STUDIES ON THE RESPIRATION OF THE "KURUMA" PRAWN,
Penaeus japonicus BATE.—II PRELIMINARY EXPERIMENTS
ON ITS OXYGEN CONSUMPTION

Syuzo EGUSA*

(Received May 26, 1961)

The present report of this series describes the results of preliminary experiments on the oxygen uptake of the "Kuruma" prawn.

From the practical point of view, the most important purpose of measuring the oxygen uptake rate of a given species of aquatic animals is to determine its ability to take up oxygen when under the influence of various external factors including gradations of the concentration of oxygen itself. Measurements of the oxygen uptake rate for this purpose require consideration of the two rates of oxygen uptake, the active and the resting. But, as to the Kuruma prawn, so far as the present author knows, no work has been done in order to determine these two rates.

As is known, this species of prawn has a habit of passing the daytime in a resting state buried in the sand (EGUSA, 1960)¹⁾. Fortunately it maintains this habit even when being confined in a experimental container of a relatively small size. Accordingly there can be no practical difficulty in determining the resting rate. Not a few crustaceans have the same habit. But, so far is known, no attempt seems to have been made to measure their oxygen uptake during burrowing, although there is an instance where the oxygen uptake of the sand crab was measured under the conditions of its being artificially covered with sand (EDWARDS & IRVING, 1943)²⁾.

On the contrary, the active rate is not so easy to determine. The disadvantage lies in the fact that it is difficult to keep prawns in a continuously active condition. The author attempted to make prawns swim or crawl at a maximum steady rate by placing them in a rotating respiration chamber, as was often attempted in the case with fish (e. g. FRY, 1957; BASU, 1959)^{3, 4)}. They did not necessarily move at a constant rate against the current of water. Occasionally they leaped violently and sometimes crouched or failed to breast the current.

Fortunately, however, it was found that prawns which were placed in a chamber without sand sufficient to permit them to hide and subjected to appropriate stimulation remained active for some considerable period of time. In the present investigation the active rate was determined under these conditions.

The investigation was carried out in September and October 1960 at the Fisheries Laboratory of the University of Tokyo located at Ikawazu, Aichi Prefecture.

* Faculty of Agriculture, the University of Tokyo. (江草周三, 東京大学農学部水産学教室)

Materials

Young prawns, regardless of sex, of weights ranging from 3 to 18 gm. were used in the experiments. They were obtained from the shoals near the Fisheries Laboratory. They were held in a retaining tank with sand on the bottom for from one to two weeks, and were fed fish and bivalve flesh every night except the night before experimentation. The temperature and chlorinity of the tank water were varying between 22 and 24°C, and between 15 and 16‰, respectively, during the period prawns were being taken for experiments.

As was mentioned in a previous report¹⁾, the Kuruma prawn hardly emerges out from the sand under the normal internal and external conditions in the daytime. Unhealthy individuals and those just before or after moulting, however, tend to remain out of the sand even by day. So care was taken not to use these "exposing" animals.

Method

Figure 1 shows diagrammatically the apparatus used for the experiment. The respiration chamber was a round glass basin about 30 cm. in diameter and 15 cm. in depth, and was provided with a removable glass cover having a stirrer. In case of determining the resting rate the bottom of the chamber was covered with approximately 8 cm. of thoroughly washed sand. Its particle-size distribution is all the same as used in the previous experiments¹⁾. While in case of measuring the active rate the bottom was covered with the same sand to a depth of only a few millimeters. Prawns placed in the chamber thus prepared frequently fan the sand backwards vigorously with their pleopods in an attempt to burrow into the sand, in addition to

Fig. 1. Apparatus for determining the oxygen uptake rate of *Penaeus japonicus*.

crawling, swimming and jumping. Moreover, the animals were stimulated by tapping the wall of the respiration chamber periodically in the course of the experiment.

The oxygen uptake rate was determined according to the following procedure. Samples of prawns were placed in the respiration chamber through which was passed aerated sea-water. The oxygen content of the inflowing and outflowing water was determined at intervals of from 20 to 30 minutes. The rate of water flow through the chamber was measured at the time when each water sample was taken. In all experiments the flow rate was adjusted so that the oxygen concentration of the effluent water might be maintained at more than 3.8 cc./l (about 70% air saturation). Experiments were carried out with from three to thirteen individuals in the chamber at the same time (number varying according to the size of animals used), an effort being made to select prawns of similar size for any one experiment. At the end of each experiment the animals were weighed.

An attempt was also made to determine the relation between oxygen uptake and external oxygen content. The procedure was much the same as mentioned above, except that the rate of water flow through the chamber was restricted in some degree. As a result of the reduction of the flow rate the oxygen concentration of water in the chamber decreased gradually with the passage of time due to the oxygen-depleting effects of the prawns' respiration.

In all experiments the water in the chamber was gently agitated throughout the experiment so that stratification of the gas content in it might be broken up. The oxygen content of water was determined by the unmodified WINKLER method, since periodic tests for nitrite in the water proved negative.

The oxygen consumption of the sand and sea-water used was measured after each actual experiment. Although they were thought to have been contaminated in some degree by experimental animals, their oxygen consumption was usually as low as less than 1.0% of that of the animals themselves. This value was deduced from the total oxygen consumption of the animals, sand and sea-water in order to give the true oxygen consumption of the animals.

Experiments were all performed at temperatures of 22-23°C and at chlorinities of 15-16‰. In each of the experiments the difference in water temperature between the retaining tank at the time when animals were taken for experiment and the respiration chamber at the beginning of experiment was under half a degree centigrade, and that in chlorinity was under 0.1‰.

Results

1) Resting rate of oxygen uptake

It is well established in the study of the respiration of fish that handling of fish required to place them in the respiration chamber raises their oxygen consumption well above the resting level, and that the excited condition may last for some time

after they are not visibly active. Therefore the measurement of standard rate of oxygen uptake requires a delay after the introduction of the fish into the respiration chamber before measurements begin (e. g. KEYS, 1930)⁵⁾. The same may be said of prawns, but experimental corroboration is lacking. To secure information on this problem measurement was started from about half an hour after the transfer of animals to the respiration chamber in the present experiment.

Representative results obtained for prawns of four different size groups are given in Fig. 2(A). (For the size of prawns used and experimental conditions, see Table 1) As will be seen from this figure, in every case the first hours after the introduction of prawns into the respiration chamber showed a high initial rate of oxygen uptake which gradually subsided. The rate of oxygen uptake did not approach a relatively constant value until about three hours after introduction. The author wishes to make clear that in all these cases all of the prawns introduced had buried themselves in the sand within a few minutes after introduction and remained buried afterwards. So the elevated initial rate of oxygen uptake seems to be partly due to the payment of an oxygen debt contracted by the prawns in their digging movements. In addition, handling of prawns must have been sufficient stimulus to cause them to consume oxygen at higher rates for some time afterwards. On the basis of these findings a steady level of oxygen uptake attained about three hours after the introduction of prawns was taken as the resting rate in this investigation. Practically the mean of several actual values of oxygen uptake in a steady phase was determined. The resting rates thus obtained for prawns of different sizes are summarized in the column headed "resting rate" of Table 1, from which it will be seen that the resting rate tends to decrease as the weight of the animals increases.

Table 1. Oxygen uptake rates of *Penaeus japonicus*.

Body weight gm.		Number of prawns used	Average temp °C	Average Cl. %	O ₂ uptake cc./kg./hr.		Approx. O ₂ level cc./l
Average	Range				Resting rate	Active rate	
3.1	2.4— 3.7	13	23.0	15.8	135	—	4.3
			22.8	15.8	—	438	3.8
5.5	4.6— 6.2	8	22.8	15.9	117	—	4.5
			22.7	15.7	—	423	3.8
11.8	11.4—12.6	5	23.0	15.7	106	—	4.2
			22.6	15.5	—	260	4.5
16.1	14.8—18.2	3	22.9	15.8	77	—	4.7
			22.6	15.6	—	312	4.2

Fig. 2. Serial determinations of the resting and active rates of oxygen uptake for *Penaeus japonicus* of different sizes. Average weights of prawns are indicated by figures in body of graph.

2) Active rate of oxygen uptake

Fig. 2(B) shows the results of determinations of active rate on the same groups of prawns as before. (For experimental conditions, see Table 1) On completion of the measurement of the resting rate the prawns of each group were transferred to their respective retaining tank. On the following day determinations of their active rate were carried out.

It is seen from the figure that the active rate changes with time, too. In the present investigation the highest point on each plot was taken as the active rate. The values obtained are set down in the column headed "active rate" of Table 1. The table shows that the active rate is usually more than three times as large as the resting rate and that it has a tendency to decrease with increase in size as in the case of the resting rate.

The above mentioned changes in oxygen uptake with time seemed to reflect changes in physical activity of the animals. In an experiment on animals 16.1 gm. in average weight, for instance, all of the animals which were active at first became extremely quiet after about one and a half hours, showing only a temporary activity when stimulated. In an experiment on animals weighing an average of 11.8 gm. only two of the five animals exhibited more or less continuous activity, though their visible activity tended to decrease with time possibly owing to fatigue. The residue remained inactive throughout the experiment. The highest activity usually occurred in the

early parts of the experiment.

3) Resting rate of oxygen uptake in relation to oxygen content

The results of experiments on prawns of three different sizes are shown in Fig. 3(A), where the oxygen uptake rates obtained are plotted against the appropriate oxygen concentrations. Data on the size of prawns used and experimental conditions are summarized as follows:

Number of prawns used	Body weight gm. Average (range)	Average temperature °C	Average chlorinity ‰
12	3.5 ₂ (3.2-4.0)	23.1	15.8
9	5.4 (4.8-5.9)	23.0	15.7
7	13.6 (11.7-14.8)	22.6	15.7

In these experiments, measurement was started about two hours after prawns were placed in the respiration chamber. During this preliminary period, which seemed to serve to cover the stage of the initial high oxygen demand, the chamber was provided with a sufficient flow of well aerated sea-water.

Fig. 3. Effect of the oxygen concentration of the medium on the resting and active rates of oxygen uptake in *Penaeus japonicus* of different sizes. Average weights of prawns are indicated by figures in body of graph.

As is clearly shown in the figure, the resting rate changed with oxygen content. It is worth noticing that the course of the relationship between oxygen uptake and oxygen content differs with the size of animals. The oxygen uptake rate of medium-

sized prawns (av. wt., 5.4 gm.) showed comparatively high values at oxygen concentrations about 3 cc./l. These high rates seem to be due to the initial high oxygen demand. Below this concentration the oxygen uptake rate remained relatively constant until the oxygen content was reduced to 1.5 cc./l. Below this level the rate rose rapidly with decrease in oxygen content, reaching a peak at about 1 cc./l. A further decrease in oxygen content was accompanied with a sudden fall in oxygen uptake. In small prawns (av. wt., 3.5 gm.) no steady phase in oxygen uptake was seen. At an oxygen concentration about 3 cc./l the rate began to rise already. It passed through a maximum when oxygen content was about 1 cc./l and finally fell off, as in the case of the medium-sized prawns. While in large prawns (av. wt., 13.6 gm.) there was found no elevation in oxygen uptake at low oxygen concentrations. The rate of oxygen uptake decreased gradually and linearly with decrease in oxygen content over the whole range tested, though it seemed to maintain a relatively constant level at oxygen concentrations from 2.5 to 1.5 cc./l.

The increase in oxygen uptake accompanying decrease in external amount of oxygen seems to reflect increase in cost of ventilation. Changes in the condition of prawns accompanying decrease in oxygen content were also observed and recorded in these experiments. The results were shown in Fig. 4, together with changes in oxygen uptake in the same way as was adopted in a previous report.¹⁾ It will be seen from this figure that in both the small and the middle-sized prawns there is a simul-

Fig. 4. Relationship between oxygen concentration, resting rate of oxygen uptake and animals' condition in *Penaeus japonicus* of different sizes. From the same experiments illustrated in Fig. 3(A). A, large prawns (av. wt., 13.6 gm.); B, middle-sized prawns (av. wt., 5.4 gm.); C, small prawns (av. wt., 3.5 gm.). Letters, A-F, denote the conditions of prawns (for explanation see a previous report¹⁾).

taneity between the beginning of the rise of oxygen uptake and that of the appearance of prawns on the surface of the sand. The large prawns seem not to differ significantly in their action from the others. Nevertheless, in these animals increased activity was accompanied by no elevation in oxygen uptake. The reason for this is not yet ascertained.

In the experiment on the small prawns an interesting fact was noticed by chance. One of the twelve prawns used began to emerge from the sand much earlier than the others. When the oxygen concentration of the medium was reduced to 3.5 cc./l, it had already protruded its respiratory siphon on the surface of the sand. When the oxygen content reached to 1.2 cc./l, it crept out of the sand. About half an hour later it died, although all the others still remained completely or partly buried in the sand. The post-mortem examination revealed that the prawn had been just in the stage of ecdysis. These observations seem to suggest that prawns in the stage of proecdysis or ecdysis are more sensitive and less resistant to low oxygen than those in the intermoult stage. It has been shown by BLISS (1953)⁹⁾ that in proecdysis oxygen consumption increases. As was mentioned above the oxygen uptake rate began to rise at as high an oxygen concentration as 3.5 cc./l in this experiment. This phenomenon may possibly have been related to the existence of the animal in question.

4) Active rate of oxygen uptake in relation to oxygen content

Experiments were conducted on the same three groups as before. The size of prawns used and experimental conditions were as follows:

Number of prawns used	Body weight gm. Average (range)	Average temperature °C	Average chlorinity ‰
11	3.5 ₄ (3.2-4.0)	22.9	15.6
9	5.4 (4.8-5.9)	22.6	15.4
7	13.6 (11.7-14.8)	22.7	15.8

The small prawns had increased in their average weight only a little (from 3.5₂ to 3.5₄ gm.), as one prawn had died during the preceding experiment as was mentioned above. The results are given in Fig. 3(B). As may readily be seen from the figure, in every case the active rate is dependent on oxygen concentration over the whole range tested. It seems likely that prawns still exhibit respiratory dependence at air saturation. The active rate of each of the small, middle and large prawns at the level of air saturation would be estimated by extrapolation to be 520, 510, and 370 cc./kg./hr., respectively.

5) Critical oxygen concentration

Various crustaceans are reported to have critical oxygen concentrations far below the air saturation level. But in some others the critical concentration is known to appear near or above it. There are even reports that no critical oxygen concentration is found in some species (cf. WOLVEKAMP & WATERMAN, 1960)⁷⁾. In regard to the

Fig. 5. Relation between resting and active rates of oxygen uptake in *Penaeus japonicus* of different sizes at different environmental oxygen concentrations. Figures in body of graph show average weights of prawns.

critical oxygen concentration in crustaceans WOLFEKAMP and WATERMAN state that the oxygen consumption in Crustacea is constant at high oxygen levels down to a P_c (critical pressure) characteristic of the specimens and conditions of experiment; below this pressure a rapid linear fall proportional to external amounts of oxygen occurs to very low levels.

The present experiment strongly suggests that the relation between oxygen supply and uptake in this species changes depending on the state or activity of animals: that is, critical oxygen concentrations appear near the air saturation level when the animals are active and near 1.0 cc. O_2/l when they are in a resting state.

Problems of this sort have been studied in detail with fish. As a result, the following view receives general acceptance nowadays: there exist two kinds of critical oxygen concentrations for fish; the incipient limiting level and the incipient lethal level³⁾. The relationships were shown intelligibly in a graphic form by SHEPARD (1955).⁸⁾

Fig. 5 is a combination of Fig. 3(A) and (B). The general shape of the curves seems to resemble to that given by SHEPARD. From the figure it may be said that the incipient limiting levels for this prawn seem to lie in the neighborhood of the air saturation level and the incipient lethal levels are found between 1.0 and 0.5 cc./l.

Summary

1) The oxygen uptake rate of *Penaeus japonicus* BATE was studied by a flowing water method both in the case in which prawns lay hidden motionlessly in the sand, and in the case in which they were prevented from burrowing, thus being in an excited, active state. Animals used ranged in body weight from 3 to 18 gm. All experiments were performed at about 23°C.

2) Immediately after burrowing into the sand prawns showed a comparatively high rate of oxygen uptake, which gradually subsided, and reached a steady resting rate after about 3 hours. The resting oxygen uptake rates per unit weight varied with the size of animals, ranging from 77 to 135 cc./kg./hr. Increase in size was accompanied by a decrease in resting rate.

3) The following relationship was observed between external oxygen content and resting oxygen uptake rate, except in the case of large prawns. Above a certain oxygen level the resting rate is independent of the oxygen content. Below this level the rate gradually increases with decrease in oxygen content, and reaches a maximum at a certain low oxygen level, below which the rate falls rapidly.

4) The active rate of oxygen uptake is more than three times as large as the resting rate. The active rate also tended to drop with increase in size.

5) The active rate is dependent on the external oxygen content over a wide range from near air saturation down to 1 cc./l.

6) From a combination of the resting and active rates of oxygen uptake it seems that the so-called incipient lethal level for this prawn lies between 1.0 and 0.5 cc./l.

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