LXXV. RESPIRATORY EXCHANGE IN FRESH WATER FISH.

PART IV.

FURTHER COMPARISON OF GOLD-FISH AND TROUT.

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IN Part II of this series of papers [1914, 2] the behaviour of trout at various temperatures under reduced oxygen tension was described, and measurements were recorded of the quantity of oxygen in the water at the asphyxial point for the various temperatures. It was found that the oxygen content of the water at the asphyxial point was much larger at higher temperatures than at lower, a result to be expected since, as was shown in Part I [1914, 1], the oxygen consumption of trout in fully saturated water increases with the rise in temperature—within the ordinary limits of temperature of natural water consumption doubles for 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.

Gold-fish as we showed in Part III [1922] require much less oxygen than trout and live at an altogether lower plane of metabolism, though their oxygen requirements are also proportional to temperature. It seemed of interest to measure the oxygen tension at the asphyxial point at various temperatures in the case of these animals.

METHOD.

The apparatus described in Part II proved unsuitable for gold-fish, as we found it impossible to reduce the oxygen in the water to the asphyxial tension within a reasonable time. We therefore used a much smaller apparatus based on the same principle, and filled with water which had previously been heated in order to get rid of the bulk of the dissolved gas and allowed to cool in a closed bottle. A six litre vacuum desiccator with slightly domed lid served for the water tank. This was filled to the edge of the ground flange with the water so that the gas space above the water was only that of the shallow dome of the lid. The tubulure of the lid was closed by a rubber stopper carrying (1) a tube leading to the bottom of the vessel and ending in a perforated bulb through which nitrogen could be sprayed through the water, (2) an exit tube for nitrogen bent over so as to dip into a beaker of water

and (3) a capillary tube fitted with a tap through which samples of the water could be drawn off as required. The desiccator stood in a thermostat. The fish were placed in the tank and the air in the water was gradually displaced by a current of nitrogen, which also served to stir the water. The compressed commercial nitrogen used contained about 0.5 % of oxygen; this quantity of oxygen was too much, and the gas was purified by passing through several wash bottles containing strong alkaline pyrogallol. In this way the percentage of oxygen in the nitrogen could easily be reduced to 0.14. A sample of the water was withdrawn for analysis at the asphyxial point, which was taken as that point at which the fish rolled over on their sides and ceased to breathe. The sample was withdrawn by displacement of mercury and analysed by the method fully described in previous papers.

Between temperatures of, say, 10° and 27° the oxygen content of the water could fairly easily be reduced to the asphyxial tension by means of a current of nitrogen, but at low temperatures it was not found possible to reduce the oxygen sufficiently by this method. In experiments at low temperatures the oxygen was displaced as far as possible by a current of nitrogen, and the apparatus was then closed and the fish made to use up the remaining oxygen themselves.

EXPERIMENTAL.

Experiment at high temperature.

Two fish, *A* weighing 53 g. and *B* weighing 58 g., were placed in the partially boiled out water at a temperature of about 22° and the tank put in the thermostat at 28°; nitrogen was then bubbled through the water. At the beginning the fish were very active, *A* being particularly violent for a gold-fish, and attempting to jump out of the water. The respirations of both animals were about 117 per minute. As the oxygen tension decreased the respiration became more spasmodic, irregular and slow. The movements of the fish gradually became less vigorous, and towards the end of the experiment the animals took short intervals of rest with scarcely any perceptible movements of the gills, alternating with short rushes round the vessel mainly near the surface. After about 90 minutes *A* remained on his side without any obvious sign of life and *B* was nearly in the same condition. A sample of water was now withdrawn for analysis, the temperature being 27.2°. The fish were now removed from the experimental vessel and placed in a trough of tap water at laboratory temperature. After two minutes *B* recovered his normal position, while *A* began to breathe and showed slight movements after five minutes, but still remained on his side. The fish were then placed in the store tank in running water and after two to three hours *A* recovered. Next day both fish appeared to be perfectly normal and healthy. On analysis 1 litre of the water was found to contain the following quantities of gas measured at 0° and 760:—free and combined CO₂ 12.25 cc., oxygen 0.422 cc. and nitrogen

8.287 cc. Taking Winkler's value 0.02727 as the absorption coefficient of oxygen at 27.2° (Landolt, Börnstein, Meyerhoffer's tables) the partial pressure of the dissolved oxygen was therefore 1.547 % of one atmosphere.

Experiment at temperature 11-12°.

Two fish, *C* 51 g. and *D* 40 g., were placed in six litres of partially boiled out water as before and purified nitrogen bubbled through the water. Both animals were fairly active at first and their respirations 80-90 per minute, the normal in fully aerated still water being between 40-50 per minute. During the first two hours of the experiment, as the oxygen gradually decreased, the fish became less continuously active, the number of respirations decreased to 40-50 per minute, and the breathing, if we can use this term, became shallower. A noticeable feature was that under these low oxygen tensions the fish indulged in spells of backward swimming. After 135 minutes the nitrogen current was stopped and the fish reduced the oxygen content of the water naturally. The breathing became shallow and very spasmodic and the respiration rate was reduced to 20-30 per minute. At the end of the fourth hour it was noticed that the fish remained inert for periods of 1-1½ minutes. This was followed by a few respirations then another period of inertness and so on. After six hours both fish were on their sides and seemed to have ceased to respire, a sample of water was therefore withdrawn for analysis. The temperature was 11.4°. One litre of the water contained 10.732 cc. free and combined CO₂, 0.397 cc. oxygen and 15.362 cc. nitrogen measured at 0° and 760. Taking the coefficient of absorption for oxygen in water at 11.4° as 0.03686, the partial pressure of the oxygen was 1.077 % of one atmosphere. The fish gradually recovered on being restored to the store tank.

Experiment at low temperature.

It was not found possible at the low temperature of the ice chest to reduce the oxygen tension sufficiently, by means of a current of nitrogen, to incommode, let alone asphyxiate, the fish. After reducing the tension as far as possible by this method we had to rely on the fish to use up the remaining oxygen, and this proved a very slow process.

Two fish, *E* 52 g. and *F* 42 g., were placed in 5½ litres of boiled out water and a rapid current of nitrogen bubbled through for two hours on March 27th. The nitrogen inlet and outlet taps were then closed and the vessel placed in the ice chest and left for a week. On the second day the fish appeared to be quite normal and during the week they remained very quiet and sluggish sometimes near the surface and sometimes at the bottom of the vessel. On April 5th they showed a rolling gait symptomatic of distress. On the morning of April 6th *F* was more or less on his side and in the afternoon appeared to be dead or nearly so. *E* was still breathing occasionally in a spasmodic manner and had a rolling gait as though finding difficulty in keeping upright. The temperature recorded by the thermometer in the upper part of the chest

varied from .3–5° during the week. A sample of water was withdrawn for analysis at 2.35 p.m. on April 6th; its temperature was 2.6°. The quantity of oxygen in the water was less than the limit of the experimental error. On analysis one litre of water was found to contain the following volumes of gas reduced to 0° and 760:—free and combined CO₂ 96.96 cc., oxygen nil, nitrogen 22.97 cc.

Comparison with trout.

Trout normally use very much larger quantities of oxygen per kilo per minute than gold-fish, and at a diminishing oxygen tension behave quite differently. The behaviour of trout was described in detail in Part II [1914, 2, p. 595]. As the oxygen diminished the respiratory movement gradually increased in rapidity and force and acquired a dyspnoeic character. The animals became more active, rushing about and jumping into the air space above the water. These periods of activity alternated with periods of rest at the bottom with the dyspnoeic respiration mentioned. The periods of rest gradually became longer than the periods of activity, and as the oxygen approached the asphyxial tension they remained nearly all the time at the bottom and ultimately quietly rolled over on their backs. In this condition they died in two or three minutes, but if oxygen were at once sprayed into the water they recovered in the course of a few minutes and afterwards appeared to suffer no ill effects. The asphyxial condition in the case of trout takes place within quite small limits of oxygen tension; at or below this tension they very quickly die but slightly above they recover. Gold-fish on the other hand could stand much lower oxygen tensions for any given temperature and are much less sensitive, so that the precise asphyxial tension is difficult to ascertain. This difference is still more marked at low temperatures. In Table I we give a comparison of the measurements for trout and gold-fish.

Table I.

Temperature	8" trout, weight 80-90 g. Oxygen in water at asphyxial point*		5" to 6" gold-fish, weight 45-55 g. Oxygen in water at asphyxial point		Normal water	
	cc. of oxygen measured at 0° and 760 per litre of water	partial pressure of oxygen in per cent. of 1 atmosphere	cc. of oxygen measured at 0° and 760 per litre of water	partial pressure of oxygen in per cent. of 1 atmosphere	cc. of oxygen at 0° and 760 per litre	partial pressure in per cent. of 1 atmosphere
2.6°	—	—	nil	nil	9.49	} app. 21 %
6.4°	0.79	1.89	—	—	8.60	
9.5 to 10°	0.81	2.19	—	—	8.00	
11.4°	—	—	0.397	1.077	7.9	
17°	1.37	4.17	—	—	6.75	
18°	1.49	4.62	—	—	6.61	
24°	1.97	6.83	—	—	5.89	
25°	2.40	8.49	—	—	5.78	
27.2°	—	—	0.422	1.547	5.52	

* Gardner and Leatham [1914, 2].

GLYCOGEN CONTENT OF TROUT AND GOLD-FISH UNDER
VARIOUS CONDITIONS.

In Part I [1914, 1] of this series it was shown that at low temperature, *i.e.* below about 6°, trout appeared to be in a condition, for a time at any rate, akin to that of hibernating animals, and their respiratory quotients were very low. These low respiratory quotients could not be directly connected with nitrogen metabolism, and it was suggested that a possible explanation might be found in the fat. If at low temperature the animals were in a state of hibernation or starvation they might be living on their fat and partially converting it into glycogen and sugar. Some such process might result in a low respiratory quotient, as indicated in the following equation:

In the hope of throwing light on this point comparative estimations of the glycogen content of starving trout at various temperatures were made. The glycogen was estimated by Pflüger's method. The precipitated glycogen, after solution and reprecipitation two or three times, was hydrolysed and the sugar estimated by Fehling. If in sufficient quantity the cuprous oxide was weighed, otherwise it was dissolved in ferric sulphate and the ferrous salt produced estimated by permanganate. The first experiments were done on four-inch trout which however proved too small for the purpose.

EXPERIMENTAL.

Experiments with four-inch trout.

(1) *Control.* Four fish weighing 44.65 g. were kept in running water in a large tank with a gravel bottom. The average temperature of the water was 19°. The fish were not fed though they may have picked up some food. After treatment by Pflüger's method and hydrolysis of the glycogen 0.0013 g. Cu_2O was obtained. Percentage of glycogen was therefore 0.0052.

(2) Four fish weighing 40.6 g. were placed in an open vessel in the ice chest and the water kept well oxygenated by a spray of oxygen. At the end of the first day the temperature of the water was 7°, at the end of the second day 6° and the third day below 5°. The fish were very inert and sluggish and scarcely moved when touched. On the afternoon of the third day they were killed and analysed. 0.0032 % of glycogen was found.

(3) Five fish weighing 57.5 g. were kept in the ice chest under the same conditions as in experiment 2 for six days. On the last day two died and as soon as this was observed the rest were killed and the whole analysed. The final temperature of the water was 3.5°. The percentage content was 0.0022.

(4) Three fish weighing 27.8 g. were kept in the ice chest for two days at reduced oxygen tension. They were then analysed but no glycogen could be detected.

As the fish were too small to give a suitable weight of material without using an inconvenient number of fish the experiments were repeated using eight-inch animals.

Experiments with eight-inch trout.

(5) *Control experiments.* The fish were kept in a large outside tank in running water for several days. They were not fed and the temperature of the water was about 16°. On analysis, fish *A* weighing 89.1 g. gave no trace of glycogen, fish *B* weighing 109 g. was found to contain 0.0012 %.

(6) Two fish were placed in separate earthenware bowls in the ice chest and a spray of oxygen passed through the water by means of circular lead pipes punctured with small holes. The fish were kept at 2.6° for two days; they were very sluggish but otherwise appeared healthy. Fish *C* weighing 114.3 g. contained no trace of glycogen nor did fish *D* weighing 134.3 g.

(7) Two fish were kept under similar conditions to those in experiment (6) for five days. Final temperature of the water was 1.7°. On the fifth day one of the animals turned over and they were therefore cut up and analysed. Fish *E* weighing 101.3 g. gave 0.1179 g. of Cu_2O and contained 0.0469 % glycogen. Fish *F* weighing 108 g. gave 0.0798 g. Cu_2O and hence contained 0.0294 % glycogen.

We do not know what is the limit of accuracy of Pflüger's method for estimating glycogen, but as the conditions—proportion of alkali, duration of hydrolysis, volume of alcohol—were kept as nearly as possible the same in experiments 5, 6 and 7 it is clear that the fish in experiment 7 contained a very much larger percentage of glycogen than the controls. This result seems to fit in with our hypothesis as to the cause of the low respiratory quotients at low temperature. At the same time we cannot pretend that the experiments are altogether conclusive.

GLYCOGEN CONTENT OF GOLD-FISH.

It seemed of interest to ascertain how glycogen content of gold-fish, which did not show abnormal quotients at low temperatures, compared with that of trout.

(8) *Control experiments.* Two gold-fish were taken from the running water tank in which they had been living many months. The tank contained plenty of food. Two fish weighing 73 g. analysed together were found to contain 0.86 % of glycogen. Temperature of the tank when the fish were caught 13–14°. Two fish weighing 90.5 g. taken from water at about 16° contained 1.57 %. Two fish weighing 61.2 g., temperature of tank 18°, contained 1.235 % glycogen.

(9) Two fish weighing together 73.3 g. were kept in an open vessel in the ice chest for seven days; the water was aerated by a current of oxygen and the final temperature was 2–3°. The fish were not fed, and on analysis they contained 1.21 %.

(10) In this experiment two fish weighing 81.8 g. were placed in the apparatus used for determining the asphyxial point and the oxygen tension considerably reduced by bubbling nitrogen through the water. The apparatus

was then placed in the ice chest and kept at about 2° for seven days. Percentage of glycogen was found to be 0.52.

(11) Two fish total weight 61.6 g. were placed in a trough of ordinary tap water through which oxygen was bubbled occasionally and kept in the ice chest for eleven days; the fish were not fed. Percentage of glycogen was found to be 0.54.

(12) Two fish weighing 60.45 g. were placed in six litres of partially boiled out tap water in the asphyxial point apparatus referred to above. Pure nitrogen was passed through the water for half an hour when the nitrogen tubes were closed and the apparatus placed in the ice chest and left for several days. The average temperature of the water was 3.7°. On the morning of the fifth day one fish was found on its side but the others were still alive and in normal position. They were killed and the whole analysed and found to contain 0.024 % glycogen.

These results appear to be in conformity with the low level of metabolism of these fish compared with trout.

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