function of temperature

The results of the study of the effect on the individual lifetime of embryos and larvae of rainbow trout at a temperature of 8 - 12 ° C are presented in Table 3.

As the results in Tables 3 show, the optical emission of the red spectrum does not exert a significant and reliable effect on the individual lifetime of embryos and larvae of rainbow trout *in vitro* in the absence of feeding at a temperature of 12 and 11 ° C and has a significant effect at a temperature of 10, 9 and 8 ° C

3. Effect of temperature on the stimulating effect of optical radiation on embryos and larvae of rainbow trout

Rainbow trout - widely distributed, cold-loving, is a stenothermic species. The rate of growth and development of fish is largely determined by environmental conditions, and primarily by temperature. The temperature of the environment is a fact that controls most of the vital functions of poikilothermic animals (Novikov 2000). As our studies have shown, the water temperature can influence the magnitude of the stimulating effect of optical radiation (Table 4-9). For example, the values of the individual life time in the control group

Table 4. Change in the individual lifetime of embryos and larvae of rainbow trout *in vitro* in the control group in the absence of feeding as a function of temperature

Groups	Mean, days	SD	SE (mean)	CV	N	Shapiro-Wilk test
Control 12	43.0	8.71	3.29	0.2	7.0	$p<0.05$
Control 11	46.5	13.3	4.7	0.3	8.0	
Control 10	51.7	13.0	4.6	0.2	8.0	
Control 9	56.7	16.7	5.6	0.3	9.0	
Control 8	63.9	17.9	6.3	0.3	8.0	

Table 5. Results of an estimation of statistical reliability of differences by Newman-Keils test between the control groups under study at different temperature regimes

	Control 12	Control 11	Control 10	Control 9
Control 12	-	-	-	-
Control 11	$p>0.05$	-	-	-
Control 10	$p>0.05$	$p>0.05$	-	-
Control 9	$p>0.05$	$p>0.05$	$p>0.05$	-
Control 8	**$p<0.05$**	$p>0.05$	$p>0.05$	$p>0.05$

Table 6. Changes in the individual lifetime of embryos and rainbow trout larvae *in vitro* exposed to LED radiation in the absence of feeding as a function of temperature

Groups	Mean, days	SD	SE (mean)	CV	N	Shapiro-Wilk test
LED 12	46.3	9.9	4.0	0.2	6.0	$p<0.05$
LED 11	51.1	11.0	3.7	0.2	9.0	
LED 10	62.6	9.9	3.3	0.2	9.0	
LED 9	70.3	5.4	1.8	0.1	9.0	
LED 8	78.9	1.1	0.4	0.0	7.0	

Table 7. Results of the statistical significance estimation of differences by Newman-Keils test between the investigated groups which were exposed to LED radiation under different temperature conditions

Groups	LED 12	LED 11	LED 10	LED 9
LED 12	-	-	-	-
LED 11	$p>0.05$	-	-	-
LED 10	$p>0.05$	$p>0.05$	-	-
LED 9	**$p<0.05$**	**$p<0.05$**	$p>0.05$	-
LED 8	**$p<0.05$**	**$p<0.05$**	**$p<0.05$**	$p>0.05$

decreased with increasing temperature (Table 4), however, only one comparison revealed significant differences (Table 5). The decrease in the water temperature in the test groups exposed to the optical radiation led to higher differences between the temperature conditions under study (Table 6-9, Fig. 2). Thus, when comparing the results of individual survival in the test groups exposed to LED and LD optical radiation, there were five significant differences in the compared

Table 8. Change in the individual lifetime of embryos and rainbow trout larvae *in vitro* which were exposed to LD radiation in the absence of feeding as a function of temperature

Groups	Mean, days	SD	SE (mean)	CV	N	Shapiro-Wilk test
LD 12	46.0	20.1	7.1	0.4	8.0	p<0.05
LD 11	54.8	4.1	1.7	0.1	6.0	
LD 10	62.7	11.4	4.7	0.2	6.0	
LD 9	72.1	6.4	2.4	0.1	7.0	
LD 8	81.0	2.8	1.3	0.0	5.0	

Table 9. Results of the evaluation of the statistical significance of the differences by Newman-Keils test between the groups under study which were exposed to LD radiation under different temperature regimes

	LD 12	LD 11	LD 10	LD 9
LD 12	-	-	-	-
LD 11	p>0.05	-	-	-
LD 10	p>0.05	p>0.05	-	-
LD 9	**p<0.05**	**p<0.05**	p>0.05	-
LD 8	**p<0.05**	**p<0.05**	**p<0.05**	p>0.05

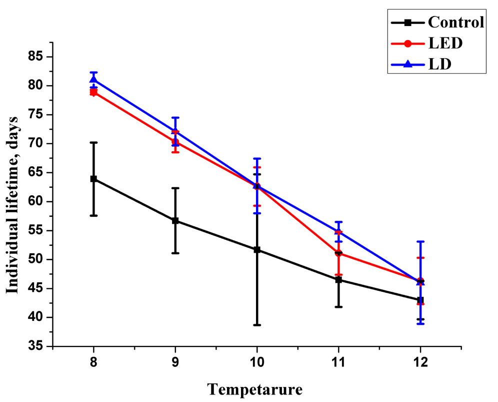

Fig.2 - Dynamics of the individual lifetime (days) of embryos and larvae of rainbow trout *in vitro* in the absence of feeding, depending on the type of optical radiation and water temperature.

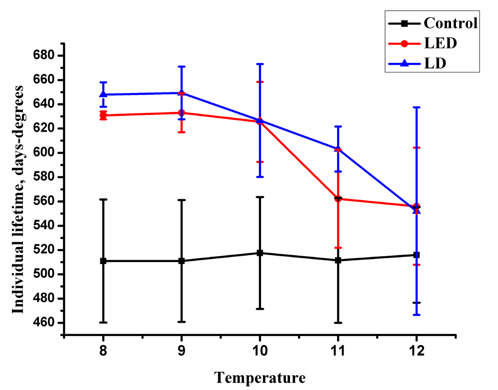

Fig. 3 - Dynamics of the individual lifetime (days-degrees) of embryos and rainbow trout larvae *in vitro* in the absence of feeding, depending on the type of optical radiation and water temperature

groups (Tables 7, 9), and the magnitude of the stimulating effect varied from 7.7 and 7.0% for LED and LD radiation, respectively at a water temperature of 12 ° C to 23.4 and 26.8% for LED and LD radiation, respectively at a water temperature of 8 ° C.

As noted by Novikov (2000), within the optimal range there is a strict dependence of the number of normally developed hatched embryos on temperature, and our data showed that within the optimal temperature range studied, the most optimal temperature was 8 ºC, as evidenced by the results of the average survival, Individual life time, etc. During the embryonic development of fish, the effect of the temperature factor is expressed primarily in changes in the rate of development. Increasing the temperature of incubation within the limits of "norm" causes a reduction in the continuity of the embryonic period both in general and its individual stages. For example, in salmon when eggs are incubated in the temperature range from 1 to 12 °C, depending on the chosen temperature, the rate of embryonic development rises by 2-4 times (Novikov 2000).

According to Golovanov (2013), the dynamics of the boundaries of the thermal stability of embryos and larvae of rainbow trout is 8 - 18 °C with an optimum of 6 - 12 °C, i .e. The temperature range used in our studies (8 - 12 °C) was within the optimum range, and the observed stimulating effect was not the results of the deviation of the growing conditions from the norm. In support of the fact that the magnitude of the stimulating effect of laser radiation was influenced by the water temperature, rather than the improvement / deterioration of optimal growing conditions, the fact of recalculation of the results of the individual lifetime in the degree days is evidence.

As is known (Benjamin et al. 2016), the term "days-degree" is widely used in the practice of ichthyology and aquaculture to assess the duration of embryonic development of fish and is defined as the product of the average water temperature by the number of days. As shown by our calculations (Fig. 3), the individual life time in the control group in all the investigated temperature regimes is at the same level and varies from 511.0 to 516.0 days-degree. In the experimental groups studied, an increase in the individual lifetime expressed in days-degrees was observed, depending on the temperature of the water. So in the experimental group, which was exposed to LED radiation, the duration of the individual life time increased from 556.0 days-degrees at a temperature of 12 °C to 633.0 and 630.8 days-degrees at a temperature of 9 and 8 °C, respectively. In the experimental group, which was exposed to LD radiation, the duration of the individual life time increased from 552.0 days-degrees at a temperature of 12 ° C to 649.28 and 648.0 days-degrees at a temperature of 9 and 8 ° C, respectively.

According to Novikov (2000), a decrease in the temperature of incubation of eggs within the optimal values led to the hatched larvae with a larger protein, as well as a lipid and carbohydrate body mass. This can explain the results of our studies, since one of the mechanisms of the action of optical radiation on biological systems is the orientation action of radiation, which induces a change in the spatial structure of the cell components responsible for the regulation of metabolic processes.

The results of our studies are consistent with the results of Wen-hwa Kwain (1975), in studies which observed mortality of embryos and larvae of rainbow trout in the temperature range 15-5 ° C under the influence of an acidic medium decreased with decreasing temperature, i.e. Survival of embryos and larvae under the influence of an unfavorable factor increased with decreasing temperature.

CONCLUSIONS

As studies have shown, the temperature regime of growing aquaculture objects, even in the ranges of optimal values, is able to exert an effect on the magnitude of the stimulating effect of optical radiation. The obtained results create prospects for more effective use of low-intensity optical radiation in aquaculture technology of valuable fish species.

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