Effects of photoperiod on molting in the brachyuran crabs, Hemigrapsus nudus (Dana, 1851) and Cancer magister (Dana, 1852)

by

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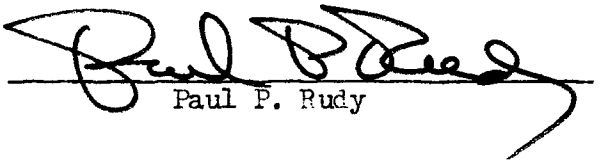
APPROVED: 

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The effects of photoperiod on the molting behavior of Hemigrapsus nudus and Cancer magister were determined by subjecting juveniles of both species to three light regimes: extended darkness (DD), long days (LD 16:8) and short days (LD 8:16).

Effects of photoperiod on the time of molting was examined for both brachyurans. A definite molting response to photoperiods was shown in Hemigrapsus nudus. Molting seemed to occur only during subjective dawn periods. Delayed shifts, due to imposed photoperiods, suggested the presence of endogenous control of dusk-molting in Hemigrapsus nudus. An aversion to molting during pre-dusk intervals was noted in Cancer magister.

Photoperiodic induction of molting in first stage post-larval Cancer magister was also examined. No photoperiodic influence was detected. Instead, the regulation of induction of molting in these juveniles was shown as being under the influence of food and other ambient environmental conditions.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

EFFECTS OF PHOTOPERIOD ON CRUSTACEAN MOLTING

Photoperiodism has been defined as the effect of the environmental photoperiodic rhythm on internal biological rhythmic processes (Beck, 1968). This phenomenon has been known to affect the behavior and physiology of Arthropods for some times. Insects have received the most attention in this area. Studies have indicated that photoperiods, in many insect species, will affect a number of physiological processes including diapause, reproduction and phenology (see reviews by Lees 1955, 1968; Danilevskii, 1965; Beck, 1968; Engelmann, 1970; Saunders 1974, 1976; Tauber and Tauber, 1976).

By contrast, photoperiodic effects in crustacean systems have been studied much less. Apart from an earlier interest in the diurinal rhythmicity of crustacean chromatophores (Abromowitz, 1937; Welsh, 1938) it was not until 1946 when other effects of photoperiod on physiological processes were first examined (Panouse, 1946). Although later papers (Löwe, 1961; Black, 1963; Bulnheim, 1966; Rao and Nagabhushanam, 1967; Burkema, 1968) began investigating different effects of light on crustacean behavior and physiology, the effects of photoperiod on the crustacean molt cycle received little attention.

Bliss (1954 a,b) and Stephans (1955) were the first to show effects of light on molting in decapods. Bliss (1954 a,b) determined the effects of light on the molt cycle of the crab, Gecarcinus lateralis by using

limb bud regeneration as an index for proecdysial (pre-molt) development. The results of these experiments (later confirmed by Bliss and Boyer, 1964) showed that constant light had an inhibitory effect on molting and in certain cases inhibited ecdysis for several months. Constant darkness, on the other hand, was observed as having a favorable influence on molting and appeared to promote ecdysis.

Stephans (1955) worked with the crayfish, Cambarus virilis, and although the results of this work were complicated by excess mortality, an apparent photoperiodic influence on molting was demonstrated. In this experiment, the crayfish were exposed to three different light conditions: a long photoperiod (20 hours of light), a normal photoperiod (9-10 hours of light) and constant darkness. The results showed a greater number of molts occurring in the long photoperiod, fewer in the normal photoperiod and virtually none in constant darkness. Stephans (1955) concluded that Cambarus responded to daily illumination with an increased tendency to molt, and that the strength of this tendency increased with increasing length of photoperiod.

Additional studies showing a certain photoperiodic influence on molting have also been reported in a number of other crustaceans, including cladocerans, macrurans, isopods, and brachyurans (Mobberly, 1963; Parker, 1966; Aiken, 1969; Weise, 1976; Mocguard et. al., 1978). Of these reports, the only study to clearly demonstrate a relationship between photoperiod, molting, and hormonal regulation has been in the crayfish, Orconectes virilis (Aiken, 1969).

In his experiments, Aiken (1969) exposed immature Orconectes virilis to short day (LD 10:14) and long day (LD 20:4) photoperiods.

The crayfish kept in the shorter light regime showed no indication of molting after several months; however, the groups exposed to longer photoperiods molted successfully after only 30 days. These results suggested that there existed a critical or threshold photophase, between 10 and 20 hours, for the induction of pro-ecdysis to occur.

Aiken (1969), also noted an altered response to photoperiods when the animals were collected at different times of the year. He then suggested that the molt inducing influence of a given photoperiod remained constant, while the resistance to this effect changed with time. Thus, in these crayfish, hormonal control of molting was temporally modified by ambient photoperiod. Aiken proposed that this photoperiodic response was advantageous as molting could be restricted to a particularly favorable time of the year.

The molt cycle of many crustaceans is recognized as being controlled by two principle hormones: molting hormone (MH) from Y organs (paired endocrine organs in the thorax) and the molt inhibiting hormone (MIH) from the X organ sinus complex of the eye stalk (Passano, 1960; McWhinnie et. al., 1972). As the X organ is located in the eyestalk, it has been proposed that it might be light sensitive (Aiken, 1969). Bliss and Boyer (1964) clearly showed that the eye was the pathway by which light affected the molt cycle in Gecarcinus lateralis. Aiken (1969) suggested the same for Orconectes virilis and proposed that long days were able to induce a molt by reducing the MIH. Here it was postulated that the level of MIH could be reduced either by inhibition of synthesis at the X organ or inhibition of release by the sinus gland, and that this inhibition could be proportional to the imposed photoperiod. In

such a mechanism, proecdysis could be induced once the MH titer built up sufficiently to overcome the inhibiting influence of the suppressed MIH.

EFFECTS OF PHOTOPERIOD ON THE TIME OF MOLT

Reports described above have pointed to a photoperiodic control in the induction of molting (induction of proecdysis) (Stephans, 1955; Aiken, 1969). Recently a photoperiodic control has also been reported for circadian rhythms in molting of certain crustaceans (Fowler et. al., 1976; Bishop and Herrnkind, 1976; Dexter, 1977). In these reports, the circadian systems were seen as time keeping mechanisms which served to control the time at which molting occurred.

Pittendrigh (1972) has proposed several models for the involvement of circadian rhythms in animals using photoperiods for measuring time. In these models, photoperiods are recognized as Zeitgebers (environmental cues) which can entrain an animals circadian system and permit the measurement of time for certain behaviors.

A number of crustaceans have been cited as molting only during night time periods, or equivalent scotophase (dark phase of an imposed photoperiod) (San Felice, 1966; Fowler et. al., 1971; Bishop and Herrnkind, 1976; Dexter, 1977). Although not all examples of nocturnal molting have been investigated, a circadian control in the time of molt has been suggested for the euphausids, Euphausia pacifica and Thysanoessa spinifera (Dexter, 1977) and the shrimp Paneus duorarum (Bishop and Herrnkind, 1976).

The involvement of photoperiods as an entrainment factor of circadian time keeping systems has been seen as a particularly valuable adaption in these animals. It has been suggested that a mechanism which dictates the 'safest time' (night time) for ecdysis to occur, can be essential in enhancing a greater chance of survival (Bishop and Herrnkind, 1976).

Previous studies have indicated that a number of factors can influence molting in different marine crustaceans. These factors include species, sex, food, temperature, crowding, injury and photoperiod (Bliss and Boyer, 1964; Aiken, 1969; Adelung, 1971; Weise, 1976; Dexter, 1977). In groups in which ambient light is an important factor, the literature described above suggests two different photoperiodic influences on molting. One effect of photoperiod is to influence the rate of molting by inducing proecdysis. The second effect is to influence the time of molt and determine (possibly through a circadian system) the time that molting is to occur.

RESEARCH OBJECTIVES

The purpose of this study is to examine the effects of photoperiod on molting in two brachyurans: Hemigrapsus nudus and Cancer magister. As mentioned above, it appears that photoperiods influence molting in two separate ways. These are: 1) an effect on the time of molting, and 2) an effect on the rate of molting.

In this study, both aspects of photoperiodic influences are examined separately, and two major questions are investigated:

1. What are the effects of photoperiod on the time of molting in Hemigrapsus nudus and Cancer magister?
2. What are the effects of photoperiod on the rate of molting in Cancer magister?

Photoperiodic effects on time of molting

Nocturnal molting has been observed in a number of different crustaceans (Fowler et. al., 1971; Bishop and Herrnkind, 1976; Dexter, 1977). It has been suggested that a circadian time keeping mechanism governs the time of molt in a number of these nocturnally molting crustaceans (Bishop and Herrnkind, 1976; Dexter, 1977).

The purpose of this study is to establish if such a time keeping mechanism is common in brachyurans, and to determine if molting in these animals occurs at a selected time within a daily photoperiod.

The two brachyurans selected for this part of the experiment belong to different marine environments: Hemigrapsus nudus from an intertidal habitat, and Cancer magister from a sub-tidal habitat.

Photoperiodic effects on the rate of molting

No reports in brachyurans have shown the existence of a critical or threshold photoperiod for the induction of a molt, as indicated in Macrura (Aiken, 1969). Bliss and Boyer (1964) speculated that the induction of molt in Gecarcinus lateralis depended mostly on whether overall environmental conditions (light, temperature, moisture, crowding) were favorable. Adelung (1971), however, worked with Carcinus maenus, and reported that growth was the main regulator, and perhaps the critical inductive force in molting. Both Bliss and Boyer (1964) and Adelung (1971) found photoperiods as having an indirect influence on the induction of ecdysis in these animals.

Perhaps one of the main reasons for a lack in evidence showing a photoperiodic induction of molt in brachyurans has been due to a lack of work with seasonally molting crabs. Aiken (1969) worked with a seasonally molting crayfish, Orconectes virilis, and pointed out that a photoperiodic influence on the induction of molt could perhaps be limited only to more specialized forms that had a need for seasonal regulation of their molt cycle. In order to test this hypothesis, and also determine if photoperiodic induction will occur in brachyurans, the seasonally molting Cancer magister was selected for this experiment.

Studies in Cancer magister have shown a seasonal molting behavior in both males and females. Molting in these animals appear to be

restricted to the summer months May through September (MacKay, 1942). Juveniles will molt several times during these months, while adults molt only once a year and peak during June (Table 9) (MacKay and Weymouth, 1935; McKay, 1942). The fact that these months, particularly June, correspond to the longest natural photoperiod, strongly suggests the possibility of a photoperiodic regulation in the summer molting behavior of Cancer magister.

MATERIALS AND METHODS

COLLECTION OF ANIMALS AND LABORATORY MAINTAINANCE IN PRE-EXPERIMENTAL STAGE

Hemigrapsus nudus

Two hundred Hemigrapsus nudus were collected from North Cove, Cape Arago, Oregon on January 20, 1979. These were all juveniles and selected between 5.5 and 8.5 mm in size (carapace width).

In the lab, the crabs were separated and placed in individual 10 oz. glass jars filled with sea water. They were then transferred into constant temperature boxes and held at 10°C in darkness for 92 days until the start of the experiment. During this time, the crabs were fed once a week with small pieces of fish meat (dover sole). The sea water was also changed once a week and always took place one day after feeding.

After 67 days Hemigrapsus nudus began molting. The molting times of the crabs in this pre-experimental stage were recorded in Figure 2a. During this period, molt checking times took place generally at 10-11 p.m. at night and 9-10 a.m. the next day (time in the field). Checking also occasionally occurred at other times of the day.

Checking times took an average of 30 seconds, and in this pre-experimental stage, was carried out with white light (General Flectric, incandescent 40 Watt bulb). As the dark periods of the constant

temperature boxes were interrupted with these checking times, it became questionable as to whether these conditions represented a true state of 'constant darkness'. This is particularly evident as light pulses of a few minutes (Brown et. al,, 1954) or even fractions of a second (Bruce et. al., 1960), can resynchronize particular behaviors in certain organisms. Accounting for this factor, the 24 hour dark period of this experiment was named 'extended darkness' rather than 'constant darkness'.

Cancer magister

Two hundred Cancer magister megalops were collected by the boat docks in Charleston, Oregon on May 31, 1979. These were brought to the lab and transferred into a 1000 liter stock tank with running sea water. Two days later the megalops began molting, and after four days, most had molted passing from the megalops stage into first stage post-larval juveniles. The juveniles were easily separated from the megalops, as the megalops would sink to the bottom of the tank and molt into juveniles. The juveniles remained in the stock tank until June 5, 1979, at which time the experiment was initiated.

EXPERIMENTAL PROCEDURE

On March 29, 1979 (92 days after exposure to extended darkness) Hemigrapsus nudus was divided into three groups and exposed to different photoperiods. One group (n=34) remained in extended darkness. A second group (n=26) was exposed to a long day photoperiod of 16 hours of light and 8 hours of darkness (LD 16:8), and a third group (n=26) was exposed to a short day photoperiod of 8 hours of light and 16 hours of darkness (LD 8:16). The long and short days corresponded to the longest and shortest natural photoperiods of the Cape Arago area (Smith and Smith, 1980).

At the start of this experiment a rigorous molt checking schedule was set. Checking was carried out four times, at 08:00, 12:00, 20:00 and 24:00 hours of each day for the three light treatments (Figure 1). This divided each 24 hour day into four intervals, two eight hour intervals and two four hour intervals, and allowed the time of molt to be estimated within a four or eight hour period. The checking times were always carried out either just before the lights went off or just after lights came on, and thus avoided the interruption of darkness. Occasionally, additional checks were run during the light phases of short and long day photoperiods.

A red light (40 Watt, G.E. I.D. #15478) was used during the checking times that interrupted dark phases (i.e. extended darkness).

A flourescent glow lamp (G.E. F40GS/WS) was used for white light during the light phases of the imposed photoperiods. Feeding and water changes were continued as described for the pre-experimental stage.

On June 5 (6 days after the megalops were placed in the stock tank), 150 Cancer magister juveniles were collected from the stock tank, separated, and placed in individual 10 oz. jars with sea water. These were then divided into three groups of 50 and placed in one of the three light treatments described above: extended darkness, short day and long day.

At this point, all experimental conditions were identical to those described for Hemigrapsus nudus. This included red light checks, feeding, water changes, and constant temperatures held at 10°C. The molting times of the Cancer magister juveniles were recorded in Figure 5, 6, and 7. In addition, lengths of intermolt periods of these first stage post-larval crabs were recorded in Figure 8. The intermolt period was defined as the duration of time that the juvenile remained in the first post-larval instar.

During this photoperiod experiment, a group of Cancer magister juveniles (n=20), remained in the stock tank. As the tank was stored in a covered courtyard, these juveniles were exposed to natural photoperiods and temperatures. These crabs were not fed as they seemed content eating the abundant supply of brown algae that had settled at the bottom of the tank. Although conditions of the stock tank differed greatly from the conditions of the light boxes (i.e. food, running water, space), the twenty stock tank juveniles were to serve as

controls in determining the intermolt period of first stage post-larval Cancer magister.

RESULTS

EFFECTS OF PHOTOPERIOD ON TIME OF MOLTING

The effects of photoperiod on the time of molting were examined in Hemigrapsus nudus and Cancer magister juveniles. This was carried out by comparing the molting times of these brachyurans in three light treatments: extended darkness, long day (LD 16:8) and short day (LD 8:16). The results of molting times in these light treatments are shown in Figures 2-7.

Results of molting times in each light regime were statistically analyzed by two different chi-square tests. One chi-square test analyzed the randomness of the distribution of molts during the overall 24 hour period of each light condition (Table 1-3, 5-7). The second chi-square test examined the randomness of the distribution of molts occurring within each individual interval of the three light regimes (Intervals I, II, III and IV; Table 1-3, 5-7).

Hemigrapsus nudus

Extended darkness

As described in the methods, 200 stock animals were placed in extended darkness for a period of 92 days prior to the beginning of the experiments. Although the checking times during this period were

irregular, the results in Figure 2a show an apparent concentration of molts occurring between the times of 08:00 and 20:00 (interval II and III). This time period corresponded with the night time hours in the field (between 20:15 and 8:15 hrs; see Figure 1).

This apparent grouping of molts became particularly evident when the experiment was initiated and more rigorous checking times were enforced (Figure 2b). The results of Figure 2b show an apparent high concentration of molts occurring in interval II and III, and an apparent absence of molts occurring in interval I and IV.

Chi-square tests examining the significance of the distribution of molts throughout the overall 24 hour period (intervals I, II, III and IV, combined) showed that the distribution of molts departed significantly from random ($p \leq 0.005$, Table 1). Further chi-square tests examining the occurance of molts within each individual interval in Figure 2a, showed that molting times of interval II and IV displayed a significantly non-random distribution (Table 1).

These results suggest that as a consequence of certain experimental conditions, the crabs in extended darkness were molting with a non-random distribution. This is particularly evident in interval II where molting was significantly occurring (observed=9, expected=3.33; $p \leq 0.025$) and interval IV where molting was significantly lacking (observed=0, expected=3.33; $p \leq 0.05$).

Long Day

The crabs that were taken from extended darkness and subjected to long day conditions (LD 16:8) appeared to respond differently. Figure

3 shows a concentration of molts occurring mainly in interval III and then later developing in interval IV. The delayed appearance (18 days after exposure to long days) of molts in interval IV suggests the possibility of a shift from interval III towards the subjected dusk (24:00 hours).

Chi-square tests show a significant departure from random distribution throughout the overall long day period ($p \leq 0.05$, Table 2). The distribution of molts within each of the individual long day intervals (I, II, III and IV) did not appear to be significant, however, comparisons between numbers of molts in individual intervals of the long day conditions with those of extended darkness did seem to show extreme differences. In extended darkness (Figure 2b) a significant number of molts appeared in interval II while no molts appeared in interval IV. This distribution was reversed in the long day conditions (Figure 3) where molts appeared in interval IV and did not occur in interval II.

Short Day

The crabs that were subjected to a short day photoperiod (LD 8:16) also showed a significant overall molt distribution that departed from random ($p \leq 0.05$, Table 3). In addition, the distribution of molts in these conditions differed from crabs in long day and extended darkness periods. The concentration of molts appeared to occur in intervals Ib and II between 04:00 and 12:00 hours (Figure 4).

Chi-square tests showed a significant number of molts occurring in intervals Ib ($p \leq 0.05$) and II ($p \leq 0.025$, Table 3). This group of

molts corresponded to the two four hour periods on either side of the subjected dusk (08:00 hours, Figure 4), and suggested that Hemigrapsus was molting either just prior to or just after the dusk period.

In order to determine if the imposed dusk and dawn had a significant effect on the distribution of molts, chi-square tests were carried out for the dusk and dawn intervals of the short day light regime. The dusk interval was defined as an eight hour period which included a four hour period before and after the imposed dusk. Similarly, the dawn interval included a four hour period before and after the imposed dawn. The results showed a highly significant occurrenace of molts in the imposed dusk interval (observed=1, expected=6.67; $p \leq 0.05$) (Table 3c). This seemed to suggest the molting was significantly occurring during dusk and reduced during dawn.

Molting During Post-Dawn and Pre-Dusk

The results in the short day light regime showed a significant distribution of molts occurring in the overall period, in the dusk interval, and in the dawn interval (Table 3). In the long day light regime, although there was a significant distribution of molts in the overall period, there did not appear to be any particular individual interval that showed a statistically significant occurrence of molts (Table 2). This was possibly due to the small numbers (n=23) of experimental crabs used in this part of the study.

In order to further establish whether an imposed dawn and dusk had an effect on the molting behavior of Hemigrapsus nudus, data from short day and long day conditions were pooled together. This increased the

number of animals (n=46) and raised the expected frequency (f=7.17) greater than 5 (Sokal and Rohlf, 1969).

The molting data, together, was arranged so as to analyze molting during post-dawn and pre-dusk periods, separately. The post-dawn period was analyzed by combining the molting time of interval II from long days (Figure 3) with those of interval Ia from short days (Figure 4, Table 4). Similarly, the pre-dusk period was analyzed by combining interval IV (long days, Figure 3) with interval Ib (short days, Figure 4) (Table 4).

Chi-square tests showed a significant occurrence of molts in the combined pre-dusk period (observed=15, expected=7.17; $p \leq 0.05$), and a significantly reduced number of molts in the combined post-dawn period (observed=1, expected=7.17, $p \leq 0.025$) (Table 4). Thus, in agreement with the previous results shown above, these results indicate that in conditions of either long or short days, Hemigrapsus nudus will most likely molt prior to the subjected dusk and most likely not molt after the subjected dawn.

Cancer magister

Apart from not being exposed to a period of extended darkness prior to the beginning of the experiment, Cancer magister was subjected to identical experimental conditions and tests as Hemigrapsus nudus.

The distribution of molts in extended darkness and short day conditions were found to be entirely random (Figure 5, 7; Table 5, 7). Chi-square tests of the molts in the dusk and dawn periods (Table 7c) and the pre-dusk and pre-dawn intervals (Table 7b) of short day

conditions showed no departure from a random distribution. In addition, a random distribution was also observed when molts from pre-dusk and pre-dawn intervals of long and short day light conditions were pooled together (Table 8). This distribution was in contrast to the results found in Hemigrapsus nudus, and indicated that these light conditions were having no influence on the time of molt in the Cancer magister juveniles.

The results in long day conditions (Figure 6) differed from extended darkness and short day conditions. The overall distribution departed significantly from random ($p \leq 0.01$), and the pre-dusk interval IV appeared to have a significantly reduced number of molts (observed=1, expected=8.17; $p \leq 0.025$). Again, this was unlike the results in Hemigrapsus nudus which appeared to have a higher concentration of molts occurring in the pre-dusk interval.

EFFECTS OF PHOTOPERIOD ON RATE OF MOLTING

In order to establish if photoperiods have an effect on the rate of molting in Cancer magister, the length of the intermolt periods of the juveniles in each light regime were compared (Figure 8).

The results showed no significant difference in the mean intermolt periods of the crabs in the three light regimes. The mean intermolt periods were 36.5, 36.8 and 39.06 days for long day, short day and extended darkness, respectively. A comparison of the distribution of molts (t-test) also showed an insignificant difference in the distributions showed in Figure 8.

The range of the intermolt periods of Cancer magister juveniles in the light boxes was between 24 and 52 days (Figure 8). This was in contrast with the range of the intermolt periods of the juveniles in the stock tank (see methods), which was between 10 and 15 days and corresponded to the 11.4 day intermolt period reported for first stage post-molt juveniles (MacKay and Weymouth, 1935). In the light of these results, it appears evident that the experimental conditions of the light boxes extended the intermolt period from the natural 11.4 day period to an overall mean of 37.5 days.

DISCUSSION

EFFECTS OF PHOTOPERIOD ON THE TIME OF MOLTING

Hemigrapsus nudus

A definite grouping of molts was observed in intervals II and III of extended darkness (Figure 2a,b). This grouping of molting times suggests the presence of a molting rhythm and might be interpreted as either the result of entrainment or possibly an indication of a free-running rhythm. There are essentially two possible explanations for the appearance of this molting rhythm.

One explanation may be that extended darkness is acting as a constant darkness environment, and that the occurrences of ecdysis in intervals II and III is in fact a molting rhythm which is under endogenous control and/or has been under previous entrainment to the natural photoperiod in the field. As nocturnal molting has been noted in a number of crustaceans (Fowler *et. al.*, 1971; Bishop and Herrnkind, 1976) and as intervals II and III (Figure 2a, b) correspond to the night time hours of the field (20:15 - 08:15 hours, see Figure 1), it is possible that the population of Hemigrapsus was still entrained to the night time of the previous natural photoperiod.

Due to the fact that extended darkness was interrupted with white light (Figure 2a) and then with red light (Figure 2b), there is a

certain amount of doubt as to whether these conditions represented a true state of constant darkness. My observations have shown that both Hemigrapsus nudus and Cancer magister juveniles were sensitive to the red light conditions used in the experiment. If these 30 second pulses (checking times) of red and white light are capable of acting as a Zeitgeber, then extended darkness cannot be considered a state of constant darkness, and the grouping of molts in interval II and III cannot be interpreted as either an endogenous molting rhythm or the result of previous field entrainment.

An alternative explanation for the distribution of molts in Figures 2a, b might be that red and white light checking times in extended darkness acted as a skeleton photoperiod. It has been reported that a phase shift and entrainment of certain behaviors can occur by exposing an organism (in constant darkness), each day and at the same time, to a light pulse of a few minutes (Bunning, 1973). It is possible, therefore, that the checking times in extended darkness (Figure 2a, b) gave rise to a skeleton photoperiod and entrained molting to intervals II and III.

Although there may be other alternative explanations, the concentration of molts occurring in these intervals (Figure 2a, b) are particularly useful as a control in estimating the effects of imposed photoperiods on the time of ecdysis. As this distribution of molts (Figure 2a, b) represents molting times prior to exposure of long and short days, it is evident that any changes in this distribution (after light conditions have been imposed), would be a consequence of a molting response to photoperiods.

A comparison to the distribution of molts in extended darkness (Figure 2a, b), long days (Figure 3) and short days (Figure 4) showed prominent differences in the molt distributions for all three light regimes. In short days, a significant concentration of molts occurred in interval Ib and II (Figure 4), while in long days, a concentration occurred in interval III and later appeared to develop in interval IV (Figure 3). These molt distributions were significantly different from the concentrations of molts (interval II and III) found in extended darkness, and strongly suggested that the imposed photoperiods were influencing the time of molt in Hemigrapsus nudus.

A comparison of the distribution of molts in long and short day conditions have also indicated the presence of a shifting of molting times away from the original phase (interval II, III, Figure 2a, b) of extended darkness. This shifting of molting times is particularly evident in interval IV of long day conditions (Figure 3). The first molt appeared to shift into interval IV, 18 days after the long day photoperiod was imposed. The apparent disappearance of molts in interval II and later delayed appearance of molts in interval IV suggests that molting in the light regime is shifting in the direction of the imposed dusk (24:00 hours, Figure 3).

A similar shift in molting times is also observed in short day light conditions. Although a delay in shift is not as prominent as long day conditions, molting appears to be also shifting towards the new imposed dusk (08:00 hours, Figure 4). It appears that molting times have shifted away from interval III and become phase locked in

two adjacent four hour intervals (Ib and II, Figure 4) one before dusk and one after dusk.

The appearance of these shifts, particularly the delayed shift in long days, (interval IV, Figure 3) could possibly be interpreted as 'transients' (Pittendrigh, 1965) and be an indication of an endogenous component in the molting behavior of these crabs. 'Transients' or non steady state cycles can occur when an imposed Zeitgeber (environmental cues, i.e. photoperiods) acts to entrain a circadian rhythm. In the case of advanced phase shift, the circadian system is seen to pass through several non steady state cycles or transients, before achieving entrainment and reaching a steady state condition (Saunders, 1977).

In the light of the apparent delayed shifting of molting times in long day conditions, it is possible that Hemigrapsus has an endogenous component which determines the time of ecdysis. Control over the time of ecdysis does not appear to be exogenous. Exogenous controlled behaviors will respond to shifted photoperiods by "instantaneously" shifting with the phase of the new imposed photoperiod, and show no indication of transients (Saunders, 1977).

The results in short and long day conditions have also implied that Hemigrapsus nudus molts during dusk periods. In this experiment, subjected dusks of long and short day photoperiods were set at entirely different times (Figure 1). In the short day light regime, dusk was at 08:00 hours (Figure 4) while in the long day light regime, dusk was imposed at 24:00 hours (Figure 3). The original phase of the distribution of molts in Hemigrapsus nudus, prior to the imposition of

the two photoperiods was between 08:00 and 20:00 hours (Figure 2a, b). Once Hemigrapsus was subjected to the two photoperiod, molting times appeared to have shifted in separate directions. In long days, molting times appeared to shift to the right, towards 24 hours (Figure 3) and in short days, molting shifted to the left and appeared to settle on either side of subjected dusk (08:00 hours, Figure 4).

The results of chi-square tests appear to support the idea that molting occurs during dusk periods. A highly significant occurrence of molts was shown for the dusk period in the short day light regime ($p \leq 0.005$, Table 3c). In contrast to this, the dawn interval showed a significant absence of molts ($p \leq 0.05$). When pre-dusk and post-dawn intervals of both long and short day light conditions were combined, similar to the results observed. The combined pre-dusk interval showed a significant occurrence of molts ($p \leq 0.025$) and the combined post-dawn interval showed a significant absence of molts ($p \leq 0.05$, Table 4).

From the results discussed above, it can be concluded that Hemigrapsus nudus will molt at a selected time of daily photoperiods. Chi-square tests suggest that Hemigrapsus will most likely molt in the later part of the day (dusk) and will not molt in the early part of the day (dawn). Evidence showing shifts toward dusk and the presence of a significant occurrence of molts during dusk (Table 3c), indicate that Hemigrapsus nudus has a strong preference for molting during dusk periods.

If Hemigrapsus does indeed cue into dusk for its molting time, then the obvious question is why? Molting is known to be a high

mortality event for crustaceans (Green, 1960; Passano, 1960). It is evident that these animals are the most vulnerable when they are undergoing ecdysis and at the time they emerge with a thin, soft exoskeleton. It would then be advantageous for these animals to remain out of the way of predators during this period.

Darkness can offer protection from predators, particularly ones that hunt during the day. If darkness plays a protective role during the molting period, then the longer that a dark period encompasses ecdysis, the greater the chances of survival.

Perhaps the main reason why Hemigrapsus nudus will molt at dusk is to benefit from a maximum period of darkness following ecdysis. Although an eight hour period is not long enough for Hemigrapsus to harden its new exoskeleton (personal observation), it may be reasonable to assume that the first few hours after molting is the most critical period of vulnerability and the best possible protection would be to molt at dusk and benefit from a full night of darkness.

Nocturnal molting has been noted in several crustaceans (San Felice, 1966; Fowler et. al., 1971; Bishop and Herrnkind, 1976; Dexter, 1977). In these reports the checking times were always at the beginning and at the end of each dark phase. Unlike the experiments in this study, the time of molting would never be narrowed to a four hour interval. Bishop and Herrnkind (1976) subjected the shrimp, Panaeus duorarum to a LD 12:12 light cycle and noted a nocturnal molting cycle. Their guess was that molting was perhaps cued to the middle of the 12 hour dark phase, and this insured that the shrimp would molt at night time.

Although it might not be true for all nocturnally molting crustaceans, it is possible that ecdysis in Panaeus duorarum and many other crustaceans, is infact taking place at the beginning of night time during the dusk period. This behavior would be especially beneficial to those crustaceans who are particularly lethargic and defenseless in the first few hours following ecdysis.

Evidence presented above has also pointed to the possible presence of an endogenous component in control of the time that ecdysis takes place. This was suggested in the light of a delayed shift (transients) in the molting times of long day conditions. If an endogenous (circadian) control of molting time does occur in Hemigrapsus nudus, it is possible that the circadian system acts as a time keeping mechanism which synchronizes the time of molt to the dusk period.

A number of animals have been noted as being dusk active, or crepuscular (Saunders, 1977). Many of these behaviors are under circadian control (Beck, 1968). The fruitfly, Dacus tryoni possesses a circadian rhythm of mating activity, which in steady state entrainment, insures that sexual activity occurs at dusk (Tyschsen and Fletcher, 1971). The authors also noted that a normal 24 hour period, a "readiness to mate" response remained zero until four hours before the beginning of dusk. At this point, it rose rapidly to a maximum which coincided with dusk, and then slowly fell back to zero by dawn of the following day.

It is possible that similar circadian mechanisms controls the molting time of Hemigrapsus nudus. In such a mechanism, the onset of ecdysis could be withheld until a few hours prior to dusk. At this

time, the circadian system would begin generating an increasing "readiness" in allowing ecdysis to occur. This "readiness" would peak at dusk and then slowly fall back to zero by the next dawn period.

Cancer magister

The results of molting times of Cancer magister differed greatly from those of Hemigrapsus nudus. No significant grouping of molts were seen in either extended darkness (Figure 5) or short day light conditions (Figure 7). It appears that these light conditions have no influence on the time of molt in juvenile Cancer magister.

Results in long day conditions (Figure 6), however, showed that the overall distribution of molts through out the period departed significantly from random ($p \leq 0.01$). In addition, a significant absence of molts was noted in the pre-dusk interval IV ($p \leq 0.025$, Table 6). This was in contrast to Hemigrapsus nudus where a significant occurrence of molts were observed in the pre-dusk intervals (Table 4, 3b).

It is difficult to explain why Cancer magister should behave differently in this light regime. Perhaps, at dusk during the summer (long days), Cancer magister juveniles are subjected to a greater amount of predation than in winter (short days), and have consequently, developed an aversion to molting during 'unsafe' periods.

MacKay (1942) reported that most of Cancer magister that were held captive in live wells, molted at night. This phenomenon was not observed in this experiment. If nocturnal molting is indeed common in Cancer magister then, in view of the fact that only first stage post-larval juveniles were used in this study, it is possible that a molting behavior response to night time does not develop until a later time in the life cycle.

The results of Figures 5, 6 and 7 also showed no indication of an entrained molting time, as discussed in Hemigrapsus nudus. Perhaps one reason for a difference in response in these two brachyurans is a difference in predatory pressure and nature of respective habitats.

Cancer magister is generally found in sandy bottoms of estuarine and ocean waters (Phillips, 1935). It has been noted that Cancer magister burrow in sandy bottoms. This behavior is particularly common in younger crabs when threatened (MacKay, 1942), and might be an important protective adaption for the vulnerable post-molt period.

Hemigrapsus nudus, in contrast, is found in an intertidal environment. Although these crabs are mostly found beneath the protective covering of rocks and crevices, they are rarely alone and are always in contact with other intertidal animals (personal observation). It may be that an intertidal environment offers a greater predatory pressure for Hemigrapsus nudus than is experienced by Cancer magister, and that the development of an endogenous control is essential in serving to increase the chances of survival.

EFFECTS OF PHOTOPERIOD ON THE RATE OF MOLTING

A photoperiodic induction of ecdysis was shown in the seasonally molting Orconectes virilis (Aiken, 1969). In order to determine if a similar photoperiodic induction was present in Cancer magister, the intermolt periods of juveniles, subjected to the three light treatments, were compared.

The results of this experiment (Figure 8) showed no significant differences in the intermolt periods of the three groups of first stage post-larval juveniles tested. The mean duration of the intermolt periods were 36.5, 36.8 and 39.06 for long days, short days, and extended darkness, respectively. This was in contrast with juveniles from the stock tank which molted with a 10-15 day intermolt period. It appeared that the experimental conditons in the light boxes extended the intermolt period from the natural mean 11.4 days (MacKay and Weymouth, 1935) to the observed overall mean of 37.5 days.

Assuming that the delaying effects of the experimental conditions did not inhibit photoperiodic responses in these animals, it would appear that photoperiods are not involved in the induction of pro-ecdysis in first stage post-larval Cancer magister. Instead, the results seemed to indicate that other factors were regulating molting in these juveniles.

The induction of pro-ecdysis in certain brachyurans has been examined by Bliss and Boyer (1964) and Adelung (1971). In their work with Gecarcinus lateralis, Bliss and Boyer (1964) showed that overall environmental conditions (i.e. light, temperature, moisture, crowding) were critical factors in determining when the onset of pro-ecdysis was to occur. It was suggested that when environmental conditions were unfavorable to molting, then ecdysis was delayed. In favorable environmental conditions, ecdysis was thought to be promoted. Adelung (1971) reported a separate regulator of ecdysis. Working with Carcinus maenas, he proposed that the molting rhythm was regulated by growth, and unless a minimum amount of tissue growth was achieved, ecdysis would not occur. During unfavorable conditions, Adelung (1971) also suggested that molting was delayed, and that this was carried out by consumption of less food.

The occurrence of a delay in intermolt periods of juveniles in the light boxes seem to correspond with the findings of Bliss and Boyer (1964) and Adelung (1971). It is evident that conditions in the light boxes differed greatly from those of the stock tank, and were probably more unfavorable for molting, i.e. little food, stagnant water, restricted space (see methods). It would appear, therefore, that molting in the first post-larval stage of Cancer magister is probably regulated by food and other ambient, environmental factors (excluding photoperiod).

Although molting does not seem to be influenced by photoperiod in this stage of the Cancer magister life cycle, it may be that a photoperiodic response develops at a later time. The development of a

seasonal molting rhythm (MacKay, 1942), particularly in the adult stage, seems to point to this possibility.

MacKay (1942) reported that molting in Cancer magister will only occur in summer from May through September. In the first summer, Cancer magister passes from the first to the fifth post-larval stage. In the second summer, it passes from the sixth to the tenth, and in the third summer (May, June), Cancer magister molts only twice passing from the eleventh to the twelfth instar. In the summers following molting occurs only once a year and generally peaks during June (Table 9).

The fact that molting in adults occurs only once a year, and mainly in June, suggests the involvement of a certain environmental cue. A feeding cue does not seem likely as feeding occurs at all times of the year and depends mainly on availability of food (MacKay, 1954). It is also doubtful that temperature is a predominating factor, as ocean temperatures in areas where Cancer magister is found, do not fluctuate greatly. Average temperatures off the Oregon coast fluctuate inconsistently between 9°C and 13°C throughout the year, and could not clearly account for peak molting in June (E.P.A., 1971).

The only other possible cue appears to be daily photoperiods. The fact that the summer months particularly June, correspond to the longest natural photoperiod strongly suggests a probably photoperiodic regulation of molting.

In view of the results described above, it is possible that the molting behavior of Cancer magister is subject to different regulating influences during its life cycle. The results in this experiment

suggest that the first post-larval stage is not influenced by photoperiods, but rather, is regulated by food and ambient environmental conditions as was reported in other brachyurans (Bliss and Boyer, 1964; Adelung, 1971). By the fifth post-larval stage, however, it may be that a photoperiodic response has developed in Cancer magister as the molting process stops in this stage and does not occur again until the following summer (Table 9). It is speculated that from this instar through to the adult stages, a photoperiodic control of ecdysis is maintained and as a consequence, molting is restricted to the summer months for the rest of the Cancer magister life cycle.

FURTHER EXPERIMENTATION

TIME OF MOLTING

One of the major problems with the study examining the effects of photoperiod on the time of molting in Hemigrapsus nudus, was a smallness in number of experimental animals. In many of the chi-square tests, expected frequencies fell below 5, and it was necessary to pool molting data in order to establish more significant results. A simple improvement to this experiment is to increase the numbers of experimental crabs. In addition to giving rise to more significant results, this would also be valuable in showing more accurate shifts of molting distributions.

A second problem in this experiment was the checking times that occurred during dark phases. It was difficult to draw any conclusions from extended darkness as there was the likelihood of entrainment everytime the crabs were checked. Perhaps an alternative approach to further testing for the presence of circadian control in the molting rhythm, is to first entrain Hemigrapsus to a light/dark cycle and then to transfer the crabs into constant light. A number of circadian rhythms in animals have been shown to persist in constant light as readily as constant darkness (Saunders, 1977), and consequently, a free-running molting rhythm may be observed in these

conditions. This would be a useful test as molt checking could occur at any time without ever interrupting the light treatment.

RATE OF MOLTING

If a photoperiodic response in molting does occur at a later stage in the life cycle of Cancer magister, then perhaps a better approach to this experiment would have been to have worked with fifth and older post-larval crabs. Inhibition of molting in these crabs, with short photoperiods during the summer months, or conversely, induction of molting with long photoperiods in winter months, would have conclusively pointed to a photoperiodic control of seasonal molting in Cancer magister.

A simple experiment would have been to subject a group of Cancer magister adults to long and short day photoperiods during the summer months. If peak molting in June is indeed the result of photoperiodic induction of molting, then the results of such an experiment should show crabs in long days molting earlier than crabs in short days.

SUMMARY

1. Photoperiods were shown to influence the time of molting in Hemigrapsus nudus.

2. It was shown statistically, in these crabs, that ecdysis will not occur in the early part of the day (dawn) but will occur during the later hours of the day (dusk).

3. From the results, it appeared that Hemigrapsus nudus cued into the dusk period for molting. It was suggested that this behavior was under endogenous control, and served as a protective mechanism which insured a maximum dark period immediately following ecdysis.

4. The molting time of Cancer magister juveniles did not appear to be influenced by photoperiod, except in the pre-dusk period of long days (summer photoperiods), when a significant absence of molting occurred. It was proposed that this was a protective response to predation.

5. As nocturnal molting was not found in first stage post-larval juveniles, but was reported in adults, it was suggested that a molting response to photoperiods developed as Cancer magister grew older.

6. Food and a combination of other environmental conditions excluding photoperiods were shown as being the main factors in regulating the induction of ecdysis in first stage post-larval Cancer magister.

7. It was postulated that a photoperiodic control of ecdysis was developed by the fifth post-larval stage, and that this control

regulated summer molting throughout the rest of the Cancer magister life cycle.

Figure 1. Light Treatments

Figure 1 demonstrates the three light conditions with respect to time, molt checking times, intervals and phases of light and dark periods of each photoperiod. The dark bands correspond to periods of darkness while the white bands correspond to the light phase of photoperiods. Note that the photoperiods of long and short days are reversed.

The hours denoted in this figure are arbitrary, with the hours 12:00 corresponding to the hours 24:15 in the field and the hours 24:00 corresponding to the hours 12:15 in the field.

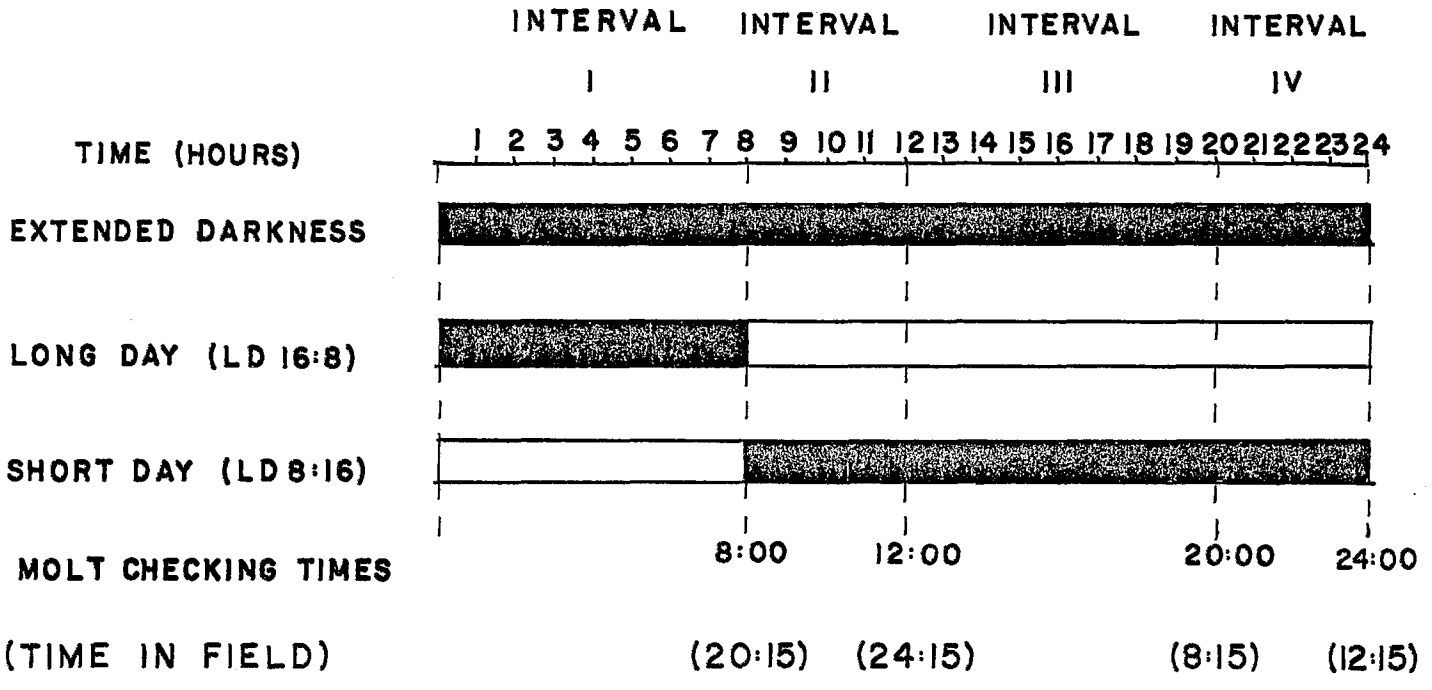

Figure 1. Light treatments

Figures 2-7

These figures show the distribution of molting times that occurred in each light condition. Each 24 hour period is broken into four intervals:

Interval I = 24:00 - 08:00 hours (8 hour interval)

Interval II = 08:00 - 12:00 hours (4 hour interval)

Interval III = 12:00 - 20:00 hours (8 hour interval)

Interval IV = 20:00 - 24:00 hours (4 hour interval)

The vertical broken lines denote checking times for Figures 2b-7, and separate each interval. The dark and white bands below the hours represent dark and light phases of imposed photoperiods, respectively.

Horizontal lines, with small circles at their midpoints, represent a time period in which molting occurred. In other words, consider a line drawn between 08:00 and 12:00 hours (Figure 2b). This would mean that when the crab was first checked at 08:00, it had not yet molted however, when it was checked again at 12:00, it had molted. A line was then drawn between these two hours (08:00 and 12:00) and indicated that somewhere in this period of time, molting had occurred. The small circle on this line represents a midpoint between the two checking times. The numbers to the right of the midpoints indicate the number of days that the crab spent in the respective experimental conditions, prior to molting.

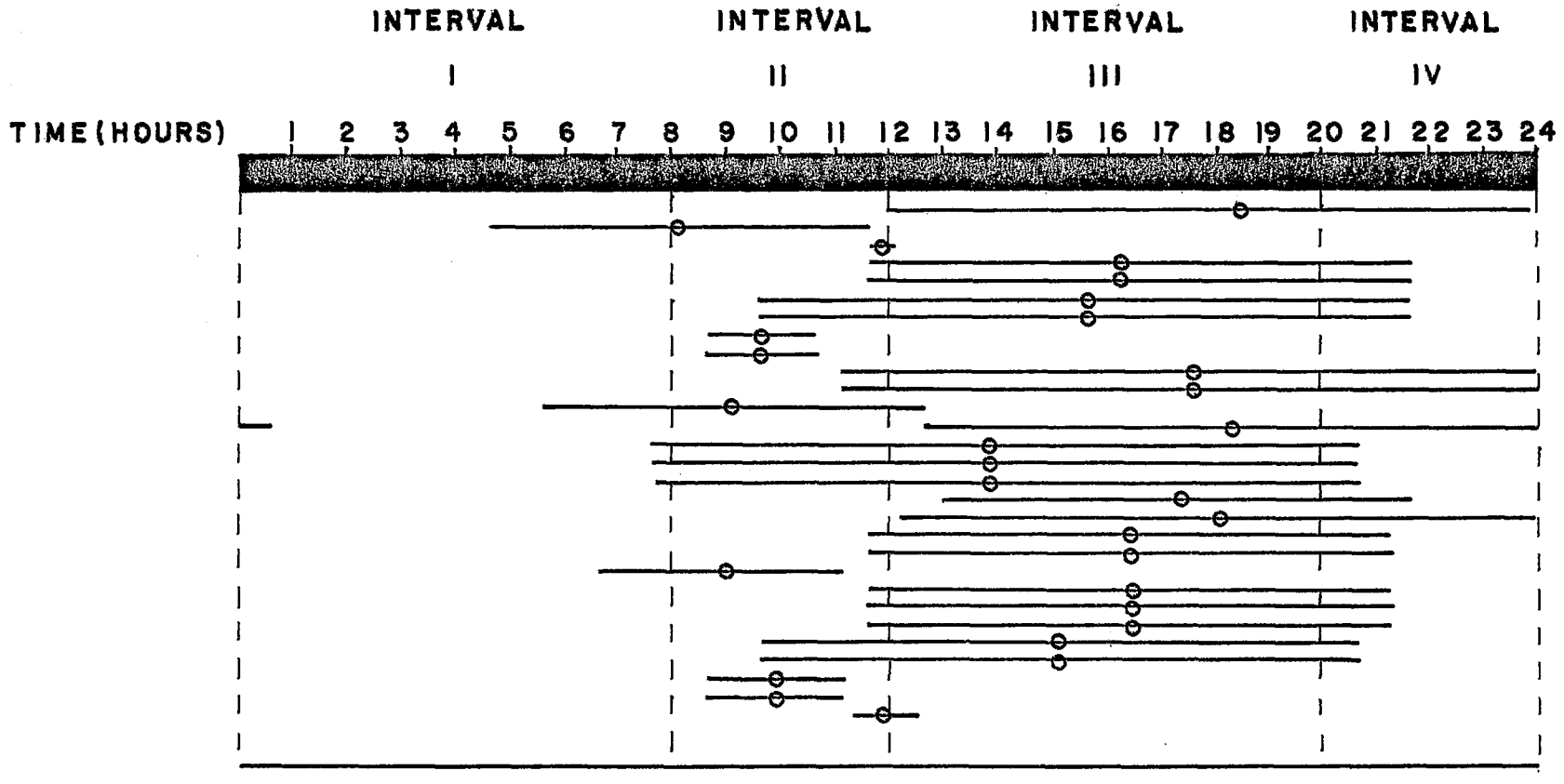

Figure 2 a. Molting times of stock animals in extended darkness.

Hemigrapsus nudus

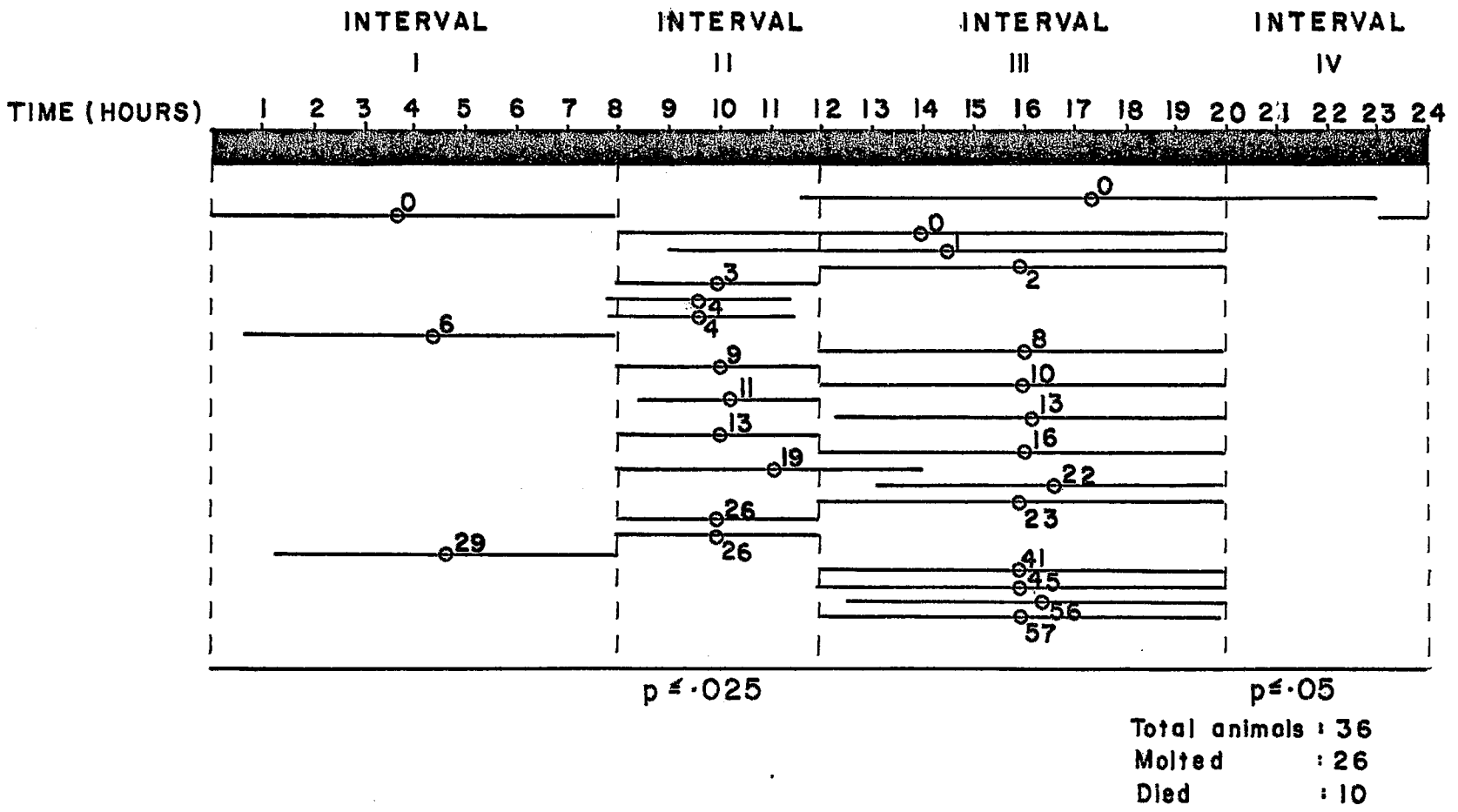

Figure 2 b. Extended darkness

Hemigrapsus nudus

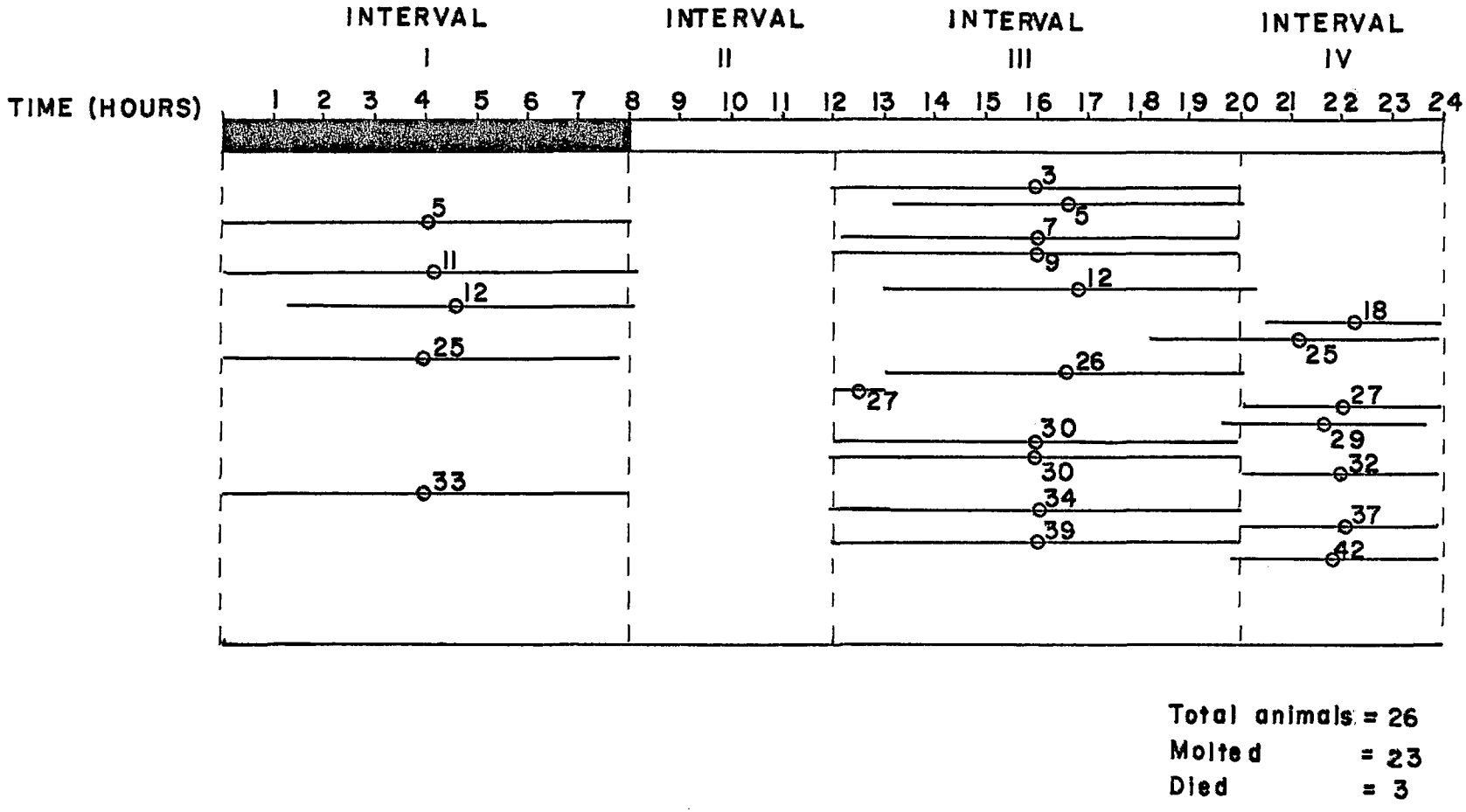

Figure 3. Long day (LD 16:8)

Hemigrapsus nudus

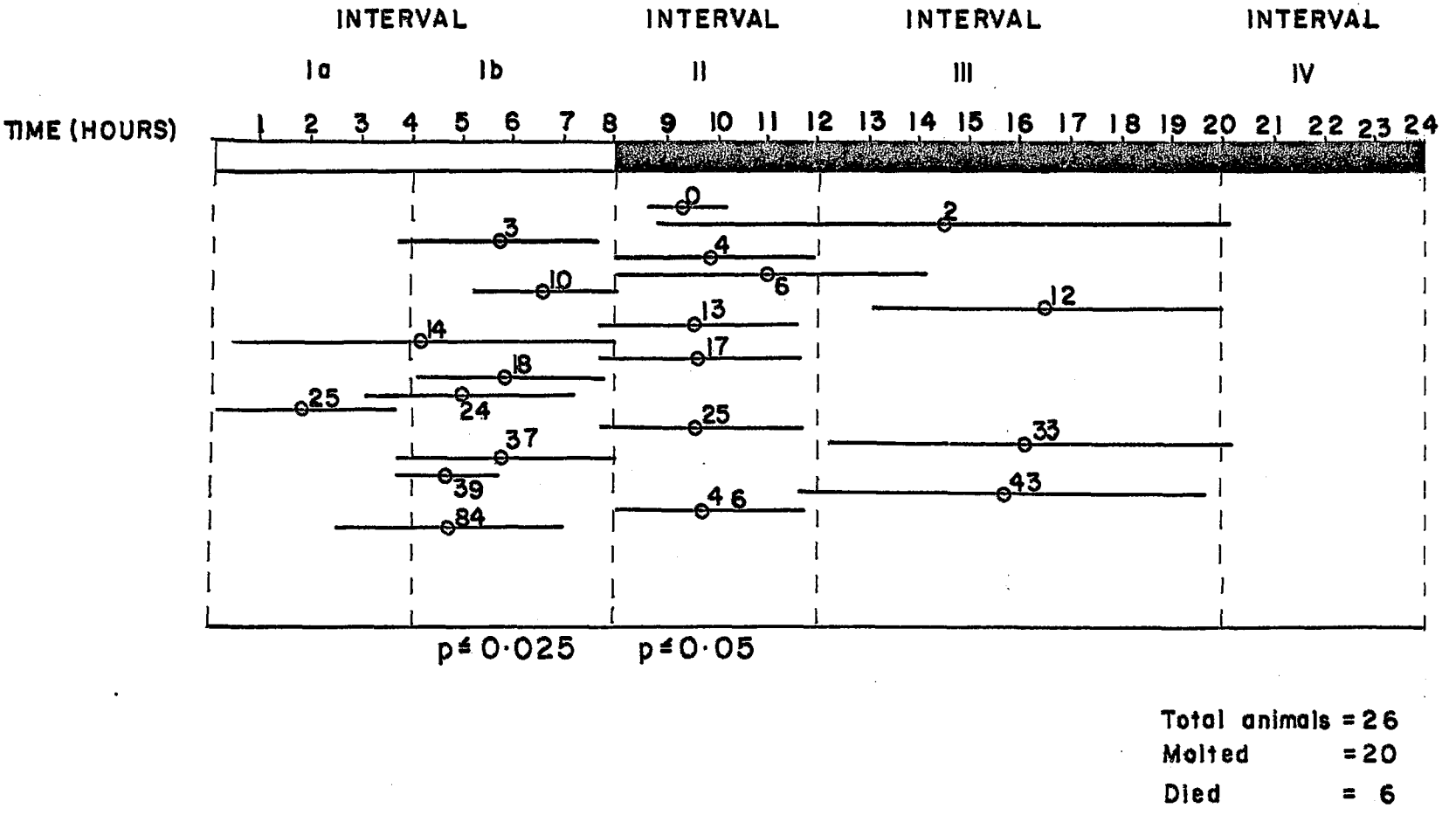

Figure 4. Short day (L D 8:16)

Hemigrapsus nudus

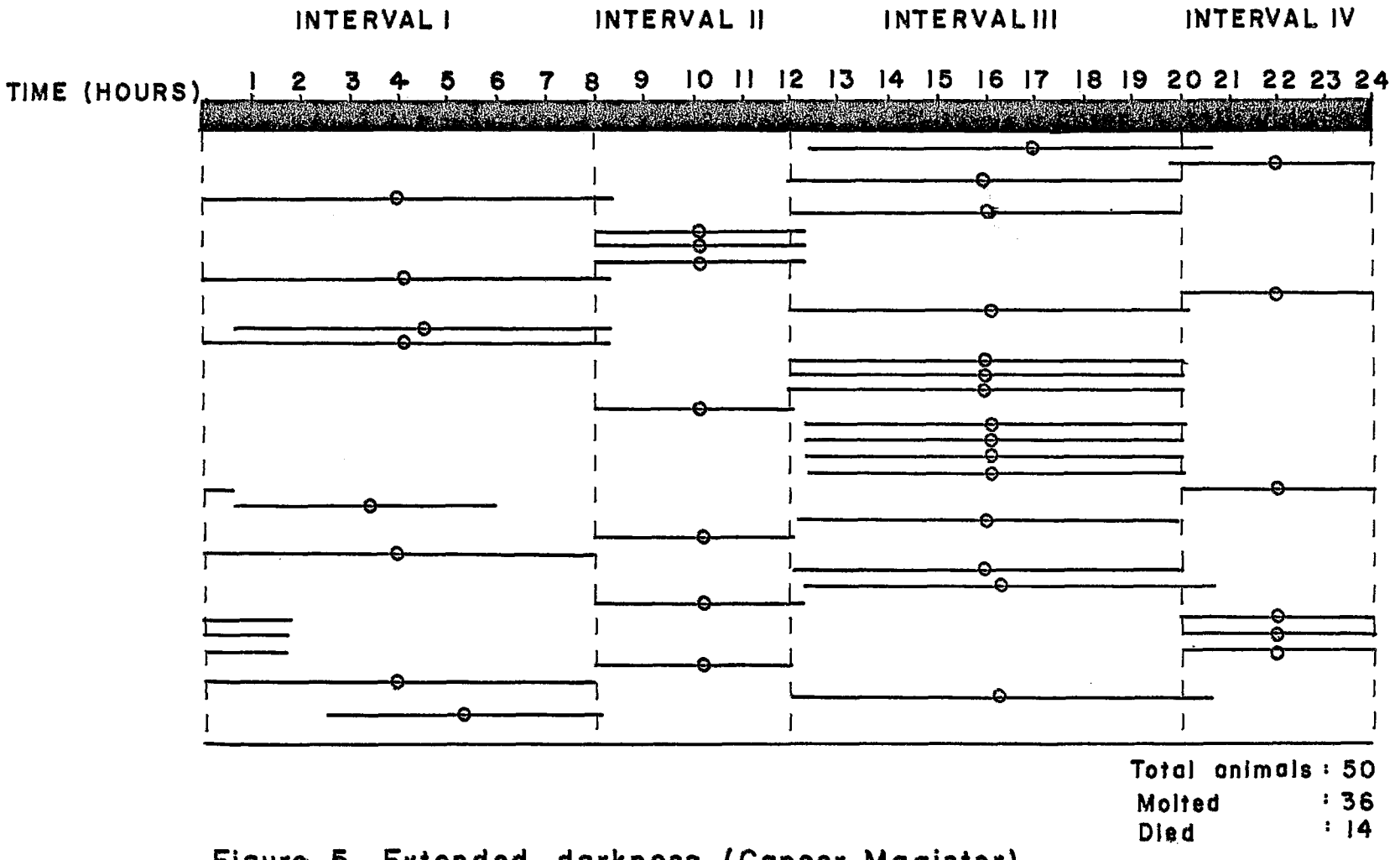

Figure 5. Extended darkness (Cancer Magister)

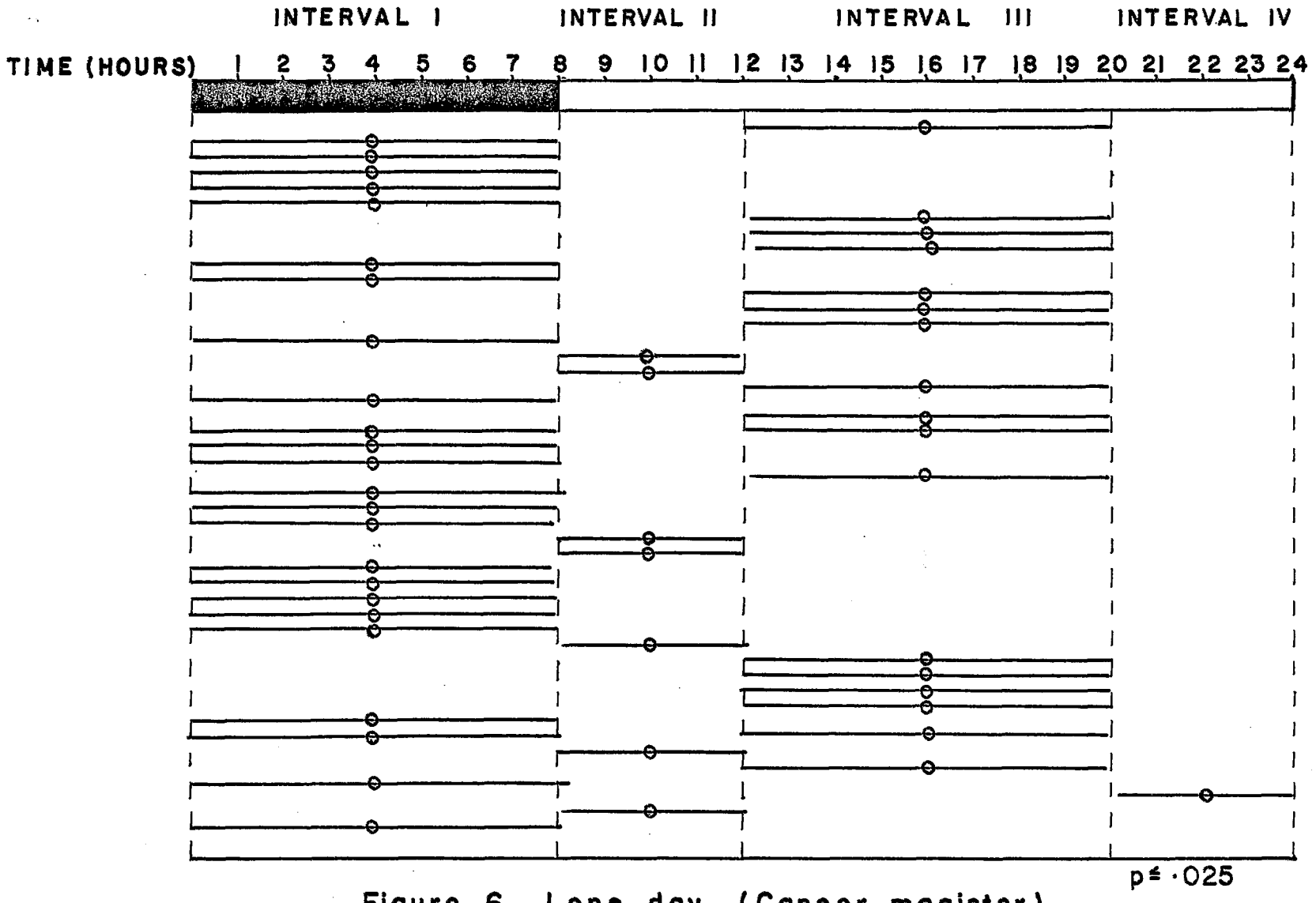

Figure 6. Long day (Cancer magister)

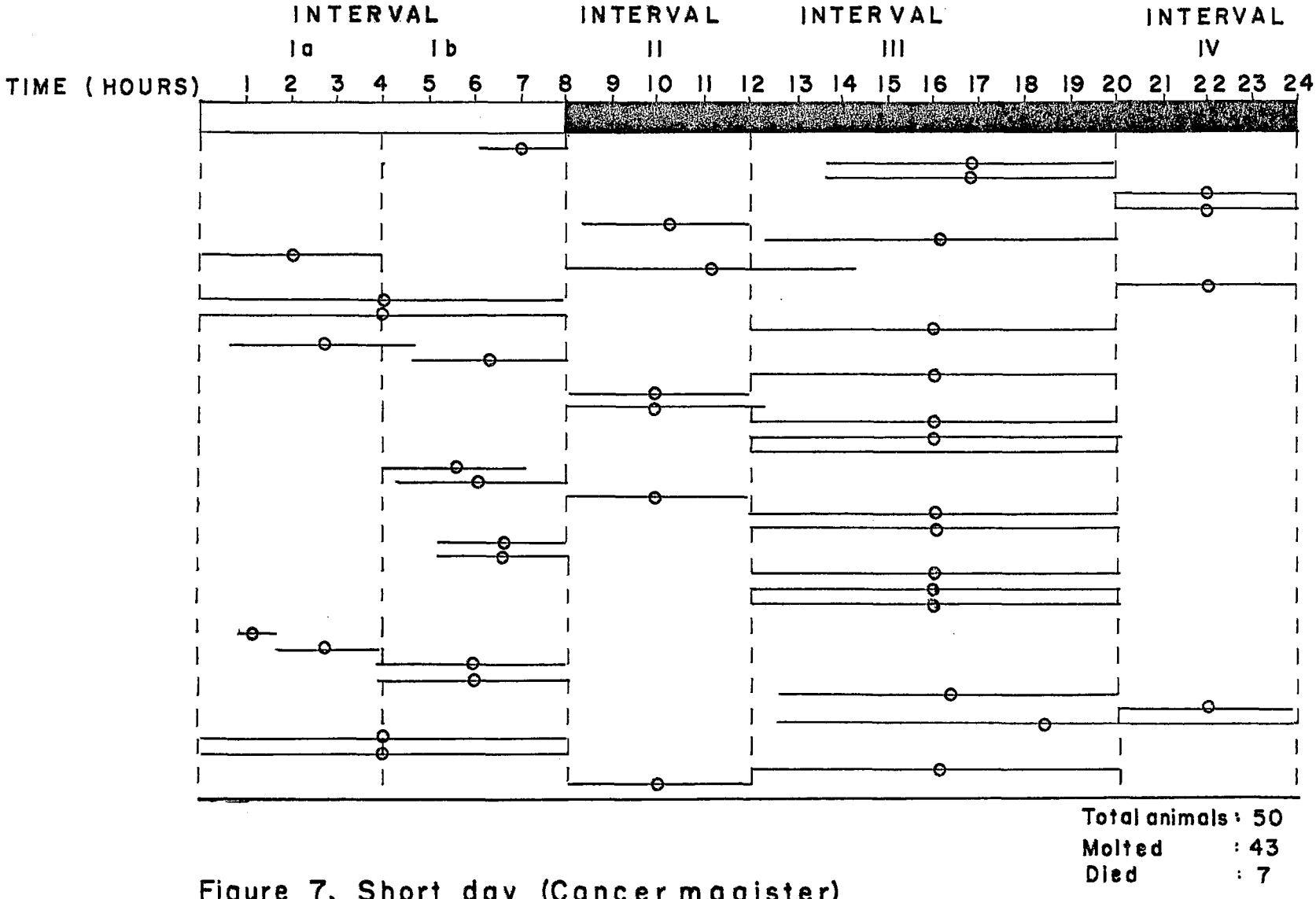

Figure 7. Short day (Cancer magister)

Figure 8

This figure shows the distribution of lengths of intermolt periods of first stage post-larval Cancer magister juveniles, that were subjected to the three light conditions. The horizontal axis denotes the number of days after the beginning of the experiment. The vertical axis represents the number of molts occurring in these days.

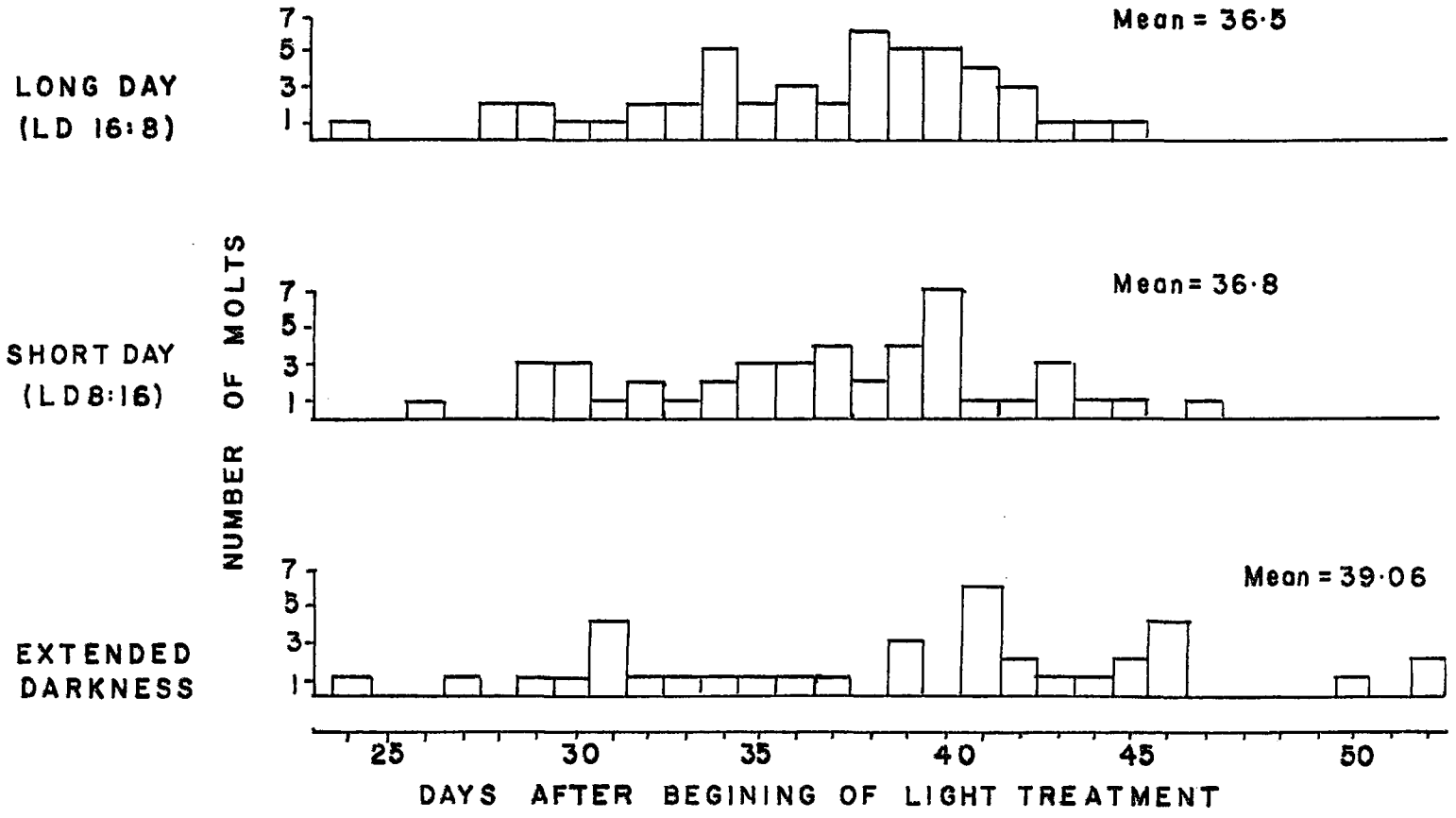

Figure 8. Distribution of molts occuring in Cancer Magister after exposure to different photoperiods.

Tables 1-3c, 5-7c

Results of chi-square tests for molting times in both individual intervals and overall periods of the different light regimes. Individual intervals are indicated by the Roman numerals (I, II, III, and IV). The dark bar represents the dark phase, and the light bar represents the light phase of the imposed photoperiod.

Dusk and dawn intervals in tables 3c and 7c represent eight hour intervals. The imposed dusk and dawn occur at the midpoints of these intervals.

Table 1. Extended darkness. Hemigrapsus nudus

	I	II	III	IV
X^2	3.17	5.03	3.28	4.83
Significant level	n.s.	.025	n.s.	.05

Overall period

X^2 = 16.35

Signif. level $p \leq .005$

Table 2. Long day.

	I	II	III	IV
X^2	0.92	3.83	1.46	2.62
Significant level	n.s.	n.s.	n.s.	n.s.

Overall period

X^2 = 8.47

Signif. level $p \leq .05$

Table 3a. Short day.

	I	II	III	IV
X^2	0.82	4.04	0.42	3.33
Significant level	n.s.	0.05	n.s.	n.s.

Overall period

X^2 = 8.61

Signif. level $p \leq .05$

Table 3b. Post-dawn and pre-dusk intervals.

	Ia	Ib	II	III	IV
X^2	1.63	6.55			
Significant level	n.s.	0.03			

Table 3c. Dusk and dawn intervals.

	Dusk Interval		Dawn Interval	
X^2	10.44		4.81	
Significant level	0.005		0.05	

Table 4. Pooling post-dawn and pre-dusk intervals of long and short day light regimes.

Hemigrapsus nudus

	POST-DAWN	PRE-DUSK
	Long day II + short day Ia	Long day IV + short day Ib
X^2	5.3	4.08
Significant level	.025	.05

Table 5. Extended darkness. Cancer magister.

	I	II	III	IV	Overall period
X^2	1.33	0.17	0.25	0	X^2 = 2.25
Significant level	n.s.	n.s.	n.s.	n.s.	Signif. level p = n.s.

Table 6. Long day.

	I	II	III	IV	Overall period
X^2	3.6	1.94	0.03	6.3	X^2 = 11.86
Significant level	n.s.	n.s.	n.s.	.025	Signif. level p ≤ .01

Table 7a. Short day.

	I	II	III	IV	Overall period
X^2	0.19	0.19	0.19	0.66	X^2 = 1.28
Significant level	n.s.	n.s.	n.s.	n.s.	Signif. level p = n.s.

Table 7b. Post-dawn and pre-dusk intervals.

	Ia	Ib	II	III	IV
X^2	0.96	0.35			
Significant level	n.s.	n.s.			

Table 7c. Dusk and dawn intervals.

	Dusk interval		Dawn interval	
	0.08		1.23	
	n.s.		n.s.	

Table 8. Pooling post-dawn and pre-dusk intervals of long day and short day light regimes.

Cancer magister

	POST-DAWN	PRE-DUSK
	Long day II short day Ia	Long day short day Ib
X^2	0.09	2.18
Significant level	n.s.	n.s.

Stages predominantly present in certain months in Boundary bay

	Stage	Predominantly present in	Present to a lesser degree in
1st. summer	Megalops.....	July, August	May, June, September
	1st post-larval.....	August	September
	2nd " "	August, September	
	3rd " "	September	October, November
	4th " "		September
	5th " "		
2nd. summer	6th " "	May	
	7th " "	June, July	May, August
	8th " "	June, July, August	September
	9th " "	July, August, September	
	10th " "	August, September	
3rd. summer	11th " "	May, June	
	12th " "	May, June	

Table.9. Occurance of Cancer magister molt stages.(After McCay,1942)

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