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THE POSITIVELY PHOTOTACTIC REACTION OF DAPHNIA MAGNA STRAUS: A CONTRIBUTION TO THE UNDERSTANDING OF DIURNAL VERTICAL MIGRATION

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CONTENTS

PART A

THE KINETICAL COMPONENT OF THE PHOTOTACTIC REACTION

PART B

THE STEERING MECHANISM

1. INTRODUCTION

> *"New ideas are thrown up spontaneously like mutations; the vast majority of them are useless crank theories, the equivalent of biological freaks without survival-value."*
>
> Arthur Koestler, "The Sleepwalkers". London 1959.

Numerous fresh water and marine species that belong to the zooplankton and the nekton, descend with arising and ascend with declining sun. This phenomenon is called diurnal vertical migration. This migration may be less than a meter in the shallow confines of a pond or it may exceed a hundred meters as was recorded for the copepod *Calanus finmarchicus* in the Atlantic Ocean (Clarke, 1954).

This vertical migration is probably not restricted to the upper water layers. Certain animals have been found to migrate vertically at depths of about 800 m (Welsh et al., 1937; Waterman et al., 1939). Clarke and Hubbard (1959) presume that a large number of animals producing luminescent flashes at 100 m and at 170 m at night migrate to levels deeper than 500 m during the day. Obviously, many species, living from the surface waters down to the very deep-sea, change their level of habitat twice each day.

It is the purpose of the present study to discover the basic laws and mechanisms that govern vertical migration.

2. PREVIOUS HYPOTHESES ON DIURNAL VERTICAL MIGRATION

Reviews dealing with experimental vertical migration have been written by ROSE (1925), CUSHING (1951), and others. The papers of some authors, representing the different approaches to the problem, will be specially discussed below. Above all, the connective line of thought, and the reason why a certain hypothesis failed to explain the phenomenon satisfactorily will be the subjects of this chapter.

Some of the difficulties encountered in the literature dealing with the reactions of animals to stimuli such as light, can be reduced to the different content various authors give to the conception of phototaxis. It is sometimes impossible to know for certain what some authors mean by it. Nevertheless the use of the term phototaxis is inevitable when experiments of previous investigators are mentioned. For this reason phototaxis is used in this paper merely to indicate a movement towards or away from light, a description that leaves enough elbow-room to apply it to the literature discussed here. In general, other terms used by authors to indicate phototaxis are given below between brackets. If necessary, further specification of the behaviour under discussion is added.

LOEB and GROOM (1890) attributed vertical migration to phototaxis (heliotropism). They thought the mechanism underlying this behaviour to be continuously acting, either in a positive or in a negative sense, thereby forcing the animals towards or away from the "light".

To explain the downward movement in the morning and the ascend in the afternoon, the sign of the phototaxis must change in the right way and at the proper time. The research of LOEB *et al.* aimed, among other things, at establishing this change.

According to them plankton animals are positively phototactic in light of low intensity, and negatively in strong light. In 1904 the positivating effect of carbon dioxide was demonstrated and in other papers acids, temperature and salinity of the water were mentioned as factors controlling the sign of phototaxis (LOEB, 1906, 1908). Taking these factors into account the vertical migration was pictured as follows (LOEB, 1908). In the morning the plankton animals at the surface are negatively phototactic under the influence of the low concentration of carbon dioxide in the water, the heating of the surface water by the sun and the high light intensity. The downward movement, away from the light, brings the animals in water layers of lower temperature and in lower light intensity in the course of the day. They now become positively phototactic. In the meantime light intensity starts decreasing, the dissimilation of the phytoplankton tends to exceed the assimilation, which makes the concentration of carbon dioxide increase. These factors guarantee the persistence of the positive reaction of the migrating animals when they arrive at higher levels in the water.

ESTERLY (1919) was one of the investigators who tried to find out what releasing stimuli might be capable of changing the sign of the phototaxis (phototropism). Temperature seemed to be such a factor. However, its effect was rather confusing. Lower temperatures, for instance, induced a negative phototaxis in the copepod *Acartia* (ESTERLY, 1919, p. 21), but a positive phototaxis in the copepod *Calanus* (p. 28). Animals from the surface reacted in a way different from that of animals of the same species caught in deeper water. In the laboratory *Acartia* from deeper water were at first negatively phototactic but they soon became positively phototactic. In *Calanus* from deeper water it was the other way round. ESTERLY (p. 14) doubted whether the results of his experiments could be applied to the events in nature: "Daily variations in surface temperature and salinity are too small to have a marked effect in causing diurnal migrations". Moreover, he suspected the phototaxis, observed during the experiments, to be an abnormal behaviour pattern: "My own opinion is that the removal of the animals from deep water to the surface temporarily upsets the physiological state, and the brief period of positive phototropism is the sign of the disturbance" (p. 67).

In 1902 PARKER had introduced geotaxis (geotropism) as a factor involved in diurnal migration. When interpreting vertical migration, this kind of orientation has been widely applied up till recent times (DICE, 1914; ESTERLY, 1919; CLARKE, 1930; KIKUCHI, 1938; HARRIS, 1953; GROSSER, BAYLOR and SMITH, 1954; WOODHEAD and WOODHEAD, 1955; BAYLOR and SMITH, 1957; SCHRÖDER, 1956, 1959), but the experimental evidence in favour of its importance and even of its existence is scarce. This forced some investigators (ROSE, 1925; CUSHING, 1951) to renounce from its use in explaining the migration pattern. It must be pointed out that the use of geotaxis in the explanation of vertical migration again includes a search for the factors capable of changing the sign in the proper sense and at the proper time.

A discussion on this kind of approach seems relevant here. To start with the sign changing factors, CUSHING (1951) affirmed ESTERLY's suspicion that the concentration of the various agents, capable of inducing a change in phototropic sign in the laboratory, is not present in nature. Evidence has accumulated that phototaxis (heliotropism, phototropism) of the kind described by LOEB *et al.* occurs only under certain artificial circumstances. It seems that such circumstances induce some condition in the animal which results in a stereotype reaction, for instance a prolonged movement toward light. Various factors are capable of inducing this condition, a reason why various authors have denoted the phenomenon by different terms. RUSSELL (1934), for instance, called it a laboratory product ("... the outcome of exposure to artificial and unnatural stimuli"), FRANZ (1912, p. 499) called it escape reactions ("meist ist sie lediglich eine Fluchterscheinung, die im Freileben nur von ganz kurzer, schnell vorübergehender Dauer sein kann") and BOHN (1906, p. 144) used the term illusions ("Dans bien des cas, les tropismes sont des illusions"). VERHEIJEN (1958), in an exhaustive study on this subject, concluded that in many laboratory experiments on phototaxis normal orientation responses are impossible because of the unnatural angular light distribution. He speaks of the "trapping effect of a light source". For planktonic animals this kind of behaviour was demonstrated by SCHALLEK (1942, 1943). On p. 120 (SCHALLEK, 1942) he states: "Some individuals, unable to get close enough to the light, hurl themselves time after time against the surface of the water". SCHALLEK was the first who recognized the importance of the light distribution and tried to adapt the distribution in the laboratory experiment to the normal conditions in nature. He observed that the copepod *Acartia tonsa* swims towards a source of light when this is placed directly above the glass cylinder in which the animals are swimming, but that they are distributed in the lower part of it when the light is more diffuse. He rejected phototaxis as a means to explain vertical migration: "Phototropism experiments in a direct beam of light need not necessarily apply to the behavior of organisms in nature", and: "Its reaction in the direct light in which experiments on phototropism are usually conducted has no bearing on its behavior in nature" (SCHALLEK, 1943, p. 104).

Since this kind of phototaxis is probably a phenomenon arising from abnormal conditions it cannot, in our opinion, play a part in vertical migration in nature.

An entirely different approach to the problem of vertical migration was made by EWALD (1910). He described regular up and down movements of *Daphnia* under constant conditions of illumination and other factors. The direction of these oscillations are in a vertical plane when the light comes from above and in a horizontal plane when the animals are illuminated from one side. Consequently locomotion seemed to be orientated with respect to some features of the light field and in EWALD's opinion to the light gradient. The oscillation was thought to be a result of a sensibilisation by the illumination. The downward movement was sometimes passive, sometimes an active one, and EWALD attributed this to the extent of the sensibilisation. He further thought that *Daphnia* has a (relative) optimum, a range in light intensity with an upper and a lower limit, which is preferred by the animals. When, in nature, the light intensity changes beyond these limits, the animals would change their positions accordingly and the result would be a vertical migration. It was not proved, however, that the oscillation is really a movement between two limits of light intensity. Nevertheless, EWALD attempted to explain vertical migration in other terms than phototaxis, and the idea of a preferendum light intensity was born.

The hypothesis of ROSE (1925) was somewhat similar to that of EWALD. With the latter author, he supposed that plankton animals are adapted to an optimum light intensity. But the permanent oscillatory movements between the two limits of light were not emphasized. As long as the animals remain in the preferendum zone their movements are in random directions. Only at the boundaries is an orientation response initiated, either a positively or a negatively phototactic (phototropic) one. The same occurs when the light intensity changes. To quote from his paper: "... on pourrait dire que l'être est adapté à une région d'intensité lumineuse telle que son phototropisme est nul ou indifferent. Si l'intensité lumineuse croît, il se développe un phototropisme négatif, qui le fait descendre dans la zône d'indifference: si elle décroît, il remonte par phototropisme positif jusqu'a cette zône" (p. 500). The important difference between LOEB's phototaxis (heliotropism) and ROSE's phototaxis (phototropism) is that the latter is not thought to be an everlasting phenomenon, but that it is initiated when a sufficient deviation of the optimum light intensity is present. However, the weak spot in this hypothesis is that the existence of such limits was not proved.

RUSSELL (1926, 1927) followed ROSE in assuming that an optimum light intensity was important. He even thought in terms of an absolute optimum intensity, not of a relative optimum as EWALD had done (RUSSELL, 1927, pp. 247, 253). His concern was to look for arguments in favour of the existence of an optimum light intensity in the field leaving it to the physiologist to find the mechanism involved in keeping the animals within their optimum zone.

After RUSSELL most research, especially in the field, has been dominated by the preferendum hypothesis. Diurnal vertical migration is "explained" by stating that the animals follow an optimum light intensity. Laboratory studies (CLARKE, 1930, 1932) emphasized, however, the predominant importance of light adaptation, and also this led to the compromise concept of a relative optimum.

CLARKE (1930) working with *Daphnia magna* "to reveal the fundamental mechanisms of phototropism" (p. 109) for the first time stated that phototaxis should be analysed, and that it is not the basic mechanism with which behaviour can be explained in the way LOEB did. As did

EWALD and ROSE great importance was attached to changes in light intensity as the cause of a swimming towards or away from a light source. CLARKE's experiments are a fine example of a systematic analysis of the behaviour of a planktonic animal. Nevertheless, it was (and is, see VIAUD, 1938; SCHRÖDER, 1956, 1959; RIMET, 1960 *a*, *b*) obviously difficult to get rid of the old heliotropism concept, for the "primary phototropism" of CLARKE is characterised by "always present", in common with LOEB's. In his own words (CLARKE, 1932, p. 185): "The primary sign of phototropism is the dominant or most persisting sign exhibited by the individual under constant environmental conditions". Besides a primary phototaxis, a primary geotaxis (geotropism) was thought to be present in the animals. Changes in the light intensity were supposed to induce a secondary phototaxis and a secondary geotaxis superimposed on the primary one. Thus an animal with a primarily negatively phototactic sign becomes temporarily positively phototactic when the light intensity decreases. However, the inhibitory action of the primary tropism would sometimes prevent the effect of the secondary tropic swimming reactions, and the value of the experimental results were doubted: "Hence it is doubtful how far this photo-geotropic effect will be found to explain diurnal migration of plankton in general" (p. 117).

It is very interesting to follow CLARKE's line of thought in the years following. In the discussion of his field observations in the Gulf of Maine CLARKE (1933, p. 433) stated: "The behaviour of plankton organisms may be regarded as a series of similar responses to changes in the intensity of light or as different responses to constant intensities of different magnitude". Clearly two possibilities are in his mind, (1) a phototactical reaction in the sense that a swimming reaction is provoked by changes in illumination, and (2) a photokinesis, which is an undirected locomotory reaction with the speed of movement depending on the absolute light intensity.

CLARKE attempted to apply the first type of mechanism in the interpretation of the migration of the copepod *Metridia lucens* in the Gulf of Maine. He calculated from his experiments with *Daphnia magna* (CLARKE, 1930, 1932) the smallest percentage decrease in light intensity capable of inducing a (secondarily) positively phototactic reaction and compared this threshold value with the decrease in light intensity in the Gulf of Maine. *Metridia* migrated upwards between 19.00 h and 19.40 h at a decrease in light intensity of 4 %, far below the threshold of 16 % determined for *Daphnia* in his laboratory experiments. His conclusion at that time was, that "Judgement on this question must be reserved until more information is available" (CLARKE, 1933, p. 433). However, a certain percentage decrease in light intensity calculated over a period of 40 minutes while the illumination is changing continuously is not necessarily the same stimulus as an instantaneous decrease of the same magnitude (see chapter 7 of this paper).

A very peculiar downward migration was observed by CLARKE in 1934. In early morning a population of *Calanus finmarchicus* was found at an illumination of 1.0 μW, "but in the next few hours the copepods descend more rapidly than the lines of equal intensity do" (p. 437). The result is that at 9.00 a.m. the animals are at a light intensity of 0.01 μW. His interpretation was: "It appears . . . (that) a higher intensity is required to initiate the migration than is required to cause it to continue" (p. 437). Obviously, CLARKE now supposes that a certain absolute value of light intensity starts the reaction. Facts are here explained by means of the second possible mechanism proposed in 1933: some type of photokinesis. The afternoon upward migration shows the same peculiarity. Now the animals swim into an intensity a hundred times the value they started with at the day depth.

Some twenty years later, CLARKE and BACKUS (1956) observed by echo sounding a scattering layer moving upwards in the afternoon into higher and higher light intensities. It is difficult to understand these movements on the basis of an absolute or relative optimum intensity. Mechanisms such as proposed by EWALD (1910) or ROSE (1925) fail to explain this phenomenon. CLARKE and BACKUS proposed some type of photokinesis that, once initiated, keeps the animals swimming. They were aware of the difficulties, however, because they suggested other unknown factors as well.

Another effort to break away from the preferendum hypothesis was undertaken by JOHNSON (1938). To quote from his paper on *Acartia clausi*: ". . . is the direction of migration such as to bring them into a region of the same light intensity as that in which they existed formerly? Or in other words, is there an optimum intensity for which the species is continually seeking?" JOHNSON's answer is no. He points to field observations which are inconsistent with the idea of an optimum intensity. His experiments suggest that *Acartia clausi* requires a change in light intensity to evoke a reaction and ". . . the more frequently the changes occur the more the animals are stimulated to move either towards or away from the light as the case may be" (p. 117).

Thereupon for some time to come the conception of a change in light intensity as the only causal factor in vertical migration came to an end.

Then a period of very different approaches begins. The apparatus and the methods are ingenious, the results rather confusing and the explanations complicated.

HARDY and PATON (1947) designed an apparatus to experiment with in nature. Their account of the results was a preliminary one. An elaborate discussion of the results of similar experiments made with the same kind of apparatus was given by CUSHING (1955). Copepods originating from various depths were put into an upper or lower compartment of a transparant tube. A set of two tubes was lowered, with closed intersection trapdoors and covered with a black cloth, to various depths in the water. The apparatus remained for some time at a given depth with open trapdoors and the black cloth removed. Then the doors were closed and the tubes hoisted to the surface. *Diaptomus* of shallow origin (0–30 m) swam towards, or remained in the upper half of the cylinders when these were lowered to depths varying from 1 tot 10 m, whereas animals caught between 30 and 50 m aggregated in the lower parts. The degree of aggregation was greatest for both kinds of animals when the apparatus was lowered to medium depth (10 m). This occurred, however, only in two out of six experiments, as follows from CUSHING's fig. 3 (p. 142), hence it may be doubted if this is a significant phenomenon. CUSHING supposed that the optimum light intensity was realised at a depth of 10 m. If this were true, it might be argued that, obviously, both kinds of *Diaptomus* were caught outside their preferendum zone. In the preferendum zone the animals are thought to swim at medium velocity and in random directions. Above this zone the swimming velocity is directly related to the light intensity, below the zone inversely related to light intensity (p. 155). According to CUSHING this photokinetical mechanism led to a difference in swimming speed in both halves of the tube and, while this is greatest at medium velocities, the observed aggregation at 10 m can be explained. In our opinion the result of such a mechanism in nature must be that the animals, moving in random directions, swim fast away from their preferendum and slowly towards it.

With regard to the general technique it seems doubtful as to whether the collecting of the animals, the covering and uncovering with the black cloth and the confinement in the experimental tubes for a certain time are less "unnatural" than laboratory experiments. In addition, the method has the disadvantage that it is impossible to observe the behaviour of the animals.

A much refined apparatus was described by HARDY and BAINBRIDGE (1954). To overcome the difficulty that animals in the laboratory experiment can only swim short distances they constructed a "plankton wheel". A circular perspex tube, turnable round a horizontal axis, permits an animal to swim up or downwards continuously as the wheel is turned in counter direction. However, while the swimming possibility had been extended to more natural conditions, other factors were neglected. For instance, the absence of a change in light intensity, normally encountered by an animal during upward or downward swimming, must have been of influence (see chapter 5, part *f* and *h* of this paper). The observations are of random character, but the authors stated that plankton animals accumulate in nature in regions with a certain optimum light intensity by means of directional swimming and not by a random movement. When this optimum has been reached, directional swimming is replaced by a "hop- and sink" type of movement.

In their experiments with *Daphnia magna* HARRIS and WOLFE (1956) tried to realize the vast change in light intensity an animal encounters in a normal vertical migration in nature. For this purpose they compressed a steep light gradient in a small tube by adding indian ink to the water. The animals were subjected to a whole illumination cycle, from darkness to darkness, with a slow increase and a slow decrease in light intensity. The result was a small-scale vertical migration. In the same year, HARRIS and MASON published an account of similar experiments with *Daphnia* of which the compound eye was extirpated. Also these animals were capable of a vertical migration with all the features present in nature. They concluded from these experiments that photokinesis must be regarded as a very important mechanism, at least with eyeless forms.

HARRIS *et al.* picture the vertical migration to occur in nature as follows. In early morning, when the animals are sufficient dark-adapted, a low light intensity stimulates the photokinetical mechanism in eyeless forms as well as in animals with eyes. The animals start to swim (the so-called "dawnrise") in the "hop and sink" manner, with the upward swimming phase dominating over the passive sinking phase. With increasing light intensity a hypothetical inhibition mechanism causes the upward swimming phase to become shorter and shorter till both phases are equal when the absolute preferendum intensity is realised. "This would represent the situation in a planktonic form without eyes of the type present in *Daphnia*" (p.290, HARRIS and MASON, 1956).

The process is thought to be somewhat different in animals with eyes. There, the "hop and sink" swimming is gradually replaced by an optical orientation reaction in which the body is pitched forward with every stroke of the antennae. When the preferendum intensity is realised the "hop and sink" is absent and the animals are swimming horizontally (dorsal light reflex), which HARRIS (1953, p.543) believes to be their normal posture in nature.

With still increasing light intensity the inhibition mechanism in the eyeless forms reduces the effect of the active phase which results in a passive downward movement of the animal.

In a form with eyes, such as *Daphnia*, the forward tilt of the body still increases, and the animals are moving actively downward.

It is not clear whether the different types of reaction were derived from the experiments. This may be illustrated by quoting HARRIS and WOLFE. On page 349 they wrote: "If the light intensity continues, the consequent eye rotation mediates a change in antennal posture which tilts the horizontal path downwards. The result is a stable linear downward movement of the animal". But on pp. 351 and 352 they interpreted their experiments with normal animals as if they were eyeless: "At a certain "optimum" level of light intensity the rise and fall are equal; and, when the light intensity in the surface begins to exceed this value, the population will descend since the photonegative fall now exceeds the photopositive rise". It seems, therefore, that both in the daphnids with extirpated eyes and in the normal daphnids the downward movement of the animals was a slow passive sinking. The ascend of the animals is equally slow when the light intensity decreases. Whether the slow swimming is due to the artificial high turbidity of the water or inherent to the reaction mechanism involved, is not clear. In case the last supposition is true it is questionable if such a mechanism can account for all features of the vertical migration as observed in nature, especially so for the fast descends and ascends sometimes observed (see, for instance, CLARKE and BACKUS, 1956).

A few general remarks should be made here. In our opinion there are two weak links in the above mentioned investigations. In the first place, a study was made of the results of the reactions (CUSHING, 1955; HARRIS and WOLFE, 1956, HARRIS and MASON, 1956), not of the reactions themselves. As with field observations, speculations had to be made with respect to the kind of reaction involved. In the second place, no definitions have been given of the stimulus to which the animals were subjected, for the mere mention of the "light factor" or even "decreasing light intensity" is far too deficient for such a complex situation. The result is a kaleidoscopic picture of hypotheses: different kinds of kineses, of phototactical responses and so on. The central thought has always been the existence of a preferendum intensity. But this final concept has never been defined, proved with experiments, or demonstrated in nature, since field observations combined with accurate light measurements are very scarce and contradictory (ULLYOTT, 1939; KAMPA and BODEN, 1954; CLARKE, 1933, 1934; CLARKE and BACKUS, 1956; SIEBECK, 1960). Most efforts were aimed at finding a mechanism which would secure the animals within the preferendum intensity. In this way, the role of the "light factor" is a static one: it represents only the upper and the lower limit of the preferendum intensity. The tremendous change in light intensity twice a day is, *ipso facto*, the indirect cause of the diurnal migration. The direct cause is the shift of the boundaries of the preferendum. Amongst other things, this may be the reason why the "light factor" was not defined. Only Clarke (1930, 1934) and Johnson (1938) considered the change in light intensity to be the direct cause of the migration.

Siebeck (1960) attempted to define exactly the change in light intensity as a causal factor. From numerous field observations, accompanied with accurate light measurements, he concluded that the rate of the change in illumination is of the utmost importance. Siebeck did not mean the rate of the relative change in light intensity, but the rate of the absolute change. He rejected the relative change as a stimulus because he observed, that, with the same percentage of decrease in light intensity, migrations took place only at high absolute light intensity, and not at low intensity.

3. INTRODUCTION TO THE EXPERIMENTS

Ecologists sometimes make objections to laboratory experiments designed to solve an ecological problem. With regard to vertical migration Hargitt (1912), for instance, asked: "Is it within the range of the calculus of probability that conclusions drawn from observations made upon an animal in the shallow confines of a fingerbowl, but whose habitat has been the open sea, are trustworthy?" These doubts about the value of experiments are based on a misunderstanding of the purpose and the limits of laboratory experiments on the one hand and of field observations on the other. Ecology tries to describe and, as far as possible, to analyse complicated phenomena in nature. This includes the indication of possible factors governing these phenomena. The indication of a factor as a possible cause is based upon correlations between factor and phenomenon. In this way ecology furnishes the physiologist with a problem, and also with the direction of research, so that it is not necessary to go into a wild goose chase, looking for causal factors. The task of the physiologist is to reveal simplified behaviour patterns that are related to the ecological problem. As soon as possible quantitive relationships must be built up between such simplified patterns and the causal agents. To do this it is necessary to subject the animals to artificial and sometimes strange conditions. The ecologist may object that the situation in which the animal is placed is too artificial to expect a normal behaviour pattern of the animal. This is right, but it must be borne in mind that the animal can only react according to its own structure. Certain reactions have been called "laboratory artefacts". The reason in most cases is that the observed behaviour can not simply be applied to the natural situation. Nevertheless, it can reveal aspects of the physiological mechanism at the base of normal behaviour which would be obscured in the natural but more complex situation. An example is a special form of phototaxis described by VERHEIJEN (1958). Honey bees were recorded walking towards a source of light in an otherwise dark room. This behaviour led to destruction due to the heat of the source and is clearly devoid of biological meaning: in nature no bee will be seen flying toward the sun. The experiment demonstrates, however, that a light source alone is not sufficient illumination for normal optical orientation as was supposed by the light compass reaction (VON BUDDENBROCK, 1914).

With proceeding analysis the gap between the ecological observation and the physiological experiment tends to widen. It is left to the proper judgment of the investigator who made an ecological problem the object of his studies to decide whether he is still busy with phenomena related to it, or whether he makes experiments for physiology's sake. At all events, the physiological mechanisms must be confronted with nature. One of the ways to do this is to construct a model and to compare it with what occurs in the field. That may be the final synthesis.

The present study is an attempt to give an outline of the basic phenomena that are involved in the diurnal vertical migration. The results are still fragmentary and a more careful analysis is needed. When an attempt is made to interpret the migration in the field, it is done to make sure by confrontation that the right road has been taken, not because it is thought that now sufficient data are available for a final synthesis.

As proposed by ethologists (TINBERGEN, 1951; BAERENDS, 1957) as well as by physiologists (MITTELSTAEDT, 1956; BURCKHARDT, 1960) the behaviour (a movement) is divided into two components, *viz.* a velocity and a direction. Therefore, at the base of such a movement two mechanisms have to be looked for: 1. a kinetical mechanism, and 2. a steering mechanism. The causal factors which initiate both mechanisms may be different too. In ethology they are called: *a*) releasing stimuli, and *b*) directing stimuli, respectively (TINBERGEN, 1951). In the present paper the releasing stimulus and the kinetical mechanism are dealt with in Part A, while the directing stimulus and the steering mechanism are treated in Part B.

Apart from the causal analysis of behaviour, finalistic aspects should be considered. The concept of a preferendum light intensity belongs to it. Sometimes the causal mechanism works in such a way that the animal remains within a certain range of illumination, whatever the change in illumination may be.

Up till now, research has been restricted to positively phototactic reactions resulting from decreases in light intensity. It is possible that other reactions to decreases in light intensity exist such as slow upward drifts when the rate of the decrease in light intensity is very small.

PART A

THE KINETICAL COMPONENT OF THE PHOTOTACTIC REACTION

4. APPARATUS AND METHODS

The experiments were performed with mature females of *Daphnia magna* Straus. The animals were cultured in the laboratory throughout the year in glass vessels of 5 liter, placed in a water bath. The temperature was regulated with a thermostat at 18° C. The daphnids were fed with *Chlorella* spec. also cultivated in the laboratory.

The essential part of the apparatus (see Plate I) consisted of a perspex cylinder (2) with a length of 65 cm and a diameter of 15 cm. In some experiments a cylinder was used with a length of 150 cm and a diameter of 14 cm. The cylinder was placed in the centre of a hardboard case (1) with partly open front and blackened walls (measurement 170 × 53 × 53 cm). The cylinder was illuminated from above with a slightly divergent beam. The light source was an incandescent lamp with a silver coated bulb front. It was put in the centre of a parabolic mirror. Lamp and mirror were placed in a light-tightbox on top of the hardboard case. The whole apparatus was mounted in a dark room.

The light intensity could be decreased in two ways: either with neutral filters or by enlarging the resistance in series with the lamp. This last method was used when the light intensity was decreased continuously. The effect of the change in spectral composition of the light which results from this method of changing the light intensity was for the greater part corrected by a liquid filter consisting of a solution of $CuSO_4$. This filter absorbed also the heat rays of the light source. The resistance could be increased gradually with the aid of a motor and an acceleration mechanism (7). The main power was stabilised with a constant voltage transformer (5).

The vertical swimming movements of one daphnid were followed with a looking device, consisting of two parallel rings, 1 cm apart and mounted round the cylinder. The looking device could be moved up and down along the cylinder by turning the wheel (3) at the outside of the case. The vertical movements of the looking device were traced on a kymographic drum (4).

The light intensity was measured with a barrier layer cell at the bottom of the cylinder and a recording microvolt-microammeter (Micrograph Kipp, Delft, Holland, 6). The light was expressed in physical units (erg/cm^2 sec). Calibration of the barrier layer cell was done on an optic bench by comparing with a calibrated thermopile.

At regular times during the experiments the light intensity was measured at different depths in the cylinder. From these measurements the extinction coefficient was estimated. It was then possible to calculate the light intensity at every depth in the cylinder. To get an exponential decrease in light intensity from surface to bottom the upper fifth part of the cylinder had to be partly blackened on the inside.

The cylinder was filled with distilled water enriched with the following salts: $CaCO_3$ 2 mg/l, $MgSO_4$ 19 mg/l, $NaHCO_3$ 100 mg/l, KCl 6 mg/l, Na_2HPO_4 18 mg/l, KH_2PO_4 2 mg/l.

This composition is based on an analysis of lake water and recommended by FLÜCHINGER and FLUCH (1949). The solution was also used as culture fluid. A certain amount of *Chlorella* spec. and sometimes indian ink was added to get a certain extinction coefficient and to provide the animals with food during the experiments. The p_H was 7.5 and the temperature 18°–21° C.

In each experiment about a hundred animals were present in the tube. They were adapted to a basic light intensity for one hour at least before the experiments started.

5. THE EFFECT OF AN INSTANTANEOUS DECREASE IN LIGHT INTENSITY

a. THE DEFINITION OF THE RELEASING STIMULUS

Daphnia magna reacts to a decrease in light intensity by swimming upward. The releasing stimulus for the kinetical component of the reaction, in the following pages mostly called the "stimulus", is defined in this paper as *the rate of the relative decrease in light intensity*, in mathematical form $\frac{1}{I}\frac{dI}{dt}$. The relativation $\left(\frac{1}{I}\right)$ is needed because of the animal's faculty to adapt itself to the large range of light intensities found in nature. For instance, a decrease of $\Delta I = 10$ erg/cm^2 sec must have another effect when the animal is adapted to a light intensity of $I = 10^4$

erg/cm^2 sec (which is about the light intensity on a cloudless day in September at a latitude of 52° N, see fig. 25), than when it is adapted to I = 10^2 erg/cm^2 sec. The best stimulus approximation at the present time seems to be dividing ΔI by I. The true relativation factor, for instance for threshold stimuli, depends on the relation between such a stimulus and the absolute light intensity.

The stimulus produced by an instantaneous decrease in light intensity may be written as the relative decrease $\frac{\Delta I}{I}$, the time needed for the decrease to occur being negligible with regard to the latent period of the reaction.

b. THE GENERAL FEATURES OF THE REACTION

An instantaneous decrease in light intensity is followed by a more or less perpendicularly upward swimming of the animal. It has been proved by CLARKE (1930) and others that the animals swim toward light. For this reason the reaction may be called a positively phototactic one. This reaction has often been described (SCHOUTEDEN, 1902; RADL, 1903; EWALD, 1910; PARKER, 1911; VON FRISCH and KUPEL-

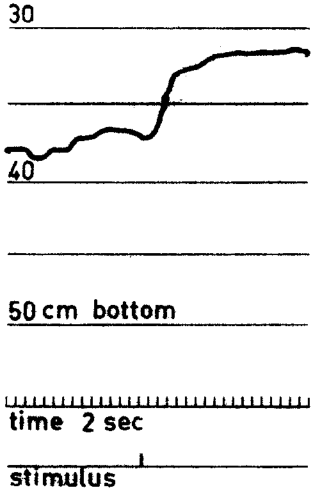


Fig. 1. Kymographic record of a positively phototactic upward swimming of *Daphnia magna* caused by a relative decrease in light intensity of 15.7%. Swimming distance 5 cm; duration of reaction 6 sec; swimming velocity 0.83 cm/sec.

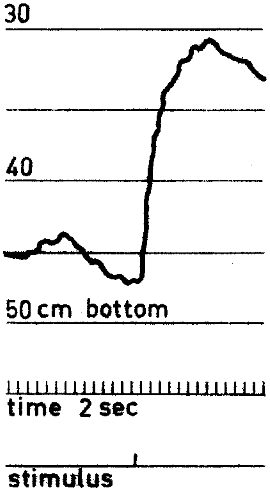
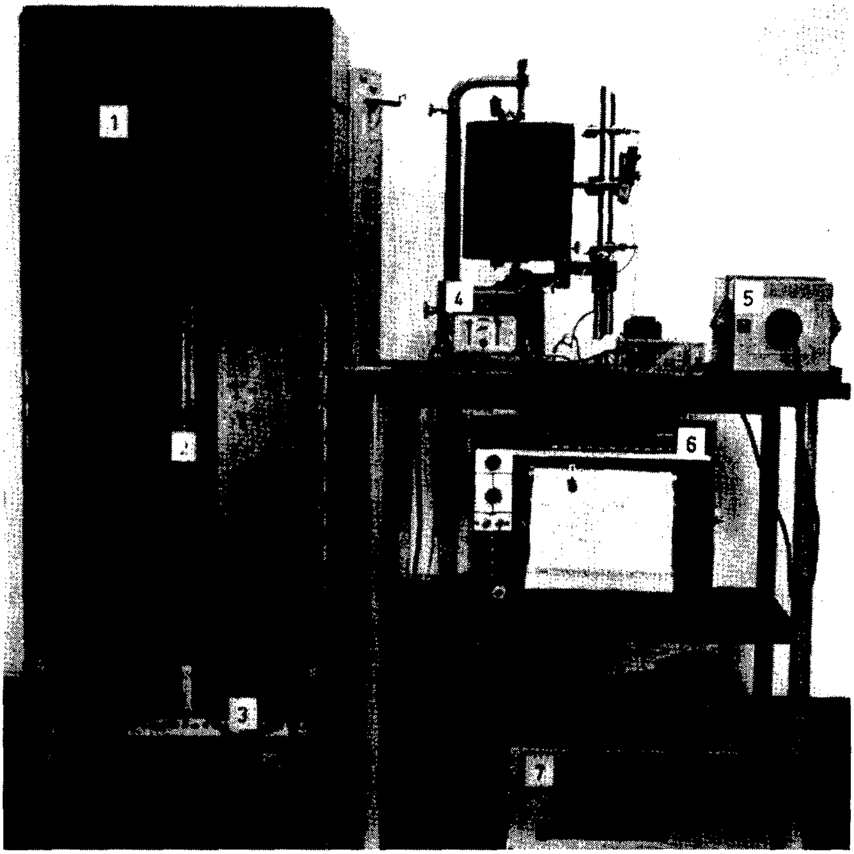


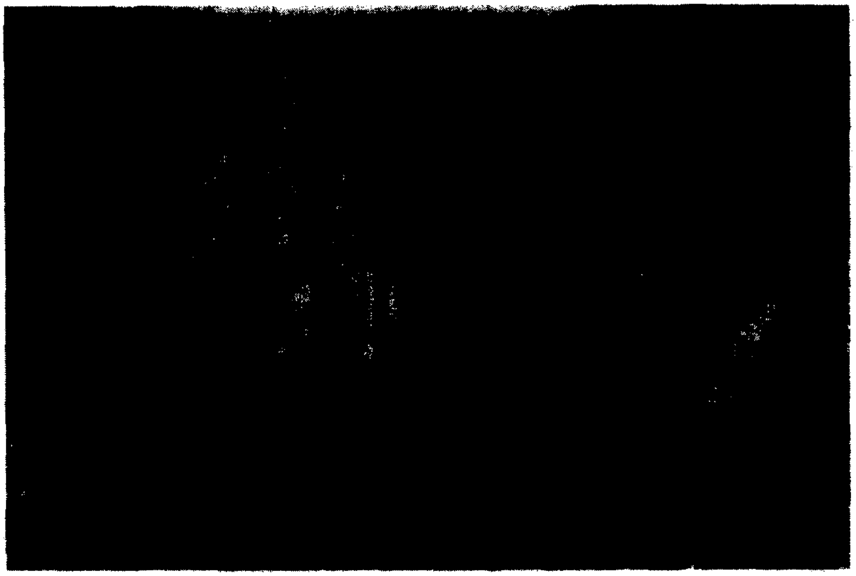
Fig. 2. See legend fig. 1. Relative decrease in light intensity of 32.7%. Swimming distance 16 cm; duration of reaction 17 sec; swimming velocity 0.92 cm/sec.

PLATE I



The apparatus: 1. hardboard case, 2. perspex cylinder, 3. wheel to handle the viewing device, 4. kymograph with smoked drum, 5. constant voltage transformer, 6. recording microvolt-microammeter, 7. acceleration mechanism.

PLATE II



The compound eye of *Daphnia magna*. The eye is turned ventrally. Note the extended dorsal eye muscles and the dorsal filament by which the eye is suspended in the orbit. The three prominent ommatidia at the dorsal side have probably the orientation function described in the text.

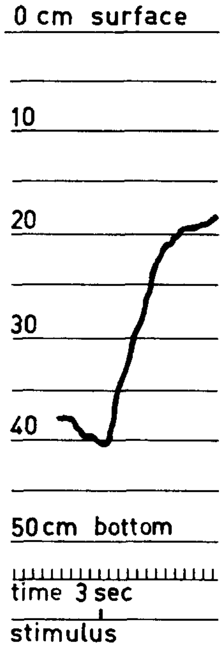


Fig. 3. See legend fig. 1. Relative decrease in light intensity of 44.4 %. Swimming distance 21 cm; reaction time 23 sec; swimming velocity 0.91 cm/sec.

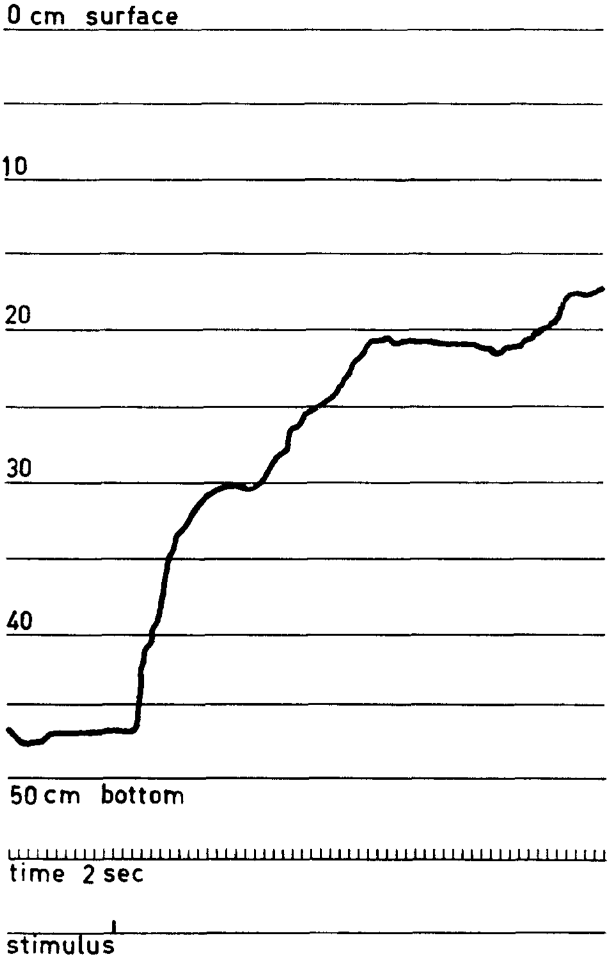


Fig. 4. See legend fig. 1. Relative decrease in light intensity of 15.4 %. Swimming distance 26 cm; duration of reaction 51 sec; swimming velocity 0.51 cm/sec. Aberrant form, see text p. 335.

WIESER, 1913; CLARKE, 1930; HARRIS and WOLFE, 1956). After a latent period of a few seconds the upward swimming reaction starts abruptly with a rather high swimming velocity. After some time it slows down before stopping (figs. 1–3). Therefore, swimming distance and the duration of the reaction are sometimes difficult to determine.

Sometimes aberrant reaction forms are observed. For instance, after a period of upward swimming the reaction may stop for a few seconds, and then start again. So a step-like swimming curve is obtained (fig. 4).

Step-like upward swimming normally occurs when the light intensity decreases continuously at a slow rate. This phenomenon is dealt with in chapter 7*c*.

Under constant light intensity some animals swim intermittantly up- and downward. Sometimes these movements fill the whole experimental tube, sometimes only a part of it. Similar oscillations were described by EWALD (1910), CLARKE (1932) and VIAUD (1938). When these up- and downward movements were analysed for harmonic components (determination of power spectrum; a full account will be published elsewhere) it was found that at least one periodicity was present. This periodicity is most expressed in vigorously up- and downward swimming animals but it is hardly demonstrable when a daphnid is swimming in the "hop and sink" way. The animals which under constant illumination swim vigorously up-and-down react only to large decreases in light intensity (50 % or more). The animals which under constant illumination show the "hop and sink" movement react to the smallest decreases. The "hop and sink" way of swimming was thought to represent the normal condition.

As far as possible—gradual transition between the two types of behaviour are of regular occurrence—animals in the "normal" condition were used for the experiments. This is emphasized because the effect of a stimulus may depend on the phase of the periodicity at the moment of stimulation (a similar suggestion is to be found in CLARKE, 1932, p. 191). No evidence is available, but part of the deviations in the reaction parameters such as the duration of the reaction or the swimming distance may be due to this factor.

c. THE SWIMMING SPEED

The swimming speed v has not a constant value during the phototactic reaction as can be seen from the figs. 1 to 3. The initial velocity slows down more or less gradually. In most cases it is possible to make a rough estimation of v by dividing the swimming distance by the duration of the reaction. When these values are made a function of the relative decrease in light intensity, a cloud of points is the result (fig. 5). To get an idea of the curvation of the function mean values were calculated for sets of 15 observations. These values are inserted in fig. 5 (circled points) and an attempt has been made to fit a line through them. There seems to be a slight increase in swimming speed with increasing stimulus.

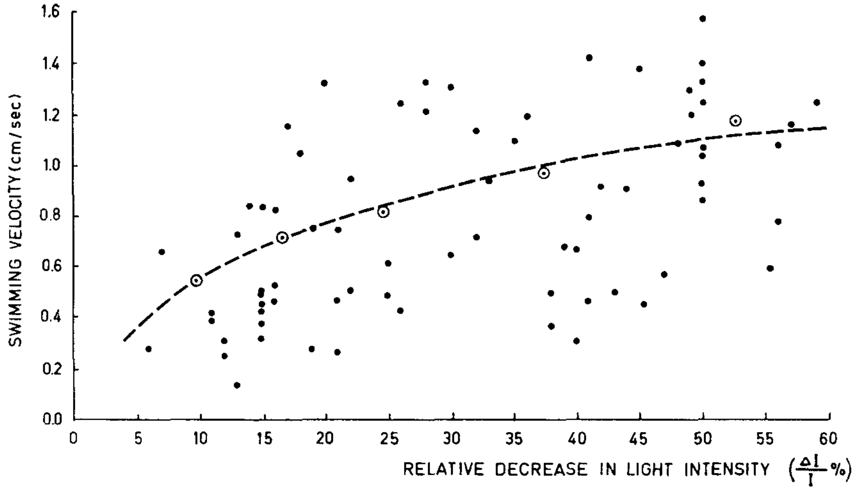


Fig. 5. The swimming velocity of *Daphnia magna* as a function of the relative decrease in light intensity. Each point represents one reaction. The circled points are means of sets of 15 observations. A smoothed curve was fitted to these points by eye.

d. THE DURATION OF THE REACTION

The duration of the reaction t is the time from the start of the upward swimming to the moment of indistinct gain of height.

Table I shows the observed values of t, expressed in seconds. There seems to be no influence of the relative decrease in light intensity upon the duration of the reaction. Nevertheless, t has not a constant value as is argued in part 5*e*.

TABLE I

The duration of the reaction in relation to the relative decrease in light intensity.
Percentage decrease in light intensity

Duration of reaction (in seconds)	6	10	14	18	22	26	30	34	38	42	46	50	54	58
34			2							1				
30			1			1				1				
26				1	1	1			1					
22			5							1		2		
18		1		2	1	1	1	1	1	2				
14	1	1	1	2	1	1		2		2	8	1		1
10				1		1		2	2	1	1		1	1
6			3			1	2							
2	1													

e. THE CORRELATION BETWEEN THE DURATION OF THE REACTION AND THE SWIMMING SPEED

When t and v are correlated, a highly significant correlation ratio η as well as correlation coefficient r is found. From the nature of the correlated quantities a nonlinear relationship may be expected. However, when the linearity is tested with the" criterion of Blakeman" (DE JONGE and WIELINGA, 1953, p. 141), it was found that a linear approximation is possible without too great an error in the area of observation.

Both regression equations were calculated: $v = -0.02\,t + 1.23$ and $t = 7.26\,v + 22.73$, and the regression lines are drawn in figure 6.

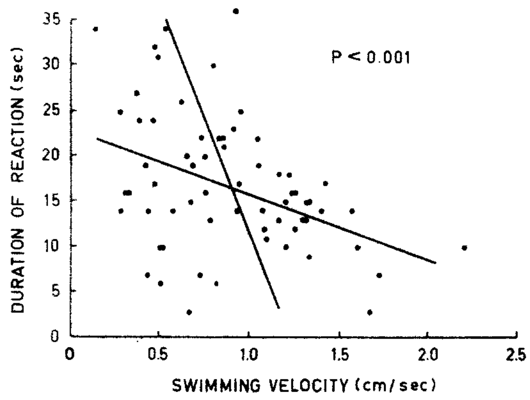


Fig. 6. The correlation between the swimming velocity and the duration of the reaction. Each point represents one reaction. The two lines are drawn according to the regression equations in part 5*e*.

High swimming velocities are thus correlated with short reaction times.

A correlation ratio $\eta = 0.55$ was found to have a probability of $P < 0.001$ that this value was found by chance. The probability that the correlation coefficient $r = -0.40$ has arisen by chance is also $P < 0.001$.

According to the "criterion of Blakeman" a linear regression can be assumed to be present if

$$\frac{\sqrt{N}}{0.6745} \cdot \frac{1}{2}\sqrt{\eta^2 - r^2} < 2.5.$$

Since a value of 2.4 was found a linear correlation seems to exist.

f. THE RESULTS OF THE REACTION

The phototactic swimming reaction of *Daphnia magna* may be considered as a correction to the disturbance of the prevailing light intensity. When the intensity (I_a) occurring at the depth where the animal is swimming just before the decrease ΔI takes place is compared with the intensity (I_r) at the depth where the reaction stops an impression is obtained of the result of the correction. Such a comparison was carried out with populations originating from two separate localities.

In fig. 7 the difference $I_a - I_r$ is represented as a function of the decrease in light intensity ΔI. The mean initial intensity in both series was $I_a = 16 \times 10^2$ erg/cm² sec. The individual values varied because of the fact that the daphnids were not always swimming at the same depth before the decreases occurred. The functions obtained with the method of least squares are strikingly the same for the two populations.

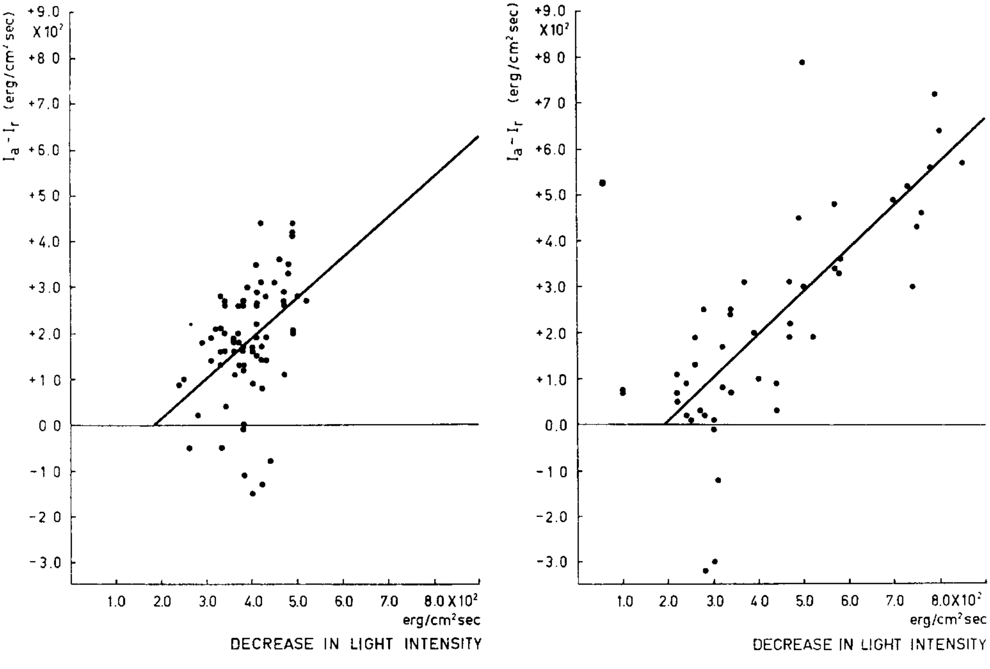


Fig. 7. The result of a swimming reaction, with regard to the maintenance of the adaptation light intensity determined with *Daphnia magna* originating from two different populations. Each point represents one reaction. Lines of best fit (method of least squares) were drawn.

They are $(I_a - I_r) = 0.917\ \Delta I - 177.5$ and $(I_a - I_r) = 0.870\ \Delta I - 160.0$ respectively. The difference may well be the result of the error of estimation.

When $I_a = I_r$ the correction is complete. This applies to a value of $\Delta I = 1.94 \times 10^2$ and $\Delta I = 1.84 \times 10^2$ erg/cm² sec respectively. When the stimulus is expressed as the relative decrease in light intensity a complete correction is obtained for $\frac{\Delta I}{I_a} = 12.1\,\%$, and $\frac{\Delta I}{I_a} = 11.5\,\%$ respectively. These are threshold values for the positively phototactic reaction (RINGELBERG, 1961).

The conclusion must therefore be that the physiological mechanism of this positively phototactic reaction is attuned in such a way that for *threshold* decreases the swimming stops at a depth with the same light intensity as at the original depth. This result is discussed in chapter 8.

When $\frac{\Delta I}{I_a}$ exceeds the threshold value, the correction shortage $(I_a - I_r)$ increases linearly. Strictly speaking, these functions hold for initial light intensities of $I_a = 16 \times 10^2$ erg/cm² sec.

g. THE CHARACTER OF THE PHYSIOLOGICAL MECHANISM: FEED-BACK OR OPEN CHAIN?

An organism is continuously exposed to influences which may upset the existing conditions. But it defends itself against disturbances of this steady state. The positively phototactic swimming of *Daphnia magna* can be considered a correction counteracting the disturbance caused by the change in light intensity. The question arises by what type of mechanism this correction is achieved. While compensation mechanisms differ as to the physiological system concerned, application of cybernetics to biology (WAGNER, 1954; MITTELSTAEDT, 1956, 1961*a*, *b*, and many others) has demonstrated that two principles may be involved in a type of reaction as the one under consideration: (1) the open chain system, and (2) the closed control system.

The first principle is the simplest, and is characterized by the so-called information flow chart of fig. 8. Its analogon in physiology is the most simple reflex circuit. The disturbance is perceived by a measuring unit (M.U.) and transmitted to the effector unit (E.U.) which performs the reaction. In an organism the measuring unit is a sense organ with its afferent nerve and ganglion, while the effector unit is a ganglion with efferent nerve and effector organ. For simplicity these organs have been omitted in the information flow chart because only the way of transmission and transformation of information in the system is essential. A steady state is guaranteed when the quantity of the reaction is properly attuned to the quantity of the stimulus.

The principle of the closed control system is illustrated in the information flow chart of fig. 9. A measuring unit estimates the deviation (*d*) from a certain, fixed value (norm) of the quantity which is to be kept constant. This deviation is transmitted as information to the effector unit which performs a correction. So far, there is no difference with the first principle. The deviation of the norm is, however, continuously perceived, also when already a correction has been made. Generally speaking, the correction is proportional to the disturbance (*d*) minus the previous correction (Y_E). That is why the information (Y_M) going to the measuring unit in the flow chart consists of a difference. The extra channel transporting this information (Y_E) is called a feed-back; it makes the system a closed one. We may say that the effect of the correction is perceived by the mechanism and influences the course of the correction. This is not so in an open chain system.

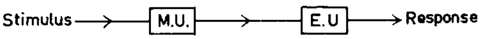


Fig. 8. The information flow chart of an open chain principle (see text p. 340).

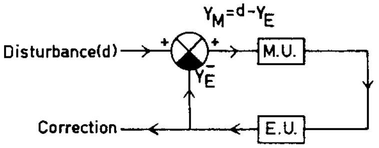


Fig. 9. The information flow chart of a closed control system (see text p. 341).

To decide according to which of the two principles the phototactic correction works in *Daphnia magna*, the following experiments were performed.

When, after a decrease in light intensity, the animal is prevented to reach a higher light intensity, the discrepancy with the norm (the light intensity the animal is adapted to) is kept constant. This interference is of no influence when an open chain is involved, for the stimulus is the decrease in light intensity itself. What occurs, once the reaction is initiated, does not matter. When a feed-back mechanism is involved, however, the deviation of the norm is the adequate stimulus, and when this deviation is kept at a constant value the correcting reaction must continue, at least theoretically.

A few daphnids were put into a perspex tube (length 75 cm, diameter 5 cm) filled with water. After sealing the tube with a perspex lid it was suspended with a nylon wire into the large perspex tube (length 150 cm, diameter 14 cm) of the normal apparatus (see chapter 4), also filled with water.

Alternately two types of experiments were made:

1) a decrease in light intensity $\left(\frac{\Delta I}{I_a} = 28.9\,\%,\ I_a = 12.0 \times 10^2\ \text{erg/cm}^2\ \text{sec}\right)$ was given, and a swimming reaction was registrated in the usual way.

2) after a stimulus of the same value the inner tube was lowered as soon as a previously chosen daphnid started to swim in an upward direction. The distance of this animal to the light source was kept constant. The movements of the inner tube were recorded in the usual way on a kymographic drum.

If a feed-back system were involved, the animals should swim till the end of the tube is reached, whereas in the case of an open chain principle no influence of the lowering of the tube is to be expected.

Forty-six experiments with the tube lowered and the same number of control experiments were performed with a number of animals. Swimming distance and the duration of the reaction were measured. The mean values of some parameters of the reaction are summarised in Table II. In the case of the lowered tube a few swimmings till the end of the tube were observed, but mostly the reaction terminated when the animals were still a fair distance away from it. The mean value of the swimming distance in the case of the lowered tube was 31.2 cm. Since the experiments were only performed with animals present in the first 15 cm above the bottom, swimming stopped, consequently, at least 28 cm from the top. This is not the result of a limiting effect of the end of the tube, for it was possible to let the daphnids swim till the end was reached with repeating decreases in light intensity.

TABLE II

The mean values of some parameters of the swimming reaction obtained in control and "lowering tube" experiment

Duration of reaction in sec		*Swimming distance in cm*		*Swimming velocity in cm/sec*		*Duration of reaction when tube lowered/duration in control*	*Swimming velocity when tube lowered/velocity in control*
Control	*Tube lowered*	*Control*	*Tube lowered*	*Control*	*Tube lowered*		
39.3	62.2	19.1	31.2	0.51	0.53	1.58	1.04

The duration of the reaction was effected by the lowering. The difference between the duration when lowering and not lowering the tube was tested statistically with the test of Wilcoxon and proved to be highly significant ($P < 0.001$). The ratio $t_{\text{experiment}}/t_{\text{control}}$ was 1.58. Preventing the animal from perceiving an increase in light intensity during its positively phototactic reaction results in a prolonged swimming. The swimming velocity is probably not affected. The ratio $v_{\text{experiment}}/v_{\text{control}}$ is 1.04.

The conclusion from these experiments must be, that neither hypothesis in the stated form is true.

Normally, an animal encounters an increase in light intensity while swimming upwardly. The lowering of the tube in the experiments prevents this increase to occur. Obviously, this fact influences the duration of the reaction. It must, therefore, be concluded that the increase in light intensity, normally encountered by an ascending animal is perceived and is of influence on the duration of the reaction. This might be called a feed-back system. It seems, however, improbably that the animal is capable of evaluating the absolute light intensity during its upward swimming and of comparing this value with the norm, the light intensity of adaptation. To overcome these difficulties a system as illustrated in fig. 10 is proposed. As a result of a decrease in light intensity a kind of information, called P-information, is formed which leads to an upward swimming of the animal. Now the animal perceives an increase in light intensity and N-information is transmitted by a separate channel in the system. It is assumed that both kinds of information are processed in the nervous system in such a way that the N-information, when increasing sufficiently, terminates the upward swimming. A more elaborate discussion of this system will be made in chapter 8 when more data of the positively phototactic reaction are available.

Evidently, still another process limits the effect of a stimulus because

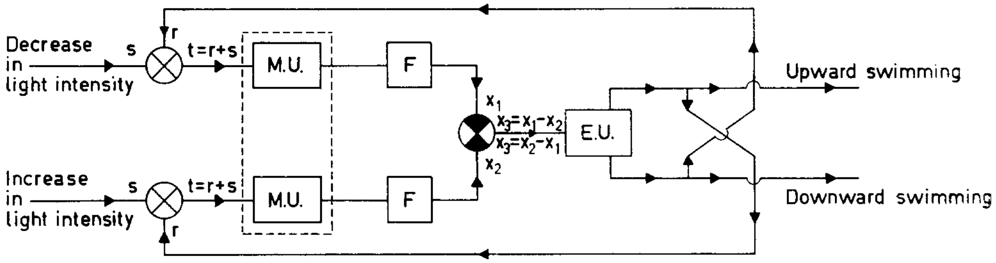


Fig. 10. The information flow chart of the (positively) phototactic reaction of *Daphnia magna* (see text p. 343). $F = \frac{cS}{c'}(1 - e^{-c't})$.

the upward swimming stops also when no N-information is formed as in the case of the experiment with the lowered tube. This process is dealt with in chapter 7*b* and 8.

The rate of increase in light intensity perceived by the animal during its ascend is a function of the swimming velocity and the turbidity of the water. So this last factor must be also of influence on the phototactic reaction.

h. THE INFLUENCE OF THE TURBIDITY OF THE WATER ON THE PHOTOTACTIC REACTION

The influence of the turbidity can also be deduced from the fact that the reaction of *Daphnia magna* to decreases in light intensity is considered a correction. After a decrease in light intensity the intensity of adaptation is realised at a shorter distance in turbid water than in clear water. This results directly from the equation $I_z = I_o e^{-Kz}$, expressing the extinction of light in water.

When

I_a = the adaptation intensity (erg/cm^2 sec),
ΔI = the absolute decrease (erg/cm^2 sec),
z_a = the distance (cm) to be travelled after a decrease ΔI, to reach I_a,

and

K = the extinction coefficient of the water

then

$$(I_a - \Delta I) = I_a e^{-Kz_a} \quad \dots\dots\dots \quad (1)$$

and

$$\frac{I_a - \Delta I}{I_a} = e^{-Kz_a}.$$

When for the relative decrease in light intensity we write $\frac{\Delta I}{I_a} = S$,

then

$$(1 - S) = e^{-Kz_a}$$

and

$$z_a = -\frac{\ln(1 - S)}{K} \quad \dots\dots\dots \quad (2)$$

The distance z_a to be swum by an animal in order to reach the light intensity to which it was adapted before the stimulus was given, is inversely proportional to the extinction coefficient of the water.

The influence of the turbidity was studied in a series of experiments in which the decrease in light intensity was held constant at a value of 30 %, but the extinction coefficient was varied. The water in the experimental tube was made turbid with a mixture of *Chlorella* and indian ink. The value of K was calculated from light measurements at different levels in the tube. For each K at least 15 recordings of the (phototactic)

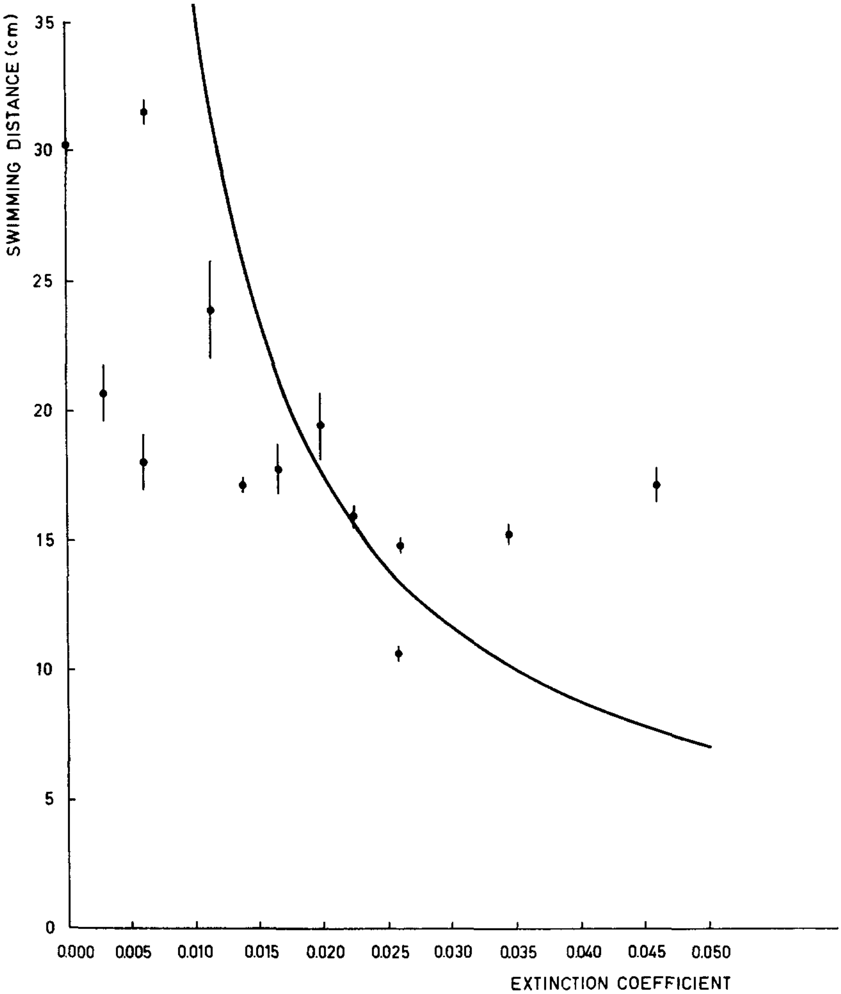


Fig. 11. The influence of the turbidity of the water on the swimming distance. The vertical bars indicate twice the standard error of the mean. The curve is drawn according to equation (2) page 344, $z_a = \frac{\ln (1-S)}{K}$.

response were made. A large number of animals was used in the experiment. The mean swimming distance z_r and its standard error were calculated. Both values are presented in fig. 11. The curve in this figure represents the theoretical swimming distance z_a as a function of K (expression (2)). The data for K = 0 were taken from the experiments with the descending tube described in the previous part (5g). The effect of the turbidity is clearly demonstrated. Comparison of the experimental data with the theoretical curve further indicates that for a certain value of K, $z_r = z_a$. In fig. 12 the difference $(z_a - z_r)$ is made a function of K. It is seen that for K = 0.020, $z_r = z_a$. At lower values of K, the animals do not reach the value of z_a, whereas at higher values they exceed this value. This means, of course, that either they do not reach, or they exceed the light intensity of adaptation I_a for these values of the extinction coefficient.

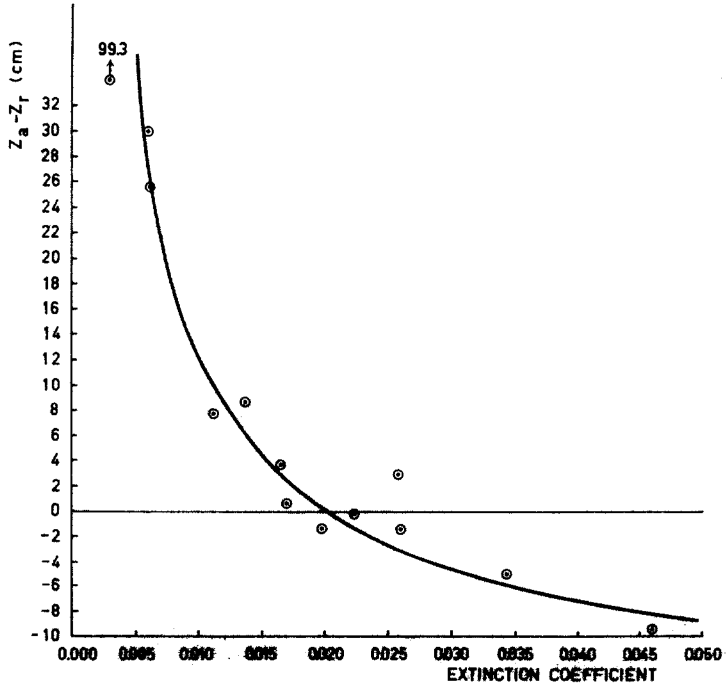


Fig. 12. A comparison of the actual swimming distance (z_r) with the theoretical distance (z_a). Each point represents the mean value of at least 15 observations. A smoothed curve was fitted to the points by eye.

6. THE REACTION TO A SERIES OF SHOCKWISE DECREASES IN LIGHT INTENSITY

When the time interval between two separate instantaneous stimuli is shortened, the chance increases that the effect of the first stimulus has not yet disappeared at the moment the second stimulus is given. Particularly interesting may be a series of instantaneous sub-threshold stimuli following each other at such a rate that each following stimulus is given before the possible effect of a previous one has disappeared. It was impossible to realize exactly the same values of ΔI with the available apparatus, but this proved not to interfere with the demonstration of the underlying principles. Within one series the time *i* between successive stimuli was kept constant, but by varying *i* for different series (fig. 13) its influence could be studied. For two values of *i* an example of the original recordings of the swimming reaction is given in the fig. 14*a* and 15 and some important data are summarized in Table III.

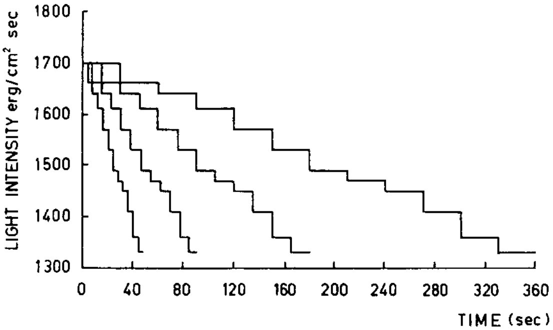


Fig. 13. The applied series of shockwise decreases in light intensity.

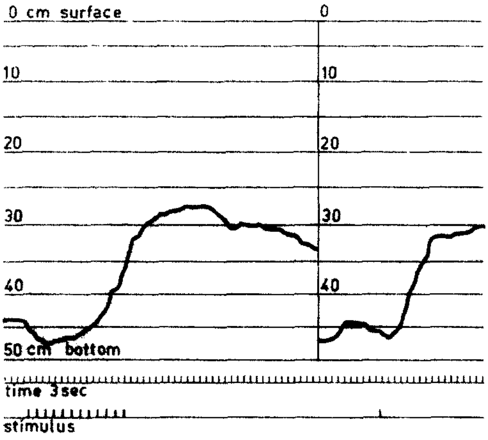
From these results the following conclusions may be drawn:

a. Sub-threshold decreases in light intensity have additional effects.

b. The longer *i* is, the greater the number of decreases that are needed to start the reaction. This means that the effect of a single stimulus diminishes with time.

c. The reaction does not start when a certain (lower) light intensity has been reached.

It can be seen from the original recordings that the reaction resulting from a series of sub-threshold stimuli stops after some time, as was ob-



a *b*

Fig. 14. *a*). Kymographic record of a positively phototactic upward swimming of *Daphnia magna* caused by a series of instantaneous sub-threshold decreases in light intensity. $i = 4$ sec.

b) As *a*). The reaction is caused by one instantaneous supra-threshold decrease of about the same magnitude as the total decrease needed to initiate a response in the previous case *a*).

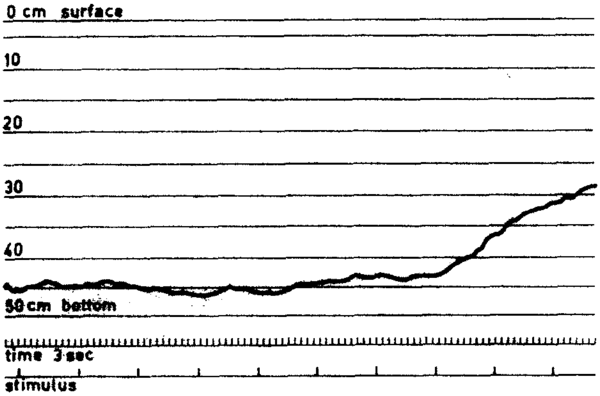


Fig. 15. See legend figure 14 *a*. $i = 30$ sec.

served after one instantaneous supra-threshold stimulus. From fig. 14*a* and *b* it can be seen that the type of swimming reaction is identical for one instantaneous stimulus above threshold value as well as for a series of sub-threshold stimuli.

When the shockwise sub-threshold stimulation is continued long enough after the reaction has stopped a renewed swimming reaction occurs. The result is an intermittent upward swimming. This phenomenon is described in detail in part 7*c*.

TABLE III

Some vital data of the reaction to the series of sub-threshold decreases

i (*sec*)	*Time* (*sec*) *before a reaction starts*	*Light intensity at the start of the reaction* (*erg/cm*^{2} *sec*)
30	231, 286	1470, 1470
15	53, 26, 37, 56	1870, 1860 1840, 1850
7.5	37	1610
4	19, 24	not measured

7. THE EFFECT OF A CONTINUOUS DECREASE IN LIGHT INTENSITY

a. INTRODUCTION

Since the upward afternoon migration of the zooplankton coincides with a gradual decrease in light intensity the study of the reactions of *Daphnia magna* to continuous decreases in light intensity seems to be of paramount importance.

A shockwise decrease in light intensity becomes merged into a continuous decrease when *i* approaches the limit d*t*. Figs. 16*a* and *b* illustrate the number and the kind of continuous decreases in light intensity that have been studied. The mathematical approximation of these decreases is the exponential equation:

$$I_t = I_o \, e^{-t/\tau} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (1)$$

I_o = the light intensity (erg/cm^2 sec) at a certain reference time,
I_t = the light intensity (erg/cm^2 sec) at time t sec later than the reference time,
τ = a time constant (sec), typical for the rate of the decrease.

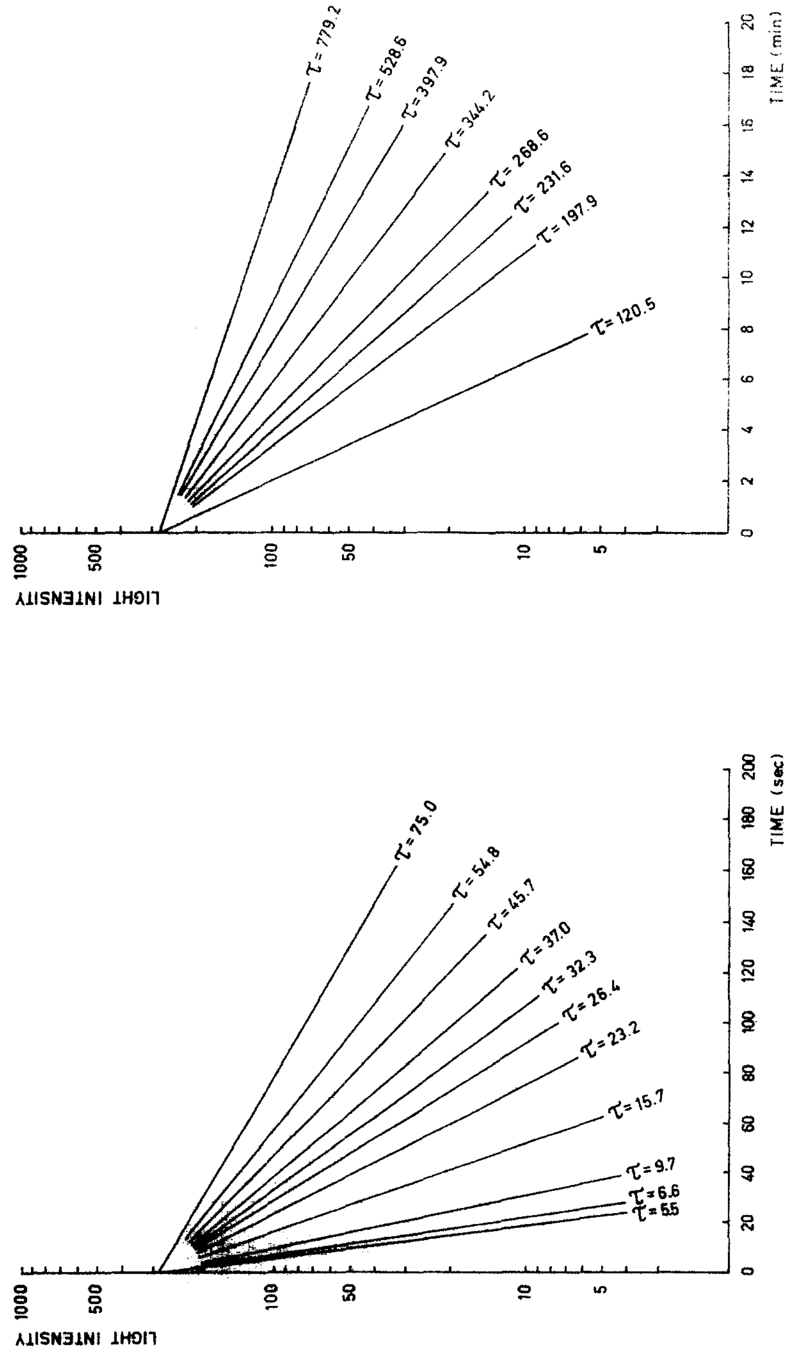


Fig. 16. *a*) and *b*). The applied continuous decreases in light intensity. The values of the time constant τ are inserted.

Differentiation of this equation furnishes the stimulus as was defined in part 5*a*.

$$\frac{dI}{dt} = -\frac{1}{\tau} I$$

and

$$\frac{1}{I}\frac{dI}{dt} = -\frac{1}{\tau} \quad \ldots\ldots\ldots\ldots \quad (2)$$

These kinds of decreases in light intensity simplify the situation because they produce stimuli of constant magnitude.

The experiments were carried out with the apparatus described in chapter 4. The extinction coefficient of the water in the experimental tube was $K = 0.037/\text{cm}$.

b. THE STRENGTH-DURATION CURVE

This term has been borrowed from the physiology of muscle and nerve (see for instance BOURNE, 1960). There the strength-duration curve results from the fact, that the threshold voltage of an electric stimulus depends on the duration of the current flow. An analogous situation exists when a continuous decrease in light intensity is applied to a daphnid. To put it the other way round, here *the minimal stimulus duration that evokes a swimming reaction is the shorter the higher the stimulus i.e. the rate of the relative decrease in light intensity.* Fig. 17 curve A illustrates this fact.

This type of curve can be approximated mathematically by assuming that a certain excitatory state (Y) is built up at a rate proportional to the stimulus strength (S). The excitatory state, however, disintegrates at a rate proportional to its momentary existing quantity. The net rate of accumulation is therefore:

$$\frac{\Delta Y}{\Delta t} = cS - c'Y \quad \ldots\ldots\ldots\ldots \quad (1)$$

The integral of this equation is:

$$Y = \frac{cS}{c'}\left(1 - e^{-c't}\right) \quad \ldots\ldots\ldots\ldots \quad (2)$$

When $t = \sim$, the value of $e^{-c't}$ is negligible:

$$Y_o = \frac{cS}{c'} \quad \ldots\ldots\ldots\ldots \quad (3)$$

$$S = \frac{c'Y_o}{c} \quad \ldots\ldots\ldots\ldots \quad (4)$$

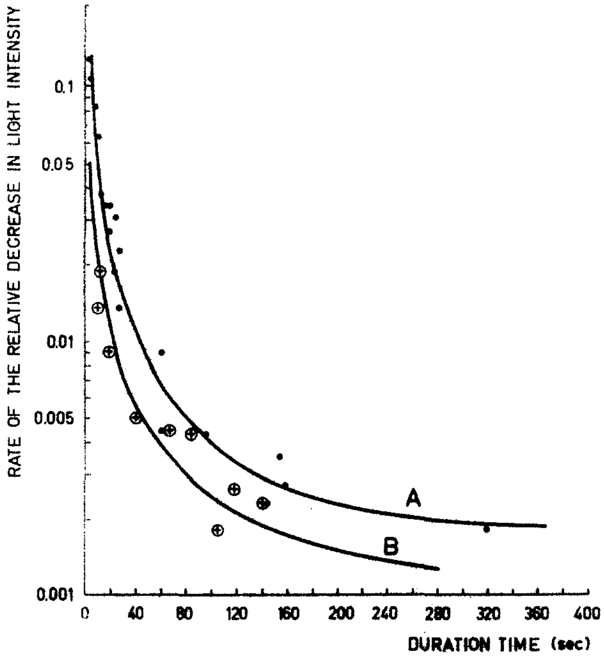


Fig. 17. The strength-duration curve for the first positively phototactic reaction (curve A, dots) of *Daphnia magna* and for the second positively phototactic reaction (curve B, crosses within circles). Each point or cross is the mean value of five to ten observations.

It follows from equation (4) that the minimal value of the stimulus evoking a reaction must have at least the value $\frac{c'Y_o}{c}$. In muscle physiology this minimal value is called the rheobase (R). Substituting R for $\frac{c'Y_o}{c}$ in equation (2), we arrive at the equations (5), (6), (7) and (8):

$$R = S(1 - e^{-c't}) \quad \dots\dots\dots\dots \quad (5)$$

$$\frac{R}{S} = 1 - e^{-c't} \quad \dots\dots\dots\dots \quad (6)$$

$$\frac{S}{S - R} = e^{c't} \quad \dots\dots\dots\dots \quad (7)$$

$$\log \frac{S}{S - R} \cdot \log^{-1} e = c't \quad \dots\dots\dots \quad (8)$$

Equation (6) represents the strength-duration curve in muscle physiology.

Substitution of the data of fig. 17 (curve A) in equation (8) ($R = 0.0017$/sec) produces a straight line (fig. 18). As (8) is derived from (6), this linear relation indicates that equation (6) can be applied to the phototactical reaction of *Daphnia magna*. This is not surprising, for the theory at the base of it is not only typical for a muscle, but valid for excitable systems in general.

Rates of the relative decrease in light intensity smaller than 0.0017/sec probably never succeed in evoking a swimming reaction. As was already observed by CLARKE (1930) the illumination can be diminished so slowly in the laboratory experiment that no swimming occurs. In twenty experiments with a stimulus of 0.0013/sec no reactions were ob-

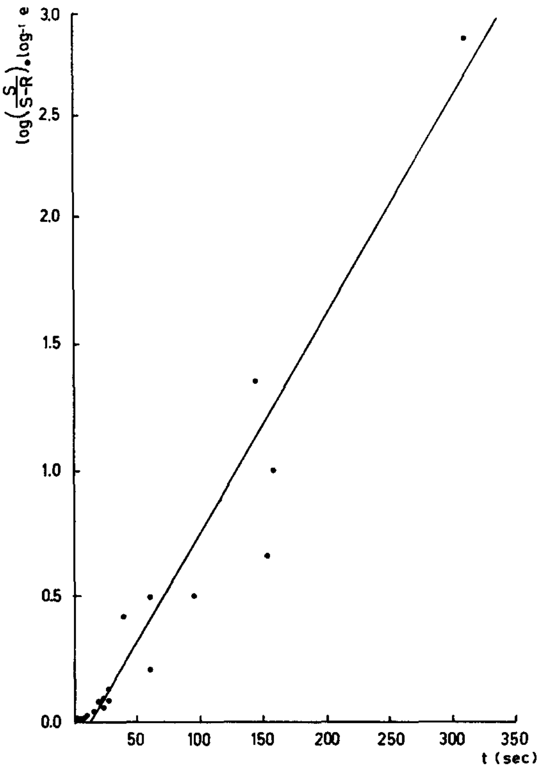


Fig. 18. The experimental data substituted in equation (8): $\log \frac{S}{S-R} \cdot \log^{-1} e = c't$. R was chosen 0.0017/sec (see text p. 353).

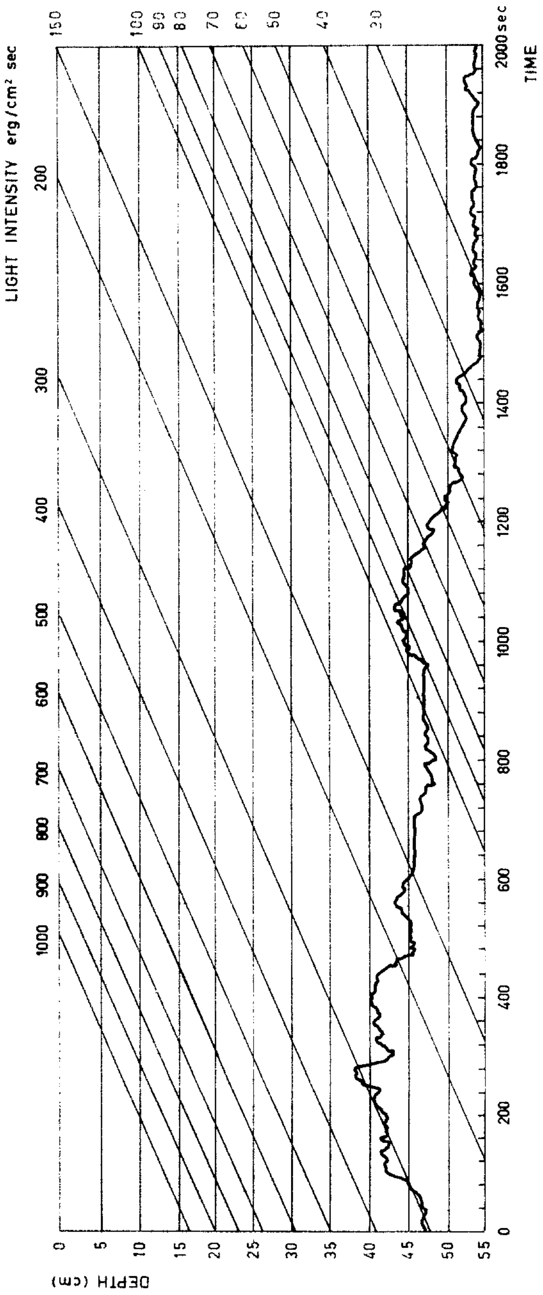


Fig. 19. The absence of a swimming response at a rate of the relative decrease in light intensity of 0.0013/sec. Lines of equal light intensity are inserted.

served within 2000 seconds. The absolute light intensity changed in these cases from 300 erg/cm^2 sec to 20 erg/cm^2 sec and less (fig. 19). In five out of eleven cases, even at a rate of the relative decrease of 0.0018/sec no reaction was observed.

The importance of the rheobase concept in vertical migration is obvious. Only decreases that exceed this value are apt to make the animals perform a positively phototactic swimming reaction. However, the complexity of the mechanism involved, prevents an allround detailed application at the moment. Complications appear as soon as a comparison is made between L_1 (the time between the beginning of the decrease in light intensity and the beginning of the first reaction), L_2 (the time between the end of the first reaction and the beginning of the second reaction), L_3, L_4 and L_5. Each following period becomes shorter as is shown in Table IV where the subsequent periods L_i are expressed as a fraction of L_1. The relative periods L_i are arranged in Table IV according to an increasing stimulus strength used in the experiments from which the durations were calculated. With increasing stimulus the relative duration time decreases for a particular period L_i. Of course, the strenght-duration curve changes also, as can be seen from fig. 17, where curve B is valid for the second phototactic reaction. Some facilitation process seems to be involved.

TABLE IV

The relative duration of the latent periods

	Relative Duration	*Mean*
First period L_1	1.00	1.00
Second period L_2	1.12–1.00–0.99–0.88–0.74–0.50–0.37–0.33–0.31–0.21	0.64
Third period L_3	0.76–0.67–0.58–0.53–0.45–0.25–0.13	0.47
Fourth period L_4	0.51–0.32–0.30–0.28–0.27–0.17	0.31
Fifth period L_5	0.29–0.28–0.25	0.27

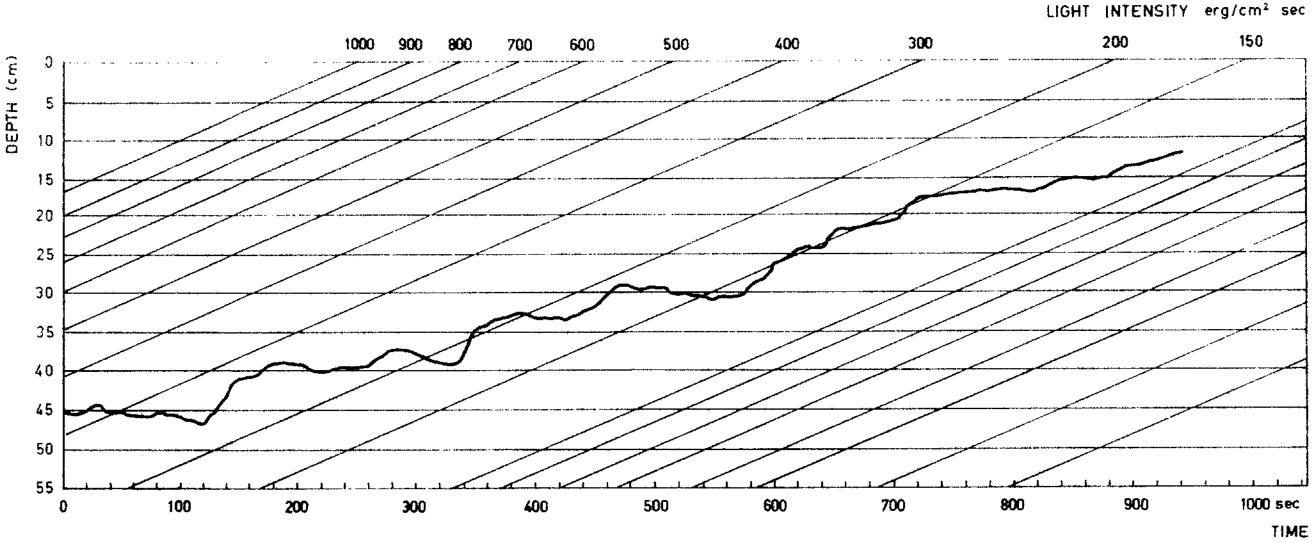


Fig. 20. The swimming response of *Daphnia magna* at a rate of the relative decrease in light intensity of 0.0025/sec. Lines of equal light intensity are inserted.

c. THE INTERMITTENT SWIMMING

The intermittent character of the swimming reaction in the case of a continuous decrease in light intensity was already mentioned in the preceding parts. Evidently, the upward swimming under conditions of continuous decreases in light intensity, consists of separate reactions of the kind performed when the change in illumination is instantaneous. Fig. 20 illustrates this behaviour. It is tempting, therefore, to suppose, that the continuous change in light intensity is processed discontinuously. This is consistent with the idea developed in the previous part (7*b*). After each reaction a new excitatory state is built up by the decrease in light intensity till the necessary quantity of excitation Y_0 is reached. With increasing stimulus intensity the periods between the separate reactions become shorter and shorter and eventually disappear. This occurs when the stimulus is about 0.020/sec. At a rate of the relative decrease in light intensity of 0.0187/sec, two out of five recordings were found with clearly observable steps in the ascending line. At the rate tried next, 0.0228/sec, and following rates, no intermittent swimming could be detected. This does, however, not mean, that the swimming is really continuous, but the periods of no upward swimming may have become too small to be noticeable in the recordings.

d. THE SWIMMING SPEED

The approximate swimming speed during the positively phototactic reaction calculated from the distance travelled and the duration of the reaction, thereby assuming that the swimming speed is uniform, appears to be constant up to a certain value of the stimulus (fig. 21). For relative decreases larger than 0.020/sec, the swimming speed increases.

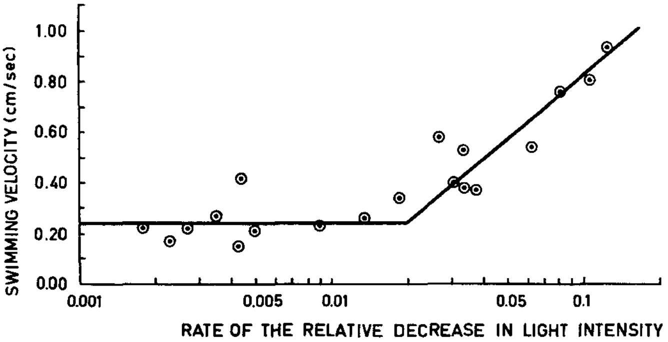


Fig. 21. The swimming velocity of *Daphnia magna* during the positively phototactic reaction. Each point represents the mean value of five to ten observations.

c. THE RESULT OF THE REACTION

As was done for an instantaneous decrease in illumination in part 5*f* a calculation was made of the light intensity reached by the animal at the end of the reaction, in order to test the hypothesis of "maintenance of a preferred light intensity" by the animal.

When after an instantaneous decrease in light intensity a daphnid swims in the direction of the overhead light the swimming animal has to do with an increase in light intensity. This need not necessarily be the case when the intensity of the overhead source decreases continuously. Whether the light intensity perceived by the swimming animal increases, remains constant or decreases, depends on (*a*) the rate of the decrease in the overhead light intensity, (*b*) the swimming speed of the animal and (*c*) the turbidity of the water. Since the turbidity of the water was held constant in these experiments the only factors to be considered are (*a*) and (*b*).[1]

a) The light intensity decreased according to an exponential function (see part 7*a*).

$$I_t = I_0 e^{-t/\tau} \quad \ldots\ldots\ldots\ldots \quad (1)$$

b) The change in light intensity as a result of an upward displacement z of the animal, relative to its original position, is also an exponential function (see part 5*h*).

$$I_z = I_0 e^{Kz} \quad \ldots\ldots\ldots\ldots \quad (2)$$

With regard to the change in light intensity the animal perceives it is clear that equation (1) holds when the animal remains at a certain depth, but the overhead light intensity decreases, while equation (2) holds when the animal swims upward, but the overhead light intensity remains constant.

Both functions have to be considered in the calculation of the change in light intensity encountered by an upward swimming animal during the decrease in light intensity of the overhead light source:

$$I = A e^{Kz - t/\tau} \quad \ldots\ldots\ldots\ldots \quad (3)$$

in which A is a constant.

---

[1] The author expresses his sincere thanks to Ir. A. SCHUYF, University of Utrecht, for his assistance with the mathematical part of this chapter.

$$\mathrm{dI} = \left.\frac{\mathrm{dI}_z}{\mathrm{d}z}\right|_t \mathrm{d}z + \left.\frac{\mathrm{dI}_t}{\mathrm{d}t}\right|_z \mathrm{d}t$$

$$\frac{\mathrm{dI}}{\mathrm{d}t} = \left(\frac{\mathrm{dI}_z}{\mathrm{d}z}\right)_t \frac{\mathrm{d}z}{\mathrm{d}t} + \left(\frac{\mathrm{dI}_t}{\mathrm{d}t}\right)_z =$$

$$= \left(\frac{\mathrm{dI}_z}{\mathrm{d}z}\right)_t v + \left(\frac{\mathrm{dI}_t}{\mathrm{d}t}\right)_z \quad \ldots\ldots\ldots\ldots \quad (4)$$

$v = \frac{\mathrm{d}z}{\mathrm{d}t}$ or the swimming velocity of the *Daphnia*. When (3) is substituted in (4):

$$\frac{\mathrm{dI}}{\mathrm{d}t} = \mathrm{AK}ve^{(\mathrm{K}z-t/\tau)} - \frac{\mathrm{A}}{\tau}e^{(\mathrm{K}z-t/\tau)}$$

$$\frac{\mathrm{dI}}{\mathrm{d}t} = \mathrm{A}\left[\left(\mathrm{K}v - \frac{1}{\tau}\right) e^{(\mathrm{K}z-t/\tau)}\right] \quad \ldots\ldots\ldots \quad (5)$$

When the displacement of the reacting animal results in a maintenance of a certain perceived light intensity, $\frac{\mathrm{dI}}{\mathrm{d}t} = 0$. Equation (5) is zero when $\left(\mathrm{K}v - \frac{1}{\tau}\right) = 0$. The speed of displacement is then

$$\mathrm{v} = \frac{1}{\mathrm{K}\tau} \quad \ldots\ldots\ldots\ldots\ldots \quad (6)$$

Since $\mathrm{K} = 0.037/\mathrm{cm}$ (see p. 351), the relation between the rate of the relative decrease in light intensity and the speed of displacement necessary to maintain the initial light intensity, can now be determined. The straight line A in fig. 22 represents this relation. Since swimming in response to stimulus values up to 0.020/sec is interrupted by periods of no upward movement the mean speed of displacement for these stimulus values had to be determined indirectly by fitting a straight line, as well as possible, through the original recordings, and by regarding this line as the graph of the displacement of the animal. For values of the stimulus above 0.020/sec the actual swimming velocity, as recorded, was used (see fig. 21). In fig. 22 the two lines B are fitted through the points that represent the experimental speeds of displacement. It is remarkable that for a stimulus of $\frac{1}{\mathrm{I}}\frac{\mathrm{dI}}{\mathrm{d}t} = 0.0056/\mathrm{sec}$ a speed of displacement was

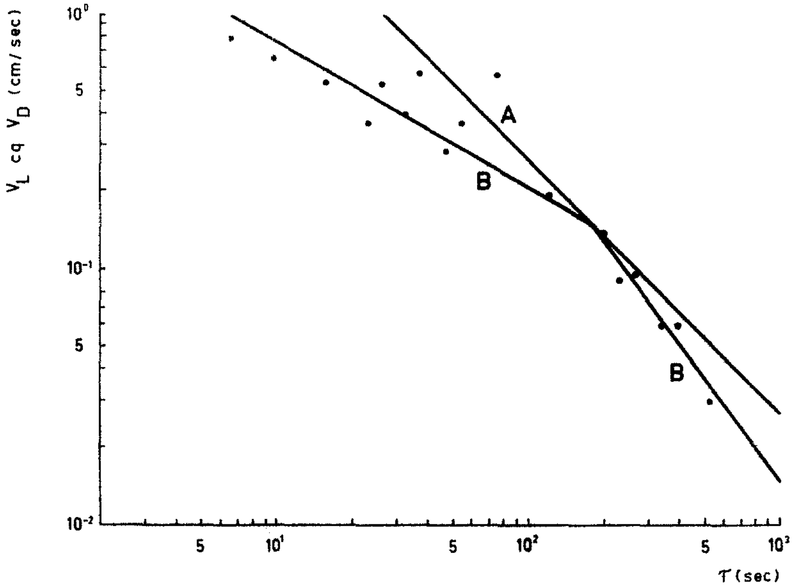


Fig. 22. A comparison between the velocity of the upward displacement of *Daphnia magna* (V_D, the dots and the line B through them) and the velocity with which a certain light intensity moves upward (V_L, line A). Both are a function of the rate of the relative decrease in light intensity.

found which is not in disagreement with the preferendum concept. In response to this stimulus value *Daphnia magna* swims at a speed such that it remains, on the whole, in the same light intensity. For all other values of the stimulus the animals gradually find themselves in lower light intensities, because everywhere the speed of displacement is smaller than the ideal ones.

A more direct demonstration of the result of the reaction is found when at a given stimulus the velocity (V_L) of the "movement" of a certain absolute light intensity in the direction of the surface is compared with the velocity (V_D) of the displacement of the animal. For every experimental stimulus lines of equal intensity were drawn on translucent paper as a function of time and depth. The same scales were used as in the original kymograph recordings of the reaction. The swimming was traced and compared with the lines of equal intensity (figs. 19, 20, 23).

The relative difference $V_L - V_D/V_L$ was calculated and is represented in fig. 24 as a function of the stimulus. Again this difference is zero for a value of about $\frac{1}{I}\frac{dI}{dt} = 0.005/\text{sec}$ which means that the displace-

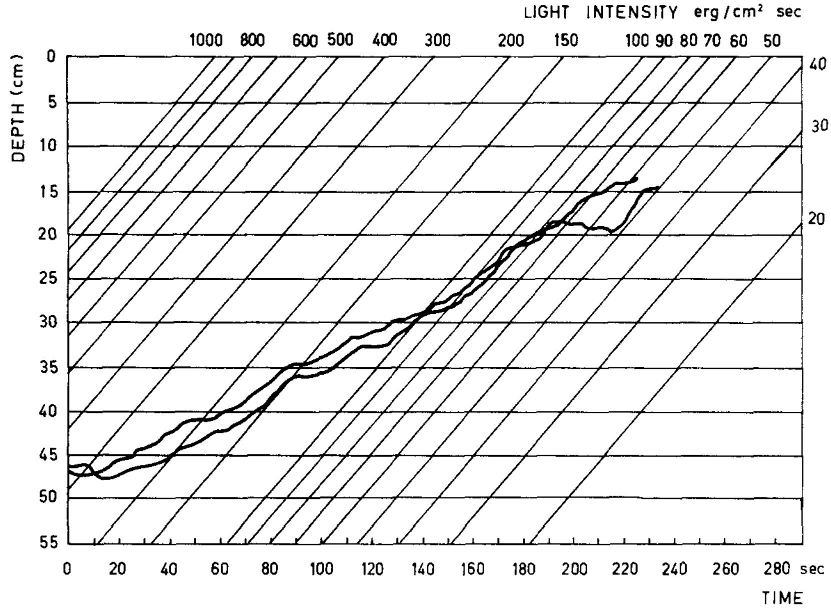


Fig. 23. Two swimming responses of *Daphnia magna* at a rate of the relative decrease in light intensity of 0.0268/sec. Lines of equal light intensity are inserted.

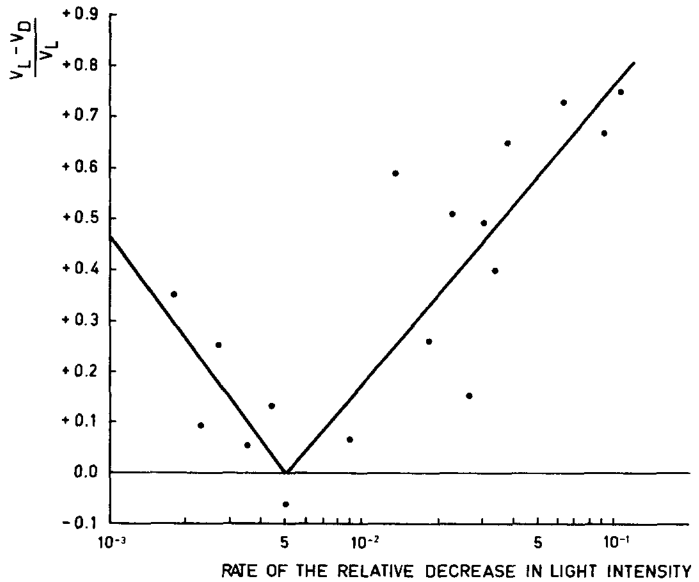


Fig. 24. The relative discrepancy between the velocity of the upward displacement of *Daphnia magna* (V_D) and the velocity of the upward displacement of a certain light intensity (V_L) as a function of the rate of the relative decrease in light intensity. Each point represents the mean value of five to ten observations. The lines are fitted by eye.

ment of the animal coincides with the "displacement" of a certain light intensity. For all other values of the relative decrease in light intensity the animals lag behind and are found in ever lower intensities.

8. DISCUSSION OF THE EXPERIMENTS

Before several characteristic features of the positively phototactic reaction can be interpreted, the underlying theory must be further elaborated. For this purpose the strength-duration characteristic (part 7*b*) is taken as the starting point.

Equation (6) (part 7*b*) was derived to describe the strength-duration relation of the stimulus. The underlying assumption for this equation was the presence of a certain state of excitation in the animal caused by the decrease in light intensity. The excitatory state is built up in proportion to the stimulus strength, and it is supposed to leak away at a rate proportional to its momentary existing amount. A certain critical level of excitation is required in order to cause a positively phototactic reaction. This level is indicated bij Y_o. Assuming that for the negatively phototactic response the same laws exist—the steering component would be the only difference between the positive and the negative reaction—it is necessary to posit two different excitatory states: one for the positive response and the other for the negative response. The corresponding threshold levels are denoted by Y_o^P and Y_o^N. The information that is received from the sense organ, and which builds up the excitatory state is called P-information and N-information, respectively. Both are assumed to be processed in the nervous system in such a way that the dominating one leads to the establishment of the corresponding excitatory state (see fig. 10).

Another attempt to interpret the phototactic response of *Daphnia magna* has been made by CLARKE (1932). This author used HECHT's theory of the nature of photoreceptor processes (HECHT, 1934). A photochemical equilibrium is disturbed by changes in light intensity, and a "stimulus substance" is formed as a result of this disturbance. As long as the equilibrium is not re-established *Daphnia* performs a phototactic reaction.

Several general features of the reaction can be explained as satisfactorily with this photochemical theory as with the theory proposed in this paper. The objection to a photochemical explanation is, however, that the complexity of a behaviour pattern is entirely transposed to the events in a sense organ. The theory proposed in this paper must therefore not be thought to replace the photochemical one, but should be regarded as a theory valid for a higher level of integration, and which makes use of a terminology suitable for this level. As VIAUD (1938) and HARRIS and WOLFE (1956) started their explanation of complex behavioural phenomena of *Daphnia magna* also from HECHT's theory the same objection may be applied to the explanations of these authors.

With this hypothesis, partly based on a mathematical description of experimental data (equation 6, part 7*b*), partly based on an analogous reasoning (the description of the negatively phototactic reaction in terms of the positively phototactic reaction), and partly based on assumptions (the processing in the nervous system), an attempt will be made to interpret the various aspects of the phototactic reaction.

When an instantaneous decrease in light intensity occurs, a certain amount of P-information is transmitted which is proportional to the stimulus. The corresponding positive excitatory state is built up and when its quantity reaches Y_o^P a positive reaction is initiated. Meanwhile two processes are started: (*a*) the positive excitatory state leaks away with time, (*b*) N-information is formed as a result of the increase in light intensity registered by the photoreceptors during the upward swimming. The effect of both processes is that after some time the positive excitatory state falls below a critical level Y_1^P (probably $Y_1^P < Y_o^P$) and the positively phototactic reaction stops.

When the increase in light intensity is absent during the upward swimming, as was the case with the descending tube experiment (part 5*g*), no N-information is produced. The result must be a lengthening of the reaction time. This was actually observed. The effect of the turbidity of the water also becomes obvious. The light gradient in the water, resulting from its turbidity, determines the increase in light intensity perceived by the upward swimming animal and consequently the formation of the negative excitatory state. In turbid water the reaction time and the swimming distance must be shorter than in clear water, provided that the turbidity does not influence the swimming velocity. These theoretical conclusions were confirmed by the experiments (part 5*h*).

A high swimming velocity seems to be correlated with a short duration of the reaction (part 5*e*). The reason could be that the rate of the perceived increase in light intensity will be higher in a fast swimming animal than in a slow swimming animal, with the result that the negative excitatory state is built up at a higher rate. With a larger instantaneous decrease in light intensity a higher swimming velocity is obtained, but the duration of the reaction is not changed by the decrease as such (part 5*e*, *d*). A larger decrease in light intensity must result in a higher level of the positive excitatory state. That nevertheless the duration of the reaction is not prolonged could be due to the higher rate with which the negative excitatory state is formed in the faster swimming animal.

The experiments described in part 5*g* were designed to decide whether a feed-back is involved in the positively phototactic reaction or not. The unsuitability of a visual system to evaluate an absolute light intensity makes it unlikely that a norm (an absolute light intensity, for instance, the light intensity of adaptation) is made use of. Changes in light intensity, however, are adequate stimuli for a visual system. In the information flow chart (fig. 10) the reaction system is not triggered by a deviation from a norm, but by a change in light intensity. Nevertheless, it was concluded from the experiments (part 5*g*) that a kind of feed-back is present. The increase or decrease in light intensity which is encountered by a reacting animal is thought to represent this feed-back. As can be seen in fig. 10 the perception of a decrease in light intensity and the flow of the information derived from this kind of stimulus on the one hand and the perception of an increase in light intensity and the information derived from that kind of stimulus on the other hand form together a symmetrical system. When the animal reacts to an instantaneous decrease in light intensity by swimming upward the encountered increase in light intensity furnishes the feed-back, and when the animal reacts to an instantaneous increase in light intensity by swimming downward the encountered decrease in light intensity furnishes the feed-back.

In part 5*f* it was described that only for threshold values of the stimulus do the animals ultimately reach the initial light intensity. It is not surprising that the reaction is attuned to this stimulus value only, for it is the only one to occur in nature. With regard to the reaction of the animals, instantaneous decreases in illumination and continuous decreases are essentially the same, as was demonstrated in chapter 6 and 7*c*. In nature changes in illumination are always of the continuous type and it is clear that the animals react to them as soon as the threshold value is reached (part 7*b*). The influence of the turbidity comes into action when the swimming response is initiated. The duration of the response is partly governed by it, and so is the light intensity that is reached. Since the effect of threshold stimuli was only investigated for one value of the turbidity, it is mere speculation to assume that the reaction is adequate (with regard to staying in a certain light intensity) for all turbidities. This should be verified by further experiments. The experiments described in part 5*h* show that also for other values of the stimulus an adequate reaction is to be expected for a given turbidity. The exact relation between stimulus and turbidity can be expressed by mathematical formulae derived from the kind mentioned in part 7*b*, but before this is possible more experiments of the same kind are needed to determine the constants and to make clear that an increase in light intensity is processed by the animal in the same way as a decrease is. However, exact knowledge of the relation between the threshold stimulus and the turbidity is not essential for the estimation of the effect of the reaction, for when the decrease is continuous other things upset the result. For instance, the departure from the initial adaptation intensity during the time the excitatory state is built up to the critical level, is not taken into account. This can be seen in the fig. 20. Each separate swimming ends at the light intensity at which it started, but the overall result is that after some swimming periods the animal is found at a lower light intensity than the initial one. This effect becomes less apparent with increasing rate of the relative decrease in light intensity, and at a certain value (in the experiments about 0.0056/sec) the animals swim as fast as the adaptation intensity "moves" towards the surface. The rate of the relative decrease to which this case applies is smaller than that for which the time between two separate responses is zero (that is for which the swimming is continuous). At least for the turbidity used ($K = 0.037/\text{cm}$); but it is quite probable that for different turbidities the reaction is adequate at other rates of the relative decrease. When the rate of the relative decrease is 0.02/sec, the swimming reaction is continuous. The critical excitatory state is supposed to be built up at the moment the previous response terminates. With still increasing rate of the change in illumination a surplus amount Y^P is superimposed. This results in an increase of the swimming speed. Nevertheless, this increase is not large enough to prevent the animal from lagging behind the initial light intensity.

There are indications that the second and following responses to a continuous decrease in illumination are initiated after a shorter time than the first one (part 7*b*, Table IV). This may be so for two reasons. In the first place because of the difference in the starting level of the positive excitatory state Y^P. The first reaction is initiated when Y^P is built up from zero to the critical value $Y_o{}^P$. Assuming that the response terminates at a lower excitatory state than it is initiated, the first reaction stops at a value of $Y_1{}^P$ somewhere between zero and $Y_o{}^P$. So the decrease in light intensity can rebuild the critical value in a shorter time.

The second reason might be some kind of facilitation somewhere in the nervous system, the nature of which must remain obscure as long as we are still without sufficient data.

Yet another phenomenon has to be mentioned in this respect. It is known that threshold values for relative changes in light intensity initiating some behaviour pattern differ for various initial light intensities (HECHT, 1919 for *Mya*; VON BUDDENBROCK, 1931 for *Balanus*; FÖH, 1932 for *Helix*). HEBERDEY and KUPKA (1942) found for *Daphnia pulex*, that the threshold values of the relative differences, needed to discriminate between two intensities, depend on the absolute intensity. Their results are, however, not directly comparable with those of the above mentioned authors, because in the experiments of HEBERDEY and KUPKA the daphnids had to choose between two intensities simultaneously present, and not to react to successive light intensities. However, when the same holds for *Daphnia magna*, which has been demonstrated in several other animals, the threshold for the positively phototactic reaction depends on the absolute intensity. Some authors, reporting on vertical migration, suppose that this is true only for instantaneous changes (CUSHING, 1951; SIEBECK, 1960). This is not the opinion of the present author. It has been demonstrated in this paper that the responses are essentially the same for both instantaneous and continuous decreases in illumination. Moreover, one mechanism can explain the effects of both kinds of stimulations. In fact, the separation into continuous and discontinuous changes in light intensity is arbitrary and when the threshold value for instantaneous decreases depends on the absolute intensity, there is no reason to suppose that the threshold value is constant for continuous decreases in light intensity.

9. THE APPLICABILITY OF THE EXPERIMENTAL RESULTS TO VERTICAL MIGRATION AS IT OCCURS IN NATURE

a. VERTICAL MIGRATION OF *Daphnia magna*

The shallow confines of the habitat of this species make the occurrence of a simple migration pattern rare. Variations in light intensity during the day caused, for instance, by incidental clouds may produce stimuli inducing the animals to swim either upward or downward. Although these stimuli are mostly of short duration they nevertheless bring the animals from the bottom to the surface or *vice versa* because of the shallowness of the water. Such small movements are hardly detectable in deep lakes or the oceans by means of the crude sampling methods applied there. But in the shallow habitat of *Daphnia magna* these movements will disturb or even prevent the observation of the actual upward afternoon migration.

Nevertheless, on 23 September 1960, a windless and nearly cloudless day, such an observation was made in the canal where the daphnids, used in the laboratory experiments, had been collected. With the help of a simple device, which needs no detailed description here, a vertical bar was towed through the water from one vertical brick bank to the other and *vice versa* at short time intervals. The depth at the experimental place was 1.20 m, the towing traject about 7 m. Five small nets (diameter 10 cm) of copper gauze were fastened to the bar at a mutual distance of 10 cm (the distance between first and second net was 5 cm), the first net sampling the upper 10 cm of the water. Obstructions, such as branches of trees, prevented sampling within the first 35 cm above the bottom. Each net terminated in a perspex tube which was closed at

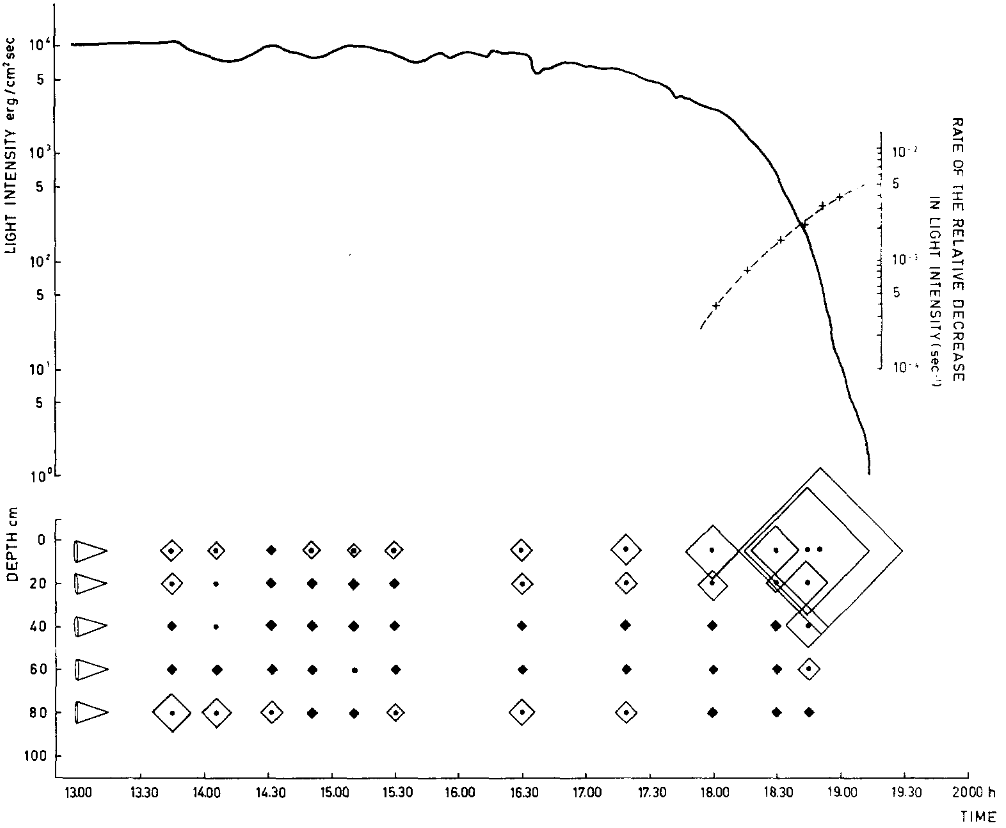


Fig. 25. A vertical migration of *Daphnia magna* in nature. The surface of the squares represents the number of animals caught at a certain depth (ordinate in the lower left hand corner) at different times of the day (abscissa). A black square indicates that fewer than ten animals were caught, a dot indicates that no animals were caught. The light intensity just below the surface (ordinate in the upper left hand corner) and the stimulus value (ordinate in the upper right hand corner) as a function of the time is also indicated.

the end with a piece of the same copper gauze. The daphnids collected by each net were counted immediately. The light intensity was continuously measured with a barrier layer cell, submerged to a depth of 10 cm and placed in the middle of the canal close to the pathway of the bar.

Sampling and light measuring started at about 13.00 h. The light intensity, apart from small decreases caused by some clouds, was constant till 17.00 h (see fig. 25). Only small numbers of animals were caught during this time and the population remained close to the bottom. This was found out by separate probing. After 17.00 h the light intensity decreased gradually, but hardly any increase in animal numbers was recorded till 18.30 h. With the next haul, fifteen minutes later, a large number of daphnids were caught, especially in the surface net. Since then, the smooth surface of the water was rippled by the numerous Cladocera. The data show that between 18.30 h and 18.45 h the animals moved towards the surface with a velocity of at least 0.1 cm/sec, which is not fast compared with the velocities obtained in the experiments (see fig. 21).

The rate of the decrease in light intensity $\frac{1}{I}\frac{dI}{dt}$, which was proposed in the experimental part of this paper as the stimulus for the positively phototactic reaction, was calculated for the time between 18.00 h and 19.00 h. This was done by considering the absolute decrease in light intensity to be exponential for short time intervals (10 minutes, decreasing to 1 minute with increasing change in illumination). The result of this is represented by the line (–x–x–x–) in fig. 25. From this figure we may notice that between 18.30 h and 18.45 h the stimulus varied from 0.0013 – 0.0024/sec. The rheobase value obtained from the laboratory experiments and estimated at 0.0017/sec for the first phototactic reaction (fig. 17) is of the same order. This supports the idea that the positively phototactic reaction studied in the laboratory is at least closely related to the natural phenomenon.

b. THE MIGRATION OF THREE SCATTERING LAYERS

Observations on vertical migrations with an echo sounder provide continuous records of the movements of animals. This method enhances the accuracy of speed calculations. From continuous measurements of the light intensity at the surface or at a certain depth the stimulus can be calculated. Light measurements within the scattering layer provide the result of vertical migration with regard to absolute light intensities.

In the paper of CLARKE and BACKUS (1956) these three important measurements are found, whereas in that of KAMPA and BODEN (1954) measurements of the decrease in light intensity are absent, so no stimulus value can be calculated. Nevertheless, an attempt was made to correlate the speed of the displacement of the top of the scattering layer, as recorded by KAMPA and BODEN, with the stimulus values calculated from the light measurements of CLARKE and BACKUS. A time correction was included, with the time of sunset as the reference point. This manipulation is not altogether correct, for KAMPA and BODEN made their observations on 30 June and about 10° south of the measurements of CLARKE and BACKUS, who did their observations on 20 and 21 July. The differences in latitude and time of year entail that for the migration observed by KAMPA and BODEN the rate of the relative decrease in light intensity was somewhat higher at a certain time from sunset.

Already a superficial comparison of the movements of the three scattering layers (see the original papers) discloses a similarity. At first a slow upward rise takes place which is suddenly accelerated some 10 minutes before sunset, then a slowing down is observed at about half an hour after sunset. This similarity suggests that (*a*) the three migrations are governed by the same factor, and (*b*) that this factor has the same magnitude at a comparable time.

Therefore a correlation was made between the stimulus, $\frac{1}{I}\frac{dI}{dt}$, and the velocity of the displacement of these scattering layers. The stimulus values were calculated from light measurements represented in fig. 1 of the paper of CLARKE and BACKUS. For this purpose the same procedure was adopted as in the case of the migration of *Daphnia magna* (see chapter 9*a*): the stimulus was calculated from τ values found by assuming the decrease in light intensity exponential for short time intervals (15 minutes) between 17.00 h and 19.00 h. For the scattering layer of KAMPA and BODEN this is between 18.16 h and 20.31 h. The velocity of the displacement was calculated for the deepest scattering layers on 20 and 21 July (CLARKE and BACKUS) and for the scattering layer of KAMPA and BODEN. Of course; the published data permitted only rough estimations. Nevertheless, the results seem to be the same for these three scattering layers (fig. 26). The relation between stimulus and displacement is simple and comparable to that obtained in my experiments with *Daphnia magna* (see fig. 21). Until the stimulus has reached a value of 0.001/sec, the velocity of the displacement increases slowly. When this value is exceeded a sudden acceleration of the velocity of the migration occurs. The time of this acceleration is the above mentioned time of about 10 minutes before sunset.

In the case of *Daphnia magna* the sudden increase in swimming veloci-

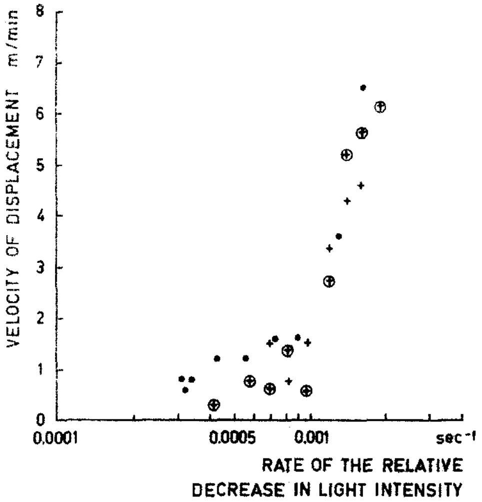


Fig. 26. The velocity of the displacement of three scattering layers as a function of the rate of the relative decrease in light intensity. The top of the scattering layer observed by Kampa and Boden (1954) is indicated by a dot; the lowest scattering layer observed by Clarke and Backus (1956) on 20 July 1955 and 21 July 1955 is indicated by a cross and a circled cross respectively.

ty was interpreted as the result of a superposition effect of the stimulus (chapter 8). Whether the same effects hold for these scattering layers is, of course, not known. But the form of the correlation (compare fig. 26 with fig. 21) suggests that the stimulus $\frac{1}{I}\frac{dI}{dt}$ is also valid for these kinds of vertical migrations. If this is true it is clear that for these animals the threshold value is less by about a factor ten.

c. DISCUSSION

The results thus far obtained seem to point to a promising application of the phototactic swimming response to diurnal vertical migration. It must be stressed, however, that the experiments have been performed with *Daphnia magna* and that the kinetical mechanism might be different in other animals. Especially so, since *Daphnia magna* is not a truly planktonic animal. Experiments with other animals may disclose whether the stimulus, the rate of the relative decrease in light intensity, $\frac{1}{I}\frac{dI}{dt}$, is of general value. Species, such as *Daphnia longispina* and *Calanus finmarchicus*, concerning which an extensive literature exists, both with regard to field observations on diurnal vertical migration and with regard to the general biology, are excellently suited for such a study.

The experiments in this paper were limited to decreases in light intensity. Therefore the applicability of the results is restricted to the upward movement of the animals in the afternoon when the light intensity is decreasing. But even then the positively phototactic response need not be their only reaction. It seems that a slow upward drift can sometimes be observed long before the stimulus of the reaction described here is of a supra-threshold nature. For instance, the scattering layer observed by CLARKE and BACKUS (1956) on 20 July 1955 shifted between 14.00 h and 14.30 h from a depth of 1250 ft to a depth of 1180 ft and between 15.00 h and 18.00 h from a depth of 1180 ft to a depth of 1020 ft.

Other factors may modify the reaction provoked by the light stimulus. The effect of the turbidity of the water has already been described. When the mechanism of the negatively phototactic reaction is similar to the mechanism of the positively phototactic reaction, it is obvious that after an increase in light intensity the reaction time is longer and consequently the swimming distance is larger in water with a low extinction coefficient than in water with a high extinction coefficient. This fact might be the reason why the depth to which the animals migrate in the morning is inversely related to the extinction coefficient (SCHRÖDER, 1959; SIEBECK, 1960).

The effect of the temperature is probably shown by the observation that a thermocline sometimes stops the vertical migration (CUSHING, 1951). The way in which the temperature can act on the animal is reported by SCHRÖDER (1962, and personal communication). This author used an echo sounder to study the vertical displacement of zooplankton in the Bodensee. *Daphnia*, *Cyclops*, *Bosmina* and *Diaptomus* were seen to accumulate in restricted water layers of steep temperature gradients. Irregular upward and downward displacements for several meters by vertical currents did not result in corrections for depth. Such corrections were, however, made by animals concentrated in layers outside the micro-thermoclines. Apparently such micro-thermoclines are physiological traps. The temperature barrier might be overcome when other stimuli become dominant. SCHRÖDER supposed that the animals reacted to the stronger light stimulus (rate of the relative increase?), and so the observation that the animals left the micro-thermocline during a particular rapid upheaval is explained.

Whereas other factors might be of influence on the pattern, the migration itself is caused by changes of the illumination, as is now generally accepted. We are of the opinion that the changes in light intensity are the direct cause for the swimming response and not the indirect cause.

A vertical migration resulting from the positively phototactic swimming response described in this paper may be pictured as follows. Relative decreases in light intensity of sufficient rapidity and of sufficient duration evoke an upward swimming during a certain short time. As long as the light intensity is decreasing at a sufficient rate new stimuli are furnished over and over again, which cause renewed reactions. When the decrease in light intensity is slow in the afternoon the upward swimming is interrupted by periods of no swimming. These periods become shorter and shorter with increasing change in illumination when time progresses. The swimming speed is constant during the periods of upward movement. However, at a certain value of the stimulus the swimming speed starts to increase. Such a detailed picture of the upward movement can not be discovered with the common sampling techniques. What may be found is for some time a slow drift to the surface, with slightly increasing speed, and then a rather sudden acceleration of the displacement.

The effect of the reaction—whether the animals lag behind, remain in, or swim faster than a certain light intensity, "moving" in the direction of the surface—depends on internal as well as external factors. Internal factors are, for instance, the constants c and c' in equation (1) part 7*b* and the proportionality factor of the relation between stimulus and swimming velocity. External factors seem to be the turbidity and the value of the stimulus itself. Whether or not the animals remain in a certain light intensity is thought to be of no importance, as might be obvious from this picture of the upward migration. The experimental fact that under appropriate circumstances the reaction mechanism enables the animal to remain in the initial light intensity furnishes no physiological argument for the preferendum concept, because this fact is independent of the absolute light intensity. The reaction might be considered a homoiostatic correction, trying to restore the initial circumstance. Moreover, a complete correction seems not necessary, when taking into consideration the logarithmical range in illumination in which the planktonic animals seem to be able to live. Perhaps a complete correction is biologically not desirable. For many days in the temperate latitudes clouds and fog intermittently screen off part of the light of sun and sky. This will result in considerable changes in light intensity. When the animals made complete corrections a good deal of energy would be spent in swimming upwards and downwards. The "strenght-duration" value of these kinds of changes in illumination furnishes few supra-threshold stimuli and consequently upward or downward swimming is limited.

At the moment, the concept of a "preferendum" does not seem, to my mind, to improve the understanding of vertical migration. Its usage supposes that the animals are found more abundantly at a certain light intensity than at other light intensities. There are but few indications that this is actually the case. A preferendum is a fixed entity and as such could only survive in the literature, dealing with field observations of vertical migration, as long as accurate determinations of the light intensity at which the animals are found were absent. Experimental evidence stressed the capacity of the animals to adapt to different light intensities and consequently the absolute preferendum was replaced by a relative preferendum. What is left seems to be merely a momentary preferendum or the light intensity to which the animal is adapted at a certain time. Little remains of the original supposition that the animals have to be found more abundantly at a certain fixed light intensity than at other light intensities. This relative preferendum is the same as the steady state which the animals "try" to preserve by performing a homoiostatic correction as has been evolved in this paper.

PART B

THE STEERING MECHANISM

10. INTRODUCTION

In chapter 3 the positively phototactic swimming reaction of *Daphnia magna* was divided into a kinetical component and a steering component. We assume that the compound eye of the daphnid is the receptor used in the steering component. This is not as self-evident as it might seem, because some authors (Viaud, 1938; Rimet, 1960 *a*, *b*) regard the dermal light sense of daphnids of similar importance for the phototactic behaviour. However, the peculiar rotations of the compound eye are claimed by most authors to underly the steering of the body. When the eye is orientated it is thought that appropriate strokes of the antennae bring the body in the "wanted" position.

In the laboratory experiment there seems to be no difficulty in indicating the stimulus to which orientation takes place, because the animals are mostly illuminated by the direct light rays from a light source. In the aquatic environment, however, light was thought to be of a diffuse nature. Loeb, for instance, supposed therefore that for orientation vertical light rays were preferred by planktonic animals to those from other directions. There is no reason why this should be so.

The question arises what properties the environmental illumination must have to be considered a directing stimulus.

a) Evidence from electrophysiology suggests that the main responses of the optical system occur when changes in light intensity are applied (GRANIT, 1956; BARTLEY, 1959). To illumination of a constant intensity the eye adapts itself and no information is transmitted to the nervous system. For this reason spatial areas of uniform light intensity do not give information and must be rejected as stimulus for the steering component. However, when over a small angular distance large differences in light intensity occur an ideal visual image can be recognized by the animal, providing, as DITCHBURN (1963) stresses, that there are spontaneous movements of the eye. These movements are necessary, because when the visual image does not move relative to the ommatidia, these receptor systems adapt themselves, so that no signals are transmitted and the contrast cannot be detected. Tremor-like movements of the compound eye may easily be observed when a *Daphnia* is studied with a microscope, and it was HARRIS (1953) who pointed out the meaning of these movements in the visual mechanism. It might therefore be concluded that spatial differences in light intensity (contrasts and gradients) are recognizable by *Daphnia magna*.

b) For the orientation component of the phototactic reaction it is not sufficient that contrasts or gradients are present, these illumination patterns have to be beacons which are fixed with regard to the environment. It is argued in the following discussion that such beacons are present in the aquatic habitat.

Light rays from the sun and especially from the sky are incident on the water surface at different angles. These light rays, entering the water, are refracted in the direction of the vertical by the surface of the water. When the surface is supposed to be smooth—as it may be in a pond, the normal habitat of *Daphnia magna*—the maximal angle of light rays penetrating into the water is 49°. An organism under water receives, therefore, direct light from the sun and the sky only within an inverted cone of rays whose semi-vertical angle is 49°.

Such a cone of sub-surface illumination was proposed by HARRIS (1953) as a unique type of visual image to which *Daphnia magna* might be orientated.

However, various factors disturb this simple illumination pattern. These factors are:

1) The radiance incident on the water surface is not equally distributed in every direction.

2) Reflexion at the water surface increases with increasing angle of incidence.

3) More oblique rays in the cone are due to heavier absorption than less oblique rays.

4) Scattering at the bottom and surface or by particles in the water makes the radiance of a diffuse nature.

5) Waves disturb the flat reflexion surface so that light rays may penetrate into the water at angles exceeding 49°.

The actual angular light distribution (abbreviated as A.L.D.) under water has been measured by several investigators (WHITNEY, 1941; JERLOV and LILJEQUIST, 1938; SASAKI *et al.*, 1958*a*, *b*, 1960*a*, *b*, 1962). These measurements were mostly done in lakes (WHITNEY) or in the oceans, but not in the habitat of *Daphnia magna*. The conditions in the superficial strata may be of interest, however.

It was demonstrated that the three-dimensional A.L.D. in a certain point is more or less "egg-shaped" with the point of the egg directed downward, which means that the highest amount of light is coming from above and the smallest amount from beneath. With a bright sky and the sun at not too low an altitude the A.L.D. becomes very elongated in the direction of the sun. The influence of the sun diminishes with depth and the A.L.D. is tilted vertical. The various forms of the light vector diagrams—as the graphical representation of the A.L.D. measured in a particular plane is called—are of little consequence in this discussion. Mostly they have one property in common, *viz.* that there are large differences in light intensity between 30°–60° with the vertical. These angular changes in light intensity may be so large that relatively sharp contrasts are present, as might be deduced from data furnished by SASAKI *et al.* (1962) or WHITNEY (1940). The exact angular position of the contrasts varies with the altitude of the sun and the direction of the plane of measurement with respect to the sun. It is obvious that A.L.D.'s with such contrasts approach the theoretical cone of illumination that is deduced from refraction by the surface of the water. With increasing depth these contrasts fade away to intensity gradients (see fig. 8 and 9 in the paper of SASAKI *et al.*, 1962).

It is concluded that the directing stimulus must be looked for in the light gradients between 30° and 60° with the vertical. Introductory experiments were performed to study the swimming behaviour of *Daphnia magna* when confronted with different kinds of A.L.D.'s. In these experiments the angular intensity differences were of a gradual nature. It was thought, however, that sharp contrasts would guarantee more precise orientations than gradients, since they have the advantage that the position of the *Daphnia* or its eye can be exactly determined in relation to the contrast edges. For these reasons we used the sharp contrasts brought about by a black screen cutting off the light from a circular fluorescent tube, though such sharp contrasts may be somewhat artificial.

II. THE MAINTENANCE OF THE NORMAL BODY POSITION IN *DAPHNIA MAGNA*

There seems to be some confusion with regard to the position *Daphnia* normally occupies while swimming. From the remarks of RADL (1901) it can be concluded that this author is of opinion that the body axis makes an angle with the vertical. This angle has been thought to be about 20° for *Daphnia pulex* (EWALD, 1910), 20°–30° (FRAENKEL and GUNN, 1961), and 20°–25° for *Daphnia longispina* (SCHRÖDER, 1959). This last mentioned author added that the body axis coincides with the vertical when the animal increases the swimming speed. HARRIS (1953) and HARRIS and WOLFE (1956), however, stated that *Daphnia magna* swims normally in a horizontal position.

Especially for *Daphnia magna* it seems not easy to indicate a certain body position as "normal" or "preferred". This species, which tends to live in the littoral zone, frequently changes its course when swimming and, consequently, its orientation. In nature as well as in the laboratory the animal may be seen cruising between aquatic plants, swimming horizontally over relatively large distances, making quite capricious movements and even crawling vertically or horizontally over the surface of plants.

Still another type of swimming may be observed in *Daphnia magna*, the so called "hop and sink" way. This mode of swimming is particularly common in pelagic forms, cladocerans as well as copepods. The way in which *Daphnia magna* performs this swimming under appropriate conditions is not so pronounced as it is in its pelagic relatives. It might be considered a relic from the pelagic habitat[1], the more so since animals swimming in the "hop and sink" way react to very small changes in light intensity with a correcting response (see part A of this paper). Such reactions are correlated with vertical migration, a typical phenomenon in the pelagic species.

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[1] WESENBERG-LUND (1926) arranged the species of the genus *Daphnia* according to increasing size, an arrangement covering the tendency to exchange the pelagic habitat for the littoral habitat.

Since the "hop and sink" way of swimming was assumed to be the starting point for the positively phototactic reaction described in part A, the orientation component of this reaction was studied in detail.

The active phase of the "hop and sink" is an antennal stroke which tilts the body in a head foremost position. The angle of the body axis with the vertical may range from 0° to 40°. The passive phase is a sinking with the antennae outspread and the body axis more or less vertical.

By means of the general applied dorsal light reflex (VON BUDDENBROCK, 1914) *Daphnia magna* is assumed by HARRIS (1953) to maintain its body in the preferred position. However, the mechanism seems to be more complicated than just turning a certain part of the body toward the incident light rays. For instance, when the light comes from above a daphnid may swim more or less vertically in the "hop and sink" way, whereas when the light is entering horizontally from one side the animal swims also upright with the back directed toward the light. Therefore, the direction of the light seems to be not always the appropriate factor for the maintenance of the body position. The following experiments may elucidate this.

A small jar (100 cc), completely filled with water and ten females of *Daphnia magna*, was placed on a thin perspex holder in the centre of a sphere of opal glass. This sphere was illuminated by fourteen incandescent light sources with opal bulbs regularly distributed round the sphere. The light intensity at the place of the jar was measured in two perpendicular planes with a barrier layer cell at the end of a long axis. The aperture of the photometer and the angle of measurement was 10°. By varying the distance of the light sources to the sphere a particular A.L.D. was produced with an accuracy of about 15 % in the planes of measurement. Only at the side of the opening in the sphere must deviations have been larger.

When the light from every direction is of equal intensity the A.L.D. is called homogeneous in this paper. The light vector diagram of a homogeneous A.L.D. is, of course, circular, or globular in the case of a three-dimensional representation of the A.L.D.

When the animals are illuminated by a homogeneous A.L.D. an estimated 60 % of them make somersaults—for the greater part in a forward direction—or lie on the bottom of the jar. The rest swims more or less in an upward position at the moment of observation, but it is quite possible that these animals are somersaulting at the next counting one minute later. Under this condition of illumination it seems to be impossible for the animals to use the optical orientation mechanism which is normally employed to regulate their body position.

That the absence of spatial differences in light intensity (contrasts) is indeed the factor which induces somersaults was found when the light intensity was increased, for instance, from above. In order to get definite and reproducable A.L.D.'s definite light vector diagrams were constructed which were realised afterwards with the aid of the apparatus described above. Starting from the circular light vector diagram the upper halves were made more and more elliptical while maintaining the circular base. In fig. 27*a* and *b* two examples are given. The solid line represents the theoretical light vector diagram, the broken line represents the light vector diagrams of the A.L.D.'s as they were actually measured after sufficient manipulation with the lamps. Six A.L.D.'s were made with a ratio I_o/I_{180} (I_o = light intensity from above; I_{180} = light intensity from below) of respectively $\frac{3}{3}$, $\frac{4}{3}$, $\frac{5}{3}$, $\frac{5.5}{3}$, $\frac{6}{3}$ and $\frac{8}{3}$. At a ratio of $\frac{6}{3}$ nearly all the animals (97 %) swam more or less normally, at least without somersaults. So it seems justified to conclude that the optical mechanism, steering the body position, needs an illumination gradient for its proper functioning.

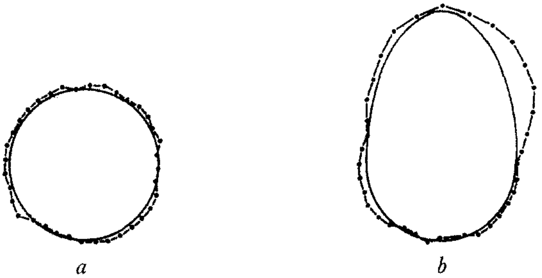

Fig. 27*a* and *b*. The light vector diagram of an A.L.D. with ratio $I_o/I_{180} = \frac{3}{3}$ (homogeneous) (*a*) and with ratio $I_o/I_{180} = \frac{6}{3}$ (*b*). The lines broken by dots represent the actual values of the angular light measurement in arbitrary units.

When the overall light intensity of a homogeneous A.L.D. is dimmed, the number of animals that make somersaults decreases. In the homogeneous A.L.D. of the apparatus described in part 12*a*, when placed vertically, a number of animals will also somersault. Other animals are temporarily orientated to the contrast in the planes oblique to the illuminated one, but these animals suddenly make loopings when they turn in the illuminated plane. Therefore it was thought possible to study the influence of the absolute light intensity with this apparatus. Five animals were swimming in the flat circular perspex box (P.B. fig. 29) and the number of somersaulting daphnids was counted at one minute intervals. The observations were made at different light intensities, measured with a barrier layer cell with an aperture of 180°. In fig. 28 the percentage of the animals performing somersaults is made a function of the light intensity. Each point is the mean value of at least 40 countings with groups of five animals. The standard error of the mean percentage is indicated by vertical bars. It can be concluded from this figure that the somersaults disappear at a light intensity of 1–10 erg/cm^2 sec. This seems to be the intensity threshold of the optical orientation mechanism involved in the orientation of the body. Because of the unknown reflexion and absorption of the perspex box with water, the intensity threshold of the optical orientation mechanism involved in the orientation of the body must be somewhat lower.

As was stated by Harris (1953), low light intensities fail to provoke eye rotations in *Daphnia magna*. Since it is generally believed that the body posture is directed by orientation responses of the compound eye (Radl, 1901; Ewald, 1910; Hess, 1910; von Frisch and Kupelwieser, 1913; Harris 1953), it is concluded that for light intensities exceeding 1–10 erg/cm^2 sec the position of the animal is governed by the orientation mechanism responding to light gradients present in the A.L.D. as registered by the compound eye. For lower light intensities, for instance at night, another mechanism seems to be responsible for

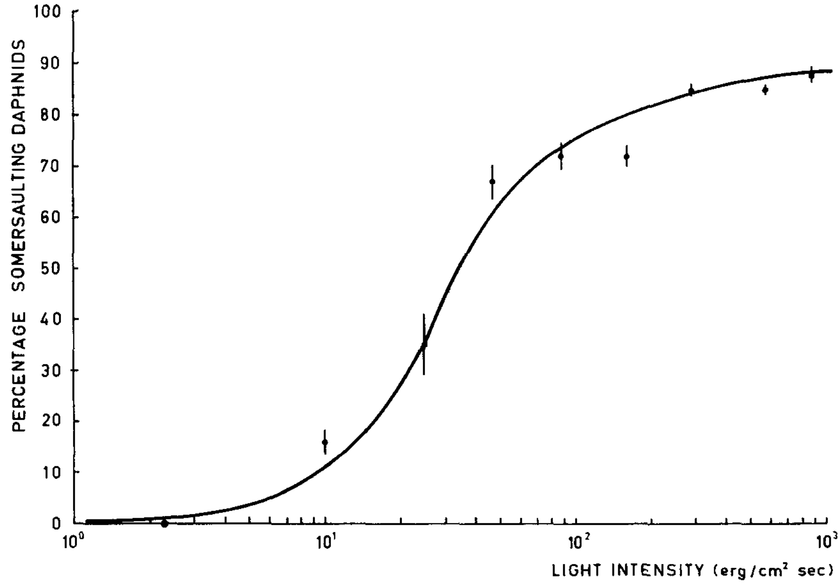

Fig. 28. The percentage of somersaulting *Daphnia magna* in a homogeneous A.L.D. of different absolute light intensity. The length of the vertical bars is equal to twice the standard error of the mean.

the maintenance of the body position. As has been pointed out earlier (Ringelberg, 1963) this mechanism may work by gravity perception (either through special receptors (Bidder, 1929) or through the "allgemeine Lagereflex" (von Buddenbrock, 1914) or it may be a passive process as a result of the position of the centre of gravity (Ewald, 1910)). Obviously this "night system" does not function at higher light intensities even when the A.L.D. is unsuitable for proper photic orientation.

12. THE ORIENTATION TO CONTRASTS

a. METHODS

To make a circular field of light of equal intensity, a circular (diameter 30 cm) fluorescent tube (fig. 29, FT) was mounted in a hardboard box (B) with circular openings in bottom and cover. A small watertight circular perspex box (PB) could be fixed on a small table (T) in the centre of the fluorescent tube. A *Daphnia magna* could be fixed by a small pin through the broodpouch in the centre of the fluorescent tube. The leg of the small table (T) in the centre of the tube could be used as an axis for a circular turntable (TT) on which a screen (S) could be fixed. It was possible to raise the turntable with the screen with a set of levers (L), thereby screening off the light from the fluorescent tube.

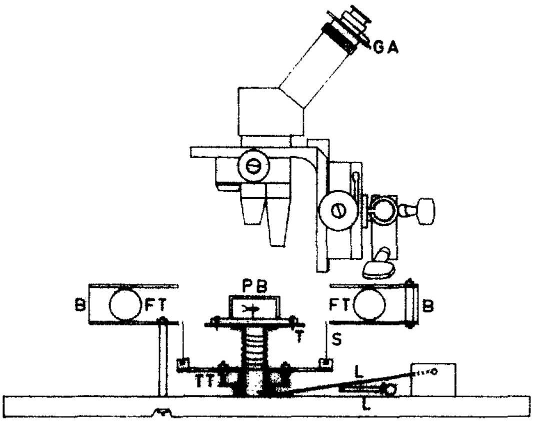

Fig. 29. The apparatus used in the study of the orientation mechanism of *Daphnia magna*. B = hardboard box; FT = fluorescent tube; GA = graduated arc; L = lever mechanism to lift the turntable with the screen; PB = circular perspex box with a pinned daphnid; S = screen; T = table; TT = circular turntable with the screen S.

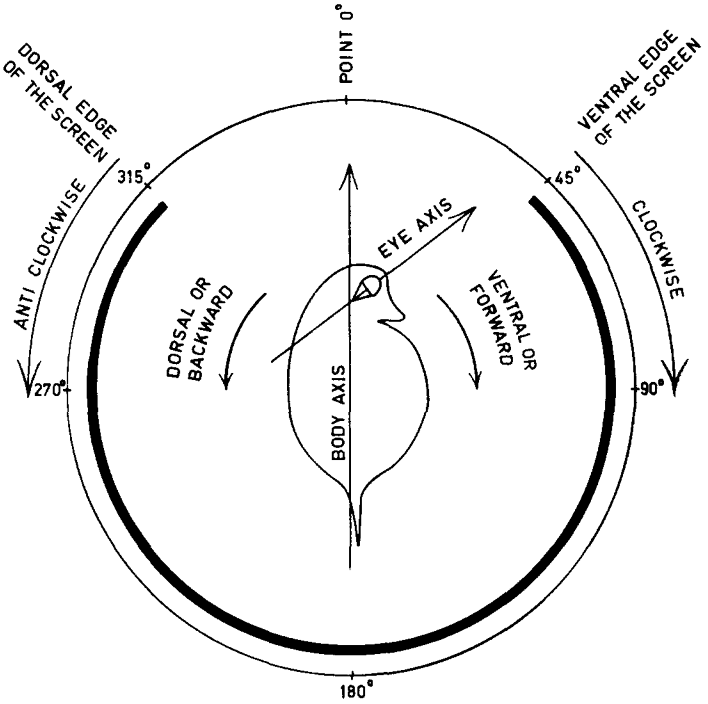

Fig. 30. The different notations used in the text.

The orientation of the daphnid was studied with a binocular microscope. By rotation of one of the oculars a hair mounted into it could be placed parallel to the body axis or eye axis, and the position of either body or eye could be read on a graduated arc (G.A.) divided in 360° and fixed to the tube of the ocular.

The body axis is a line more or less parallel to the dorsal surface of the animal, the eye axis is a line through the point of insertion of the nerves and the centre of the eye (see fig. 30). In its normal position the complex eye of *Daphnia magna* is more or less bean-shaped, with the flattened surface centripetally (see Plate II). In this case the eye axis is at right angles to this flattened surface. Sometimes it was found easier to place the hair over two prominent ommatidia, especially when extreme rotations of the eye were studied and the flattened surface or the insertion of the nerves was obscured by a slight rotation in a lateral plane. From such measurements the position of the eye axis was afterwards calculated.

The principal notations used in the following discussion of the experimental results are shown in fig. 30. The position of the screen (edges), the experimental animal, its eye etc. were recorded with respect to an arbitrary reference point 0°. Measuring was always done in a clockwise direction. The experimental procedure—local screening of light—was started at the moment that the rotating animal passed point 0°. With the animal in the point 0° position the edge of the screen at the dorsal side of the animal was called the dorsal edge, the edge at the ventral side the ventral edge of the screen. When the animal had turned for instance to 180° the edge of the screen now at the ventral side of the animal was still called the dorsal edge. The animal was always pinned according to the position in fig. 30, thus forward rotation is clockwise rotation.

To make possible a free rotation round the pin a hole, fairly large, was made in the carapax of the broodpouch. A thin needle was inserted into this hole and the needle with the animal was placed in a glass capillary filled with wax and mounted in the perspex box. When rotating in a horizontal plane the animals rested mostly on the capillary, but well above the bottom of the perspex box in order to ensure free movements of the antennae. The location of the pin was found to have no influence on the direction, forward or backward, of the animal's rotation. When, however, the pin was placed too far to the centre of the animal it appeared to be not strong enough to overcome the friction of the pin in the hole.

When the pinning was done carefully the animals remained alive in this position for days. Once an animal cast its skin when in the perspex box during observation. The moulting succeeded with the help of the investigator and the experiments were continued. Another animal, with several embryos in the broodpouch, liberated normal instars a few days after having been pinned for half a day.

With the apparatus described above the animal is thought to be illuminated in the medial plane. The A.L.D. is consequently two-dimensional. When no screen is used the light intensity is more or less of equal intensity in every direction in that plane and the A.L.D. is called homogeneous. When a screen of 270° is used the resulting A.L.D. is called homogeneous over 90°.

b. THE ORIENTATION OF THE EYE AXIS AND THE BODY AXIS

The position of the eye and body were recorded as indicated above. The experiments of 1962 and 1963 are described separately since the results differ in some respects.

1962 Experiments (January–February)

Daphnids pinned through the broodpouch and placed with the medial plane in the homogeneous A.L.D. of the fluorescent tube may rotate in a dorsal or in a ventral direction. This direction of rotation is neither the result of the place of the pin in the carapax nor an accidental occurrence. Animals rotating with a steady movement, for instance, in a dorsal (backward) direction, mostly have the compound eye turned in a dorsal position. Animals rotating in a ventral direction mostly have the compound eye turned in a ventral position.

When the circular fluorescent tube was screened off suddenly for 270 degrees with a black paper screen the rotations, whether dorsal or ventral, stopped immediately. Now the following movements took place in a rapid succession. The eye was turned dorsally when not already in that position. With a few strokes of the antennae the animal moved either in the original direction (in this case the previous stopping may be altogether absent) or in the other direction till a certain position was reached. As may be noticed from fig. 31 two positions were predominant. The body axis may be directed toward the lighted area, between 355°

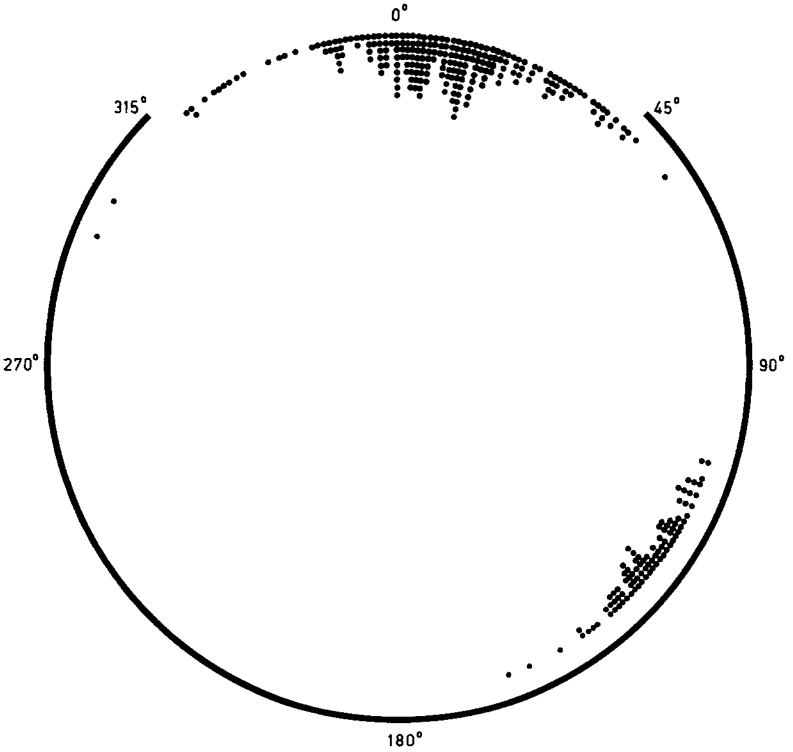

Fig. 31. The body positions of *Daphnia magna* when pinned in the centre of a circular fluorescent tube which is screened off for 270°.

and 20°, or it may be directed toward darkness, between 110° and 140°. Maintaining their position, the daphnids made frequent beats with the antennae for some time.

Animals previously rotating backward in the homogeneous A.L.D. generally directed their body axis toward the lighted area, while those previously rotating forward pointed the body axis toward the darkened area. It was clearly observed that the eye was turned in a dorsal position for both kinds of body orientations.

As a beacon for orientation three aspects of the A.L.D. homogeneous over 90° were considered:

(1) the dorsal contrast (the dorsal edge of the screen),
(2) the ventral contrast (the ventral edge of the screen),
(3) both contrasts (both edges of the screen).

To decide whether the dorsal, the ventral or both edges of the screen were used as a beacon for orientation either one or both edges of the screen were shifted over 30°. In the case both were shifted they were shifted simultaneously, but in opposite directions. If the dorsal edge of the screen is used for orientation (1) an animal with the head pointed toward the lighted area will correct its position when either the dorsal edge or both edges are shifted. If the ventral edge is used for orientation (2) the animal in question will correct its position when either the ventral edge or both edges are shifted. If both edges are used for orientation (3) the animal will correct its position, perhaps over only half the number of degrees when the dorsal or the ventral edge is shifted, and, perhaps not at all, when both are simultaneously shifted over the same angle, but in opposite directions. In Table V the theoretical number of

TABLE V

Expected and observed corrections of the body position after shifts of the screen edges

Expected correction when	*Dorsal edge/ /Ventral edge − 30°/ + 30°*	*Dorsal Edge + 30°*	*Dorsal Edge − 30°*	*Ventral Edge − 30*	*Dorsal Edge + 30*
Dorsal edge used for orientation (1)	−30	+30	−30	0	−30
Ventral edge used for orientation (2)	+30	0	0	−30	0
Both edges used for orientation (3)	0	+15	−15	−15	+15
Observed corrections	−26	+39	−51	+ 8	+28

degrees an animal would move according to the three possibilities is compared with the observed movement. A clockwise shifting of the edge of the screen is denoted positive, an anti-clockwise shifting negative. Accordingly, a ventral movement of the animal is denoted positive and a dorsal one negative. The table demonstrates that the observed turning of the animal is in accordance with the first possibility. Consequently, the dorsal contrast is used as a beacon for orientation in animals directing their body toward the lighted area.

Two more series of experiments were carried out in order to test the basis for orientations: one series with animals rotating backward, and one series with animals rotating forward in a homogeneous A.L.D. The procedure was as follows. The screen of 270° was presented in an arbitrary position to the rotating animal while the latter passes through point 0°, and the body position was recorded (1). After that the screen edges were shifted, each time followed by a recording of the body position, in the following sequence: the dorsal edge was shifted for −30° (2); the dorsal edge was moved back for +30° (3); the ventral edge was shifted for +30° (4); the ventral edge was moved back for −30° (5). Now the screen was lowered and the animal was confronted again with the homogeneous A.L.D. After some time the screen, now in another position with regard to point 0°, was presented again to the animal while rotating through point 0° and the procedure was repeated. The results of both series of experiments are shown in the Tables VI and VII. It may be noticed that an animal directed toward the lighted area reacts to a shifting of the dorsal edge of the screen, while an animal directed toward the darkened area reacts to a shifting of the ventral edge of the screen.

In both cases the eye axis is turned dorsally and the edge of the screen can be projected on a dorsal area of the compound eye. As regards this projection the mode of orientation seems to be the same. However, while the rest of the eye is illuminated in an animal reacting to the dorsal contrast, it is in darkness in an animal reacting to the ventral contrast.

1963 Experiments

From 8 to 12 June 1963 a few experiments were repeated to verify the results obtained in 1962. The experiments were made with *Daphnia magna* originating from the same laboratory culture. A different behaviour was observed, however. The animals showed variable, sometimes incoherent orientations of body axis as well as eye axis. From only five animals (numbered I–V) consistent behaviour was observed.

TABLE VI

The reaction of a light-directed animal to shifts of the screen edges

Centre of lighted area at start	*Body position of* Daphnia *at start*	*Shifting of screen edges*				*Observed corrections*			
		Dorsal −30°	*Dorsal +30°*	*Ventral +30°*	*Ventral −30°*				
		Body position after shift of screen edges							
	(1)	*(2)*	*(3)*	*(4)*	*(5)*	*(1)–(2)*	*(2)–(3)*	*(3)–(4)*	*(4)–(5)*
135	143	121	156	160	151	−22	+35	+ 4	− 9
315	327	299	324	314	307	−28	+25	−10	− 7
315	335	321	330	334	327	−14	+ 9	+ 4	− 7
315	327	318	333	334	318	− 9	+15	+ 1	−16
315	316	298	320	333		−18	+22	+13	
315	325	287	315	330	334	−38	+28	+15	+ 4
345	6	338	355	338	345	−28	+17	−17	+ 7
345	345	312	344	328	351	−33	+32	−16	+23
345	27	17	32	42	19	−10	+15	+20	−23
15	43	15	18	10	15	−28	+ 3	− 8	+ 5
45	39	27	42	45	12	−12	+15	+ 3	−33
45	32	349	27	34	33	−43	+38	−17	− 1
315	–	306	326	324	325	–	+20	− 2	+ 1
45	–	352	32	32	40	–	+40	0	− 8
Mean observed corrections						−23.6	+22.4	+1.7	−3.7
Expected corrections						−30	+30	0	0

TABLE VII

The reaction of a dark-directed animal to shifts of the screen edges

Centre of lighted area at start	*Body position of* Daphnia *at start*	*Shifting of screen edges*				*Observed corrections*			
		Dorsal −30°	*Dorsal +30°*	*Ventral +30°*	*Ventral −30°*				
		Body position after shift of screen edges							
	(1)	*(2)*	*(3)*	*(4)*	*(5)*	*(1)–(2)*	*(2)–(3)*	*(3)–(4)*	*(4)–(5)*
65	189	189	189	221	195	0	0	−32	−26
35	155	146	147	180	144	− 9	+1	−33	−36
335	79	90	89	107	87	+11	−1	−18	−20
335	90	83	86	101	81	− 7	+3	+15	−20
305	69	69	69	103	69	0	0	+34	−34
275	52	51	54	67	50	− 1	+3	+13	−17
155	282	273	271	295	270	− 9	−2	+24	−25
125	246	246	246	275	248	0	0	+29	−27
Mean observed correction						−1.9	+0.5	+24.8	−25.6
Expected corrections						0	0	+30	−30

In the same period determinations of threshold values for decreases in light intensity were performed in the large perspex tube described in part A. While during April and May 1963 most animals swam quietly more or less in the "hop and sink" manner and reacted to very small decreases in light intensity (10% and less) in June the daphnids swam vigorously upwards and downwards, or in horizontal circular pathways, and reacted only to large changes in light intensity (30% or more).

When placed in a homogeneous A.L.D. the pinned animals did not rotate as vigorously as the daphnids did in 1962, and mostly in a forward direction. The eye was in the normal position or slightly turned ventrally.

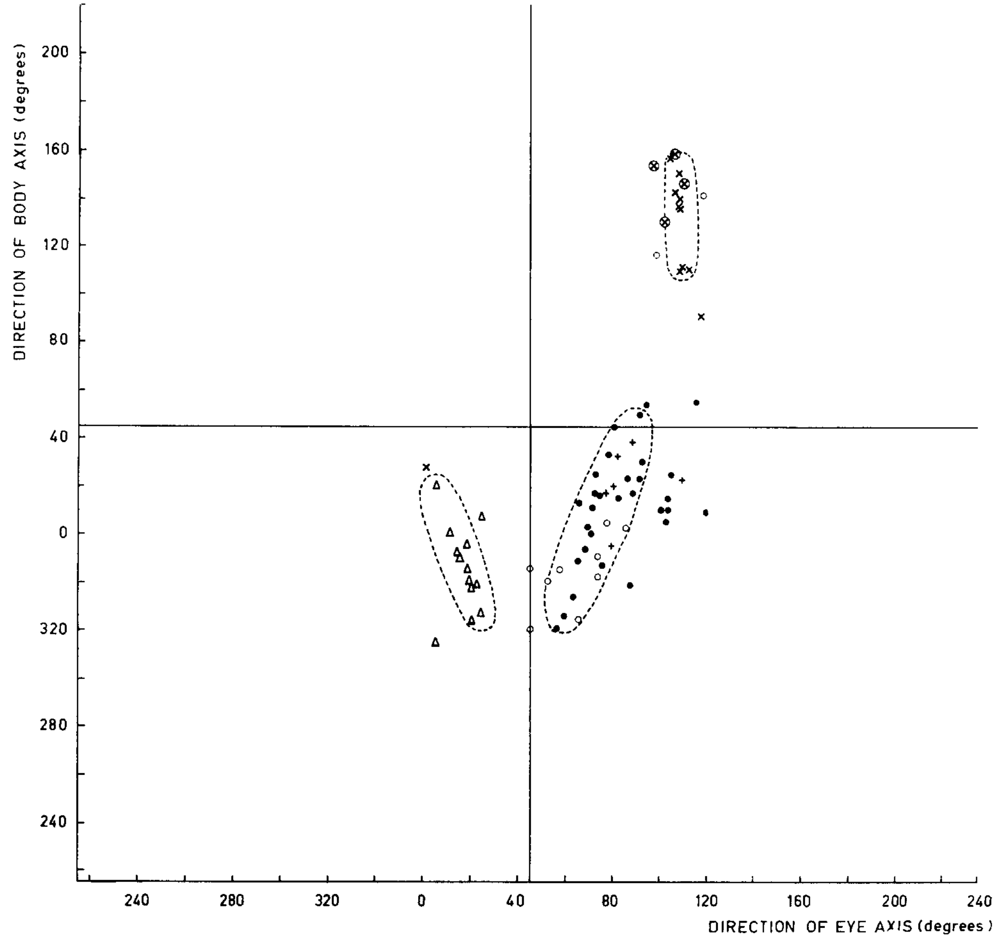

Fig. 32. The correlation between the eye axis and the body axis of *Daphnia magna* when pinned in the centre of a circular fluorescent tube which is screened off for 270°. Experiments performed in 1963. Animal I + ; animal II × ; animal III ●; animal IV and animal V ○; animal VI Δ.

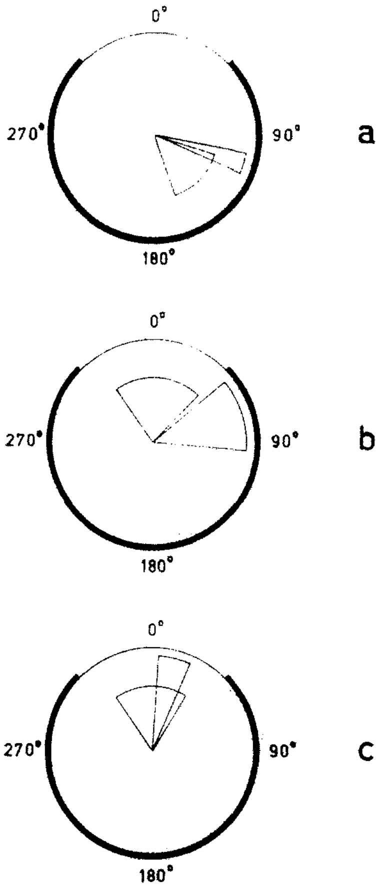

Fig. 33. The three principal orientations of the body axis (segments with short radius) and the eye axis (segments with the long radius) taken from 1963 experiments.
a) The eye axis is turned to 100°–115° while the body axis is turned to 105°–160°.
b) The eye axis is turned to 50°–95° while the body axis is turned to 325°–45°.
c) The eye axis is turned to 5°–25° while the body axis is turned to 325°–30°.

When confronted with a screen of 270° while passing point 0°, the rotation stopped and the eye was turned dorsally. The next phase, the orientation of the body axis, showed much variation. Whenever a certain position was adopted, it was frequently observed that a few strokes of the antennae brought the body axis into another position, while the eye remained in the same position with respect to the light field.

In fig. 32 the direction of the eye axis is correlated with the direction of the body axis.

On 26 June 1963 amongst several daphnids reacting to a shift of the ventral edge of the screen, one animal (VI) was found to react to the dorsal edge of the screen. In some aspects this daphnid resembled the animals from 1962, in other aspects the animals from 1963. The results of a few measurements of body axis and eye axis of this animal when confronted with a screen of 270° are also represented in fig. 32.

From this figure three major types of orientation might be abstracted. These types are schematically represented in fig. 33. Orientation type (*a*) is identical with the orientation of 1962 animals directed to the darkened area. The animals react to a shift of the ventral edge of the screen. In orientation type (*b*) the body axis is turned to the lighted area, but the eye axis is turned ventrally and not dorsally as was observed in 1962 for animals directed to the light. Moreover, the body axis moved when the ventral screen edge was shifted and not—as was demonstrated for 1962 animals—when the dorsal edge was shifted. In orientation type (*c*) the body axis is also turned to the lighted area, but the eye is turned dorsally as was observed in 1962. The difference with the 1962 animals is the large variation in body position. In orientation type (*b*) and (*c*) a relation seems to exist between the eye axis and the body axis. It was found that animals (orientation type (*b*)) in the extreme positions (body axis toward 325°, eye axis toward 60°; the observed maximal difference between body axis and eye axis is about 95°, see fig. 35) no longer reacted to a shift of the ventral edge of the screen, but to a shift of the dorsal edge. So this orientation type (*b*) seems to become merged into orienta-

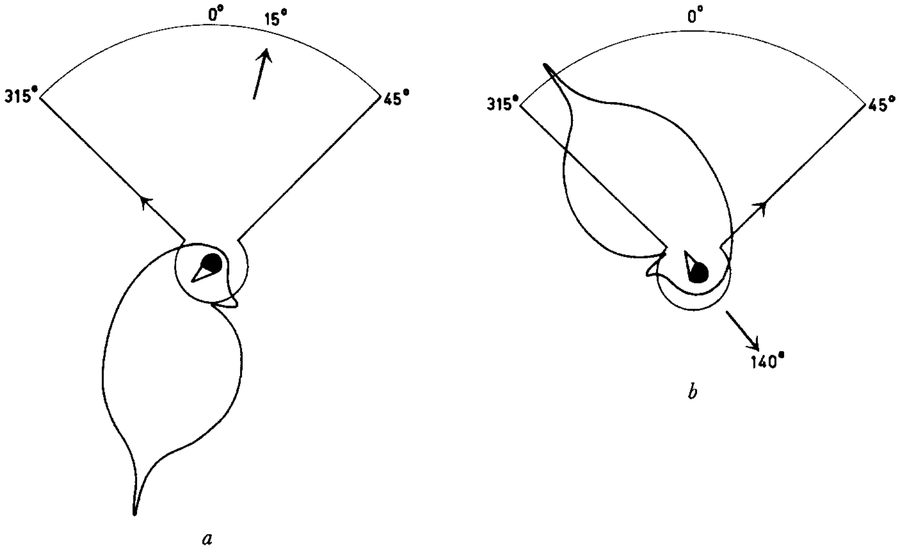

Fig. 34. The light vector diagram of an A.L.D. homogeneous over 90° with a schematical representation of the two types of orientation performed by *Daphnia magna*.

a) The positively phototactic orientation with the dorsal beacon contrast (315°) as the directing stimulus.

b) The negatively phototactic orientation with the ventral beacon contrast (45°) as the directing stimulus.

tion type (*c*). It was reported earlier (RINGELBERG, 1963) that *Daphnia magna* directed the body axis either to the ventral or to the dorsal screen edge. While these directions may be observed, especially with orientation types (*b*) and (*c*), they are not the greatest in number.

When the results of 1962 and 1963 are summarized, it seems to be possible to propose at least two types of orientation mechanisms: First, a body directed to darkness while the eye is orientated to the ventral (now dorsal) contrast (fig. 34*a*). Second, a body directed to the lighted area while the eye is orientated to the dorsal contrast (fig. 34*b*). In the extreme cases (1962 animals) the behaviour is very consistent. The body is directed towards a restricted area in the light (355°–20°) and the eye is orientated to the dorsal contrast or the body is directed to darkness (120°–140°) and the eye is orientated to the ventral (now dorsal again) contrast. 1963 animals seem to represent a transitory state between these extremes. The behaviour is ambivalent in the homogeneous A.L.D. as well as when orientations are concerned.

Ambivalent behaviour of another kind was sometimes observed in 1962 animals. A particularly illustrating example was a daphnid moulting when pinned through the broodpouch. Before the casting of the carapax—which succeeded with the help of the experimentator—this animal rotated very regularly in a backward direction in the homogeneous A.L.D. and orientated its body axis toward the lighted area when confronted with the screen. After the moulting, however, it had changed in a forward rotating animal and directed its body axis toward darkness when confronted with the screen.

C. FURTHER INFORMATION ABOUT THE ORIENTATION MECHANISM

The following experiments were performed in 1962 with a female of *Daphnia magna*. The animal was fixed with a pin through the broodpouch in the point 0° position without the possibility of body rotation. After a few minutes of adaptation to the homogeneous A.L.D. the 270° screen was presented in a certain position. Then the orientation of the eye axis was recorded and the screen removed. The screen was rotated 20° and after a few minutes presented again. Eighteen positions of the screen round the animal were tested in this way. Subsequently, the animal was liberated from the pin, the hole in the parapax was made larger and the animal was replaced in the homogeneous A.L.D. now with the possibility to rotate. The procedure with the screen was repeated and

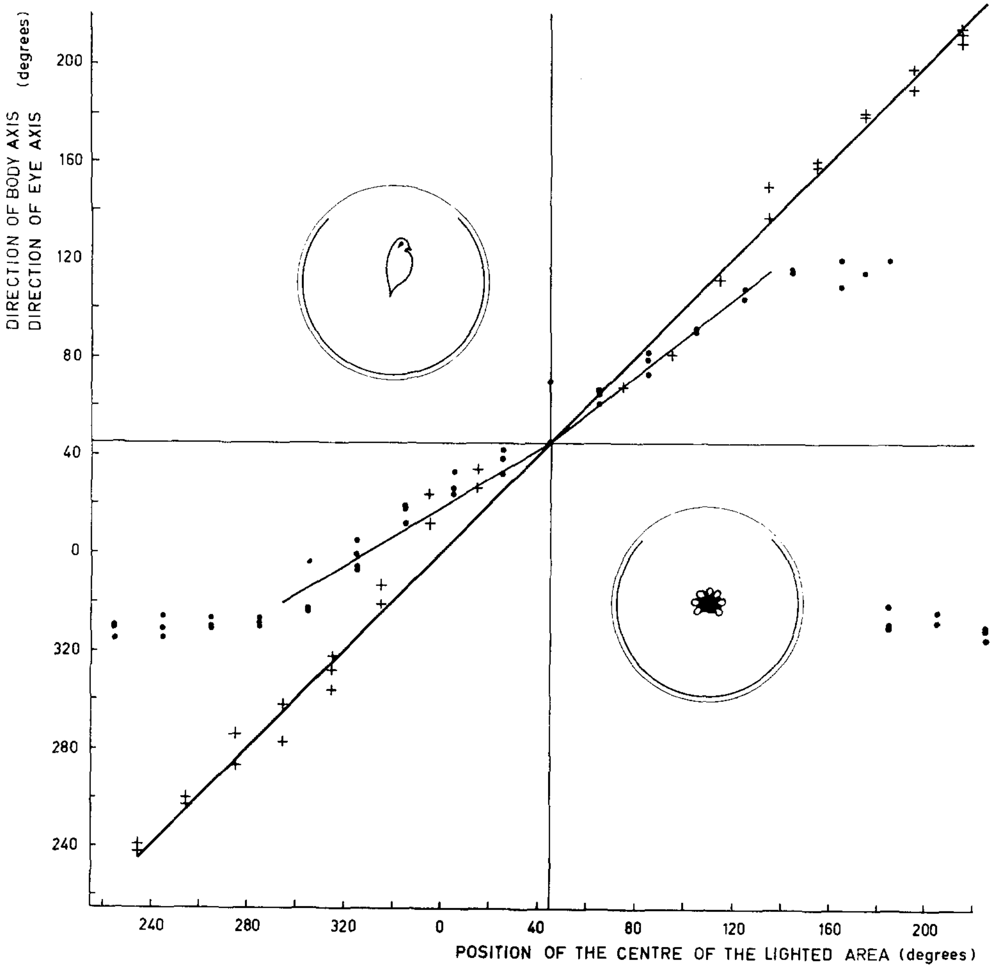

Fig. 35. The direction of the eye axis (dots) to different positions of a screen of 270° (denoted by the centre of the lighted area) when the animal is fixed in the point 0° position and the direction of the body axis (crosses) of the same animal to different positions of the same screen when this animal can rotate freely round the pin. Insets: orientated position of the compound eye and the body with regard to the dorsal screen edge.

the position of the body axis measured. The results are presented in fig. 35. It is obvious that the animal was directed toward the lighted area.

First the eye movements are discussed. When the screen is presented to the fixed animal with the centre of the lighted area (C_L) to 45°, hardly any movement of the eye is observed and the eye persists in the normal position of about 45° with the body axis. When the screen is presented again, moved in a clockwise or anti-clockwise direction, the eye rotates in the same direction as the screen, but lags behind while doing so. The maximal rotations of the eye, which seem to be 70° in the ventral direction and 70°-80° in the dorsal direction, are reached when the centre of light is 135° respectively 295° from point 0°. When the screen is presented still further away the eye rotation does not exceed these values.

When C_L reaches 185° the two extreme alternative positions of the eye axis may be obtained: the eye turns either to a ventral position (135°) or to a dorsal position (335°). Still further movement of the screen only leads to dorsal rotation of the eye.

It may be obvious that throughout the whole cycle of orientations one of the edges of the screen can be projected on the dorsal surface of the compound eye. For positions of C_L from 45° to 185° the dorsal edge can be projected on this area of the compound eye, from 185° onwards the ventral edge becomes nearer to the dorsal area of the eye when this eye is turned in a dorsal direction and when C_L is at the back of the animal the dorsal edge of the screen takes over the task of the ventral screen edge.

Also from these experiments it may be concluded that contrast areas are of crucial importance for the orientation of the compound eye.

The recordings of the body position reveal a remarkable phenomenon. As long as the screen is presented to the animal with C_L between 105° and 345° the body axis is directed exactly to the centre of the lighted area. When the screen is presented with C_L between 345° and 105°, that is 60° dorsally or ventrally from the normal position of the eye axis at 45°, the body axis lags behind the centre of the lighted area in the same way as the compound eye did. Of particular interest with regard to this phenomenon is the position of the screen with C_L at point 0°, the circumstance in which most previous experiments have been conducted. According to the measurements the body axis points to 15°, which means that about the same deviation from point 0° is obtained as can be noticed from fig. 31.

The results of comparable experiments performed by RADL (1901), HESS (1910), EWALD (1910) and VON FRISCH and KUPELWIESER (1913) are the following. These authors studied the orientation responses of the compound eye of daphnids to the direction of the light rays. They all mentioned rotation of the compound eye when the light source is rotated round the animal or when the animal is rotated with regard to the light source. They also recorded ambivalent rotation from dorsal to ventral and *vice versa* when the light source is in a certain position to the animal.

Detailed measurements of the rotations have not been made by these authors, but VON FRISCH and KUPELWIESER made a picture of various positions of the body and eye with regard to the light source. They used a narrow beam which can be considered parallel. With regard to the position of the eye VON FRISCH and KUPELWIESER stated that the dorsal part of the eye is directed to the light source and that the eye tries to persist in this position when the body of the animal is turned (page 540): "... und der Scheitel S des Auges ist der Lichtquelle zugekehrt. Diese Lage sucht das Auge auch bei allen folgenden Körperstellungen möglichst beizubehalten ...". From fig. H in the paper of VON FRISCH and KUPELWIESER a normal body position of about 60° with the light source can be measured. When the light source in this experiment is supposed to have the same beacon function as the dorsal contrast in my experiments and when the same co-ordinates are applied as in use in this paper a body position of 60° — 45° = 15° from point 0° is calculated from the experiment of VON FRISCH and KUPELWIESER. As this direction of the body axis of 15° agrees with the body directions obtained in my experiment (see figs. 34*b* and 31) a similar orientation must be supposed.

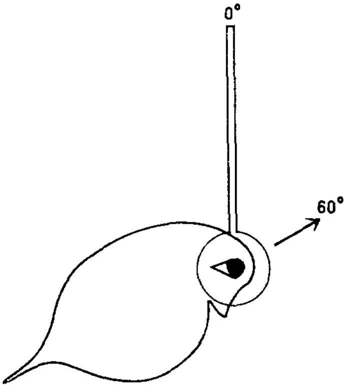

Fig. 36. A schematical representation of the light vector diagram of an A.L.D. originating from a parallel beam of light. The body and eye position of *Daphnia pulex* in such an A.L.D., as found by VON FRISCH and KUPELWIESER (1913), is indicated.

At this point the A.L.D. of a light source should be discussed. When the light source is assumed to be a point, the A.L.D. at a certain distance is the same as in the case of a parallel beam. The light vector diagram of these situations is schematically presented in fig. 36. It is assumed that some scattering of the light of the source or beam causes light to come from the other directions. The light vector diagram of the A.L.D. homogeneous over 90°, resulting from the fluorescent tube and the screen is schematized in figs. 34*a*, *b*. Light from the tube is reflected by the black screen, hence the low intensity from the screen part.

It is obvious that in both cases the directing stimulus is to be found in the contrast (see for argumentation part 12*b*). In the case of a pointed light source or a parallel beam, one such beacon contrast is present; in our experimental set up there are two.

It might be clear now why VON FRISCH and KUPELWIESER (1913), studying the orientation of eye and body to a light source, obtained the same result as we did investigating the orientation to contrasts. The stimulus of the steering mechanism is the same in both cases. Whether the beacon is called a light source or a contrast seems to be trivial. However, we prefer the term "contrast" or "gradient" for the optical beacon used by the animal as a directing stimulus for orientation.

13. THE EFFECT OF A DECREASE IN LIGHT INTENSITY ON THE POSITION OF THE COMPOUND EYE

Several authors have reported that a decrease in light intensity evokes a dorsal rotation of the eye, whereas an increase in light intensity evokes a ventral rotation of the eye (RADL, 1901; EWALD, 1910; VON FRISCH and KUPELWIESER, 1913). Nevertheless, the same authors doubted the existence of this phenomenon. This was clearly expressed by EWALD (1910, p. 52): "Die Reaktionen waren zudem so unsicher, dass ich von einem genaueren Eingehen auf diese Frage Abstand nehme."

This doubt originates from the observation that the direction of the eye rotation in response to a decrease in light intensity depends on the position of the animal—and its eye—with respect to the light source (see for instance VON FRISCH and KUPELWIESER, 1913). It seems, therefore, that two factors may cause a turning of the compound eye, (*a*) orientational factors, and (*b*) changes in light intensity. When these two factors operate independently it must be possible to separate their influence by, for instance, decreasing the light intensity while the possibility of an orientation is absent. This can be done by an overall decrease in light intensity of a homogeneous A.L.D.

A pinned *Daphnia magna*, without the possibility to rotate, was placed in the centre of the homogeneous A.L.D. When the light intensity was decreased by a circular screen of transparent paper or by a circular filter of gelatine made turbid with indian ink the eye of most animals made a vigorous movement in a dorsal direction. While the animal made vigorous beats with the antennae the eye drifted back to the original position. The same occurred when the screen was removed, but now the eye rotated to ventral and drifted back from this position to the initial one. These experiments were performed in 1963 and some of the animals did not react at all. This is not surprising to anyone familiar with the caprices of daphnids in general (the reader is referred to VON FRISCH and KUPELWIESER, 1913, page 548, or KOEHLER, 1924, page 105).

14. DISCUSSION

In the introduction to this part it has been argued that only spatial differences in light intensity—contrasts or gradients—are considered as stimuli for the steering mechanism. While *Daphnia magna* is unable to maintain its body position in a homogeneous A.L.D., but re-assumes normal swimming when the light intensity from above is increased, we may assume that spatial differences are also necessary to guarantee normal swimming. We have seen, however, that it is difficult to say what body position must be called the "normal" one in *Daphnia magna.* This is due in the first place to the fact that the littoral habitat requires more variation in the mode of locomotion than the relatively uniform pelagic habitat. The variation in body position might therefore be seen as an adaptation to the variable conditions present in the littoral. Secondly, the A.L.D. of the littoral habitat may differ from place to place, and consequently the beacon stimuli may have variable spatial positions, so that even in the case of a rigid, stereotype orientation mechanism different body positions may be observed. An example is, for instance, a phenomenon called the "Uferflucht" observed by SIEBECK (lecture delivered at the International Congress of Limnology in 1962, and personal communication). *Daphnia longispina* leaves the littoral zone, swimming in a horizontal position at a time in early morning when members of the same species are swimming downwards in the pelagic area. SIEBECK supposes that this difference in swimming posture is due to differences in the A.L.D. of these two habitats.

SASAKI *et al.* (1962) demonstrated that even in the pelagic habitat the angular position of the beacon contrast may vary, depending on the depth of the water and the position of the sun. Consequently, the position of the daphnids may vary, not only at different depths, but also in different vertical planes.

Maintenance of the body position by optical steering seems to be possible only when the absolute light intensity exceeds a certain threshold. Below this threshold value another orientation mechanism replaces the optical one.

It was demonstrated by the experiments with an A.L.D. homogeneous over 90° in what manner eye and body are orientated to the beacon contrasts. Both the dorsal and the ventral contrast is used for orientation (Tables V, VI, VII). It will be argued below that the dorsal contrast is used by *Daphnia magna* in a positively phototactic orientation, while the ventral contrast is used in a negatively phototactic orientation.

First argument. The animals directing the body axis to the lighted area are generally orientated to the dorsal contrast (Table VI). These animals would probably swim toward the light when not pinned through the broodpouch. If so, such reaction must be called a positively phototactic one.

The animals directing the body axis to the darkened area are orientated to the ventral contrast (Tabel VII). When liberated these animals would probably swim obliquely downward (the experiments with the screen might have been performed in a vertical plane without influencing the results). Such a reaction away from light must be called a negatively phototactic one.

Attention is called to the oblique position of the body axis in the darkness-directed animal. When freely swimming daphnids illuminated from above perceive an increase in light intensity, they also move obliquely downward, often in a zigzag course. These different body orientations—more or less perpendicularly upward in the positively phototactic response, obliquely downward in the negatively phototactic response—result from the position of the beacon contrast and the anatomical limits restricting the angle between body axis and eye axis. When, for instance, the contrast toward which orientation takes place is at 45° with the vertical and this contrast is fixed with the dorsal orientation area, the eye axis is directed to $45° + 90° = 135°$. The maximal angular difference in a dorsal direction between body axis and eye axis is about 25°. Consequently the body axis might be directed to not more than $25° + 135° = 160°$ with the vertical.

Second argument. In a light-directed animal the dorsal orientation area of the compound eye is pointed to the dorsal edge of the screen. Consequently the ommatidia on other parts of the eye are illuminated. In dark-directed animals the orientation area is pointed to the ventral edge of the screen, and accordingly the ommatidia on the other part of the eye are not illuminated. Also this difference: illuminated eye—non illuminated eye might be seen as an argument that there is a positively and a negatively phototactic reaction involved.

While it is generally accepted (see part A this paper) that decreases in light intensity induce a positively phototactic reaction, it might be thought peculiar that partial screening of the fluorescent tube which results also in a decrease in light intensity, evokes a positively as well as a negatively phototactic reaction. However, the phototactic reaction encountered in the experiment with the screened fluorescent tube is not the same as the phototactic response caused by changes in light intensity. It should be kept in mind that daphnids sometimes perform oscillatory movements up and down an experimental tube or persist in staying for a long time at either end of the tube.

CLARKE called the underlying mechanism the primary phototropism, as opposed to the secondary phototropism caused by changes in light intensity. These oscillatory or persistent movements are present under constant conditions of illumination, and were thought to be of internal origin. They may be triggered by an environmental or internal disturbance (see part 5*b*).

We therefore suppose that the positively and negatively phototactic orientations exhibited in the homogeneous A.L.D. of 90° is identical with this phototaxis of internal origin, and not with the phototaxis caused by changes in illumination. The fact that light directed orientation is preceded by backward rotation in the homogeneous A.L.D., whereas darkness directed orientation is preceded by forward rotation in the homogeneous A.L.D. indicates that the "phototaxis" is present under constant illumination and before orientation to the screen edges is possible.

A probably comparable case of positively phototactic animals making backward somersaults, and negatively phototactic animals making forward somersaults is reported by SEIFERT (1932) for *Artemia salina.*

The orientation mechanism proposed by HARRIS (1953) and MITTELSTAEDT (1961*a*) differs from the one evolved from our experiments and will be described first.

Although the first author tried to explain that *Daphnia* is confronted with a cone of light, originating from refraction at the water surface, he did not make use of the contrast as a beacon for orientation. Presumably led astray by the idea that, according to the dorsal light reflex, the daphnid has to turn its dorsal side toward the light from above, he suggested that *Daphnia magna* is swimming in a horizontal position in the cone of illumination. If so, the typical form of the light field is of no importance to the animal, because it would swim in the same position when the cone had other proportions, for instance 179°. HARRIS was aware of the existence of a dorsal orientation area of the eye. Since the orientation is to the light from above this area must be directed perpendicularly upward. The eye axis was consequently designed parallel with the body axis in his fig. 2. This is erroneous because the eye axis makes an angle of about 45° with the body axis when the eye muscles are of equal tension as HARRIS supposed that they were.

Moreover, HARRIS' theory of the orientation mechanism of the positively and negatively phototactic reaction as a result of changes in light intensity made him assume a horizontal starting position for the animal.

For instance, when a decrease in light intensity causes the dorsal eye muscles to contract the body is tilted, according to HARRIS, by appropriate strokes of the antennae into such a position that the tension in the muscles is equal again. This provides a body position directed upward and the animal starts swimming toward light. As a consequence of this turning, however, the orientation area of the eye is no longer directed toward light and we must therefore assume that the animal is not orientated whilst performing a positively phototactic reaction, which seems strange. The crucial point is that HARRIS assumes the eye to be in the normal position during the positive (upward) swimming reaction. It has been demonstrated in this paper that this view is not correct. It was, furthermore, argued that the orientation is probably to the edges (contrasts) of the cone and not to the light from above.

MITTELSTAEDT (1961*a*) thinks the orientation of *Daphnia pulex* to be as follows. The eye axis in the normal position of the eye is parallel to the body axis, as HARRIS assumed, but the daphnid does not swim in a horizontal but in a vertical position. The reason is obvious, because MITTELSTAEDT assumes that the "Scheitelpunkt" (this is the point opposite the insertion of the nerves) of the eye is and remains directed toward the light. For instance, when the animal starts to swim away from light the animal, still in a vertical position, turns the eye dorsally. In order to keep the "Scheitelpunkt" directed toward the light source, the body has to rotate forward. Finally the daphnid is swimming perpendicular downward with the eye still in the same position, that is with the "Scheitelpunkt" directed to the light above (see fig. 1 of the paper of MITTELSTAEDT 1961*a*).

This theory does not hold. In the first place *Daphnia* does not orientate with the "Scheitelpunkt" (neither does *Daphnia pulex*, see VON FRISCH and KUPELWIESER, 1913), in the second place an eye axis directed vertically upward and a body axis directed vertically downward is an anatomical impossibility.

Although our knowledge of the steering of *Daphnia magna* during the phototactic response is sadly insufficient the following picture of the steering mechanism may be proposed.

The absence of normal swimming in a diffuse A.L.D. suggests that some optical orientation underlies the "hop and sink" movement but as long as no direct observations of eye positions in freely swimming animals have been made, this cannot be known for certain. The orientation probably takes place during the active phase. It seems unlikely, however, that the dorsal eye muscles are contracted in the passive sinking phase. When the body is tilted out of its momentary "normal" position, for instance by turbulences in the water, the eye will rotate to keep the beacon contrast on the dorsal orientation area, just as in the experiments of von Frisch and Kupelwieser where the body was rotated with respect to the light source. Subsequently the body position is corrected.

A dorsal rotation of the compound eye was observed when a decrease in light intensity was perceived (chapter 13) or, more precisely, when the critical level of the excitatory state Y_o^P (chapter 8) has been reached. In this case the orientation area of the eye is turned toward the dorsal contrast. After that, the body is tilted by appropriate strokes of the antennae in the vertical position, and an increase in antennal beats (also observed in the experiments) propels the animal upward. During this upward swimming the dorsal eye muscles remain contracted and the eye remains orientated to the dorsal contrast. In contradiction to what Harris believes, the body is not rotated into such a position that the eye muscles are again of equel tension. In my opinion the asymmetrical tension of these muscles is, on the contrary, an indication that the animal is reacting.

Von Frisch and Kupelwieser (1913) observed a slackening of the eye muscles about half a minute after their contraction in answer to a stimulus. This slackening process may be the announcement of the end of the reaction. In our experiments with a homogeneous A.L.D. (chapter 13) the return of the eye to the normal position sometimes occurred immediately. This might be the result of the absence of a directing stimulus. Also freely swimming animals somersaulting in a diffuse A.L.D. do not visible react to decreases in light intensity, probably because orientation is impossible.

An increase in light intensity is followed by a ventral rotation of the compound eye, by which means the orientation area is turned toward the ventral contrast in the environment. Strokes of the antennae next rotate the body till the eye is in a dorsal position with respect to the body. Now the body points obliquely downward and an increase in antennal beats this time propels the animal downwards.

The change in illumination, a decrease or an increase, thus determines which contrast is to be the beacon. A control to the animal that the eye is really directed to the right beacon contrast may be present in such a form that the rest of the ommatidia are either illuminated or in (relative) darkness.

During the active phase of the "hop and sink" swimming the angle between eye axis and body axis has probably not the same value as during the positively phototactic reaction, because the animal swims in a different position. Especially in *Daphnia magna* internal factors may change the "prescribed" angle between body axis and the orientated eye. In this way we may suppose that different swimming courses are established. Owing to lack of reliable experimental evidence it seems wise not to venture on this subject.

No experiments have been made to disclose the exact nature of the orientation of the eye. Near to the median plane of the eye, at the left and the right side, three ommatidia are present in the dorsal area of the eye. These three pairs of ommatidia seem to form the orientation area. Whether all three pairs have a function is unknown, because the nature of the orientation is unknown. The irregular twitches of the eye, which can easily be observed with a microscope, might take care that a certain pair of ommatidia charged with the orientation function is alternately pointed to the lighted and the darkened side of the contrast. This is a possible way to register whether or not such ommatidia are still directed toward the contrast. A similar function of the scanning movements of the eye was supposed by HARRIS (1953).

Inspection of the body positions of the animals which are directed to the light in fig. 31, reveals that these positions form two distinct and symmetrical distributions. This might be an indication that it is not one pair of ommatidia that has an orientation function, but that there are two. More experiments, especially with light gradients as the directing stimulus for the orientation mechanism, are necessary to gain still better insight into this intriguing mechanism.

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15. SUMMARY

The present investigation was aimed at gaining more insight into the physiological mechanism underlying the diurnal vertical migration of planktonic animals. The experiments were carried out on *Daphnia magna*. Only reactions to decreases in light intensity were studied.

Kinetical aspects.

Experiments with instantaneous decreases in light intensity revealed that the reaction to this stimulus is a positively phototactic swimming, the speed of which increases with increasing stimulus intensity. For

threshold stimuli the light intensity reached when the reaction stops is equal to the initial intensity. The swimming distance depends on the turbidity of the water. There is experimental evidence that a kind of feed-back is involved.

Series of sub-threshold decreases in light intensity may lead to a phototactical swimming reaction, which demonstrates the additional effect of these sub-threshold decreases.

Continuous decreases in light intensity lead to a series of separate responses that are identical with the positively phototactic reactions caused by instantaneous decreases. With increasing rate of the relative decrease in light intensity these separate responses merge into a continuous upward swimming. The period of time after which the first reaction is initiated depends on the rate of the continuous decrease in illumination; a relation is found between the logarithm of the stimulus strength and the duration of the stimulus needed to initiate a reaction, comparable to such a relation valid in muscle stimulation. The swimming speed is constant as long as the reactions are separated, but as soon as they flow together the swimming speed starts to increase linearly with the logarithm of the stimulus intensity.

At a small rate of the relative decrease in light intensity the reacting animals lag behind the initial light intensity. With increasing rate this lagging behind disappears gradually, but at a certain rate the lagging behind increases again. A theory is built up, which tries to account for several observed phenomena.

An observed migration of *Daphnia magna* in nature demonstrated the applicability of the experimental results to the natural phenomenon. Also the interpretation of the migration of three scattering layers in terms of the theoretical concepts proved to be promising.

Directional aspects.

It is argued that spatial differences in light intensity, such as contrasts and gradients, might be proper orientational beacons for *Daphnia magna*. In the angular light distribution of the aquatic habitat relatively large changes in light vectors (contrasts or gradients) are present between 30° and 60° with the vertical. Therefore, the orientation responses of *Daphnia magna* to contrasts were studied experimentally. *Daphnia magna* proved to be unable to swim normally when the light from every direction is of equal intensity, but increasing the light intensity from one direction enables the animal to reassume a normal body position. Below a certain light intensity normal swimming is possible, independently of the angular distribution.

When a *Daphnia magna*, transversally pinned through the broodpouch and lying on its side, is illuminated in the medial plane by light of equal

intensity from every direction in this plane, forward or backward rotations are observed. Screening off the light for 270° stops these rotations, the formerly forward rotating animals direct the body axis toward darkness, the formerly backward rotating animals direct the body axis toward the lighted area. It was proved that the animals are orientated in these two cases to different edges of the screen (beacon contrasts). In adjusting the compound eye and the body axis to displacements of the beacon contrasts the animal lags behind.

A decrease in total light intensity of an angular light distribution with light of equal intensity coming from every direction evokes a dorsal eye rotation in *Daphnia magna*. This fact demonstrates that no optical beacons are needed to induce eye rotations.

It is concluded that the directing stimulus for the phototactic reaction is a contrast or a gradient present in the angular light distribution. Previous hypotheses on the orientation mechanism in *Daphnia* are compared with the one developed in this paper.

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