J. mar. biol. Ass. India, 1976, **18** (3) : 441-447

DIURNAL VARIATIONS IN THE POTENCY OF CHROMATOPHOROTROPINS OF THE NEUROSECRETORY SYSTEM OF THE PRAWN *CARIDINA WEBERI*

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ABSTRACT

The freshwater prawn *Caridina weberi* was found to show a diurnal rhythm of colour change when placed on a specially devised 'neutral' background in natural light. The red and white pigments were dispersed during day time and fully concentrated at night. A daily cycle of chromatophorotropin contents was noticed in eyestalk, supraoesophageal ganglia and circum-oesophageal commissures kept in such a condition. Among these, the red pigment dispersing hormone (RPDH) of supraoesophageal ganglia and the white pigment concentrating hormone (WPCH) of circumoesophageal commissures were more in amount during day time while the RPDH of eyestalk was more in the night time. The red pigment concentrating hormone of various nervous tissues changed little in amount during the 24-hour cycle.

INTRODUCTION

THE DAILY RHYTHM of movement of pigments within the chromatophores of crustaceans have attracted the attention of many biologists. Among the decapods much attention has been paid to the rhythmical colour changes in the Brachyura (Fingerman, 1963). However, very little attention has been paid to the circadian rhythm of colour change in the natantian decapods.

Scheer (1960) reported a diurnal rhythm of colour changes in the monochromatic erythrophores of *Palaemon*. The prawns were almost colourless in the day light while at night the body took bright red colour. Humbert (1965) observed a similar diurnal rhythm of colour change in the marine species, *Palaemon serratus*. This rhythmic phenomenon was also observed by Aoto (1966) in the freshwater prawn, *Palaemon paucidens*. In view of the rapidly increasing data on the rhythmic behaviour of colour changes in the crustaceans, it was thought reasonable to study the diurnal rhythm of colour changes in the freshwater prawn, *Caridina weberi*. Besides, an attempt was made to show the relationship between the daily rhythm of colour change and the amount of chromatophorotropins present in the central nervous organs.

The authors wish to express their sincere thanks to Prof. S. Mehdi Ali, Head of the Zoology Department for kindly providing facilities to carry out the work.

Material and Methods

Adult male and female specimens of the freshwater prawn, *Caridina weberi* were collected from the Kham river near Aurangabad, India. The prawns after being brought to the laboratory were maintained in glass aquaria containing aerated tap water. The animals were used from this stock for the experimental work irrespective of sex. In addition to the white and black backgrounds, a special neutral background was used to find the diurnal variation in colour changes in the animal. A glass trough was taken which was covered on the sides and at the bottom with sheets of paper checkered with white and black squares of 3 cm. The trough containing 30 intact animals was placed in an unlit room and no incident light was allowed to illuminate the trough directly. Similarly 60 intact animals were taken and distributed equally in different white and black enamel pans containing fresh water and kept for observation. The animals were maintained in such a condition from 18th to 21st December 1967. The water was changed every day and the pans were put back in original positions. The chromatophores on the dorsal portion of the carapace just above the heart was staged using the Hogben and Slome Scheme (1931). The average stages of the experimental animals were determined on 18th December at 1030 hrs before placing them in their respective backgrounds. The chromatophore stage of white and red pigments was determined at 1200 hrs, 1600 hrs, 2000 hrs and 2400 hrs. The same procedure was repeated on 19th December at four hours interval commencing from 0400 hrs to 2400 hrs and it was continued upto 21st December.

In order to compare the activity of the chromatophorotropins of the central nervous organs at different times of a day, five animals on the netural background were killed at one time and their eyestalks, supraoesophageal ganglia and circum-oesophageal commissures were dissected out at every four hours interval beginning from 0400 hrs to 2400 hrs. Each lot of organs was triturated and suspended in 0.3 ml of distilled water adjusted to pH 7.2 to make the final concentration one-third of an organ or a pair of eyestalks per 0.02 ml of extract. The activity of any organ from animals killed at different times of a day was assayed simultaneously. Only intact prawns were used as assay animals and each of them received a dose of 0.02 ml of extracts. Injection experiments, performed on five black-adapted and five white-adapted animals, were repeated once and the average chromatophorotropic activity was calculated for each extract using the method of Sandeen (1950).

Results

Diurnal rhythm of colour change

Diurnal rhythm of colour changes was observed and the results are presented in Tables 1 and 2. It is clear that the white and red chromatophores have a definite diurnal rhythm on the neutral background, the pigment being dispersed at noon and fully concentrated at midnight. Maximum dispersion of white pigment to stage 4.0 was observed at noon whereas the red pigment dispersed only to stage 2.5. However, the degree of concentration was the same in both the pigments reaching the punctate stage at midnight. Most specimens were found to have the pigments fully concentrated during night from 2000 hrs to 0400 hrs. In the experiments conducted with the white and black background no variation in the migration of either the white or red pigment was seen.

TABLE 1. *Diurnal variation in the white chromatophores of* C. weberi *in the neutral background*

Day of observation	Chromatophore stage and time (hrs)					
	0400	0800	1200	1600	2000	2400
18.12.1967 ..	—	—	3.4	3.0	1.8	1.0
19.12.1967 ..	1.5	3.1	3.8	3.6	1.0	1.0
20.12.1967 ..	1.0	3.0	4.0	3.5	1.0	1.0
21.12.1967 ..	1.0	3.4	4.1	3.0	—	—

TABLE 2. *Diurnal variation in the red chromatophores of* C. weberi *in the neutral background*

Day of observation	Chromatophore stage and time (hrs)					
	0400	0800	1200	1600	2000	2400
18.12.1967 ..	—	—	2.4	2.0	1.0	1.0
19.12.1967 ..	1.0	1.8	2.5	2.1	1.0	1.0
20.12.1967 ..	1.0	2.0	2.3	1.6	1.0	1.0
21.12.1967 ..	1.0	1.8	2.4	1.5	—	—

Diurnal variation of chromatophorotropins

The data on the diurnal variation in the amounts of chromatophorotropins present in the eyestalk (ES), supra-oesophageal ganglia (BR) and circum-oesophageal commissures (CC) are presented in Tables 3, 4 and 5. The red pigment dispersing hormone (RPDH) of the eyestalk increased during the night time when the animals had normally their red pigments fully concentrated (Table 3). However, variation of the RPDH present in the eyestalk was not as remarkable as that present in the supraoesophageal ganglia. The RPDH of the supraoesophageal ganglia was more during the day time than at night (Table 4). This hormone reached the maximum at noon, maintained high titre throughout day time and two hours after the sunset and then decreased gradually throughout the night. With regard to the red pigment concentrating hormone (RPDH), little change was observed in the amount throughout the 24-hour cycle in all the central nervous organs examined.

TABLE 3. *Diurnal variation in the chromatophorotropins of the eyestalk of* C. weberi

Name of the hormone	Chromatophorotropic activity					
	Time (hrs) of observation					
	0400	0800	1200	1600	2000	2400
Red pigment dispersing hormone	2.5	1.5	1.0	1.6	2.1	3.5
Red Pigment concentrating hormone	0.6	0.4	0.7	0.5	0.5	0.8
White pigment dispersing hormone	3.7	3.5	4.0	3.2	3.3	3.0
White pigment concentrating hormone	—	—	—	—	—	—

TABLE 4. *Diurnal variation in the chromatophorotropins of the supra-oesophageal ganglia of* C. weberi

Name of the hormone	Chromatophorotropic activity					
	Time (hrs) of observation					
	0400	0800	1200	1600	2000	2400
Red pigment dispersing hormone	2.6	2.8	4.8	4.5	3.5	2.5
Red pigment concentrating hormone	3.0	3.4	3.7	3.0	3.2	3.1
White pigment dispersing hormone	9.5	14.5	17.6	15.4	12.6	8.5
White pigment concentrating hormone	0.2	—	0.3	0.5	—	—

With respect to the white pigment, almost complete parallelism to the RPDH in the supraoesophageal ganglia was noticed of the white pigment dispersing hormone (WPDH) (Table 4) and of the white pigment concentrating hormone (WPCH) in the CC (Table 5), both being contained in great amount during the day time than at night. It may be concluded that the dispersion of white pigments during day

time is normally brought about by the WPDH actively produced and released immediately from the BR and that their concentration at night is brought about by the WPCH which is continuously produced but released only at night from the CC.

TABLE 5. *Diurnal variation in the chromatophorotropins of the circum-oesophageal commissures of* C. weberi

Name of hormone	Chromatophorotropic activity					
	Time (hrs) of observation					
	0400	0800	1200	1600	2000	2400
Red pigment dispersing hormones	0.5	0.8	0.6	0.7	0.4	0.6
Red pigment concentrating hormone	4.5	4.5	5.0	5.3	5.0	4.8
White pigment dispersing hormone	6.8	7.0	7.5	7.6	7.0	7.2
White pigment concentrating hormone	1.0	1.9	3.0	2.5	1.8	1.0

DISCUSSION

In the prawns kept on a specially devised neutral background the white and red pigments showed a 24-hour rhythm of migration, the pigments being dispersed during day time and concentrated in the night. However, red and white pigments of *Caridina* kept on white and black backgrounds did not show the findings of Aoto (1966) on *Palaemon*. In *Caridina* placed in natural light on a black or white background, the red pigment remained either in a fully dispersed or concentrated state according to the colour of the background during a 24-hour cycle of light. On the other hand, the white pigment of a white adapted prawn that was fully dispersed in day time tend to concentrate in night time whereas on a black background the white pigment showed a persistent maximum concentration. Thus in *Caridina* that are placed on either a black or a white background no appreciable daily variation of chromatophoral behaviour is seen. However, these two background colours appear rather usual for the prawns to encounter in their natural habitats.

Studies on long term background adaptation have shown to affect not only the chromatophorotropin titres in the crayfish (Fingerman and Aoto, 1958) and *Palaemon* (Aoto, 1961) but also the morphological colour change in the crab (Green, 1964) and in five species of natantians (Chassard, 1965). Hence, it seems reasonable to assume that the rhythm of colour change exhibited by the prawns on the neutral background could be regarded as a ' standard ' background which would affect little morphological colour change. The dispersion of white and red pigments during

day time may serve as a protective mechanism. Dispersion of pigments may result in the protection of the protoplasm of the animal, the dispersed region preventing the transmission of light to deeper tissue areas (Brown and Sandeen, 1948).

The experiments on the bioassay of eyestalks, BR and CC at different times of the 24-hour cycle revealed that there are three categories of the hormones in terms of their relationship to the daily rhythm of colour change, namely (a) those which show high titre during day time and a low titre at night, such as the RPDH and WPDH of the BR and the WPCH of the CC, (b) those which show a high titre at night than by day such as RPDH of the eyestalk and (c) those which show little or no change in titres throughout the 24-hour cycle, such as the RPCH of ES, BR and CC and the RPDH and WPDH of the CC.

In crustaceans the chromatophorotropins are synthesized in the neurosecretory system. The function of the system involves the production, transport, storage and release of the secretory materials. In *Caridina* a greater amount of the RPDH in the supraoesophageal ganglia during day time seemed most closely related to the dispersed condition of red pigment during that time. Therefore, if this hormone plays a principal role in yielding dispersion of red pigments in the rhythm, it is considered that following synthesis this hormone is released promptly into the circulation. The RPDH of ES also shows definite cycle but is less pronounced than that of BR. Contrary to the physiologically corresponding substance in the BR, it was contained in greater quantity during night time than in day time. At least two explanations seem possible, (1) the eyestalk RPDH is derived from the RPDH of BR which, continuously synthesized but not used in the night time, is transported to and accumulated in the ES or (2) the ES has an independent rhythm of production of the RPDH which is to be released readily for momentary colour changes. In the CC the level of the RPCH exhibited little change in amount throughout the cycle whereas that of the WPCH was higher during day time than at night. It is assumed that the RPCH is probably synthesized continuously at the same rate through the cycle and is reserved for a temporary colour change. The high level of the WPCH during the day time when the white pigments are more dispersed than at night may be explained by that, besides the pigments' primary response to change of light-intensity (Knowles, 1940), the WPCH when not used is accumulated in the tissue. The present findings are in agreement with those made earlier by Aoto (1966) in another freshwater prawn, *Palaemon paucidens*.

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