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FEEDING BEHAVIOR OF FISH. INFLUENCE OF LIGHT DEPRIVATION ON THE EFFECTS OF SEROTONIN IN CARP Cyprinus carpio L.

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Abstract. The effect of light deprivation on the dynamics of locomotor activity and the diet in carp underyearlings under the influence of serotonin (5-HT) was studied. It was shown that the rate of the feeding reaction and the diet under the influence of 5-HT are more strongly reduced in fishes that were kept at an illumination of 405 lux in comparison with fish kept at an illumination of 0.8 lux. The mechanisms of this phenomenon are discussed.

Keywords: *food behavior, diet, light deprivation, carp.*

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1. Introduction

It is known that 5-HT is involved in the regulation of many physiological processes, including food motivation and feeding intensity in fish (Kuz'mina, 2015). When studying the effect of 5-HT on food intake, an anorectic effect was detected under its central administration (De Pedro *et al.*, 1998). Under the peripheral injection of 5-HT, the reduction of the diet as well as the rate of feeding reaction of fish (Kuz'mina & Garina, 2013) is proved. A significant decrease in the content of 5-HT in the intestine of carp in autumn and winter was found in comparison with the summer period (Terenina & Gustavsson, 2003). An increase of the temperature from 14 to 19°C leads to an increase in the level of 5-HT in the brain of European eel *Anguilla anguilla* by almost 20% is also shown (Sebert *et al.*, 1985). However, the decrease of the concentration of 5-HT in fish tissues can be caused by not only by the water temperature, but also by the decrease in the duration of the daylight and the intensity of the light flux. Indeed, the effect of photoperiod on the serotonergic activity of the hypothalamus and other parts of the fish brain is known (Olcese *et al*., 1980; Khan & Joy, 1988; Senthilkumaran & Joy, 1993). The highest level of 5-HT in the hypothalamus of the spotted snakehead *Channa punctatus* was detected in November, and the most significant diurnal variations were in February (Khan & Joy, 1988).

At the same time, the content of 5-HT in the hypothalamus and the forebrain in the fossil cat *Heteropneustes fossili* in February and March at night is significantly higher than in the daytime (Senthilkumaran & Joy, 1993). Data on the effect of photoperiod on locomotor reactions and diets fish in the available literature are absent.

The aim of this work is to study the dependence of serotonin effects on various aspects of carp feeding behavior under light deprivation.

2. Material and methods

The carp *Cyprinus carpio* L. underyearlings were object of investigations.

Experimental conditions. Carp underyearlings were obtained as a result of natural spawning with subsequent cultivation during the summer period in the ponds of the field research laboratory of the IBIW RAS "Sunoga". After the capture at the end of September, the fish was transported to a laboratory where they were kept in an aquarium of 200 L with running tap water (temperature 18-20 °C, pH 7.0-7.3, total hardness 4.6 mmol / L, Ca^{+2} 3.1 , Mg^{+2} - 1.5, Na^{+} - 2.0, K^{+} - 0.13, Cl^{-} - 0.08, SO_4 - 0.19 mmol / L), where they were preserved until the beginning of the experiments. The fish were fed twice a week *ad libitum*. The feed consisted of a carefully mixed fillet of *Theragra chalcogramma* (86g) crushed with a blender (MMR08A1 Corporation Robert Bosch Corporation, City, Slovenia) and trout feed (14g) which was filled with 200 ml of 7.5% gelatin solution. Feed composition: proteins - 17.3%, fats - 1.7% and carbohydrates - 0.1% based on the raw weight.

The body weight of the control and experimental fish at the beginning of the experiment was 8.5 ± 1.0g and 9.0 ± 1.0g, respectively. Three weeks prior to the experiment, the fish were placed in 4 stagnant aquaria of 40 L (bottom 30 x 60 cm) with forced aeration (water temperature 20 ± 2 °C). In 2 aquariums fish (5 specimens in each aquarium) were kept in non-dark aquaria, under conditions of variable illumination. In 2 aquariums fish (5 specimens in each aquarium) were kept in darkened aquaria. The illumination on the water surface corresponded to 405 lux (light) and 0.08 lux (darkness). The illumination of the fish in the control group was 6:18, the experimental group was 0:24. Fish kept in daylight in illuminated aquariums were treated as controls, in unilluminated aquariums - as an experiment.

Method. It was used the model of the benthic type of fish feeding. The fishes were placed into a chamber constructed of transparent perforated walls (the starting chamber). The size of the chamber was 10 x 5 x 6 cm. This chamber was installed near the back wall of the aquarium. The front wall of the chamber could be raised. At the opposite wall of the aquarium, the forage (30 frozen chironomid larvae, individual weight 7.5 mg) was placed. When the front wall of the chamber was raised, the fishes could leave the chamber to search for food and consume it. During two weeks before the start of the experiment, the fish were trained for two weeks to find the food in the experimental conditions described above. Within the time of the experiment, the fishes could get food only when the registration took place. Fish could receive food after registering the parameters studied only. 0.1 mL of Ringer's solution for poikilothermic animals (109 mM NaCl, 1.9 mM KCl, 1.1 mM $CaCl_2$, 1.2 mM $NaHCO_3$) was administered intraperitoneally to the fishes of the control group 1 hour prior to the experiments, and the fish of the test group received an equal amount of serotonin hydrochloride (Sigma), at a dose of 10 μg/g body weight, prepared on the same Ringer's solution.

The following temporal (motion) parameters were registered: latency to leave the starting chamber after its front wall was raised (t_1) and latent time of feeding (t_2), i.e., the time period from moving the fish off the chamber until approaching the food: this parameter is inversely related to the feeding reaction rate ($1/t_2$). Time was measured using a stopwatch. Moreover, the ration (R), i.e. the quantity of eaten chironomid larvae for 3 min of observation, was taken into consideration. The experiments took place 2-3

times a day for 3 or 5 days. The ½ volume of water in the aquaria was changed daily 1 hour before the beginning of the experiment.

Data were statistically processed using the EXCEL supplement of the MS Office'XP program. Statistical significance of revealed differences was assessed using the Student's t test for small samples at $p \leq 0.05$.

3. Results

In intact fish kept under conditions of variable illumination, t_1 values corresponded to 0.5 ± 0.1s, t_2 - 3.1 ± 0.1s, R - 10 ± 0.6 specimens of chironomid larvae. In intact fish contained in conditions of light deprivation, the values of t_1 corresponded to 0.7 ± 0.1s, t_2 - 3.9 ± 0.4s, R - 8.4 ± 1.1 specimens of chironomid larvae. During the experiment, these parameters changed in both experimental and control fish (Fig.1). At the same time under the influence of 5-HT in the vast majority of cases, the values of t_1 and t_2 increased, R - decreased. After 1 hour the value of t_1 in control fish contained in conditions of variable illumination increased by 2 times and by 4.3 times in experimental fish. In fish kept in conditions of light deprivation, the value of this parameter increased by 1.4 and 5.4 times, respectively.

The values of t_2 in the control fish contained in the conditions of variable illumination increased at the same time 2.1 fold, in the experimental ones are 37.7 times. In specimens kept in conditions of light deprivation, the parameter values increased by 2.6 and 32 times, respectively. The R values under the influence of injections, on the contrary, decreased, most significantly in fishes kept in conditions of variable illumination, especially 1 h after injection. In control fish R decreased by 1.3 times, in experimental fish by 8 times, in individuals kept under conditions of light deprivation, by 1.1 and 2.8 times, respectively.

It should be noted the stable trend the return towards the end of the experiment of the values of all the investigated parameters to that of intact fish. At that, the values of t_2 in experimental fish contained in light deprivation conditions were significantly ($p < 0.05$) higher than those of fishes kept in conditions of variable illumination in the period of 24-72 hours, R in the period 48-96 hours.

4. Discussion

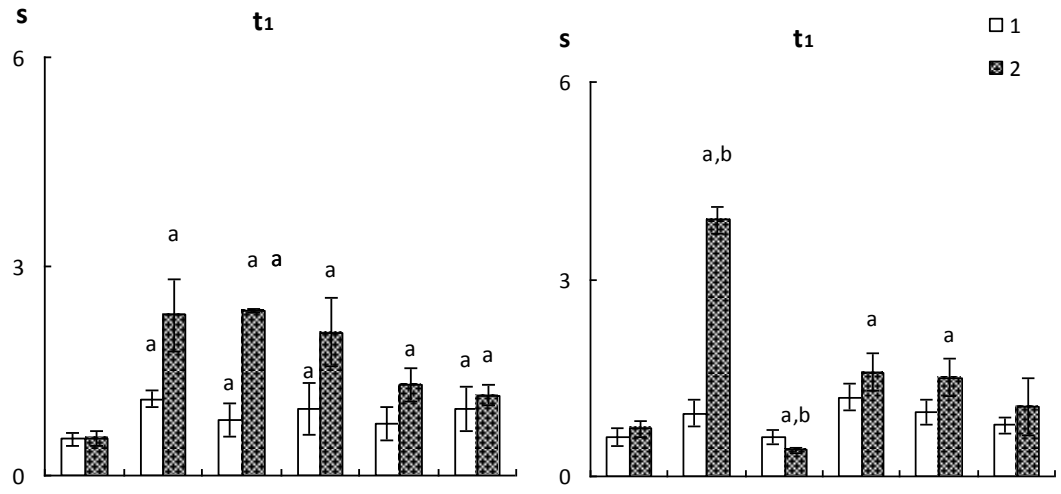
The results of the experiments confirmed previously obtained data on the inhibitory effect of peripherally introduced 5-HT on the feeding behavior of fish (Kuz'mina & Garina, 2013). In contrast to the short-term anorectic effect of 5-HT on food intake under its central administration (De Pedro *et al*., 1998), under it intraperitoneal administration, as in previous experiments (Kuz'mina & Garina, 2013), its prolonged effect was observed.

The reduction of locomotor activity of carp fry correlates well with data indicating a decrease of exploratory behavior and the spontaneous locomotor activity in Arctic charr *Salvelinus alpinus* after pharmacological stimulation of brain serotonergic activity (Winberg *et al.*, 1993). Also these data are in good agreement with the results of oral administration of gelatin capsules containing 5-HT, to seabass *Dicentrarchus labrax*, which caused an increase in plasma levels of serotonin almost 2-fold with a maximum effect after 20-45 minutes after pellet consumption, accompanied by a decrease of food consumption by fish (Rubio *et al*., 2006). The latter may be due to the fact that 5-HT is

produced by enterochromaffin cells, which are localized in the intestinal epithelium of the fish (Holmgren & Nilsson, 1983; Terenina & Gustavsson, 2003). However, the greater part of the 5-HT is not associated with mucosal enterochromaffin cells, but is associated with serotonergic fibers (98%) disposed in the walls of the intestine (Caamaño-Tubío *et al*., 2007).

The obtained data confirm the involvement of 5-HT in the regulation of feeding behavior of fish not only through central, but also through peripheral mechanisms. The demonstration of a decrease in the effect of 5-HT on fish locomotor activity and diet of fish under the influence of it intraperitoneal injections in the condition of light deprivation is most importantly. In all probability, this is due to the fact that under these conditions the level of endogenous 5-HT in the fish body was reduced due to the synthesis of melatonin from it. The leading role in this, apparently, is played by the relationship of the analogue of the epiphysis, namely paraphysis, which possesses photosensitive cells, and the hypothalamic-pituitary system (Andreeva & Obukhov, 1999). In addition, 5-HT amacrine retina cells found in a number of fish species plays an important role (Jaffe *et al*., 1987; Tornqvist *et al*., 1983). At that, as in the case of circadian rhythms, the light-dependent cryptochromes CRY1 and CRY2, whose genes are also expressed in the retina (Sancar, 2004), can participate in these processes.

Thus, light deprivation causes a decrease in the effect of 5-HT, introduced to carp underyearlings intraperitoneally. The locomotor activity under light deprivation is reduced under the influence of 5-HT to a greater extent, the diet decreases to a lesser extent than in fish kept in conditions of variable illumination. It has been suggested that the decrease in the inhibitory effect of exogenous 5-HT on feeding and locomotor activity under light deprivation is due to the synthesis of melatonin from it under dark conditions, as well as due to the participation of light-dependent retinal cryptochromes.



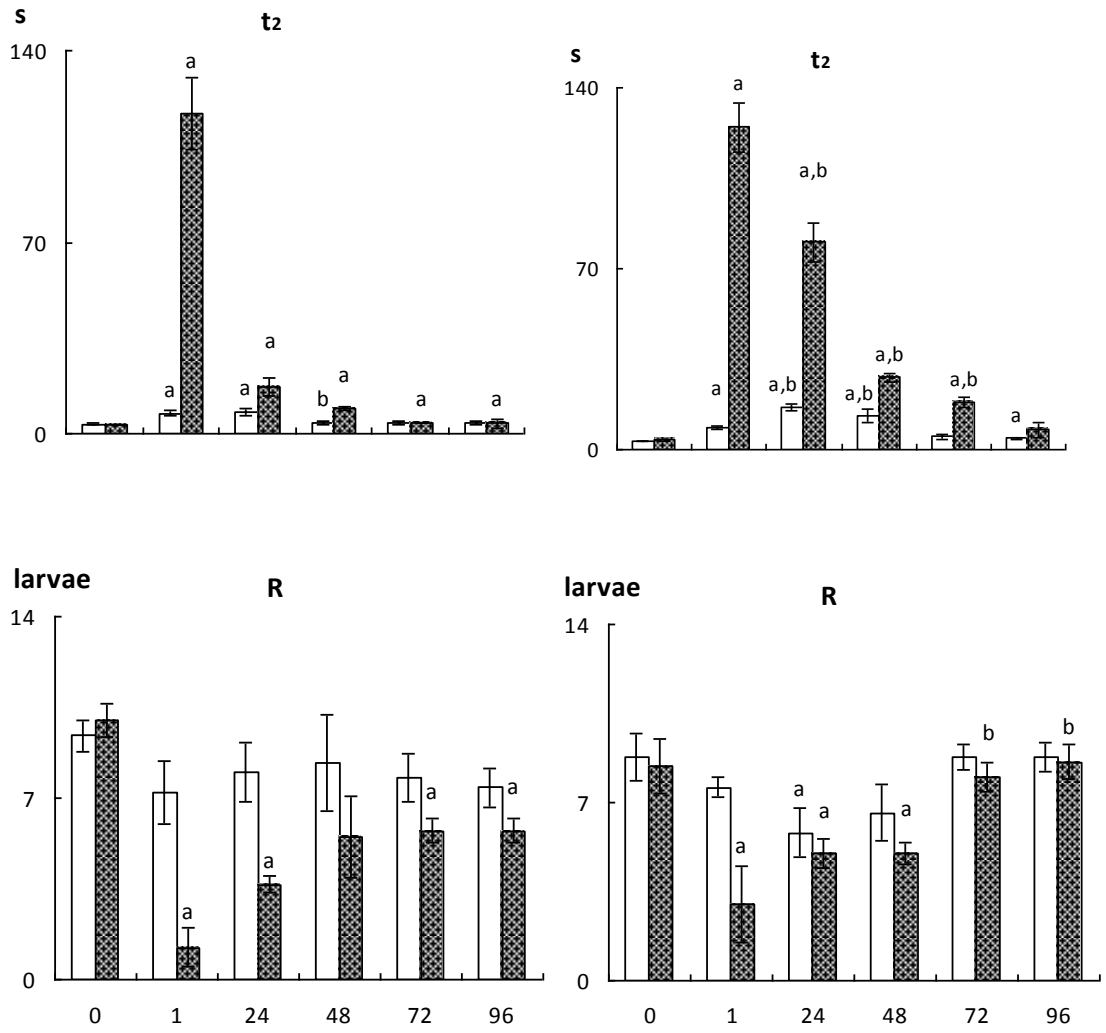


Figure. Influence of intraperitoneal injections of serotonin on the presence of carp in the chamber (t_1, s), latent period of feeding (t_2, s) and diet (R, specimen of chironomid larvae) under variable illumination (left) and light deprivation (right).
Designations: 1 - control, 2 - experiment, a - statistical significance of revealed differences in comparison with intact fish at $p \leq 0.05$, b - reliability of differences between fishes kept in conditions of variable illumination and light deprivation at $p \leq 0.05$.

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