

Preliminary Observations on the Relative Importance of the various Factors Responsible for the Death of Fishes in Polluted Waters.

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INTRODUCTION.

WITH the growth of modern industry the problem of the influence of polluted waters on the bionomics of fishes is becoming very important. Fortunately, biologists anticipated this and many works on the subject have appeared, especially during the last twenty-five years. The polluting substances can be divided into two main classes, namely, (i) those that are directly poisonous to organisms, e.g. gas liquor, sulphites, oils, etc., and (ii) those that give rise to poisonous substances or otherwise make the water undesirable after putrefying, e.g. organic matter in solution and the decomposing bodies of plants and animals. The substances in the first category act in a relatively straightforward manner, and numerous investigators have studied them from several aspects (Shelford, '17; Matthews, '04; Powers, Wells, '15, etc.); it is with regard to those in the second class that many questions are yet unsettled and the present communication deals. We roughly know that the effect of the putrefying substances is to increase the hydrogen ion concentration and decrease the oxygen content of water. It has also been suggested that the by-products of putrefaction themselves may be poisonous to animals. It is still an open question as to which of these factors is chiefly responsible for the death of fish; some investigators emphasize the influence of hydrogen ion concentration, others the importance of the deficiency of oxygen, and so on. One reason for this state of things seems to be that practically no systematic attempt has been made so far to isolate the various factors, and note their influence on the organisms separately. Therefore it was thought desirable to undertake a study on these lines, and some preliminary experiments have been performed, which are reported in the following pages.

A short bibliography of papers concerning factors affecting freshwater life is given at the end of this communication.

MATERIAL AND METHODS.

The freshwater fish, *Gasterosteus aculeatus*, the common "stickleback," found round about Plymouth, was chosen for the experiments. It is quite a hardy species. This selection is against the suggestion of Shelford ('18), who thinks that a sensitive individual should be taken for such investigations. But in the opinion of the writer, a hardy species affords a more definite and hence more reliable reaction. Moreover, if a certain factor is found to be toxic to a strong race, it should be all the more toxic to sensitive ones, and therefore have a wider application.

The average weight of the above fish was 0.8-1.2 gms. ; some specimens weighed as much as 1.8 gms. Fishes weighing 0.30-1.5 gms. behaved similarly, and all the experiments were performed on them ; those below 0.30 gms. or above 1.5 gms. died rather quickly.

To start with, three substances, all proteins, namely, peptone, albumen of eggs, and casein of milk, have been examined as putrefying substances.

The hydrogen ion concentration determinations were made by the colorimetric methods. The oxygen content was measured by Winkler's method. The "alkali reserve" was determined by titrating with 0.01N. H_2SO_4 , using methyl orange as indicator.

EXPERIMENTAL.

OPTIMUM HYDROGEN ION CONCENTRATION AND CO_2 PRESSURE.

On an average the pH of the waters in which sticklebacks were found in the field was 7.5-8.0 (not corrected for the salt error). The alkali reserve of these waters was 0.0045 N. In the Laboratory these fishes lived quite comfortably between pH 6.0-8.5. Outside this range, an increase on the acid side was more harmful than on the alkaline one. In water at pH 4.5 "stickles" died in about twelve hours, at pH 3.0 they did so within three quarters of an hour.

Jewell ('24) found several freshwater fishes flourishing in waters having pH 4.4.

As regards the pressure at which CO_2 becomes harmful it was noticed that as long as the concentration of this gas was not above 10.0-13.0 c.c. per litre the fish did not suffer any harm. Many freshwater fishes have been found to withstand even a higher concentration of CO_2 , up to 40-50 c.c. per litre (Reuss, '10 ; Wells, '13, etc.).

RESISTANCE TO LOW CONCENTRATION OF OXYGEN.

As elsewhere shown in detail by the present writer (p. 741 of this Journal), if pH and other conditions are suitable, sticklebacks can live without any harm till the oxygen content of water goes down as low as 0.25-0.50 c.c. per litre.

It appears that the power to withstand low pressures of oxygen is quite common amongst the freshwater fishes. Birge and Juday point out several cases in which a concentration of 1.0 c.c. per litre apparently did not prove dangerous to the individuals. Powers ('21) cites numerous authors to show that fishes do not suffer from oxygen want till its concentration reaches 1.7-0.4 c.c. per litre.

COURSE OF CHANGES IN THE PHYSICAL AND CHEMICAL CONDITION OF WATER ON THE ADDITION OF PUTREFYING SUBSTANCES.

Observations on this subject, as mentioned above, are based on the decomposition of peptone, albumen, and casein, under both aerobic and anaerobic conditions. These substances were dissolved in Plymouth tap water (pH about 7.0 and alkali reserve .0005N) in the proportion of 1 gram to a litre. The solutions subjected to aerobic conditions for the growth of bacteria were kept in wide shallow bowls of 6 litres capacity and were aerated at intervals of four hours in the daytime (9 am.-11 p.m.), no aeration being applied during the night. In the mornings, while traces of oxygen were often found in the solutions, the oxygen had sometimes been exhausted altogether. Winchester bottles were used for the solutions, which were kept out of contact with the atmosphere so that anaerobic putrefaction would proceed. All cultures were kept at room temperature, 65°-70° F.

Hydrogen Ion Concentration, etc.

The first noticeable result of decomposition is the production of CO₂ and organic acids which increases the hydrogen ion concentration. As above mentioned, the aerobic solutions were aerated frequently, which naturally interfered with the normal course of pH change. Therefore to ascertain accurately the changes in this factor, two separate solutions of peptone and casein were kept undisturbed in bowls, 6 litres and 500 c.c. capacity respectively. The data obtained from these solutions are given in Table I, an examination of which will show that during the first three or four days the pH fell, being 6.5 (casein) at the end of that period. Then it began to rise steadily and went up to 8.0. After reaching this point, the pH fell again and remained about 7.6 till the experiments were discontinued at the end of the forty-fifth day. The course of these changes resembles closely that observed in the solutions (Table II) which were aerated frequently.

In the anaerobic solutions, as in the above, the pH went down during the first few days, and did so much more, reaching the figure 6.0-6.1. Then it began to rise, but the rate of rise was much slower than that in the aerated solutions (Table II).

The interesting point that the above account brings out is that the pH after falling for a short time begins to rise, suggesting thereby that basic substances are being produced in the putrefying solutions. As will be discussed at a later stage, this has a very significant bearing on the

TABLE I.

COURSE OF CHANGES IN PH.

Solutions in wide shallow bowls. Undisturbed.
Room Temperature.

Age of solution in days.	Peptone pH.	Casein pH.	Remarks.
1	7.15		
2	6.8	6.5	
3	6.95	7.0	Oxygen totally exhausted in Casein solution.
4	7.2	7.6	Oxygen totally exhausted in Peptone solution.
5	7.3	7.65	
6	7.4	7.75	
7	7.45	7.8	
8	7.5	7.9	
9	7.55	8.0	
10	7.55	8.0	
11	7.65	7.7	
12	7.7	7.65	
13		7.7	
14		7.8	
18	7.7	7.65	
21	7.65	7.7	
28	7.75	7.7	
33	7.8		
45	7.7		

problem under investigation. The production of alkalinity during bacterial growth is a well-recognised phenomenon, Reddie ('23) having observed it in numerous cases.

The second point that may be emphasised in connection with the above-described variation in hydrogen ion concentration is that the pH does not go much below 6.0, both in the aerated and anaerobic solutions. As the

writer showed in the case of hay infusions ('26), the lowest pH that is reached in fermentations depends upon the alkali reserve of the water used, being minimum in the case of distilled water. The Plymouth tap water in having the low alkali reserve of 0.0005N is almost similar to distilled water. Hence it is fair to conclude that in natural waters,

TABLE II.
CHANGES IN pH IN AEROBIC AND ANAEROBIC SOLUTIONS.

Age of the solution in days.	Aerobic.				Anaerobic.		
	Peptone A.	Peptone B.	Albumen C.	Casein D.	Peptone E.	Albumen F.	Casein G.
3	6.6			6.7			6.5
4		6.6				6.4	
7			7.0	7.7			
8		7.3			6.45		6.2
9	7.4						
10				8.1	6.55	6.3	6.1
11			7.25	8.1			
12	7.65					6.15	6.3
13	7.75	7.8	7.3		6.65		
14		7.85	7.5	7.85			6.5
17		8.1	7.9			6.2	6.65
19					6.9	6.35	6.85
21					7.0	6.5	6.85
22		7.75	7.85			6.6	7.2
24					7.25	6.5	
26					7.45	6.5	7.2
30						6.5	7.8
34						6.6	7.7
38						6.6	

which invariably have some alkali reserve, the pH under similar conditions will not reach even 6.0.

Oxygen Content.

The changes in the oxygen content were also observed in the solutions kept undisturbed in wide bowls. The solutions when started had about 6.5 c.c. per litre of oxygen. There was a steady fall, and within three to four days the whole of the oxygen had been exhausted. When the supply

of oxygen was renewed, as in the case of the aerated solutions, it was noticed that the rate of oxygen decrease was not greatest in the first few days, but after the expiry of about a week. Moreover, this rate was not the same in the different solutions, being far higher in the fermenting casein than in the others.

Production of Toxic Substances.

The last important change to be observed in the nature of the water is the appearance of toxic substances, which are obviously produced as a by-product of putrefaction. To study this factor, the killing power of each solution at different stages in its growth was ascertained after it had been thoroughly aerated and its pH adjusted at 7.0-8.0, to exclude the harmful influence of hydrogen ion concentration, high carbon dioxide pressure, and the deficiency of oxygen. These experiments were performed in the 500 c.c. bowls, and generally each observation was based on the dying time of three specimens.

The data under this section are given in Table III. An examination of the same will show that the solutions become most poisonous when they are 7-15 days old, the anaerobic ones taking a few days longer; then they gradually lose their toxic power. Of the three kinds of solutions studied, namely, peptone, albumen, and casein, the last named is the first to acquire toxicity and becomes most poisonous, its shortest killing time being only half an hour, as compared with two and six hours for peptone and albumen respectively. It is interesting to add that the putrefying substance remaining the same, a solution becomes equally toxic whether aerated at intervals or kept under anaerobic condition, though under the latter, as mentioned above, it acquires toxicity more slowly.

As to the nature of the toxic substances, this is rather a complex problem and is left for further research, only a few remarks being made here. The fact that the solutions after becoming highly poisonous gradually lose their toxic power suggested that the toxic substances may be of a volatile nature. To verify this hypothesis the following experiments were performed: about 500 c.c. of the various solutions were boiled to half the volume, cooled, and water subsequently added to bring them to their original volume. Then they were aerated and their pH was adjusted at 7.0-8.0. The killing power of the solutions so treated was ascertained. It was very much reduced; for example, the peptone solution which killed the fish in two hours before the above treatment, did so in twenty-five hours after it. In this connection it was also thought desirable to ascertain the effect of the distillates from solutions which had putrefied and were distilled in both alkaline (pH 8.5) and acid (pH 4.0)

TABLE III.

APPEARANCE OF THE TOXIC SUBSTANCES IN THE 0.1% SOLUTIONS.

Age of the solution in days.	Killing time in hours after thorough aeration and adjustment of hydrogen ion concentration.						
	Aerobic.			Anaerobic.			
	Peptone A.	B.	Albumen C.	Casein D.	Peptone E.	Albumen F.	Casein G.
3	130			18			
4		72	60			18	
5							
6				2		18	
7			48	$\frac{1}{2}$			5
8		72		$\frac{3}{4}$	30	20	$\frac{1}{2}$
9	12				30		
10	3			6	30	20	$\frac{1}{2}$
11			48	7			
12	2						
13	$2\frac{1}{2}$	12	18		30		1
14		3					
15							3
16							
17			6		28		
18		6	9	10		20	
19		7					
20						7	
21							
22				15		3	
24		10			18		
25		11	10				
26		11			9		
27			10		4		
28							
29							
30					4		

conditions. In Table IV are given the killing times of the solutions (before distillation), of the residues and of the distillates. It will be observed that the residues in some cases are only very slightly toxic, while the distillates were decidedly toxic, although less so than the original putrefied solutions. They should have killed the fish almost instantly if all the toxic substances were volatile. This means that only some of the toxic substances are volatile, and that the residues lose the toxic power probably due to the mere action of heat. To examine this possibility some putrefied casein solution was heated at 70° C. for twelve hours; after this treatment the solution did not kill the fish in six days even (when the experiment was stopped), while originally its killing power was only one hour.

The other fact that the above experiments reveal, and which is likely

TABLE IV.

Putrefying substance.	Killing time in hours.			Remarks.
	Solution.	Distillate.	Residue.	
Peptone	15	48	79	Distilled in alkaline condition.
Peptone	3	5	60	Distilled in acid condition.
Casein	$\frac{1}{2}$	18	240	Do.
Casein	$\frac{3}{4}$	48	300	Distilled in alkaline condition.
Albumen	6	12	60	Do.

to have a bearing on the problem of the nature of the toxic substances, is that the stage of highest toxicity in the case of each putrefying solution was preceded by a period of rapid oxygen consumption.

RELATIVE INFLUENCE OF VARIOUS FACTORS.

After describing the optimum conditions and those that occur in water containing putrefying substances, some concluding remarks may be made as to the comparative significance of the various factors concerned.

The fact that pH does not go below 6.0 and that after falling for a short time begins to rise steadily, suggests strongly that hydrogen ion concentration as such has not got such a great importance in polluted waters as many investigators seem to think. On the other hand, carbon dioxide accumulates as has been described above. Sometimes, as in

the case of solutions putrefying under anaerobic conditions, a high pressure of CO_2 may occur sufficient to prove harmful to fish life, especially in still water. It is legitimate to emphasize its importance and urge the field worker to measure this gas even when the pH may be optimum.

All the infusions at some stage showed a high rate of oxygen consumption; therefore this gas, in spite of the fact that many freshwater fishes can withstand its low concentrations, is likely to be a factor of importance in polluted waters, especially in the bottom layers.

But the most important fact that the above experiments bring out is the great significance possessed by the toxic substances which appear as by-products of putrefaction, a factor hitherto almost ignored. Water containing any of the putrefying substance examined becomes highly toxic and kills the fish in $\frac{1}{2}$ -6 hours, even when it is saturated with oxygen has an optimum pH and a low pressure of CO_2 . But, as has already been pointed out, the toxicity varies in the case of different substances, and it will therefore be a fruitful problem to investigate, from a comparative point of view, the toxicity resulting from the decomposition of various proteins, carbohydrates, etc., which are likely to be thrown in waters inhabited by food fishes. On the other hand, the problem of the nature of the toxic substances is bound to be of great interest to bio-chemists.

In conclusion, I must thank Dr. E. J. Allen, the Director of the Plymouth Laboratory, and Dr. W. R. G. Atkins, the head of the Physiological Department, who arranged for all the facilities for performing the experiments. My indebtedness is also due to Mr. H. W. Harvey of the same Laboratory, who took a keen interest in this investigation and made some valuable suggestions. Mr. H. O. Bull rendered me assistance in various ways, for which I am grateful.

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