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Title RESPIRATION OF CRAYFISH IN DIFFERENT SALINITIES

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The respiration of the western North American crayfish, Pacifastacus leniusculus, in different salinities was studied. The animals were acclimated in 0, 20, 40, 60, 80 and 100 percent sea water and for each salinity, the metabolic rates of the whole animal and of the excised gill, green gland and gut were measured. Results obtained for the whole animal indicated that changes in respiration rate in the different salinities might be a reflection of other activities stimulated by the stress, e.g. locomotory activity in attempting to escape from the medium, rather than the osmotic work alone. The gut tissue showed a somewhat higher oxygen consumption at 60 and 80 percent sea water, as compared to the freshwater controls, indicating a possible function for osmoregulation in the higher salinities. The oxygen consumption for both the gill and the green gland maintained at about the same level from 0 to 40 percent sea water, with a significant increase at 60 percent. In the higher salinities, there was a considerable drop in the metabolic rate of the green gland and it

dropped to significantly below normal at 100 percent. The gill, however, showed a steady increase in oxygen uptake from 60 to 100 percent sea water. The probable reasons for these changes in respiration were discussed.

RESPIRATION OF CRAYFISH IN DIFFERENT SALINITIES

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

RESPIRATION OF CRAYFISH IN DIFFERENT SALINITIES

INTRODUCTION

One of the principal problems faced by fresh-water animals is the constant influx of water by osmosis through the permeable boundary membranes and the loss of salts from the body fluids to the external medium. Beadle (4, p. 176-178) suggested that this problem has been met in two ways (1) by a reduction in surface permeability to salts and water; and (2) by the adaptation of tissues to a lowered blood osmotic concentration which can be more easily maintained, and the simultaneous development of renal salt reabsorption resulting in the production of a hypotonic urine. Shaw (47, p. 153-162) has shown that the acquisition of an efficient sodium uptake mechanism may diminish the necessity for a great decrease in the permeability of forms living in dilute media. He suggested that the adaptation of crustacea to fresh water involves not only a gradual reduction in the permeability of the body surface to salts but also a reduction in the critical concentration for ion uptake. Basically, adaptation to fresh water has involved mechanisms for maintaining the body fluids hypertonic to the medium and adaptation of the cells to function normally in that dilution of the body fluid that does occur. If an animal maintains a steady-state body fluid concentration in a dilute medium, water must be excreted from the body fluid at the same rate as it is entering and solute must be taken up at the same

rate as it is leaving (10, p. 158; 26, p. 294). These processes, which involve regulation against concentration gradients, will require that energy be expended.

Energy expenditure on osmoregulation[1] in fresh water animals can be reduced to reasonable proportions by the ability to tolerate an exceeding low blood concentration, thus reducing a water flux through the body (36, p. 628; 10, p. 166; 26, p. 293). According to Lockwood (26, p. 293) a decrease in the blood concentration has a multiple effect on the work performed in ion transport. It diminishes both the diffusion loss of ions and the urine volume, and decrease in urine volume will inevitably restrict ion loss through this route. A fall in blood concentration may also diminish the work on ion transport at the cellular level, and by decreasing the electrochemical gradient tending to drive sodium into cells, the energy expenditure on sodium extrusion may be expected to decline in consequence.

Potts (36, p. 630) has calculated that in a semi-permeable[2] form in fresh water, the reduction of the urine concentration to the point at which it is isotonic with the medium can reduce the osmotic

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[1]Osmoregulation refers to the ability of an animal to maintain a blood osmotic concentration different from that of the external medium.

[2]Potts assumed that the surface of the animal is permeable to water but impermeable to salts so that extra-renal salt loss is negligible.

work by as much as 90 percent, and even a moderate reduction of the urine concentration, so that the urine is hypotonic to the blood but many times more concentrated than the medium, greatly reduces the osmotic work and is compatible with high osmoregulatory efficiency. Croghan (10, p. 163), however, has some doubts on the energetic advantage of hypotonic urine production unless the mechanism responsible for ion transport differ at body surface and in the excretory tubule. Lockwood (26, p. 294) concluded that until further evidence is available, the production of a hypotonic urine in general is perhaps more satisfactorily regarded as being related to the conservation of ions already within the body and as a means of fine control over the rate of ion loss, rather than as a means of energy conservation.

In crustaceans, the chief organs and tissues concerned in ionic and osmotic regulation are the gills, the antennal glands, and in some species, the gut. Gills are the most permeable part of the integument and are the site of the continuous absorption of ions which replace those lost in the antennal gland secretion in marine species and those lost in the urine and by outward diffusion through the integument in brackish- and fresh-water forms. The absorption involved is active in the sense that most of the ions in the first case and all the ions in the second are taken up against concentration gradients, with the expenditure of energy (50, p. 336). The functioning of the

gut in water balance has been shown in at least one form, the salt-lake and brine-pool anostracan Artemia, which continuously swallows the medium (9, p. 243-249). Antennal glands are also part of the mechanism of ionic regulation excreting selectively certain ions, chiefly Mg^{++} and SO_4^{--} (37, p. 19; 50, p. 336). Only in the fresh water decapod family Astacidae, however, has the urine been shown to be markedly hypotonic to the blood, thus functioning in osmotic regulation. The crayfish green gland consists, proximal to distal, of a coelomic sac, a sponge-like labyrinth, an elongated nephridial tubule and a bladder which empties to the exterior by a short duct (37, p. 19; 24, p. 88). Peters, as cited by Riegel and Kirschner (40, p. 302), concluded from his experiments and from observations on the microscopic anatomy of the crayfish green gland that the primary urine, in the form of a blood filtrate, was formed in the coelomic sac and modified by reabsorption of salts (and possible water) in the parts distal to coelomic sac. Despite the absence of a well defined site in the crayfish green gland at which filtration[1] can occur, Riegel and Kirschner (40, p. 307) concluded that because inulin is excreted and glucose causes glucosuria, filtration does occur in that organ. In a recent study, Kamemoto (21, p. 81-87) has found

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[1] The term "filtration" usually is used quite specifically to indicate the bulk movement of a solution through a porous membrane by the application of a head of pressure (40, p. 302).

cholinesterase present in the tubule and bladder, and suggests that it is functional in the sodium reabsorption mechanism.

Very little work has been done in the oxygen consumption of excised organs or tissues dealing with osmotic or ionic regulation. The gills of Carcinus and Mytilus were found to swell in dilute sea water and showed a definite increase in metabolism (24, p. 77). Peters, as reported in Krogh (24, p. 93), has found an increase in metabolism also of other tissues such as muscles, liver and kidneys, on exposing Astacus to osmotic stress.

In contrast to the limited amount of work pertaining to tissue metabolic rate during osmotic stress, respiration of the whole animal in various crustaceans, especially the marine and brackish-water forms, has been extensively studied. A relationship between the oxygen consumption and the salinity of the medium has been demonstrated in several crustaceans, an increase in oxygen consumption being noticed with increased departure from media nearest in osmotic pressure to the body fluids of the organisms concerned (13, p. 259-273; 17, p. 43-63; 38, p. 307-313; 30, p. 75-81; 39, p. 188-192). Schlieper (41, p. 478-514) has proposed the theory that homoiosmotic marine forms respire more rapidly in dilute than in normal sea water, and suggested that the energy expended in resisting the osmotic inflow of water is derived from oxidative mechanism. This has been demonstrated by Schlieper (41, p. 478-514), Beadle

(3, p. 211-227), Schwabe (42, p. 183-236), Lowenstein (28, p. 217-221), Eliassen (11, p. 1-18), Gilchrist (16, p. 54-65), Lofts (27, p. 730-736) and Lumbye (29, p. 245-262). Most of these metabolic studies on the whole animal have generally been interpreted to mean that increased work is being done by the osmoregulatory organs in animals under osmotic stress. Potts (36, p. 618-630), however, has shown that the thermodynamic work done by the gills and kidneys of these organisms represents only a very small proportion of their total energy turnover. In the crab Eriocheir sinensis, for example, Potts (36, p. 626) has calculated that the energy used for osmoregulation is only 0.5 percent of the total metabolic energy when the animal is in fresh water. In view of the small proportion of the total metabolic energy which seems to be involved in osmotic work, Robertson, as cited in Watermann (50, p. 337), pointed out that the increases in oxygen uptake of such homoiosmotic decapods as Carcinus and Ocypode, when kept in water of lower than normal salinity, cannot be due entirely to increased osmotic work at the gills, but may be due in great part to the increased oxygen utilization by the hydrated tissues, especially the muscle and hepatopancreas. Gross (17, p. 55) suggested that such metabolic increases may be caused in part by activities of the organism other than osmotic regulation. Furthermore, Munday's (34, p. 277-287) work on mitochondria isolated from the hepatopancreas of the crab Carcinus maenas.

indicated that changes in intracellular osmotic pressure could directly regulate mitochondrial size and structure and in turn mitochondrial activity, with respect to cellular adaptation in animals under osmotic stress. Munday (34, p. 287) claimed that such an effect may explain the elevation of respiration often found in animals under osmotic stress. In a study of active ion transport by frog skin, Zerahn (55, p. 316) found that the oxygen consumption bears a constant ratio to the amount of sodium transported and that the osmotic gradient is of only secondary importance.

In its normal environment of fresh water, the crayfish is able to maintain a blood osmotic concentration higher than the external medium (25, p. 149-161; 24, p. 86; 6, p. 83; 37, p. 18; 22, p. 24). Relatively little work has been done on the ability of crayfish to tolerate increased salt concentrations. The European genus Astacus can osmoregulate up to about 50 percent sea water, and in higher concentrations above this, the blood becomes iso-osmotic with the medium (37, p. 17). Astacus have been kept for a month in 66 percent sea water and for three months in 50 percent sea water (24, p. 93). Lienemann (25, p. 149-161) found that the crayfish Cambarus clarkii did not survive well in an isotonic solution of sodium chloride. Since she did not use sea water dilutions, the animals might very likely have died from the lack of some essential ions in the medium. According to Kerley (22, p. 22-42), the western North American

crayfish, Pacifastacus leniusculus, is able to hyperregulate up to 60 percent sea water while above this, it begins to show hyporegulation.

The ability of crayfish to osmoregulate over a wide range in salinities presumably involves the expenditure of energy for the transport of ions and/or water against chemical gradients. It seemed to us, therefore, that a metabolic study on salinity-stressed crayfish might provide useful information regarding the mechanisms of osmoregulation. Peters, as cited in Krogh (24, p. 92), has examined the respiratory metabolism of Astacus acclimatized in fresh water and in 15 parts per thousand sea water respectively and found the metabolism lowered 40 percent in the sea water. However, no complete study on the relationship of osmotic stress to metabolism has apparently been done on crayfish. In the present study, an attempt was made to investigate the respiration (metabolic rate) of crayfish in different salinities, and, furthermore, to observe the correlations, if any, in metabolic rate between the whole animal and certain excised tissues presumably doing osmotic work.

MATERIALS AND METHODS

Collection and Maintenance of Animals

On the basis of a key by Miller (33, p. 144-147), the crayfish used in the experiments were determined to be Pacifastacus leniusculus. They were collected in Mary's River around the Corvallis area by traps baited with fish. After collection, they were kept in a laboratory aquarium with running charcoal-filtered tap water, for a period of at least two days before any of them were used for experimentation. The water was constantly aerated and maintained at a temperature between 16 and 20° C. Beef-liver slices were fed to these animals every two days and 48 hours before each experimental run. Under the above conditions the animals were observed to live for a long period without apparent adverse effects.

Males between 30 and 45 grams were used in the experiments. In order to avoid abnormal osmoregulation as demonstrated by Stage D animals (2, p. 531-544; 22, p. 33) care was taken to use only intermolt animals, which were recognized by the non-compressibility of the carapace and the absence of gastroliths.

Experimental Procedures

Acclimation of animals to various salinities. Sea water

brought in from the coast was filtered immediately through glass wool to remove the algae and debris present, and pollution of the sea water was thus avoided.

Since the salinity of sea water at the coast varied from time to time, sea water with 31.93 parts per thousand NaCl, a value obtained from Copenhagen sea water, was taken as 100 percent. However, the coastal sea water was usually lower in salinity than this designated value. On the other hand, filtered tap water was taken as 0 percent sea water.

Sea water of 0, 20, 40, 60, 80 and 100 percent was used. Except for 100 percent and 0 percent the various desired salinities of sea water were prepared by dilution of the filtered sea water with dechlorinated tap water. To prepare the 100 percent sea water, concentrated sea water was employed to bring the salinity up to 31.93 parts per thousand. All salinity estimations were made by titration of a known volume of sample water against standardized silver nitrate solution, with potassium chromate as indicator.

For each complete run, 18 animals were used, three for each of the salinities mentioned above. It was found advisable, however, to begin with more than 18 animals since the survival rate in 100 percent sea water was about 50 percent. The animals were first weighed and then put in filtered tap water. They were weighed to the nearest 0.5 gram on the heavy-duty beam balance in a suitably

sized beaker after the excess water was removed by shaking and blotting. To avoid problems arising from crowding, the animals were isolated in single flasks. To prevent the escape of animals from the containers, each flask was fitted with a rubber stopper, through which plastic tubing was also passed for aeration.

The crayfish were saline-stressed in gradual steps. After 48 hours in containers of filtered tap water, the metabolic rates for three animals, of the whole animal and the excised tissues of gill, green gland (kidney) and gut were measured. The remaining animals were fed and half an hour later transferred to 20 percent sea water. This procedure was repeated, in increments of 20 percent until 100 percent sea water was reached. The total time for acclimation was 12 days.

Oxygen consumption of whole animals. Metabolic rate of the whole animal was determined by a modified sealed-jar method. Before each test, the experimental jars (which were also the acclimation jars) were flushed with water of the desired salinity for three hours. They were then sealed with a stopper-siphon arrangement and the animals allowed to consume oxygen for a half-hour period. To insure that the animals were disturbed as little as possible by persons moving about the laboratory, the jars were wrapped in dark cloth. To prevent stratification of oxygen in the water, the jars were gently rotated from time to time. After 30 minutes, a water sample for

oxygen determination was siphoned from the experimental jars, and from a blank jar containing no animals. The difference in oxygen content between the blank and the experimental jars represented oxygen consumed in the measured period. From this, and the wet weight of the animal, the rate of oxygen consumption was computed in cc/Kgm/hour.

Oxygen content was estimated by the Winkler method as outlined in Ellis (12, p. 5-25). Titrations were performed on 50 ml aliquots using a 10 ml semi-microburet.

Oxygen consumption of excised tissues. Before the animals were sacrificed for tissue metabolic studies, they were weighed again to detect possible changes in weight after the acclimation period; no noticeable changes were found.

The oxygen consumption of gill, green gland and gut tissue was determined by the direct method of Warburg, as outlined by Umbreit et al. (48, p. 11-17). The center well of each flask contained 0.2 ml of 10 percent KOH and a strip of accordian-shaped folded filter paper. The main compartment contained 3 ml of van Harreveld solution, an appropriately balanced medium isosmotic to the blood of crayfish (53, p. 120).

After the various tissues were removed from the animal, they were blotted to remove excess body fluid. The kidney and gut were then sliced freehand with a razor blade. All the tissues

obtainable from each animal, approximately 180 mg of gill and 70 mg each of kidney and gut in wet weight, were placed separately in three flasks.

All flasks were allowed to equilibrate in the constant temperature bath for 15 minutes at 23° C. Manometer readings were taken at 30-minute intervals for two and one-half hours. After the final reading, the tissues were retained for dry-weight determinations. Tissue oxygen consumption was expressed in microliters per milligram dry weight per hour.

RESULTS AND DISCUSSION

When the crayfish, Pacifastacus leniusculus, is experimentally stressed to a wide range of sea water dilutions, under the same conditions as in the present study, the blood osmotic concentration, though increasing slightly, shows little change from 0 to 80 percent sea water (22, p. 28-33). It was thought, therefore, that studies of the respiration of tissues active in osmoregulation, in animals osmotically stressed, might reveal something of the mechanisms involved in maintaining this blood homeostatic condition. The main purpose of this work was to measure the metabolic rate of the whole animal, as well as the excised tissues of green gland, gill and gut in animals exposed to different salinities. The tissues used are either known, or suggested, to be involved in osmotic and ionic regulation. The results obtained on respiration of tissues (expressed as microliters per milligram dry weight per hour) are shown in Figures 1, 2, 3 and Tables 1, 2 and 3. Data on the metabolic rate of the whole animal are presented in Figure 4 and Table 4.

Kidney tissue from animals exposed to salinities from 0 to 40 percent sea water respired, in vitro, at about the same rate (Figure 1). Over this range of sea water dilutions, there is a pronounced decline in the osmotic gradient between the blood and the medium sustained by the animal, but this is not apparently reflected in any

marked change in energy requirements, in terms of oxygen consumption rate, of the green gland excretory apparatus. That the crayfish green gland functions on a filtration-reabsorption basis, in the production of a hypotonic urine, seems well established (35, p. 355-381; 25, p. 149-161; 40, p. 296-307; 21, p. 81-87). Preliminary studies on Pacifastacus (22, p. 36) indicate that from 0 to 40 percent sea water, urine volume declines somewhat, but the osmotic concentration of the urine remains as hypotonic as in fresh water.

A rise in oxygen consumption of the green gland occurs in 60 percent sea water (Figure 1). Under the same stress condition, the blood osmotic concentration of Pacifastacus is about iso-osmotic with the medium (22, p. 28-33) and thus almost no osmotic gradient between the blood and the medium exists. Why green gland should be expending more energy when there seems to be no osmotic load imposed on the animal is not clear. However, several investigations on crayfish (5, p. 174; 25, p. 155; 22, p. 36) have shown great reduction in urine volume flow and a corresponding increase in urine osmo-concentration at higher salinities. In Pacifastacus specifically, Kerley (22, p. 31) has shown that a very great reduction in urine flow occurs in salinities higher than 40 percent sea water; so much, in fact, that it is often difficult to get a sample of urine at all. The urine solute concentration in 60 percent sea water, however, increases only very slightly over that in animals exposed to 40 percent,

and the urine is still decidedly hypotonic to the blood (22, p. 28). The reduction in urine flow in 60 percent sea water would be due mainly to the reduced influx of water from the medium at this salinity. Thus, if the animal in 60 percent sea water is still filtering considerable quantity of fluid, then we might postulate that reabsorption of water, as well as salt, occurs through the green gland tubules in order to prevent dehydration. An increased energy expenditure associated with water reabsorption could then at least partially explain the increased oxygen consumption observed. The absence of quantitative information on filtration rate and urine volume flow at the higher salinities makes further comment on this point unjustified. It should also be noted that no direct evidence of water reabsorption by green gland tubules in crayfish is yet available.

Respiration rate of green gland tissue from animals exposed to 80 percent sea water is less than that from animals stressed to 60 percent (Figure 1). Pacifastacus is regulating hypo-osmotically in this salinity stress (22, p. 28), although it is not known how long the animal may be able to maintain its blood osmotic concentration lower than the medium. In any event, a considerable salt load is imposed and, in view of the not unduly great energy expenditure by the green gland, it is possible that other routes, such as the gills, are used more extensively for getting rid of the excess salt.

In 100 percent sea water, green gland respiration falls far

below the level of the controls (0 percent sea water). Survival is poor in this stress and it is likely that the low respiration rate merely reflects a general breakdown in regulatory mechanisms of the animal. This is born out by the extremely low value for respiration of the whole animal in this stress (Figure 4).

The oxygen consumption of the gill tissue, like that of the green gland, is relatively constant from 0 to 40 percent sea water (Figure 2, Table 2). At 60 percent sea water, oxygen uptake of excised gill tissue shows a significant rise, is maintained at about the same level at 80 percent, and again increases in 100 percent sea water. The higher oxygen consumption of gill tissue at salinities greater than 40 percent sea water may indicate that an energy requiring mechanism, possibly salt extrusion, is operating in the gill to actively remove excess salt from the body fluid. It is believed that in its normal environment an active uptake of salts in crayfish occurs, mainly through the gills (24, p. 90; 31, p. 152-156; 7, p. 100-108). To our knowledge, there has been as yet no direct demonstration of salt extrusion from the crayfish gill at higher salinities. Bryan (8, p. 113-128), however, has shown that sodium rapidly appears in the medium from the NaCl- loaded crayfish, a sizeable fraction via the urine and the remainder through the body surface.

Very little work has been done on the crustacean gut pertaining

to its possible function in osmoregulation. The brine shrimp, Artemia salina, continuously swallows its medium (9, p. 243-249) and it is considered that the gut of Artemia has become adapted as a mechanism for the active uptake of water, controlling water balance and preventing dehydration in hypertonic media. Although crayfish do not ordinarily drink the medium in their normal environment, Maluf (32, p. 153) states that in a hypertonic medium, the crayfish do swallow some water. Maluf (31, p. 287) further claims that the considerable constancy in weight of the animals in hypertonic media may be brought about by an active intake of water through the gut, making up for a loss through the gills. Since the active uptake of water requires energy, one might expect the gut tissue to have a higher metabolic rate in the higher salinities. In the present study, the gut tissue does indeed show a slightly higher oxygen consumption at 60 and 80 percent sea water (Figure 3, Table 3). However, the standard errors overlap the values obtained at other salinities, and in the absence of additional information on water and salt movements across the gut epithelium, further comment seems unjustified.

The previous discussion indicates that the respiration rates of tissues involved in osmoregulation in the crayfish do not necessarily bear any simple or direct relation to the osmotic gradient between the body fluid and the external medium. The same may be

said of the metabolic rate of the whole animal, which is illustrated in Figure 4. A very decided and inexplicable rise in oxygen consumption occurs in the animals exposed to 20 percent sea water, compared with the fresh water controls. In this same stress, blood osmotic concentration (22, p. 28), and respiration of gills, kidney and gut (this study) are similar to the levels in freshwater controls. We can only suggest that the sudden transfer from freshwater to one-fifth sea water may have "triggered" an increase in metabolic rate which failed to stabilize after 48 hours acclimation. Measurements of respiration after more extended acclimation to this stress may clarify this point. One further comment may be appropriate. In its natural habitat, the crayfish may show a lower oxygen consumption, in spite of the great osmotic gradient sustained, through a mechanism acquired for adaptation to its environment after the invasion of freshwater. This explanation has been suggested by Rao (38, p. 307-313) in connection with his study of responses to osmotic stress in the prawn, Metapinaeus monoceros.

The mean metabolic rate values following stepwise acclimation to 40, 60 and 80 percent sea water, are not considered to differ significantly, either among themselves, or from the mean value in freshwater (Figure 4). Although an upward trend in metabolic rate over this salinity range is apparent, the overlap in standard errors is considerable. Peters, as cited by Krogh (24, p. 92), found a 40

percent reduction in metabolic rate of the European crayfish, Astacus, acclimated to 15 parts per thousand salinity (corresponding roughly to a 40 percent sea water stress). In our case, there was indeed a somewhat lower metabolic rate at a similar stress, but differences in acclimation time in the two studies, plus the probably insignificant lowering of the metabolic rate in the present study, would make further comparison unjustified.

In 100 percent sea water, the extremely low metabolic rate (Figure 4) is probably mainly a reflection of the viability of the animals in this medium. They were observed to be sluggish and survival was poor. As mentioned earlier, a similar precipitous fall in respiration was found in green gland tissue taken from animals exposed to this medium. It has been suggested by several workers (45, p. 160; 13, p. 272; 26, p. 264) that metabolic depression probably occurs when the blood osmotic concentration rises above a critical level. In Pacifastacus, the blood osmotic concentration does show a decided rise in 100 percent sea water (22, p. 28) and may be at least partly responsible for the low metabolic rate observed.

Many factors are known to affect metabolic rate, and in the present study an attempt was made to control as many of these as feasible. Only male animals of a limited size range were used, and to avoid possible differences related to molt cycle, the experiments

were restricted to summer animals in the intermolt condition. No measurements of locomotor activity were made, however, and some of the changes in oxygen consumption rate observed may be attributable to activity changes. Gross (17, p. 60), in analysing the responses to osmotic stress of a number of decapod crustaceans, states that observed changes in respiration at different salinities may often be more closely related to processes not involved in osmoregulation, in particular to changes in behavior patterns and locomotor activity. It would be particularly interesting to know the degree of activity exhibited by Pacifastacus in 20 percent sea water, compared with the other salinity stress used.

The results of the present study do not lend support to the view expounded by Schlieper (41, p. 478-514) and Flemister and Flemister (13, p. 259-273), that increases in metabolic rate under osmotic stress are due to added osmotic work performed by osmoregulatory organs. There is no correlation between the changes in metabolic rate of the whole animal and those of the tissues used. Furthermore, because of the small size of the organs, it is doubtful that anything but very great changes in their oxygen consumption would add appreciably to the total metabolism of the animal, a point made by Potts (36, p. 626) and Gross (17, p. 60). Robertson, as cited in Waterman (50, p. 337), has suggested that hydration (or dehydration) of tissues could bring about changes in their energy

requirements which might explain changes in the whole animal respiration. Recently, Munday (34, p. 287), working on mitochondria isolated from the hepatopancreas of the crab Carcinus maenus, has shown that changes in intracellular osmotic pressure bear a direct relation to mitochondrial activity. In exploring this problem further, thus, it might be of some interest to measure the respiration of tissues which make up a large share of the total body mass, such as muscle and hepatopancreas.

Table 1. Respiration of crayfish green gland tissue in different salinities. Oxygen consumption values with standard errors, reported as microliters per milligram dry weight per hour.

Percent Sea Water	No. of Animals	Oxygen Consumption (μl/mg dry weight/hour)
0	16	0.514 ± 0.034
20	14	0.485 ± 0.016
40	15	0.482 ± 0.021
60	16	0.616 ± 0.056
80	17	0.516 ± 0.026
100	9	0.367 ± 0.025

Table 2. Respiration of crayfish gill tissue in different salinities. Oxygen consumption values with standard errors, reported as microliters per milligram dry weight per hour.

Percent Sea Water	No. of Animals	Oxygen Consumption (μl/mg dry weight/hour)
0	17	0.237 ± 0.008
20	15	0.239 ± 0.012
40	16	0.223 ± 0.010
60	17	0.263 ± 0.016
80	18	0.278 ± 0.012
100	9	0.313 ± 0.023

Table 3. Respiration of crayfish gut tissue in different salinities. Oxygen consumption values with standard errors, reported as microliters per milligram dry weight per hour.

Percent Sea Water	No. of Animals	Oxygen Consumption (μl/mg dry weight/hour)
0	17	0.310 ± 0.028
20	16	0.260 ± 0.012
40	16	0.297 ± 0.021
60	18	0.353 ± 0.035
80	15	0.342 ± 0.016
100	9	0.283 ± 0.014

Table 4. Total metabolic rate of crayfish in different salinities. Oxygen consumption values with standard errors, reported as cc/Kgm wet weight/hour.

Percent Sea Water	No. of Animals	Oxygen Consumption (cc/Kgm wet weight/hour)
0	16	68.46 ± 5.15
20	18	85.61 ± 7.94
40	16	61.6 ± 5.85
60	17	68.98 ± 6.79
80	17	79.07 ± 8.55
100	11	37.62 ± 5.38

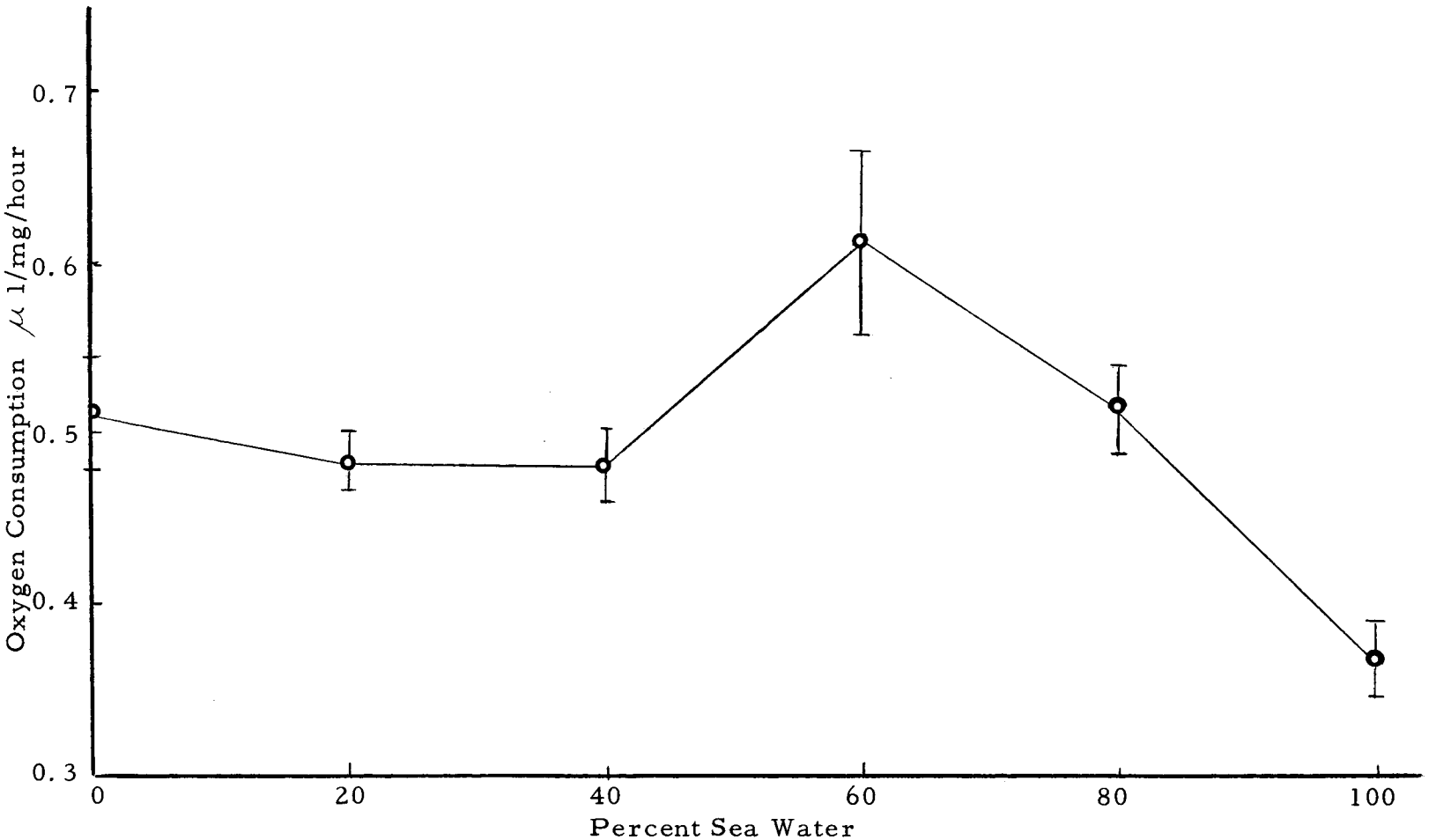

Figure 1. Respiration of crayfish green gland tissue in different salinities.

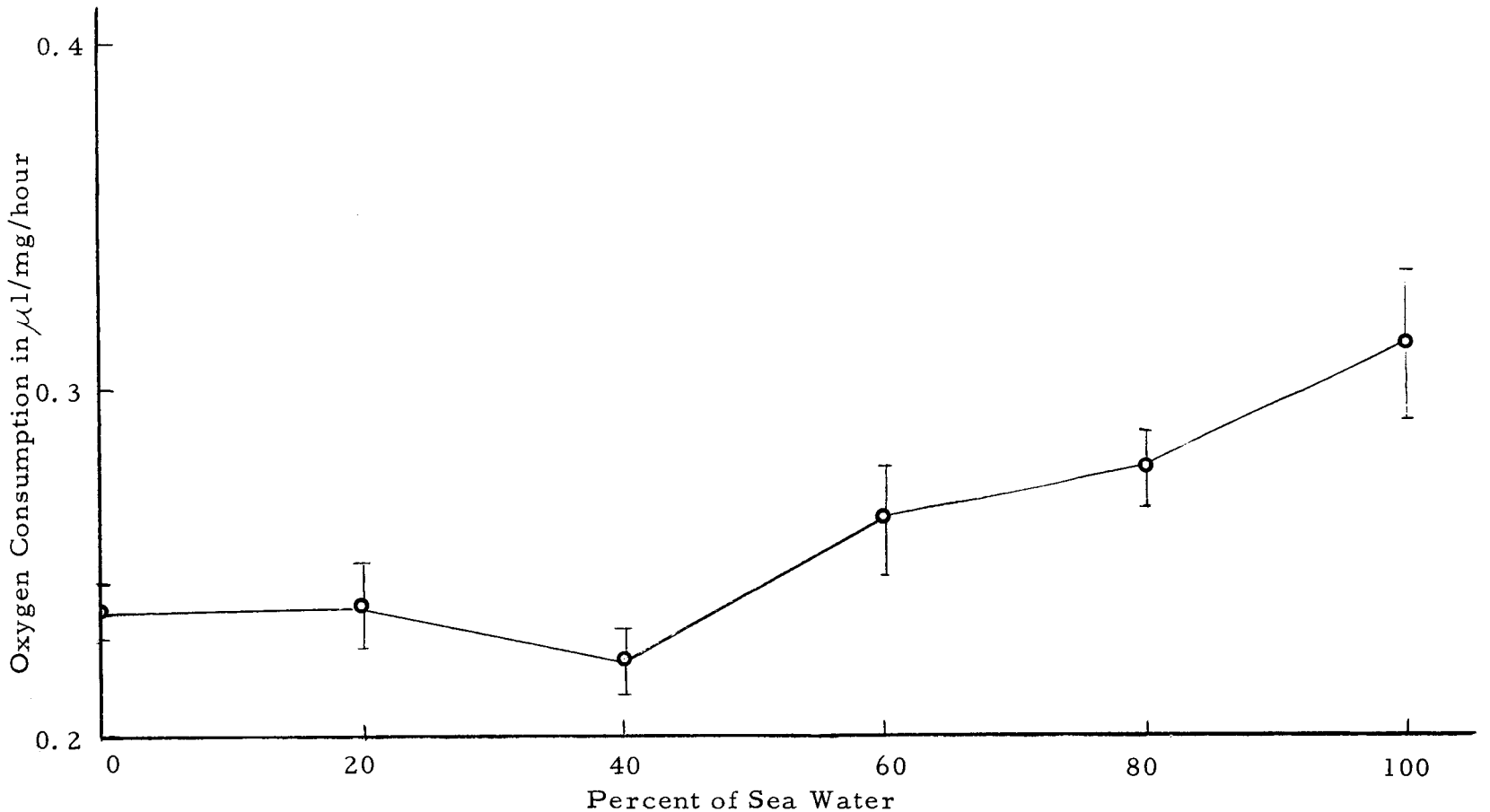

Figure 2. Respiration of crayfish gill tissue in different salinities.

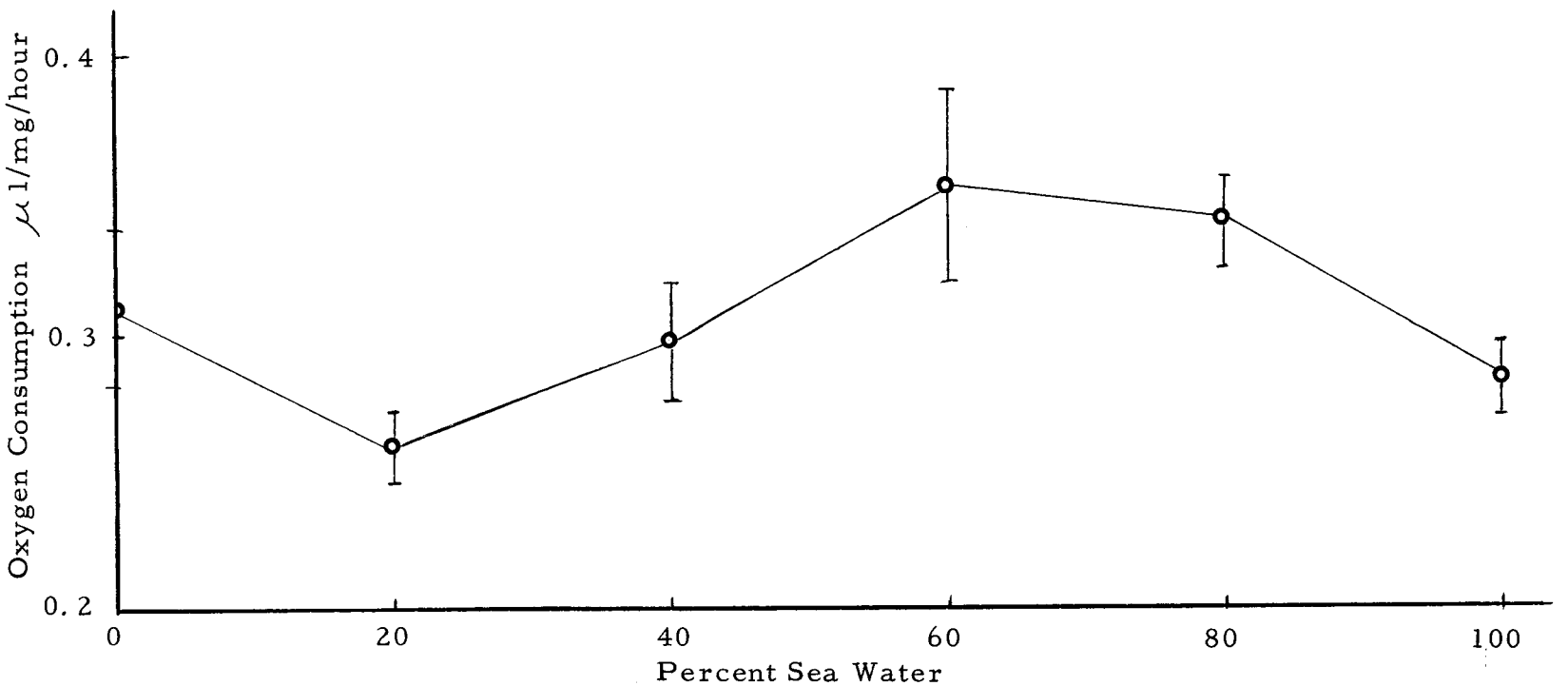

Figure 3. Respiration of crayfish gut tissue in different salinities.

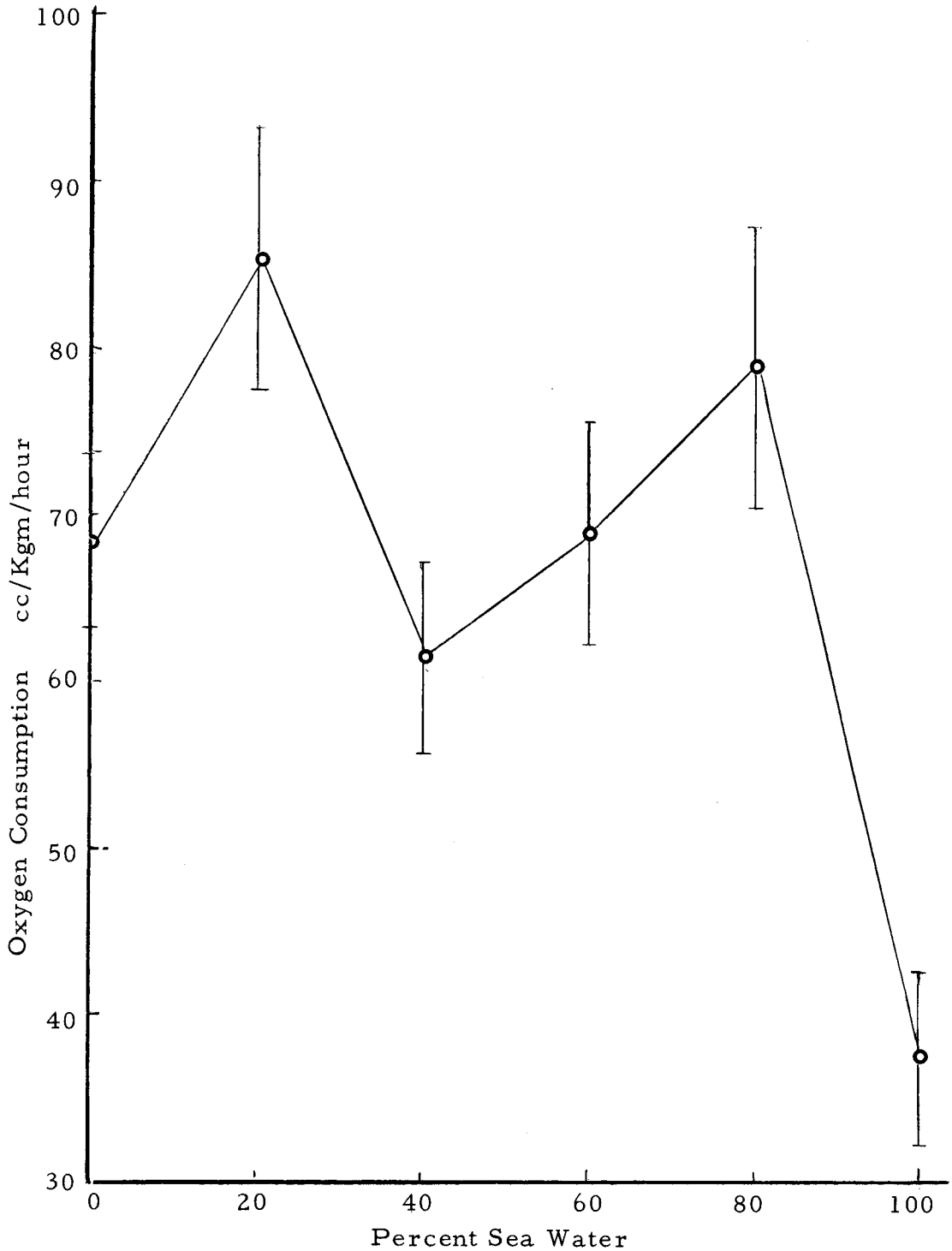

Figure 4. Metabolic rate of crayfish in different salinities.

SUMMARY

1. The respiration of the western North American crayfish, Pacifastacus leniusculus, in different salinities was studied. The animals were acclimated in 0, 20, 40, 60, 80 and 100 percent sea water and for each salinity, the metabolic rates of the whole animal and of the excised gill, green gland and gut were measured.

2. Results obtained for the whole animal indicated that changes in respiration rate in the different salinities might be a reflection of other activities stimulated by the stress, e. g. locomotory activity in attempting to escape from the medium, rather than the osmotic work alone.

3. The gut tissue showed a somewhat higher oxygen consumption at 60 and 80 percent sea water, as compared to the freshwater controls, indicating a possible function for osmoregulation in the higher salinities.

4. The oxygen consumption for both the gill and the green gland maintained at about the same level from 0 to 40 percent sea water, with a significant increase at 60 percent. In the higher salinities, there was a considerable drop in the metabolic rate of the green gland and it dropped to significantly below normal at 100 percent. The gill, however, showed a steady increase in

oxygen uptake from 60 to 100 percent sea water. The probable reasons for these changes in respiration were discussed.

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