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Fish physiology

**IMPACT OF THE COLOUR OF AQUARIUM ON THE LEVELS OF ACTH  
AND CORTISOL IN THE BLOOD OF CARP (*CYPRINUS CARPIO* L.)**

**WPLYW KOLORU AKWARIUM NA POZIOMY ACTH I KORTYZOLU  
WE KRWI KARPIA (*CYPRINUS CARPIO* L.)**

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A 14-day-long stay of carp in blue, yellow, and red aquaria caused statistically significant increase of ACTH and cortisol in the blood of the fish studied, compared to the initial state and to the aquarium in natural colours.

INTRODUCTION

Light plays a special role in biology. Depending on the kind of radiation (length of light wave) being at the same time radial energy—it modifies, triggers, or promotes important biochemical processes in living organisms. It has been known that stimulating action of light is not limited to affecting sight organs—specialized light receptors. To such organs light, above all, plays an informative role, that does not require big amounts of transmitted energy (sight, photoperiodism, phototropism and others) (Grabikowski and Murkowski 1984). Light acts also through entire surface area of the body playing a role of energy carrier (photosynthesis, photodestruction).

Impact of light on an organism, particularly of defined wavelengths is well known and used in medicine in so called phototherapy. In animal production, stimulating action of red light was used for increasing milking capacity of cows or egg yield of poultry. Attempts were made to assess impact of defined wavelengths of light on fish organisms (Blaxter 1964; Avery et al. 1982; Douglas 1986). Some of them concerned the rate of development (Ostrowski 1989), the others—the growth rate and increments of the body weight (Kuliński and Styczyńska-Jurewicz 1993).

The aim of this work was to test impact of the color of aquarium in which the fish were kept on the concentrations of ACTH and cortisol. The above hormones, through changes in their concentrations inform about the influence of selected factors on the organism.

## MATERIAL AND METHODS

The experiment was conducted in the experimental aquarium facilities of the Faculty of Marine Fisheries and Food Technology, Agricultural University of Szczecin on a total of 75 clinically healthy two-year-old carp. The fish weighing  $260 \pm 45$  g originated from a fish farm located in the discharge canal of post-cooling waters of the Dolna Odra power plant. The carp were placed in 400-l aquaria, 15 individuals in each. The aquaria were in four colors: natural, blue, yellow, and red and they were illuminated from above with 60-W light bulbs. The temperature of the water, constantly aerated four days prior and during the experiment, was identical with the temperature of the fish-farm canal and it amounted to 20°C. The photoperiod was 12 hours.

The blood for the studies was collected from the caudal vein between 07:30 and 08:00 hours before bringing the fish in and on day 14 of the experiment. The blood serum was examined for ACTH and cortisol concentrations—using a radio-immunological method, with labeled  $^{125}\text{J}$  ACTH and  $^{125}\text{J}$  Cortisol of the sets manufactured by CIS Bio International France (ACTH) and by Orion Diagnostica Finland (cortisol).

Readings of the illumination intensity and the density of the photon flux were conducted applying the sensor of the meter to the frontal, devoid of color in this area, wall of aquarium in its mid-length at the level of 15 cm above the bottom. The illumination intensity was measured using a luxometer Type L-01, Poland, while the density of the photon flux—using a phytophotometer Type FF-01, for a range of wavelength 400–700 nm.

The acquired results were processed statistically using a computer program Statgraphics v. 6.0. The significance of differences was tested using the Duncan test.

## RESULTS

**Table 1**

Readings of illumination intensity and density of photon flux in aquaria of different colors

Parameter	Colour of aquarium			
	natural	blue	yellow	red
Illumination intensity [Lx]	50	44	60	47
Density of photon flux [ $\mu\text{mol (photons)/m}^{-2}/\text{s}^{-1}$ ]	0.7	0.5	0.6	0.9

Readings of the illumination intensity and the density of the photon flux are shown in Tab 1. The highest intensity of illumination affecting a given area was stated in the yellow aquarium. The highest density of the photon flux—describing

the power of action of radial energy of light on an area unit in a time unit—was stated in red aquarium.

It was stated that the 14-day-long stay of the fish in naturally colored, blue, yellow and red aquaria caused in each of the analyzed cases a statistically significant ( $P < 0.01$ ;  $0.05$ ) increase of ACTH and cortisol levels in the blood of the fish. The differences were observed in relation to the initial state as well as between the aquaria—Tab. 2.

**Table 2**

ACTH and cortisol levels in the blood of carp kept in aquaria of different wall colours ( $\bar{x} \pm SD$ ),  $n = 15$

Color Aquarium	ACTH pg/ml	Cortisol nmol/l
Before (a)	48.6 ± 18.3	178.6 ± 30.9
Natural (b)	78.2 ± 31.4	236.1 ± 62.3
Blue (c)	367.1 ± 138.0	1012.1 ± 221.7
Yellow (d)	1539.4 ± 240.9	700.6 ± 196.2
Red (e)	181.4 ± 72.9	611.2 ± 154.5
Significance of differences	a-b*, a-c**, a-d**, a-e** b-c**, b-d**, b-e* c-d**, d-e**	a-b*, a-c**, a-d**, a-e** b-c**, b-d**, b-e** c-e*

\*, \*\* – Significance of differences ( $P < 0.05$ ;  $0.01$ ).

from yellow aquarium—1539.4 ± 240.9 pg/ml, while the lowest—in fish from red aquarium—181.4 ± 72.9 pg/ml. The highest concentration of cortisol occurred in fish from blue aquarium—1012.1 ± 221.7 nmol/l, while the lowest—in the fish from red aquarium—611.2 ± 154.4 nmol/l.

Comparing the concentrations of ACTH and cortisol in the blood of fish from the aquaria, with the data collected before the experiment, it can be concluded that the extent of the changes of the hormones must have been affected not only by the light of a given wave length, but also by the change of place. It was evidenced by a statistically significant increase of the levels of both hormones in the blood of the fish from naturally colored aquarium.

In the course of the experiment, on day 12 two fish from red aquarium died. Possible presence of parasites was ruled out through parasitological examination. The necropsy revealed extensive hyperemia and numerous ecchymosia in the internal organs of the fish.

Analysis of the acquired results prompted a statement, that the extent of the changes of ACTH in the blood of the fish was not always correlated with the extent of cortisol changes. The highest level of ACTH was stated in the fish

## DISCUSSION

The results acquired indicate that different color of the surrounding, associated with a defined wavelength inflicts significant impact also on fish. Presently stated very high increase of the levels of ACTH and cortisol in the blood of fish from colored aquaria suggests that the effect of a color associated with a defined energetic value is similar to effects of the other factors of environmental aggression, causing stimulation of pituitary-interrenal system (Friedrich 1996; 1997).

Classical definition of stress elaborated for homeothermal animals and humans finds its confirmation also in fishes (Donaldson 1981). Action of the factors of environmental aggression causing a number of functional (Eddy 1981), biochemical (Kłyszajko 1986; Prost et al. 1995), and morphological changes in the tissues and systems of fish organism has impact on triggering regulatory mechanisms. In such processes, one of the major functions is played by hypothalamus-pituitary-interrenal system. The mechanism of the reaction to the action of environmental aggression factors in fishes has yet been unknown. However, the participation of ophthalmic lobes as structures associated with transmission of information from the perimeter to hypothalamus has already been confirmed (Thomas 1990). Important role is also attributed to exteroceptors, which decide about receptive ability and transmission of effective information on the hypothalamus-pituitary-interrenal pathway (Thomas 1990; Węgrzynowicz and Górczyńska 1982).

In the present experiment statistically significant increase of ACTH and cortisol concentrations in naturally coloured and artificially-coloured aquaria indicates also a role of ophthalmic lobes and exteroceptors in triggering adaptive reaction of the organism, with important action of ophthalmic lobes. It is confirmed by extensively higher concentrations of the studied hormones in the fish from coloured aquaria. The results of numerous studies proved that all animals express sensitivity of their sight to the wavelengths same or very similar to those perceived by humans (Schmidt-Nilsen 1992). It seems interesting in this context that the highest levels of ACTH occurred in fish from yellow aquarium. This may be linked to hindering impact of illumination intensity on the excretory activity of pineal gland, which generally promotes increase of amounts of hormones released from pituitary. It was stated, that the illumination intensity in this aquarium was higher than in the other coloured aquaria, but not that much as to justify 4 to 8.5 fold higher level of this hormone. This relationship cannot be also confirmed, because in the fish from the naturally coloured aquarium, where illumination intensity was higher than in blue and red aquaria—the concentration of ACTH was lower than that in the fish from the latter aquaria. Very high concentrations of ACTH in the fish from yellow aquarium cannot be also justified by the amount of energy associated with the density of photon flux, because it was lower, than that observed in red and natural aquarium. In human medicine it has been demonstrated

that yellow light has minimal thermal action (contrary to red light) and it almost does not penetrate inside the tissues (Świrski 1994). The question is whether it can be assumed that in fishes, like it is in land animals, yellow colour is a warning colour and its strong stimulating activity consists in causing emotional stress? Similarly interesting and difficult to explain is the lack of correlation between concentrations of ACTH and cortisol in fishes from this aquarium.

On the other hand fish mortalities in red aquarium and the presence of hyperemia and echymosia in their internal organs, positively correlated with the highest density of photon flux observed in this aquarium—confirms the known fact of strong parameters of red colour capable of penetrating deeper layers of tissues. This feature has been used in human medicine for warming up deeper layers of organism, which causes their hyperemia, enhances processes of oxygenation of tissues as well as circulation of the blood and lymph (Świrski 1994). Apparently, in the presently described experiment, the exposure time for such strongly acting colour was too long (fish mortality) and the lowest level of ACTH observed in fish of this aquarium could indicate the occurrence of the third stage of GAS (general adaptation syndrome) so called exhaustion stage. Such explanation could confirm the lowest, among the fish from coloured aquaria, cortisol level occurring in the fish from this aquarium.

In the present experiment the least questionable was impact of blue colour on levels of the studied hormones. Fish from this aquarium exhibited the highest—among all fish—cortisol level, but its concentrations were comparable with the increase of ACTH levels

## CONCLUSIONS

1. Presently observed high increase of ACTH and cortisol levels in the blood of fish kept in blue, yellow, and red aquaria compared to the initial state and to the fish from naturally coloured aquarium gives evidence that the colours used act similarly to the other factors of environmental aggression.
2. The mechanism of this influence seems to be complex and it is probably linked to :
  - a) the defined wave length of light perceived as colour;
  - b) intensity of illumination and the associated functions of the organism;
  - c) density of photon flux being energetic carriers of the radiation used.

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STRESZCZENIE

Doświadczenie przeprowadzono w sali akwaryjnej Wydziału Rybactwa Morskiego i Technologii Żywności Akademii Rolniczej w Szczecinie, na 75 karpach, w drugim roku życia, o masie ciała  $260 \pm 45$  g, umieszczonych w akwariach w kolorach: naturalnym, niebieskim, żółtym i czerwonym. Celem pracy było zbadanie wpływu koloru akwarium, w którym ryby przebywają, na stężenie ACTH i kortyzolu, hormonów, których zmiany stężeń informują o charakterze wpływu wybranego czynnika na organizm.

Stwierdzono, że 14-dniowe przebywanie karp w akwariach spowodowało statystycznie istotny wzrost stężenia ACTH i kortyzolu we krwi wszystkich badanych karp, w stosunku do stanu przed doświadczeniem. Zmiany te statystycznie istotne przy  $P < 0.05$  w akwarium naturalnym, u ryb z akwariów kolorowych wielokrotnie przekraczały nie tylko stan wyjściowy ale i poziom obserwowane u ryb z akwarium naturalnego. Najwyższy poziom ACTH stwierdzono u ryb z akwarium żółtego, najniższy u ryb z akwarium czerwonego. Najwyższy poziom kortyzolu stwierdzono u ryb z akwarium niebieskiego, a najniższy u ryb z akwarium czerwonego.

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