STUDIES ON THE FAUNA OF CURAÇAO AND OTHER CARIBBEAN ISLANDS: No. 98.

THE DAILY COLOUR RHYTHM OF THE FIDDLER CRAB UCA RAPAX ON CURAÇAO

by

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Rhythm is an inherent characteristic often encountered in animal physiology and behaviour (CLOUDSLEY THOMPSON 1961; *Cold Spring Harbor Symposia* 1960). In animals like insects, crustaceans, amphibians many of the metabolic processes, sexual behaviour, locomotor activity and colour change are found to be synchronized with daily, synodial or yearly rhythms. Whether these organisms have innate biological clocks or are influenced and regulated by their ambient physical environment is still a challenging problem (BROWN 1959).

The colour rhythms of fiddler crabs, genus *Uca*, have been widely investigated. ABRAMOWITZ (1937) first described the colour rhythm of *Uca* as consisting of two phases: a light-coloured one during the night and a dark-coloured one in daytime, both being independent of background, temperature or the intensity of illumination. Many species, besides showing a daily colour rhythm, exhibit also a lunar or tidal rhythm which manifests itself during the day at low tide by a deepening of the day-time black colour. Particularly in the North-American species *Uca pugnax* and *Uca pugilator* (BROWN et al. 1953, FINGERMAN 1959) and, more recently, in some species from equatorial Brazil (BARNWELL 1963) as well, these rhythms have also been found to persist under constant laboratory conditions.

Colour changes in *Uca* are caused by pigment migrations in the chromatophores, and the endocrine system plays an essential role in the regulation of these phenomena. In *Uca*, the hormones responsible are present in the sinus gland of the eyestalk and are stored in

granules with a diameter of 0.1 to 0.3 micron (PEREZ-GONZALEZ 1957). Extirpation of the eyestalk results in persistent blanching of the animal, and injection of an eyestalk extract in eyestalkless animals causes a temporary darkening. However, the amount of the darkening principle in eyestalks has not been found to vary synchronously with the daily colour rhythm (BROWN in WATERMAN 1961).

The present experiments were performed with the tropical fiddler crab *Uca rapax* (Smith) during my stay at the Caribbean Marine Biological Institute at Curaçao, during the months of January through June, 1964 (Fig. 55).

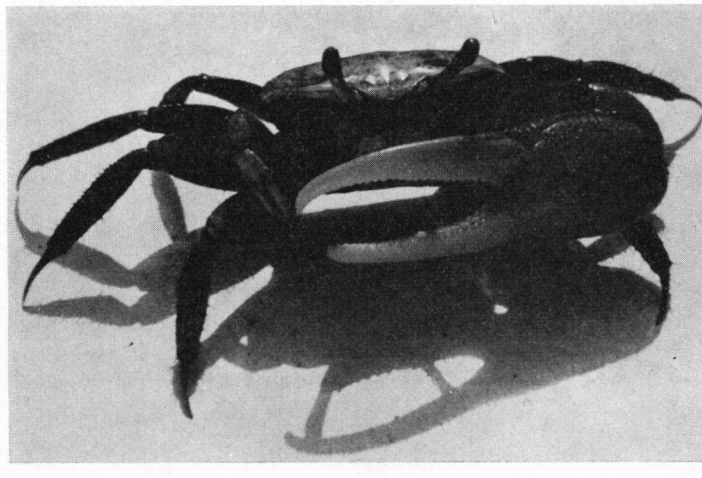

Fig. 55. Male *Uca rapax* from Piscadera Baai, CURAÇAO.

I am greatly indebted to Dr. A. C. J. BURGERS for his constant help, critical evaluation and stimulating enthusiasm. The director and personnel of the Caribbean Marine Biological Institute made my stay in Curaçao both fruitful and pleasant. I am grateful for their assistance. I wish also to thank Dr. L. B. HOLTHUIS for his identification of the crabs, Dr. J. H. WESTERMANN for his sincere interest in my work, and Dr. P. WAGENAAR HUMMELINCK and Dr. T. C. LEE for reading the manuscript.

Tide recordings obtained continuously at the nearby Annabaai harbour were kindly placed at our disposal by the officials of the Annabaai Havenbureau, Willemstad, Curaçao.

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Material and Methods

Specimens of *Uca rapax* were collected near the high tide level of the mud flats along the inner part of Piscadera Bay (Curaçao) (Plate VII). The animals were kept in aquaria containing seawater with depths varying from 0.5 to 2 cm along a sloping plane, and were fed with tinned meat (dog's food). During the experiments the animals were kept separately in small glass dishes containing small amounts of seawater.

Rhythm studies were done in a laboratory room where temperature and light variations were similar to that outdoors. To provide constant conditions for study, an air-conditioned darkroom maintained at an average temperature of 25°C was used. For constant illumination, the animals were illuminated by a bulb. The light received ranged from 1200 to 1500 Lux.

Eyestalk extracts were prepared from dried eyestalks which were crushed in a mortar and extracted with seawater for three hours. Thereupon, the liquid was centrifuged (1500 r.p.m.) for 3 minutes and the supernatant used. Blood was collected by suction after

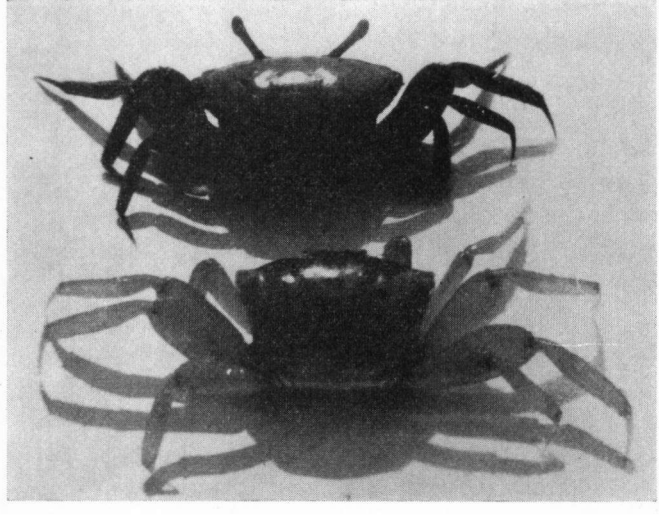


Fig. 56. Normal and eyestalkless female *Uca rapax* from Piscadera Baai, Curaçao. – The normal animal shows the dark day stadium of the colour rhythm. Eyestalkless animals are light during day and night.

cutting the legs of the crabs. The blood was either frozen for storage or directly used.

The colour rhythm was followed by observing changes in the melanophores on the dorsal part of the merus of the two posterior walking legs (Plate VIIIa). With a stereomicroscope (magnification 40×) the dispersion of the pigment was determined according to the HOGBEN & SLOME (1931) index. According to this index stage 1 (M.I. 1) represents fully concentrated, and stage 5 (M.I. 5) represents fully dispersed, pigment granules (Plate VIIIb). For these observations it was necessary to illuminate each animal for about 30 seconds.

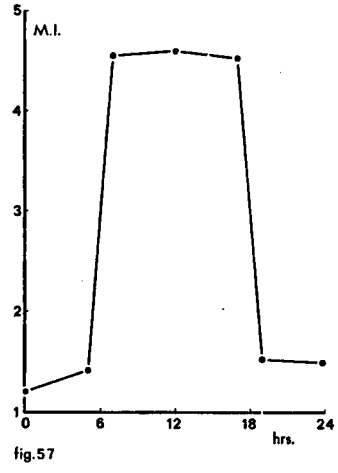


Fig. 57. Daily colour rhythm of the melanophores of *Uca rapax* under normal laboratory conditions during a 24 hour period. M.I. 1: black pigment fully concentrated; M.I. 5: black pigment fully dispersed. Each point is the average of 30 animals.

The dispersing activities of eyestalk extracts and blood samples were tested by injecting 0.1 cc blood or 0.1 cc of various dilutions of eyestalk extracts into the third right walking leg between the basis and the ischium of six eyestalkless animals (3 males and 3 females). The eyestalks were extirpated 24–48 hours before the test (Fig. 56), and the injections were given at noon. The reactions of the melanophores on the dorso-lateral side of the fourth left walking leg of the animals were observed and recorded.

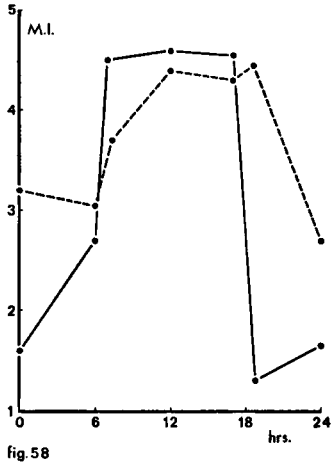
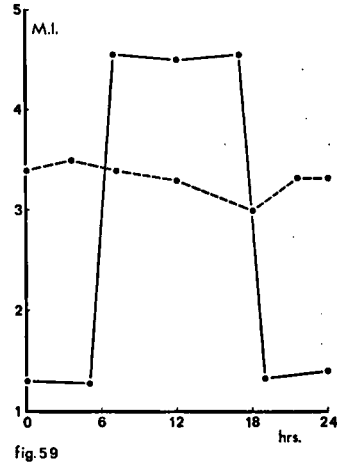


fig. 58



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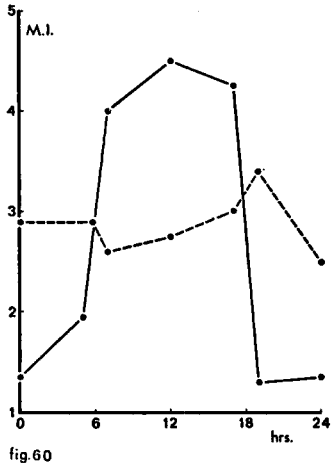

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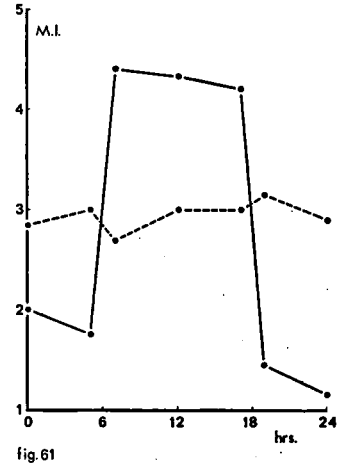


fig. 61

Fig. 58–61. Colour rhythm of *Uca rapax* under constant illumination (dashed lines) compared with the rhythm of animals placed under laboratory conditions (solid lines). Each point is the average of 10 animals. Represented are data obtained on day 2 (Fig. 58), day 19 (Fig. 59), day 34 (Fig. 60) and day 54 (Fig. 61).

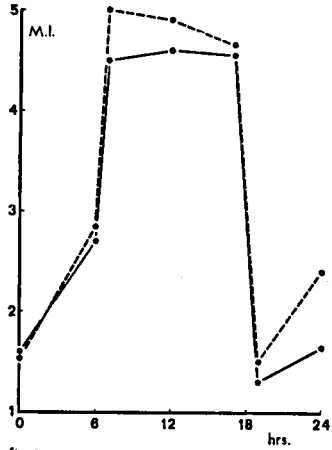
Results

Rhythm

From a group of 39 animals 9 were selected at random and their eyestalks extirpated. Thereupon the whole group was placed in a laboratory room for 8 days and the melanophore-indices of these animals were, as a rule, determined according to the following schedule: at midnight, 5 AM, 7 AM, noon, 5 PM, and 7 PM (Fig. 57). On the ninth day (this day will now be called number 1 for convenience) 10 normal crabs, 5 males and 5 females, were brought under constant illumination at 7 AM. The same number was also placed in constant darkness at 7 PM. The remaining 10 normal animals (control group) remained under the previous conditions. Most of the 9 eyestalkless animals died after repeated moulting, the rest of these animals was removed. The colour changes in the control group and in the group kept in constant darkness were followed from day 1 (January 30th) until day 132 (June 30th). Changes in the group living under constant illumination were followed until day 54 (March 23th). The average results obtained with animals placed under constant illumination can be seen in Figs. 58–61, and those of animals kept in constant darkness in Figs. 62–65.

The animals of the control group maintained the colour rhythm during the 132 days of observation under laboratory conditions (Figs. 58–65). However they showed some variation in dispersion during daytime, but on evaluation of these small differences according to the high and low tide levels occurring during the day, no clear correlation could be seen.

Eyestalkless animals did not show any colour rhythm at all. The black pigment constantly stayed in a fully concentrated stage. Animals under constant illumination showed beginning on day 2, the maximum of darkening shifting from 12 to 6 PM, and the minimum from 24 to 6 AM. Later on, the maximum shifted to 10 PM at day 8 and to 3 AM at day 18. From day 2 till day 12 an average day-night period of 24.9 hours can be calculated. The differences between maximum and minimum M.I. decreased progressively during the experiment, and at day 54, no differences could be further detected. The melanophores remained persistently in a stage of



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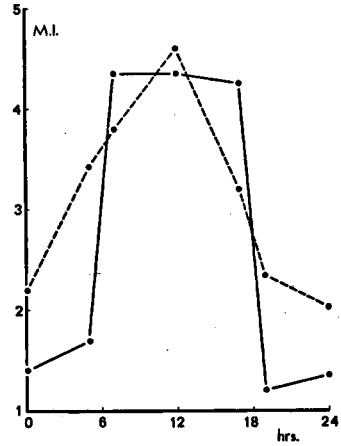

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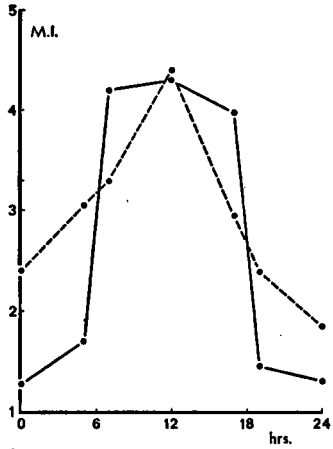

fig. 64

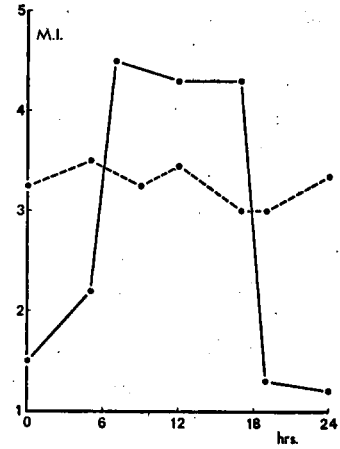

fig. 65

Fig. 62–65. Colour rhythm of *Uca rapax* in constant darkness (dashed lines) compared with the rhythm under laboratory conditions (solid lines). Each point is the average of 10 animals. Represented are data obtained on day 2 (Fig. 62), day 47 (Fig. 63), day 90 (Fig. 64) and day 132 (Fig. 65).

intermediate dispersion both during day and night. No changes could be correlated with changing tide levels.

Animals in constant darkness became initially darker in daytime than the controls, but after a few days, they also became darker at night than the control animals. The extreme darkening at daytime, as well as the higher minima at night, became restricted to a few

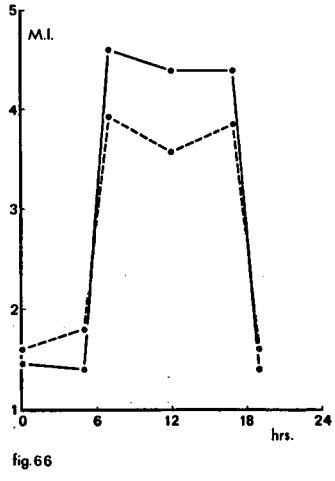

Fig. 66. Colour rhythm of *Uca rapax* the second day under normal laboratory conditions after a 132 day stay in constant darkness (dashed line) as compared to the animals which stayed under normal laboratory conditions during the same time (solid line). Each point is the average of 10 animals.

hours. The rapid day-night colour change vanished, indicating an impeded rate of concentration and dispersion of pigment. The colour change was evident untill about day 110, and at day 132, no detectable differences could be further observed. At that time the animals were brought back to normal laboratory room day-night conditions. Under these conditions the animals showed on the second day already a rhythm similar to that of the controls (Fig. 60). lation could be seen with tide levels within this group also.

Injections with eyestalk extracts

Eyestalks were cut from male and female animals at noon on different days and dried for 48 hours at 40°C. Extracts were prepared

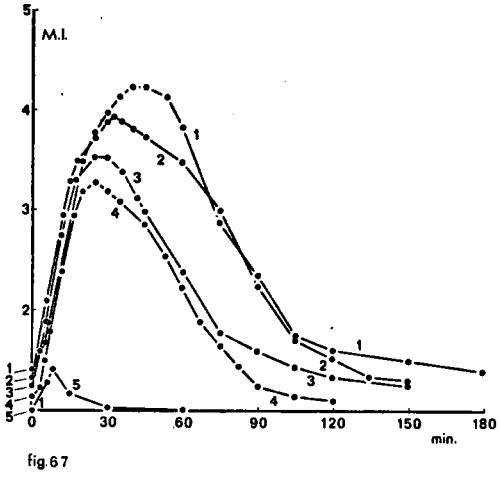

Fig. 67. Dispersion of melanophores after injection of different concentrations of eyestalk extracts in eyestalkless animals.
1: 0,1 mgr eyestalk extract per animal (average of 10 animals).
2: 0,05 mgr per animal (average of 14 animals).
3: 0,02 mgr per animal (average of 35 animals).
4: 0,01 mgr per animal (average of 10 animals).
5: 0,1 cc vehicle (seawater) per animal (average of 12 animals).

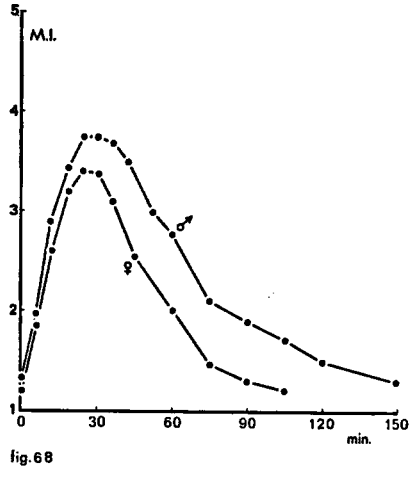

Fig. 68. Dispersion of melanophores after injection of 0.02 mgr eyestalk extract in male (average of 17) and female (average of 18) eyestalkless test animals.

from 6 to 10 eyestalks. Fig. 67 shows the responses of eyestalkless animals after injections of extracts of different concentrations (the calculations are based on dried-weight of the starting material). The injected volume was always 0.1 cc per animal. It is evident that a dose-response relationship exists for extracts prepared in this manner. The injection of the vehicle gave a very transient and slight darkening.

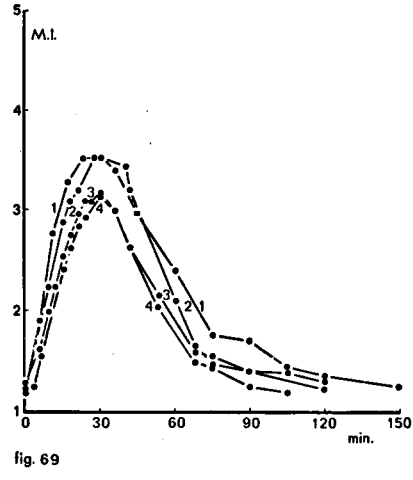

Fig. 69. Dispersing activity of eyestalk extracts, prepared from eyestalks extirpated at different times of day and night.
1: eyestalk extract of 12 AM (average of 35 animals).
2: eyestalk extract of 12 PM (average of 22 animals).
3: eyestalk extract of 6 AM (average of 18 animals).
4: eyestalk extract of 18 PM (average of 18 animals).

The responses of male and female eyestalkless animals after injections of the same dose of extract (0.02 mgr/0.1 cc per animal) are shown in Fig. 68. The M.I. in the males reached a higher maximum, and the reaction was of longer duration than in the females. Animals having the same carapax-width were selected, but due to the large male chela the body weight of the male was about twice that of the female (average male: 3.0 gr, average female: 1.7 gr). Because of these sex differences in body size, the greater sensitivity of the male in response to a given dose of extract becomes surprisingly

even more significant when the injected material is considered to be presumably more diluted in the male than in female.

Fig. 69 shows the results obtained following injections into eyestalkless animals of 0.02 mgr of eyestalk extract prepared from eyestalks extirpated at different times of day and night. From this graph it is clear that the amount of pigment-dispersing hormone present in eyestalks is more or less the same at the different time periods studied, both during the day as well as during the night.

Injections with blood

It was assumed that the daily colour change may be regulated by hormonal material originating from the eyestalk and circulating in blood. However, since we were not able to demonstrate a difference in pigment dispersing activity in eyestalks extirpated in daytime or at night, we suspected that a greater amount of pigment dispersing activity might exist in blood taken in daytime than in blood taken at night. In order to investigate this, attempts were undertaken to determine the pigment dispersing activity in blood.

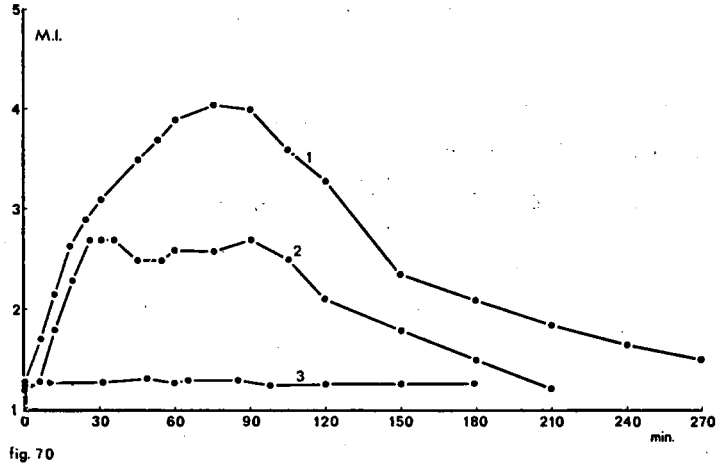

Fig. 70. Dispersing activity of blood collected at noon and prepared in three different ways before injection in eyestalkless animals.
1: blood frozen and thawed (average of 12 animals).
2: blood kept outside the body for 45 minutes (average of 12 animals).
3: blood injected directly after collection (average of 6 animals).

In the first experiments blood was collected from a group of normal animals and immediately frozen and stored at —5°C. After defrosting, different amounts were injected in eyestalkless animals. Blood taken from normal animals either at noon or at midnight had a high pigment dispersing activity. Since we had not expected this degree of activity in blood taken from light (midnight) animals, we therefore further tested blood taken from animals which had their eyestalks extirpated two days previously. Surprisingly, we found this blood also to be very active. Injections of a 1.3 M saccharose solution which is isotonic with *Uca* blood (PEREZ-GONZALEZ 1957) in volumes equal to blood injections showed no dispersing activity. Blood sampled from normal animals at noon and injected immediately into eyestalkless animals (0.2 cc blood per animal) also had no black pigment dispersing activity. On the other hand, blood kept outside the body for 45 minutes at room temperature contained a high activity, just as the frozen blood. Fig. 70 shows the results of injections in eyestalkless animals of blood collected from normal animals at noon and prepared according to the three different methods described. It is concluded that blood which was either frozen or kept outside the body for 45 minutes has a non specific effect on the pigment of melanophores. In blood of normal animals no real pigment dispersing activity could be found.

DISCUSSION

The colour rhythm of *Uca rapax* in normal day-night conditions in the laboratory can be observed for at least 132 days, the period of observation in the present study. The colour change is shown to be very rapid and follows the rapid day-night successions in tropical areas. This finding agrees with the results obtained by BARNWELL (1963) with *Uca mordax*, *Uca maracoani* and *Uca rapax* of Brazil. In constant darkness *Uca rapax*, in the present experiments, demonstrated a persistence of colour change which lasted for a much longer period (110 days) than the period of 2 to 4 weeks which has been described for *Uca pugnax* (BROWN & WEBB 1948). The rhythm under constant illumination is comparable to that described by BARNWELL (1963) for *Uca rapax* of Brazil. This author mentions a phase shifting

of the maximum darkening from 12 (noon) to 18 PM in a period of 6 days. *Uca pugnax* also shows a maximum shifting to 18 PM (BROWN et al. 1953). In our experiments this phenomenon was already evident in the first two days of constant darkness. Later on, the maximum was further shifted to 3 AM. Observations of any further shifting was not possible because of the decreasing amplitude of change from maximum to minimum darkening. BROWN et al. (1953) and FINGERMAN (1956a) showed persistent tidal and semilunar rhythms of colour change in *Uca pugnax*, *Uca pugilator* and *Uca speciosa*. This was not observed by us in *Uca rapax* of Curaçao, probably due to the irregularity of the tides in Curaçao (DE HAAN & ZANEVELD 1959) and to the fact that the habitat of *Uca rapax* is around and above the high tide level.

Although the existence of neurosecretory particles in the sinus glands of *Uca* has been determined (PEREZ-GONZALEZ 1957), it was not possible in the present experiments, to demonstrate a difference in dispersing activity in extracts of eyestalks removed either at day or at night. WEBB et al. (1954) postulated a 24 hour rhythm in eyestalkless animals which is presumably due to cyclically varying quantities of black pigment concentrating hormones circulating in blood. Since we always injected the eyestalk extracts in eyestalkless animals at noon, this concentrating hormone could not likely therefore, have interfered with the results obtained on the dispersing activities of eyestalk extracts. The dispersing activity of the eyestalk extracts used in our experiments was found to be similar to the activity found by BURGERS (1958) working with the same species, also at Curaçao. FINGERMAN & FITZPATRICK (1956) demonstrated that the pigment in the melanophores in female specimens of *Uca pugilator* was more dispersed than the pigment in males. Removal of the large chela from the male resulted in approximately equal coloration for both sexes. We demonstrated that after injection of eyestalk extracts there is a more pronounced darkening in the males than in the females, although the circulatory volume in the male is presumably larger than in the female. Our results showed the greater sensitivity of the melanophores of eyestalkless males.

FINGERMAN (1956b) demonstrated in *Uca pugilator* that the period of blanching of isolated perfused legs is longer when 0.05 cc of

blood of dark animals is added to the perfusion fluid. This effect could not be seen, when blood of light animals instead of dark animals is added. He suggested the existence of a black pigment dispersing hormone in blood of dark animals and a black pigment concentrating hormone in blood of light animals. In our experiments, it was not possible to demonstrate a dispersing activity in blood of dark specimens of *Uca rapax* when a volume of 0.2 cc of blood was injected in eyestalkless animals. However, we found an intense black pigment dispersing activity in blood which had been kept outside the body for a relatively short duration (45 min.). Although these findings have no apparent physiological meaning, it is clear that pigment cells may be influenced by substances or manipulations other than hormonal materials. The slight darkening after injecting seawater in eyestalkless animals may be of significance in this respect.

SUMMARY

1. *Uca rapax* of Curaçao showed a daily colour rhythm. The animal is dark during the day and light during the night. This rhythm persisted in animals which were placed in constant darkness for 110 days, and in those which were placed in constant illumination for 40 days. The latter condition caused a gradual phase shift of the maximum darkening from 12 (noon) to 3 AM within a period of 18 days. The maxima and minima of animals kept in constant darkness were higher than in control animals. No correlation between tide levels and colour change was found.

2. Eyestalk extracts were found to contain a pigment dispersing activity. There was no difference in the amount of dispersing material between the extracts of eyestalks removed either in daytime or at night. Males reacted more strongly upon injection of eyestalk extracts than females.

3. Blood taken from eyestalkless or normal animals had no dispersing activity, although a "non-specific" dispersing activity can be demonstrated in blood kept outside the body for 45 minutes.

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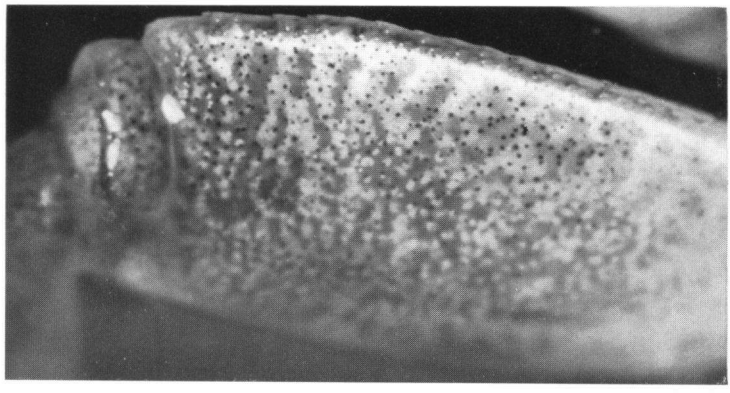
PLATE VII

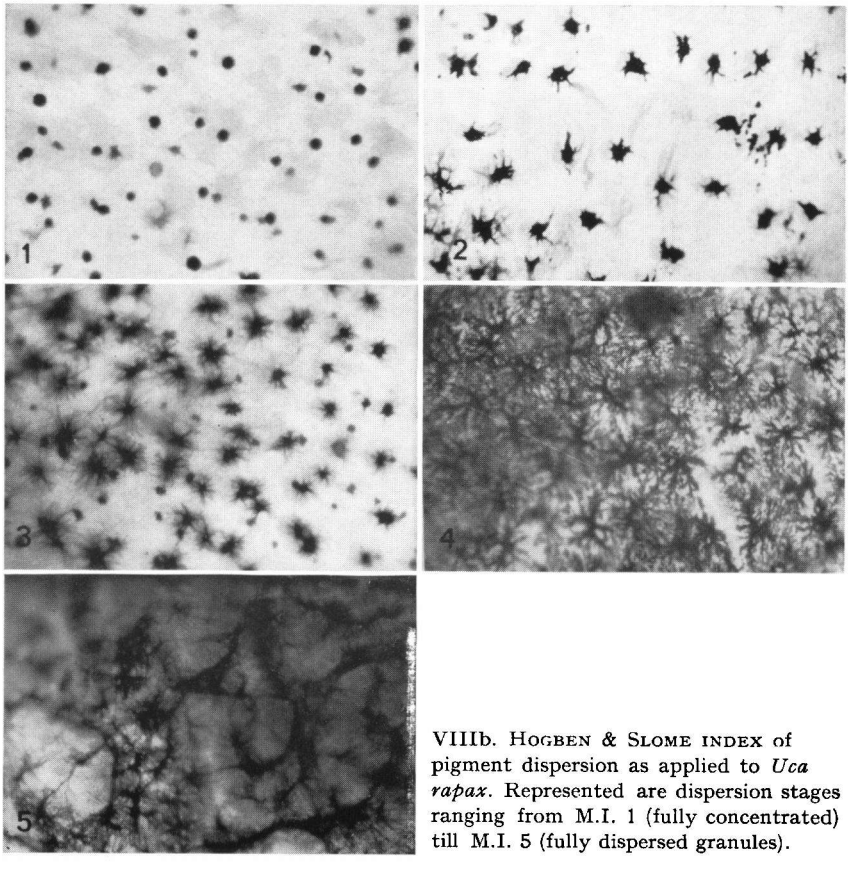
VIIa. Collecting sites of *Uca rapax* along the mangrove belt of northeastern Piscadera Baai, as seen from Veerisberg, CURAÇAO.

VIIb. Collecting site of *Uca rapax* at Piscadera Baai, CURAÇAO. Openings of burrows can be seen.

PLATE VIII



VIIIa. Merus of the last walking leg of an eyestalkless *Uca rapax*. Pigment granules are fully concentrated.



VIIIb. HOGBEN & SLOME INDEX of pigment dispersion as applied to *Uca rapax*. Represented are dispersion stages ranging from M.I. 1 (fully concentrated) till M.I. 5 (fully dispersed granules).