OXYGEN UPTAKE OF TISSUE FROM NORMAL AND DESTALKED CRAYFISH (PACIFASTACUS SP.) BOTT

by

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INTRODUCTION

The metabolism of crustacean arthropods has been investigated by many workers. Measurements of the oxygen uptake of the whole animal, and of slices and homogenates of specific tissues, have provided valuable indices indicating the manner in which certain intrinsic and environmental factors influence metabolism. It was recognized, as early as 1803, that oxygen was utilized by crustaceans, and the results of investigations since that time indicate that the oxygen uptake of these animals is not notably different from that of other similarly sized poikilotherms (26, p. 213). The influence of such factors as geographical variation (effect of terrestrial or aquatic habitat, latitude), and specific environmental changes (temperature, osmotic pressure, respiratory stress, seasonal changes, etc.), have been studied and the results of these effects described in some representatives of Crustacea (12, p. 395-405).

The process of molting, with its cyclical morphological and physiological changes by which the crustacean periodically sheds its exoskeleton, usually with an increase in size, is associated with marked changes in

metabolism (26, p. 213).

The intermolt cycle was originally classified into stages, by Drach (1939), and since then has undergone several revisions so as to describe the molt stages found in specific groups of Crustacea more accurately (7, p. 95-100; 22, p. 474-495; 27, p. 4-8). Current intermolt cycle classification describes these stages: Stage A (early postmolt), Stage B (late postmolt), Stage C_a (early intermolt), Stage C_b (late intermolt), Stage D_0 (beginning premolt), Stage D_1 (early premolt), Stage D_2 (middle premolt), Stage D_3 (late premolt), and Stage D_4 (final premolt) closely followed by ecdysis itself (Ibid., p. 4-5).

Ecdysis was previously thought of as an isolated incident separated by relatively long intermolt periods. With further study, this original concept has been replaced by a new realization that molting, so fundamental to the growth of the animal, occupies an essential role in the entire life of the individual. Following molt, metabolic processes are initiated which begin to prepare the animal for the next molt. Except in cases of terminal molt, there is no time when the crustacean entirely escapes the dominating influences of the molting process (22, p. 473-474).

There is abundant evidence to indicate that

hormones from the eyestalk, and from certain areas outside the eyestalk, influence the intermolt cycle. In 1905, Zeleny, working with the fiddler crab Uca pugilator, observed that removal of the stalked eye resulted in precocious molting (47, p. 81-84). Similar effects, resulting from eyestalk removal were again noted in 1912 by Megušar (referred to in 18, p. 175). Several investigators in the years 1938 to 1941, rediscovered the early observations of Zeleny and Megušar, reporting that removal of the eyestalk from the specific crustacean studied resulted in accelerated molting: Uca pugilator (1, p. 278-296; 2, p. 326-327; 3, p. 179-188; 19, p. 145-149), Cambarus immunis (6, p. 104-114), Cambarus clarkii (34, p. 145-152). Drach (11, p. 40-62), investigating the prawn Leander serratus, and Guyselman (15, p. 115-136), working with the fiddler crab Uca pugilator, produced evidence indicating that removal of the eyestalks from these crustaceans resulted in accelerated molting only if the operation was performed prior to the early premolt stage.

Scudamore (32, p. 187-208) was the first to observe an elevation in the oxygen uptake immediately prior to molt, in the crayfish Cambarus immunis and virilis. Following Scudamore's 1947 observation, several investigators have examined the oxygen uptake of other

representative crustaceans, and in general obtained similar results. Frost et al. found that removal of the eyestalks, from the crayfish Astacus, increased the oxygen consumption of the whole animal (14, p. 572). Bliss (1953), working with Gecarcinus lateralis, observed an increase in oxygen uptake and a decrease in respiratory quotient in proecdysis (5, p. 275-296). Scheer and Scheer (1954) examined the effects of eyestalk removal on both molting interval and oxygen uptake in Leander serratus, and found no acceleration of molting, and only a transient increase in oxygen consumption during the premolt stages D_1 and D_3 only (29, p. 397-418; 30, p. 419-426).

The studies heretofore described, investigating the effects of eyestalk removal and molting on oxygen consumption, have been done predominantly on whole animals. Few studies have been made on the oxygen uptake of isolated tissues, especially with regard to variations in the oxygen uptake of specific tissues following eyestalk removal and/or molting. Fox and Wingfield determined oxygen uptake values of muscle tissue from Pandalus borealis and P. montagui (13, p. 275-282). Vernberg determined the level of oxygen consumption in nine crustacean decapods which ranged from marine aquatic to intertidal to terrestrial environments,

examining both gills and hepatopancreas (41, p. 227-234). Belding et al. (4, p. 75-88) and Weymouth et al. (45, p. 50-71) determined the oxygen consumption to body weight relationship for hepatopancreas from the kelp crab Pugettia producta. Roberts determined the oxygen uptake levels for the crab Pachygrapsus crassipes, examining brain and muscle tissues (25, p. 242-255). More recently, Vernburg (1960) has examined the effect of temperature acclimation on isolated tissue preparations from closely related species of the fiddler crab, Uca (43, p. 399).

In none of these examples of measurements of oxygen uptake from isolated tissues was there an attempt made to determine the effects of either eyestalk removal or varying molt stages. Scheer and his group (1952) determined the oxygen uptake of hepatopancreas and muscle tissue removed from unoperated control and destalked lobsters, 24-30 hours after the eyestalking operation had been performed. They found decreased levels of oxygen uptake in hepatopancreas from male animals, with no Q_{O_2}* change in females. They observed no alteration of oxygen uptake in muscle tissue, and no variation in respiration

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*Q_{O_2} is oxygen uptake or consumption usually expressed in units of microliters oxygen per unit tissue weight per unit time (μl O_2/unit tissue weight/unit time).

of any tissue examined attributable to varying stages of the molt cycle (31, p. 327-338). Skinner (referred to in 22, p. 486) demonstrated an increase in the level of epidermal tissue oxygen consumption from 0.49 to 0.85 microliters oxygen per milligram dry weight per hour during the D_2 Drach stage. This work was done on the land crab, Gecarcinus lateralis.

While it has been repeatedly shown that oxygen consumption of the intact animal increases during the premolt stages, there is limited information regarding changes in the level of oxygen uptake of specific tissues. Changes in the Q_{O_2} presumably represent symptoms of the metabolic processes, and it would seem likely that metabolic changes associated with molting would be reflected in variations in the Q_{O_2} of the tissues. With this in mind, the levels of oxygen consumption of two tissues, the hepatopancreas and the epidermis (both of which are known to occupy a central role in the molting process), were examined for a period of 30 days following eyestalk removal. The results on oxygen consumption of tissues from destalked animals were compared with the oxygen consumption of tissues from unoperated control animals.

METHODS AND PROCEDURES

Crayfish, Pacifastacus sp. Bott (24, p. 447-469) were obtained from Callaway Lake in the Peavey Arboretum, about six miles north of Corvallis, Oregon. Following capture, in February and April 1961, the animals were maintained in holding tanks for an acclimation period of two weeks. Holding tanks were constructed of galvanized metal coated with fiberglass and were supplied continuously with air and charcoal filtered tap water. Water temperature in the holding tanks was 15 ± 5°C during the entire experiment. Ground beef liver was fed at varying intervals (see below). Two identical series of determinations, each lasting 30 days, were performed on two separate groups of animals, designated as Group I and Group II. Only males were used. During the period of acclimation, the crayfish were individually identified (by numbering with fingernail polish on the carapace), sexed, weighed, and a small section of one uropod was removed for staging as described by Scheer (27, p. 3-18). Ninety crayfish, ranging in weight from 30-70 grams (mean 49 grams), were selected for Groups I and II. For each group, bilateral eyestalk removal was performed on 30 animals, leaving 15 crayfish as unoperated controls. Animals to be destalked were

pre-cooled to 10°C in an ice bath and transferred individually to a special holding apparatus designed expressly for the destalking operation.

The holding apparatus consisted of an 8 x 12 x 2 inch stainless steel pan, having a plexiglass platform on the bottom, with a series of opposing hooks accomodating a row of horizontally placed rubber-bands (see Figure 1). The holding apparatus was filled with ice-water, and air supplied by means of an air stone placed at the anterior end of the crayfish. Such an arrangement allowed little movement of the animal during the operation, yet did not subject the crayfish to excessive desiccation or hypoxia.

The eyestalk was clamped behind the crystalline lens using locking forceps, and extended by the elasticity of a rubber-band extending from the forceps to a fixed post securely mounted on the holding board. With the eyestalk in such an extended position, a ligature (3-0 silk thread) was fastened securely around the base of the eyestalk, and the eyestalk removed by a scapel cut distal to the point of ligation. The remaining stub of the original eyestalk was lightly cauterized with the fine tip of an electrical cautery instrument. The destalked crayfish were allowed a short period of accomodation immediately following eyestalk removal, and then

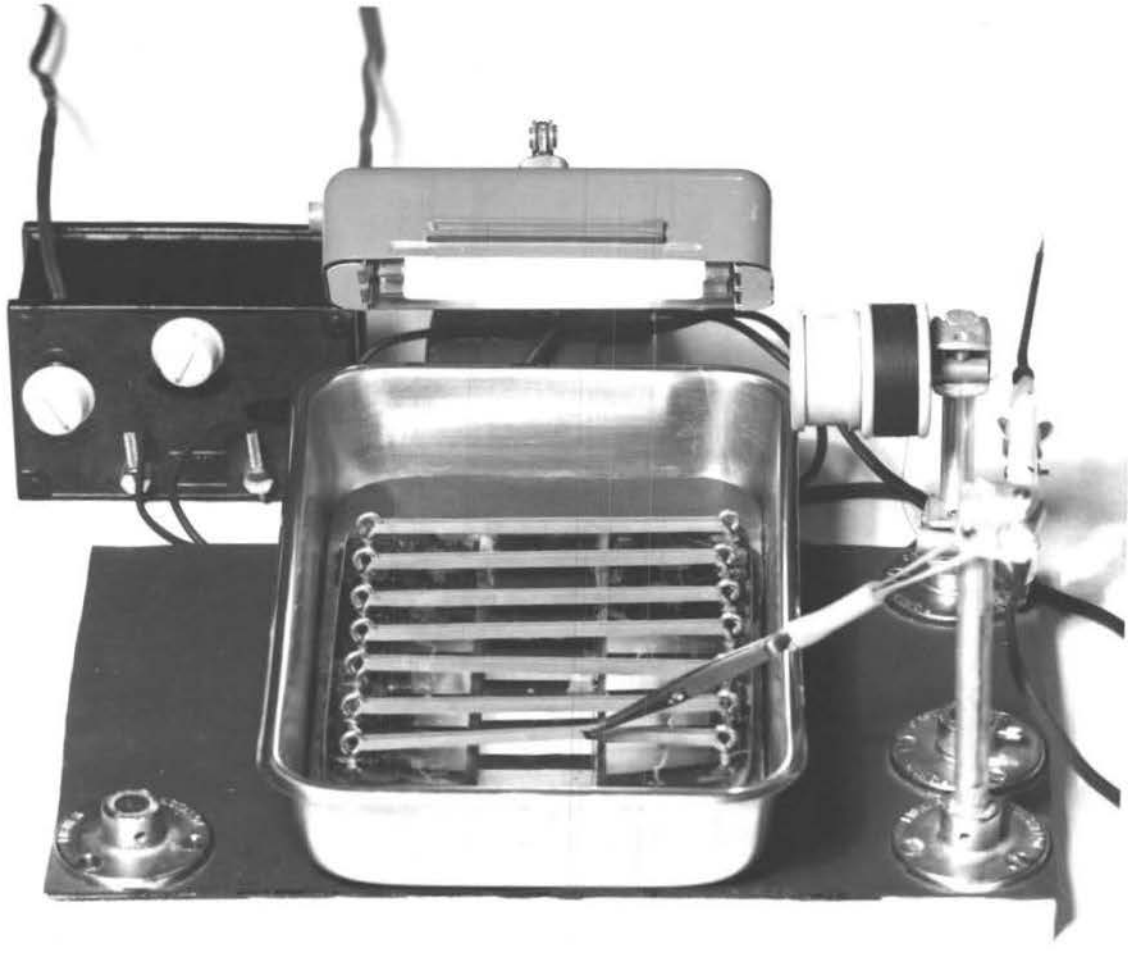

FIGURE 1. Destalking Apparatus

transferred to a small container of ice-water. The operated animals remained in this cooled condition for approximately one hour and were then held in small polyethylene holding tanks for 24 to 48 hours. During this period of time the destalked crayfish were observed for signs of mortality; obviously weak animals were discarded. Finally, the destalked crayfish were placed in the holding tank with the controls.

The control animals received no operation, and were placed in the original holding tank, being separated from the destalked animals by a perforated plexiglass partition. This partition allowed free interchange of water, presumably creating equal environments for the two groups of animals.

Feeding was irregular at first, but soon it was realized that eyestalked animals required regular and frequent feeding. Frequency of feeding was maintained at every other day during the latter half of the experiments.

Two days following eyestalk removal, the first of a series of eight determinations (runs) was made, of the oxygen uptake of two tissues, the hepatopancreas and the epidermis, removed from the destalked and unoperated control crayfish. For each run, two crayfish were selected from the destalked group, and one crayfish from the

control group. Selection of animals was based on corresponding weights between these three crayfish. These animals were weighed and uropod samples obtained immediately before removing the tissues to be analyzed.

The hepatopancreas was removed *in toto*, following a midventral incision, extending from the anterior margin of the abdomen to the base of the antenulles. The entire gland, consisting of two equal elongate lobes (40, p. 40-54), was quickly placed in a Syracuse watch glass containing 10 ml. of van Harreveld's solution (39, p. 428-432; 44, p. 161). The end portion of each lobe of the hepatopancreas was removed with scissors and discarded. A second cut was made across each lobe, the resulting tissue slice weighing approximately 150-300 mg (wet weight after blotting). The paired tissue samples, one from each lobe, constituted duplicate samples of tissue from each individual animal.

For removal of the epidermis, a second incision was made on the mid-dorsal surface of the carapace, longitudinally extending from the posterior margin of the carapace to a point near the eyes. After removing the hepatopancreas, the two halves of the carapace were separated from the animal and immersed in van Harreveld's solution in a Syracuse watch glass. The epidermal tissue was obtained by careful scraping of all the soft,

non-calcified tissue in the area of the carapace ordinarily covering the gills. The two tissue pieces obtained, one from either half of the carapace, were placed in van Harreveld's solution immediately upon separation from the carapace, and represent the duplicate samples of epidermal tissue for each individual animal.

Gastroliths were frequently present, particularly in animals from which the eyestalks had been removed for more than one week. Whenever present, gastroliths were removed, placed in ethyl alcohol (70%) or dioxan, later dried and mounted for comparison.

After the tissue samples had been removed, each sample was individually transferred, after uniform blotting on filter paper, to a 15 ml Warburg flask containing 3.0 ml of van Harreveld's solution (pH 7.5) with 0.2 ml of 10% KOH in the center well.

Flasks containing individual tissue samples were connected to manometers and allowed to equilibrate to temperature (25 $\pm$ 0.01°C), with shaking (amplitude 4.0 cm/frequency 108 oscillations per minute) in an air atmosphere. Following equilibration for 30 minutes, readings were taken at 15 minute intervals for 2.5 hours, following the procedures outlined in Umbreit *et al.* (38, p. 1-7, 64-78).

Upon completion of manometric analysis, the total

contents of each Warburg flask were transferred quantitatively to individual Kjeldahl flasks for determination of total nitrogen. Each Kjeldahl flask contained 2.0 ml H_2SO_4 (specific gravity 1.84), 0.66 g of digestion mixture of K_2SO_4 and HgO (4:1) (17, p. 354-358; 36, p. 134-155), and two small glass beads. The contents of the Kjeldahl flasks were digested until clearing occurred (4-6 hours), cooled, the sides of the flask washed down with 10.0 ml of glass distilled water, and redigested until cleared a second time. After second clearing, 20.0 ml of glass distilled water was added after the flasks were cool. Flasks in this condition were often stoppered and allowed to sit overnight, being finished the following day.

Distillation of hydroxide-released-ammonia was accomplished using modified Iowa State Kjeldahl traps which coupled to standard straight tube condensors. Ten ml of 50% NaOH was layered down into each flask, the flask connected to the condensor, and the contents mixed by gentle swirling. The resulting ammonia was distilled into a 2% boric acid-indicator receiving solution (16, p. 552). Distillation was continued until the first evidence of bumping, at which time the heat was immediately reduced and the condensor inlet moved to a position slightly above the surface of the receiving solution.

The sides of the receiving flask were rinsed with glass distilled water, and the flask contents titrated with 0.014 $\underline{N}$ H_2SO_4 to determine the amount of nitrogen present. The thermobarometer flask was treated as a "Blank" and this nitrogen value subtracted from the nitrogen values obtained for each of the other flasks containing tissue samples.

The rate of oxygen uptake was calculated for each of the ten manometric readings taken during the 2.5 hour interval, and the results expressed in units of microliters oxygen per milligram of nitrogen per minute (μl O_2/mg N/min). The first two readings were discarded as they frequently showed marked deviations from subsequent readings. The average of the eight remaining readings was obtained for each tissue sample, and this value was used to represent the respiratory rate for that specific sample, providing one of the individual variates for statistical analysis (see below).

The same procedure, as described above, for one run, was followed in each of eight successive runs, made subsequently at four day intervals, for a total of 30 days following eyestalk removal.

The results of Group I and Group II were combined, assuming no variation between the two Groups despite the difference in seasons (see Table 1 for confirmation of

the validity of this assumption). Considering only one tissue, and only samples from destalked animals, then a total of eight oxygen uptake values were obtained in each run (each value representing the mean of eight manometer readings). The same procedure was followed for the controls, except that only four values were obtained in each run, since there were only half as many unoperated as operated animals. The results of oxygen uptake from the other tissue were handled in an identical manner.

A multiple classification analysis of variance (35, p. 253-317) was performed for each tissue, to test whether or not there was a significant variation between the tissue from destalked and unoperated control animals.

RESULTS

Destalked crayfish became quiescent immediately following eyestalk removal, and usually within 10 to 15 days demonstrated gross morphological signs of entering the premolt stages, which have been well described (9, p. 466-468; 22, p. 484-487).

The destalked animals eagerly responded to food soon after the operation. Feeding had to be done at very regular and frequent intervals or the mortality rate increased sharply. One of the striking changes signalling the approach of molt was the softening of the carapace, indicating the resorption of calcium and other components of the organic matrix composing the cuticle. Gastroliths were found consistently in the destalked animals six days following eyestalk removal.

The hepatopancreas gave no gross morphological evidence indicating changes because of eyestalk removal. There were occasional, but inconsistent, variations in the gross structure, relating to the degree of definition of the individual tubules of this gland, but nothing that seemed to be related to the molting process, or eyestalk removal.

The epidermis did show definite changes as the premolt stages progressed in the eyestalked animals.

There was a distinct change in the quality and quantity of epidermal pigmentation; the epidermis from intermolt animals was pink, while that of the late premolt animals was dark brown. The thickness of the epidermis increased so markedly, from the fifteenth day post eyestalk removal to the end of the 30 day period, that tissue samples of epidermis from crayfish in late premolt stage tended to be very much enlarged in comparison with corresponding tissues from control animals. Such a thickening of this tissue in response to the preparation for molting has been frequently described (9, p. 466-468; 22, p. 485-487).

Figures 2 and 3 illustrate the variation in oxygen uptake in response to eyestalk removal. In Figure 2, it can be seen that, with the exception of the results of Run 1 (on the second day following eyestalk removal), the level of oxygen consumption of the digestive gland from eyestalked forms is always a little higher than that of the control hepatopancreas. The analysis of variance (Table 1) indicates that this difference is not significant.

Figure 3 illustrates the change in the level of oxygen consumption of the epidermis tissue following eyestalk removal. At no point, during the 30 day period, does the level of oxygen uptake of epidermis from

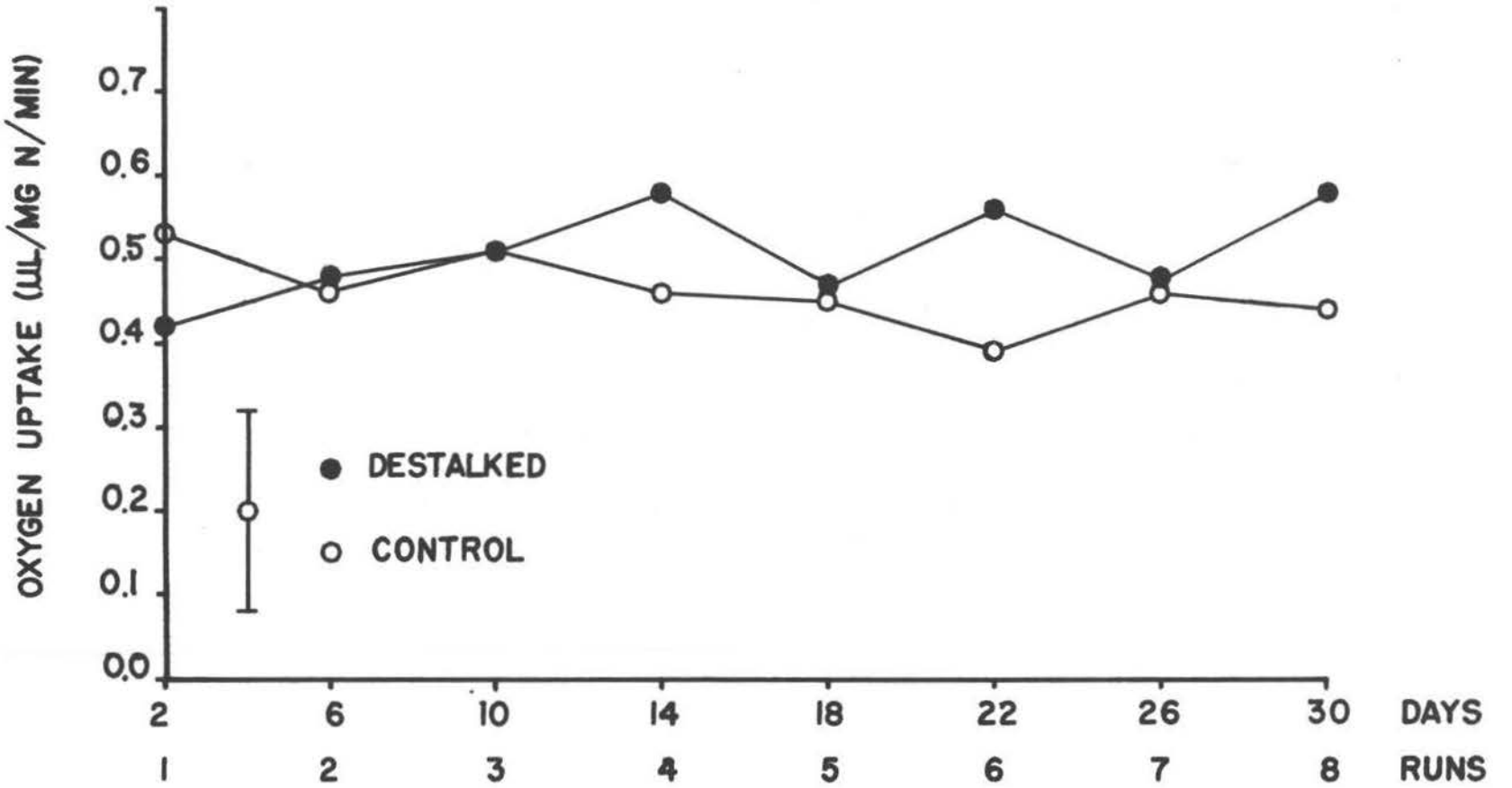

FIGURE 2. OXYGEN UPTAKE OF HEPATOPANCREAS TISSUE FROM DESTALKED AND UNOPERATED CONTROL CRAYFISH PLOTTED AGAINST DAYS FOLLOWING EYESTALK REMOVAL. VERTICAL LINE REPRESENTS STANDARD ERROR.

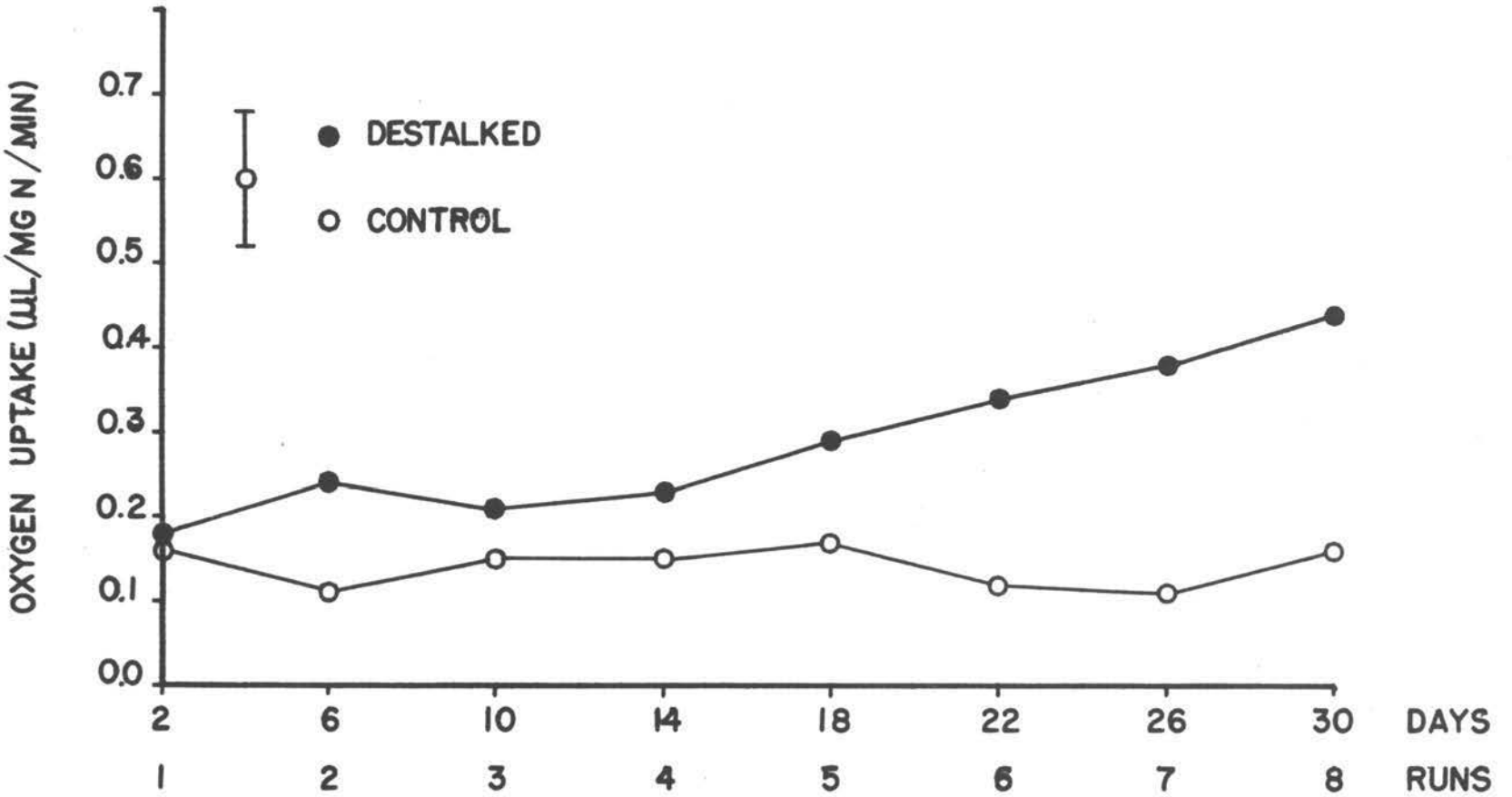

FIGURE 3. OXYGEN UPTAKE OF EPIDERMAL TISSUE FROM DESTALKED AND UNOPERATED CONTROL CRAYFISH PLOTTED AGAINST DAYS FOLLOWING EYESTALK REMOVAL. VERTICAL LINE REPRESENTS STANDARD ERROR.

TABLE 1. Analysis of variance of oxygen uptake of hepatopancreas and epidermal tissue samples from destalked and unoperated control crayfish.

Source of Variation	Hepatopancreas				Epidermis			
	Degrees of Freedom	Sum of Squares	Mean Square	F	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	95	1.086960	0.011442		95	1.415945	0.014905	
Group	1	0.000308	0.000308	0.02	1	0.008951	0.008951	0.94
Treatment	1	0.054136	0.054136	3.93	1	0.468865	0.468865	49.30**
Time	7	0.079674	0.011382	0.83	7	0.298494	0.042642	4.48**
Treatment x Time	7	0.158328	0.022618	1.64	7	0.171691	0.024527	2.58
Group x Treatment x Time*	15	0.206413	0.013761		15	0.142655	0.009510	
Between Animals	16	0.367082	0.022943		16	0.192646	0.012040	
Between Duplicate Tissue Samples	48	0.221019	0.004605		48	0.132643	0.002763	

* Experimental error
** Significant variation at the 1% level

destalked animals fall below that of the corresponding tissue from control animals. Table 1 indicates a significant difference in the level of tissue respiration as a result of the destalking treatment.

DISCUSSION

The results of this study indicate that there is a correlation between the oxygen consumption of the epidermis tissue of crayfish and their stage in the molt cycle. No significant change was observed in the oxygen uptake of the hepatopancreas at various molt stages.

The oxygen uptake level of the hepatopancreas from the destalked animals was usually greater than the Q_{O_2} of this tissue from control crayfish. The results obtained in Run 1 were the exception. Scheer et al. (31, p. 327-338) determined oxygen uptake of hepatopancreas and muscle slices from the lobster, Panulirus, 24-30 hours following removal of the eyestalks. They found no change in muscle tissue, whereas hepatopancreas showed a highly significant decrease in oxygen uptake. Since their measurements were made 24 hours earlier than the first determinations in the present study, a direct comparison may not be justified. It is interesting, however, to note that the oxygen uptake values for the hepatopancreas from destalked animals in both Groups were lower at the time of the first run, and that subsequently oxygen consumption rose to a slightly higher and constant level (Figure 2 and Appendix Table 2). There is the possibility, therefore, of a significant decrease in

oxygen uptake of this tissue within the first 48 hours following eyestalk removal, which would remain undetected in these experiments.

The only other study directly pertinent to the present one is that of Skinner (1958), in which it was shown that the oxygen uptake of the epidermis of the land crab, Gecarcinus lateralis, increased in the D_2 stage of the intermolt cycle. This was published in abstract form, and later mentioned in the review of Passano (22, p. 486).

Other investigators have determined the oxygen uptake of the hepatopancreas from several representatives of Crustacea (4, p. 75-88; 41, p. 227-234; 45, p. 50-71). In none of these studies, however, were the effects of either eyestalk removal or various intermolt stages analyzed.

In adding to this literature, one must take into account sources of variation suspected or known to affect tissue oxygen consumption. Probably the main factors to consider in crustacean work are size of animal, sex of animal and stage in the intermolt cycle.

The influence of animal size, as it affects the oxygen consumption of whole animals, has been studied by several investigators (42, p. 227-234; 45, p. 50-71; 46, p. 43-47). Some studies have also been made to

determine the effects of body size on the oxygen consumption of isolated tissues (4, p. 75-88; 20, p. 249-269). These investigations indicate that the level of oxygen consumption of isolated tissues decreases as the size of the animal increases. The range of animal weight in the present study was 30 to 70 g, and may have introduced some error in the results obtained. In each run, the three crayfish (two destalked and one control) were selected on the basis of similar weights. The results obtained within each run were, therefore, not influenced by variation in animal size, although some of the fluctuation between successive runs might result from this variation in animal weight.

During the latter part of the study the crayfish were approaching the time of their normal spring molt. It was very difficult to obtain intermolt crayfish, of stages C_a and C_b, to use as unoperated controls. Several control animals, upon removing the tissues, were found to possess small gastroliths, indicating that they had progressed at least as far as D_0 (37, p. 74). This condition for the control animals was undesirable, yet unavoidable at that particular time of the year. Such variation would presumably increase the oxygen uptake of the control tissues in the direction of values obtained from tissues of the destalked animals, masking any true

variation between treatments.

Scheer et al. (31, p. 327-338) find a significant difference in the respiration of hepatopancreas tissue from male and female Panulirus. To eliminate possible sexual differences, only male crayfish were used in these experiments.

Most of the studies to date have reported oxygen uptake in terms of tissue wet weight or dry weight. Wolvekamp and Waterman (46, p. 42) suggest that the measurement of oxygen uptake of the epidermis, on the basis of microliters oxygen per gram wet (or dry) weight per unit time, may be in error due to the large proportion of inert material contained in this tissue. This may be true, especially as the epidermis thickens in the late premolt stages. For this reason, oxygen uptake in the present study was determined on the basis of the nitrogen content of the tissues. The amount of tissue was thus estimated on a more physiological basis which presumably eliminated the error contributed by inert material.

With the recent advancements in the knowledge of endocrine control in crustaceans, it is possible to correlate the hormonal aspects of eyestalk removal and the respiratory variations observed in this investigation. The role of the antagonizing hormones, the molt

inhibiting hormone of the X-organ, and the molt accelerating hormone from the Y-organ has been described in some detail (22, p. 497-504). Eyestalk removal provides a relatively crude means of eliminating the site of production (X-organ) and release (sinus gland) of the molt inhibiting hormone. In the absence of this inhibiting substance, the molt accelerating hormone is free to exert its full influence, rapidly preparing the animal for molt. According to Passano (23, p. 89-95), removal of the Y-organ has no effect on the oxygen consumption of the crab, Sesarma reticulatum, whereas removal of the eyestalk causes the usual marked increase. This is interpreted to mean that the effect of eyestalk removal on oxygen consumption is separate and distinct from the "activation" of the Y-organ following loss of the molt inhibiting hormone. This still, of course, leaves unanswered the important question of whether the molt inhibiting hormone and the hormone which regulates oxygen consumption are indeed one and the same. It would be most interesting to investigate the effects of Y-organ removal on tissue respiration.

The observed increase in the rate of oxygen consumption by specific tissues, whether produced by eyestalk removal or normal premolt preparation, presumably reflects increased metabolic demands by the tissues

associated with preparation for molt. There is little known as to the actual mechanism by which hormones exert such an influence on metabolism at the cellular level. Scheer states, "in the arthropods there has accumulated a considerable body of evidence that interference with the neuroendocrine system has consequences in the metabolic processes of the animals. There has been no satisfactory general evidence that basic metabolic events are under neurohumoral control" (28, p. 184).

The elucidation of the manner in which hormones exert their influence has provided the stimulus for much of the past and present investigation on crustacean metabolism. The pursuit of this subject has gained impetus recently with the application of radioisotopic tracer techniques, which, coupled with increased interest in working with Crustacea as an experimental organism, should provide valuable information within the years to come.

SUMMARY

1. Removal of the eyestalks from crayfish resulted in morphological and physiological changes simulating normal molting (appearance of gastroliths, inactivity, and a softening of the exoskeleton).
2. There were no consistent changes in the gross appearance of the hepatopancreas correlating with the various intermolt cycle stages examined. The epidermis did show marked changes in its gross appearance; the change in color from pink to a dark brown associated with a gradual thickening of this tissue paralleled the transition from intermolt to premolt stages.
3. The statistical comparison of the oxygen consumption of the hepatopancreas removed from the destalked and unoperated control animals revealed no significant difference in the Q_{O_2} as a result of the destalking operation.
4. It was shown that the level of oxygen uptake of epidermal tissue from destalked crayfish increased as the animals approached molting, the molting preparation having been stimulated by eyestalk removal. This increase was statistically shown to be of significance when compared to the oxygen uptake of the same tissue removed from unoperated control crayfish.

BIBLIOGRAPHY

1. Abramowitz, A. A. and R. K. Abramowitz. On the specificity and related properties of the crustacean chromatophorotropic hormone. The Biological Bulletin 74:278-296. 1938.

2. Abramowitz, R. K. and A. A. Abramowitz. Moulting and viability after removal of the eyestalks in Uca pugilator. The Biological Bulletin 77:326-327. 1939.

3. _____. Moulting, growth, and survival after eyestalk removal in Uca pugilator. The Biological Bulletin 78:179-188. 1940.

4. Belding, Harwood S. et al. Studies on the metabolism of marine invertebrate tissue. I. Respiration of the midgut gland of the kelp crab (Pugettia producta). Physiological Zoölogy 15:75-88. 1942.

5. Bliss, Dorothy E. Endocrine control of metabolism in the land crab, Gecarcinus lateralis (Fréminville). I. Differences in the respiratory metabolism of sinus-glandless and eyestalkless crabs. The Biological Bulletin 104:275-296. 1953.

6. Brown, F. A., Jr. and Ona Cunningham. Influence of the sinusgland of crustaceans on normal viability and ecdysis. The Biological Bulletin 77:104-114. 1939.

7. Carlisle, D. B. and P. F. R. Dohrn. Sulla presenza di un ormone d'accrescimento in un crostaceo decapode, la Lysmata seticaudata Risso. Ricerca Scientifica, Torino 23:95-100. 1952.

8. Carlisle, D. B. and F. Knowles. Endocrine control in crustaceans. London, Cambridge University Press, 1959. 119 p.

9. Dennel, Ralph. Integument and exoskeleton. In: The Physiology of Crustacea, ed. by Talbot H. Waterman, vol. 1. New York, Academic Press, 1960. 670 p.

10. Drach, P. Mue et cycle d'intermue chez les crustacés décapodes. Annales de l'Institut Océanographique, Monaco 19:103-391. 1939.

11. _____. Étude preliminaire sur le cycle d'intermue et son conditionnment hormonal chez Leander serratus (Pennant). Bulletin Biologique de la France et de la Belgique 78:40-62. 1944.

12. Florkin, Marcel. Blood chemistry. In: The Physiology of Crustacea, ed. by Talbot H. Waterman, vol. I. New York, Academic Press, 1960. 670 p.

13. Fox, H. Munro and C. A. Wingfield. The activity and metabolism of poikilothermal animals in different latitudes. Proceedings of the Zoological Society of London 107A:275-282. Sept. 1937.

14. Frost, R., R. Soloum and L. H. Kleinholz. Effects of sinus gland and of eyestalk removal on rate of oxygen consumption in Astacus. Anatomical Record 111:572. 1951.

15. Guyselman, J. Bruce. An analysis of the molting process in the fiddler crab, Uca pugilator. The Biological Bulletin 104:115-137. 1953.

16. Hawk, Philip B., Bernard L. Oser and William H. Summerson. Practical physiological chemistry. 13th ed. New York, Blakiston, 1954. 1439 p.

17. Kirk, P. L. Kjeldahl method for total nitrogen. Analytical Chemistry 22:354-358. 1950.

18. Kleinholz, L. H. Endocrinology of invertebrates, particularly crustaceans. In: Recent Advances in Invertebrate Physiology, ed. by Bradley T. Scheer, Eugene, Oregon, University of Oregon Publications, 1957. 304 p.

19. Kleinholz, L. H. and E. Bourquin. Effects of eyestalk removal on decapod crustaceans. Proceedings of the National Academy of Sciences, Washington 27:145-149. 1941.

20. Krebs, H. A. Body size and tissue respiration. Biochemica et Biophysica Acta 4:249-269. 1950.

21. Megušar, F. Experimente über den Farbwechsel der Crustacean. Archiv für Entwicklungsmechanik der Organismen 33:462-465. 1912.

22. Passano, L. M. Molting and its control. In: The Physiology of Crustacea, ed. by Talbot H. Waterman, vol. I. New York, Academic Press, 1960. 670 p.

23. _____. The regulation of crustacean metamorphosis. American Zoologist 1:89-95. 1961.

24. Pennak, Robert W. Fresh-water invertebrates of the United States. New York, Ronald Press, 1953. 769 p.

25. Roberts, John L. Thermal acclimation of metabolism in the crab, Pachygrapsus crassipes Randall. II. Mechanisms and the influence of season and latitude. Physiological Zoölogy 30:242-255. 1957.

26. Scheer, Bradley T. The hormonal control of metabolism in decapod crustaceans. In: Recent Advances in Invertebrate Physiology, ed. by Bradley T. Scheer. Eugene, Oregon, University of Oregon Publications, 1957. 304 p.

27. _____. Aspects of the intermoult cycle in natantians. Comparative Biochemistry and Physiology 1:3-18. 1960.

28. _____. The neuroendocrine system of arthropods. In: Vitamins and Hormones, ed. by Robert S. Harris and Dwight J. Ingle, vol. 18. New York, Academic Press, 1960. 615 p.

29. Scheer, Bradley T. and Marlin Ann Ray Scheer. The hormonal control of metabolism in crustaceans. VII. Moulting and color change in the prawn Leander serratus. Pubblicazioni della Stazione Zoologica di Napoli 25:397-418. 1954.

30. _____. The hormonal control of metabolism in crustaceans. VIII. Oxygen consumption in Leander serratus. Pubblicazioni della Stazione Zoologica di Napoli 25:419-426. 1954.

31. Scheer, Bradley T., Calvin W. Schwabe and Marlin Ann Ray Scheer. Tissue oxidations in crustaceans. Part III of the hormonal regulation of metabolism in crustaceans. Physiologia Comparata et Oecologia 2:327-338. 1952.

32. Scudamore, Harold H. The influence of the sinus glands upon molting and associated changes in the crayfish. Physiological Zoölogy 20:187-208. 1947.

33. Skinner, Dorothy M. The molt cycle of the land crab, Gecarcinus lateralis. Anatomical Record 132: 507. 1958.

34. Smith, Ralph I. Studies on the effects of eyestalk removal upon young crayfish (Cambarus clarkii). The Biological Bulletin 79:145-152. 1940.

35. Snedecor, George W. Statistical methods. 4th ed. Ames, Iowa, The Iowa State College Press, 1955. 485 p.

36. Steyermark, Al. Quantitative organic microanalysis. New York, Blakiston, 1951. 389 p.

37. Travis, Dorothy F. Matrix and mineral deposition in skeletal structures of the decapod crustacea. In: Calcification in Biological Systems, symposium presented at the Washington meeting of the American Association for the Advancement of Science, Dec. 29, 1958, ed. by Reidar F. Sognnaes. Washington, D.C., 1960. 511 p.

38. Umbreit, W. W., R. H. Burris and J. F. Stauffer. Manometric techniques. Minneapolis, Minnesota, Burgess, 1959. 338 p.

39. van Harreveld, A. A physiological solution for fresh water crustaceans. Society for Experimental Biology and Medicine Proceedings 34:428-432. 1936.

40. van Weel, P. S. Processes of secretion, restitution, resorption in gland of mid-gut (glandula media intestini) of Atya spinipes Newport (Decapoda - Brachyura). Physiological Zoölogy 28:40-54. 1955.

41. Vernberg, F. John. Study of the oxygen consumption of excised tissues of certain marine decapod Crustacea in relation to habitat. Physiological Zoology 29:227-234. 1956.

42. _____. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus Uca. II. Oxygen consumption of the whole organisms. The Biological Bulletin 117:163-184. 1959.

43. _____. The influence of temperature on the tissue metabolism of fiddler crabs from the temperate and tropical zones. Anatomical Record 137:399. 1960.

44. Welsh, John H. and Ralph I. Smith. Laboratory exercises in invertebrate physiology. Rev. ed. Minneapolis, Minnesota, Burgess, 1960. 179 p.

45. Weymouth, F. W. et al. Total and tissue respiration in relation to body weight; a comparison of the kelp crab with other crustaceans and with mammals. Physiological Zoology 17:50-71. 1944.

46. Wolvekamp, H. P. and Talbot H. Waterman. Respiration. In: The Physiology of Crustacea, ed. by Talbot H. Waterman, vol. I. New York, Academic Press, 1960. 670 p.

47. Zeleny, Charles. Compensatory regulation. The Journal of Experimental Zoology 2:1-102. 1905.

APPENDIX

TABLE 2. Oxygen uptake (μl O_2/mg N/min) of hepatopancreas tissue samples from destalked and unoperated control crayfish. Each oxygen consumption value listed represents the mean of eight manometric readings taken for that particular tissue sample.

Treatment	Destalked								Unoperated Controls			
Group	I				II				I		II	
Animal	A_I		B_I		A_{II}		B_{II}		A_I		A_{II}	
Tissue Sample	1	2	1	2	1	2	1	2	1	2	1	2
Time												
1	0.531	0.538	0.394	0.415	0.424	0.442	0.357	0.286	0.426	0.501	0.605	0.584
2	0.255	0.257	0.492	0.547	0.487	0.477	0.583	0.758	0.372	0.543	0.422	0.486
3	0.481	0.479	0.500	0.470	0.476	0.463	0.491	0.731	0.480	0.452	0.533	0.589
4	0.693	0.671	0.498	0.681	0.418	0.445	0.516	0.685	0.462	0.452	0.407	0.503
5	0.461	0.474	0.339	0.603	0.419	0.506	0.493	0.487	0.461	0.580	0.402	0.369
6	0.530	0.455	0.590	0.590	0.466	0.538	0.667	0.655	0.430	0.405	0.371	0.340
7	0.331	0.440	0.551	0.493	0.267	0.457	0.594	0.732	0.488	0.544	0.373	0.441
8	0.499	0.509	0.629	0.697	0.527	0.570	0.602	0.644	0.398	0.588	0.378	0.381

TABLE 3. Oxygen uptake (μl O_2/mg N/min) of epidermal tissue samples from destalked and unoperated control crayfish. Each oxygen consumption value listed represents the mean of eight manometric readings taken for that particular tissue sample.

Treatment	Destalked								Unoperated Control			
Group	I				II				I		II	
Animal	A_I		B_I		A_{II}		B_{II}		A_I		A_{II}	
Tissue Sample	1	2	1	2	1	2	1	2	1	2	1	2
Time												
1	0.193	0.202	0.132	0.106	0.246	0.156	0.162	0.225	0.216	0.168	0.130	0.121
2	0.219	0.194	0.140	0.184	0.250	0.232	0.338	0.342	0.106	0.136	0.098	0.087
3	0.211	0.203	0.202	0.180	0.276	0.232	0.204	0.216	0.143	0.133	0.134	0.182
4	0.316	0.280	0.172	0.231	0.201	0.160	0.333	0.185	0.216	0.097	0.078	0.214
5	0.221	0.243	0.159	0.175	0.266	0.563	0.383	0.342	0.139	0.166	0.220	0.162
6	0.262	0.219	0.427	0.254	0.334	0.423	0.395	0.390	0.198	0.087	0.058	0.143
7	0.538	0.579	0.285	0.254	0.257	0.204	0.500	0.390	0.144	0.122	0.105	0.057
8	0.464	0.442	0.407	0.463	0.401	0.375	0.507	0.469	0.210	0.212	0.113	0.118