PROXIMAL RETINAL PIGMENT RESPONSES AND THEIR RELATIONSHIP TO TOTAL PHOTOMECHANICAL ADAPTATION IN THE DWARF CRAYFISH, *CAMBARELLUS SHUFELDTI*[1]

MILTON FINGERMAN
and
R. NAGABHUSHANAM,
Department of Zoology, Newcomb College, Tulane University, New Orleans, Louisiana

The compound eye of crustaceans has three retinal pigments, distal, proximal, and reflecting, that are used for photomechanical adaptation in response to changes in intensity of illumination. An appreciable amount of information exists concerning the physiology of the distal retinal pigment, but a relatively small amount of data is available for the other pigments. The most recent review of the literature concerned with these pigments is that of Kleinholz (1961).

In the few species of crayfishes whose eyes have been studied in detail, the distal and proximal pigments migrated in response to illumination changes but the position of the reflecting pigment was fixed (Welsh, 1939; Kleinholz, 1949, 1961). Migration of the distal pigment is under endocrine control, but crayfishes show diversity in the mechanism of regulation of the proximal pigment.

Welsh (1939) and Kleinholz (1949) appear to be the only investigators to have studied the controlling mechanisms for the proximal pigment in crayfishes. Welsh reported that eyestalk extracts caused light-adaptation of the proximal as well as the distal pigment in *Cambarus bartoni*. However, a higher concentration of eyestalk extract was required to activate the proximal pigment than the distal pigment. This observation was interesting in view of the earlier report of Kleinholz (1936) that the distal and reflecting pigments of the prawn *Palaemonetes vulgaris* migrated toward the light-adapted positions after injection of eyestalk extract, but that the proximal pigment was unaffected. In another crayfish, *Pacifastacus trowbridgi*, the proximal pigment seems to be an independent effector (Kleinholz, 1949). This hypothesis was based on the observation that the proximal pigment of isolated eyestalks maintained in moist chambers continued to respond to light and darkness by appropriate migrations.

The distal retinal pigment of the dwarf crayfish, *Cambarellus shufeldti*, the animal used in this investigation, was first studied by Fingerman (1957). He showed that the eyestalks and supraesophageal ganglia contain the distal retinal pigment light-adapting hormone. Fingerman also determined (1) the rate of migration of the distal pigment and (2) the relationship between the position of this pigment and intensity of illumination. In 1959, Fingerman, Mobberly, and Sundararaj showed the existence of a distal retinal pigment dark-adapting hormone in the optic ganglia of the eyestalks and other central nervous organs of *Cambarellus*.

In view of the small amount of information available concerning the proximal and reflecting pigments of crustaceans in general and crayfishes in particular, the present study was undertaken with the dwarf crayfish. Among the experiments was a determination of the migration rate of the proximal pigment to provide a basis of comparison with the distal pigment. This rate has never been accurately determined for the proximal pigment in any crayfish. An attempt was also made to understand the mechanism controlling migration of the proximal pigment in *Cambarellus*, in view of the strikingly different mechanims postulated for *Cambarus* and *Pacifastacus*.

Materials and Methods

Specimens of the dwarf crayfish, *Cambarellus shufeldti*, found in roadside ditches at Hickory, Louisiana, were used in these experiments. In the laboratory the crayfish were kept in aerated tap water at 72-75° F.

The positions of the retinal pigments were determined by observation of sectioned eyestalks. To fix the retinal pigments in position, the crayfish were killed rapidly by immersion in boiling water for at least 15 seconds. Both eyestalks were then removed

[1] This investigation was supported by Grant No. B-838 from the National Institutes of Health.

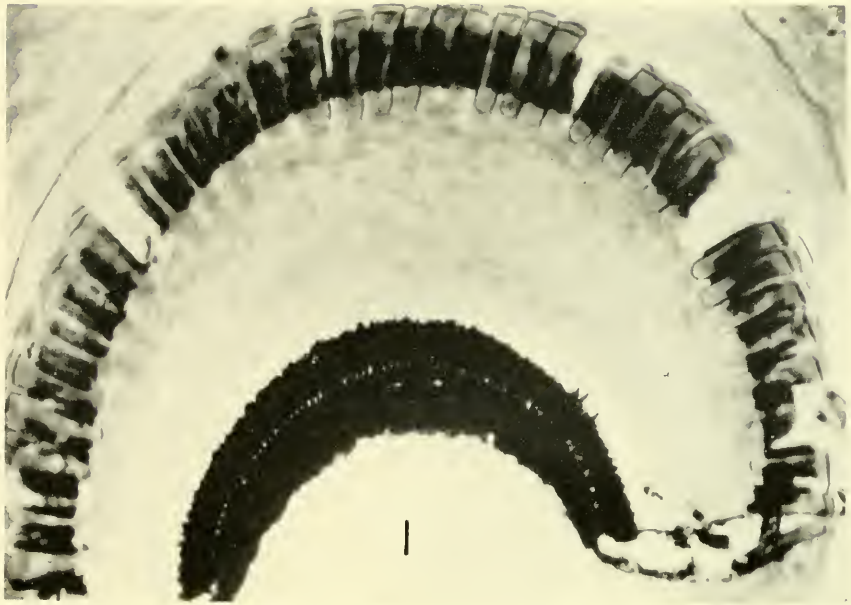
and place in Bouin's solution. After the eyestalks were dehydrated and embedded in paraffin, sections 20 μ thick were prepared. The compound microscope was arranged so that both transmitted and reflected light shone on the eyestalk sections. In this manner, the three pigments could be distinguished readily from one another. To express the positions of the pigments in quantitative terms, four measurements were made with the aid of an ocular micrometer. The technique of measurement was similar to that employed by de Bruin and Crisp (1957) for use with eyes of marine crustaceans. The measurements were: (A) distance from outer corneal surface to the distal edge of the proximal pigment, (B) distance from outer corneal surface to the distal edge of the reflecting pigment, (C) distance from the outer corneal surface to the basement membrane whose position is fixed, and (D) width of the band of distal pigment. The ratios A/C and B/C were called the proximal and reflecting pigment indexes respectively. Use of ratios minimized the effects on the measurements of size differences among the eyestalks. At the magnification used to observe the pigments, 100X, each unit of the ocular micrometer represented 10.9 μ.

Experiments and Results

Relationship between time in light and dark and the positions of the proximal and reflecting pigments.—The objectives of this group of experiments were (1) to learn whether the proximal and reflecting retinal pigments of *Cambarellus* migrate, and if so (2) to determine the times required for migration between the maximally light-adapted and maximally dark-adapted positions. For use in the first experiment five white pans each containing at least seven crayfish were placed in a darkroom for two hours. At the end of this period the animals in one pan were killed. The remaining four pans were placed under an illumination of 120 ft. c. Light intensities were measured with a General Electric photometer calibrated at the Department of Physics, Newcomb College. The animals in one of the four pans were sacrificed after exposure to illumination for five minutes. Successive groups were killed after exposures of 10, 15, and 30 minutes. In this manner the time required for light-adaptation of the pigments was determined. To learn the time necessary for dark-adaptation, the reciprocal experiment was performed with five groups of crayfish that had been exposed to an illumination of 120 ft. c. for two hours. Then the crayfish in one of the white pans were killed while the remaining four groups were placed in the darkroom. Successive groups were sacrificed 15, 30, 60, and 90 minutes after having been put in the darkroom. The eyes were sectioned and the positions of the pigments observed. Both experiments were performed twice.

In a dark-adapted eye (fig. 1) the distal pigment lies just below the cornea and surrounds the crystalline cone; the proximal pigment migrates proximally exposing the distal portions of the retinula cells. The reflecting pigment cells lie against the basement membrane, the row of light dots near the middle of the black band at the base of the eye, and extend distally. This pigment appears black by transmitted light. The distal portion of the black band at the base of the eye is reflecting pigment; the proximal portion, proximal pigment. In a light-adapted eye (fig. 2) the distal pigment migrates proximally and the proximal pigment moves distally. The proximal pigment lies within the retinula cells and migrates to fill the distal portion of these cells; some proximal pigment, however, always remains proximal to the basement membrane. The distal pigment in a light-adapted eye forms a sleeve completely enveloping the crystalline cone stalk. As the distal pigment migrates proximally, the band of pigment becomes progressively wider.

In a maximally dark-adapted eye the proximal pigment index was 1.0; in a maximally light-adapted eye approximately 0.65 (fig. 3). Light-adaptation required 10 minutes (fig. 3A); dark-adaptation 60 minutes (fig. 3B). Each point in Figure 3A represents the mean index of 12 eyestalks, each from a different crayfish; in Figure 3B, eyestalks from 13 different crayfish. The average distance from the outer corneal surface to the basement membrane in 41 eyestalks, each from a different individual, was 345 μ. The mean maximum distance the proximal pigment migrated above the basement membrane in 40 fully light-adapted eyes was 107 μ. Comparison of the position of the distal pigment in 20 light-adapted eyes with the position in a like number of



Figures 1-2. **1.** Sagittal section of a dark-adapted eyestalk of *Cambarellus*, X180. **2.** Saggital section of a light-adapted eyestalk of *Cambarellus*, X110. See text for descriptions.

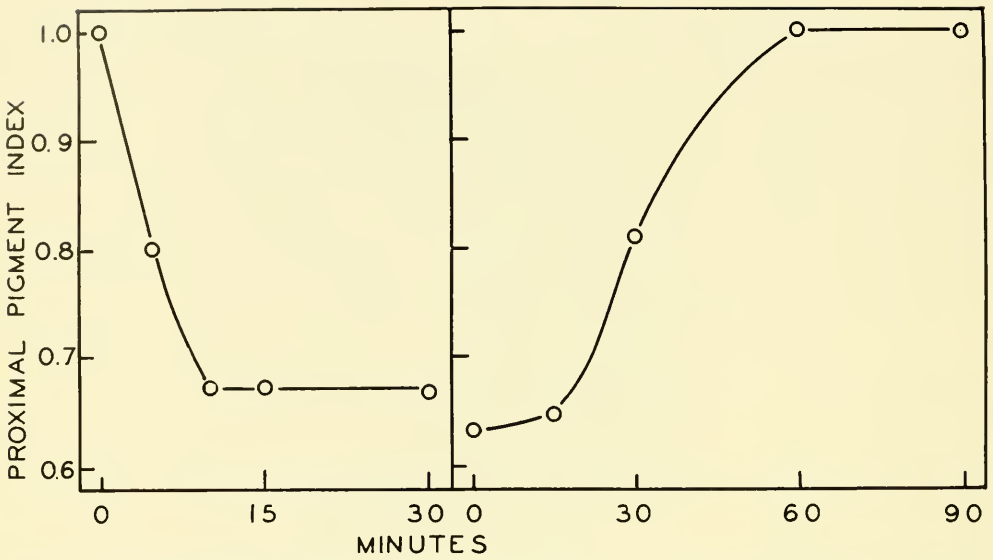


Figure 3. Relationships between proximal pigment index and time; **A** (left) in light, **B** (right) in darkness.

dark-adapted eyes revealed that the distal edge of the distal pigment migrated a mean distance of 92 μ between these two states.

The mean reflecting pigment indexes of the same eyestalks from which the proximal pigment indexes of Figure 3 were obtained are presented in Table 1. Inspection of the data in this table reveals that the reflecting pigment does not migrate. The mean width of the band of reflecting pigment in 32 eyestalks was 49 μ.

TABLE 1.
Reflecting pigment indexes (R. P. I.) of dwarf crayfish kept in light (120 ft. c.) or darkness for different periods of time.

In light		In darkness	
Minutes	R. P. I.	Minutes	R. P. I.
0	0.85	0	0.86
5	0.85	15	0.87
10	0.86	30	0.87
15	0.86	60	0.85
30	0.87	90	0.87

Relationship between proximal pigment index and light intensity.—The object of this experiment was to determine the light intensity at which the proximal pigment became maximally light-adapted. White pans containing dwarf crayfish were exposed to incident illuminations of 17, 29, 75, and 120 ft. c. for two hours. An additional pan of crayfish was placed in the darkroom for two hours. Thirteen proximal pigment indexes, each representing one eyestalk from a different crayfish, were determined for each intensity. The means of the indexes were used in the preparation of Figure 4. Maximal light-adaptation of the proximal pigment required an illumination of approximately 75 ft. c.

Effect of extracts of eyestalks and supraesophageal ganglia with the circumesophageal connectives attached upon the position of the proximal retinal pigment.—Extracts having a concentration of 100 eyestalks per ml of Van Harreveld's solution (Van Harreveld, 1936) were prepared. The dose per crayfish was 0.02 ml. The assay animals were maintained for two hours in black containers under an illumination of 29 ft. c. prior to injection of the extract. A preliminary experiment had revealed that under these conditions the proximal pigment would be close to midway between the maximally light-adapted and maximally dark-adapted positions. Each crayfish in a control group received 0.02 ml Van Harreveld's solution. The animals were killed 45-60 minutes after injection of the eyestalk extract or saline. As anticipated, the distal pigment of the crayfish injected with eyestalk extract showed a light-adaptational response. However, the proximal pigment showed a dark-adaptational response; this

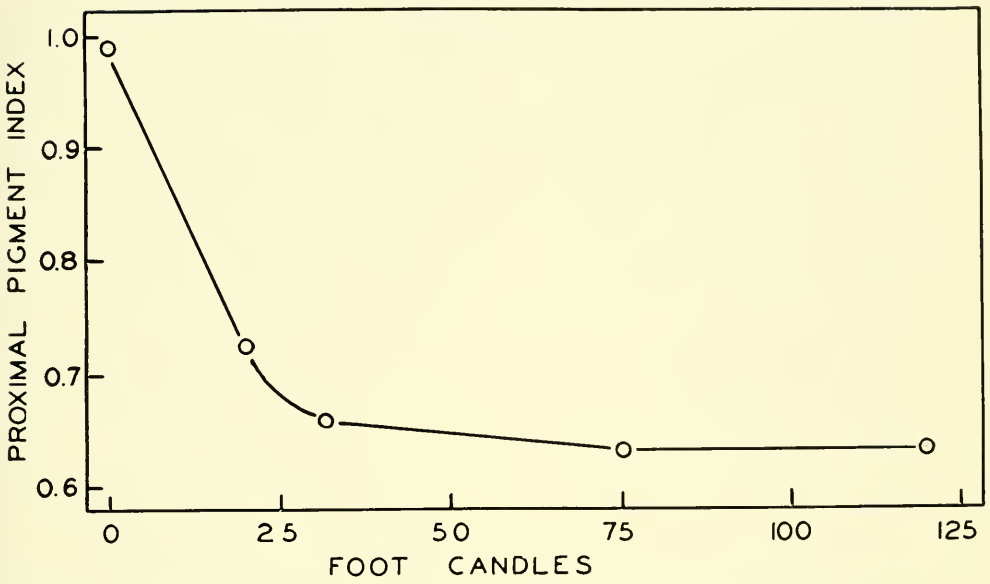


Figure 4. Relationship between proximal pigment index and incident light intensity.

pigment had migrated proximally. The experiment was done three times with the same results. The proximal pigment indexes of the crayfish used in the three experiments are shown in Figure 5. The mean proximal pigment index of the controls was 0.73; that of the experimentals was 0.88. Student's *t* test revealed that the difference between the means was highly significant ($p < 0.001$). The eyestalk extracts had caused the proximal pigment to migrate 56 percent of the mean distance between the position of the pigment at the start of the experiment and the fully dark-adapted position. Eyestalk extracts injected into dark-adapted crayfish had no effect on the proximal pigment, but, as expected, light-adapted the distal pigment. In two experiments extracts of supraesophageal ganglia with the circumesophageal connectives attached, 50 organs per ml, were injected into dwarf crayfish maintained under the same conditions as those that received eyestalk extract. Here also, a dark-adaptational response of the proximal pigment was observed. Thirteen animals received extract and a like number served as controls. The mean proximal pigment indexes for the experimentals and controls were 0.86 and 0.74 respectively. The response was not as large as observed with eyestalk extracts. However, Student's *t* test revealed that the difference between the means was highly significant ($p < 0.001$).

Discussion

Light-adaptation of the proximal retinal pigment of *Cambarellus* required 10 minutes and dark-adaptation 60 minutes. Comparison of these values with previously determined times (Fingerman, 1957) for light-adaptation (30 minutes) and dark-adaptation (90 minutes) of the distal pigment in the same crayfish, reveals that both processes are slower for the distal pigment than the proximal pigment. Furthermore, with both pigments, dark-adaptation is slower than light-adaptation. The rates of migration of these pigments have not been determined in any other crayfish. However, de Bruin and Crisp (1957) determined the times required for light-adaptation of the distal and proximal pigments in the prawns *Palaemon serratus* and *Pandalus montagui*, and the mysid *Praunus flexosus*. The times required by the proximal pigment in these three crustaceans were 4, 4-6, and 4 minutes respectively. Corresponding times for the distal pigment were 90, 40, and 20 minutes. Just as observed herein with *Cambarellus* the distal pigment migrated slower than the proximal pigment in the three species. The times required for dark-adaptation were not deter-

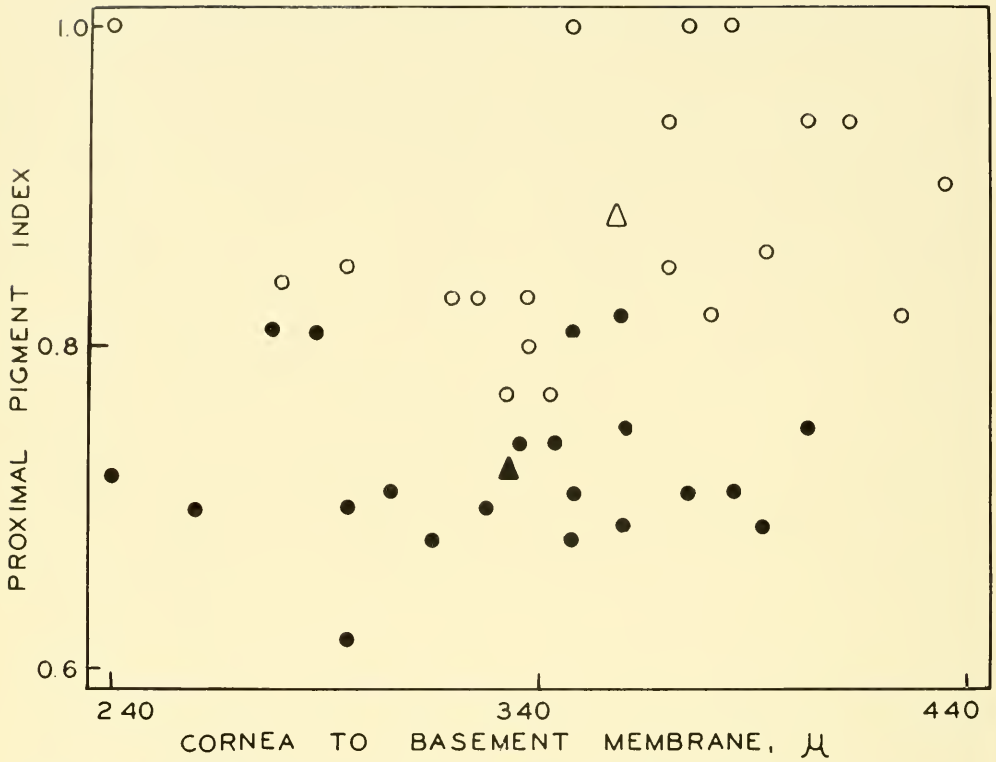


Figure 5. Relationship between proximal pigment index and distance from outer corneal surface to basement membrane. Circles = eyes of crayfish that had received eyestalk extract; dots = eyes of control specimens; triangles = means of the two sets of data.

mined by de Bruin and Crisp who obtained complete light-adaptation of both pigments with an illumination of 1.1 ft. c., a very low value compared with what has been found for other crustaceans. For example, with *Cambarellus,* an illumination of approximately 75 ft. c. was required for complete light-adaptation of the proximal pigment (fig. 4) and about 3500 ft. c. for the distal pigment (Fingerman, 1957). Sandeen and Brown (1952) found that 50 ft. c. was required for complete light-adaptation of the distal pigment of the prawn *Palaemonetes vulgaris.*

Bernhards (1916), apparently the first investigator of retinal pigment migration in a crayfish, found that the distal pigment of a dark-adapted European crayfish, *Astacus astacus,* resided in the region of the crystalline cone of each ommatidium, just as in *Cambarellus.* However, in a light-adapted eye the distal pigment still abutted against the cornea but extended proximally beyond the middle of the crystalline cone stalk, forming a broader band than in the dark-adapted condition. In contrast, in *Cambarus bartoni,* reported by Welsh (1939), and in *Cambarellus* (fig. 2) the distal pigment migrated proximally in light with the result that the portions of the distal pigment cells among the crystalline cones were devoid of pigment. In these three crayfishes all of the proximal pigment lay proximal to the basement membrane in the dark-adapted condition. However, in *C. bartoni* all of the proximal pigment migrated distal to the basement membrane in light, whereas in light-adapted *Cambarellus* and *Astacus astacus* some of the proximal pigment always remained proximal to the basement membrane. The position of the reflecting pigment cells, however, was the same in the three crayfishes.

Welsh (1930) noted that the band of distal pigment in the prawn *Palaemonetes vulgaris* is wider in the dark-adapted condition than in the light-adapted one, but in crayfishes the opposite situation is found.

For example, the mean width of the distal pigment in 15 light-adapted eyes of *Cambarellus* was 99 μ, but only 61 μ in 15 dark-adapted eyes, whereas in *Palaemonetes* the values presented by Welsh (1930) were 70 μ in darkness and 35 μ in light. This difference may depend upon the manner in which the distal pigment migrates. Welsh (1930) believed that migration of the distal pigment in *Palaemonetes* is accomplished by shortening of contractile fibrils whereas in crayfishes protoplasmic streaming may be responsible (Kleinholz, 1961).

The mean proximal pigment index for *Cambarellus* in a white pan at 29 ft. c. was 0.66 (fig. 4). At the same light intensity, the mean proximal pigment index of crayfish in a black pan was 0.73 (fig. 5), *i.e.* the pigment was closer to the dark-adapted position in specimens in black pans than in white ones. A similar situation was observed with the distal pigments of *Palaemonetes* by Sandeen and Brown (1952) and of *Cambarellus* by Fingerman (1957). The difference between the positions of the distal pigments was due to the fact that a black background decreased the brightness of the visual field and was not a true background or albedo response. Further investigation is required, however, before one can say with certainty that this explanation also applies to the proximal pigment of *Cambarellus*.

Evidence was presented herein for a principle in the eyestalk and supraesophageal ganglia with the circumesophageal connectives attached that dark-adapted the proximal pigment of *Cambarellus*. As mentioned above, Kleinholz (1936) noted that eyestalk extracts did not cause migration of the proximal pigment in *Palaemonetes*. Welsh (1939), on the other hand, observed that eyestalk extracts of *Cambarus bartoni* light-adapted the proximal pigment.

The immediate responses to the eyestalk extracts were light-adaptation of the distal pigment but dark-adaptation of the proximal pigment. When extracts of the optic ganglia from eyestalks of *Cambarellus* were assayed on the distal pigment, the dark-adapting hormone expressed itself only after the light-adapting principle had run its course (Fingerman, Mobberly, and Sundararaj, 1959). Herein, because of the apparent absence of a light-adapting principle for the proximal pigment, the dark-adapting substance for the latter pigment was able to operate immediately.

Summary and Conclusions

1. The distal and proximal retinal pigments of the dwarf crayfish, *Cambarellus shufeldti*, migrate in response to changes in intensity of illumination. The position of the reflecting pigment is fixed.

2. The width of the distal pigment layer is greater in light-adapted eyes than in dark-adapted ones.

3. The times required for maximal light-adaptation and maximal dark-adaptation of the proximal pigment were determined. Maximal light-adaptation occurs faster than maximal dark-adaptation.

4. The relationship between the position of the proximal pigment and light intensity was determined.

5. Evidence is presented for a principle that causes dark-adaptation of the proximal retinal pigment.

6. The data are discussed in relation to the findings of other investigators.

References Cited

Bernhards, H. 1916. Der Bau des Komplexauges von *Astacus fluviatilis* (*Potamobius astacus* L.). Ein Beitrag zur Morphologie der Decapoden. *Zeit. wissen. Zool.* 116: 649-707.

de Bruin, G. H. P. and D. J. Crisp 1957. The influence of pigment migration on vision of higher Crustacea. *Jour. Exper. Biol.* 34: 447-463.

Fingerman, M. 1957. Regulation of the distal retinal pigment of the dwarf crawfish, *Cambarellus shufeldti*. *Jour. Cell. and Comp. Physiol.* 50: 357-370.

_____, W. C. Mobberly, Jr. and B. I. Sundararaj 1959. Hormonal regulation of the distal retinal pigment of crayfishes, and the effects of long exposure to light and darkness. *Amer. Midl. Nat.* 62: 429-439.

Kleinholz, L. H. 1936. Crustacean eyestalk hormone and retinal pigment migration. *Biol. Bull.* 70: 159-184.

_____ 1949. Responses of the proximal retinal pigment of the isolated crustacean eyestalk to light and to darkness. *Proc. Nat. Acad. Sci. Wash.* 35: 215-218.

_____ 1961. Pigmentary effectors. *In The Physiology of Crustacea, Vol. II, Sense Organs, Integration, and Behavior*. T. H. Waterman, ed. Academic Press, Inc., New York.

Sandeen, M. I. and F. A. Brown, Jr. 1952. Responses of the distal retinal pigment

of *Palaemonetes* to illumination. *Physiol. Zool.* 25: 222-230.

Van Harreveld, A. 1936. A physiological solution for fresh water crustaceans. *Proc. Soc. Exper. Biol. and Med.* 34: 428-432.

Welsh, J. H. 1930. The mechanics of migration of the distal pigment cells in the eyes of *Palaemonetes*. *Jour. Exper. Zool.* 56: 459-494.

———— 1939. The action of eyestalk extracts on retinal pigment migration in the crayfish. *Cambarus bartoni*. *Biol. Bull.* 77: 119-125.

Abstract

Photomechanical adaptation of the compound eye of the dwarf crayfish, *Cambarellus shufeldti*, in response to changes in intensity of illumination is described. The reflecting pigment does not migrate, whereas the distal and proximal pigments show considerable movement. Evidence is presented for a proximal pigment dark-adapting hormone in the eyestalk and supraesophageal ganglia with the circumesophageal connectives attached. Maximal light-adaptation of the proximal pigment requires 10 minutes; 60 minutes are necessary for maximal dark-adaptation. The relationship between the position of the proximal pigment and incident light intensity is also described. An illumination of approximately 75 ft. c. is necessary for complete light-adaptation of the proximal pigment. The width of the distal pigment band is greater in light-adapted than in dark-adapted eyes. These observations are discussed in relation to the data of other investigators.