Ann. Zool. Fennici 20: 239-244. 1983

Absence of changes in colour discrimination ability of goldfish when reared in monochromatic light

Ernst Mecke

Mecke, E. 1983: Absence of changes in colour discrimination ability of goldfish when reared in momochromatic light. — Ann. Zool. Fennici 20: 239-244.

The study represented an attempt to clarify whether goldfish, after rearing in monochromatic light, show deviations from normal in their colour discrimination ability. Goldfish were made to spawn, the spawn then being distributed to three rearing basins which were illuminated with red (687 nm), blue (434 nm), or white, light. After 14-19 months, fish of all groups were tested for their colour discrimination ability by means of blue-green and red-green discrimination training. No significant differences between the rearing groups were found. Further, for fish of all rearing groups, blue-green discrimination was significantly more difficult than red-green discrimination. Spontaneous colour preferences were not observed. Neither did lightmicroscopic investigation of the retinae reveal differences — the shape and pattern of distribution of the discernible cone types were the same in all groups and corresponded to the descriptions given by other authors.

Ernst Mecke, Dept. of Zoology, Univ. of Helsinki, Arkadiankatu 7, SF-00100 Helsinki10, Finland.

1. Introduction

It is a known fact that the rearing or keeping of animals under conditions of unusual visual stimulation can produce abnormal development in the visual system of mammals (Hirsch & Leventhal 1978, Ganz 1978), as well as of lower vertebrates (Keating & Kennard 1976, Chung et al. 1973, Yoon 1975, Marotte et al. 1979, Wye-Dvorak et al. 1979). It is also known that among lower vertebrates the cyprinid fishes have good trichromatic colour vision (e.g. Muntz & Cronly-Dillon 1966). This has been used as a basis for a great deal of further research(e.g. Marc & Sperling 1976, Kaneko 1979, Stell 1975, 1978). The question, though, of whether vision in lower vertebrates would develop normally under conditions of unusual stimulation of the colour discrimination system has apparently been investigated only once (Knoll 1953). In that study the animals (frogs), after rearing in monochromatic light, exhibited changes in their spectral sensitivity (interpreted as changes in the composition of the visual pigments). However, the experimental procedure (test for optomotor response in a rotating striped drum) did not permit the elucidation of changes in the ability to discriminate between colours.

In the present study goldfish were reared from spawn in monochromatic light and afterwards tested for function of their colour discrimination system. Red and blue monochromatic lights were used since with red light there is maximum probability that some types of receptors are not stimulated at all, and blue light stimulates all types of receptors while still excluding colour contrast. Operant conditioning was chosen as the method to test the animals after rearing for their colour discrimination ability, so that any innate or acquired preference for an offered colour stimulus would have an opportunity to manifest itself. A further requirement of the behavioural method selected was that it show any effect caused by the rearing conditions irrespective of its place, peripheral or central in the visual pathway. Finally, a histological investigation of the animals' eyes was carried out.

2. Materials and methods

2.1. Rearing

Two pairs of goldfish fit for spawning were provided by the botanical gardens of the University of Helsinki, Deartment of Botany. Under favourable conditions (described by Yamamoto et al. 1966) the animals spawned without hormone treatment. Within 8 h from spawning, all spawn was distributed in separate rearing basins. The basin of the "red" group received light through a filter with maximum transmission at 687 nm, that of the "blue" group at 434 nm, both lights being of high spectral purity (Schott & Gen. double interference filters). The basin containing the "control" group received white light from a tungsten source. Moreover, the control fish were provided with a multi-coloured environment. The light intensity was low in all basins (although sufficient to stimulate the cone types sensitive to the respective colours — cf. Marc & Sperling 1976), being at the most well-illuminated point (at water surface level under the lamp) in the "red" basin $60 \cdot 10^{12}$ q/cm^2/sec, at the corresponding points in the "blue" basin $0.49 \cdot 10^{12}$ q/cm^2/sec, and in the "control" basin 120.4 lux. All light measurements were made with an Airam UVM-8 photometer which, for quantal measurements, was equipped with a Telefunken BPW 21 -detector, for lux measurements with a detector whose sensitivity was exactly the same as that of the photopic human eye. The daily light period was 16 h, the temperature 23—25°C. The fish were fed on infusoria during the first week, then on artemia up to about the 80th day, after which the diet was gradually changed to "Sponda" (Vitakraft) which constituted all of the food from about the 150th day onward. At the time of the colour discrimination tests (14—19 months of age), the size of the fish was in the range of 3.5-5.0 cm standard length.

2.2. Training

Fig. 1 shows the essential parts of the training apparatus as seen from above. The main units are the training basin *TB* and the stimulus boxes *SB*, in which the light stimuli were produced. In the course of a test run, the fish had to leave the starting-compartment *sc* by the starting-door *sd* (from which both stimulus windows' *sw* were visible), cross the middle compartment *mc*, and enter one of the goal compartments *gc* by its door *gd*. If the fish had chosen the goal compartment illuminated by the correct light stimulus (produced in the stimulus box *SB* by means of Wratten filters *Wr* in the light beam and projected from outside on to the stimulus window *sw*), first the correctness of the choice was indicated to the fish by a "cue splash" of water on to the water surface, and then a small food reward was poured into the compartment by hand. After a short break, the fish was removed from the goal compartment by means of a ladle and transferred back to the starting-compartment or to the waiting-basin (not shown) where it spent the time between experiments. The middle compartment was covered by an opaque lid *co* so that the fish should not see the observer (in position *Ob*) while making its choice. The fish were light-adapted before experiments and kept so during the runs by white background light. While being trained, the fish obtained their food exclusively through being rewarded.

Pretraining took place in two stages. In the first stage, all four fishes of a training-group were trained together in such a way that on the first day they were put into one goal compartment and received food after a "cue splash" and while the compartment was illuminated with white light through its stimulus window. During the next six days they had to find their way to the illuminated goal compartment (before receiving food there), first from the middle and later from the starting compartment. During the second stage, each fish was individually given a total of 12 runs from the starting-compartment to one of the goal compartments which was illuminated by a stimulus light of the same colour and brightness as those stimuli which the fish was supposed to choose later on (during colour training), the other goal compartment not being illuminated.

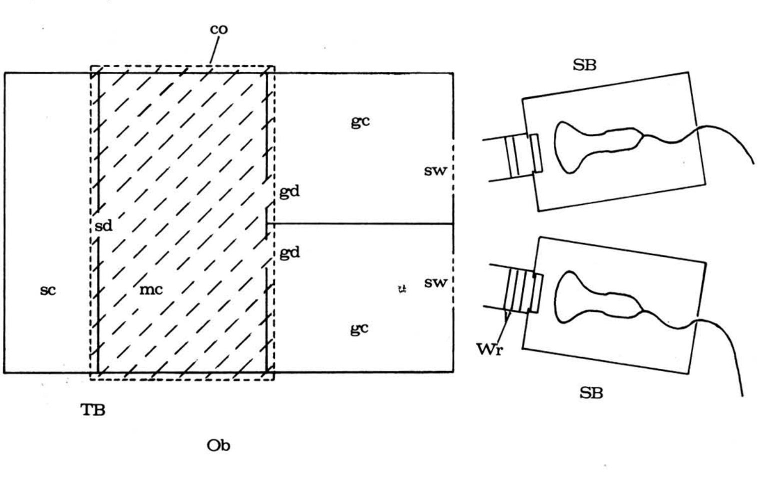
When an error occurred, the fish was corrected. It was given time to move by itself to the correct goal compartment. If this did not occur, the fish was offered the same choice as previously, this procedure being repeated until it had found its way to the correct goal compartment and received a food reward. Correction was also applied in the colour training. There the entire procedure from the first wrong choice up to the successful one was recorded as a single error.

All through pretraining the fish were, in effect, rewarded for choosing the brightest light stimulus available (which in the second stage was also of the correct colour).

During colour training, both correct and incorrect goal compartments were illuminated by one coloured light stimulus each, and the fish had to learn to make its choice correctly on the basis of colour. For a given training group the stimulus lights were always either green and blue (Wratten filters No. 61 and 47B, with, for the fish, maximum intensities at 530 and 454 nm) or green (as described) and red (Wratten filter No. 29, maximum intensity for the fish at 630 nm). Each of the stimulus lights was offered at two intensities; i.e. they were adjusted by means of Wratten neutral density filters in such a way that any one of the pair of stimulus lights should appear to the fish either brighter, or less bright, than the other one. Both direction and intensity of a stimulus light were varied according to a random numbers sequence.

The brightness of the stimuli to the fish was calculated on the basis of the spectral sensitivity curves given by Cronly-Dillon & Muntz (1965) and Yager (1967). Further, the possibility was considered (on the basis of Marks 1965) that

Fig. 1. Training apparatus (from above). *SB* stimulus boxes; *TB* training basin; *co* opaque lid covering middle compartment; *gc* goal compartments; *gd* doors to goal compartments; *mc* middle compartment; *Ob* place of observer; *sc* starting-compartment; *sd* starting-door; *sw* stimulus windows; *Wr* Wratten filters. In the stimulus boxes, light stimuli of desired colour and brightness are produces by means of the Wratten filters, and projected from outside on to the stimulus windows. The fish is put in the starting-compartment, leaves it by the starting-door, and swims into one of the two goal compartments, of which one is illuminated, through its stimulus window, by the correct light stimulus.



during rearing in monochromatic light some types of receptors might not have acquired their normal function. Another problem was that a second study involving the fish used here (Mecke & Reuter, in preparation) produced evidence of a shift in the relation of the vitamin A_1- to the vitamin A_2-based pigments in the visual receptors of some fish (cf. Tsin & Beatty 1978, 1979), so that the above calculations had additionally to be checked for the possible effects of this type of pigment change. In this, the necessary computations were based on the curves provided by Tsin et al. (1981).

Each training group consisted of four fish, which were trained singly. In order to make the effects of possible spontaneous colour preferences obvious (for later correction, if necessary), two fish were always rewarded for choosing one colour, the other two for choosing the other colour. Each fish had ten runs per day. For blue-green training a total of 150 runs were made per fish, for red-green training (with the exception of one group) only 120 runs per fish. A regression analysis was performed on the results.

2.3. Histology

For histological investigation of the retinae, the eyes (in very small fish the entire heads) were fixed in Bouin's solution. Beforehand the lens together with a ring of the iris and some of the vitreous humor was removed from eyes more than 3.0 mm in diameter. The eyes were then washed and embedded in paraffin according to standard procedures. Sagittal and tangential sections, 7 μ in thickness, were treated in 0.25 % $KMnO_4$ and 2 % oxalic acid solution in order to bleach part of the melanin, then stained in Mayer's hematoxylin and Fast Green FCF, and investigated microscopically.

3. Results

3.1. Colour discrimination training

The results of the colour discrimination training are shown in Fig. 2, each point showing the average result of a training group of four fish during one day (= 40 runs altogether).

The first and most obvious conclusion from the results is that fish of all groups were able to discern colours and to use them as a clue; i.e. their colour vision had developed under the rearing conditions. Furthermore, a comparison between the blue-green and the red-green discrimination curves in Fig. 2 shows that for fish of all rearing groups red-green discrimination was easier to learn than blue-green; the difference in the slope of the curves is highly significant (confidence limits 0.5 %). This result agrees well with the findings of other authors (Muntz & Cronly-Dillon 1966, Powers & Easter 1978). Additionally, in this study the intensity differences between bright and less bright stimuli were especially great in blue-green discrimination, which could have made it more difficult for the fish to change from a possible brightness preference in pretraining to a colour preference. Thirdly, a comparison of the curves of either the blue-green or the red-green discrimination indicates that there were no clear differences between the learning speeds of different rearing groups. The regression analysis shows in some cases differences which are significant within confidence limits of only 25 % (in blue-green discrimination between the "control" and the "red", the "control" and the "blue" fish, in red-green discrimination between the "control" and the "red", the "control" and the "red-pretrained red" fish), but otherwise it brings out no differences at all. The "red-pretrained red" group had been trained using extra precautions in order to make possible differences existing between the groups clearer; during pretraining the fish were exclusively exposed to red light (in which the fish had also been reared), so that differences which might have existed when the fish were taken out of their rearing basin should not be lost by exposure to white light during the pretraining period. The outcome was, however, that this "red-pretrained red" group performed in the same discrimination slightly better than the "red" group which had been trained in the normal way. This fact notwithstanding, it can be seen that in all cases where there was any difference at all (low significance), the control fish were more successful. Finally, statistical analysis did not show signs of a blue-preference in a blue-green choice as reported by Muntz & Cronly-Dillon (1966), nor were any other spontaneous colour preferences observed. (Hence, a training day's result of a group of four fish could be shown as one point instead of two).

3.2. Histology

Microscopic investigation of the retinae was hindered by the fact that most of the available eyes were very small, so that the cones were standing so packed as to be unable to assume the typical shapes described by Stell & Hárosi (1976). In sagittal sections of eyes from all the rearing groups double cones as well as miniature cones were identified, however. There were no differences between the groups in either the thickness of the retinal cell layers or in the density of cell nuclei. Tangential sections of eyes of all the groups showed that the cones were standing in the characteristic "square" pattern as described by Marc & Sperling (1976). From this it was concluded that the cones in the typical positions of the pattern belonged to the corresponding cone types and, consequently, that cones of all of the types which make up the pattern in the goldfish retina were developed and present in their usual respective numbers. In no case was the square pattern developed over all of the retina, a situation agreeing well with the observations of

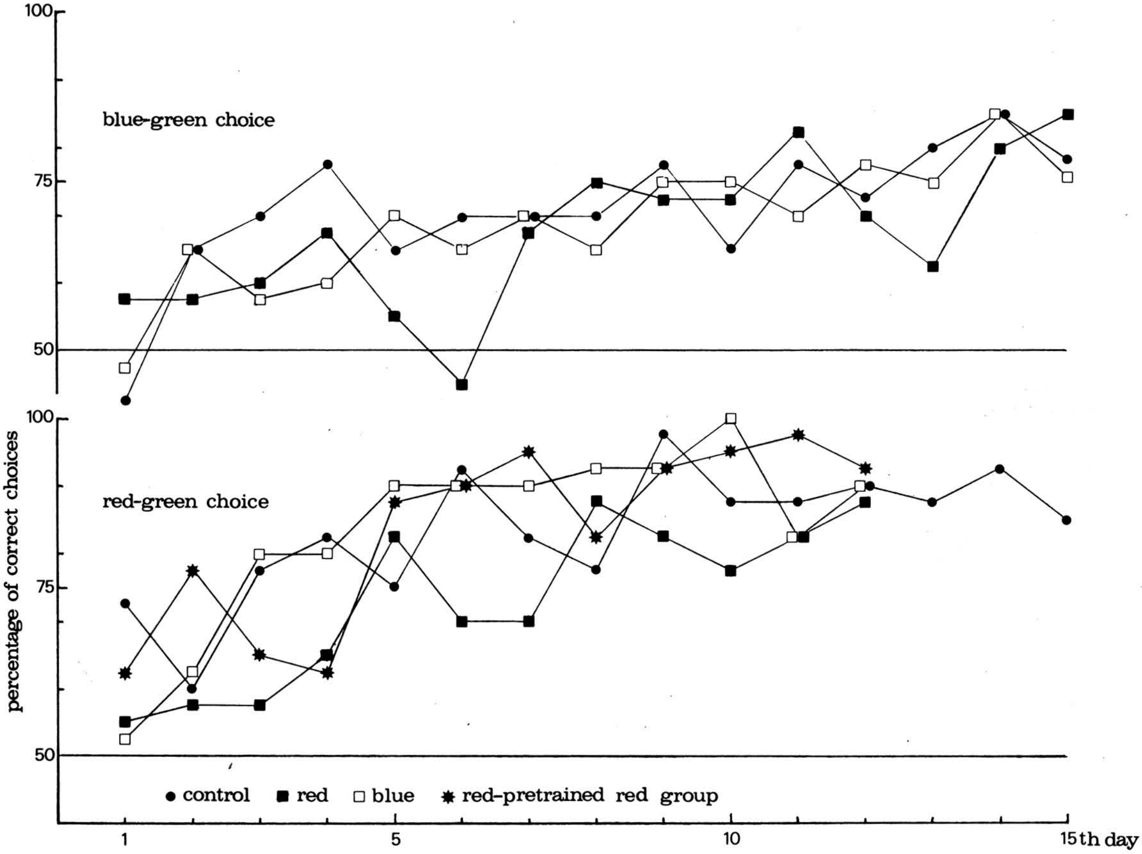


Fig. 2. Results of colour discrimination training (see text).

other authors (Ahlbert 1975, Engström 1960). It was not apparent that the areas with a developed square pattern were larger or smaller in one group than in any other. Altogether the histological investigation did not, through the light microscope, show any clear differences between the different rearing groups. It may be noted that Eakin's (1965) electron microscopic investigation of the receptors of dark-reared frogs did not show deviations from normal either.

4. Discussion

In the histological investigation, no differences between the rearing groups were found. The colour discrimination training did not show significant differences either, although whenever there were differences (non-significant ones), it was the control fish which were more successful. The strongest difference appeared in the red-green choice when comparing the performance of the "red" and the "control" fish, but it was not confirmed by the additional training of an extra group of "red-pretrained red" fish under rigid conditions. Altogether it would appear that rearing in monochromatic light produced no pronounced differences.

In detail this result (following Hemilä et al. 1976) would indicate that under the given rearing conditions the fish had produced all the usual visual pigments (each of them separately in its usual type of receptor), formed the receptor-specific couplings allowing two receptor types to affect some retinal neurones in an antagonistic (colour-opponent) fashion, developed the decoding system of the brain (making it possible to interpret the signals from the retina in terms of different colours), together with all the other nervous formations necessary for the storing of perceived colour impressions, their recognition on later occasions and their use as clues. Such a degree of genetical determination may seem surprising, but the result is supported by the findings of several authors in regard to warm-blooded animals — Ganz & Riesen (1962) reared monkeys in the dark, while Kovach (1971) reared chicks, and Peterson (1962) ducklings, in monochromatic light.

The result does not preclude, indeed the persistent (though statistically non-significant) superior performance of the control fish even suggests, some minor differences between the rearing groups. Verification of such differences possibly could be achieved in a number of ways, each of which would have gone beyond the confines of the present study.

One way is suggested by the studies on warm-blooded animals mentioned above. In these the authors found in all cases a deviation from normal in the generalization of colour stimuli, i.e. in the animals' tendency to show such reactions for which they had been rewarded when showing them towards stimuli of one colour, spontaneously towards stimuli of other colours as well. Consequently, an attempt could be made to study colour generalization in specially-reared goldfish too.

Another way would be to rear and train considerably larger numbers of fish and to obtain (possibly) thereby statistically significant results.

Making the task put to the fish more demanding could constitute a third way. In the present study the fish had to discriminate between pure-coloured stimuli whose energy maxima were at least 75 nm apart in the spectrum, so that even fish with slightly impaired colour vision may have had no difficulties in discriminating between them. In rats, differences in depth perception between dark-reared animals and controls became apparent (after previous tests had not revealed any differences) when in the task (avoidance of a visual cliff) the two depth to be discriminated between were made more similar (cf. Slomin& Pasnak 1972, Walk et al. 1957, Walk & Bond 1968). Similarly, in the colour discrimination training of goldfish one could attempt to make the colour stimuli progressively more similar, for instance, with the hope that in controls discrimination ability would fail at a different point than in specially-reared fish.

A point to be considered in all studies of goldfish reared under special light conditions is the animals' tendency to develop mixtures of vitamin A_1- and A_2-based visual pigments in their photoreceptors. This varies with the rearing-conditions, in particular according to temperature, the daily light period, and the overall light intensity (cf. Tsin & Beatty 1979). The same can be said concerning frogs. Knoll's (1953) observation that tadpoles and young frogs reared in the dark or in red light showed a reduced photopic sensitivity to red light can probably be explained as an effect of low light intensity which had caused an increase in the relative proportion of the less red-sensitive vitamin A_1-based visual pigments (cf. Bridges 1974a, b).

Acknowledgements. I am deeply grateful to S. Hemilä, J.-H. Kock and T. Reuter for essential help during the investigation, to G. Roth, Bremen, for organizational help and a critical reading of the manuscript.

Note. This paper is a shortened version (40 % of its original length) of a Ph.D. thesis which was accepted by the Dr. Ing./Dr. rer. nat. graduation committee of the university of Bremen, Federal Republic of Germany. In the graduation procedure the reports concerning the thesis were written by W. Himstedt (Darmstadt) and G.Roth (Bremen). The final examination took place on February 1st, 1982.

References

Ahlbert, I.-B. 1975: Organization of cone cells in the retinae of some teleosts in relation to their feeding habits. — Ph. D. thesis, Stockholm.

Bridges, C. D. B. 1974a: Effects of light and darkness on the visual pigments of amphibian tadpoles. — Vision Res. 14: 779–793.

—»— 1974b: Spectral sensitivity of the system controlling visual pigment composition in tadpole eyes. — Vision Res. 14: 929–935.

Chung, S. H., Gaze, R. M. & Stirling, R. V. 1973: Abnormal visual function in Xenopus following stroboscopic illumination. — Nature (London), New Biol. 246 Nb, 154: 186–189.

Cronly-Dillon, J. & Muntz, W. 1965: The spectral sensitivity of the goldfish and the clawed toad tadpole under photopic conditions. — J. Exp. Biol. 42: 481–493.

Eakin, R. M. 1965: Differentiation of rods and cones in total darkness. — J. Cell. Biol. 25: 162–165.

Engström, K. 1960: Cone types and cone arrangement in the retina of some cyprinids. — Acta Zool. 41: 277–295.

Ganz, L. 1978: Innate and environmental factors in the development of visual form perception. — In: Held, R., Leibowitz, H. W. & Teuber, H. L. (eds.), Handbook of sensory physiology. VIII. Perception: 437–488. Springer, Berlin, Heidelberg, New York.

Ganz, L. & Riesen, A. H. 1962: Stimulus generalization to hue in the dark-reared macaque. — J. Comp. Physiol. Psychol. 55: 92–99.

Hemilä, S., Reuter, T. & Virtanen, K. 1976: The evolution of colour-opponent neurones and colour vision. — Vision Res. 16: 1359–1362.

Hirsch, H. V. B. & Leventhal, A. G. 1978: Functional modification of the developing visual system. — In: Jacobson, M. (ed.), Handbook of sensory physiology. IX. Development of sensory systems: 279–336. Springer, Berlin, Heidelberg, New York.

Kaneko, A. 1979: Physiology of the retina. — Ann. Rev. Neurosci. 1979, 2: 169–191.

Keating, M. J. & Kennard, C. 1976: The amphibian visual system as a model for developmental neurobiology. —

In: Fite, K. V. (ed.), The amphibian visual system: a multidisciplinary approach: 267-315. Academic Press, New York, London.

Knoll, M. 1953: Über das Tages- und Dämmerungssehen des Grasfrosches (Rana temporaria L.) nach Aufzucht in veränderten Lichtbedingungen. — Zeitschr. Vergl. Physiol. 35: 42-70.

Kovach, J. K. 1971: Interaction of innate and acquired: color preferences and early exposure learning in chicks. — J. Comp. Physiol. Psychol. 75: 386-398.

Marc, R. E. & Sperling, H. G. 1976: The chromatic organization of the goldfish cone mosaic. — Vision Res. 16: 1211-1224.

Marks, W. 1965: Visual pigments of single goldfish cones. — J. Physiol. (London) 178: 14-32.

Marotte, L. R., Wye-Dvorak, J. & Mark, R. F. 1979: Retinotectal reorganization in goldfish — II. Effects of partial tectal ablation and constant light on the retina. — Neuroscience 4 (6): 803-810.

Muntz, W. R. A. & Cronly-Dillon, J. R. 1966: Color discrimination in goldfish. — Anim. Beh. 14: 351-355.

Peterson, N. 1962: Effects of monochromatic rearing on the control of responding by wavelength. — Science 136: 774-775.

Powers, M. K. & Easter, S. S. 1978: Wavelength discrimination by the goldfish near absolute visual threshold. — Vision Res. 18: 1149-1154.

Shefner, J. M. & Levine, M. W. 1976: A psychophysical demonstration of goldfish trichromacy. — Vision Res. 16: 671-673.

Slomin, V. Jr. & Pasnak, R. 1972: The effects of visual deprivation on the depth perception of adult and infant rats and adult squirrel monkeys (Saimiri sciurea). — Vision Res. 12: 623-626.

Stell, W. K. 1975: Structural studies of functional pathways in goldfish retina. — In: Ali, M. A. (ed.), Vision in fishes: new approaches in research: 81-90. Plenum Press, New York.

—»— 1978: Inputs to bipolar cell dendrites in goldfish retina. — Sensory Processes 2: 339-349.

Stell, W. K. & Hárosi, F. I. 1976: Cone structure and visual pigment content in the retina of the goldfish. — Vision Res. 16: 647-657.

Tsin, A. T. C. & Beatty, D. D. 1978: Goldfish rhodopsin: P. 499_1. — Vision Res. 18: 1453-1455.

—»— 1979: Scotopic visual pigment composition in the retinas and vitamins A in the pigment epithelium of goldfish. — Exp. Eye Res. 29: 15-26.

Tsin, A. T. C., Beatty, D. D., Liebman, P. A. & Drzymala, R. 1981: Rod and cone visual pigments in the goldfish. — Vision Res. 21: 943-946.

Walk, R. D., Gibson, E. J. & Tighe, T. J. 1957: Behavior of light- and dark-reared rats on a visual cliff. — Science 126: 80-81.

Walk, R. D. & Bond, E. K. 1968: Deficit in depth perception of 90-day-old dark-reared rats. — Psychonom. Sci. 10: 383-384.

Wye-Dvorak, J., Marotte, L. R. & Mark, R. F. 1979: Retinotectal reorganization in goldfish — I. Effects of season, lighting conditions and size of fish. — Neuroscience 4: 789-802.

Yager, D. 1967: Behavioral measures and theoretical analysis of spectral sensitivity and spectral saturation in the goldfish, Carassius auratus. — Vision Res. 7: 707-727.

Yamamoto, K., Nagahama, Y. & Yamazaki, F. 1966: A method to induce artificial spawning of goldfish all through the year. (In Japanese with English abstract.) — Bull. Jap. Soc. Scient. Fish. 32: 977-983.

Yoon, M. G. 1975: Effects of post-operative visual environments on reorganization of retinotectal projection in goldfish. — J. Physiol. (London) 246: 673-694.

Received 2.III.1983
Printed 13.XII.1983