Light Influence on Chromatophores Activity in *Poecilia reticulata*

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Abstract

A total of 30 fish were examined during 15 days (in April - March 2013) in three similar tanks with different illumination conditions: control group, total darkness and 40W incandescent light bulb exposure. Every 3 days the fish were examined under the optic microscope (4x) and images were taken for further analysis and interpretations. For the microscopic examination the fish lots were separately placed in a drop of water on a Petri dish. Central nervous system projection was considered to be most relevant due to the higher number of chromatophores in this area. The analysis of the images revealed the total area coverage of the chromatophores in the selected area and also the light influence over chromatophores activity.

Key Words

fish, pigment, chromathophores, MSH, fish physiology, Poecilia reticulata.

INTRODUCTION

Recent studies revealed the importance of the environment over the pigmentation of fish. It is known that fish can adapt to different environments by changing colour intensity to avoid predation. The speed of this adaptation process is vital to the survival of the species facing pollution, fishing, collection, competition from non-native fish species and also the drastic environment changes (Hărşan and Petrescu-Mag, 2008).

It is vital to reveal the adaptive processes that can provide information for further anticipation of wild living fish populations. Pigment distribution and intensity can become a vital characteristic of fish when facing serious changes of the environment. Continued understanding of melanocyte contributions to skin biology will hopefully provide new opportunities for the prevention and treatment of skin diseases (Lin and Fisher, 2007). Light intensity is important for fish and larvae, which must be reared in a specific light range, depending on the developmental stage and the species (Boeuf and Le Bail 1999). Better understanding of the early adaptation mechanisms in the skin development of the juvenile fish can improve technology in the fish farming sector. Only by understanding the non-stressful, comfort zone of the spawn to light intensity we can adapt rearing systems to the optimal conditions for every different fish species. Certain additives can improve the survival and growth rate of the larvae, vitamin C is able to protect the cells from oxidative stress during UV-B irradiation. A combination of vitamin C and seeds may be more UV-B protective for carp larvae. (Moirangthem *et al*., 2013)

MATERIALS AND METHODS

Our observations were made on 30 *Poecilia reticulate* fry, Red Blond variety during their first 15 days of life. Before including them in the experiment we evaluated the physiological status, removing any undersized or unhealthy fish. Fish were fed three times a day: 8:00 a.m., 12:00 a.m., 20:00 p.m. Optimal microclimate conditions were kept 5.5- 6.6 ph and temperatures varying

between 22 and 26 C°. Daily maintenance and cleaning of the tanks kept the nitrites values low. The fish tanks were 3 plastic boxes for food storage with sizes: 15 cm. height; 25 cm. length; 17 cam. wide. Fish were divided in three groups, of ten individuals, 2 experimental groups and 1 control group. Experimental groups were offered different light intensity conditions, for one group light was added using a 40W incandescent bulb, 12h per day, and the other experimental group was kept in total darkness by covering the tank with a box. Every 3 days the fish were examined under the optic microscope (4x) and images were taken. Fish restraining was accomplished by placing every group in a Petri dish, each fish was individually placed in a drop of water (Fig.1). The use of anaesthesia is not an option because of the lateral or upside-down position of the fish during anaesthesia that would not allow the examination of the dorsal area.

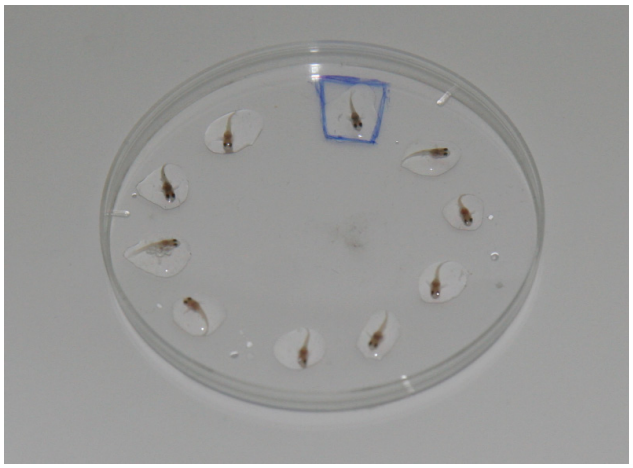

Fig.1 Guppy fry restrained on a Petri dish for microscopically investigation of the cromatophores. *The technique allows the operator to investigate 10 or more individuals without anaesthesia.*

The selected area for our study was the dorsal view of the central nervous system due to the high number of cromatophores in this area. All the images were taken by the same operator, with the same camera and microscope settings, during the entire experiment in order to reduce errors. The central nervous projection area was selected by using Adobe Photoshop CS6 and further on the total dark pigment coverage area was calculated for each individual. The measurements were made on 150 images 4032 x 3024 pixels.

RESULTS AND DISCUSSIONS

Quantification of the total area coverage revealed variations of the mean values between 14.97% and 21.02%, absolute values varying between 4.93% and 43.41%, for the group kept in total darkness. The control group mean values of the total area coverage varied from 9.95% to 15.6% with absolute values from 4.54% to 33.31%. The light exposure group mean values of the total area coverage varied from 12.1% to 16.8% with absolute values from 6.29% to 35.48%. For the 15 day observation period we consider the 4x magnification optimal, fish size allowing the investigation, the entire selected area being visible from the first (Fig.2) to last day of study (Fig. 3), further adjustments not being necessary.

The graphics (Fig. 4, 5, 6, 7, 8) show the daily evolution of the coverage percentage, descending from one measuring to another, up to 3.5-6% to the last day of the monitoring. The decrease of the coverage rate reveals a dynamic process with the highest protection against UV radiations in day 0. Proportionally with the growth of the fish, spaces between cromatophores grew, exposing more nervous tissue to the light radiations.

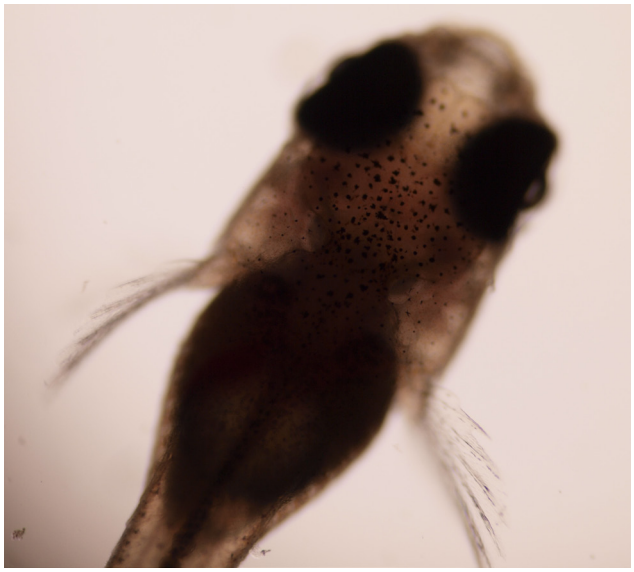

Fig. 2 Dorsal view over the cromatophoric system of the Guppy fish *Poecilia reticulata* newborn 4x. CNS is visible at this stage trough bone transparency.

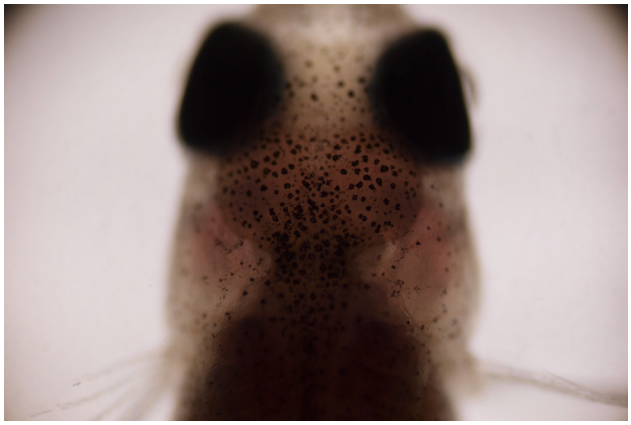

Fig. 3 Dorsal view over the cromatophoric system of the Guppy fish *Poecilia reticulata* in the 15-th day of monitoring 4x. The transparency Of the bone structures allows the delimitation of the central nervous system.

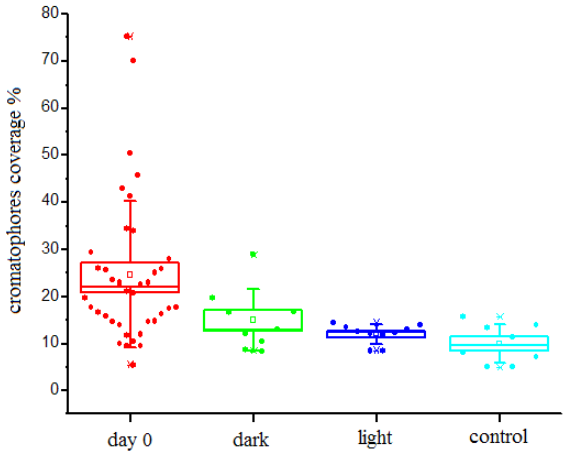

Fig. 4 Dynamics of the cromatophores coverage on 3 days old Guppy fish CNS

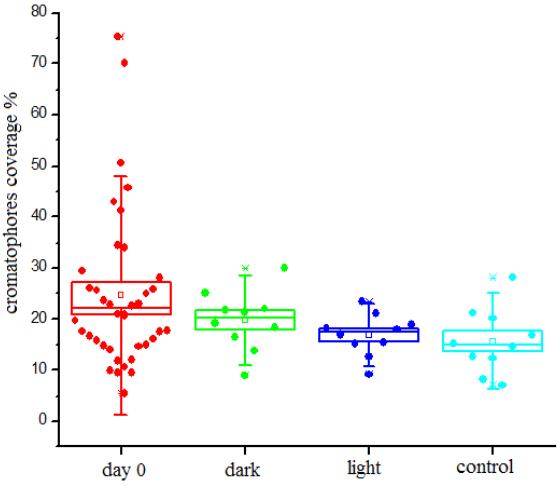

Fig. 5 Dynamics of the cromatophores coverage on 6 days old Guppy fish CNS.

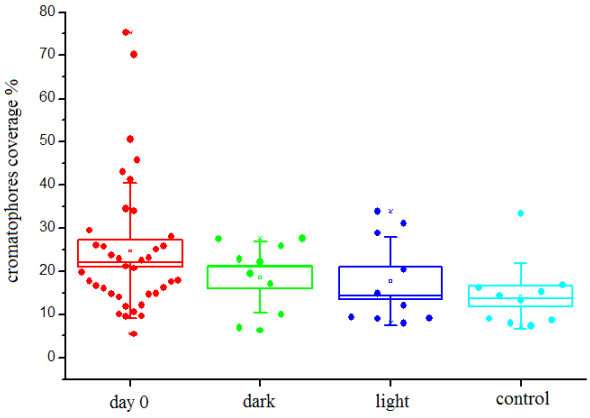

Fig. 6 Dynamics of the cromatophores coverage on 9 days old Guppy fish CNS.

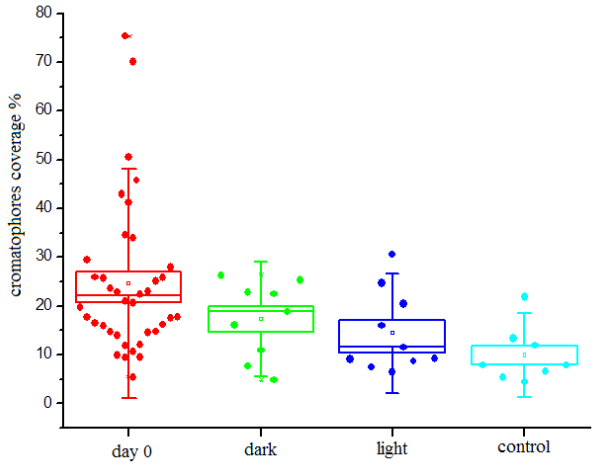

Fig. 7 Dynamics of the cromatophores coverage on 12 days old Guppy fish CNS

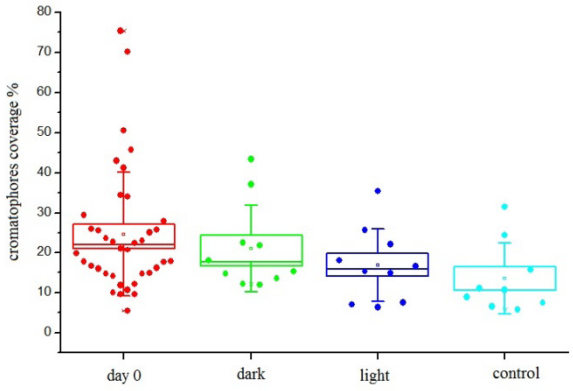

Fig. 8 Dynamics of the cromatophores coverage on 15 days old Guppy fish CNS.

The graphics reveal the dynamic tendency to uniformity of the mean values of the three lots. The main decrease of 6% was observed in the dark group, fact that can be associated to the privation of light causing the inhibition of the MSH secretion known to be strictly related to the forming and expansion of the cromatophoric cells.

Although the high macroscopically selection of the fish seemed to produce a homogenous group, the microscopically study revealed different cromatophores sizes, suggesting that they can actually be further on selected on the dominant cromatophoric cell type.

The uniformity of the mean values registered during the 15 days of observation reveals that the adaptation of the „Guppy-fish" to different light intensity is poor and high intensity light radiation can affect the newborns welfare.

CONCLUSION

For the first 15 days of life of *Poecilia reticulata* Red Blond variety, the maximum protection level can be considered the moment of birth. The results can be used, as reference values, in future studies of the cromatophoric system.

Further studies must be carried, with a prolonged period of monitoring in order to reveal the influence of different illumination conditions.

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