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The Effects of Temperature & Photoperiod on Molting in Seasonal Populations of the Crayfish Orconectes Rusticus Rusticus (Girard)

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THE EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON MOLTING IN SEASONAL POPULATIONS OF THE CRAYFISH ORCONECTES RUSTICUS RUSTICUS (GIRARD)

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THE EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON MOLTING IN SEASONAL POPULATIONS OF THE CRAYFISH ORCONECTES RUSTICUS RUSTICUS (GIRARD)

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ABSTRACT

Immature Orconectes rusticus rusticus (Girard) were collected from Doe Run, Meade Co., Ky. on January 16, June 1, and October 21, 1972, for the purpose of examining the effects of three different temperatures (14C, 18C, and 22C) and three different photoperiods (6L:18D, 15L:9D, and 24LL) on seasonal molting patterns. For each seasonal experiment, 144 crayfish were placed in 3 X 3 experimental units and maintained for a 90-day period.

In Experiment 1 (January 18-April 18), 98 molts were attempted with 98% of the molts being successfully completed. Arrangement of these molts within the separate treatments of this experiment indicated that there was a highly significant linear relationship between increasing temperature and increasing populational molt frequency as well as a significant linear relationship between increasing length of photophase and increasing molt frequency. No crayfish molted during the first 15 days of this experiment and animals in the warmest temperature molted an average of 17.06 days earlier than those in the coldest temperature.

Crayfish in Experiment 3 (October 23-January 20) molted in similar patterns with respect to temperature and photoperiod but molted in fewer numbers (43 molts attempted) than those in Experiment 1. Animals molted immediately

upon initiation of this experiment and crayfish kept at 22C molted in highest numbers toward the end of the experiment. Ninety five percent of the molts attempted by these crayfish were successfull.

Crayfish in Experiment 2 (June 3-September 1) molted in the highest total number of all of the experiments (144 molts attempted) but with the lowest percentage of molts successfully completed (30%). Although molt mortalities were high in this experiment, they appeared to occur independent of temperature and photoperiod treatments. Crayfish molt frequency showed a significant quadratic relationship with temperature but no relationship between successful or unsuccessful molt frequencies and increasing photophase was noted.

Two auxiliary experiments were also completed in which 48 adults collected in June were tested for molt frequency in three different light intensities (10, 40, and 110 f.c.) and 48 adults collected in October were compared directly with the molt frequency of 48 immatures. In both experiments, adults molted in fewer numbers than immatures tested at the same time, the greatest difference being found in the October experiment (3 adult molts vs. 19 molts by immatures). No relationship between light intensity and molt frequency was found.

No sex differences or interactions between treatments were found in any of these experiments.

Discussion of these results centered around the characterization of a proposed molt control mechanism for this population of O. r. rusticus. Such a mechanism was characterized as being most sensitive, on a populational level, to temperature and photoperiod during the spring and fall seasons and less sensitive or insensitive to these same signals during the summer. The linear aspect of the relationship between treatments and molt frequencies in Experiments 1 and 3 was interpreted as an indication that either this molt control mechanism is less sensitive to environmental signals than the one previously characterized for more northern populations of O. virilis or that more individual variation exists in this population of O. r. rusticus.

The large number of mortalities that occurred in Experiment 2 was discussed but not explained.

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INTRODUCTION

The objectives of this study were to determine in controlled conditions the extent of the effects of temperature and photoperiod upon molting patterns in one species of crayfish, Orconectes rusticus rusticus (Girard). Three different temperatures (14C, 18C, and 22C) and three different photoperiods (6L:18D, 15L:9D, and 24LL) were tested on immature crayfish obtained at three different times of the year (January 16, 1972; June 1, 1972; and October 22, 1972) from Doe Run, Meade Co., Kentucky. The effects of these treatments upon the crayfish used were measured by observing the frequency and success of individual molts (ecdysis) as well as the temporal arrangement of molting within the 90-day experiments.

Molting is one of the most important aspects of crustacean physiology. The presence of an exoskeleton for protection and muscle attachment is advantageous but such a rigid calcified structure places severe restrictions on growth. For animals with exoskeletons, molting is the only means of growth. The periodic replacement of integument directly and indirectly affects most aspects of the life of a crustacean, including metabolism, reproduction, behavior, and other processes.

At one time, the term, molting, referred only to the actual exuviation (ecdysis), but more recently the term has come to include pre-molt, molt, post-molt, and inter-molt (Drach, 1939; Passano, 1960; Kurup, 1963; Aiken, 1968b). This cycle is continuous, since recovery from one molt is immediately followed by preparation for the next. The actual ecdysis (Stage E) takes only a short time compared to the whole cycle, but this period presents mechanical, physiological, and biological danger.

Mechanical difficulty occurs as the animal actively withdraws from the old exoskeleton (Passano, 1960; Lockwood, 1967). Aiken (1968b) divided ecdysis (Stage E) into four distinct terminal subcategories: E1, E2, E3, and E4. Animals that became entangled in the old exoskeleton and were unable to complete exuviation were termed E1, E2, or E3, depending upon the degree of withdrawal previous to termination. All of these unsuccessful molts resulted in the death of the crayfish involved. A molt which was successfully completed with the animal totally withdrawing from the old carapace and surviving to initiate another molt cycle was termed E4.

Physiological problems arise from the heavy uptake of water that occurs following ecdysis and the associated dilution of the total ion concentration of the body. Permeability changes in the softened cuticle prior to deposition of chitin and calcium also present problems. Finally, biological danger arises from the increased susceptibility to

disease and predation of the newly molted animal (Lockwood, 1967).

The molt cycle of crustaceans is currently believed to be controlled by two principle hormones: molting hormone (MH) and molt inhibiting hormone (MIH)(Carlisle and Knowles, 1959; Passano, 1960; Mobberly, 1963; Lockwood, 1967; Costlow, 1968; Aiken, 1969b). Molting hormone, produced and released by the Y-organ located in the thoracic region of crayfish, is apparently responsible for initiating ecdysis. Molt inhibiting hormone is produced by the X-organ of crayfish and stored for release in the sinus gland complex of the eyestalk. It is believed that the presence of this hormone inhibits the secretion of MH. Activation of the Y-organ by a reduced concentration of MIH causes the release of MH into body circulation, bringing about the pre-molt phase. It is believed that, once initiated, the molting process will then continue until the completion of one full molt cycle is achieved. During intermolt, high levels of MIH will inhibit the activation of the Y-organ until sufficient metabolic reserves are stored to start the cycle again. McWhinnie, et al. (1972), suggested that several molt control hormones may be involved in this cycle.

The possible effects of environmental conditions upon the endocrine MH-MIH balance have received little attention in the literature. Some consideration has been given to the possible effects of environmental and mechanical variations on the speed and degree of completion of molt cycles in

crustaceans. Limb loss stimulated molt activity in laboratory experiments on the land crab, Gecarcinus lateralis (Skinner and Graham, 1970), and the presence of another individual inhibited successful molting (Bliss and Boyer, 1964). An inhibition of molting by the presence of unnatural environmental conditions and soil humidity was also demonstrated (Bliss and Boyer, 1964).

Limb loss (Mobberly, 1963; Stevenson and Henry, 1971), eyestalk removal (Scudamore, 1947; Mobberly, 1963), and the removal of eggs from "in berry" females (Scudamore, 1947), accelerated the molt cycles of crayfishes. These factors, however, are primarily dependent upon population density and predator-prey relationships and would, as a result, be more important on the individual molt level than they would be to the overall populational molt condition.

A population existing in a temperate region would be exposed to seasonal variations in meterological and climatic conditons and would find certain times of the year undesirable for the initiation of the critical period of ecdysis. Signals used for the coordination of the molt cycle, then, should properly reflect the changing seasons so that proper avoidance of unfavorable molting conditions can be made (Pittendirgh and Minis, 1964; Adkisson, 1966). Such signals would be most valuable if they remained constant from year to year. Above all, these environmental keys must allow the population to detect and take advantage of favorable environmental conditions as well as avoid unfavorable con-

ditions. Temperature and photoperiod are two possible environmental variables that would seem likely to fulfill proposed requirements.

The role of temperature on molting patterns of crustaceans has, for the most part, been neglected. The majority of work has been done in laboratory studies of other metabolic activities. Orconectes virilis acclimated at 21C had higher levels of oxygen consumption than at 1C and 11C (Jungreis and Hooper, 1968). In this study, increased circulating blood volumes and decreased intercellular water content were also observed at the higher temperature. Orconectes virilis responded to increased temperatures with linear quantitative changes in overall metabolism (McWhinnie and O'Connor, 1967; Jungreis and Hooper, 1968). Aiken (1969a) found that increased spring water temperature induced egg laying in female O. virilis as long as there was an associated long-day photoperiod.

In examining the effects of temperature on the molting phenomenon of crustaceans, Hess (1941) found that temperature changes were important in regulating the diurnal molt cycle in the shrimp, Crangon armillatus. Temperature influenced molt cycles have also been proposed for the crab, Gecarcinus lateralis (Bliss and Boyer, 1964), the Louisiana crayfish, Faxonella clypeata (Mobberly, 1963), an Illinois population of Orconectes propinquus (Van Deventer, 1937), and a Kentucky population of O. r. rusticus (Prins, 1968).

Temperature, as a major seasonal control mechanism, has a great deal to do with the regulation of cycles, but wide fluctuations of temperature found within a specified season could cause the termination or initiation of a critical cycle at the wrong time. Stephens (1955), for example, found that large percentages of photoperiod-controled molts resulted when animals were maintained in a temperature (21C) abnormally warm for the October collected crayfish.

Daily light cycles, however, are fixed by a synchronized yearly cycle and lend themselves as accurate control signals year after year. There is extensive literature on photoperiod and its implications, but most of the work has been done with insects. Photoperiodic controls in insects have been implicated in growth, metabolism, diapause induction and termination, and many other factors (Beck, 1968; Adkisson, 1966; Lees, 1966; Lutz, 1968; Kamm, 1971). By comparison, the crustacea have been studied very little (Aiken, 1969b).

Apparently, for crayfish as well as for other arthropods, both duration of illumination and intensity of light affect molting. Mobberly (1963) found that constant light reduced pre-molt activity in F. clypeata from Louisiana. Stephens (1955) stated that in O. virilis from Canada, collected during winter conditions, almost no molting occurred in complete darkness and that these crayfish responded to increased daily illumination (10L:14D and 20L:4D) with increased numbers of molts. His work, however, was marked by

high mortalities. Aiken (1969b), repeating the work of Stephens (1955), recorded minimal deaths with a high number of successful molts completed by experimental animals and was able to support the proposed photoperiodically controlled molt cycle of this crayfish.

An associated relationship between light intensity and molting regulation was demonstrated in the crab, G. lateralis. In this animal, light intensities greater than 10 lux inhibited molt activity (Bliss, 1954).

It has been demonstrated that temperature and photoperiod are independently important to molting cycles; however, interactions between these signals may also exist. Aiken (1969a) indicated that long-day photoperiods must be associated with high temperatures for the proper coordination of egg producing cycles of female O. virilis. Interactions have not been examined with regard to molt cycles.

The impact of these environmental signals may be seasonally variable in northern populations. Aiken (1969b) found that the photoperiodic molting response in O. virilis was seasonally dependent. Crayfish in northern latitudes have been observed to cease molting during winter conditions with a resumption of molt activity associated with the advent of spring conditions (Ortmann, 1906; Creaser, 1934; Van Deventer, 1937; Tack, 1941; Smart, 1962). These patterns might be geographically and environmentally limited since crayfish found in relatively constant temperature environments do not demonstrate such clear-cut seasonal

patterns (Penn, 1943; Prins, 1968).

Variations in molting activity have also been demonstrated for different age-size groups of crayfish. Prins (1968) found that O. r. rusticus individuals reduce their yearly molting frequency with increased age, an observation earlier made with O. propinquus (Van Deventer, 1937). Similar inverse relationships between molt frequency and age have been reviewed by Passano (1960) who proposed that, in crustaceans in general, intermolt stages recur with progressively longer durations, presumably correlated to the increasing mass of the organism.

Immature O. r. rusticus were therefore chosen for primary study in the experiments presented herein since higher numbers of molts would be expected from them and this would allow for more precise statistical analyses. Adult molting was not ignored in this study, however, and two experiments compared their molting patterns with immatures.

This study was initiated to further clarify the effects of temperature and photoperiod on the molting cycles of the crayfish, O. r. rusticus, collected in different seasons. Additional experiments were developed to examine the effects of different light intensities on adult crayfish molting patterns and to further research the different patterns of adult and immature molt cycles. Reactions of males vs. females to all treatments were also examined along with any possible interactions between treatments.

Collections of members of an established population of the stream crayfish, O. r. rusticus, were made about two miles from the spring source of Doe Run, Meade Co., Ky. (Station III, Prins, 1968). Besides describing the physical condition of the collection site on a seasonal basis, Prins also gave detailed field observations on the occurrence and ecology of this species.

METHODS AND MATERIALS

Crayfish were collected in January, June, and October, 1972, for use in each of the three 90-day seasonal experiments. For each, crayfish were obtained by seine and 144 sub-adults (e.g., those with 14-17 mm carapace length; Prins, 1968) were kept and transported to laboratory facilities at Western Kentucky University. Field temperatures were taken at the time of each collection and the water used for the transportation of crayfish was allowed to adjust to ambient laboratory temperature (approx. 22C) prior to weighing, measuring, and assigning animals to experimental positions. Other than requiring equal sexes in each compartment, assignments were made randomly.

Three experimental units, modified from Harrison (1964), were used in each of these experiments. Each unit contained its own temperature controlled water supply which circulated throughout its six subdivisions. These subdivisions comprised the compartments among which two duplications of each of the three photoperiods (6L:18D,15L:9D, and 24LL) were randomized. The three temperature units (14C, 18C, and 22C), each containing the three photoperiods, were arranged in a 3 X 3 completely random design with a factorial arrangement of treatments (Cochran and Cox, 1957).

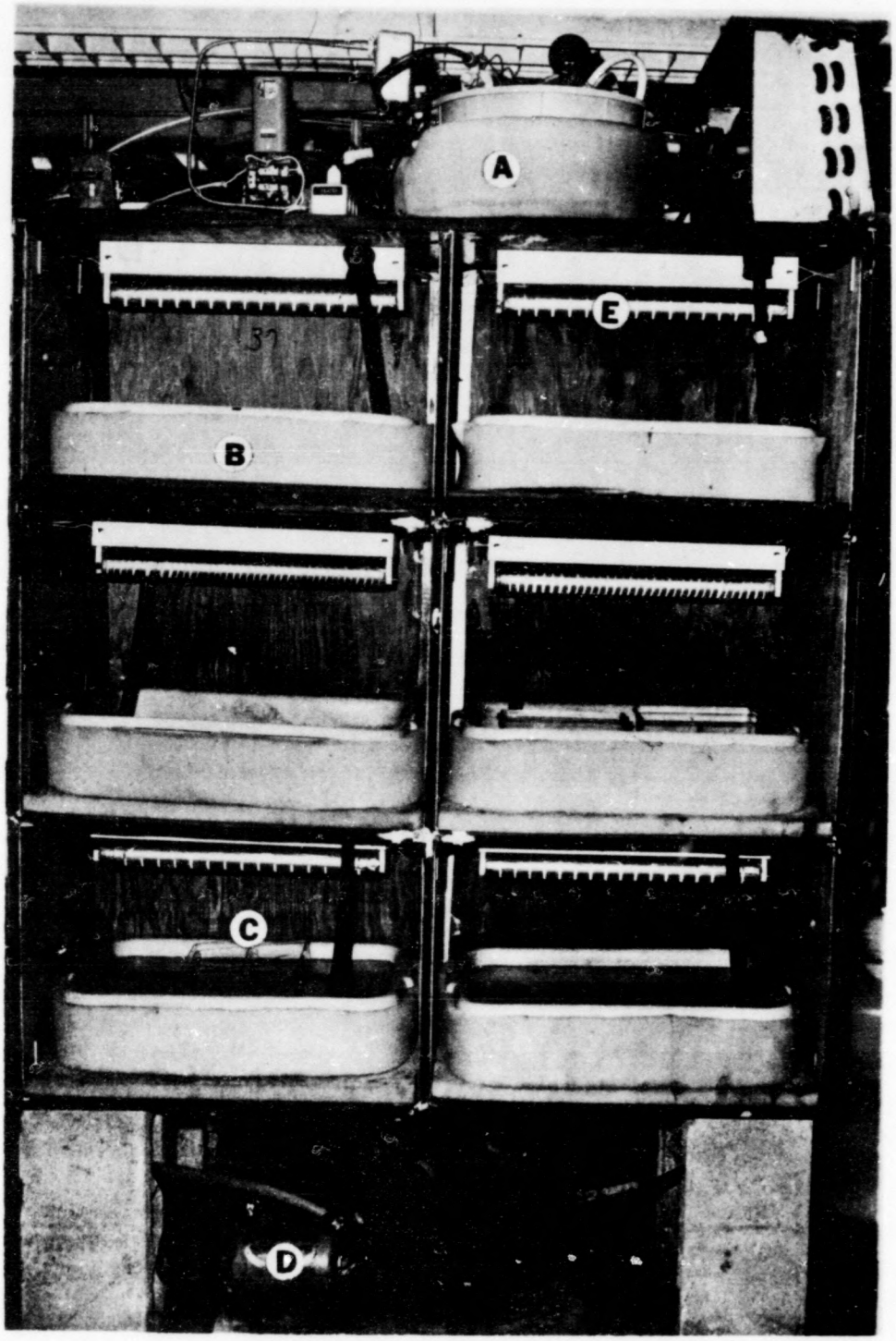
Each experimental unit contained approximently 75 l of water. Water flow was maintained within a unit by a gravity system powered by a pump located beneath. Water collected at the bottom was pumped to the top of the unit where it flowed through a glass wool, activated charcoal filtering system and then into the temperature control reservoir (Fig. 1A). Here, a thermostatically controlled freon cooling system alternated with an immersion heater to maintain the designated temperature within 0.5C. Water then entered the top two compartments and flowed (at approximately 6 l/min) into plastic basins (Fig. 1B).

Crayfish were arranged within basins by being confined in one of two partitioned plastic trays which were partially immersed in water (Fig. 1C). These trays provided 585 mm^2 of crawling surface and 200 ml of water directly available to each crayfish. Holes were drilled through the trays to allow for free flow of water and transparent plastic tops were placed on each tray to prevent animals from crawling out.

Gravitational water flow then continued through the middle and lower compartments, below which water was once again pumped (Fig. 1D) to the top for recirculation. Tap water was used and the water level was adjusted frequently to correct for evaporation. The entire water supply was replaced every three weeks.

The three photoperiods used in these experiments were maintained by time-controlled fluorescent light within the

FIGURE 1. One environmental chamber used for testing Orconectes rusticus rusticus. (A) Reservoir containing thermostatically controlled heating/cooling system. (B) Individual compartment. (C) Receptacle for 4 crayfish. (D) Water pump. (E) Fluorescent light wrapped with opaque tape to reduce light intensity.


A
E
B
C
D

compartments. In each compartment, one 43 cm General Electric Cool White 15 watt bulb (F15T12•CW) was suspended 22 cm above water level (Fig. 1E). Intensity was measured at the water surface level and adjusted to 40 f.c. by partially wrapping bulbs with opaque cloth tape. Trays were randomly rearranged weekly to insure equal reception of light intensity by experimental animals.

Tray tops were wiped daily and reduced the visible spectrum light intensity less than 10%. The ultraviolet light band, however, was reduced by approximently 58% while passing through the plastic. Each 90-day experiment was initiated 48 hours after placement of crayfish in the experimental units. Animals were placed in the units the same day on which they were collected. During each testing period, individuals were checked daily and molts (along with sexual form and molt condition) were recorded. Molt categorization was accomplished by consolidating the terminal molt stages proposed by Aiken (1968b). Molt stages E1, E2, and E3 were termed unsuccessful for the purposes of this study and E4 molts were termed successful.

Exuviae were removed following the completion of each molt. Dead crayfish were removed, weighed, and measured as the experiment proceeded. All living crayfish which were remaining at the termination of experiments were also weighed and measured.

Every five days, crayfish were fed approximently 0.5 g of an experimental high protein diet. Uneaten food was re-

moved and weighed after desiccation. Fecal material was removed daily from the bottom of each plastic basin.

Upon the termination of these experiments, molting data were analyzed by means of the Analysis of Variance (Cochran and Cox, 1957). A linear and quadratic regression analysis was further performed on treatment sums of squares for more specific information. Sex differences in molting patterns were also analyzed along with any possible interactions between treatment effects which might have occurred. The average number of days until animals within each treatment accomplished their first molt was computed along with standard deviations. In Experiment 2, analysis of variance and regression analyses were also performed on data arranged in categories of successful and unsuccessful molts.

In Experiment 3 a 5:3 male to female ratio was utilized in each compartment as opposed to the 1:1 ratio used in the other experiments. This was due to a shortage of female crayfish in the collections made on October 21.

Supplementary experiments were conducted with the following modifications in experimental methods. During the collection of animals used in Experiment 2 (June 1, 1972), 48 recently matured crayfish (average carapace length 22.6 mm) were retained for use in Experiment 4-A. In this experiment, animals were placed in one experimental unit which was maintained at 18C and had two duplications of three different light intensities (10, 40, and 110 f.c.) randomized within. All light intensities were on a 15L:9D photoperiod.

In another experiment (5-A), 48 adult and 48 immature crayfish (collected October 20, 1972) were placed in one 18C experimental unit. This required individual compartments to hold 16 crayfish rather than the usual number of 8. The three photoperiods discussed above were randomized within.

RESULTS

The highest number of molts recorded in any of the seasonal experiments occurred during Experiment 2 (Table 1, Fig. 2). One hundred twenty five of the 144 crayfish molted at least once with a total of 145 molts accomplished. The fewest molts occurred in Experiment 3 with only 40 of the crayfish molting at least once for a total of 43 molts. In Experiment 1, 98 molts were observed with 85 animals completing one or more.

Animals collected in June (Experiment 2), although registering the greatest number of molting attempts, had the lowest degree of success in molting. Of the 145 molts recorded in this experiment, only 30% of the animals survived ecdysis. Mortalities associated with molting did not exceed 5% in any of the other experiments--including Experiment 4-A where 48 adults, collected and tested at the same time as the immatures in Experiment 2, completed 43 molts without a single associated mortality. In fact, the analyses of data from all experiments except Experiment 2 indicated the same statistical significances even when only the successful molts were considered.

Major differences in the temporal molting patterns occurring in the three seasonal experiments were noted (Table 2, Fig. 3). These differences were most apparent

Table 1. Total, successful, and unsuccessful molts by Orconectes rusticus rusticus and mortalities not associated with molting. Numbers in parentheses indicate the number of animals that molted twice.

Experiment	Total No. Animals	Total Molts	Animals molting at least once	E4 Molts	E1,E2, and E3 Mortalities	Unexplained Mortalities
1.	144	98(13)	85	96	2	9
2.	144	145(20)	125	43	102	13
3.	144	43(3)	40	41	2	15
4-A.	48	43(8)	35	43	0	4
5-A. Immatures	48	19(2)	17	18	1	3
Adults	48	3(0)	3	3	0	4

FIGURE 2. Total number of molts completed by immature Orconectes rusticus rusticus over ninety-day periods of Experiments 1 (January 18-April 18), 2 (June 3-September 1), and 3 (October 23-January 20).

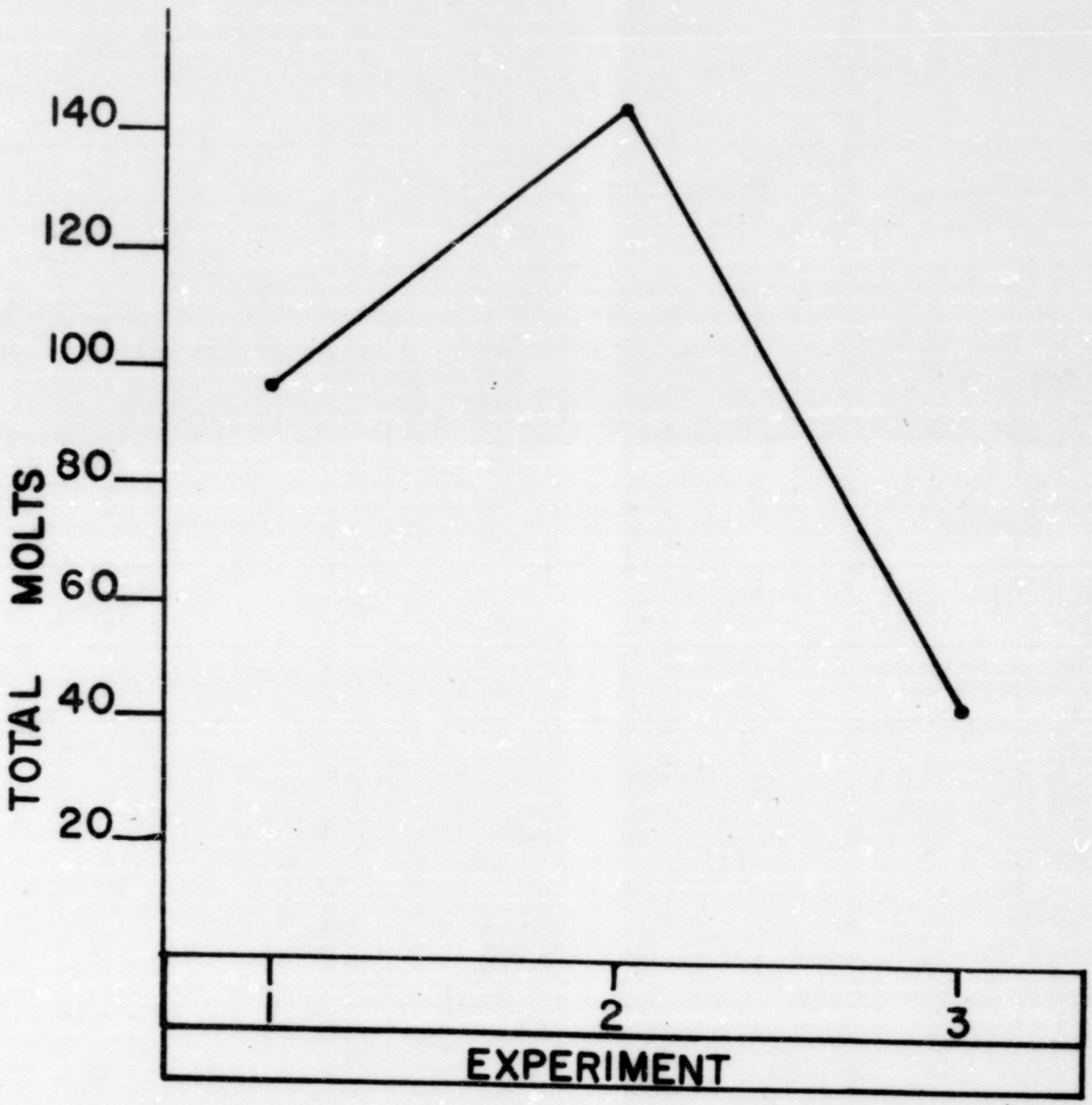
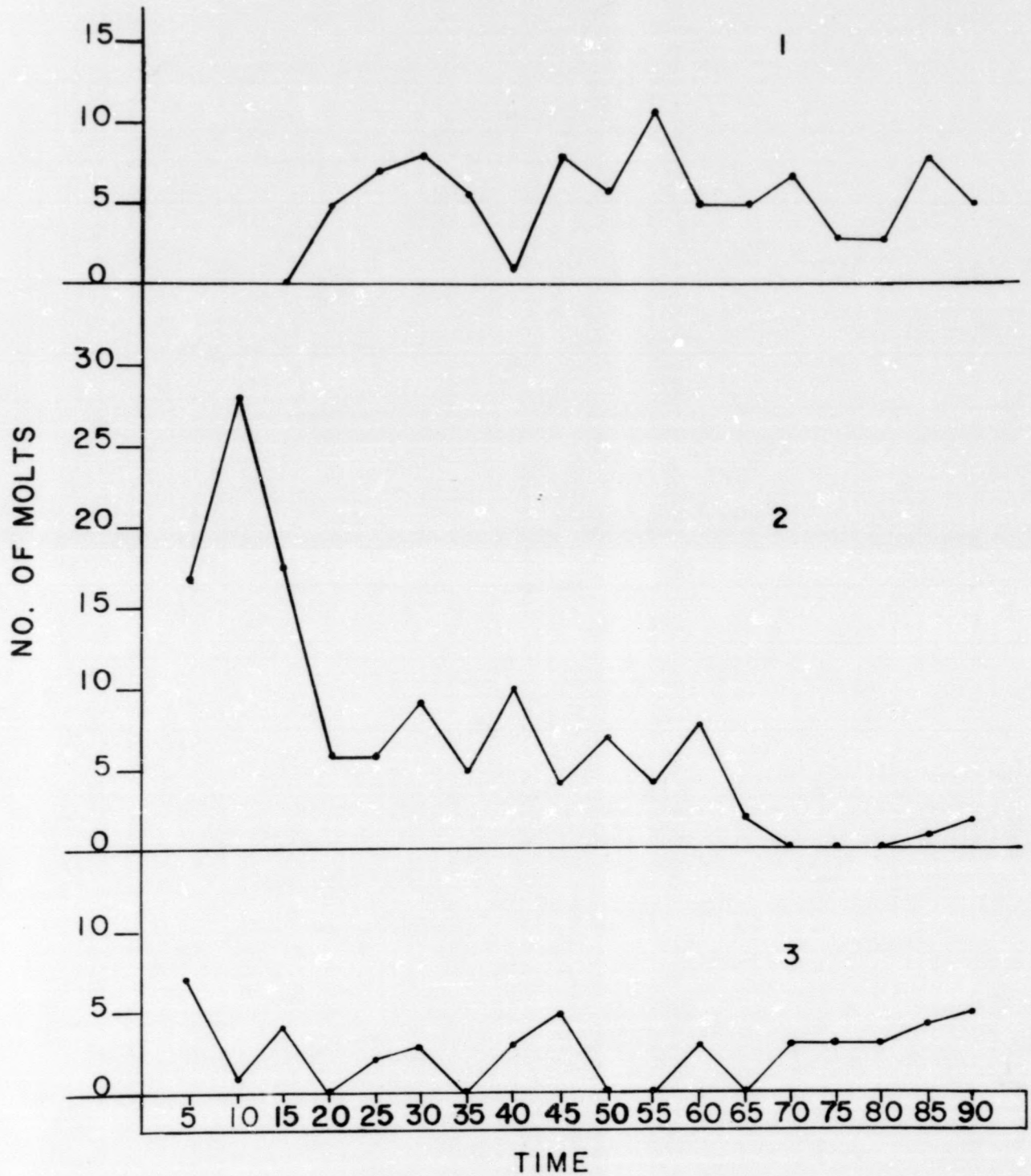

140
120
100
80
60
40
20
TOTAL MOLTS
2
3
EXPERIMENT

Table 2. Temporal occurrence of molting by Orconectes rusticus rusticus in three seasonal experiments. Categories are arranged in five-day intervals.

Days		5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90
Experiment 1.	14C	0	0	0	1	0	0	1	1	1	1	1	1	0	2	3	0	1	1
(January)	18C	0	0	0	0	1	3	2	0	1	1	6	2	3	3	0	2	6	4
	22C	0	0	0	4	6	5	3	0	6	4	4	2	2	2	0	1	1	0
	Total	0	0	0	5	7	8	6	1	8	6	11	5	5	7	3	3	8	5
Experiment 2.	14C	1	7	9	1	0	1	2	6	1	1	4	3	1	0	0	0	0	2
(June)	18C	5	12	5	4	4	5	1	2	1	2	0	3	1	0	0	0	1	0
	22C	11	9	2	1	2	3	2	2	2	4	0	2	0	0	0	0	0	0
	Total	17	28	16	6	6	9	5	10	4	7	4	8	2	0	0	0	1	2
Experiment 3.	14C	0	1	2	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0
(October)	18C	3	0	1	0	0	2	0	0	1	0	0	1	0	0	1	0	2	2
	22C	4	0	1	0	1	1	0	1	3	0	0	1	1	1	1	2	2	4
	Total	7	1	4	0	2	3	0	2	5	0	0	2	1	2	2	2	4	6

FIGURE 3. Number of molts per five-day period for Experiments 1 (January 18-April 18), 2 (June 3-September 1), and 3 (October 23-January 20).


15
10
5
0
1
30
25
20
15
10
5
0
2
10
5
0
3
NO. OF MOLTS
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
TIME

when the first fifteen days of each experiment were compared and when the last twenty days of each experiment were compared. For example, during the first 15-day intervals, no molts were accomplished by animals in Experiment 1, 61 molts (42% of the total number molting) were completed by animals in Experiment 2, and Experiment 3 animals molted a total of 12 times (28% of the total number molting).

In the last 20-day periods of the experiments, only 3 molts (2% of the total) were completed during Experiment 2, while 26 molts (27% of the total) and 16 molts (37% of the total) were recorded in Experiemts 1 and 3, respectively. Only 46 of the original 144 crayfish survived to the last 20 days of Experiment 2, however.

These temporal molting patterns were reflected in the values for the mean number of days until individuals first molted in each experiment and the standard deviations from these means (Table 3). Crayfish collected in January molted within an average of 58.78 days after the initiation of the 90-day experimental period, the longest average of the three experiments, while animals collected in June averaged the shortest period of time prior to molting (25.82 days). Crayfish collected in October molted in equal numbers during the first and last halves of their experimental period for a mean of 45.00 days.

Standard deviations from these means further clarified the comparative consistency of molting patterns. Large blocks of experimental time devoid of molt activity in Ex-

Table 3. Mean number of days and standard deviations to the completion of the first molt of *Orconectes rusticus rusticus*.

Experiment	Temperature	Mean	Standard Deviation
1. (January)	14C	58.14	20.6
	18C	62.49	21.1
	22C	41.08	17.9
	Overall	58.78	23.6
2. (June)	14C	34.63	23.3
	18C	22.83	19.1
	22C	20.45	18.3
	Overall	25.82	20.9
3. (October)	14C	29.67	23.3
	18C	46.23	10.9
	22C	48.36	10.0
	Overall	45.00	10.0

periments 1 and 2, resulted in high standard deviations of 23.6 and 20.9 days, respectively. The lowest standard deviation (10.0) was found in Experiment 3 where molting was more consistent throughout this experimental period than it was in the others.

A breakdown of the occurrence of molts within the separate treatments comprising the seasonal experiments (Table 4) indicated that the various temperatures affected animals differently during the three seasons in which they were tested (Fig. 4). For example, in Experiment 1, 13, 33, and 52 animals molted at 14C, 18C, and 22C, respectively. This represented a highly significant (0.01) linear molting response to temperature. Similarly, there was a linear effect of temperature in Experiment 3 with 7, 14, and 22 molts occurring at the three progressively warmer temperatures. Statistical significance could be placed only at the 0.05 level of confidence in this experiment, however.

When the total molts initiated within the June experiment were considered, it appeared that crayfish molt activity responded to temperature in a significant (0.05) quadratic manner. In this experiment, the highest numbers of molts were initiated at 18C (55 molts) rather than at 22C. Only 48 molts were attempted by crayfish in the 22C water and a substantial number of molts (41) were found at 14C.

When only successful molts completed by crayfish were considered, a different statistical pattern was found (Table

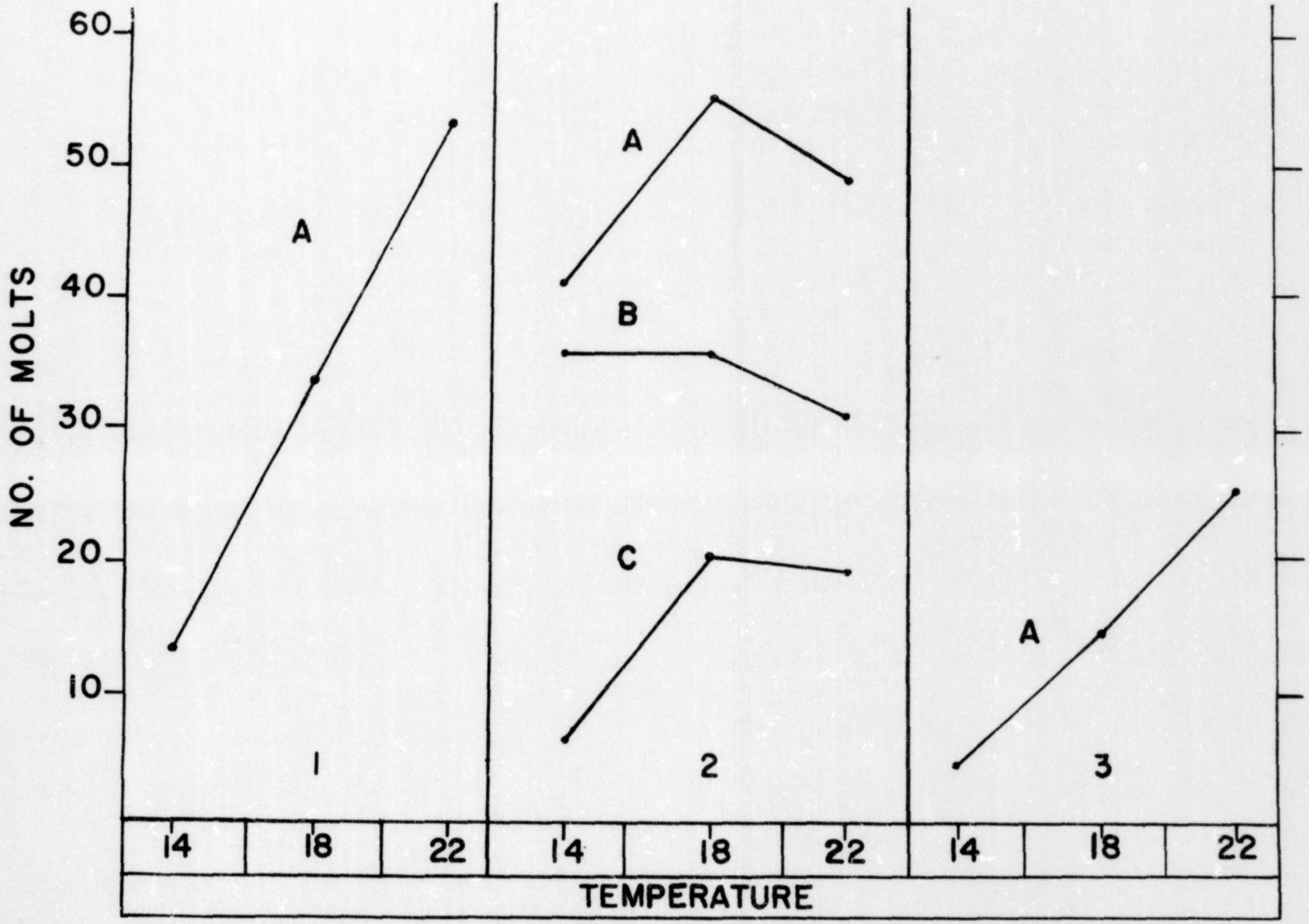
Table 4. Numbers of molts occurring within experimental treatments of Experiments 1 (January 18-April 18), 2 (June 3-September 1), and 3 (October 28-January 20) by Orconectes rusticus rusticus. * denotes significant linear effect (0.05), ** denotes highly significant linear effect (0.01), ' significant quadratic effect (0.05), n.s. nonsignificant effect, x overall molt totals.

			Photoperiods (PP)			Molt Totals
Experiment	Molt Type	Temperature	6L:18D	15L:9D	24LL	(Temp.)
1.	Total Molting	14C	1	4	8	13**
	Attempts	18C	8	12	13	33**
		22C	16	18	18	52**
	Molt Totals (PP)		25*	34*	39*	98 x
2.	Total Molting	14C	15	11	15	41'
	Attempts	18C	17	20	18	55'
		22C	16	18	14	48'
	Molt Totals (PP)		48 n.s.	49 n.s.	47 n.s.	144 x

Table 4. Cont'd

Experiment	Molt Type	Temperature	Photoperiods (PP) 6L:18D	15L:9D	24LL	Molt Totals (Temp.)
2. (Cont'd)	Successful	14C	1	3	2	6*
	(E4)Molts	18C	5	8	6	19*
		22C	5	6	7	18*
	Molt Totals (PP)		11 n.s.	17 n.s.	15 n.s.	43 x
	Unsuccessful	14C	14	8	13	35 n.s.
	(E1,E2,E3)	18C	11	12	12	35 n.s.
	Molts	22C	11	12	8	31 n.s.
	Molt Totals (PP)		36 n.s.	32 n.s.	33 n.s.	101 x
3.	Total Molting	14C	2	0	2	4*
	Attempts	18C	0	5	9	14*
		22C	5	9	11	25*
	Molt Totals (PP)		7*	14*	22*	43 x

FIGURE 4. Number of molts of immature Orconectes rusticus rusticus within 14C, 18C, and 22C treatments of Experiments 1 (January 18-April 18), 2 (June 3-September 1), and 3 (October 23-January 20). (A) Total molts initiated. (B) Unsuccessful (E1, E2, and E3) molts. (C) Successful (E4) molts.


NO. OF MOLTS
60
50
40
30
20
10
A
A
B
C
A
1
2
3
14
18
22
14
18
22
14
18
22
TEMPERATURE

4). More animals successfully completed molts at 18C (19 E4's) than at 14C (6 E4's). This relationship between increasing temperature and increasing molt success, although statistically a linearly significant (0.05) effect, was not the same relationship found in the overall analyses of Experiments 1 and 3. In Experiment 2, an increase from 18C to 22C did not result in as great of increase in the number of successful molts as did the difference between 14C and 18C.

Although there was a greatly reduced number of E4 molts in the 14C treatment of Experiment 2, unsuccessful molts were not initiated with a greater frequency than in the 18C treatment (35 unsuccessful molts attempted in each). In fact, there were no statistically significant differences among the molt mortalities in any of the treatments used in this experiment; thus, unsuccessful molting appeared to occur randomly throughout these treatments. No such suggestions could be made for the other two seasonal experiments because of the highly successful molting records of these animals.

The temporal arrangements of molting within the separate temperature treatments of the three seasonal experiments (Table 3) revealed only one pattern that was consistent from experiment to experiment. Standard deviations from the mean days to molt in the 22C treatments were lower in all three experiments than the standard deviations in the 14C and 18C treatments. For example, in Experiment 2, standard deviations

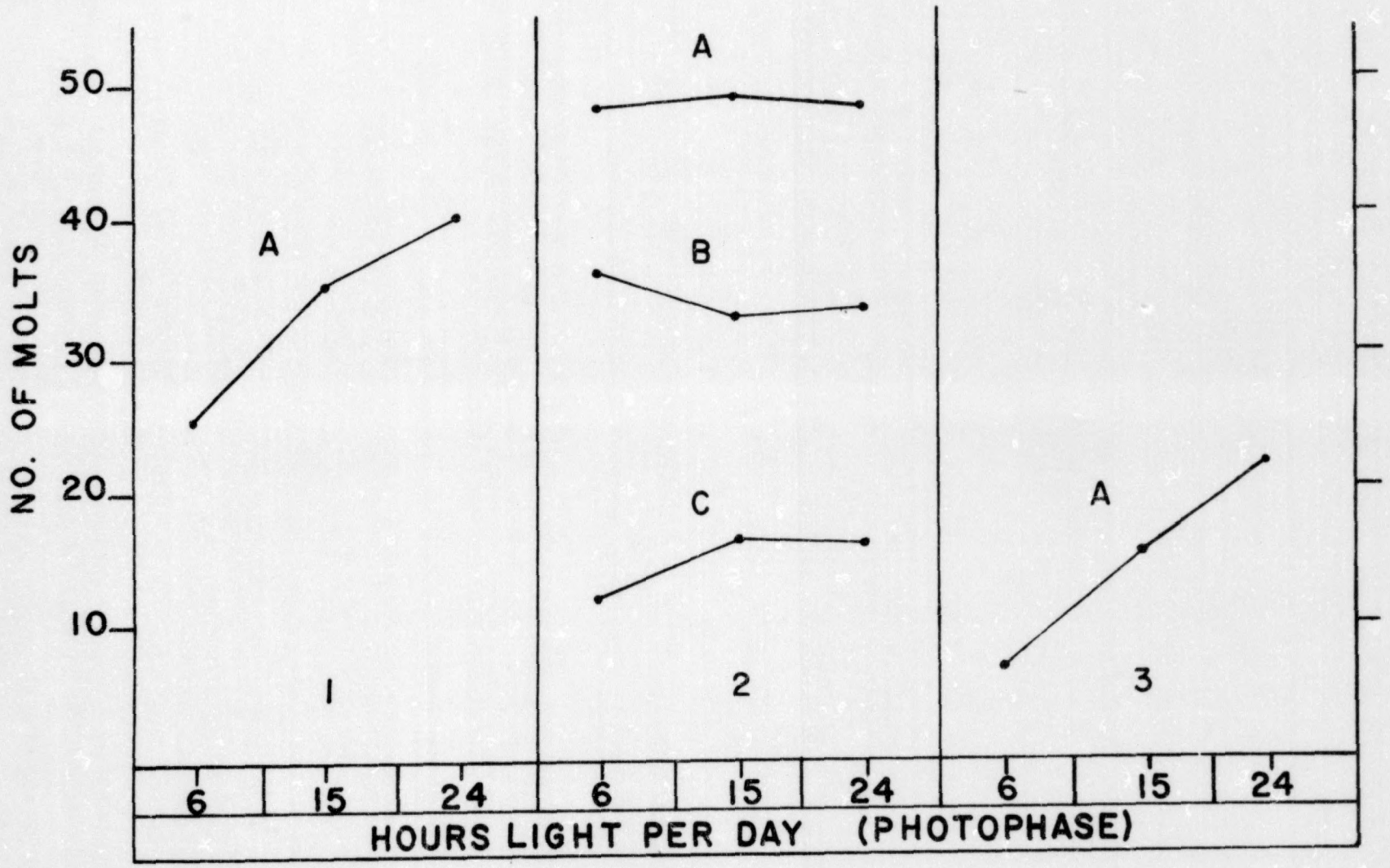
of 23.3, 19.1, and 18.0 days were found in the 14C, 18C, and 22C treatments, respectively. Other than a slight discrepancy in data from Experiment 1 (where a standard deviation of 21.1 days was calculated from crayfish molting dates in the 18C treatment and a deviation of 20.6 was found at 14C), the general trend of the degrees of coordination of crayfish molting was that the warmer the temperature that was used, the lower the standard deviation, or, in other words, the closer to the mean that individuals molted.

The values for the mean number of days until molt within the temperatures of each experiment were not in the consistent patterns found in the values for the deviations. For example, a linear increase in the mean number of days until crayfish molting was correlated with increasing temperatures in Experiment 3, but not in the other two (Table 3).

The effects of the three different photoperiods used in Experiments 1 and 3 were similar in pattern to the temperature effects (Table 4, Fig. 5). In both experiments, statistically significant linear increases in molting frequency correlated with increases in photophase. In 6L:18D, 15L:9D, and 24LL, animals collected in January molted 25, 34, and 39 times, respectively; the animals collected in October molted 7, 14, and 22 times in these same treatments.

In Experiment 2, however, no significant effects of photoperiod were noted regardless of whether total molts (48, 49, and 47 molts arranged in the linear order of the photoperiods), successful molts (11, 17, and 15 E4 molts),

FIGURE 5. Number of molts of immature Orconectes rusticus rusticus within 6L:18D, 15L:9D, and 24LL treatments of Experiments 1 (January 18-April 18), 2 (June 3-September 1), and 3 (October 23-January 20). (A) Total molts initiated; (B) Unsuccessful (E1, E2, and E3) molts; (C) Successful (E4) molts.


NO. OF MOLTS
50
40
30
20
10
A
A
B
C
A
1
2
3
6
15
24
6
15
24
6
15
24
HOURS LIGHT PER DAY (PHOTOPHASE)

or unsuccessful molts (36, 32, and 33 molt mortalities) were considered, indicating statistical randomness of molting within the different photoperiods.

Results of Experiment 4-A indicated that light intensities of 10 (14 molts), 40 (15 molts), and 110 (14 molts) foot candles had no significant effect on the molting frequency of adult O. r. rusticus collected in June.

A comparison of the results of Experiment 4-A with those obtained from immatures tested over the same period of time and at the same temperature (18C treatment of Experiment 2) indicated that adults completed only 12 fewer molts (78%) than the same number of immatures. Adults in Experiment 5-A, however, exhibited a greatly reduced molt frequency when compared with immatures tested in the same experiment at the same time (October). Here, adults completed only 3 molts (16%) compared with 17 molts by immatures. In addition, when data from immatures in this experiment were substituted for the data collected from the same temperature treatment of Experiment 3, all statistical significances in Experiment 3 remained.

Immature crayfish consistently gained more carapace length per molt than did the adults. Immatures increased in length an average of 5.41-6.96% with a single molt in the experiments completed in this study while adults increased in length an average of only .47-.83% with their molt (Table 5).

Table 5. Average gain in carapace length of Orconectes rusticus rusticus undergoing one successful molt. All measurements in mm. * denotes an average from three molts only.

Experiment	Stage	Carapace length	Average length	Average Gain with Molt	% Gain
1. (Jan.)	Immatures	13.5-21.3	18.1	1.26	6.96
2. (June)	Immatures	12.3-20.0	15.7	.85	5.41
3. (Oct.)	Immatures	14.2-23.0	17.8	1.02	5.73
4-A.	Adults	18.3-27.4	22.6	.21	.83
5-A.	Adults	23.7-31.9	27.5	.13*	.47*
	Immatures	14.9-22.7	18.1	.98	5.41

No significant differences in the molt frequency or success of male and female crayfish were found in any of the experiments.

No interactions were found between any of the treatments used in this study, indicating that the effects of temperature and photoperiod, when noticed, were separate from each other and did not contribute toward any modification in the effects of the other. Extended photoperiod and increased temperatures in Experiments 1 and 3, for example, independently caused an increased molt frequency in populations tested without influencing the magnitude or pattern of the effects of the other.

DISCUSSION

The seasonally variable molt frequencies found in the populations of immature O. r. rusticus tested in this study can be at least partially supported by the literature. Aiken (1969b) found differences in the seasonal molting patterns of O. virilis and proposed that populations which had experienced different environmental conditions prior to placement in an experiment would be expected to exhibit different molting characteristics.

Prins (1968) indicated that the active molt period for O. r. rusticus in Doe Run extended from May through August and that the molt frequency within the population was reduced following this period with a total cessation of activity over the winter months. Similar reductions of molt activity were observed in an Ohio fish pond population of O. rusticus (Langlois, 1936) as well as in O. propinquus in Illinois (Van Deventer, 1937). The low frequency of molting observed in crayfish collected in October (Fig. 2) might be attributed to the naturally occurring fall activity of a molt inhibition mechanism which was actively reducing molting activity at this time in anticipation of the harsh winter period. Evidence also exists indicating that this proposed mechanism was sensitive to localized environmental variations, a concept that will be discussed later

under headings of Temperature and Photoperiod.

The reduced frequency of molting among crayfish collected in January, when compared with the high number of molts by animals collected in June, would appear to contradict an observed field pattern of these crayfish which indicated that at least the adult population in Doe Run exhibited a more synchronized mass molt upon being released from the winter condition than at other seasonal times (Prins, 1968). This might, however, be attributed either to the shortness of time that crayfish collected on January 16 had spent in actual winter conditions prior to testing, or to an unnatural stimulation of molt activity among the Experiment 2 crayfish which resulted in abnormally high frequencies.

The first possibility can be supported by reference to Aiken (1969b) who observed that the longer the period of time that a population of O. virilis was maintained in the winter torpid condition, the greater was the percentage of individuals molting upon being released from this condition. This concept suggests that crayfish, or at least O. virilis, possess a molt control mechanism which actively suppresses molt activity until a certain dormant period has passed. Aiken further proposed that the increased molt activity of cold-adapted crayfish was due more to a reduction of molt resistance in individuals rather than a specific molt induction. If the same mechanism were responsible for the frequencies observed in this study, then the occurrence of

molts by January collected crayfish would seem to represent a reduced expression of the full molt potential caused by a lack of sufficient winter conditioning to completely eliminate molt inhibition.

The second proposal that the difference between the frequencies obtained in Experiments 1 and 2 was due primarily to abnormally high molt activity by animals collected in June can also be supported, at least in terms of the unnaturalness of the molting in this experiment. This concept, suggested by the high numbers of mortalities associated with molting in Experiment 2, will be discussed later.

The temporal pattern of molting within the different seasonal experiments (Fig. 3) also suggests the influence of environmental pre-conditioning. When animals were collected in January, they had been exposed to at least some period of reduced temperature (the water temperature at Doe Run on January 16 was 11C), and the lack of molt activity in the initial stages of this experiment suggests that an "acclimation period" was required before animals, recently released from cold conditions, could once again initiate molt cycles.

Animals used in Experiments 2 and 3, on the other hand, were collected at times (June and October, respectively) when molting in the field was found to be normally occurring (Prins, 1968) which indicates the reason for the immediate expression of molting by these crayfish when placed under experimental conditions.

Temperature

Crayfish used in Experiments 1 and 3 exhibited linear increases in molt frequency when maintained in progressively higher temperature treatments (Fig. 4), a response possibly related to metabolic rates. Although little work has been done recently concerning the influence of temperature on the crayfish molt cycle, Mobberly (1963) and Passano (1960) both acknowledged the possibility that low temperatures suppress molting in crayfish by decreasing overall metabolic rate. Metabolism is among the poikilothermic physiological processes believed to react relatively linearly to change in temperature (Scholander, et al., 1953). However, Bullock (1955) indicated that metabolism does not always follow a strictly linear pattern in poikilotherms, being subject to seasonal variation and variation in environmental conditions.

Thus, a correlation between metabolic and molt activity cannot be ruled out even in Experiment 2, where linear increases in temperature did not result in linear increases in molt activity. The proposed unnatural occurrence of molts in this experiment, the lack of any information regarding the metabolic activity of the crayfish used in the seasonal experiments, and the lack of any information concerning either the effects of temperature on the proposed MH-MIH hormonal levels or possible differences between the metabolic patterns of adults and immatures, all contribute to the current lack of understanding of the specific mechanism involved.

Regardless of the nature of the mechanism, temperature apparently did have a direct effect on the molt frequency of immature O. r. rusticus specimens collected in January and October. Temperature signals would be expected to be more effective sources of information for the stimulation or inhibition of molt cycles at these experimental periods (late winter and late fall) than during the summer period. A strict reliance upon temperature information would not normally be required by summer crayfish since low temperatures (below 13C) are not normally experienced by Doe Run O. r. rusticus during this period (Prins, 1968). Therefore, although the coldest treatment in Experiment 2 reduced molt frequency, the two warmer treatments were evidently interpreted the same by the crayfish molt control mechanisms and comparably large numbers of molts were completed by each group. Although the 18C treatment was 4C lower than the highest temperature treatment, this temperature would not have been expected to signal the onset of winter conditions since summer pre-conditioning would have virtually assured crayfish a safe period for full molt cycle expression even though the local temperature was somewhat lower than the seasonal optimum.

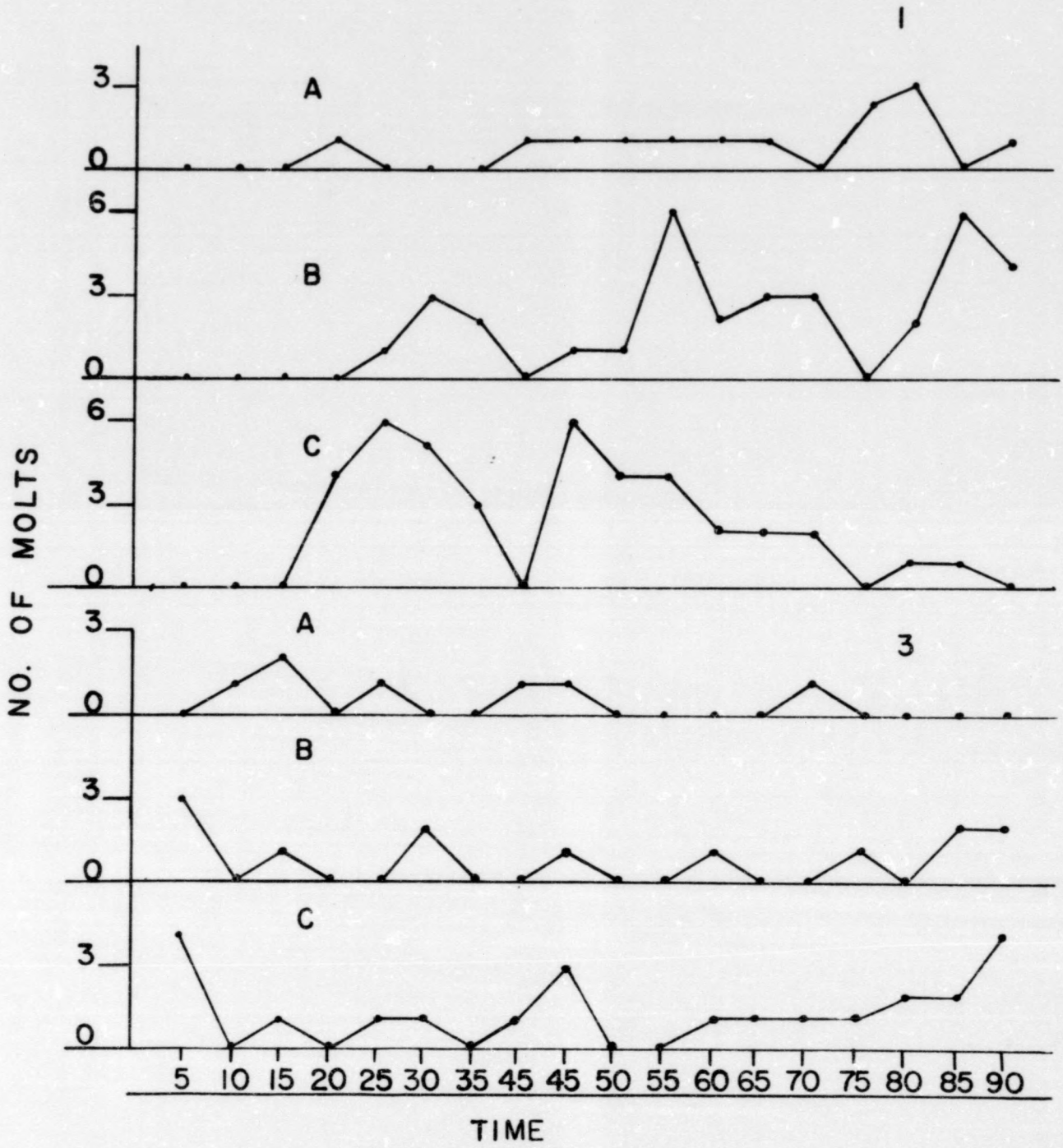
On the other hand, variations in temperature might have provided vital information regarding the progression of the seasons to crayfish in Experiments 1 and 3--information needed for the avoidance of unfavorable conditions. As such, the proposed molt control mechanism might have

resulted in reduced molt frequencies in crayfish at 18C even though molts could have been safely attempted at this temperature.

This suggestion can be further supported by reference to the temporal arrangement of molting within the temperature treatments of Experiments 1 and 3 (Fig. 6). The crayfish in these two experiments molted in essentially opposite temporal patterns. Crayfish collected in January initiated molts within progressively shorter times when maintained in progressively warmer temperatures while those collected in October molted in a reverse pattern.

The animals collected in January were facing the onset of the spring season, and may have had low resistance to signals indicating a safe period for molt preparation (Aiken, 1969b). Increased temperatures, then, could have brought about earlier expressions of molting activity than those in the colder temperatures. Crayfish collected in October, on the other hand, were facing severe winter conditions at the time of collection and already may have possessed high resistance to the initiation of molt. Thus, crayfish in warmer temperatures would have required time before this resistance could be overcome and molting once again be initiated on a populational level. A molt control mechanism that would immediately interpret warm temperatures as a safe period for molting would place many individuals in jeopardy, especially at this time of the year. A mechanism keyed only to this one environmental signal might be

FIGURE 6. Number of molts per five-day period within the 14C (A), 18C (B), and 22C (C) treatments of Experiments 1 (January 18-April 18) and 3 (October 23-January 20).


1
A
3
0
B
6
3
0
C
6
3
0
NO. OF MOLTS
A
3
3
0
B
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C
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5 10 15 20 25 30 35 45 45 50 55 60 65 70 75 80 85 90
TIME

subjected to a great deal of local disruption since unseasonal temperatures could easily cause a misinterpretation of the seasonal character. Further relationships found in this study tend to confirm the existence of a multi-faceted environmental information gathering mechanism.

Photoperiod

A variety of proposed photoperiodic models (Pittendrigh and Minis, 1964; Adkisson, 1966; Aiken, 1969b) consider that the operation of molt control mechanisms, where they exist, is essential during the spring and fall, when the greatest danger of improperly initiated molts exists. During the summer months, however, animals are usually insensitive to photoperiodic phenomena since this period represents an interval of almost unlimited molt potential with minimal danger of getting caught in unfavorable environmental conditions at some critical stage of the molt cycle. Animals tested in this study fit this photoperiodic model since significant differences in molts within photoperiod treatments were found only in Experiments 1 and 3 and were completely absent from Experiment 2.

The arrangement of molts within the photoperiod treatments of Experiments 1 and 3 did not, however, fit that found in another species of crayfish from a different geographic location. Stephens (1955) found that individuals of O. virilis in Canada that were collected in October successfully completed only 1 molt at photoperiods shorter than

20L:4D but completed 6 molts at that photoperiod. Aiken (1969b) supported the existence of a minimal photoperiod threshold in Canadian O. virilis collected in October and proceeded to demonstrate that this threshold decreased with the exposure of crayfish to prolonged winter conditions. It would appear that these animals demonstrated a "trigger" response to photoperiod with molt activity tightly geared to the detection of daylength (and consequently, to the detection of seasonal progress). This all-or-none photoperiod-controlled molting response was in sharp contrast to the strictly linear molting pattern found in individuals of O. r. rusticus collected in January and October and tested at the different photoperiods of this study (Fig. 5). The information supplied by photoperiod, then, was not acted upon as universally in O. r. rusticus as it was in O. virilis suggesting a greater variability in genetic response to photoperiod signals in O. r. rusticus.

The more southerly location of the O. r. rusticus used in this study when compared with the northern O. virilis might provide some insight into these different patterns. The temperate environment of O. r. rusticus from Doe Run would provide less selective pressure against crayfish initiating molt prematurely in the spring or late in the fall than would the severe winter experienced by more northern crayfish. Thus, the necessity for each individual to refer strictly to photoperiod signals as the most stable indication of the progression of seasons would not be as great in O.

r. rusticus. The lack of any direct comparisons of these populations does not allow for any firm conclusions.

Molt Mortalities

The high occurrence of molt mortalities only in Experiment 2 cannot be explained in terms of an expected seasonal variation in mortalities by these animals. Field observations at Doe Run (Prins, 1968) revealed that although immatures consistently suffered higher mortalities than adults, no major reduction in the occurrence of immature O. r. rusticus was recorded during the summer months. The explanation of these mortalities would therefore appear to be related to artificial experimental conditions.

Aiken (1969b) suggested that molt mortalities recorded in experiments, such as Experiment 2 of this study, might be caused by an interaction and imbalance of the Molt Hormone (MH) and the Molt Inhibitory Hormone (MIH). He felt that this imbalance was the result of improper environmental signals stimulating the production of MIH during some critical time in the molt cycle, thereby terminating it prematurely. McWhinnie, et al. (1972), found that increased levels of crustecdysone (the proposed MH) influenced the progress of a molt already initiated but did not change the number of molts themselves (which might be evidence that the high number of molts attempted by crayfish in Experiment 2 did not represent abnormal numbers of molts stimulated by unnatural conditions as was earlier suggested).

The random occurrence of molt mortalities within the known treatments of this experiment could suggest the presence or absence of an undefined experimental condition which was effective in causing asynchronous hormone levels in all experimental treatments. Other suggestions, however, might be equally plausible since no work has yet been completed on the physiology of molt mortalities. For the present, it would appear that this mortality was due to an unnatural cause of unknown value.

Light Intensity

Results of Experiment 4-A provide little information concerning the role of light intensity as an environmental signal. Although adults tested in this experiment did not appear to molt in relation to intensity, it must be noted that immatures tested during the same period in Experiment 2 (June 3-September 1) were not significantly influenced in their molt frequency by photoperiod differences. It therefore appears possible that a molt control mechanism not relying on photoperiod signals for molt control during the relatively safe summer period, would also not be expected to rely on light intensity signals. In addition, differences in the mechanism of adults and immatures might exist, a concept that will be discussed next.

Adults *vs.* Immatures

Adult and immature individuals of *O. r. rusticus* displayed several differences in molting patterns in the exper-

iments where they were directly compared. None of the differences contradicted the well-accepted concept (Passano, 1960; Aiken, 1969b; and others) that molting in adults, unlike that in immatures, is not primarily a growth and development mechanism. Carapace gain with molt, for example, fits this model (Table 5).

Further support was demonstrated in the seasonal differences in molting percentages of adult and immature animals. Although adult molting was comparable in frequency to that of immatures in Experiment 2, adults collected in October molted at a much lower frequency than immatures. If this reduction of adult molting is the result of a seasonal molt inhibition, then it would appear that the mechanism responsible goes into effect at an earlier time and to a greater degree than that existing in immatures tested over the same period. This is in line with the molt function proposed for adults in that it would not be advantageous for an adult population to extend its molt activity into a seasonal period where low temperatures might be experienced. Immatures, on the other hand, would molt as long as relative safety was available since growth and development would be of vital concern.

Interactions

The lack of interactions among any of the treatments tested in this study suggests that individuals of O. r. rusticus posses a molt control mechanism which is not dependent

upon the coordination of separate environmental signals, but capable of refering to the signals individually . With the addition of enough information (for example, long photoperiods and warm temperatures), the full populational molt potential could be expressed. No one environmental signal was acted upon strictly enough to either influence or override the reaction to other signals. This is in sharp contrast to the overpowering photoperiod reaction found in O. virilis (Stephens, 1955; Aiken, 1969b). It might once again be proposed that the temperate population of O. r. rusticus would have less need for a totally reliable seasonal indicator than O. virilis and might, as a result of decreased selective pressure, be a population containing greater variation in molting response than O. virilis. Thus, many environmental signals could be used for the determination of the seasonal character.

SUMMARY

1. This study was designed to examine possible seasonal differences in the molting response of experimental populations of O. r. rusticus maintained at three different temperatures (14C, 18C, and 22C) and three different photoperiods (6L:18D, 15L:9D, and 24LL).

2. Immature O. r. rusticus collected on January 16 and October 21 (and tested from January 18-April 18 and October 23-January 20, respectively) responded to linear increases in temperature and photophase with linear increases in the percentage of experimental populational molting.

3. Immature O. r. rusticus collected on June 1 and tested from June 3-September 1, responded to linear increases in temperature with a quadratic increase in molt frequency and did not respond to photoperiod treatments at all. Molt mortalities were 70% in this experiment.

4. No molts were attempted by crayfish collected in January during the first 15 days of the experiment and animals at warmer temperatures averaged shorter times until molting attempts than did those at colder temperatures. Immatures collected in October molted immediately upon being placed at experimental conditions and averaged a longer time until initiation of molt at warmer temperatures than at colder temperatures.

5. Two auxillary experiments indicated that adults molt in fewer numbers than immatures with the greatest difference found with adults and immatures collected in October. In addition, adults collected in June did not react to different light intensities with changes in molt frequency.

6. Consideration was given to a proposed molt control mechanism for O. r. rusticus, characterized as being less strictly dependent upon photoperiod signals than the mechanism earlier proposed for more northern populations of O. virilis (Stephens, 1955; Aiken, 1969b). It was suggested that this difference could be the result of less severe winter conditons in the temperate environment of O. r. rusticus which would contribute to greater variation in individual response.

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