The Effects of Total Darkness and Black and White Backgrounds on the Development of Melanophores In Brachydanio rerio

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A review of the literature concerning teleost color change reveals that the actual mechanisms controlling melanophore behavior are far from being satisfactorily understood.

The purpose of this experiment was to determine the effect of albedo upon the pigmentation of *Brachydanio rerio* (Hamilton), reared under the following conditions: (1) total darkness, (2) black background, and (3) white background.

It is an observed fact that fishes living in total darkness are devoid of pigment and those living in environments ranging from dimly-lighted to lightless habitats are paler than species living in well-lighted, clear waters. If a direct environmental effect could be expected, a species raised in the total absence of light should be devoid of pigment and those individuals experimentally reared under progressively diminishing albedos should develop less pigment than specimens reared in normally lighted habitats.

Sumner (5) refers to the work of Babak who used larval salamanders and concluded that the nervous system, under the influence of the light relations of the environment, controls not only the movements and the mass of the pigment in the chromatophores, but also influences the division or multiplication of the chromatophores.

Odiorne (2,3) experimenting with Fundulus, found that fishes kept in light surroundings over long periods of time become unable to adapt themselves to dark surroundings to the usual degree. It was shown that this inability was the result of degeneration of some of the melanophores, followed by a loss of pigment contained in them. He found that the melanophores of fish placed in white surroundings soon contracted and remained so. After a few days, examination revealed that the melanophores were degenerating, for clumps of pigment granules were visible and counts revealed a decrease in the number of melanophores. As this process went on, the pigment granules formed large aggregations which accumulated at the edges of the scales. These granules were later lost at the surface of the body.

Sumner (4) working with flatfishes, regarded the determining factor in color changes as not the absolute intensity of light reflected from the background, but rather the ratio between this and the overhead illumination.

Cole (1) concluded that the light from the background stimulates the retina of the eye which initiates nervous impulses carried by the sympathetic and parasympathetic nerves to the melanophores.

The experiments reported herein are in agreement with the works of others in that the melanophores of the zebra danio react to light and background in the usual manner. Melanophore numbers in a measured area of integument are definitely affected by variations in light and background.

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The eggs of *B. revio* were collected on October 26, 1951, from aquaria kept in the laboratory of Dr. Roy W. Jones. The writer is indebted to Dr. Jones and his assistants Waymon Gibson and Robert W. Ingersol for materials and advice.

Equal numbers of developing embryos were placed in four different aquaria. One aquarium was painted black and covered with a black box in order to prevent any light from reaching the embryos. The only light to which the fish were subjected was a 10-watt ruby photographic light, used at short intervals of only a few minutes for observation or feeding.

The second aquarium was painted black and a 75-watt Mazda bulb was constantly burned close above it throughout the experiment.

The third aquarium was painted white and lighted in the same manner as aquarium two.

The fourth aquarium was used as a control and kept under ordinary laboratory conditions.

During the first month of life, the fish were fed infusoria daily. Later, Micrograin constituted the major portion of the diet with occasional feedings of plankton and dried shrimp. The aquaria were cleaned at weekly intervals by siphoning the water. After cleaning the aquaria, the original water was returned and water was added to replace loss by evaporation. Complete change of water was effected twice during the course of the experiments.

On January 12, 1952, two fish, each easily recognized by abnormal caudal fins, were transferred to different aquaria. One from the black aquarium was transferred to the white aquarium. The other fish was transferred from the white aquarium to the black one. Frequent observations were made during the following 24-hour period after which each fish was returned to its original aquarium.

In order to test the conditioning effect of the first transfer, the same two fish were again reciprocally moved after a 24-hour period. Observations were made at 10-minute intervals for the first two hours and thereafter at hourly intervals for another 24-hour period. Color changes were recorded at each observation time and the fish were killed in 10 per cent formalin for further study. Non-transferred fish from each aquarium were also killed in the same manner.

The fish kept in total absence of light died within one month without appreciable growth. Evidently, these fish were able to find sufficient food to maintain life temporarily, but insufficient amounts for growth. The danio is therefore interpreted as a visual feeder, quite dependent upon light in order to find sufficient food. The fact that life was maintained for one month might be explained by assuming that some food, in addition to yolk, was obtained by accident.

Although fish raised in total darkness died without appreciable growth, the primary integumentary larval pigment areas could be seen to be as follows: a pair of vertebral stripes, a pair of stripes along the ventral lines of the caudal peduncle, and scattered melanophores on the yolk sac. In addition, the choriodal pigment was well developed. All pigment cells, except those of the choriod coat of the eye, appeared as tiny black dots.

The fish raised on the black background (not transferred) possessed very dark and distinct longitudinal stripes on the sides and vertical fins.

Although these fish were larger at the end of the experiment than fish in the other aquaria, the small number of individuals does not permit the conclusion that black background is conducive to maximum growth rate. However, it seems reasonable that food particles would be more easily seen against a black, rather than a white background since the food used was of a light color.

The fish raised on white background (not transferred) were very light in color. Only one poorly-developed lateral band was visible. Bands were not visible on the vertical fins (dorsal, caudal, and anal).

The specimen raised on black background and transferred to white was light in color, although all bands were visible on the sides and vertical fins.

The fish kept on white background and transferred to black was practically the same color as the fish transferred from black to white, the difference being that the melanophores in the white-to-black transfer were greatly expanded, whereas, the black-to-white transferred fish possessed more numerous but contracted melanophores (Table I).

It is evident from Table I that fish reared on black background developed more melanophores per unit area than did any others, in fact, nearly three times as many as those on white background, and averaged twice as many, per unit area, as the white-to-black transferred-fish.

Two abnormal conditions were observed among these fish. Two fish kept on black background were devoid of pelvic fins, as was one of the fish on white background. One fish raised in the control aquarium had no dorsal fin.

The same experiments should be carried out on a more elaborate scale, using a species of fish which is able to feed in darkness. A study of the effect of light and background upon growth would be of value and integest.

1	NON-TRANS	FERBED FISH	TRANSFERBED FISH				
BODY REGION	BLACK	White	Black to White	White to Black	CONTROL		
MIDNUCHAL	26	8	27	11	13		
DOBSAL OBIGIN	35	10	36	16	20		
BACK OF ANGLE OF							
GILL CLEFT Directly over	19	9	19	10	16		
PELVIC INSERTION	15	1	13	7	10		
CAUDAL BASE	22	16	21	14	20		
ANAL FIN BAND	29	10	28	14	25		
SNOUT MIDLATERAL BAND	11	9	11	10	10		
ABOVE ANAL FIN Dorsolateral band	22	5	19	10	17		
ABOVE ANAL FIN	19	8	16	10	17		
BEHIND EYE	3	Ō	2	0	8		
PECTOBAL	4	1	3	0	2		
AVERAGES	18.6	7.0	17.7	9.3	13.9		

TABLE I

Counts	of	Melanophores	in	.09	Square	Millimeter	of	Surface
		in Various	Bod	y R	egions o	f B. rerio	•	•

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