XLIII. ON THE RESPIRATORY EXCHANGE IN FRESH WATER FISH.—Part I: ON BROWN TROUT.

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In the course of an inquiry, commenced some time ago by one of us, on the toxic effects of various impurities in river water on fish life, data were required on the relative amounts of oxygen absorbed by different varieties of fresh water fish, on the least quantities of dissolved oxygen compatible with healthy fish life, and on the influence of temperature on these quantities. On searching through the literature we were unable to find the systematic information required, and though a number of observations had been made with carp, perch, roach, etc., respiratory exchange had been mainly studied on marine animals. We therefore decided to investigate systematically the common British fresh water fish, and in this paper we give an account of some of our experiments with brown trout (*S. Fario*).

The respiration of fishes was first studied by G. Humboldt and Provençal [1807]. The fishes were placed in a flask of water, the gaseous contents of which had been analysed, and then after an interval a sample of the water was examined and the alteration in its gases determined. The quantity of water was measured, and thus it was possible to estimate the amount of gases absorbed and discharged by fish. A similar method has been used by Vernon [1896] for the measurement of the respiratory exchange in marine invertebrates. Vernon was the first we believe to make use of pumps to determine the oxygen, nitrogen and carbon dioxide. Baumert [1855] improved Humboldt and Provençal's method by passing a stream of water through the flask containing the animals; the gases contained in samples of the water entering and leaving the flask were determined. In recent years a somewhat similar method has been made use of by August Pütter [1908, 1909].

The first elaborate apparatus for measuring the respiratory exchange of fish was devised by Jolyet and Regnard [1876], and was based on the principle of the well-known apparatus of Regnault and Reiset for land animals. This apparatus in improved form has also been made use of by J. P. Baunhiol [1905]. The largest and most complicated apparatus is that devised by Zuntz [1901], who also describes an ingenious apparatus for automatically supplying oxygen as it is used up by the fish.

The method we employed does not differ much in principle from those already used by various observers.

Fig. 1.

The animals were placed in a hermetically closed vessel containing known volumes of water and air, both of which were carefully analysed at the beginning of the experiment. The air was then pumped through the water continuously for a definite period, the oxygen absorbed by the animal being gradually replaced from a reservoir. At the end of the period the air and water were again analysed. The differences in carbon dioxide and oxygen content give the oxygen used by the animal and the carbon dioxide produced in the given time. The apparatus used is simpler than some of the more complex forms described and presents points of novelty.

APPARATUS EMPLOYED.

The apparatus is shown in diagrammatic form in Fig. 1. The receiver A, in which the animal was placed, consisted of a large glass bottle of some

50 litres capacity, and with a neck sufficiently large to admit of the animals being easily introduced. This was fixed on a lead base sufficiently heavy to sink it in the water of the thermostat, and provided on the outer edge with stout upright steel rods, which served the double purpose of keeping the bottle firmly in position and affording convenient handles for lifting the apparatus. The bottle was carefully calibrated by weighing it empty and full of water on a delicate machine capable of reading to a gram. The bottle was placed in a thermostat consisting of a large wooden tub *B* containing water. The temperature of the water in the tub was regulated by means of ice or warm water circulating in a coil of pipe, or by an electric lamp—whichever happened to be most convenient for any particular experiment. The water was stirred by bubbling air through from a cylinder. These accessories are not shown in the diagram for the sake of clearness.

The neck of the bottle was closed by a stout rubber bung, through which passed (1) a capillary tube *C*, for withdrawing samples of air, fitted with a three-way tap, so that the capillary could be filled with mercury; (2) a capillary tube *D* for withdrawing samples of water, also fitted with a tap and reaching to the middle of the water in the bottle; (3) a delicate manometer *E* which could be closed by a tap, shown diagrammatically; (4) a thermometer *F*, of the kind used in incubators, for recording the temperature of the air; (5) a tube leading to the oxygen reservoir *G*; (6) two tubes communicating with the pumping mechanism *H*, *I* and *J*. One of these tubes connected the end of the valve chamber *H* with the air in the bottle and the other, connected to the opposite end of *H*, reached to the bottom of the water and terminated in a perforated glass spray *K*. The mechanism for pumping the air in the bottle *A* through and through the water consisted of a cylindrical valve chamber *H* of about 80–100 cc. capacity containing two valves opening as indicated. Each valve was made by a disc of thin sheet rubber fitting over a brass nose, and proved very effective. The body of the valve chamber was connected to one limb of a large U-tube of the shape indicated containing mercury, the other limb of which was connected by a brass tube to an accurately made single action brass pump *J*. By means of this pump the mercury was made to rock up and down in the U-tube, and by means of the valves in *H* ultimately sucked air from the upper part of *A* and forced it through the spray *K* in the lower part. The amount of displacement of mercury was regulated by the length of stroke of the pump, which was varied by clamping the end of the connecting rod further from or nearer to the axis of the wheel *L*. The wheel *L* was geared by means of a reducing pulley

(not shown in diagram) to main shafting. In most experiments it was found convenient to arrange so that the piston of the pump gave 25 strokes per minute, each stroke displacing approximately 100 cc. of mercury in the U-tube. The side tube *M*, fitted with a tap, afforded a convenient fine adjustment. The oxygen reservoir *G* consisted of an accurately measured tube containing the amount of oxygen required in any particular experiment. Reservoirs of various sizes were provided. The oxygen can be as a rule most conveniently delivered by hand, by raising the companion tube *G'* containing water and opening the tap, a few cc. at a time at regular intervals, or if desired the tap can be left open and the oxygen delivered continuously at any desired rate by dropping water into *G'* by means of a water clock or other arrangement. Usually, however, it was found quite satisfactory to do this by hand.

The volume of the gas in the valve chamber *H*, with its various tubes, and in the left hand limb of the U-tube *I*, when the mercury was drawn, as described later, to a certain mark *Z* on the narrow upper part of the right hand limb, was accurately determined, and also the volume of the glass of the various tubes dipping into the bottle.

In performing an experiment, the bottle *A* was weighed, and water poured in and its volume determined by weighing again. The bottle was then placed in the thermostat and left for the temperature to adjust itself. The temperature of the water was then read, also that of the thermostat, which should be approximately the same. The fish to be experimented on were then taken from their tank, washed with pure water and weighed. They were then put into the bottle. If the temperature of the water in the tank is much different from that to be used in the experiment, the temperature of the fish must be slowly brought to that required in the experiment while in the pure water serving to wash them. This precaution seems to be very necessary. The stopper was then adjusted as in the diagram, and samples of air and water withdrawn from *C* and *D* by displacement of mercury. The apparatus for collecting the sample of water is given in Fig. 2. It consisted of a cylindrical vessel *a* fitted with a three-way tail tap at *b*, and at the other end with a capillary delivery tube *c*: *a* and its tubes were filled with mercury and connected at *b* with the tube *D* of the fish bottle. By suction through the tail of the tap *b* the capillaries were filled with the water and the taps adjusted. The bottle *d* was then fixed to the delivery tube *c* by a rubber tube and by sucking gently at *e* the mercury flowed from *a* and was displaced by the water from the fish bottle. The sampling tube *a* was

accurately calibrated and thus a known sample of the water was obtained quite free from air bubbles and protected from contact with air. After taking samples, the tap *D* (Fig. 1) was closed, and by suction at the tap *M* by means of a small hand pump (not shown) the mercury in *I* was drawn to the point *Z*. The manometer tap was opened to see that the gas in the bottle was at atmospheric pressure, and the tap of the air tube *C* was closed. The thermometer *F* was then read, and the time taken. The pump *J* was now started, and the air of the bottle *A* sprayed through the water. During the experiment the oxygen used by the fish was renewed from *G* as described, care being taken that the pressure in *A* as recorded on the manometer remained approximately the same. At the end of the experiment the pump was stopped,

Fig. 2.

and the mercury in *I* sucked again to *Z* and the manometer read. The temperature recorded by *F* was again taken, and samples of air and water withdrawn as before. The time at which the water sample was taken was noted, and the temperature of the water in *A* and in the thermostat read. The gases in the water samples were pumped out by means of the modified form of Toepler pump without taps or joints, devised by Buckmaster and Gardner [1910] for blood-gas work. The samples of water were introduced into the pump through the barometer direct from the sampling tube (Fig. 2) by displacement of mercury in the manner described in the paper referred to. As it was found better to determine the whole of the carbon dioxide, free and combined, in the water samples, 10–15 cc. of 80 % phosphoric acid were

introduced into the pump before the water samples. This pump, with some further modifications introduced by Buckmaster and Gardner [1912], proved very suitable and convenient for this work, as it was not found necessary to cut the apparatus down after each experiment for cleaning purposes as in the case of blood work.

The gas analysis apparatus employed for the analysis of the air samples and the gases pumped from the water was the well-known form of Frankland, as modified by W. A. Bone.

Various difficulties were encountered in perfecting the apparatus, and of these we may mention one, an apparent "diffusion" of nitrogen through the rubber cork and rubber joints of the apparatus. This was more noticeable when there was any difference of nitrogen partial pressure on different sides of the rubber, as for instance in experiments in which oxygen, or mixtures of oxygen and nitrogen richer in oxygen than air, were used in the bottle. This was obviated by covering the upper and lower faces of the bung with tin foil, and by thickly painting the bung and all rubber joints with the acid proof enamel used by electricians for painting the connections of accumulators. We also found it very necessary to take the utmost care in the measurement of the temperature and pressure of the air in the bottle, and to arrange that the temperatures of the inner water and the thermostat were very nearly the same all through. In some of our earlier experiments we modified the chamber *H* by introducing a device by which the carbon dioxide could be absorbed from the air passing through, and thus the accumulation of carbonic acid in the water prevented. This carbon dioxide was afterwards estimated chemically. It was found however that comparatively little carbon dioxide was swept out of the water by the air current, and that we had merely introduced another estimation of somewhat doubtful accuracy with little if any beneficial effect. Further the experiments of Krepzis [1901], and also of Hans Winterstein [1908], have shown that fish such as perch and roach are not apparently affected by far larger quantities of this gas than were ever produced in our experiments. The procedure was therefore discontinued.

An example will make clear the method of calculation :

Weight of water taken 29526 g.; volume of air above the water at the beginning of experiment 17299.7 cc.; temperature 21.2° C., barometer 762.5 mm. The volume of dry air at 0° and 760 mm. was therefore 15710.9 cc. 13 fish were used weighing 300 grams and the experiment was commenced at 11.15 a.m.

A sample of the air taken for analysis measured 20.09 cm. at 20.42° and constant volume; after treatment with potash it measured 20.00 cm. at 20.40°; and after alkaline pyrogallate 15.86 cm. at 20.32°. The total air therefore contained 69.3 cc. of carbon dioxide, 3234.4 cc. of oxygen and 12407.2 cc. of nitrogen at 0° and 760 mm.

53.5 cc. of the water yielded to the pump 8.39 cm. of gas at 20.25° and constant volume (48.65 cc.); after potash the pressure was 1.53 cm. at 20.20° and after alkaline pyrogallate, 1.13 cm. at 20.20°.

The total volume of water (29526 cc.) therefore contained 2257.2 cc. of carbon dioxide (free and combined), 130.4 cc. of oxygen and 371.7 cc. of nitrogen at 0° and 760 mm.

The experiment was continued until 6.15 p.m., and during this time 278.96 cc. of commercial oxygen, measured at 21.2° and barometric height 762.5, were added. 13.97 cm. of this oxygen at 20.3°, after treatment with pyrogallate, measured 0.42 cm. at 20.3°. The dry gas added contained therefore 245.7 cc. and nitrogen 7.6 cc. at 0° and 760 mm. After the end of the experiment the volume of the gas above the water was 17299.7 cc., the temperature was 21.2° and the barometric height 762.5. The manometer showed a positive pressure of + 0.15 cm. The volume of the dry gas at 0° and 760 mm. was therefore 15698.1 cc.

11.9 cm. of the air at 20.88° measured after treatment with potash 11.74 cm. at 20.7°, and after alkaline pyrogallate 9.42 cm. at 20.8°.

The total volume of gas therefore contained 201.6 cc. of CO₂, 3066.5 cc. oxygen and 12429.9 cc. of nitrogen. 53.5 cc. of the water yielded to the pump 9.01 cm. gas at 20.8° and constant volume (48.65 cc.), which after treatment with potash measured 1.38 cm. at 20.7°, and after alkaline pyrogallate 1.09 cm. at 20.55°.

The total water therefore (29526 cc.) contained 2504.5 cc. CO₂, 95 cc. oxygen and 358.1 cc. nitrogen at 0° and 760 mm.

Adding therefore the volumes of carbon dioxide, oxygen and nitrogen together we get:

	At beginning of experiment	At end of experiment	Difference
CO ₂ ..	2326.5	2706.1	+ 379.6 cc.
Oxygen ..	3610.5	3161.5	- 449.0 "
Nitrogen ..	12786.5	12788.0	+ 1.5 "
Oxygen consumed per kilogram per hour = $\frac{449}{0.3 \times 7} = 213.8$ cc.			
Oxygen consumed per fish per hour = $\frac{449}{13 \times 7} = 4.63$ cc.			
Respiratory quotient = $\frac{379.6}{449} = 0.84$.			

Sources of error. In respiration experiments care should be taken that towards the end of the experiment there should be no oxygen deficiency. It is well-known that fish can stand a moderate diminution of the oxygen content of the water without harm, but it is not advisable to allow the oxygen to diminish during the experiment by more than one-third of its original value, and even this should be avoided if possible. It might be thought that a rapid spraying of the air, in which oxygen was continually renewed, through the water, as in our experiments, would effectively prevent this diminution of the oxygen content of the water. This was not however the case, as diffusion of a gas through water is a slow process. If the oxygen content of a bulk of water such as was used in our experiments is allowed to diminish, it takes a surprisingly long time before equilibrium is again established by even a very vigorous spray. It is important therefore not to place too many fish in a given bulk of water. The experiment quoted in detail above illustrates this difficulty. The number of fish was rather too large, and they used up the oxygen at a somewhat greater rate than could be counteracted by the spraying. Other and more serious sources of error might arise from the consumption of oxygen by organic matter and bacteria in the water, and bacteria introduced into the water by the bodies of fish. The observations of Knauthe [1898] on the oxygen destruction in water in which fish have lived lend strong support to this fear, and any investigation on the oxygen consumption of water animals must reckon with this error and either dispose of it in some way or estimate it quantitatively.

In a botanical metabolic investigation, for instance with germinating seeds, it might be necessary to collect the metabolic products during the course of, say, a week, and from analysis of such products argue as to the changes in the seed. In such a case, obviously, rigidly sterile conditions would be necessary. If, however, in an animal investigation, the necessity for such rigid conditions be postulated, no metabolic researches would be possible, since there are no germ-free animals. Fortunately in our experiments the duration was not so long, and in one, two or three hours and particularly at lower temperatures it did not seem likely that sufficient development of bacteria could take place to introduce any serious error into the results. This conclusion is borne out by the extended series of observations of August Pütter [1909], Zuntz [1901], and others.

To ascertain however, for ourselves, how far serious error due to these causes entered into our results, we made a number of blank experiments under conditions rigidly similar to those in our actual fish experiments, using

both fresh London tap water, and tap water in which trout had been living for from 1·5 to 2·5 hours. The figures are given in the following Table I. As the duration of different experiments varied considerably, the results are reduced to one hour.

TABLE I.

Kind of water	Temperature of water	Duration of expt. in hours	Oxygen lost or gained p. hr. in cc. at 0° and 760	Carbon dioxide lost or gained per hr. in cc. 0° and 760	Nitrogen lost or gained per hr. in cc. at 0° and 760
Tap	10°	3·5	-2·18	+3·94	-1·03
Tap	15	4·7	-0·25	-7·32	-6·60
Tap	16	4·5	-2·13	-5·67	-0·11
Tap	9	2·75	+4·10	+5·21	-33·60
Tap	16·5	3·42	-5·30	+0·63	+13·00
Tap in which fish had lived	15	3·0	-0·07	-6·78	-23·44
"	10	4·17	-2·08	+1·50	+4·80
"	10	3·70	-3·30	-8·00	-4·84
"	10	3·0	+1·97	—	+1·97
"	12	2·32	+3·70	-2·02	-1·84

The differences should of course be nil, but a glance at the table will show that they are irregularly + and —, so that the source of error in question did not seriously affect our experiments. Nevertheless we adopted all the precautions we could think of to reduce such error. London water, as is well known, is very efficiently filtered and is remarkably free from bacteria, so much so that probably the bulk of those in a sample drawn from the tap are post-filtration organisms. It contains of course a good deal of organic matter in solution, but this appears to be in stable conditions, and on the whole it proved a very suitable medium for the work. In all experiments at summer temperatures we took the precaution to keep the water used in fully oxygenated condition for a day or two in closed vessels before use. The fish were always washed by being allowed to swim about in a considerable bulk of pure water before being placed in the experimental bottle.

The method of pumping out the gases from a small sample of water by means of the blood-gas pump and analysis by the method described proved very satisfactory, and the results of consecutive experiments were sensibly identical. The errors set out in the above table may appear to be very large, but this must of necessity be the case in metabolic experiments of this kind, however accurate the analytical methods adopted. A very minute error in the analysis of the gas from 50–100 cc. of water appears of considerable magnitude when the results are multiplied up to give the gas in 30 litres.

The errors in the oxygen and nitrogen we believe are largely due to the difficulty of determining the exact average temperature of the gas above the water. The carbon dioxide determinations, as will be seen from the table, are less satisfactory than those of oxygen. This is mainly due to the difficulty of estimating very exactly the small quantities of carbon dioxide in the air above the water. Further, in nearly all our experiments the second sample of water was put into the same pump which had been used for the first sample, and which still contained the pumped out water of that sample. The second sample was thus diluted, prior to pumping by its own volume of water, and thus the difficulty of pumping out every trace of carbon dioxide was somewhat increased.

In most of the experiments, as in the one quoted above, when a considerable volume of oxygen was absorbed and carbon dioxide produced, the errors are not serious and make very little difference in reckoning the respiratory quotients. In some of the low temperature experiments, however, when the volume of carbon dioxide produced is small the effect is considerably more marked.

Other chemical methods of estimating carbon dioxide did not seem likely to give as satisfactory results, and indeed are rather put out of court by the fact that brown trout appear to secrete some acid substance which affects the composition of hard water. We have not yet investigated the nature of this substance, nor are we aware of any experiments by other observers. We repeatedly noticed, however, that four or five eight inch trout in some 30-40 litres of water, kept well aerated for a couple of hours, produced enough of this substance markedly to interfere with the action of potassium chromate used as indicator in the estimation of chlorides by silver nitrate, an effect not observed in the case of fish such as dace. The production of this acid appears to be well known to the trout breeders, and Mr F. G. Richmond informs us that our observation fits in with his experience of travelling fish in hard and soft water. The hard water apparently neutralises acids given off by the fish, and this secretion from brown trout is more acid than that from other varieties. We have not yet had an opportunity of experimenting with other varieties than brown trout.

EXPERIMENTAL RESULTS.

Two series of fish were taken for experiment, approximately four inches and eight inches long respectively. The small fish were fairly uniform in weight, 20-24 grams each, but the large fish showed very considerable variations,

70–150 grams. The small fish were kept in a tank in the laboratory, and the temperature of the water was pretty constant—about 14°–15° C. They were fed on a commercial fish food, consisting mainly of ground up fish, and appeared to eat well.

The large fish were kept in a tank outside, and as the experiments were performed during the winter months, the average temperature of the water was lower and varied considerably. They were fed on live minnows, and this was the only food they would take at this period.

The results of our experiments are gathered together in the following Table II. We do not give the full protocol of each experiment, since, as the same apparatus was used all through, the details do not differ very much from those in the example already given in full.

DISCUSSION OF RESULTS.

Influence of temperature. The oxygen absorbed appears within limits to be proportional to the temperature, and is approximately doubled for an increase of ten degrees. This is best seen in the case of the small four inch trout, partly because under the conditions of experiment each small animal had a freer range of movement, but mainly because the same animals were used in each experiment and in relatively large numbers, so that the influence of individual idiosyncrasies was to a considerable extent got rid of.

In the case of the larger fish, experiments 5–20, the results are very variable. The eight inch trout differed from one another much more in weight than the four inch, only two or four animals were used in each experiment, and in different experiments different animals were of necessity used. These were taken from the stock in the tank indiscriminately, the only precaution being that only healthy fish were used. Our tank accommodation and water supply did not permit of our keeping more than eight or ten of these fish at a time in healthy condition, in fact it was found better to have a smaller number than this. The fish were therefore frequently renewed from the farm as they died off or were killed for experimental purposes. Some of the fish used were therefore fresh from the farm and had not yet got used to their surroundings, others had been in the tank for a considerable time. This would no doubt have an influence. Most of the experiments were done in the winter months, and the animals were kept in an open tank with running water outside. On the very cold days the fish were sluggish and did not feed much, but even in the warmer periods when they appeared more ravenous they would only take live minnows. In the spring and summer they would

TABLE II.

No. of experiment	Temperature of water °C.	No. of fish	Total weight of fish in grams	Average weight per fish in grams	Oxygen absorbed per kilo per hour in cc. at 0° and 760	Oxygen absorbed per fish per hour in cc. at 0° and 760	Carbon dioxide evolved per kilo per hour in cc. at 0° and 760	Respira- tory quotient	Remarks
1	7.5- 7.8°	12	276.6	23.0	89.04	2.05	49.4	0.55	Experiments performed end of May and beginning of June, and fish kept indoors and fed on commercial fish food.
2	15.8-16°	11	264.0	24.0	117.40	2.82	129.0	1.09	
3	21.2-21.4°	13	300	23.0	213.80	4.63	180.8	0.84	
4	25 -25.1°	11	240	21.8	258.87	5.64	209.2	0.81	
Experiments with eight inch brown trout									
5	4.1- 4.5°	4	423	106	144.36	15.26	32.18	0.22	Large fish in a tank kept outside in running water.
6	6.3- 6.6°	2	235	118	93.21	10.95	11.43	0.12	Fresh fish.
7	5.9- 7.2°	2	235	118	105.26	12.37	1.13	0.01	Dec.
8	6.3- 6.5°	3	285	95	130.6	12.40	17.79	0.14	Dec. Same fish as in (6).
9	6.3- 6.4°	3	322	107	71.58	7.68	27.78	0.39	Nov.
10	6.1- 7.0°	4	354	88.5	96.44	8.53	93.19	0.96	Dec. Fish in tank several days and tank at same temp. as experimental water
11	6.3- 7.4°	5	403	80.6	76.20	6.14	74.3	0.98	Dec.
12	10°	3	285	95	230	21.85	172.4	0.75	Jan.
13	12.5-12.6°	3	322	107	149.76	16.07	129.3	0.86	Nov.
14	16 -16.6°	4	325	81	206.45	16.77	131.5	0.64	Dec.
15	16.8°	3	267	89	224.78	20.45	213.6	0.95	Nov. New fish.
16	16.1-16.6°	4	384	96	188.44	18.09	118.1	0.63	Nov. Taken on warm day following a spell of cold in which they had not been feeding.
17	20.1-20.4°	3	307	102	189.5	19.39	147.8	0.78	Jan.
18	22 -22.7°	3	267	89	218.1	19.40	103.8	0.47	Dec.
19	21.4-22.2°	4	410	102	186.06	19.0	160.3	0.87	Nov. Same as (15). One fish died during experiment towards end
20	21.9-22.3°	4	367	92	223.3	20.5	204.5	0.91	Nov.

readily take other food such as worms, flies, etc., but not in the winter. If we take experiments 5-11, done below 7° C. the results are very variable and show nothing as to the influence of temperature, similarly if we take 14, 15 and 16 at 16°, or 17-20 at 22°. If, however, we take the average value of each series, as shown in the following Table III, it will be seen that the

TABLE III.

Number of experiments	Temp.	Average oxygen consumption cc. per kilo per hour
7	4.1- 7.4°	102.5
3	16.1-16.8°	206.5
4	20.1-22°	204.2

oxygen consumption at 16° is double that at about 6°, i.e. it is doubled for a rise of 10° as in the case of the four inch fish. On the other hand the increase appears to stop at this temperature, and the average at 20-22° is approximately the same. It would appear from this that the organism in the case of the larger fish ceases to react to temperature above the limit of 16 or 17°, but it would seem probable that this is more apparent than real, for as is well known, and as indeed we shall ourselves show in a subsequent paper, fish can live and keep well for long periods at a very much lower level of oxygen partial pressure than that corresponding to full saturation; and further the large fish were somewhat restricted in their movements in the experimental tank compared with the small animals, and were consequently less favourably situated for assisting the pumping of water through the gills by swimming movements. If we make the assumption that a fish absorbs the whole of the oxygen from the water passing through its gills, then calculating on the basis of Winkler's figures [1905] for the oxygen content of water saturated with air at various temperatures, a four inch trout at 7-8° would need to pump 242-248 cc. of water through its gills per hour, or about 4 cc. per minute, while at 21° he would need 743 cc. per hour or 12.4 cc. per minute, and at 25°, 977 cc. per hour or 16 cc. per minute. On the same basis an eight inch trout would pump, at 6°, 1207 cc. per hour or 20 cc. per minute, while at 16° he would require 2676 cc. per hour or 44-45 cc. per minute. The above assumption is perhaps scarcely warrantable, so that these figures must be regarded as minimum values. It is therefore perhaps not surprising that the large fish should apparently cease to react to increases of temperature at a lower temperature than the small fish. On the other hand higher temperatures appear to be dangerous or fatal to trout. On

attempting to determine the oxygen absorption for eight inch trout at 25° C., to compare with result No. 4 for four inch trout, of the three fish used in the experiment one died in about two minutes, though the water was fully oxygenated, and the other two turned over on their backs in about ten minutes, and would no doubt have died, but on adding cold water they recovered completely and rapidly and appeared quite well the next day. This result was not due to any sudden change of temperatures as the fish were carefully warmed up beforehand during the course of one hour. It must be noted, however, that the small fish which stood the 25° temperature during an experiment of several hours duration without apparent harm had been living for some days in a tank in the laboratory in relatively warm water (14–18°), whereas the large trout had been living in a tank outside at ordinary winter temperatures of 9–10° or thereabouts. It is possible that had they been living under summer conditions for a long time they might have stood the high temperature better. In another experiment owing to the accidental use of an imperfect thermometer, four eight inch fish were plunged into well oxygenated water at 33° C. The fish gave a few violent leaps and collapsed, as one might perhaps imagine a warm blooded animal doing on falling into boiling water, and were all quite dead in under one minute. The water afterwards was found to be quite frothy as though it contained some saponaceous material.

Influence of size of animal. The oxygen absorption appears to be approximately proportional to the body weight at a given temperature but we have not sufficient data for small animals to show precisely what the average relation is. As far as the experiments go, the rate of oxygen used per kilo per hour at 7° for four inch and eight inch fish is 1 : 1.13, and at 21–22°, 1 : 0.95; at 16° the ratio is different 1 : 1.75, but probably the average of a larger number of experiments would bring this mean to 1 : 1.

Respiratory quotients. If we except experiments 5–9 done at low temperature, the average value of the respiratory quotient is 0.81. The variations lie between 0.5 and 1.01, but the majority lie round 0.8. This value is of much the same order as that found for carp and tench, and for various kinds of marine fish by Jolyet and Regnard, and others, and the variations fall within similar limits (*vide* table in Schäfer's *Text-book of Physiology*, vol. 1, p. 703).

In experiments 5–9 done at low temperatures, and on fish which had been living in a tank outside during a spell of cold weather, the respiratory quotient shows a remarkable drop, and its average value in the five experiments is only 0.18. We do not profess that any of the five individual values

possesses any high degree of accuracy, for the quantities of carbon dioxide produced are very small and therefore the error of experiment would be very large, but we think there is no doubt about the drop in the value of the quotient, if for no other reason than that after the first anomalous result the utmost care was taken with the other experiments. The change appears to take place somewhere about 6-6.5° C., and below this temperature the fish appear to be in a condition akin to that of a hibernating mammal. We noticed that the fish in our tanks during cold weather were very sluggish and as far as we could tell did not eat. They left minnows in the tank severely alone, whereas on somewhat warmer days they took them readily. We do not know whether this is the experience of other observers, or whether fish in a state of nature get into this condition. We have, however, made many inquiries amongst fishermen, river watchers and others interested in fish, but have not been able to obtain any definite evidence on the point.

In the hope of ascertaining whether this phenomenon had anything to do with the nitrogen metabolism, estimations were made of the saline and "albuminoid ammonia" contained in the water before and after fish had been kept in it for definite periods at various temperatures (by the conventional methods of water analysis). The results are given in the following table :

TABLE IV.

Temp. of water	Wt of fish used in g.	Saline ammonia given off per kilo per hour	Albuminoid ammonia per kilo per hour
3-4°	329	0.0038	0.002
4-5°	345	0.0058	0.0007
1.8-2.2°	354	0.0058	0.003
	mean	0.0051	0.0019
6.4-6.8°	341	0.0071	0.0045
6.9-7°	383	0.0087	0.0012
	mean	0.0079	0.0028
13-15°	352	0.0064	0.0049
13.7-13.8°	351	0.0118	0.0042
	mean	0.0091	0.0045
21.6°	366	0.0162	0.0039
20.6-21.2°	346	0.0187	0.0061
	mean	0.0117	0.0050

The figures in the last column do not represent of course the total nitrogen excreted, other than saline nitrogen, but merely that portion of the soluble organic nitrogen evolved as ammonia on boiling with alkaline permanganate under conventional conditions. The figures throw no light on the cause of

the low respiratory quotients at low temperatures, though they generally confirm the conclusion that the metabolism of fish increases with rise of temperature.

Estimations were also made of the saline ammonia, urea and water-alcohol soluble substances containing amino-nitrogen in the whole fish in case of animals kept for about a week at definite temperatures. For this purpose the animal was rapidly cut into lumps which were dropped into hot water, rendered faintly acid with acetic acid (1 cc. 50 % acetic per litre). After heating on a water bath for about half an hour, the lumps were pounded up in a mortar and repeatedly extracted with hot acid water, after each extraction the solid matter being filtered on the pump. The aqueous extracts, usually about a litre in volume, were evaporated to small bulk *in vacuo* at a temperature below 40° and made up to a definite volume. Aliquot portions were taken for ammonia and urea estimations which were made by the urease method as described by Plimmer [1914]. The remainder of the solution was mixed with ten times its volume of absolute alcohol and allowed to stand overnight. The alcoholic liquor was then filtered, the insoluble residue washed with alcohol, and after being rendered alkaline, the alcohol was distilled from the filtrates *in vacuo*. The alkaline aqueous solution so obtained was then made up to a definite volume and analysed for amino-nitrogen by van Slyke's method. The results are given in Table V.

TABLE V.

Temp. average during week	Amino-nitrogen of alcohol soluble substances in g. of N. per 100 g. of fish	Urea-nitrogen in g. per 100 g. of fish	Saline-nitrogen as ammonia in g. per 100 g. of fish
4°- 5° C.	0.082	0.00028	0.0045
4°- 5° C.	0.077	0.0033	0.0194
8°- 9° C.	0.0886	0.0007	0.0139
9°-10° C.	0.081	nil	0.015
10° C.	0.0798	0.0027	0.0226

How far the urea and ammonia figures have any value we cannot say, owing to the risk of hydrolysis during the prolonged vacuum distillations.

The amino-nitrogen figures are very constant at the various temperatures, and the ammonia figures fairly so. We think it is evident from these experiments that low respiratory quotients have no direct connection with the nitrogen metabolism or the quantities of soluble nitrogenous substances in the tissues.

A possible explanation may perhaps be found in the fat. If at the low temperatures the animals are in a state of hibernation or starvation, they may 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 :

Owing to the fact that we had no spell of frosty weather this year after the end of January we were unable to test the validity of this hypothesis, but we hope to be able to investigate this question more closely during the coming winter.

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