OXYGEN REQUIREMENTS OF FRY OF THE INDIAN MAJOR CARP, *LABEO ROHITA* (HAMILTON)

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(Communicated by B. S. Bhimachar, F.N.I.)

(*Received April 4; read October 7, 1957*)

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INTRODUCTION

In view of a general awakening to the culture of fish in various parts of the country, Central Inland Fisheries Research Station organised a Fish Seed Syndicate at Calcutta in 1952 for supply of fry and fingerlings of Indian major carps to deficit States for piscicultural purposes. But, for successful execution of any large scale programme of fish culture, it is essential to evolve improved methods of transport of fry over long distances. It was therefore considered necessary to obtain data on oxygen requirements of fry which would be of basic importance in devising such methods. The present paper communicates the results of experiments relating to oxygen requirements of fry of *Labeo rohita* (Hamilton) under laboratory conditions.

MATERIAL AND METHODS

Fry of *L. rohita*, ranging in total length between 22 and 46 mm, were supplied by the Syndicate from their nurseries which were being maintained along scientific lines. On arrival in the laboratory the fry were immediately placed, 500 to a container, in large, hardened earthen containers of 15-gallon capacity and acclimatised to the chlorine-free corporation tap water of *p*H 8.0—8.2. The dissolved oxygen of water ranged between 6.0 and 6.4 p.p.m. and temperature between 27.0 and 30°.0C. Free chlorine from the water was eliminated by vigoroulsy agitating it over a period of several hours. Tap water subjected to this treatment and kept overnight was used in all the experiments described here. Planktonic organisms (Diatoms, Desmids, Rotifers, Copepods etc.) in proper quantities were given to the fry once in 48 hours. The feeding times were so regulated, that at least 24 hours lapsed between them and the time when the fry were used in experiments. Only normal healthy specimens were selected from the stock and no fish kept in the containers for more than six days were used in these experiments.

Oxygen was determined by the basic unmodified Winkler's method as recommended by American Public Health Association (1946).

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EXPERIMENTS

(*i*) *One-litre sealed bottles*

Two series, each having twelve 1-litre reagent glass bottles, were filled with water of known oxygen content. A fixed number of fry, all of about the same size, was introduced in each bottle and the bottle sealed immediately. The bottles in one series only were covered with thick opaque paper to shut off penetration of light. Two bottles, one each from the two series, were unsealed at 20-minute intervals and samples of water collected for determination of dissolved oxygen. The test fry from each bottle were removed immediately, fixed in 5 per cent formaldehyde, dried on a blotting paper and weighed to the nearest milligram. The experiments in these series were repeated four times. The results of the four series of experiments are summarised in Table I.

TABLE I

Summarised results of the four series of experiments in closed bottles

Serial number of bottle	Duration of experiment in minutes	Oxygen consumed (in mgm) by 100 gm. of *L. rohita* fry per hour							
		A – Covered series				B – Exposed series			
		Exp. I-A	Exp. II-A	Exp. III-A	Exp. IV-A	Exp. I-B	Exp. II-B	Exp. III-B	Exp. IV-B
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	20	69.06	64.23	68.17	69.61	65.76	65.90	68.69	70.52
2	40	66.42	69.79	69.01	69.32	68.16	68.17	66.69	71.12
3	60	70.26	66.36	67.03	69.09	64.29	68.92	70.61	66.92
4	80	64.68	61.82	63.42	66.91	69.36	69.64	60.78	56.10
5	100	68.16	65.56	65.16	67.51	62.82	58.04	65.57	65.11
6	120	60.39	59.85	67.45	65.40	67.98	64.86	64.11	62.71
7	140	55.38	60.36	59.26	60.60	64.02	56.83	62.91	67.55
8	160	52.95	54.77	59.43	54.31	61.56	59.29	58.29	60.09
9	180	55.53	53.16	52.92	51.60	64.77	64.74	61.26	64.51
10	200	51.37	51.62	54.26	50.40	68.34	62.54	56.84	66.91
11	220	50.43	48.75	51.11	52.51	54.81	59.19	61.89	–
12	240	51.06	52.26	51.47	52.20	59.28	63.48	58.68	–
Temperature °C	C	29.60	28.20	28.40	28.80	29.60	28.10	28.20	28.80
	T	30.50	29.00	30.20	29.40	30.60	29.30	29.80	29.80
D.O. of the medium (p.p.m.)	C	6.70	6.75	6.58	6.72	6.68	6.75	6.58	6.72
	T	2.32	2.41	2.09	1.98	1.93	1.89	1.82	1.98
Size range of fry (mm.)		17–30	17–30	17–30	16–31	17–30	17–30	17–30	17–30
Weight range in fry in each bottle (gm.)		1.89–3.12	1.97–2.72	1.78–3.15	1.69–3.25	1.81–2.35	1.78–3.05	1.73–2.98	1.60–2.79

C = At the commencement of experiments.
T = At the termination of experiments.

(ii) Continuous-flow system

The apparatus used in this series was similar in design and operation to that of Keys (1930) except that in the present apparatus five respiratory chambers instead of two were provided and were all properly covered with thick opaque paper. The flow of water in the chambers was maintained at a rate, which was considered slow enough to enable measurement of the amount of oxygen removed by test fry and also sufficient to maintain a supply of fresh water in the chambers, so that diminished oxygen would not enter as a limiting factor. The fry remained in the chambers for full two hours before observations were taken. Once the observations were commenced, water from the respiratory and control chambers was allowed to pass into collecting jars for exactly 20 minutes and then by-passed to the sink. Known volume of water from all the collecting jars was taken for determination of dissolved oxygen and the balance drained off and measured. The by-pass lines were then shut off and the whole procedure begun again, this being repeated from 8 to 10 times over a period of about 10 hours. At the conclusion of the last observation, fry were removed from each chamber, fixed in 5 per cent formaldehyde and weighed. A preliminary check on *p*H and CO_2 of water, entering and leaving the respiratory chambers, showed no significant change. Temperature of water during the experiments ranged between 28°.2 and 30°.4C.

Results of twenty-one sets of experiments are summarised in Table II. In each set, at least 8-10 observations each of 20 minutes' duration were made over a period of at least 10 hours. It can be seen clearly that the values of respiration rate, obtained in 8-10 observations, do not show any significant difference and therefore the mean of all the values in a particular experiment has been calculated to show the average respiration rate for 20-minute period.

Discussion

The method of measuring respiration rates in sealed bottles has been criticized specially when the duration of experiments is rather long, because of the possible accumulation of CO_2 and other katabolic products in the experimental bottles. The other serious defect in this type of experiment is the progressive depletion of oxygen in the water and consequent reduction in the oxygen available for respiration. At the end of four hours (maximum period allowed in bottle experiments) the dissolved oxygen of the medium was never less than 1.82 p.p.m. (cf. Table I) and since Basu (1949) has stated that Indian carp fry can normally live for at least 24 hours without any apparent ill-effects in water with D.O. between 1 and 1.5 p.p.m., it would seem unlikely that the depleted oxygen in the experimental bottles would have been a limiting factor for normal respiration.

The first point of interest that emerged from "sealed bottle" experiments was that the values for the first hour or so, showed a persistent increase over subsequent values. Keys (1931) experimenting with *Fundulus parvipinnis*, observed that the maximum respiratory exchange was attained in the first two or three hours and the rate subsequently tended to be constant. According to Keys (*op. cit.*) at least part, if not all, of the explanation of the phenomenon must be that in the excitement and struggling incident to transfer to the respiratory chambers, the fishes acquire a considerable "oxygen debt". Wells (1932) and Black *et al.* (1939) among others, have also recorded that handling of fish raises their oxygen consumption well above the resting level and that the excited condition may last for some time even after the fish is not visibly active. Graham (1949) calculating the maximum active and standard respiratory rates of speckled trout (*Salvelinus fontinalis*), noted that the handling of the fish, as it was first put into a chamber, was evidently sufficient stimulus to activity and need for further agitation during the next hour

TABLE II

Results of experiments in continuous-flow system apparatus showing oxygen consumption rates of fry of Labeo rohita *(Ham.) at resting level*

Sl. No.	No. of fish per chamber	Size range mm.	Average size mm.	Combined wt. of fish per chamber (gm.)	Number of 20-minute observations	Oxygen consumed in 20-minutes (mgm.)	Mean oxygen consumed in 20-minute period (mgm.)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1.	15	23—27	25.50	1.900	8	0.319 0.318 0.321 0.325 0.320 0.332 0.340 0.327	0.325
2.	15	23—27	26.13	1.941	8	0.391 0.390 0.366 0.381 0.357 0.372 0.350 0.360	0.371
3.	5	37—42	38.80	2.442	9	0.397 0.382 0.358 0.368 0.371 0.367 0.368 0.375 0.373	0.373
4.	5	37—42	40.00	2.690	10	0.533 0.490 0.491 0.498 0.504 0.504 0.489 0.508 0.507 0.498	0.502
5.	20	23—28	25.90	2.911	10	0.444 0.502 0.424 0.478 0.469 0.490 0.508 0.467 0.490 0.462	0.473
6.	20	25—28	26.25	3.095	10	0.486 0.490 0.515 0.498 0.545 0.530 0.555 0.528 0.540 0.532	0.522
7.	5	39—46	44.00	3.100	8	0.552 0.522 0.535 0.550 0.535 0.546 0.511 0.538	0.536
8.	14	27—33	29.70	3.220	9	0.616 0.620 0.616 0.602 0.641 0.614 0.570 0.610 0.614	0.611
9.	30	23—28	25.90	4.272	8	0.796 0.789 0.726 0.776 0.758 0.746 0.790 0.796	0.772
10.	8	37—42	39.10	4.322	10	0.590 0.642 0.625 0.614 0.636 0.652 0.638 0.644 0.652 0.614	0.632

TABLE II—(*contd.*)

Sl. No.	No. of fish per chamber	Size range mm.	Average size mm.	Combined wt. of fish per chamber (gm.)	Number of 20-minute observations	Oxygen consumed in 20-minutes (mgm.)	Mean oxygen consumed in 20-minute period (mgm.)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
11.	10	35—42	38.10	4.743	10	0.825 0.846 0.808 0.836 0.860 0.842 0.795 0.820 0.830 0.806	0.827
12.	25	26—33	30.16	5.652	9	0.944 0.948 0.930 0.918 0.891 0.942 0.967 0.944 0.960	0.938
13.	40	23—28	26.10	5.940	10	0.996 0.927 0.985 0.936 0.924 0.964 0.930 0.938 0.912 0.946	0.946
14.	10	38—44	41.10	6.103	8	0.987 0.961 0.961 0.973 0.958 0.955 0.948 0.962	0.963
15.	10	23—34	26.30	6.661	10	1.012 1.146 1.138 1.118 1.065 1.158 1.214 1.162 1.100 1.146	1.126
16.	15	36—42	38.60	6.930	10	1.128 1.248 1.245 1.234 1.172 1.241 1.203 1.250 1.200 1.225	1.215
17.	60	22—29	25.43	8.090	9	1.462 1.430 1.494 1.448 1.516 1.536 1.530 1.503 1.518	1.493
18.	20	34—40	36.60	8.402	9	1.522 1.575 1.590 1.558 1.590 1.582 1.540 1.536 1.532	1.558
19.	60	24—30	26.05	8.500	10	1.466 1.451 1.439 1.492 1.470 1.478 1.456 1.495 1.500 1.465	1.471
20.	20	36—45	41.10	11.530	10	1.915 1.935 1.887 1.905 1.935 1.896 1.787 1.890 1.933 1.924	1.910
21.	20	37—46	40.70	11.942	10	1.887 1.998 1.827 1.872 1.899 1.868 1.816 1.896 1.814 1.906	1.878

or so, which was otherwise necessary to obtain the maximum active respiratory rate, was not felt.

It is thus obvious from the results obtained in "sealed bottle" experiments that the values of at least the first three to five observations represent maximum active respiratory rate. The subsequent values are lower and although on the whole steady, these cannot possibly be representative of respiratory rate at resting stage of fish because these values are computed averages of all the observations made in a particular series and therefore include the values representative of maximum active metabolism also. By prolonging the duration of experiments in sealed bottles, it would be perhaps possible to obtain values of respiratory rate at resting stage, but in that case the possibility of progressive depletion of oxygen as a limiting factor for normal respiration, will have to be taken into consideration. Thus the "bottle" experiments, at best, can only give an idea of active respiratory rates.

The other point of interest was that the values on respiratory rates obtained in the "uncovered" series of "bottle" experiments, although rather inconsistent (Table I, panel B.), were nevertheless comparable to those obtained in the initial stages of experiments in "covered" series which represent active respiratory rate. As has already been stated, the active respiratory rate is attributable to the handling of fish and since subsequent values in the "uncovered" series were also comparable to initial values, it follows that in these series the fry were in a constant state of excitement even to the end of experiments. The state of excitement evinced by fry in "uncovered" series may perhaps be ascribed to very frequent and irregular variation in the intensity of light in the experimental bottles incident to constant and continuous movement of workers in the room.

In the experiments conducted in continuous-flow system, the test fry were therefore allowed to remain in the respiratory chambers for a period of two hours before the observations were taken. It may also be noted that all the respiratory chambers were properly covered with thick opaque paper so that external movement could not act as a stimulus. It is therefore reasonable to assume that the values obtained in these experiments represent respiratory rate at resting stage of fry. Since the difference in the respiratory rates (on the basis of 20-minute observations) among 8-10 observations made in a particular series, was found to be insignificant (cf. Table II) it would appear that the oxygen consumption of *L. rohita* is, perhaps a constant function under controlled laboratory conditions, at least within the duration of present experiments. Glausen (1936) experimenting with fresh water fishes, however, concluded that the rate of oxygen consumption varies from hour to hour in the same fish and shows differences between individuals of the same species.

The respiratory chambers provided in the continuous-flow system were of the same volume (one litre) and the number of individuals in each chamber varied from 5 to 60 (cf. Table II) so that the volume of water available to an individual was not always the same. It is therefore natural to expect that grouping of fish would, perhaps, depress the respiratory rate (Schuett, 1933; Schlaifer, 1939). But the results seem to show that the respiratory rate is dependent only on the mass of fishes irrespective of the number of fry in a chamber. Consequently the volume of space available to an individual fish in a chamber cannot be considered a limiting factor for normal respiration, at least within the size range and the number of individuals used in these experiments.

The results of twenty one sets of experiments summarised in Table II, show that the oxygen consumption rate of fry of *L. rohita* increases with increasing weight. It has therefore been possible to reduce the data to a mathematical formula as follows :

$$\log O_2 = 2.24764 + 0.9667676 \log W/10^8 \quad \ldots \text{(i)}$$

where O_2 = oxygen consumed (mgm.) in 20 minutes at resting stage of respiration, and

W = combined weight of fry (gm.) in a respiratory chamber.

To test whether the difference between observed and theoretical values, as calculated from formula (i), is significant or not, χ^2 ("chi square") test was made. The value of χ^2 being 0.516 with 20 d.f. (Table III) it follows that the fitted curve represents the observed data. The small value of χ^2 may presumably be attributable to the counter balance of the positive and negative errors with each other.

TABLE III

Comparison of observed and calculated values of oxygen consumed by L. rohita (*Ham.*) *fry in 20 minutes*

($\log O_2 = 2.24764 + 0.9667676 \log W/10^8$)

Exp. No.	Weight of test fish (gm.)	Observed values of O_2 consumed in 20-minutes (mgm.)	Calculated values of O_2 consumed in 20 minutes (mgm.)	P.C. difference*	χ^2 test
(1)	(2)	(3)	(4)	(5)	(6)
1.	1.900	0.325	0.329	+ 1.230	
2.	1.941	0.371	0.336	− 9.434	
3.	2.442	0.373	0.419	+12.332	
4.	2.690	0.502	0.460	− 8.367	
5.	2.911	0.473	0.497	+ 5.074	
6.	3.095	0.522	0.527	+ 0.958	
7.	3.100	0.536	0.528	− 1.487	
8.	3.221	0.611	0.548	−10.311	
9.	4.272	0.772	0.720	− 6.736	
10.	4.322	0.632	0.728	+15.190	
11.	4.743	0.827	0.797	− 3.628	$\chi^2 = 0.516$
12.	5.652	0.938	0.944	+ 0.640	
13.	5.940	0.946	0.990	+ 4.640	
14.	6.103	0.963	1.016	+ 5.504	
15.	6.661	1.126	1.106	− 1.776	
16.	6.900	1.215	1.149	− 5.433	
17.	8.090	1.493	1.335	−10.582	
18.	8.402	1.558	1.385	−11.104	
19.	8.500	1.471	1.400	− 4.827	
20.	11.530	1.910	1.880	− 1.571	
21.	11.942	1.878	1.945	+ 3.567	

*Difference between calculated and observed values expressed as percentage of the latter.

For practical utility of the results, it was considered necessary to study length-weight relationship of fry of *L. rohita*, since the trade actually engaged in transport of fry in oxygen containers, understands better, if the number and size of fry to be transported in a container are given, rather than their combined weight. For this purpose over 300 fry of *L. rohita*, ranging in size between 21 and 45 mm. in total length, were examined. The fry for this study came from the nursery tanks

which provided material for the experiments described above. The correlation between length and weight is found to be of high magnitude with the value of r being 0.96368. The general relationship has been calculated from the formula $W=CL^n$, C and n being determined by the method of least squares. The relationship is expressed by :

$$W = 0.205385 \times 10^{-5} \times L^{3.432022} \quad \ldots \text{(ii)}$$

In the case of young and adult of *L. rohita* from ponds, Jhingran (1952) found the relationship to be

$$W = 1.554 \times 10^{-5} \times L^{3.0140028}$$

On the basis of the two equations (i) and (ii) above the oxygen requirements per hour of 1000 fry of different size ranges at the resting stage and at the active stage of respiration has been calculated and given in Table IV.

TABLE IV

Oxygen consumption per hour by 1,000 fry of L. rohita *(Ham.) of different size ranges at resting and active levels of respiration*

Length of fry : (mm.)	Weight of 1000 fry (gm.)	Oxygen consumption per hour by 1000 fry at			
		Resting level		Active level	
		mgm.	litre at 35°C and normal pressure	mgm.	litre at 35°C and normal pressure
(1)	(2)	(3)	(4)	(5)	(6)
20	60	27.31	0.0191	40.57	0.0284
25	123	55.99	0.0391	83.18	0.0582
30	240	109.25	0.0765	162.31	0.1136
35	398	181.17	0.1268	269.16	0.1884
40	645	293.60	0.2055	437.21	0.3060

For calculation of active respiratory rate, averages of all the values obtained in the first 60 minutes in "covered" series of bottle experiments, were taken into consideration.

ACKNOWLEDGEMENTS

The authors like to express their sincere thanks to the Fish Seed Syndicate for providing material for this study and to Messrs. S. J. Karamchandani and P. Roy for their assistance in the laboratory. Mr. K. H. Sujansinghani was mainly responsible for the statistical aspect of this work. They are indebted to Dr. H. Srinivasa Rao, F.N.I., for his encouragement and helpful criticism during the progress of work and to Dr. B. S. Bhimachar, F.N.I., for going through the manuscript critically.

Summary

Oxygen consumption rates of fry of *Labeo rohita* (Ham), ranging in sizes from 22 to 46 mm., at resting and active stages of respiration have been studied. It was observed that oxygen consumption rate appeared to be dependent on the total mass of test fry rather than their number, suggesting that "grouping" effect on the respiratory rate was not apparent. The data have been reduced to a mathematical equation : $\log O_2 = 2.24764 + 0.9667676 \log W/10^3$ (where O_2 is the oxygen consumed in mgm. at resting level in 20 minutes and W is the combined weight of fry in grams). For practical utility of these results, length-weight studies relating to fry of *L. rohita* were made by examining over 300 specimens. The relationship is expressed as : $W = 0.205385 \times 10^{-5} \times L^{3 \cdot 432022}$. On the basis of the two equations, a table has been prepared for guidance of the trade engaged in transport of fry, which depicts the oxygen requirements (in volume) at resting and active stages of respiration of 1000 fry of sizes between 20 mm. and 40 mm. at normal pressure and 35°C.

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Issued November 24, 1957.