THE RELATION OF RESPIRATION OF FISHES TO ENVIRONMENT. XII. CARBON DIOXIDE TENSION AS A FACTOR IN VARIOUS PHYSIOLOGICAL RESPIRATORY RESPONSES IN CERTAIN FRESH WATER FISHES

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I. INTRODUCTION

Many results obtained through laboratory experimentation on fishes are seemingly paradoxical to field observations on the same and other species of fishes. It was with a desire to clear up this apparent paradox that the experiments here described were undertaken at the Franz Theodore Stone Laboratory during the summers of 1935 and 1936. The experiments were to a certain extent repetitions of various experiments described by the senior author during the past few years. More recently developed apparatus and technique were employed.

Since the time of the publication of the paper, *An experimental study of the movements of herring and other marine fishes*, by Shelford and Powers (1915) there has developed a better understanding of the problems involved. There are still problems not yet appreciated (Shelford, 1934). It is not the observations of man that are at fault but his interpretation of them.

II. MATERIALS AND METHODS

One to three or more fishes, generally three, the number depending upon the size, were placed in one of the ten-gallon glass bottles made large mouthed by sawing off the necks and made to fit a number 15 rubber stopper. The bottles were filled with water dipped up out of the lake. Various carbon dioxide tensions were produced in the experimental water by either adding various amounts of normal sodium hydroxide or various amounts of gaseous carbon dioxide. These methods were in a way crude. It takes large expenditures and time to develop elaborate apparatus. This, however, was not necessary since the apparatus at hand and the methods used were satisfactory for the purpose of these experiments. The lowering of the carbon dioxide tension of the water by adding sodium hydroxide is never entirely satisfactory. Additional difficulties were met with, due to the nature of substances in solution in the lake water. Perhaps more serious difficulties would have been encountered had any other method been

used to reduce the carbon dioxide tension of the water. This will be discussed in the body of the paper.

All the different kinds of observations were made as far as possible on each individual fish and with each experimental water.

The pH of the water was determined colorimetrically and the pH of the blood was determined by a quinhydrone electrode the first summer (1935). These blood pH determinations are not included in Table 1 and Figure 1, as explained later. The second summer (1936) the Beckman pH meter was used to determine the pH of the water, the blood, and the serum. A modified Van Slyke Manometric Blood Gas Analysis Apparatus was used to determine the carbon dioxide volume per cent of the experimental water. The modified Winkler Method (Kemmerer, Boyard and Boorman, 1923, and Amer. Pub. Health Assoc., 1933) was used in determining the oxygen content of the water. The method reported by Birge and Juday (1911) was employed to determine the water alkalinity except pH 4.2 was taken as the end point with brom cresol green as an indicator.

III. Experimental Data and Discussion

1. The carbon dioxide tension of the experimental water

It has been shown by Powers (1927, 1930) and Powers and Bond (1927, 1928) that the carbon dioxide tension of natural waters can be determined by the equation $(-)pH = -ne - nlogP$ where pH = pH of the water at a particular carbon dioxide tension P and n and e are constants that must be determined for each natural water. They must also be determined for each modification of a natural water, providing the modification is by the addition or removal of alkali in solution in the water. If ne and n were constant for all water the carbon dioxide tension of the water could be determined directly by finding the difference between the pH of the water unaerated and aerated. If the pH of the water is lowered when aerated, the carbon dioxide tension of the water is less than the carbon dioxide partial pressure of the atmosphere. When the aerated water pH is greater than the unaerated water pH the carbon dioxide tension of the water is greater than the partial pressure of the atmosphere. When the two pH's are equal the carbon dioxide tension of the water is the same as the carbon dioxide partial pressure of the atmosphere. Theoretically, the pH of the aerated lake water should always be the same except for variations in temperature. In that case the carbon dioxide tension of the water below or above the carbon dioxide partial pressure of the atmosphere could be measured by the pH units above or below the pH reading of aerated water (see columns marked pH Difference in Tables 1 to 3). This could not be held to for two reasons. First, the addition of sodium hydroxide to the water naturally increases the alkalinity of the water, and thus the pH reading of the aerated modified water. This increased pH effect was to a certain

TABLE 1. *The Rock Bass, Ambloplites rupestris (Rafinesque). The data given for any one experiment were obtained from the same fish. Several observations were made on individual fish rather than attempt to repeat conditions on different fish for different observations. This also applies to the data recorded in Tables 2 and 3. All data are arranged in order of the pH of the water at the end of the experiment.*

Expt. No.	Time in Hrs. of Expt.	Total Weight in grs. and Number of Fish	pH of Experimental Water			Alkali Reserve of Experimental Water		CO_2 in Molar Concentration in terms of NaOH or CO_2 Added to Water	Total CO_2 Vol. % at End	O_2 in ml. per L. in Water at End	O_2 Consumed in ml. per Kilo per Hr.	Swim Bladder Gas of Fish at End of Expt.		pH of Blood at End	pH of Serum			Hemoglobin in Blood grs. %
			at End	Aerated at End	Difference	Before Adding NaOH or CO_2	At End of Expt.					% O_2	% CO_2		pH at End Unaerated	pH at End Aerated	pH Difference	
45	.75	224.9 (4)	11.68	11.31	−.37	39.40	97.27	−2.40x 10−3	3.21	4.74 (4.76) *	5.77	7.39	2.34	.....	.....	.....	.....	9.87
47	1.16	173.5 (2)	11.48	11.00	−.48	36.18	84.69	−2.27x 10−3	2.53	5.46 (5.77) *	82.70	2.38	.80	6.34 6.35	6.36 6.34	.....	.....	.....
43	1.50	215.4 (5)	11.38	11.00	−.38	39.58	76.10	−1.91x 10−3	2.80	4.06 (4.69) *	82.00	.....	.....	.....	.....	.....	.....	.....
44	2.50	218.0 (4)	11.28	10.87	−.31	39.54	56.19	−1.77x 10−3	2.94	3.95 (4.84) *	76.00	5.93	.30	7.07	.....	.....	.....	10.59
60	.....	211.5 (2)	11.00	10.78	−.22	39.85	57.46	−1.67x 10−3	2.54	5.35 (6.85) *	.....	3.69	1.94	.....	.....	.....	.....	.....
48	4.00	246.0 (2)	11.00	10.78	−.22	41.81	86.73	−2.40x 10−3	3.58	4.48 (5.05) *	28.34	1.39	.16	7.08	7.07	6.91	−.16	11.80
59	14.00	218.0 (2)	10.72	10.36	−.32	40.31	63.55	−1.51x 10−3	2.68	3.45 (6.74) *	54.40	4.45	.43	6.70	6.70	6.82	+.12	.....
42	7.50	388.0 (2)	10.60	10.18	−.42	39.11	62.20	−1.43x 10−3	3.22	1.04 (4.48) *	57.80	6.08	.93	6.54	6.82	.....	.....	9.71
41	7.00	484.0 (3)	10.50	10.10	−.40	38.99	62.83	−1.39x 10.3	3.21	.95	43.40	6.04	.76	6.83	6.82	7.07	+.15	13.75

39	8.30	309.5 (2)	10.31	9.70	−.61	37.41	50.20	−1.08x 10−3	3.00	.73	91.30	7.33	1.55	6.47	6.47	.....	.....	9.16
0f	5.62	506.5 (3)	10.25	9.80	−.45	37.41	54.09	−1.18x 10−3	3.19	.74	52.20	10.67	.78	.....	.....	.....	.....	.....
58	16.50	289.0 (2)	10.20	9.82	−.38	39.76	55.69	−1.18x 10−3	3.01	.93	60.60	10.77	.40	6.52	6.72	6.93	+.21	9.16
74	14.50	388.0 (2)	10.15	10.02	−.13	40.20	54.90	−1.18x 10−3	3.09	.71	48.90	4.23	1.70	6.77	6.79	7.01	+.22	7.01
38	6 00	441.5 (2)	10.15	9.76	−.39	37.32	47.37	−1.11x 10−3	3.07	.60	73.30	9.60	.38	6.72	6.86	.....	.....	9.75
75	6.00	370.0 (2)	9.90	9.59	−.31	39.15	48.19	−1.11x 10−3	3.09	.46	50.08	7.86	.83	.....	.....	.....	.....	.....
37	8.00	363.5 (2)	9.84	9.60	−.24	37.29	41.70	−8.40x 10−4	2.97	.62	95.40	6.59	1.02	.....	.....	.....	.....	.....
57	13.50	338.5 (2)	9.75	9.48	−.27	40.90	47.37	−1.11x 10−3	3.27	.40	59.50	6.60	.42	6.54	.....	.....	.....	.....
35	6.00	405.5 (2)	9.45	9.10	−.35	36.49	66.28	−8.92x 10−4	2.87	.72	102.70	7.74	.83	.....	.....	.....	.....	.....
56	12.80	340.0 (2)	9.40	9.17	−.23	40.84	41.32	−7.59x 10−4	3.45	.36	59.80	6.01	.41	6.28	6.61	6.60	−.01	9.87
30	.....	325.5 (2)	9.40	8.93	−.47	35.75	38.95	−7.00x 10−4	3.15	.72	.....	.....	.....	.....	.....	.....	.....	.....
33	5.00	429.0 (2)	9.38	9.16	−.22	37.80	38.62	−7.00x 10−4	3.05	.67	114.80	8.80	.81	.....	.....	.....	.....	.....
32	4.80	461.9 (2)	9.38	9.20	−.18	36.12	35.10	−6.32x 10−4	3.05	.57	107.0	11.00	.54	6.78	6.40	6.48	+.08	.....
36	4.50	512.0 (2)	9.36	9.00	−.36	36.03	35.07	−6.94x 10−4	3.00	.73	121.2	11.33	.83	6.68	6.30	.....	.....	10.52
34	7.80	379.0 (2)	9.30	9.06	−.24	36.11	31.47	−6.41x 10−4	3.02	.61	83.1	10.79	.90	.....	.....	.....	.....	.....
28	.....	..... (2)	9.26	9.03	−.23	37.60	36.12	−5.70x 10−4	3.19	.80	.....	.....	.....	.....	.....	.....	.....	.....
31	5.16	437.7 (2)	9.22	8.85	−.37	36.49	32.45	−5.70x 10−4	2.97	.69	105.30	10.91	1.02	.....	.....	.....	.....	.....
26	7.60	251.0 (2)	9.06	8.94	−.12	37.20	37.88	−5.56x 10−4	3.40	.83	106.80	11.00	.95	6.65	.....	.....	.....	5.69

TABLE 1—(*Continued*)

Expt. No.	Time in Hrs. of Expt.	Total Weight in grs. and Number of Fish	pH of Experimental Water			Aklali Reserve of Experimental Water		CO2 in Molar Concentration in terms of NaOH or CO2 Added to Water	Total CO2 Vol. % at End	O2 in ml. per L. in Water at End	O2 Consumed in ml. per Kilo per Hr.	Swim Bladder Gas of Fish at End of Expt.		pH of Blood at End	pH of Serum			Hemoglobin in Blood grs. %
			at End	Aerated at End	Difference	Before Adding NaOH or CO2	At End of Expt.					% O2	% CO2		pH at End Unaerated	pH at End Aerated	pH Difference	
27	12.50	155.0 (4)	9.04	8.77	−.27	37.20	.....	−5.50x 10−4	3.27	.55	122.00	15.72	.66	6.70	.....	.....	.....	.....
24	10.40	342.0 (2)	8.96	8.89	−.07	37.88	43.73	−4.78x 10−4	3.53	.36	70.60	11.29	.93	6.89	.....	.....	.....	9.16
23	9.10	302.0 (2)	8.88	8.70	−.18	36.61	38.49	−4.80x 10−4	3.55	.38	85.20	9.62	.36	6.62	.....	.....	.....	7.80
25	9.80	262.0 (2)	8.82	8.67	−.15	38.96	39.57	−4.99x 10−4	3.45	.45	91.70	11.05	.97	.....	.....	.....	.....	.....
11**	6.50	535.0 (3)	8.80	8.78	−.01	.....	.....	−1.04x 10−3	3.05	.27	.....	9.17	.96	.....	.....	.....	.....	.....
55	16.50	225.5 (2)	8.64	8.30	−.34	40.93	36.49	−4.75x 10−4	3.38	.33	75.20	10.06	.73	6.47	6.36	6.52	+.16	9.02
15	9.50	494.0 (2)	8.61	8.51	−.10	40.50	45.12	−2.63x 10−4	4.29	.30	53.80	5.91	.97	.....	.....	.....	.....	.....
14	.....	411.0 (2)	8.57	8.62	−.05	38.79	44.60	−2.52x 10−4	4.32	.32	.....	6.41	1.24	.....	.....	.....	.....	.....
13	.....	515.0 (2)	8.57	8.71	+.26	38.18	47.15	−2.14x 10−4	4.42	.40	.....	3.23	.52	.....	.....	.....	.....	.....
16**	6.00	528.0 (3)	8.55	8.40	−.15	.....	.....	−5.79x 10−4	3.36	.30	.....	8.39	.77	.....	.....	.....	.....	.....

12	14.00	301.0 (2)	8.54	8.52	−.02	39.26	45.30	−1.89x 10−4	4.42	.46	53.07	4.44	.63					5.90
22	7.90	387.0 (2)	8.50	8.38	−.12	38.49	35.84	−4.20x 10−4	3.99	.72	74.50	9.49	.68					8.42
18	15.80	289.0 (2)	8.42	8.28	−.14	38.18	37.88	−3.35x 10−4	3.78	.30	57.00	8.41	.45	6.58				
17	10.60	365.0 (2)	8.38	8.30	−.03	38.18	39.79	−3.28x 10−4	3.85	.29	64.6	14.19	.89					
51	15.85	249.5 (2)	8.32	8.33	+.01	40.80	35.20	−1.79x 10−4	4.39	.30	65.5	13.30	.35	6.37	6.68	6.70	+.02	9.71
9	12.50	345.0 (2)	8.32	8.38	+.06	39.45	37.26	−1.66x 10−4	4.57	.63	42.70	17.18	.44					6.31
16	11.50	419.0	8.30	8.24	−.06	38.12	39.75	−2.83x	3.99	.26	55.08	15.47	.70	6.50				
12**	7.16	498.0 (3)	8.12	8.22	+.10			−2.59x 10−4	4.04	.30	63.51	7.43	.61					
19	12.16	293.0	8.10	8.16	+.06	38.18	35.12	−3.56x 10−	3.53	.32	124.50	9.70	.83	6.58				7.80
50	14.00	284.5 (2)	8.09	8.15	+.06	40.07	44.20	−1.25x 10−4	4.39	.29	63.70	6.90	.49	6.22	6.50	6.60	+.10	11.10
11	17.50	290.3 (2)	8.08	8.15	+.07	42.78	44.10	−1.18x 10−4	4.41	.27	53.20	8.65	.85					6.31
8	11.30	395.0 (2)	8.03	8.18	+.15	39.42	44.97	−1.51x 10−4	4.59	.27	47.50	8.74	.68					4.99
54	27.00	181.7 (2)	7.91	8.27	+.31	40.03	35.90	−3.58x 10−4	3.33	.35	63.03	7.53	.69	6.39	6.34	6.58	+.24	
10	12.60	351.0 (2)	7.89	8.13	+.24	39.35	39.39	−1.11x 10−4	4.42	.20	54.10	7.60	.90					6.07
53	18.50	198.5 (2)	7.84	8.20	+.36	40.40	40.46	−3.16x 10−4	3.81	.32	76.60	4.58	.93	6.33	6.52	6.74	+.12	6.51
52	13.20	327.0 (2)	7.60	8.18	+.58	40.42	35.59	−2.30x 10−4	3.84	.30	69.6	8.85	.81	6.20	6.52	6.59	+.07	10.52
14**	6.00	518.0 (3)	7.60	8.08	+.48			−2.08x 10−4	3.90	.40	77.31	8.33	1.24					
7**	5.00	584.0	7.55	8.03	+.48				4.60	.26	76.54	12.16	2.24					
7	6.50	351.0 (3)	7.53	7.99	+.45	39.17	43.12	−7.10x 10−5	4.49	.42	94.8	15.28	.63					

TABLE 1—(*Continued*)

Expt. No.	Time in Hrs. of Expt.	Total Weight in grs. and Number of Fish	pH of Experimental Water			Alkali Reserve of Experimental Water		CO2 in Molar Concentration in terms of NaOH or CO2 Added to Water	Total CO2 Vol. at End	O2 in ml. per L. in Water at End	O2 Consumed in ml. per Kilo per Hr.	Swim Bladder Gas of Fish at End of Expt.		pH of Blood at End	pH of Serum			Hemoglobin in Blood grs. %
			at End	Aerated at End	Difference	Before Adding NaOH or CO2	At End of Expt.					% O2	% CO2		pH at End Unaerated	pH at End Aerated	pH Difference	
46	12.70	145.0 (2)	7.50	8.15	+.65	39.42	40.50	0	4.24	.43	145.8	13.75	.49	.....	.....	.....	.....	.....
49	14.70	275.3 (2)	7.49	8.07	+.58	40.50	42.24	−5.90x 10−5	4.45	.31	64.1	10.25	.66	.....	.....	.....	.....	.....
3	8.00	..... (2)	7.44	7.73	+.31	39.73	42.65	−5.05x 10−5	4.44	.28	.....	9.27	1.16	.....	.....	.....	.....	.....
5**	9.25	473.0 (2)	7.40	8.02	+.62	.....	.....	.....	4.76	.24	53.19	7.64	1.59	.....	.....	.....	.....	.....
1	9.00	337.0 (2)	7.20	7.60	+.40	39.73	40.77	0	4.24	.29	58.50	7.05	1.82	.....	.....	.....	.....	.....
76	8.50	510.0 (3)	7.19	8.10	+.91	39.33	37.55	0	4.20	.36	66.50	6.26	1.15	6.80	6.52	6.79	+27	9.57 8.21
61	16.60	214.5 (2)	7.12	8.18	+1.06	39.45	40.68	+6.60x 10−5	4.48	.37	74.09	11.55	.73	.....	.....	.....	.....	.....
62	39.00	216.5 (2)	7.08	8.15	+1.07	42.59	42.22	+1.41x 10−4	4.67	.51	31.16	14.90	1.60	.....	.....	.....	.....	.....
2**	3.53	695.0 (3)	7.05	8.07	+1.02	.....	.....	.....	4.00	.36	95.87	7.53	3.09	.....	.....	.....	.....	.....
63	22.16	166.5	6.97	8.08	+1.11	39.29	41.60	+2.00x 10−4	4.71	.35	72.10	11.04	1.52	.....	.....	.....	.....	.....
4**	4.00	574.0 (3)	6.90	8.06	+1.16	.....	.....	.....	4.77	.41	102.7	10.47	3.37	.....	.....	.....	.....	.....
64	13.60	305.5 (2)	6.78	7.91	+1.13	39.87	40.40	+2.73x 10−4	4.83	.34	79.20	8.32	1.65	6.12	.....	6.52	.....	8.55
13**	7.50	501.0 (3)	6.75	8.03	+1.28	.....	.....	+3.11x 10−4	4.64	.20	50.65	8.95	2.62	.....	.....	.....	.....	.....

65	9.16	386.5	6.73	8.00	+1.27	39.11	41.42	+3.64x 10–4	4.93	.52	72.20	9.49	2.90	.....	.....	.....	.....	.....	
66	12.00	327.0 (2)	6.71	8.02	+1.27	38.96	40.31	+5.09x 10–4	5.09	.39	65.10	7.00	2.85	6.33	6.05	.....	.....	8.42	
3**	5.50	486.0 (3)	6.70	8.05	+1.30	.....	.....	.....	5.03	.37	85.61	6.21	4.53	.....	.....	.....	.....	.....	
77	4.50	246.5 (2)	6.67	8.27	+1.90	39.39	40.54	.....	5.26	.34	.....	5.07	2.99	6.43	6.81	7.29	+.48	.....	
67	12.75	298.0 (2)	6.63	8.06	+1.43	39.36	40.16	+5.58x 10–4	5.02	.30	75.04	7.60	2.20	6.19	6.16	6.26	+.10	8.21	
1**	5.00	599.0 (3)	6.60	8.06	+1.46	.....	.....	.....	5.07	.27	74.89	6.21	4.52	.....	.....	.....	.....	.....	
68	13.75	231.7 (2)	6.58	7.93	+1.35	39.23	40.37	+1.01x 10–4	5.23	.31	83.10	8.40	2.80	.....	6.55	6.80	+.25	.....	
69	15.75	248.5 (2)	6.56	7.93	+1.37	39.25	40.06	+6.19x 10–4	5.26	.38	70.20	6.09	3.10	6.50	6.31	6.83	+.52	6.23	
70	14.16	314.5 (2)	6.53	8.20	+1.67	39.82	40.93	+7.14x 10–4	5.49	.37	50.90	9.50	3.60	6.47	6.51	6.82	+.31	9.57	
71	14.34	292.0 (2)	6.37	8.20	+1.83	39.88	41.45	+1.15x 10–3	5.27	.45	63.05	10.10	3.80	6.25	6.60	6.84	+.24	8.21	
72	13.17	384.6 (2)	6.29	8.18	+1.89	39.79	41.27	+1.43x 10–3	7.22	.40	55.30	7.76	4.92	6.09	6.60	7.31	+.71	9.87	
														6.17	6.64	7.11	+.67	6.94	
78	2.30	297.5 (3)	6.29	7.94	+1.65	38.92	39.44	.....	7.51	.45	.....	6.10	8.09	6.67	7.27	8.00	+.73	.....	
73	17.18	265.0 (2)	6.20	8.06	+1.86	40.56	42.75	+2.59x 10–3	7.82	.42	55.30	7.46	8.19	7.02	7.56	8.28	+.72	.....	
														6.35	6.63	7.55	+.93	.....	
79	.....	481.2 (2)	6.10	7.84	+1.74	38.53	40.38	.....	8.68	.53	57.61	9.65	6.11	6.36	6.55	7.20	+.65	.....	
6**	6.50	530.0 (3)	6.05	8.02	+1.97	.....	.....	.....	9.48	1.70 (4.76) *	40.64	16.66	8.41	.....	.....	.....	.....	.....	
9**	.16	555.0 (3)	5.55	8.06	+2.51	.....	.....	.....	18.73	3.56 (4.62) *	59.78	8.83	12.76	.....	.....	.....	.....	.....	
8**	1.16	493.0 (3)	4.15	8.01	+3.83	.....	.....	.....	13.46	4.17 (4.28) *	6.25	13.36	9.57	.....	.....	.....	.....	.....	

extent counteracted when small amounts of sodium hydroxide were added. Seemingly an almost molecular equivalent of calcium carbonate was precipitated. See the two columns under the heading *Alkali Reserve of Experimental Water*. Before titrating against hydrochloric acid the water was filtered. This removed all precipitates and suspensions. The calcium bicarbonate precipitated as carbonate liberates molecular equivalents of H_2CO_3. This unites with the sodium hydroxide to form sodium bicarbonate without increasing or decreasing the free carbon dioxide. This is true, of course, only in so far as the reaction $NaOH + Ca(HCO_3)_2 = CaCO_3$ (precipitated) $+ NaHCO_3 + H_2O$ tells the whole story. The reduction of the carbon dioxide tensions of the water to which sodium hydroxide has been added is more apparent than real. This is shown by the *pH Difference* curve in Figure 1. Despite the precipitated $CaCO_3$, the pH of the aerated treated water was raised, due to sodium being a stronger alkali than calcium. This increased alkalinity is not apparent as increased alkali reserve when it is determined by titrating against hydrochloric acid to a pH 4.2 (see Tables 1 and 2, the two columns marked *Alkali Reserve of Experimental Water*). Second, the alkalinities of the unaerated and aerated waters to which sodium hydroxide had been added were increased with a corresponding increase between the pH's of the waters unaerated and aerated (see columns marked *pH Difference* under *pH of Experimental Water*, Tables 1, 2, and 3, and the *pH Difference* curves, Figures 1, 2, and 3). In Table 1 it is seen that the pH of aerated experimental water was raised from approximately pH 8.10 to pH 11.68 without showing more than — .61 pH units difference between unaerated and aerated water.

In Tables 1, 2, and 3, and Figures 1, 2, and 3, the pH's of the aerated experimental waters are taken as a basis of comparison. The actual carbon dioxide tension of the water as determined by the formula, $(-)pH = - ne - n\log P$, is taken as the basis of comparison in Tables 6 and 7 and Figures 4 and 5. The pH values of unaerated experimental waters are compared to the actual carbon dioxide tension in Tables 3 and 7. In these two tables a corresponding number is one and the same experiment. There is a slight uncorrected error in calculating the carbon dioxide tension in that the value of n was not changed to correspond to the variations in the values of the pH's of aerated experimental waters.

2. The ability of fishes to extract oxygen from water at various carbon dioxide tensions.

A. The Rock Bass, Ambloplites ruprestris (Rafinesque). In experiments on the rock bass and in all other experiments two of the three fish in a bottle were allowed to die, and the third—or last, if fewer or more than three—was taken alive for observations on blood, swim bladder gas, etc.

When Table 1 and Figure 1 are examined it is seen that oxygen could be absorbed more efficiently at pH's from about 8.00 to 7.00 or 7.50, *i. e.*, about 1.5 to about 4.4 or 4.9 mm. Hg carbon dioxide tension. There seemed to be very little loss of efficiency in absorbing oxygen by the fish up to about pH 9.00 or down to .16 to .26 mm. Hg carbon dioxide tension. This is very striking since .23 to .30 mm. Hg tension equals the carbon dioxide partial pressure of the atmospheric air and 4.88 mm. Hg would be the carbon dioxide tension of the water if a fish having a respiratory quotient of .80 should absorb all the oxygen from a saturated solution in the water and turn it back as carbon dioxide. This calculation is based upon .759 as the absorption coefficient of carbon dioxide and .028 as the absorption coefficient of oxygen in water at 25°C.

From these observations it follows that the rock bass cannot absorb oxygen from water at low oxygen tensions when the carbon dioxide tension is greater than it would be if the oxygen were turned back into the water as carbon dioxide by the fish with a respiratory quotient of .80. Thus, it seems that the fish has a mechanism to counteract

TABLE 1A *(Supplement to Table 1). The pH of the blood of the Rock Bass as determined by the quinhydrone electrode. All blood was oxalated. The pHs are variable which means that there were different degrees of aeration of the oxalated blood or different amounts of oxalate. This might explain the variations in the pH or cH of blood reported by Barcroft (1934).*

Number of Experiments Averaged	pH of Water at End	pH of Blood	Experiment No.	pH of Water at End	pH of Blood
16	8.55	7.03	1	6.60	7.37
12	8.12	7.37	9	5.55	7.30
7	7.55	7.70	8	4.15	7.53
4	6.90	7.50			

the ill effect of the carbon dioxide liberated in the closed system when absorbing oxygen at a low level. This is not the limit of adjustment, as these experiments show that the rock bass can still absorb oxygen at low tensions when the carbon dioxide tension of the water has been raised from about 3.59 to about 12.9 times this value (4.88 mm. Hg) (pH 6.00, experimental water)—approximately 17.5 to approximately 43.00 mm. Hg tension.

When the carbon dioxide tension is raised very little above this point the rock bass die very rapidly in the experimental water. Fish will not live in natural waters if the carbon dioxide tension is raised to a very much less high value (Davidson, 1933). This apparent paradox will be discussed in connection with other experiments to be described later.

The fish died very rapidly in modified experimental water with a pH of about 11.00 and a carbon dioxide tension of about .12 to .13 mm. Hg. The ill effect is due either to the low carbon dioxide tension

or to the high active alkalinity of the water. The rapidity with which the fish died at high and low carbon dioxide tensions is indicated in Table 1 by giving the oxygen content of the water at the beginning and at the end of the experiment. This is also shown in Figure 1 by joining with a solid line the points indicating the oxygen at the end and the beginning of an experiment.

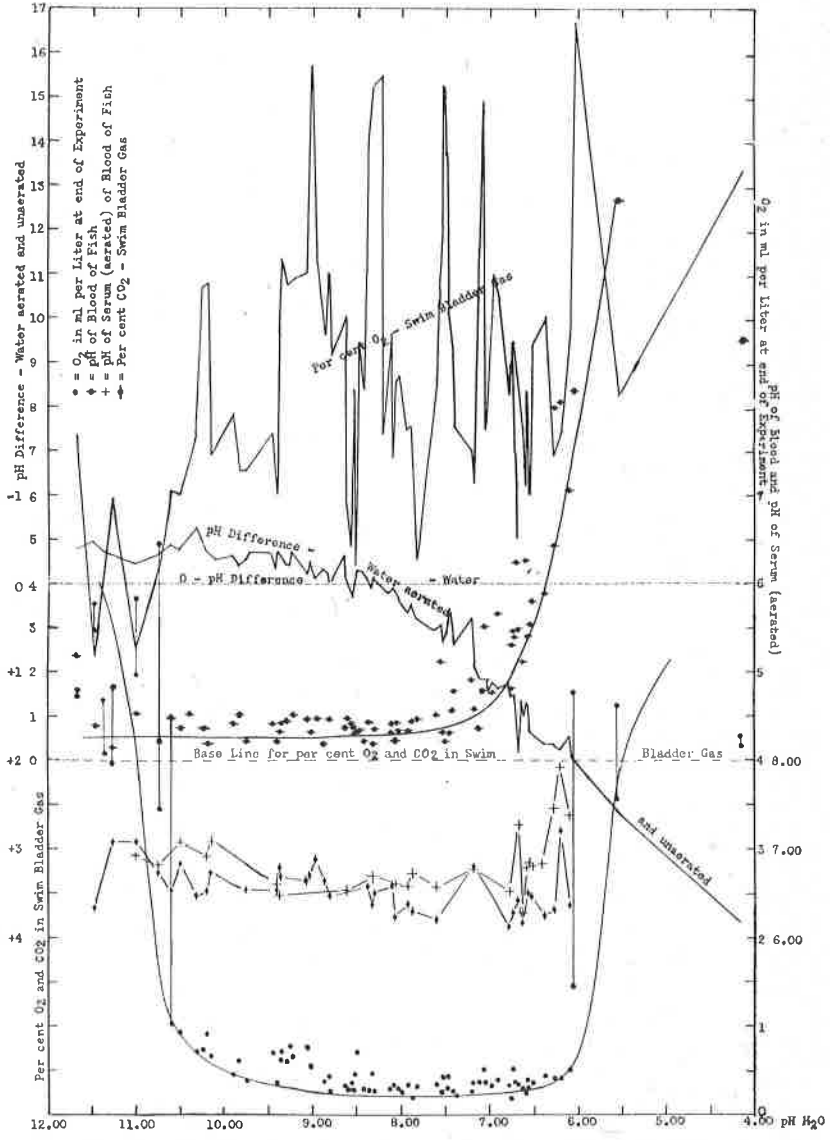

Fig. 1. Graphic Representation of the Data Given in Table 1.

TABLE 2. *The Small-mouth Bass, Micropterus dolomieu (Lacepede) (For explanation see Table 1).*

Expt. No.	Time in Hrs. of Expt.	Total Weight in grs. and Number of Fish	pH of Experimental Water			Alkali Reserve of Experimental Water		CO_2 in Molar Concentration in terms of NaOH or CO_2 Added to Water	Total CO_2 Vol. % at End	O_2 in ml. per L. in Water at End	O_2 Consumed in ml. per Kilo per Hr.	Swim Bladder Gas of Fish at End of Expt.		pH of Blood at End	pH of Serum			Hemoglobin in Blood grs. %
			at End	Aerated at End	Difference	Before Adding NaOH or CO_2	At End of Expt.					% O_2	% CO_2		pH at End Unaerated	pH at End Aerated	pH Difference	
6	.....	194 (1)	10.05	9.80	−.25	38.65	48.74	−9.80x 10−4	2.76	1.10 (6.74)*	.....	.....	.....	.....	6.68	6.50	−.18	14.02
3	6.00	558.5 (1)	9.40	9.05	−.35	40.34	40.63	−7.35x 10−4	3.34	.82 (5.41)*	65.11	11.50	.45	6.62	6.59	6.65	+.06	11.47
4	8.33	339.5 (1)	7.58	8.13	+ .55	40.63	43.15	−6.07x 10−5	4.39	.44 (5.58) *	83.80	12.80	.85	6.58	6.68	6.82	+.14	10.52
9	.....	812.0 (1)	7.12	8.12	+1.08	40.50	41.11	0	.....	.40 (5.74) *	.....	11.80	1.60	6.54	6.61	7.09	+.48	8.20
5	9.50	403.0 (2)	6.43	7.83	+1.40	39.76	41.41	+8.20x 10−4	6.66	.28 (5.26)*	62.09	.....	.....	6.58	6.66	7.23	+.57	9.56
8	12.00	278.0 (1)	6.24	8.15	+1.91	39.11	40.63	CO_2 Bubbled	7.09	.78 (6.86) *	87.50	.....	.....	.....	6.60	7.50	+.90	12.13
2	2.50	630.0 (1)	6.05	8.05	+1.97	.....	.....	CO_2 Bubbled	8.20	1.55 (3.44) *	42.27	16.74	4.44	.....	.....	.....	.....	.....
1	1.30	580.0 (1)	5.69	8.03	+2.34	.....	.....	CO_2 Bubbled	16.71	3.90 (.....) **	.....	17.97	11.74	6.58	6.88	7.50	+.62	11.47

*O_2 at beginning of experiment. **O_2 sample was not taken but obviously the O_2 was not much greater than at the end. See O_2 content at beginning of preceding experiment.

At a pH from 9.00 to 9.45 are a group of experiments in which the fish did not absorb the oxygen to as low a point as would be expected from the observations of the other experiments. At pH 8.50 is another high point. Without exception these high points are of experiments numbers 36 and below. During the first experiments a technique had not been sufficiently developed to prevent the leaking of air into the bottles around the rubber stoppers. At first thought it would be supposed that the fish would last longer and absorb oxygen down to a lower point when allowed to obtain additional air at intervals. But such is not the case. This paradox will be discussed with other series of experiments.

B. The Small-mouth Bass, Micropterus dolomieu (Lacepede). Only nine small-mouth bass could be obtained for experimental purposes. However, with our observations on the rock bass we were able to distribute the carbon dioxide tension of the water so as to show the physiological responses of this fish to the carbon dioxide tension of the water.

Table 2 and Figure 2 indicate that the small-mouth bass is very efficient in absorbing oxygen from the water at low tensions at comparable carbon dioxide tensions, as was the rock bass. The number of observations were too few to enable one to mark off the carbon dioxide tension range of the water at which the small-mouth bass could absorb oxygen most efficiently at low tensions.

C. Yellow Perch, Perca flavescens (Mitchill). The yellow perch, as shown by Figure 3, has a more restricted pH range (carbon dioxide tension range) at which oxygen can be absorbed at low tensions, than either the small-mouth bass or the rock bass, it being from about pH 6.25 to about 9.00 +-. From about pH 9.25 the ability of the yellow perch to absorb oxygen at low tensions falls rapidly but more gradually than in the rock bass. The lower pH limits (high carbon dioxide tension) were not reached in experiments with this fish. The yellow perch, like the rock bass and small-mouth bass, has a narrow pH range (carbon dioxide tension range) at which it is most efficient in absorbing oxygen at low tensions.

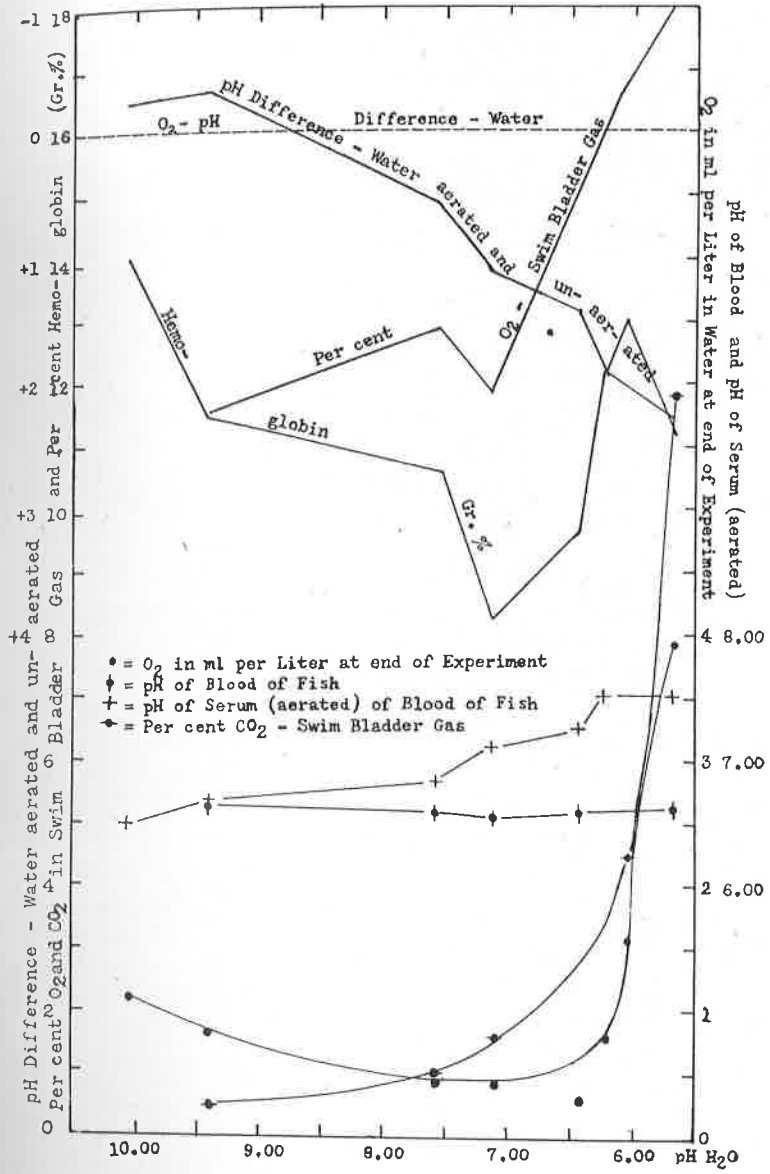

Fig. 2. Graphic Representation of the Data Given in Table 2. The base line for the percentage of carbon dioxide and of oxygen has been moved up to avoid confusion.

IV. The Reactions and the Alkali Reserve of the Blood under the Various Carbon Dioxide Tensions of Water

Only venous blood was obtained and used in these experiments. The most interesting facts determined were that the reactions of the blood of each of the species of fishes were fairly constant under the various carbon dioxide tensions. Barcroft (1934) gives, Figure 2, page 5, cH of $3x10^{-8}$ to $2.5x10^{-7}$ (pH 7.52 to 6.60) as normal for fish (Scup).

TABLE 3. *The Yellow Perch, Perca flavescens (Mitchell) (For explanation see Table 1).*

Expt. No.	Time in Hrs. of Expt.	Total Weight of Fish in grs.	pH of Experimental Water			Alkali Reserve of Experimental waters		CO2 in Molar Concentration in terms of NaOH or CO2 Added to Water	Total CO2 Vol. % at End	O2 in ml. per L. in Water at end	O2 Consumed in ml. per kilo per Hr.
			At End	Aerated at End	pH Difference	Before Adding NaOH or CO2	At End				
23	12.20	78.0	10.57	10.22	−.35	38.68	54.74	-1.22x10-3	2.40	5.15	46.23
22	14.30	60.0	10.27	10.00	−.27	38.68	48.20	-9.84x10-4	2.48	(6.07)* 4.02	113.70
21	16.25	73.0	9.65	9.43	−.22	38.68	39.11	-7.29x10-4	2.72	(6.07)* 1.77	174.50
1	24.00	18.0	9.22	9.02	−.20	38.26	35.75	-6.39x10-4	3.90	(6.07)* .84	456.50
2	25.00	38.00	9.02	8.90	−.12	38.26	34.64	-5.48x10-4	3.12	1.05	207.50
3	22.15	63.00	8.84	8.67	−.17	38.26	34.33	-4.98x10-4	3.31	.54	155.50
18	14.15	85.50	8.62	8.42	−.20	40.87	36.93	-4.89x10-4	3.44	.29	194.20
4	.....	96.00	8.60	8.52	−.08	38.26	35.03	-4.20x10-4	3.30	.37	.....
5	19.00	108.00	8.39	8.33	−.06	38.26	33.87	-3.67x10-4	3.53	.50	108.20
9	15.00	104.00	8.19	8.30	+.11	38.72	42.81	-1.27x10-4	4.44	.33	145.27
17	.....	63.50	8.08	8.23	+.15	40.87	37.14	-3.69x10-4	3.57	.23	.....
14	12.30	86.8	7.93	8.20	+.27	41.11	46.35	-1.22x10-4	4.36	.35	215.20
6	15.00	72.0	7.92	8.20	+.28	38.72	34.39	-3.04x10-4	3.74	.29	212.10
16	9.50	59.8	7.90	8.05	+.18	40.86	40.90	-2.43x10-4	3.90	.36	410.15
7	17.00	77.0	7.88	8.18	+.30	38.72	36.03	-2.45x10-4	3.81	.45	168.00
10	18.00	21.0	7.82	8.30	+.48	38.72	35.18	-2.41x10-5	4.42	.56	577.20
8	18.00	90.0	7.81	8.17	+.36	38.72	36.92	-2.45x10-4	3.87	.30	152.01
12	27.5	68.0	7.78	7.96	+.18	41.11	42.22	0	4.47	.43	145.27
19	12.25	114.0	7.55	8.21	+.66	39.42	41.24	0	4.15	.32	214.20
20	20.45	121.0	7.53	8.17	+.64	39.42	40.39	-2.44x10-5	4.10	.34	120.12
24	17.40	45.0	7.42	8.22	+.80	39.42	40.04	+2.08x 10-6	4.15	.47	367.20
15	28.50	77.0	7.40	8.02	+.62	41.11	40.76	-6.22x10-5	4.48	.31	101.80
38	22.00	146.0	7.38	8.11	+.73	.....	.....	.....	4.08	.26	.....
11	18.00	22.00	7.38	8.15	+.77	38.72	39.05	0	4.36	.46	563.40
37	18.50	107.0	7.34	8.13	+.79	.....	.....	.....	4.08	.26	.....
30	19.00	121.0	7.30	8.22	+.92	47.29	48.72	+2.22x 10-4	5.53	.41	133.56
26	.....	68.9	7.26	8.30	+1.04	40.65	41.24	+2.23x x10-4	4.37	.30	.....
31	14.60	141.0	7.16	8.13	+.97	47.29	48.55	+1.88x x10-4	5.94	.58	142.40
13	11.30	148.0	7.00	8.06	+1.06	40.50	42.29	+1.23x x10-5	4.51	.27	157.20
32	13.00	173.0	7.00	8.15	+1.15	47.29	49.69	+2.04x 10-4	7.12	.27	136.20
28	14.40	114.0	6.86	8.11	+1.24	39.50	44.32	+2.05x 10-4	4.68	.17	189.55
33	17.35	107.0	6.74	8.05	+1.31	46.81	49.11	+4.10x 10-4	4.02	.28	168.45
29	16.10	107.0	6.62	8.05	+1.43	39.50	41.27	+4.33x 10-3	5.58	.32	172.20
34	16.20	138.0	6.61	8.21	+1.60	46.81	49.65	+6.43x 10-4	5.53	.30	129.55
35	15.00	185.0	6.50	8.17	+1.67	47.01	62.89	+6.89x 10-4	5.94	.32	104.83
36	16.00	168.5	6.30	8.05	+1.75	48.72	51.75	+1.97x 10-3	8.89	.40	113.40
37	9.25	405.3	6.17	8.03	+1.86	.....	.....	.....	6.69	.53	57.61

The greatest variations in the reactions of the blood under the extreme carbon dioxide tensions tested were with the rock bass. Since the reactions of blood of the yellow perch, and especially of the small-mouth bass, were so much more nearly constant than in the rock bass, the authors are inclined to attribute the greater variations in the pH of the blood of the rock bass not to the actual variations in the reaction of the blood itself, but to the less well developed technique at the beginning of the summer. The Beckman pH meter was new to the operator. Contamination of the very small amount of blood obtained from the rock bass was difficult to avoid. Clotting was also more troublesome with the blood of the rock bass than with the other two species of fishes.

Both blood and serum were untreated. The technique was to take the blood from one of the cavities of the heart by means of a small syringe after the pectoral girdle had been parted. The pH of one portion of the blood was determined before coagulation. The one drop Gramercy receptacle was used for the very small amount of

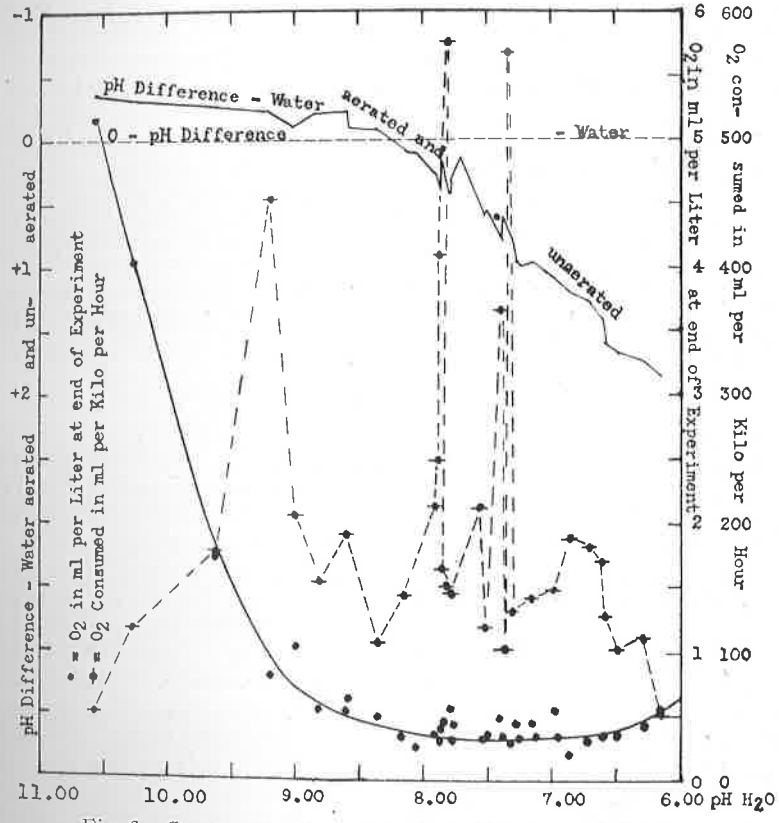

Fig. 3. Graphic Representation of the Data Given in Table 3.

blood and serum obtained. Another portion was started centrifuging in small tubes. The corpuscles and the fibrin, as fast as formed, were thrown to the bottom of the tubes. The serum was taken from the top above the clot and the pH determined unaerated and aerated. A very troublesome factor was the contamination of the small amounts of blood obtained with small amounts of body fluid. This contamination caused both a rapid coagulation of the blood and a laking of the corpuscles. The yellow perch and the small-mouth bass and the rock bass at the very low and very high carbon dioxide tensions were tested in the latter part of the summer. Naturally a better technique in handling blood and the Beckman pH meter was developed. More consistent results were obtained. In addition, comparatively large amounts of the blood could be obtained from the small-mouth bass and thus its handling was less a problem.

In each of the three species of fishes the alkali reserve of the serum as measured by the pH of aerated serum increased with an increase and decreased with a decrease in the carbon dioxide tension of the water. This is more plainly shown with the small-mouth bass than with the other two species. Again, this is due not so much to a more sensitively responding mechanism, though it may be more sensitive; but to the fact that much more blood could be obtained from this fish than from the other two, thus making the whole technique much less difficult. The pH of the unaerated serum of the small-mouth bass was fairly constant, the extremes being pH 6.54 to 6.62.

In experiment 3, Table 2, the pH of the blood is 6.62. This no doubt is slightly in error since the unaerated serum is pH 6.59 and is lower than either the same blood or same serum aerated. If this blood pH be called 6.59, the pH of the blood would be quite uniform. There would be three bloods with pH 6.58, one pH 6.54 and the other pH 6.59. The pH of the unaerated serum was less uniform than the blood. This would be expected since a certain amount of aeration would take place during centrifuging. The pH of all serum unaerated, with the exception of experiment 3, was higher than the blood pH. The blood of fish (experiment 6) was bad. The pH could not be obtained. The pH 6.68 unaerated serum and the pH 6.50 are both perhaps high. The pH 6.88 of unaerated serum, experiment 1, was more than likely due to aeration since the aerated serum had a pH of 7.50. pH 7.50 aerated serum of this experiment is low as compared with other aerated serums of fish in lower carbon dioxide tension water (Tables 2 and 5). When these tables are examined it is found that in each of the three species of fishes, the rock bass, the small-mouth bass and the yellow perch, the carbon dioxide tension of the blood at the low water carbon dioxide tensions was always higher than the carbon dioxide tension of the experimental water. At the higher carbon dioxide tensions of the water, the reverse was always true. There are two explanations. One, the serum was not being completely aerated. This was realized. The small-mouth bass serum

was more completely aerated. Aside from a conscious effort to more completely aerate the serum there was more serum to aerate. More blood was obtained from the small-mouth bass than from the other two species. Table 6 shows a higher carbon dioxide tension of the blood in comparison to the carbon dioxide tension of the water as compared with the other two species of fishes. The second explanation is that the fish when taken out of the experimental water was wrapped with a moist cloth during the interval of time required to part the pectoral girdle, manipulate the apparatus and draw blood from the heart. During this time the fish was readjusting the alkali reserve of its blood to a new and much lower carbon dioxide tension, *i.e.*, the carbon dioxide partial pressure, .30 mm Hg, of the air. The fact that a fish can change its alkali reserve rapidly has been found in connection with other experiments and field observations—to be described later. The explanation offered here is further emphasized by the fact that the pH of the blood of each of the three species of fishes was quite uniform under wide variations of experimental water carbon dioxide tensions. This uniformity in the pH of the blood is in spite of the difficulty in maintaining a uniform pressure in the syringe when drawing the blood. Notes were made of difficulties run into but they have not been included since an inclusion would greatly increase the volume of this paper.

These experiments show that the pH of the blood of each of the three species of fishes tested was quite uniform. This uniformity is maintained under various conditions by modifying the alkali reserve of the blood. They also show that the fishes are not able to control the carbon dioxide tension of their blood, but are in turn able to compensate by rapidly changing the alkali reserve of their blood. The rapidity with which the alkali reserve can be changed in the

TABLE 4. *The Rock Bass. The data taken from Table 1. Note the relations between the carbon dioxide tensions of the experimental water and the blood of the fish.*

Number of Experiments Averaged	Carbon Dioxide Tension of the Water, in mm. Hg at End of Expt.		Carbon Dioxide Tension of Blood in mm. Hg	Number of Experiments Averaged	Carbon Dioxide Tension of the Water, in mm. Hg at End of Expt.		Carbon Dioxide Tension of Blood in mm. Hg.
	Range	Average			Range	Average	
5	.12–.19	.15	.65	3	1.14-4.05	2.81	1.21
3	.23–.35	.29	1.04	3	6.76-8.10	7.28	1.21
2	.62–.79	.70	1.11				

blood of a fish may be found to have a more profound influence on the movements of fishes than the *so called homing instinct.*

The fish in experiment 1, Table 2, lived only one hour and 20 minutes and absorbed but little oxygen from the water (down to 3.90 ml. per liter) and perhaps did not completely compensate its blood

alkali for the very high carbon dioxide tension, or the fish had begun to reach the limit of tolerance or compensation for high carbon dioxide tension. No doubt each factor played its part. If we disregard experiment 1 we have an interesting relationship between the carbon dioxide tension of the water and the carbon dioxide tension of the blood of the fish.

The relation shown between the pH of the water and the pH of the aerated serum is qualitative and not quantitive. By applying the formula $(-)pH = -ne - n\log P$ and referring to Figure 1 and Table II (Powers, 1930, pp. 348-349), it will be seen that n for the blood of fish would approximate .60 and 1.00 for the experimental water. All calculations for carbon dioxide tensions were made on this basis. Tables 5 and 6 show a quantitative relation between the carbon dioxide tension of the blood of the fish and the carbon dioxide tension of the water. In each of the three species of fish, the carbon dioxide tension of the (venous) blood is, with only one exception,

TABLE 5. *Experiments on the Small-mouth Bass in which the carbon dioxide tensions of the water and the blood and partial pressure of the swim bladder are compared.*

Expt. No.	pH of Water at End	Carbon Dioxide Tension in mm. Hg at End		Partial Pressure in mm. Hg of Swim Bladder Gases	
		Water	Blood	Dioxide	Oxygen
6	10.05	.17	.22		
3	9.40	.13	.39	2.60	87.40
4	7.53	.74	.76	6.46	97.28
9	7.12	3.63	2.46	12.16	89.68
5	6.43	7.56	3.66		
8	6.24	24.30	10.26		
2	6.05	28.20		33.74	127.22
1	5.69	66.00	10.26	89.92	136.57

TABLE 6. *The Yellow Perch. The carbon dioxide tensions of the water and the blood and the partial pressure of the swim bladder are compared. The numbered experiments are the same experiments of the corresponding numbers in Table 3.*

Expt. No.	pH of Water at End of Expt.	pH of Blood at End	pH of Serum			Carbon Dioxide Tension in mm. Hg at End	
			At End Unaerated	At End Aerated	pH Difference	Water	Blood
18	8.62	6.24	6.48	6.54	+.06	.19	.38
16	7.90	6.62	6.60	6.62	+.02	.46	.32
12	7.78	6.56					
19	7.55	6.72	6.53	6.62	+.09	1.80	.42
20	7.53	6.39	6.39	6.52	+.13	1.31	.50
24	7.42	6.33	6.18	6.47	+.29	1.89	.94
13	7.40	6.53					

higher than the carbon dioxide tension of the water, when the water carbon dioxide tension is below .70 mm.Hg. The one exception is experiment 16, Table 6. This experiment seems to be in error. This is due to the fact that the tissues of the fish absorb oxygen from the blood and turn it back as carbon dioxide (.80 — R. Q.). This obviously raises the carbon dioxide tension of the venous blood above that of arterial blood. On the other hand, when the carbon dioxide tension of the water was high, the carbon dioxide tension of the blood without exception was lower than the higher carbon dioxide tensions of the water (Tables 4, 5 and 6).

What we want to make clear is, that the lower carbon dioxide tension of the blood of the fish, when in high carbon dioxide tension water, is not due to a mechanism that enables a fish to maintain a carbon dioxide tension of the blood below that of the carbon dioxide tension of the water in which the fish is living. This could be true only if there were a one way passage of carbon dioxide through the gill membranes. A one way passage is not in keeping with the present day conception of permeability. These experiments show, if they show anything, that there is a two way passage of carbon dioxide through the gill membrane between the blood and the water in which the fish is placed. First, the carbon dioxide tension of the blood of the fish does increase with an increase in the carbon dioxide tension of the water. Second, the pH of the blood of the fish is quite uniform over a wide range of carbon dioxide tensions of the water. The pH of the blood was determined outside the body by means of a Beckman pH meter glass electrode. The question can reasonably be asked:

TABLE 7. *The Rock Bass. Large numbers of experiments are averaged and the carbon dioxide tension of the water is compared with the partial pressures of the carbon dioxide and the oxygen of the swim bladder of the experimental fish. Data from Table 1.*

Number of Experiments Averaged	Carbon Dioxide Tension of Water in mm. Hg at End of the Experiment		Partial Pressure in mm. Hg of Swim Bladder Gases	
	Range	Average	Carbon Dioxide	Oxygen
25	.10–.19	.14	6.14	7.95
11	.21–.29	.24	6.32	9.64
7	.30–.38	.34	5.16	10.33
10	.43–.90	.69	8.23	8.36
4	1.14–1.34	1.22	6.77	10.12
7	3.15–4.35	3.66	15.37	10.02
8	5.51–8.67	6.66	21.08	7.49
3	14.04–16.50	14.52	46.09	7.69
2	20.31–21.75	21.03	44.96	8.78
2	23.33–23.75	23.54	30.00	6.41

why does the drawn blood not lose its carbon dioxide if it is at high carbon dioxide tension, and become alkaline? The first answer to this question is that the pH of the blood was determined very quickly, *i.e.*, before the blood had had time to coagulate. This had to be done or

the pH could not be taken. Second, the carbon dioxide does not pass into and out of solution as rapidly as was at one time thought. River waters retain a high or low carbon dioxide tension after miles of flow (Powers and Hickman, 1928, and Powers, 1928).

Due to the high absorption coefficient of carbon dioxide it would tend to be given off rapidly from solution. On the other hand, the kinetics of the reaction, $H_2CO_3 = CO_2 + H_2O$, retards the liberation

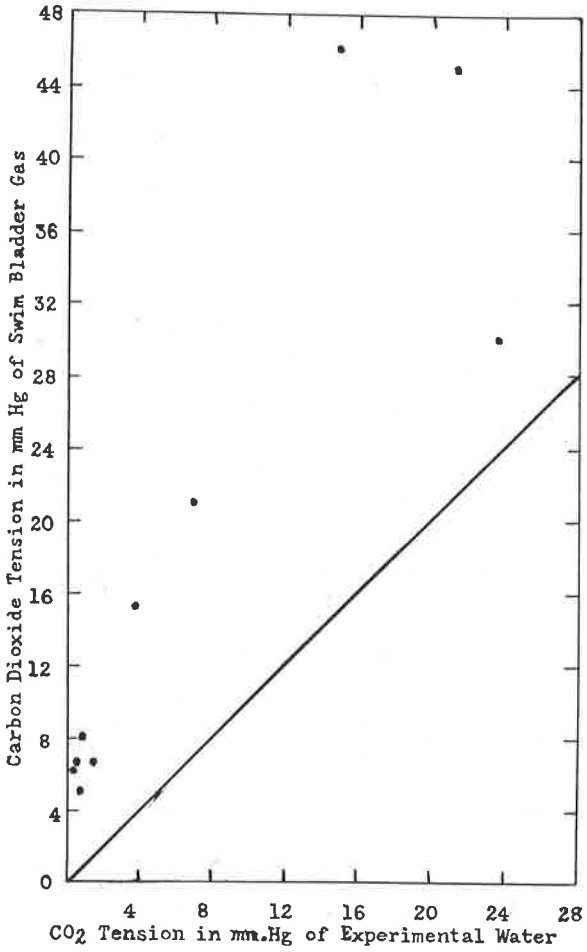

Fig. 4. Graphic Representation of the Data Given in Table 7 (see also Table 1 and Figure 1). The straight line in this figure and in Figure 5 represents a one to one ratio between the carbon dioxide tension in the swim bladder of the fish and the experimental water.

of carbon dioxide from solution. These facts were never fully appreciated by all until the discovery of carbonic anhydrase by Rough-

ton and associates (1935). The carbonic anhydrase which is located, at least in the higher forms, in the red blood corpuscles, would tend to facilitate the carbon dioxide exchange of the thin-layered blood in the gills over that in the drawn blood of larger volumes. The gills were exposed to the carbon dioxide partial pressure of the atmosphere. Thus the blood in the gills was exposed to low carbon dioxide both more effectively and during a much greater interval of time than the drawn blood of which the pH was being taken. Even though the time was much shorter and the carbon dioxide exchange in the blood volume was less efficient, some of the blood was slightly aerated before the pH could be taken with the Beckman pH meter glass electrode.

If the fish did not change the alkali reserve of its blood rapidly, it would stand to reason that even the slightest variation of carbon dioxide tensions met by the fish in natural waters would be quite detrimental to them. Fish do meet with variations in carbon dioxide tensions in waters in passing up either one of the forks of large rivers (Powers and Hickman, 1928, and Powers, 1928). Again it will be found that this physiological reaction of the fishes is one of the fundamental factors in the migratory movements of fishes.

This will be taken up again in a discussion based upon experimentation and field observations of the factors involved in the sudden mortality of fishes.

TABLE 8. *The gaseous composition of swim bladders of fishes taken from "Normal Conditions." All except the last record, a Yellow Perch, and the next to last, a Small-mouth Bass, are Rock Bass.*

Weight of Fish in grs.	Swim Bladder Gas of Fish in per cent of total		Date	Remarks
	Oxygen	Carbon Dioxide		
124.0	20.67	1.10	6/29/36	From Live Box in Lake
88.0	18.44	1.30	6/29/36	From Live Box in Lake
.....	2.06	4.06	7/ 7/36	From Holding Tank in Laboratory
151.3	18.88	.50	7/16/36	From Live Box in Lake
128.5	16.67	.77	7/16/36	From Live Box in Lake
156.0	20.11	.72	7/16/36	From Live Box in Lake
127.0	16.06	.90	7/16/36	From Live Box in Lake
.....	12.69	1.62	7/17/36	From Live Box in Lake
.....	17.24	1.56	7/17/36	From Live Box in Lake
.....	24.61	2.05	8/24/36	From Live Box in Lake
.....	15.16	1.23	.....	From Live Box in Lake

V. The Composition of the Swim Bladder Gas

When Figure 1 is examined it is seen that the percentage of carbon dioxide in the swim bladder gas parallels the pH Difference—water aerated and unaerated curve. The pH Difference—aerated and unaerated—shows the increase in the carbon dioxide tension by the dip of the curve, and is logarithmic.

In Tables 5 and 7 and Figures 4 and 5, the relations between the carbon dioxide tension of the experimental water and the carbon dioxide partial pressure of the swim bladder are shown. Without exception the carbon dioxide partial pressure of the swim bladder gas increased with an increase in the carbon dioxide tension of the experimental water. In all cases the carbon dioxide partial pressure of the swim bladder was higher than the carbon dioxide tension of the water. The oxygen partial pressure of the swim bladder gas

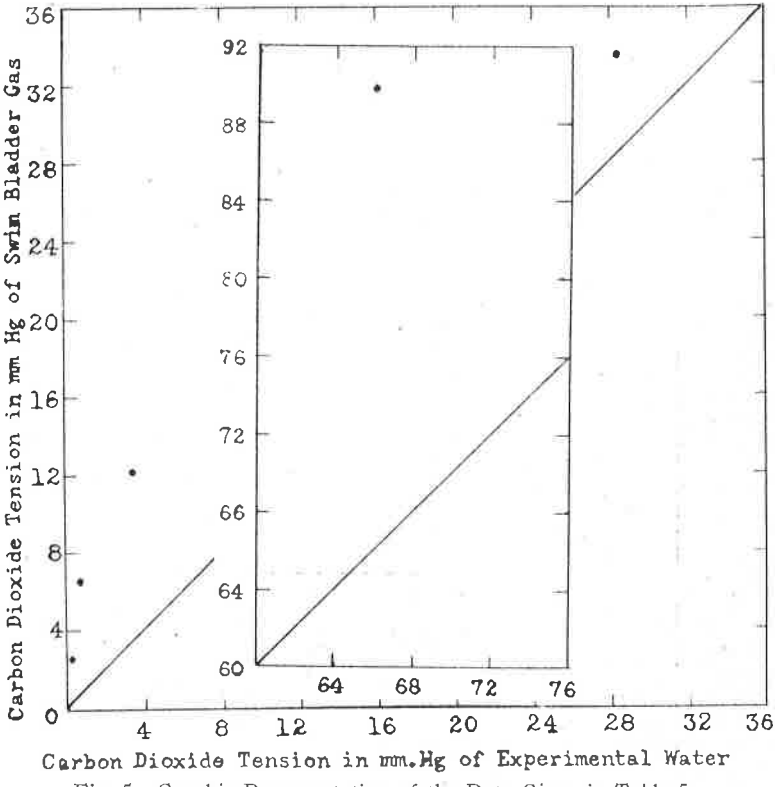

Fig. 5. Graphic Representation of the Data Given in Table 5.

was more variable than the carbon dioxide partial pressure but it also increased with the carbon dioxide tension of the experimental water (Tables 1, 2, 5, 7; Figs. 1, 2, 4, 5). Figure 1 shows more strikingly

the oxygen increase with the carbon dioxide tension of the water than does Table 7. Table 8 shows a striking uniformity in the swim bladder gas complex of fishes taken from any one condition. This emphasizes the inter-relation between the carbon dioxide tension of the water and the carbon dioxide partial pressure of the swim bladder gas. The carbon dioxide partial pressure of the swim bladder gas in turn affects its oxygen partial pressure.

The swim bladder gas complex is modified more by the carbon dioxide tension of the water than by the oxygen content. This is not through an equilibrium but through a mechanism. This is in keeping with the explanation (Powers, 1932) of the deposition of gases into the swim bladder of fishes.

VI. The Gram per cent Hemoglobin in the Blood

The hemoglobin content of the blood was determined by the Newcomer Modified Colorimetric Method. The percentage of error in this method is at best large. From the data obtained, it is seen that the greatest hemoglobin content is found when conditions are most adverse to the absorption of oxygen by the fish, *i.e.*, at the low and the high carbon dioxide tensions. This is not in keeping with the views of physiologists in general. However, this is in keeping with what would be expected of a mechanism the function of which is to absorb and transport oxygen. What is the factor that calls forth red blood corpuscles into the blood? Is it oxygen want?

VII. The Oxygen Consumed in ml. per Kilo per Hour

It is here clearly stated to prevent misunderstanding that the oxygen consumed is not oxygen requirement for basal metabolism. It is the oxygen consumed by the fish under the conditions existing in a particular experiment.

When Tables 1, 2 and 3 are examined it is found that less oxygen per kilo hour is consumed when conditions are less favorable for oxygen absorption, *i.e.*, at the low and high carbon dioxide tensions. This is more plainly seen in Figure 3 where the data of Table 3 have been plotted.

VIII. Conclusions

1. Fishes are able to absorb oxygen at a low oxygen tension through a wider range of carbon dioxide tensions than found in the natural waters in which they live.

2. The pH of the blood of the rock bass, the small-mouth bass and the yellow perch is fairly constant for each species over a wide range of carbon dioxide tensions of the water.

3. These fishes are not able to control the carbon dioxide tension of their blood. The carbon dioxide tension of the venous blood is

determined by the carbon dioxide tension of the water plus the metabolic carbon dioxide.

4. The constancy of the acidity of the blood is maintained at different carbon dioxide tensions by increasing or decreasing the alkali reserve of the blood.

5. The carbon dioxide partial pressure of the swim bladder gas increases with an increase and decreases with a decrease in the carbon dioxide tension of the water. This is not a simple membrane diffusion equilibrium mechanism.

6. In the swim bladder the oxygen partial pressure is more dependent upon its carbon dioxide partial pressure than upon the carbon dioxide tension of the water.

7. There are indications that the hemoglobin is increased and that the rate of oxygen utilization is decreased near the extremes of carbon dioxide tolerance of the fish.

8. The carbon dioxide tension is the effective factor and not the pH of the water. The pH is incidental to the carbon dioxide tension and alkali reserve of each natural water.

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