LIX. ON THE RESPIRATORY EXCHANGE IN FRESH-WATER FISH. Part II: ON BROWN TROUT.

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The influence of diminution of oxygen content of water on fish life has been investigated by many observers, notably by Duncan and Hoppe-Seyler [1893], Chopin and Nikitin [see Kupzis, 1901] and König and Hünneke [1901]. The results generally indicate that fish—of very different varieties, including trout—die when the oxygen content of the water reaches 1–0·4 cc. per litre.

In the method employed by most observers the fish were placed in a closed vessel, and the gradual diminution of the amount of dissolved oxygen was brought about by the breathing of the animals themselves. When asphyxia took place samples of the water were withdrawn and analysed.

The subject has been more recently dealt with by Hans Winterstein [1908], who made use of roach (*Leuciscus erthyrophthalmus*). In his experiments the tension of the dissolved oxygen was reduced by passing through the water a continuous stream of nitrogen, and his method was not very dissimilar from that adopted in the experiments to be described in this paper.

Winterstein found that in the case of roach an oxygen content of 0·7 cc. per litre, or a tension of 2·2 % of an atmosphere, was compatible with life, but when the oxygen was reduced to 0·4–0·5 cc. per litre, *i.e.* a tension of 1·3–1·5 % of an atmosphere, asphyxia took place.

In Part I of this series of papers [1914], we showed that trout use more oxygen per kilo. per hour than some other varieties of fresh-water fish, and further that within the limits of temperature of our climate the oxygen absorbed was a function of temperature. Between 10° and 20° C. the oxygen absorption per kilo. per hour was approximately doubled. As the volume

of water that must be pumped through the gills is inversely as the oxygen content of the water, it seemed likely that the asphyxial tension would be greater at higher temperatures than at lower, and this difference would be dependent on the facts that at the higher temperatures the fish require more oxygen, and that under such conditions more work has to be performed to obtain the oxygen. It seemed of interest therefore to investigate the effects of diminishing tension of oxygen in the case of trout, and to determine the least quantity of oxygen per litre compatible with life at various temperatures.

The method adopted was a modification of that described in our earlier paper. The animals were placed in an hermetically closed vessel containing known volumes of water and air. The air was displaced by a *continuous*

Figure 1.

current of nitrogen, or a mixture of nitrogen and oxygen of known composition. The gas above the water was then pumped through the water continuously. If the gas above the water consisted of nitrogen, the quantity of oxygen dissolved in the water was gradually reduced. Samples of water were withdrawn when the fish entered into the asphyxial condition, or at any desired point prior to this, and analysed. If the gas above consisted of any definite mixture of oxygen and nitrogen, the oxygen in the water was gradually reduced until equilibrium was attained between the gas above the water and that in solution, and the fish could then be observed under any desired oxygen tension.

APPARATUS EMPLOYED.

The apparatus used was that fully described in Part I with some modifications. The essential parts of the apparatus with the modifications introduced

are shown in Figure 1, but the pumping mechanism is omitted, as it was exactly the same as in the earlier experiments.

In these experiments a much larger volume of water was used—about 45 litres—and the gas space above the water was consequently only 2–3 litres in capacity. Only one fish of about eight inches in length and approximately 90–100 grams in weight was used in each experiment. The reason for using only one animal and a relatively very large volume of water was to prevent any appreciable increase in the carbon dioxide pressure during the experiment. The oxygen reservoir in the old apparatus was replaced by a small mixing bottle *G*, of about 0.5 litre capacity, connected through two gas measuring instruments *P* and *Q* with cylinders containing respectively compressed oxygen and nitrogen. In this way pure nitrogen or any desired mixture of oxygen and nitrogen could be delivered continuously into the gas space above the water in *A*.

The excess of gas escaped through the tube *R* which was just closed by a water seal *S*. The meters *P* and *Q* were “Rotameters” made by the Deutsche Rotawerke Co. of Aachen. The “Rotameter” consists of a glass tube slightly increasing in diameter from below upwards and divided in a “litres-per-hour” scale. The gas on entering from the lower opening sets in rapid rotation, about a vertical axis, a cylindrical float on the side surface of which a spiral groove is cut. The float rotates freely and visibly in the gas stream without coming in contact with the walls of the tube, and rises in the tube to a height corresponding to the volume of the gas passing through. The tube is marked with an empirically graduated scale giving the litres per hour. The oxygen meter was graduated to read from 0 to 50 litres per hour, and the nitrogen meter from 0 to 100 litres. Analyses of the mixed gases showed that the instruments gave reasonably accurate results—quite as accurate as would be obtained by the use of any of the ordinary forms of volume meters—and they were much more convenient. In performing an experiment the bottle *A* was filled to the desired point with tap water and left in the thermostat *B* to attain the temperature required. A weighed fish was then introduced; the air in the gas space was displaced by nitrogen or any desired mixture of oxygen and nitrogen, and a current of this gas passed through at a known rate. The gas in the space above the water was then pumped through and through the water by means of the pumping mechanism *I*, *H*, and spray *K* at a rate of about 100 cc. per few seconds. The rate could be varied by adjustment of the pumping mechanism. In this way the composition of the gas dissolved in the water was

gradually changed to that corresponding to the partial pressure of the gases in the space above. This was a slow process, owing to the large bulk of water employed, and, even when pure nitrogen was passed through the gas space, it took several hours, the exact time depending on the rate and efficiency of spraying, to get rid of the oxygen in the water. Measured samples of water were withdrawn by displacement of mercury in the manner described in our former paper, at desired periods. The gases were pumped out of the samples by means of the pump described by Buckmaster and Gardner [1910, 1912] and analysed in the usual way. An example will make clear the mode of calculation. A fish weighing 80 g. was placed in the apparatus at 1 p.m., and the current of nitrogen started at 65 litres per hour. The temperature of the water was 6° C. The pump was now started and at 4.40 p.m. the fish turned over on his back in an asphyxial condition. A sample of water was now withdrawn, measuring 53.5 cc. This sample yielded to the pump gas which after standing over potash to get rid of carbon dioxide measured 1.83 cm. at 14.15° and constant volume (48.65 cc.). After treatment with alkaline pyrogallate it measured 1.76 cm. at 14.10°. The water therefore contained 0.79 cc. of oxygen and 20.02 cc. nitrogen, measured at 0° and 760 mm., per litre. Taking Winkler's value 0.04181 as the absorption coefficient of oxygen at 6° (Landolt, Börnstein, Meyerhoffer's tables) the partial pressure of the oxygen is $\frac{0.79}{10 \times 0.04181} = 1.89$ per cent. of an atmosphere.

The results, we believe, are accurate. They do not depend, as in the experiments described in Part I, on analytical differences before and after a period of time, and are not therefore affected by the sources of error there described.

EXPERIMENTAL RESULTS.

It was easy, of course, to determine the gas content of the water at the point at which the animal entered into the asphyxial condition, but it was very difficult to find out at what point prior to this the animal began to suffer inconvenience.

Observations on the behaviour of trout under diminishing oxygen pressure showed that between 10° and 25° the animals exhibited strikingly similar behaviour. With fully saturated water the animals moved about continually in leisurely fashion, breathing in a normal manner. As the oxygen diminished the respiratory movements gradually increased in rapidity and force, and acquired a dyspnoeic character, as has been described by Baglioni [1908]

and other observers. At the beginning the animals spent the greater part of their time moving about gently. As the oxygen gradually diminished, they became more active, rushing about and jumping into the gas space above the water. These periods of activity alternated with periods of rest at the bottom with the dyspnoeic respiration noted. Gradually the periods of rest became longer relatively to the periods of activity, and ultimately the fish remained for the bulk of the time at the bottom with occasional short rushes to the surface. As the oxygen approached the asphyxial tension they remained all the time at the bottom and ultimately quietly rolled over on their backs. In this condition they died in the course of three or four minutes, but if at this stage oxygen was sprayed through the water they recovered in the course of 5 to 10 minutes, and afterwards appeared to suffer no apparent ill effects. The asphyxial condition evidently takes place within quite small limits of oxygen tension. At or below this they die, but slightly above they recover. Their behaviour can, we think, be explained by the increased volume of water the animals have to pump through their gills to get the necessary oxygen, as the tension of the gas diminishes below a certain value, necessitating the expenditure of a considerable amount of energy.

In quantitative experiments samples of water were withdrawn when the animals turned over on their backs and were in an asphyxial condition. Before reaching this point it was difficult to decide exactly when the animal began to suffer inconvenience, but an arbitrary point was selected, viz. when the animal's periods of rest at the bottom became well established, and the alternating periods of activity were quite short. The point arbitrarily selected was when the periods of rest were about three minutes, the active periods occupying only a few seconds.

At temperatures below 6° or thereabouts the animals behaved rather differently. At these low temperatures the animals, at the beginning, remained quietly at the bottom, with only occasional excursions round the bottle and to the surface. This continued for a considerable period and until the oxygen tension was very considerably reduced. No marked dyspnoeic respiration was noticed. As the oxygen tension further diminished the fish became very active, and ultimately spent most of their time rushing round near the surface, and often jumping and thrusting their noses out into the gas above the water. Asphyxiation generally took place during a period of activity and the fish fell over on their backs and gradually sank. Death took place rapidly, but again if oxygen was sprayed through recovery took

place in a very few minutes. At these low temperatures samples were taken at the asphyxial point only.

The results of a number of experiments are given in Table I.

TABLE I.

Weight of fish	Temp. of exp. ° C.	Arbitrary initial pt.		Asphyxial point		Normal	
		c.c. of oxygen at 0° and 760 in water per litre	Partial pressure in % of 1 atm.	c.c. of oxygen at 0°, 760 in water per litre	Partial pressure % of 1 atm.	c.c. of oxygen 0°, 760 in water per litre	Partial pressure in % of 1 atm.
90 g.	6.4°	—	—	0.79	1.89	8.6	} app. 21 %
90 „	9.5°-10°	3.75	9.86	0.81	2.19	8.0	
80 „	18°	3.19	9.9	1.49	4.62	6.61	
80 „	17°	5.05	15.4	1.37	4.17	6.75	
90 „	24°	3.55	12.33	1.97	6.83	5.89	
88 „	25°	3.18	11.23	2.4	8.49	5.78	
1	2	3	4	5	6	7	8

In a number of other experiments fish were kept for the greater part of a day at tensions which analysis showed were between the arbitrary points given in column 3 of the above table and the asphyxial points without any apparent subsequent ill effects, though judging by their dyspnoeic breathing the animals were in a state of distress. How long the animals could have existed in such a condition we did not attempt to determine. Winterstein kept roach under like conditions at a tension above the asphyxial point for about a week, but we did not push our experiments with trout to such lengths. Experience showed us that trout do not take kindly to being kept in a confined space for long periods, and to keep them healthy a very considerable space per fish is necessary, and it was felt that if conclusive results were to be obtained by keeping fish in the above apparatus for long periods a very large number of experiments would be necessary, and the fish would have to be observed after returning to the aquarium for long periods further. Our trout accommodation did not permit of this.

DISCUSSION OF RESULTS.

A glance at columns 5 and 6 of Table I will show that the oxygen content of the water when asphyxiation took place was much higher than was found by Winterstein in the case of roach. The oxygen content at 6°-10° was of much the same order as that at which he was able to keep roach alive for a week.

In our earlier paper we showed that the oxygen consumption of trout in fully saturated water increases with increase in temperature—within the ordinary limits of temperature it doubles itself for a rise of 10° ,—and consequently the fish would have to pump a much larger volume of water through their gills at a higher temperature than at a lower to obtain the necessary amount of oxygen. Hence as pointed out at the beginning of this paper we should expect the oxygen content at the asphyxial point to be greater at higher temperatures than at lower. This conclusion is fully borne out by the figures in columns 5 and 6. The rate of increase with temperature of the figures in column 5 appears to be somewhat greater than the rate of increase in oxygen consumption under normal conditions of fully saturated water. This we think can be well explained by the increased work done in respiration under low tension of oxygen at higher temperatures. The figures in column 3 at the arbitrary point selected do not appear to show much variation, but we know nothing of the other factors governing them.

From a consideration of our own work and that of other observers there can be no doubt that trout can exist at tensions of oxygen considerably lower than normal, but judging by the type of respiration and the general behaviour of the animal, the trout under conditions between those represented in columns 3 and 5 were in a condition of distress which even in their natural environment would have a very deleterious effect. Such conditions of oxygen content might of course be readily produced by pollution of the natural water.

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