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Photoperiod, Endocrinology and the Crustacean Molt Cycle

Seasonal changes in endocrine levels may alter the effect of photoperiod on the molt cycle of the crayfish.

D. E. Aiken

More than 2000 years ago Herodotus wrote: "Exposure to the sun is eminently necessary to those who are in need of building themselves up and putting on weight."

Whatever the merits of his counsel to mankind, he spoke the gospel for an assortment of lesser creatures. Directly or indirectly, most animal species respond to the influence of the sun. Dormant animals become active, lean animals become fat, small ones become larger, and complex activities associated with growth and reproduction wax and wane in harmony with the solar rhythm.

The timing of sunrise and sunset is a predictable event in an otherwise fickle environment, and this is extremely important to the many animal species which must time their vital functions to coincide with the appropriate seasons. Thus it is not surprising that so many have come to rely upon the relative lengths of the light and dark phases—photoperiod—for information on progression of the seasons. In the natural environment these light-dark cycles always approximate 24 hours, and this fact has a profound effect upon living systems. The physiologic functions of virtually all organisms, with the exception of bacteria and blue-green algae, show periodic oscillations (*1*). Such oscillations are termed "endogenous" if they persist in the absence of cues from the external environment, and "circadian" (*2*) if they have a period of about 1 day. Environmental stimuli "phase-set" these oscillations to keep them properly tuned to progression of the seasons. The concept of circadian periodicity has generated intense investigation into the nature of the timing mechanism (the biological clock) involved in the regulation of such cycles, and this subject appears to be at the root of a complete understanding of photoperiodism.

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Photoperiodism and Endocrinology: History and Definitions

The concept of photoperiodism had its genesis back in 1920 in the then little appreciated work of Garner and Allard (*3*). Their suggestion that some spring-blooming plants flowered in response to long days was not well received at the time because the view that flowering was controlled by temperature was then generally accepted. A few years later Marcovitch (*4*) demonstrated that sexual form in aphids was photoperiodically regulated, and his findings were followed by Rowen's convincing work on avian photoperiodism (*5*). There was then little doubt that photoperiodism was a widespread and important phenomenon.

Although the present literature on photoperiodism in animals is extensive, most of the work has been done on birds and insects. In insects alone, photoperiod has been implicated in the regulation of metabolism, growth rate, diapause induction, diapause termination, form differentiation, and gametogenesis (*6*). By comparison, the Crustacea have been studied very little. Although there was early interest in diurnal rhythmicity of crustacean chromatophores, the possibility that long-term physiologic cycles were controlled by light was ignored until 1946 (*7*), and not fully examined until 1952 (*8*).

Bliss (*9*) and Stephens (*10*) were the first to examine the relationship between light and the molting cycle of decapods. Stephens' data were complicated by excessive mortality and relatively few molts, but it was amply clear from the work of both Stephens and Bliss that photoperiod was, in some way, involved in certain aspects of the molt cycle. Since that time a number of papers describing the effects of light on various crustacean functions have been published (*11*), but, with a few exceptions (*12–15*), photoperiodic control of the crustacean molt cycle has been ignored.

In the scientific literature terms have a way of evolving until their original precision is lost. The terms *photoperiod* and *photoperiodism* are good examples. As Beck (*16*) has recently pointed out, the term *photoperiod* has been interpreted by some as referring to only the light phase in a light-dark cycle, and by others as including both the light and dark phases. In the past this has caused some confusion. The tendency now is to define *photoperiod* as a cycle consisting of a light and a dark phase, and to speak of the light portion of that cycle as the photophase and the dark portion as the scotophase.

The term *photoperiodism* has also achieved a measure of ambiguity. Some authors have considered virtually all light-dependent functions to be manifestations of photoperiodism. At the other extreme, Farner (*17*) uses the word *photoperiodism* only to designate long-term (usually annual) physiologic cycles that are maintained in phase by the *changing length* of the natural daily photoperiod. A number of problems arise when this definition is strictly applied. In my opinion the most flexible (and probably the most useful) concept of photoperiodism was recently proposed by Beck (*16*), who considers photoperiodism to be the effect of the environmental photoperiodic rhythm on internal biological rhythmic processes.

The history of crustacean endocrinology contrasts sharply with the rather limited work on photoperiodicity in the Crustacea. A great deal has been written concerning hormonal control of molting, reproduction, and other physiologic processes; much of this has been summarized by Kurup (*18*).

Genuine interest in crustacean endocrinology developed during the period 1928 to 1939, when hormonal control of both molting and integumentary chromatophores was demonstrated (*19, 20*). Since that time a great deal of work has revealed that the molt cycle of many crustaceans is controlled by two principal hormones: molting hormone (MH) (*21*), from the so-called Y-organs (paired endocrine organs in the thorax, comparable to the prothoracic glands of insects), and molt-inhibiting hormone (MIH), from the X-organ sinus-gland complex of the eyestalk (Fig. 1). In addition there appear to be other accelerating or inhibiting neurosecretory substances which are elaborated under specific sets of conditions (*22*).

What we need to investigate at present is not so much the endocrinology of the system as the integration of endocrine function and environment, to obtain a complete picture of the molt cycle. It is important to know that a molt-inhibiting and a molt-promoting hormone exist, but it is equally important to know why the effect of a particular hormone is apparent at one time of year and not at another. If the molt of a species is restricted to a specific season, something in the environment may be modifying the endocrine balance in such a way as to bring this about. This is a subject that deserves more attention than it has received in the past.

Genesis of a Research Problem

In September 1963 I began working with a population of the crayfish *Orconectes virilis* from the east-central region of Alberta, Canada. This is an aggressive and adaptable crayfish which is found in lakes and streams throughout a large part of North America (*23*). A number of these crayfish were maintained in the laboratory throughout the winter at a temperature of 20°C, on a schedule of 16 to 20 hours of light daily. No attempt was made to control the photoperiod precisely since these crayfish (all immature) were stock animals that were being held for an entirely different purpose. However, the molting activity that I observed was entirely different from that previously reported by Stephens (*10*), who also worked with immature individuals of this species. Under long-day conditions he had obtained exceptionally high mortality and relatively few successful molts. In contrast, there were no deaths in my experimental groups and all the animals successfully completed three or four molts (*24*) during the 4 months spent under these laboratory conditions. Because of this conflict in experimental results, a new program was designed to examine the ways in which environment affected the molt cycle of this crayfish.

In the study which resulted, only immature crayfish were used because maturity complicates the molting pattern. Adult males molt only twice each year, both molts being associated with changes in breeding condition, and egg-bearing females usually molt only once. In contrast, immature crayfish, with carapace length of 14 to 21 millimeters, normally complete up to four molts in a single season.

Light was the principal parameter of study, but since this is only one aspect of the total environment, the effects of temperature, density, food, aggressive interaction, protective cover, and so on, could not be ignored. In order to minimize the effect of unnatural conditions the habitat was made as nearly natural as laboratory conditions would permit. The value of this approach was dramatized by the work of Bliss (*25, 26*),

who found that any of several unfavorable environmental situations would inhibit molting in the land crab *Gecarcinus*.

The following October, groups of immature *Orconectes virilis* were collected and exposed to daily photophases of either 10 or 20 hours. In the groups exposed to 20 hours of light, all the animals molted successfully within 30 days, but there was no indication of impending molt in the crayfish exposed to 10 hours of light daily, even after 3 months of such exposure. Since the water temperature was rigidly maintained at 20°C for all groups and there were no other apparent environmental differences, it seemed obvious that photoperiod did control the molt cycle of this animal, and that the critical or threshold photophase for induction of proecdysis (the period of rapid physiological preparation for molt) was between 10 and 20 hours of light daily. At this point I was certain that the basic principles were clearly established and that only the details remained to be worked out. The problem, unfortunately, was not that simple.

Changing Seasonal Response

In insects, where photoperiodism has been most extensively studied, induction of a physiological event such as diapause begins when the photophase (or the scotophase) reaches a critical length. This is the basic reason for reliance on photoperiodic information: the animal can complete vital functions before seasonal changes bring about an unfavorable environment. In Alberta, the crayfish population I studied commenced molting about the first of June and ceased molting in the latter part of August. Water temperature decreases rapidly in late August, and by September the water is cold enough to interfere with the molt cycle (*27, 28*). Thus it is to the animal's advantage to have finished molting before the temperature becomes unfavorable in late summer, and to be ready to molt when it again becomes favorable in the spring. From the natural photoperiod in Alberta I estimated that the critical or threshold photoperiod for this particular population would be about 16 hours of light and 8 hours of dark. This would be long enough to induce early molt activity in the spring, but short enough to suppress molting activity after the middle of August.

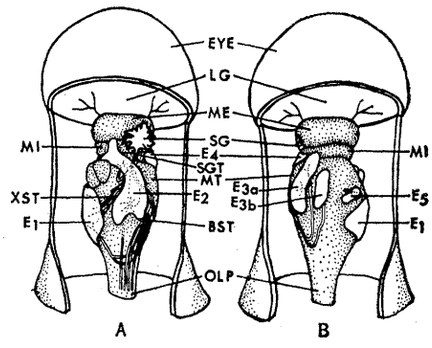

Fig. 1. (A) Dorsal and (B) ventral views of the neurosecretory structures of the right eyestalk of *Orconectes virilis*. (E_1 through E_5) Regions of neurosecretory cells which constitute the X-organ; (*SG*) sinus gland; (*SGT*) sinus-gland tract; (*XST*) X-organ, sinus-gland tract; (*BST*) brain, sinus-gland tract; (*LG*) lamina ganglionaris; (*ME*) medulla externa; (*MI*) medulla interna; (*MT*) medulla terminalis; (*OLP*) optic lobe peduncle. [From Bliss, Durand, and Welsh, *Z. Zellforsch. Mikroskop. Anat. Abt. Histochem.* **39**, 520 (1954)].

In the early experiments I explored the effects of increasing, decreasing, and unchanging photoperiod schedules, and the results generally supported my original impression that molting activity was induced by day lengths in which the photophase was longer than 16 hours. However, when some of the early experiments were repeated, the results were inconsistent. With photoperiod schedules between 14L–10D and 24L–0D (L and D represent hours of light and dark, respectively), good molting results were obtained from all experimental groups. Similar results were later obtained with photoperiod schedules between 9L–15D and 16L–8D, even though I had originally held animals of comparable size three times as long on a schedule of 10L–14D with no sign of molt. Why did some groups molt rapidly in a 9-hour photophase while others showed no sign of molt in a 10-hour photophase?

The answer seemed to be connected with the history of the experimental animals. Although most had been collected at the same time of year (October), it was not possible to conduct all of the experiments simultaneously. As a result, the "stock" crayfish for succeeding experiments were held in constant darkness at 4°C. Disturbance (and exposure to light) occurred only when crayfish were removed for use in the experiments, and this amounted to only a few minutes every couple of months. Since, under the holding conditions, the animals promptly retired to hibernacula and refused food, no feeding disturbance was necessary and their existence in the holding tank (never more than 6 months) was similar to what it would have been in the natural environment.

After analyzing the molting results I concluded that the crayfish that molted in the shortest photophases were those that had spent the longest time in the winter conditions of the holding tank. When the experiments were repeated with crayfish freshly collected in May, the results were in no way comparable to those obtained with crayfish collected in October: those collected in May responded more rapidly and to much shorter photophases. Finally, a group collected between May and July was held in darkness at 4°C until October and then matched against comparable groups of crayfish taken directly from the stream. The results (Table 1) show that at all photoperiods, from 3L–21D to 16L–8D, there

Table 1. Difference in the response of crayfish collected in the spring and in the autumn to various photoperiods, at 20°C. Crayfish collected in the spring (S) and held in darkness at 4°C for 3 to 5 months were matched with crayfish collected in the autumn (A).

Photoperiod	Season collected	Successful molts	Molt mortality			Died* (*N*)	Molt period (days)	Molt attempt (%)
			E_1	E_2	E_3			
16L–8D	A	0	0	0	0	0		0
16L–8D	S	1	2	0	0	1	26–36	60
11L–13D	A	0	0	0	0	0		0
11L–13D	S	0	4	0	0	1	23–38	80
9L–15D	A	0	0	0	0	0		0
9L–15D	S	0	3	0	0	1	23–24	60
7L–17D	A	0	0	0	0	0		0
7L–17D	S	2	1	1	0	1	22–29	80
5L–19D	A	0	0	0	0	0		0
5L–19D	S	1	3	0	0	1	15–33	80
3L–21D	A	0	0	0	0	1		0
3L–21D	S	2	2	0	0	1	26–33	80

* Deaths not obviously associated with ecdysis.

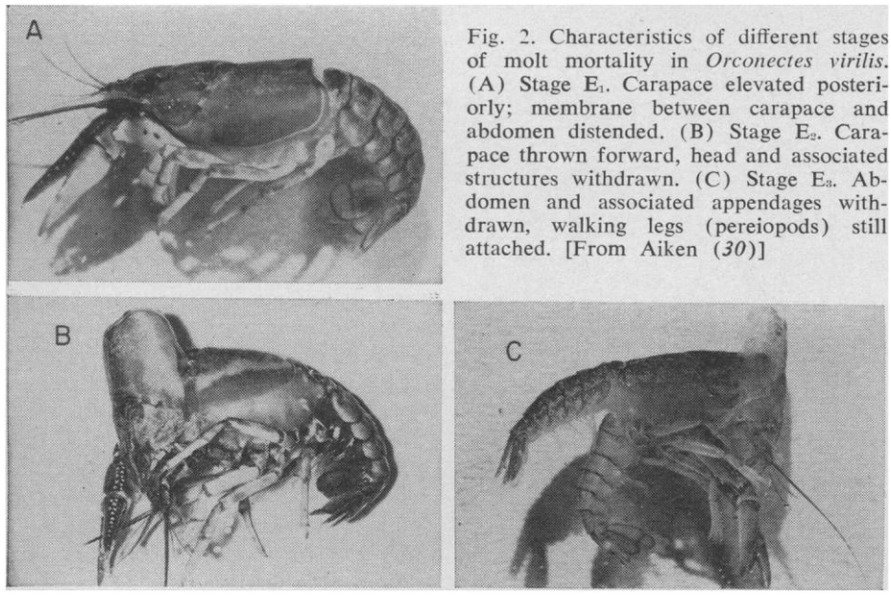

Fig. 2. Characteristics of different stages of molt mortality in *Orconectes virilis*. (A) Stage E_1. Carapace elevated posteriorly; membrane between carapace and abdomen distended. (B) Stage E_2. Carapace thrown forward, head and associated structures withdrawn. (C) Stage E_3. Abdomen and associated appendages withdrawn, walking legs (pereiopods) still attached. [From Aiken (*30*)]

were successful and unsuccessful molts among the crayfish collected in May, *but no indication of molt among those collected in October.*

In Table 1, molts that were not successfully completed are classified as E_1, E_2, or E_3 mortalities. The crustacean molt cycle is divided into five stages—A through E (*29*)—ecdysis being designated stage E. As these experiments progressed it became apparent that molt mortalities could be grouped into types, a finding which suggests that stage E consists of several distinct physiological steps. The different types of molt mortality are shown in Fig. 2. (For a complete description, see *30*; see also *31*.)

In the autumn a photophase as long as 20 hours is required to induce a molt. In the spring the animal will molt in response to a photophase of 3 hours or less. What happens during this critical winter period? Is there a gradual change in response, or is there a sudden reversal in photosensitivity at some point in the winter period?

In an effort to find out, I collected approximately 200 crayfish on 9 October and held them in darkness at 4°C. Groups were removed at 30-day intervals from 9 November through 7 February, a span of 120 days. Half of the crayfish in each group were exposed to 16 hours of light daily; the other half, to 7 hours. Thus all the groups received exactly the same exposures; only the time spent in winter conditions prior to exposure differed for the various groups. For the sake of convenience this experiment is referred to hereafter as "experiment 15."

The result, summarized in Table 2, shows how response to a given photoperiod is altered by the time spent in winter conditions. In November, both the 7-hour and 16-hour daily photophases were too short to induce a molt. By December the 16-hour photophase was long enough to cause 2 of 20 crayfish to attempt a molt, but both of these died at stage E_2. A month later the 7-hour photophase was still too short, but the 16-hour photophase caused 29 of 30 crayfish to attempt a molt. Most important, 28 of the 29 died at stage E_3. This suggests that the different types of molt mortality result from an interaction between the environment and the physiological state of the animal.

In February the 16-hour photophase induced rapid and successful molting, but the crayfish exposed to 7 hours of light did not fare so well. Apparently the 7-hour photophase was close to the critical length at this time, because only 70 percent of the crayfish attempted a molt and only 40 percent were successful.

Although the January group on the 7L–17D photoperiod schedule had shown no tendency to molt during the 30-day period of observation, they were maintained on this schedule for an additional 35 days for comparison with the February group on the same schedule. Surprisingly, both groups began molting at the same time. This result is important because it suggests that the molt-inducing influence of a given photoperiod remains relatively constant whereas the *resistance* to this effect changes with time.

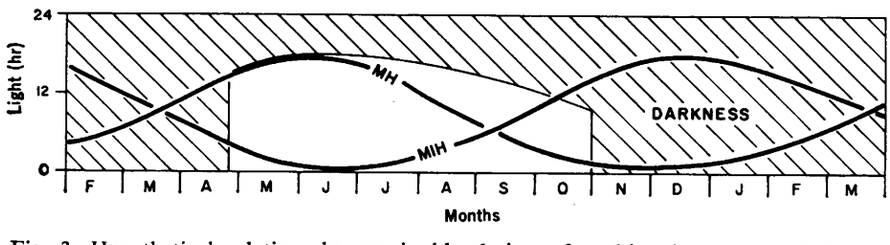

Fig. 3. Hypothetical relative changes in blood titer of molting hormone (*MH*) and molt-inhibiting hormone (*MIH*) in *Orconectes virilis* in Alberta, as inferred from laboratory molting results. Synthesis or release of MIH is assumed to be inhibited by light. The unshaded region indicates the hours of light experienced by this species at various times of the year as determined from behavioral and photoperiod data.

Photoperiod and Endocrine Activity

The relationships between environment and endocrine activity are complex and poorly understood. Long days will induce molt during the winter, when molting would not normally occur, and removal of both eyestalks produces the same result (*27*, *32*). Similarly, the effects of both photoperiod and eyestalk ablation differ according to the season of the year. This indicates that long days induce molt in the same way that eyestalk ablation does—by reducing the titer of MIH. Bliss (*25*) suggested that photoperiod controls MIH secretion in the land crab *Gecarcinus*, but Jegla (*33*) has apparently found a seasonal cycle of MIH in a cave crayfish that is never exposed to light.

It is commonly stated that MIH is primarily an inhibitor of the Y-organs (*25*, *34*, *35*) and that Y-organ synthesis of MH commences when the titer of MIH is reduced. However, Carlisle (*34*; see also *35*) has demonstrated that MH and MIH may be present at the same time, and the results from experiment 15 (7L–17D, groups C and D) suggest that the titers of MH and MIH may even be independent of one another. This could mean that Y-organ activity

is directly stimulated by light. In some insects light acts through the central nervous system to cause production of ecdysone by the prothoracic glands (*37*). Since molt-accelerating substances have been found in the central nervous system of crayfish and other crustaceans (*38*), and since there are a number of similarities in the molting physiology of insects and crustaceans, photoperiodic control of Y-organ activity is certainly a possibility.

Experiments which I am at present conducting suggest that the molt cycle of the lobster (*Homarus americanus*) is not as sensitive to photoperiodic control as the molt cycle of the crayfish. In addition, attempts to accelerate the lobster molt cycle by bilateral eyestalk ablation have not been very successful. Since *Homarus* is not truly a seasonal breeder, it may be that MIH is not present in this animal (*22*). *Homarus* may represent the primitive condition, MIH and photoperiodic influence being found only in more specialized forms which have a need for seasonal regulation of the molt cycle. If MIH is the endocrine substance which has been evolved for seasonal regulation of molting, and if light is the parameter upon which this regulation is based, it seems logical that the effects of light should be expressed through regulation of MIH. This is especially true since the X-organ sinus-gland complex is intimately associated with the eye, and Bliss and Boyer (*12*) clearly showed that the eye is the pathway by which light affects molting in at least one crustacean.

Although there are a number of possible explanations, the limited experimental results favor the following hypothesis of photoperiodic control of ecdysis in *Orconectes virilis*. Long days inhibit synthesis (by the X-organ) or release (by the sinus gland) of MIH, and the inhibition is proportional to the length of the photophase. Maximum titer of MIH occurs in short days or constant darkness, but the X-organ sinus-gland complex eventually becomes refractory and MIH titer decreases. MIH controls the molt cycle principally by preventing the tissues from reacting to MH, and proecdysis is induced when the endocrine balance shifts in favor of MH.

As yet there is no solid evidence that MIH does not regulate Y-organ synthesis of MH, but recent experiments by Lowe, Horn, and Galbraith (*39*) showed that injected 20-hydroxyecdysone (crustecdysone) will accelerate

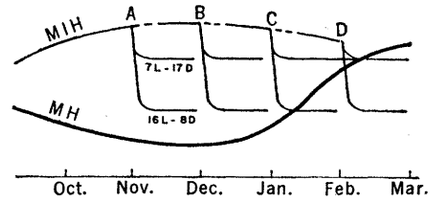

Fig. 4. Possible changes in titer of molting hormone (*MH*) and molt-inhibiting hormone (*MIH*) in crayfish exposed to photoperiods 7L–17D and 16L–8D in experiment 15 (see text). Inhibition of MIH is assumed to be proportional to the length of the photophase.

proecdysis in crayfish from which the eyestalks had been removed but is ineffective in intact crayfish (that is, crayfish in which MIH is still present). This finding supports the suggestion that MIH inhibits molting by interfering with the action of MH.

The annual relative titers of MIH and MH in a natural population of *Orconectes virilis* would be similar to those shown in Fig. 3, where the titer of MH is maximal during spring and summer and minimal in autumn and winter. Long days of spring and early summer would hold MIH secretion to a minimum, but the titer would increase gradually through late summer and autumn and reach a maximum by December, after which time the eyestalk complex would become refractory to further stimulation.

When this scheme is applied to the results of experiment 15, the hormonal changes shown in Fig. 4 may be expected. Here the degree of inhibition of MIH is assumed to be proportional to the length of the two photophases used (7 and 16 hours). In groups A and B no molting occurred because of the great difference in hormonal titers in November and December, but by January this difference was not so great and molting was induced by 16 hours of light (although all attempted molts were unsuccessful). By February the relative titers of MH and MIH had changed and molt was induced by both photophases, although there was a pronounced difference: those on the 16L–8D schedule molted rapidly and successfully, but 60 percent of those on the 7L–17D schedule died during the molt. Significantly, crayfish from the January group on the 7L–17D schedule also began molting at this time, and 79 percent of these molts, also, were unsuccessful.

Molt Mortalities

Thirty years ago Brown and Cunningham (*20*) removed the eyestalks from two groups of crayfish, then implanted a single sinus gland into each individual of one group and eyestalk tissue without the sinus gland into individuals of the other group. Through the work of Passano (*40*) and several others, we know that both implants would have released MIH, but that the sinus-gland tissue would probably have liberated more than the eyestalk tissue alone. In the Brown and Cunningham experiments the group that received the greatest amount of MIH showed only the first signs of ecdysis, while the group that received lesser amounts of MIH progressed further: some of the animals withdrew completely from the exuvium before dying. The results of Brown and Cunningham suggest that molt mortalities obtained with ab-

Table 2. Relationship between time spent in winter conditions (darkness, at 4°C) and molting response to short- and long-day photoperiods at 20°C. Data are for 30-day observation periods.

Beginning date of experiment	Photoperiod	*N*	Successful molts	Molt mortality			Died* (*N*)
				E_1	E_2	E_3	
		Group A					
9 November	16L–8D	10	0	0	0	0	0
9 November	7L–17D	10	0	0	0	0	0
		Group B					
9 December	16L–8D	20	0	0	2	0	0
9 December	7L–17D	20	0	0	0	0	0
		Group C					
8 January	16L–8D	30	0	0	1	28	1
8 January	7L–17D	30	0	0	0	0	1
		Group D					
7 February	16L–8D	20	20	0	0	0	0
7 February	7L–17D	20	8	4	2	0	2

* Deaths not obviously associated with ecdysis.

normal photoperiods might be caused by the interaction of MH and MIH.

I have cited evidence that MH and MIH may be present in the blood at the same time. If proecdysis is induced when the influence of MH exceeds that of MIH, it may be that problems arise if the titer of MIH subsequently becomes the more influential. Ecdysis proceeds through a series of distinct steps, and the pattern of molt mortalities indicates that each of these steps is controlled by a different physiologic process during proecdysis. If, after the onset of proecdysis, the titer of MIH interferes with premolt preparation for one or more of these molting steps, the molt attempt is terminated at the point where the affected step occurs. A lesser degree of interference from MIH may simply prolong proecdysis, whereas a greater degree may suspend it indefinitely.

The pattern of successful and unsuccessful molts in experiment 15 suggests that the 16-hour photophase was of subthreshold length in November and December, of marginal length in January, and clearly above threshold length in February. Likewise, a 7-hour photophase was subthreshold from November through January and marginal in early February. If mortalities result from the interaction of MH and MIH, and if photoperiod controls synthesis or release of either of these hormones, then molt mortalities are an even better indicator of photoperiod-endocrine interaction than the molt itself. Instead of the all-or-none indication provided by the molt, the molt mortality provides a *graded* response, which tells as much about the status of the endocrine system as it tells about the inductive nature of the photoperiod.

Some General Principles

The molt cycle of *Orconectes virilis* is regulated by environmental factors, especially photoperiod, so that molting occurs at the most propitious times of the year. However, the endocrine system of this animal modifies the inductive effect of photoperiod in an interesting way. During seasons of the year when molting would be disadvantageous, photophases much longer than those that occur in nature are required for induction. Conversely, during those seasons which are most favorable for growth of the animal, successful molting can be induced by extremely short photophases. The significance of this fact must be emphasized, for it means that the *history* of an experimental animal is of paramount importance. Isolated experiments, and experiments made with animals whose precise history is not known, are bound to be misleading. Similarly, the reaction of the animal to its total environment cannot be ignored. Other experiments, not described here, clearly showed that molting response to inductive photoperiods is adversely affected by such unnatural conditions as lack of adequate cover or crowding of experimental animals, and that in extremely crowded conditions the mortality is excessive. Large experimental groups are useful from a statistical point of view, but they may yield results which are meaningless unless the ecological requirements of the species are met.

Heavy mortality during ecdysis occurs in crayfish on abnormal photoperiod schedules, and in those on schedules in which photophase is close to a critical or threshold value. Protracted intermolt times and high incidence of unsuccessful molt also occur when a photophase which is initially above threshold is later reduced to a subthreshold length. Molt mortalities were found to occur at definite stages during ecdysis, and there seemed to be a cause-effect relationship involving the history of the animal and the length of the photophase to which it was exposed.

The experimental results suggest that photoperiod controls the molt cycle by regulating synthesis or release of MIH, which in turn alters tissue response to the inductive effect of MH. Proecdysis is induced when the balance between these two hormones shifts sufficiently for the influence of MH to overcome that of MIH. If the titer of MIH subsequently rises to an inhibitive level, attempted molts may be incomplete or proecdysis may be either protracted or indefinitely suspended.

Implicit in this hypothesis is the assumption that the molt cycle is controlled by two hormones—MH and MIH. Lowe *et al.* (*39*) have suggested that the Y-organ may produce additional hormones which are essential for the initiation of proecdysis, and, as Mobberly (*13*) has pointed out, MIH may in fact be a series of hormones. New techniques will undoubtedly provide answers to these questions.

The problems of time measurement, photoperiodism, and endocrine activity in *Orconectes virilis* are apparently more complex than had been originally thought. Few of the principles of time measurement which have been worked out for photoperiodic regulation in insects (*41*) and birds (*42*) can be applied to this crustacean because of its constantly changing sensitivity to photoperiodic stimulus. Much work will have to be done on both endocrine and environmental aspects before the Crustacea can be added to the select list of animal groups whose photoperiodic responses are reasonably well understood.

Note added in proof: After this manuscript was submitted, Krishnakumaran and Schneiderman (*43*) published results which were diametric to those which I have cited by Lowe, Horn, and Galbraith (*39*). Although both groups used the same genus of crayfish and injected the same synthetic hormone, Krishnakumaran and Schneiderman induced molting in intact animals by injecting crustecdysone in concentrations nearly 50 times the *lethal* dosage reported by Lowe *et al.* The reasons for those conflicting results are not clear at present, but it is obvious that more work of this nature is required.

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15. Mobberly (*13*) worked with the crayfish *Faxonella*, and was concerned with photoperiodic influence on gastrolith production rather than with the actual molt. It should be remembered that gastrolith formation is only one physiologic step in the premolt process, and that conditions that will cause gastrolith formation may not be suitable for molt. Bliss and Boyer (*12*) studied the land crab *Gecarcinus*, and their work is a classic. Parker's paper (*14*) contains limited data on photoperiodic influence on molting in *Daphnia*.
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