FACTORS INFLUENCING THE RATE OF OXYGEN CONSUMPTION OF THE DWARF CRAWFISH, *CAMBARELLUS SHUFELDTII*[1]

(DECAPODA, ASTACIDAE)

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The comparative physiology of respiration has been reviewed recently by Zeuthen (1955). Sex, weight, endocrines, and a daily rhythmicity are among several factors which may influence the rate of oxygen consumption of an arthropod.

Edwards (1946) demonstrated that the male imago of the housefly, *Musca domestica,* had a higher rate of metabolism than the adult female. The higher rate of the male was due to the sexual difference and not to a weight difference. In 1950 Edwards found no sexual difference in the metabolic rate of the fiddler crab *Uca pugilator.*

In general, within a single species on a unit weight basis small animals have a higher rate of oxygen consumption than large animals. Edwards (1946) found that this relationship held for the mole crab, *Emerita talpoida,* and the amphipod *Talorchestia megalopthalma.*

The role that endocrines play in the control of the metabolic rate of crustaceans has been investigated by several workers. Scudamore (1947) demonstrated that removal of the sinus glands from within the eyestalks of the crawfish *Orconectes immunis* led to an increase in the rate of oxygen consumption. Sinus gland extracts decreased the rate of oxygen consumption and central-nervous-tissue extracts increased the rate. Bauchau (1948) observed that the increase in the metabolic rate of the crab *Eriocheir sinensis* with a rise in temperature was greater following eyestalk removal. He concluded that the sinus glands of this poikilotherm normally operate in the control of metabolic rate as a partial temperature compensator. Edwards (1950), working with *Uca pugilator,* and Bliss (1953), working with the land crab *Gecarcinus lateralis,* showed an increase in the metabolic rate following eyestalk removal. Scheer and Scheer (1954) showed that ablation of the eyestalks from the prawn *Leander serratus* is not followed by a rise in the rate of oxygen consumption. *Leander* was the first crustacean investigated for which no change in metabolic rate following eyestalk removal has been reported.

Edwards (1950) demonstrated a daily rhythm of oxygen consumption in the fiddler crab *Uca pugilator.* This 24 hour cycle of oxygen consumption corresponded to the daily activity rhythm of the species. More recently, Brown, Bennett, and Webb (1954) confirmed the daily rhythm of oxygen consumption in normal *Uca pugilator.* The rate is maximal at 6-8 a.m., minimal about noon and midnight; and,

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a secondary maximum occurs about 10-11 p.m. The latter investigators also described a daily rhythm of the rate of oxygen consumption in eyestalkless individuals of this species. The daily form of the latter rhythm differed slightly from the rhythm of normal animals.

The current investigation was initiated to determine (1) the effect of eyestalk removal upon the rate of oxygen consumption of the dwarf crawfish, *Cambarellus shufeldtii,* (2) the influence of sex and size upon the rate of oxygen consumption, and (3) the rate of oxygen consumption throughout a 24 hour period.

Materials and Methods

Adult specimens of the dwarf crawfish, *Cambarellus shufeldtii* (Faxon), identified by Dr. George H. Penn, were collected in the vicinity of New Orleans, Louisiana, for use in the experiments. The total length of these crawfish is 15 to 25 mm, the female being larger than the male. This species, whose physiology has not been investigated previously, is one of the more common crawfish in this vicinity.

The rate of oxygen consumption of the crawfish was measured by means of (1) a continuously recording respirometer designed by Brown (1954) and (2) a Warburg respirometer. The former instrument was used for measuring the metabolic rate over 24 hour periods, the latter for intervals up to three and a half hours.

The respirometer designed by Brown consists of a collapsible plastic bag attached to 100 ml Soxhlet flask via a capillary connection. The plastic bag contained sufficient oxygen for at least 72 hours. Weights were affixed to the exterior of the flask so that the respirometer would sink just below the surface of the water in a constant temperature bath maintained at 29°C. As oxygen was consumed by an animal in the flask the specific gravity of the respirometer increased and the buoyancy decreased. The respirometer, therefore, sank deeper. Increase in specific gravity was recorded by means of a lever system attached to ink pens recording on a drum moving at the rate of 0.29 cm per hour. The decrease of the oxygen volume in the respirometer caused the observational pen to trace a line which continuously approached closer to a fixed base line. The respirometer increased one gram in weight for every milliliter of oxygen consumed. Therefore, the lever system could be calibrated and the volume of oxygen consumed per hour calculated. Data for three hour intervals were lumped. The method of analysis and presentation of the data was the same as described in detail by Brown, Bennett, and Webb (1954).

For each determination the following were placed into each of eight flasks: a vial of 20 percent potassium hydroxide (carbon dioxide absorbent), a vial of saturated cupric sulfate (ammonia absorbent), and a volume of aerated tap water sufficient to allow the crawfish to swim. Seven of the respirometers had one crawfish in each of them, the eighth served as a control. Throughout the periods of observation only one-third of the records for the 24 hour periods could be used because the animals in some of the flasks died and

some of the pens did not record between midnight and 6 a.m. The respirometers were maintained in a laboratory in which the blinds were drawn. The light intensity of the surface of the water in the bath never exceeded two ft.-c. The small changes in light intensity could not have accounted for the results to be described because the changes in light intensity that occurred in the laboratory were arrhythmic. On several evenings the laboratory lights were on until midnight with no apparent effect upon the results.

The Warburg respirometer was used in the conventional manner. Each flask contained 20 percent potassium hydroxide (carbon dioxide absorbent) and a filter paper wick in the center well, 50 percent sulphuric acid (ammonia absorbent) in the side-arm, and one ml of aerated tap water plus a crawfish. The water bath was maintained at 28.5°C.

Following removal from either respirometer, each crawfish was weighed and sexed. Total wet weight was determined by blotting each crawfish in paper towelling to remove as much of the external liquid as possible and weighing the animal to the nearest one-hundredth of a gram by means of a chainomatic analytical balance. The rates of oxygen consumption were expressed as milliliters of oxygen consumed per gram of tissue per hour (ml/g/hr).

Statistical analysis of the data which established (1) the nature of the daily rhythm and (2) the effect of eyestalk extracts on the metabolic rate was not necessary because the metabolic rates of individual crawfish were not compared with one another, but each crawfish served as its own control. Individual crawfish were considered over 24 hour intervals. Individual variation influenced only the amplitude of the daily rhythm curve but not the shape of the curve, *i.e.* the character of the daily rhythm, which was the primary object under investigation. The character of the daily rhythm was the same in all the crawfish, but the amplitude of the rhythm varied because of sex and size differences which are discussed later. The metabolic rate of each crawfish was determined before it received the extract of eyestalk. The effect of the extract upon the previously determined metabolic rate was then determined.

Experiments and Results

Daily rhythm of oxygen consumption of normal Cambarellus.— Normal animals, of which 75 percent were males, were placed in the respirometers and the rate of oxygen consumption over 24 hour intervals was determined. One crawfish was in each respirometer so that the metabolic rate of any one crawfish could be followed, rather than compare the rate of oxygen consumption of different crawfish from different times of day. These observations were made from midnight to midnight on nine days between March 30 and May 12, 1955. The data for the individual crawfish for the nine days were averaged (fig. 1). Data obtained on three other days within this period were similar to the data for the nine days but did

not include a midnight to midnight 24 hour interval and, therefore, could not be averaged with the data of the nine days. As is evident from Figure 1, the metabolic rate of the individual crawfish was continuously changing throughout the 24 hour period. The respiratory rate was maximal about 6 a.m. with a secondary peak in the

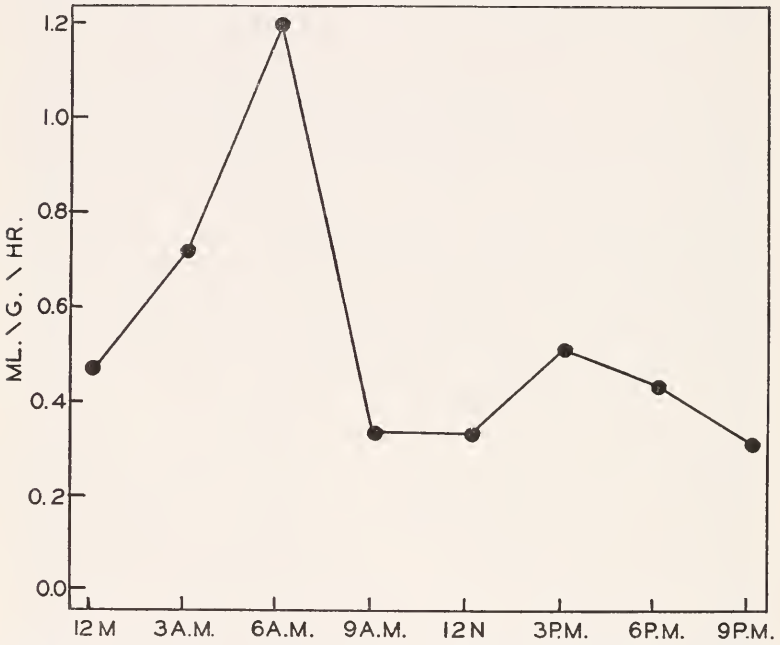

Figure 1. The daily rhythm in rate of oxygen consumption of normal *C. shufeldtii.*

afternoon (3-6 p.m.). Minima occurred between 9 a.m. and noon and 9 p.m. and midnight. No sexual difference in the character of the daily rhythm was observed.

The maximal rate of oxygen consumption was three times the minimal rate. Normal variations of this sort can explain the diverse rates of oxygen consumption in the literature for any one species. This rhythmic variation of the metabolic rate of individual crawfish is in all probability due to an endogenous activity rhythm. Mildred E. Lowe, a graduate student, observed that this species is most active in the laboratory about 7 a.m.

Daily rhythm of oxygen consumption of eyestalkless Cambarellus.— Both eyestalks were removed by transection at their bases and the wounds cauterized to minimize bleeding. Females whose eyestalks had been removed at least 24 hours previously were placed in the respirometers and the rate of oxygen consumption of these eyestalk-

less crawfish was determined. Data were obtained from midnight to midnight on three days between April 14 and May 9, 1955 and averaged (fig. 2). Results obtained on three additional days were rhythmically similar but did not include a midnight to midnight period. A daily rhythm of oxygen consumption in eyestalkless *Cambarellus* is evident from this figure. The daily pattern of the eyestalkless crawfish differs only slightly in form from the pattern of normal animals. The maximal and minimal metabolic rates occurred at approximately the same times in both normal and eyestalkless crawfish.

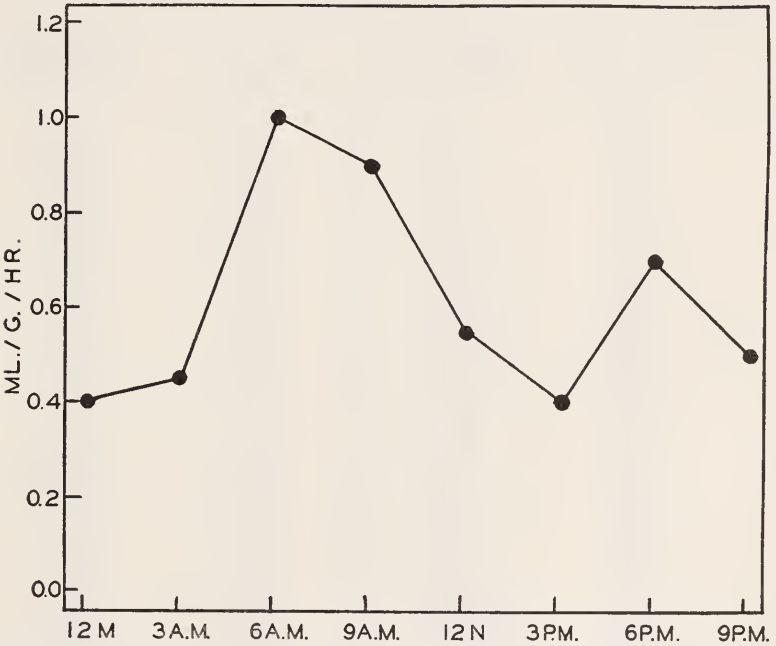

Figure 2. The daily rhythm in rate of oxygen consumption of eyestalkless *C. shufeldtii.*

Brown, Bennett, and Webb (1954) found the same is true for the daily metabolic rhythms of normal and eyestalkless *Uca pugilator.* The amplitude of the curve for eyestalkless animals is 16 percent greater than for normal crawfish.

Influence of sex and weight upon the rate of oxygen consumption in normal Cambarellus.—The rate of oxygen consumption of normal male and female *Cambarellus* was determined for one hour intervals by means of the Warburg respirometer. The data are presented in Table 1. *Cambarellus* possibly has a metabolic rhythm with a tidal frequency as found in *Uca* (Brown, Bennett, and Webb, 1954). If true, then one could not be certain that the crawfish would be rhyth-

mically similar at the same hour each day. The decision was made therefore to randomize the data with respect to time of day. Data for both sexes were collected at several times of day and night to randomize the effects of the daily rhythm. The metabolic rates of males and females could be compared with one another because the metabolic rates of individuals from both sexes were determined at

TABLE 1.
OXYGEN CONSUMPTION OF NORMAL *C. shufeldtii*

Males		Females	
Weight (g)	Oxygen Consumption (ml/g/h)	Weight (g)	Oxygen Consumption (ml/g/h)
0.12	0.140	0.10	0.280
0.12	0.197	0.11	0.279
0.13	0.277	0.11	0.293
0.13	0.300	0.11	0.331
0.13	0.323	0.12	0.179
0.13	0.654	0.14	0.161
0.15	0.238	0.14	0.182
0.15	0.264	0.15	0.286
0.15	0.268	0.16	0.210
0.15	0.480	0.26	0.307
0.17	0.205	0.28	0.196
0.17	0.307	0.28	0.261
0.17	0.312	0.29	0.238
0.18	0.339	0.30	0.267
0.19	0.203	0.30	0.280
0.19	0.221	0.31	0.164
0.19	0.313	0.31	0.222
0.20	0.369	0.31	0.229
0.21	0.472	0.33	0.152
0.22	0.245	0.33	0.165
0.22	0.427	0.33	0.170
0.23	0.330	0.33	0.245
0.24	0.278	0.35	0.211
0.24	0.279	0.35	0.214
0.24	0.307	0.35	0.342
0.31	0.171	0.36	0.292
		0.38	0.121
		0.38	0.203
Average 0.18	0.305	0.26	0.230

all times of day. Twenty-six males had an average wet weight of 0.18 grams and an average metabolic rate of 0.305 ml/g/hr. Twenty-eight females had an average wet weight of 0.26 grams and an average metabolic rate of 0.230 ml/g/hr.

The males weighed less and had a higher metabolic rate than the females. The difference in metabolic rate was probably due in part to the inverse relationship between metabolic rate and weight. This relationship also seems to hold if the sexes are considered separately. There was probably also a sexual component to the difference in

metabolic rate between the males and females. A comparison of males and females of similar weight suggested that males had the higher metabolic rate.

Effect of eyestalk removal upon the rate of oxygen consumption of Cambarellus.—The metabolic rate of male and female specimens of *Cambarellus* was determined for one hour by means of the Warburg respirometer. The animals were then weighed and their eyestalks

TABLE 2.
INFLUENCE OF EYESTALK REMOVAL ON OXYGEN CONSUMPTION

Weight (g)	Oxygen Consumption After Eyestalk Removal (ml/g/hr)				
	0 Days	1 Day	2 Days	3 Days	4 Days
		Males			
0.18	0.339	0.367			
0.19	0.221	0.289			
0.21	0.472	0.299			
0.22	0.427	0.518			
0.23	0.330	0.369			
0.13	0.323	0.446	0.867		
0.19	0.313	0.242	0.248		
0.31	0.171	0.236	0.220		
0.13	0.277	0.408	0.215	0.277	
0.13	0.300	0.553	0.492	0.423	
0.15	0.264	0.438	0.402	0.264	
0.24	0.278	0.395	0.373	0.410	
		Females			
0.30	0.276	0.311			
0.33	0.165	0.246			
0.33	0.170	0.224			
0.33	0.245	0.251			
0.35	0.214	0.286			
0.36	0.292	0.288			
0.38	0.203	0.224			
0.10	0.280	0.254	0.230		
0.28	0.261	0.225	0.268		
0.29	0.238	0.286	0.249		
0.35	0.211	0.091	0.191		
0.28	0.196	0.197	0.243	0.247	
0.31	0.164	0.187	0.155	0.139	
0.31	0.229	0.239	0.206	0.231	
0.33	0.152	0.182	0.206	0.215	
0.38	0.121	0.131	0.198	0.198	0.195

removed. The metabolic rate of these animals was subsequently determined at 24 hour intervals until death occurred. The observed data are presented in Table 2. To prepare Table 3, the data of Table 2 were averaged according to the number of days the animals of each sex survived. The values were then converted to the percentage of the metabolic rate determined prior to eyestalk removal. For example, the inital value for the animals in the "Destalked 72 hours" category

TABLE 3.

OXYGEN CONSUMPTION OF *C. shufeldtii* BEFORE AND AFTER EYESTALK REMOVAL IN TERMS OF ML/G/HR AND PERCENTAGE OF THE ORIGINAL VALUE

Sex	Number Specimens	Normal Values *	Values after Destalking *			
			24 hrs	48 hrs	72 hrs	96 hrs
		Destalked 24 hrs				
♂	12	0.310	0.380	.....	.....	.....
		100	122.5			
♀	16	0.214	0.226	.....	.....	.....
		100	105.6			
		Destalked 48 hrs				
♂	7	0.275	0.388	0.402	.....	.....
		100	141.1	146.2		
♀	9	0.206	0.199	0.216	.....	.....
		100	96.7	104.9		
		Destalked 72 hrs				
♂	4	0.280	0.449	0.371	0.344	.....
		100	160.4	132.5	122.9	
♀	5	0.172	0.187	0.202	0.206	.....
		100	108.8	117.4	119.8	
		Destalked 96 hrs				
♀	1	0.121	0.131	0.198	0.198	0.195
		100	108.2	163.6	163.6	161.2

* upper figure = ml/g/hr; lower figure = percent of original value.

was based upon the initial metabolic rate of animals which survived the operation at least 72 hours. As is evident from these averages, the metabolic rate increased following eyestalk removal. In every case where a comparison could be made, the males showed the greater percentage increase. This fact supports the contention of a basic sexual difference in metabolic rate. The percentage increase of the rate of oxygen consumption for both sexes was combined in Figure 3. There was a general trend for the animals surviving longer to have the larger increase in metabolic rate.

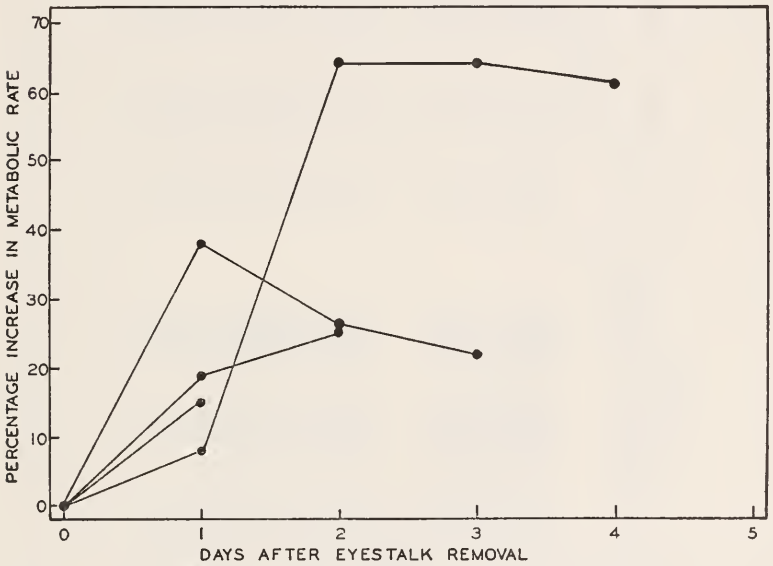

Figure 3. The effect of eyestalk removal upon the rate of oxygen consumption. The rate of oxygen consumption is expressed as the percentage increase over the rate determined prior to eyestalk removal. The results have been plotted according to the number of days the eyestalkless crawfish survived.

Effect of eyestalk extract upon the metabolic rate of Cambarellus.—Two experiments involving 11 animals were performed. The metabolic rates of six control and five experimental animals whose eyestalks had been removed at least 24 hours previously were determined for one hour by means of the Warburg respirometer. The experimental group received immediately after the initial determination of the metabolic rate an injection of the equivalent of one eyestalk in 0.025 ml of extract which was prepared in the following fashion. The eyestalks were removed from several *Cambarellus,* triturated, and resuspended in a sufficient volume of van Harreveld's solution, which is isotonic with crawfish blood, so that each 0.025 ml of

TABLE 4.

METABOLIC RATES OF EYESTALKLESS CRAWFISH INJECTED WITH EYESTALK EXTRACT (EXPERIMENTALS) AND SALINE (CONTROLS)

Minutes	Controls (ml/g)						Ave.	Experimentals (ml/g)					Ave.
10	0.027	0.030	0.046	0.032	0.035	0.043	0.036	0.048	0.030	0.031	0.034	0.048	0.038
20	0.058	0.070	0.080	0.082	0.084	0.097	0.078	0.092	0.067	0.064	0.072	0.107	0.080
30	0.067	0.097	0.114	0.129	0.119	0.140	0.111	0.130	0.100	0.092	0.107	0.159	0.118
40	0.097	0.145	0.157	0.179	0.157	0.170	0.153	0.168	0.140	0.128	0.141	0.200	0.155
50	0.127	0.190	0.197	0.227	0.208	0.220	0.195	0.200	0.187	0.168	0.175	0.245	0.195
60	0.148	0.227	0.240	0.271	0.251	0.271	0.244	0.241	0.232	0.200	0.220	0.252	0.231
30	Injected with Saline							Injected with Eyestalk Extract					
10	0.033	0.043	0.054	0.038	0.041	0.046	0.043	0.048	0.039	0.036	0.026	0.024	0.035
20	0.061	0.080	0.094	0.080	0.078	0.103	0.083	0.085	0.065	0.050	0.076	0.052	0.066
30	0.088	0.120	0.140	0.132	0.113	0.149	0.124	0.122	0.097	0.069	0.118	0.086	0.098
40	0.109	0.150	0.172	0.170	0.162	0.203	0.161	0.152	0.112	0.075	0.153	0.172	0.124
50	0.140	0.195	0.220	0.206	0.192	0.249	0.200	0.197	0.148	0.103	0.186	0.162	0.159
60	0.172	0.235	0.254	0.238	0.241	0.294	0.239	0.241	0.177	0.117	0.225	0.206	0.193
70	0.176	0.280	0.300	0.290	0.294	0.344	0.280	0.259	0.203	0.141	0.251	0.248	0.220
80	0.212	0.315	0.334	0.325	0.330	0.374	0.315	0.289	0.229	0.156	0.286	0.303	0.253
90	0.249	0.387	0.374	0.368	0.373	0.428	0.363	0.313	0.249	0.170	0.319	0.331	0.277
100	0.285	0.420	0.408	0.402	0.392	0.480	0.398	0.344	0.274	0.189	0.350	0.365	0.304
110	0.321	0.455	0.443	0.449	0.443	0.534	0.441	0.368	0.300	0.205	0.399	0.410	0.336
120	0.357	0.492	0.486	0.474	0.478	0.572	0.477	0.403	0.338	0.239	0.413	0.442	0.367

extract contained the equivalent of one eyestalk. The control group immediately received 0.025 ml of van Harreveld's solution.

The averaged results of the two experiments were used to prepare Figure 4. The oxygen consumption of each crawfish used in this

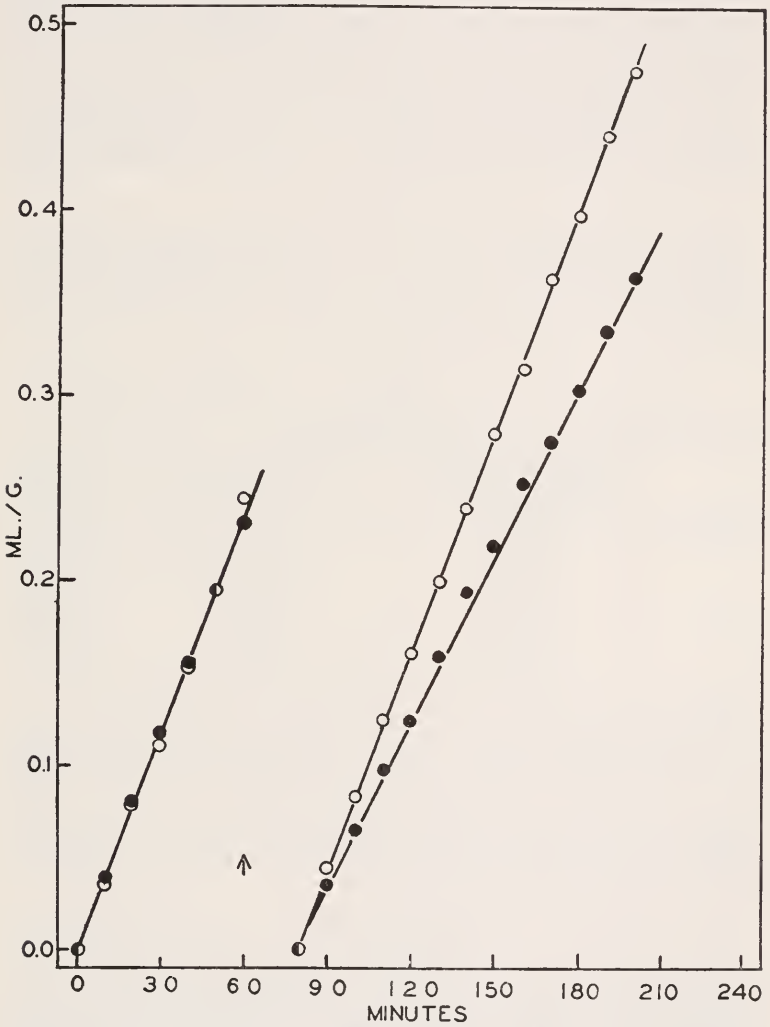

Figure 4. The influence of eyestalk extract upon the rate of oxygen consumption of eyestalkless *C. shufeldtii*. Circles = control animals which received injections of saline; dots = animals which received an injection of eyestalk extract; arrow indicates when the injections were administered; ordinate shows the milliliters of oxygen consumed per gram of body weight.

series of experiments is presented in Table 4. In this experiment comparison was made of the metabolic rate of the same crawfish before and after injection, rather than a comparison of the metabolic rates of different crawfish. The metabolic rates of the controls and experimentals were identical prior to the injections. Following the injections the controls showed no change in metabolic rate. The line representing the rate of the control animals paralleled the line drawn with the data obtained prior to the injection.

The experimental group showed a decrease in metabolic rate as evidenced by the decrease in slope. One hundred fifty minutes following the injections the metabolic rate of the experimental group was 23 percent less than the rate of the controls.

Lack of effect of stimulation of the eyestalk stubs.—The metabolic rates of six eyestalkless animals were determined for one hour by means of the Warburg respirometer. The eyestalk stubs were then stimulated by means of an electric cautery and the metabolic rates again determined. The results are presented in Table 5.

TABLE 5.
INFLUENCE OF CAUTERY OF EYESTALK STUBS UPON RATE OF OXYGEN CONSUMPTION

Animal	Oxygen Consumption Before Cautery (ml/g/hr)	Oxygen Consumption After Cautery (ml/g/hr)	Percentage Change
1	0.246	0.261	+ 6
2	0.191	0.137	—28
3	0.248	0.279	+12
4	0.224	0.276	+23
5	0.251	0.224	—12
6	0.239	0.312	+31

There was no constant effect. Four of the animals showed an increase in rate, two a decrease. The average change was a 6.4 percent increase. Scudamore (1947) found a 56.8 percent increase in metabolic rate following cauterization of the eyestalk stubs of *Orconectes immunis*. Experiments designed to test the effects of extracts of the supraesophageal ganglia with the circumesophageal connectives attached were equally inconclusive.

DISCUSSION

The existence of a daily rhythm of oxygen consumption in eyestalkless *Cambarellus* demonstrated that the sinus gland and x-organ of the eyestalk are not the centers of rhythmical activity in this species. The slight difference in the daily patterns of normal and eyestalkless *Cambarellus* might have been a modification of the basic metabolic rhythm due to the loss of the endocrine sources in the eyestalks.

The higher metabolic rate of eyestalkless crawfish is evident from (1) the greater amplitude of the daily rhythm of eyestalkless indi-

viduals and (2) the observations of the effect of eyestalk removal upon the metabolic rate of the same individual from day to day. The crawfish used in the determination of the daily rhythm of eyestalkless animals were females which were shown to have a lower metabolic rate than males. Seventy-five percent of the animals used in the studies of the daily rhythm of normal crawfish were males. The observed increase in amplitude of the daily rhythm of eyestalkless crawfish would probably have been greater than the observed 16 percent if animals of the same sex had been compared. The observed effect of eyestalk extract upon the metabolic rate confirmed the earlier observations of Scudamore (1947) who used a different genus of crawfish.

Summary

1. The rate of oxygen consumption of the dwarf crawfish, *Cambarellus shufeldtii,* has been continuously recorded for 24 hour periods.

2. Analysis of the data revealed a daily rhythm of rate of oxygen consumption. The rate was maximal around 6 a.m. with a secondary maximum at 3-6 p.m. Minima occurred from 9 a.m. to noon and from 9 p.m. to midnight.

3. Eyestalkless *Cambarellus shufeldtii* also had a persistent daily rhythm of metabolic rate. The daily rhythm of eyestalkless individuals differed slightly in pattern from that of normal crawfish.

4. Males weighed less and appeared to have a higher metabolic rate than females. The higher rate was probably due to both the sexual and weight differences.

5. Removal of the eyestalks resulted in an increase in the metabolic rate.

6. Extracts of eyestalks decreased the metabolic rate.

7. The metabolic rate of males increased more than the metabolic rate of females after eyestalk removal. This fact is further evidence in favor of a sexual difference in metabolic rate in *Cambarellus.*

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