PHOTOTACTIC REACTIONS AND THEIR REVERSAL IN THE MAY-FLY NYMPHS HEPTAGENIA INTERPUNCTATA (SAY).

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Reactions to Light.

When nymphs of the may-fly *Heptagenia interpunctata* are placed in a long glass dish of water near a window, they immediately swim away from the light, and upon reaching the end of the dish they repeatedly collide with and claw against the invisible obstruction. These movements may be kept up, with intervals of rest, for a whole day. When quiet, the nymphs do not always face away from the light, nor when they swim do they always go in a straight line away from it. Often if there are large dark objects in the room, they will swim against the side of the dish in the direction of the objects.

As the behavior of the nymphs is often much affected by objects in the neighborhood, most of the experiments on the reactions to light were performed in a dark room in a large, evenly colored box constructed of white cardboard, thus shutting off all objects and undesirable light. The light usually employed was derived from an ordinary sixteen candle power incandescent lamp attached to a wire and movable about at will. Almost without exception, the nymphs swam away from the light. Occasionally, some specimens after repeatedly clawing against the transparent barrier and not securing a hold would fall down on their backs with their legs sticking up, remaining in that position sometimes for hours. If a stone was placed in the end of the basin nearest to the light, the nymph would attach itself and remain there for days. When the light was brought near the stone and moved about, the insect would seek the shaded area, but light even much more intense would seldom force it to desert the stone entirely.

When a piece of white cardboard is placed on the water in a small glass dish, the nymphs soon attach themselves to the lower side of it. By illuminating that side of the paper they soon crawl upon the top which is shaded. Then, when the strong light is again thrown from above, they move below, and in that manner can be made to move back and forth repeatedly, though not with such precision after the experiment is kept up for some time. The nymphs respond to light most readily when occupying the upper side of the paper, and the best results are obtained when white paper is used. Quite similar results are obtained by using gray or black paper, but in such cases the nymphs take a much longer time to move from one surface to another.

I have performed quite a number of series of experiments with different groups of nymphs using two lights, differing in intensity, one at each end of a glass basin twenty-four inches long. I noticed that by placing the nymphs in the water half way between the two lights they would often continue going in the direction in which they first started, regardless of the intensity of the light, though many of them manifested much promptitude in going towards the end of the dish from whence came the light of less intensity, even when there was a difference of only a few candle powers. The excitability apparent in many specimens at the instant of being placed in the water, which is largely responsible for their reckless behavior was almost totally reduced by placing the nymphs in a narrow tunnel made of glass strips, which was placed in the middle of the large basin at right angles to the rays of the two lights. The tunnel was sufficiently wide to enable the specimens to go forward, but not wide enough to enable them to turn around in it. Then the nymphs were put in at one end and allowed to pass through two or three inches of the tunnel, thus getting the full force of the two lights at the same time, and also a chance to resume a more normal attitude. By the time the end of the tunnel was reached, which was in line with the two lights, it was only a matter of choice which direction was to be taken. Sometimes the nymphs would manifest no choice, but remain in the middle of the dish for several minutes, especially when the two lights differed little in intensity. But, generally the insects evinced considerable precision in choosing the end of the basin from which came the light of less intensity. The results obtained in some of the experiments are given in the following tables.

TABLE I.

Series.	Turns Toward 80 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	0	0	10	10
2	0	0	10	10
3	0	0	10	10
4	0	0	10	10
5	0	0	10	10
6	0	0	10	10
Totals	0	0	60	60

TABLE II.

Series.	Turns Toward 32 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	0	2	23	25
2	0	0	25	25
3	1	3	25	25
4	2	0	23	25
5	0	0	25	25
6	1	1	23	25
Totals	4	6	140	150

TABLE III.

Series.	Turns Toward 10 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	1	2	7	10
2	1	3	6	10
3	0	3	7	10
4	0	4	6	10
5	2	3	5	10
6	1	3	6	10
Totals	5	18	37	60

In further experiments an apparatus essentially the same as that used by Strasberger in his work on *Protista* was employed. It consisted of a prism of a solution of India ink placed over a long glass trough the sides and bottom of which were covered over with white paper. Ten specimens were placed in the end of the trough under the thin portion of the prism, and when fairly quiet the light was reflected perpendicularly through the prism giving a perfect gradation in the intensity of light from one end of the trough to the other. Three of the specimens immediately swam toward the dark end of the trough; four others followed within half a minute; and the remaining three collected into a group remaining that way for a few minutes when they were separated, and within another minute also left for the dark area. Sometimes a specimen would return to the less obscure end of the dish and not finding anything favorable to its thigmotactic inclination, it went back whence it came from. This experiment was repeated several times, always with similar results.

The light was next permitted to enter the trough obliquely, the thicker end of the prism being next to the light. Again five of the individuals made for the darker end of the trough almost immediately, this time going against the rays of the light. About half a minute later two other specimens followed and within three minutes all had deserted the light area. This experiment was repeated many times with ten different sets, and always in less than one minute a large majority of the specimens were in the dark portion of the trough. There were almost always a few slow specimens which required a longer time, from one to ten minutes and even much longer, to be moved. If left in the trough, almost invariably at the end of a few hours the nymphs, with some exceptions, would gather into a group in the obscure region and remain there indefinitely. If the prism and light were reversed, leaving the entangled colony undisturbed in the more illuminated end, the colony would slowly break up and within several hours again form in the more obscure territory.

Another apparatus was so arranged that the length of the prism could be increased or lessened by tipping the trough holding the solution, at various angles, and thus augmenting or diminishing the contrast between the two ends of the trough. It was observed that the proclivity of most nymphs to choose one end of the dish in preference to the other varied directly in proportion to the contrast in the illumination of the two extreme regions of the trough. Here again, we find that some individuals react far more readily than others.

It was also observed that some nymphs under ordinary light manifest no signs of agitation when in a uniformly lighted area, yet when an area in their neighborhood becomes slightly shaded they soon aggregate there.

Control of Phototactic Reactions by Chemicals.

It was found in the experimental work on the reactions to light that *H. interpunctata* nymphs are practically all negatively phototactic, but that the different individuals vary in the intensity of their negative response. Also, out of about five or six hundred specimens several were quite indifferent, and a few were even weakly positive. This obvious variability in response among the different individuals, in an identical external environment, is apparently due to the variability in the internal conditions of the various specimens.

Loeb[1] says that in all probability light produces chemical changes in the eye or skin of the animals, and that these changes are responsible for the heliotropic reactions. In a brief preliminary communication[2] on the control of heliotropic reactions in fresh water crustaceans by chemicals, he says that specimens of *Gammarus pulex*, which are naturally negatively heliotropic can be made positive by specific chemical substances. For example, if carbon dioxide is allowed to bubble through the water, or if the animals are thrown into water previously charged with carbon dioxide, they at once become positively phototactic. The same results were obtained with other acids, as hydrochloric, oxalic and acetic, but boracic acid produced no such effect. Among the salts, he found that only those of ammonium could be compared in their effects with those of the acids.

Jackson[3] experimented with another amphipod, *Hyalella knickerbockeri*, which is also negatively phototactic, and obtained results similar to those of Loeb, except, that *Hyalellas* were made positive by boracic acid. Tartaric acid, however, produced no change in their reaction. With the salts and alkalies it was again found that there seemed to be no relation between the class of chemical used and the reactions.

Mast found that *Arenicola* larvæ which are normally positive, become negative in solutions containing various narcotics, acids, alkalies or neutral salts, and he claims that the sense of the reaction in these forms seems to be due to the general state of the

[1] Loeb, J., "Comparative Physiology of the Brain and Comparative Psychology," 1910.

[2] Loeb, J., "The Control of Heliotropic Reactions in Fresh-water Crustaceans, by Chemicals, especially CO_2" (University of California Publications), "Physiology," Vol. 2, p. 1, 1904.

[3] Jackson, H. H. T., "The Control of Phototactic Reactions in *Hyalella* by Chemicals," *The Journal of Comparative Neurology and Psychology*, Vol. 20, No. 3, pp. 259-263, 1910.

organism as a whole, rather than to the specific effect of acid or other solutions on the chemical state of some postulated substance.

My experiments on the effects of chemicals on phototaxis in *H. interpunctata* nymphs were performed in a dark room and the source of light was an incandescent bulb of seventy-five candle power. In each experiment twenty fresh specimens decidedly negative in their reaction to light were put in a long and narrow glass basin, three and a half by twenty-six inches, of about 2,000 c.c. capacity half filled with distilled water. The concentration of the solution was then slowly and gradually increased by adding constant small amounts of the given chemical at about ten or twelve minute intervals. A careful record was kept of the number of insects positive and the number negative at the different concentrations throughout the experiment. It was noticed that as the concentration of the solution was increased there was a gradual increase in the number that became positive. For example, in the experiment with hydrochloric acid at 01 per cent. solution, of the twenty specimens four became positive; at .02 per cent. eight were positive; at .03 per cent. fifteen were positive; and at .04 nineteen were positive. It was the same with the other chemicals employed. As a rule, specimens that once became positive remained positive throughout the experiment, and usually the insects that seemed to be the least annoyed by the light in fresh water were the first to manifest their positive phototaxis in the solution. In general the specimens that evinced their dislike for the light most obviously in fresh water were the ones that made the most frantic efforts to get at the light when they once became positive. However, the specimens as a rule were very persistent in their normal reaction, remaining negative until death occurred.

Of the different classes of chemicals employed the acids were the most effective in reversing the phototaxis of *H. interpunctata* nymphs. A large majority, and in some cases all of the twenty specimens became positive in the acid solutions. When CO_2 was bubbled through the water, again, a large percentage of the specimens became positive. The concentrations sufficient for this effect with the different acids used are,—hydrochloric .04 per cent., tartaric .04 per cent., nitric .03 per cent., sulphuric .03 per cent., boracic .05 per cent., and acetic .07 per cent. Similar results were obtained with the salts, although the number of specimens which became positive was somewhat smaller. However, all the salts employed effected a reversion, and the concentrations necessary to do this are as follows: potassium iodide .3 per cent., potassium chloride .3 per cent., ammonium bromide .3 per cent., sodium chloride .3 per cent., sodium sulphite .06 per cent., and sodium sulphate .08 per cent. The alkalies were less effective, only about half of the specimens becoming positive in these solutions. In general all of the specimens became quite inactive, and when specimens which failed to manifest a positive response were placed in the end of the basin nearest to the light, they seldom made any effort to get away. All three of the alkalies, sodium hydroxide, potassium hydroxide, and ammonium hydroxide, which were employed, produced similar results at about the same concentration, which was .09 per cent. Alcohol, too, at a concentration of about .8 per cent. produced a reversal of the reaction in a large percentage of the specimens.

In their natural environment these insects live on the under side of stones and even in the aquaria I have never seen them on the upper surface. When a certain amount of any of the above mentioned chemicals was added to the water in the aquaria, the nymphs would soon crawl up on top. The acids, again, were particularly effective in this change, and although ordinary daylight was sufficient to bring the nymphs up, the results became more obvious when the aquaria were taken in the dark room and a strong light was suspended over them.

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