The Effect of Oxygen and Carbon dioxide On the Development of Certain Cold Blooded Vertebrates

By

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THE EFFECT OF OXYGEN AND CARBON DIOXIDE ON THE DEVELOPMENT OF EGGS OF THE TOAD, *BUFO AMERICANUS*[1]

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Introduction

The success of a species in a given habitat depends largely upon the part which environment plays in the development of the individual, and the location of the most sensitive periods in the life history. The toad, *Bufo americanus* Le Conte, lives and breeds in warm, temporary, stagnant pools, thick with suspended mud and decaying matter, subject to many fluctuations in temperature and all other environmental factors, while the whitefish,[2] must have clean cold water comparatively free from decaying materials, presenting the minimum of daily fluctuations. This paper is an attempt to determine the effects of variations in dissolved gases, of a magnitude common in the toad habitat, upon development, and to locate the sensitive stages in the early life history. An index of sensitivity was also sought in the amount of oxygen released from hydrogen peroxide.

Materials and Methods

It was found that the toads at Urbana, Illinois, breed in a variety of places from barnyard pools thick in manure and mud, to temporary pasture puddles. A plentiful supply also breed in a small artificial lily pond on the campus of the University of Illinois where the adults may be easily captured. They begin laying early in the morning and will continue until noon or after when brought into the laboratory. This affords a continuous supply of freshly fertilized eggs throughout the morning, and is more satisfactory than artificial insemination. A further advantage that these eggs possess for such a study as this is the fact that the time from fertilization to the functioning of the internal gills at 16° C. is very short, about twenty days. This makes it possible to watch their growth from hour to hour and to see the effect of changing the conditions. The eggs are large enough to see the stages well under a hand lens.

The pH was determined by the use of Hynson, Westcott and Dunning indicator sets with additional tubes for high and low values. The oxygen determinations were made by the Winkler method. As the amounts of

[1] Contribution from the Zoological Laboratories of the University of Illinois. 244

[2] Studied for comparison, results to be published later in Ecology.

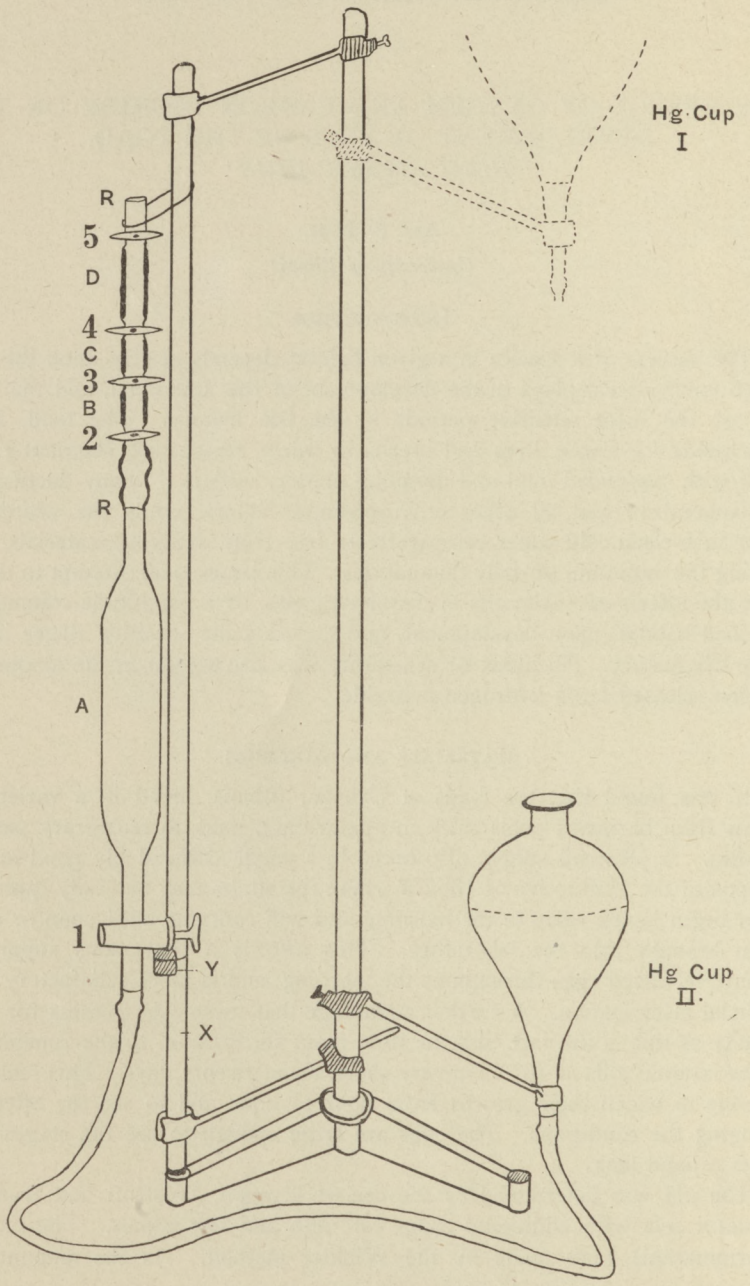

FIG. 1. Apparatus for measuring the oxygen content of small amounts of water by the Winkler method. **A**, large tube 2.2 cm. diameter by 6.5 cm. length, capacity 26 cc., total length from 1 to 2 is 17 cm. **R** to **R** heavy black rubber tubing enclosing glass rings **B C** and **D**, diameter 1 cm. outside, .6 cm. inside.

water in the experimental dishes were small, 200 to 400 cc., the use of 250 cc. bottles was impracticable. A piece of apparatus was therefore devised that would handle small amounts of water (Fig. 1). By raising and lowering the mercury cup, water was siphoned into the apparatus from the experimental dish, and the cocks 1 and 2 closed. **B** was filled with manganous chloride (sol. I, Winkler method), **C** with potassium hydroxide-iodide (sol. II), and **D** with concentrated hydrochloric acid, and clamps 3, 4, and 5 closed after each addition. The clamps were then removed one at a time in the same order and the liquids mixed by means of gravity, and a glass bead inserted with solution I. The 26 cc. of water which the apparatus holds was then titrated against N/40 sodium thiosulphate and the oxygen in cc. per liter calculated.

Experimental Data

A. Physiological Life History from Fertilization to Internal Gill Stage

In this work on physiological life history several types of experiments were performed: (1) Keeping the pH constant (8.0) the oxygen content was varied, four concentrations, .4 cc., .9 cc., 1.41 cc. and 4.64 cc. per liter being used. (2) Keeping the oxygen about the same in all dishes, .4 cc. to .8 cc. per liter, the pH was varied from 6.1 to 8.0. (3) Eggs were sealed into

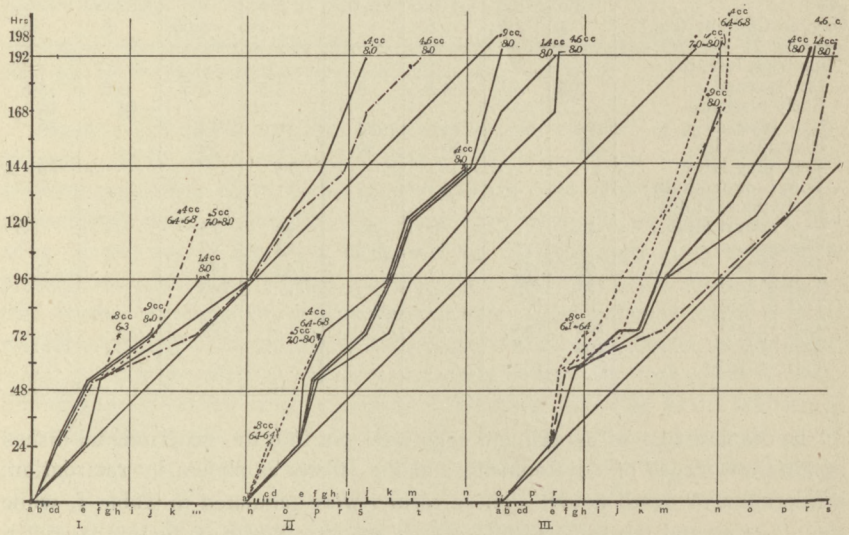

Fig. 2. Toad eggs put into experimental conditions: I at 1 cell stage, II 2 cell stage, and III 4 cell stage.

a fertilization, **b** 2 cell, **c** 12–16 cell, **d** early blastula, **e** late blastula, **f** blastopore lip, **g** yolk plug large, **h** yolk plug medium, **i** yolk plug small, **j** early neural folds, **k** late neural folds, **m** elongation, **n** tail differentiating, **o** tail 1/4 body, **p** tail 1/3 body, **r** tail 1/2 body, **s** tail 2/3 body, **t** tail equal to the body (7 days), **u** 8 days, **v** 9 days, **x** point of death.

known quantities of water with differing oxygen content and the time and extent of development noted.

Time for normal development of toad eggs at 16° C., plotted as a straight line in graphs of figs. 2 and 3, is as follows: Fertilization 0 hrs.; first cleavage (2 cell), 3 hrs. and 20 min.; 4 cell, 4 hrs. and 30 min.; 8 cell, 6 hrs.; 12 to 16 cell, 8 hrs.; early blastula, 10 hrs. and 30 min.; late blastula, 23 hrs. and 30 min.; blastopore lip, 28 hrs.; yolk plug-large, 31 hrs. and 30 min.; yolk plug medium, 41 hrs.; yolk plug small, 48 hrs.; early neural folds, 51 hrs.; late neural folds, 62 hrs.; elongation, 72 hrs.; out of jelly—tail differentiating, 95 hrs.; tail 1/4 length of body, 114 hrs.; tail 1/3 length of body, 123 hrs.; tail 2/3 length of body, 143 hrs.; tail equal to body, 168 hrs.; gills three, 192 hrs.

TABLE I. *Time in hours for reaching various stages of development in the toad experiments*

Read down the columns; embryos died where the number is underscored.

	Series I. Put in 25 Min. before 1st Cleavage				Series II. Put in at 2 Cell Stage			
	A–1	*A*–4	*C*–1	*C*–2	*A*–1	*A*–4	*C*–1	*C*–2
pH	8.0	8.0	5.8–6.6	6.0–6.4	8.0	8.0	5.8–6.6	6.0–6.4
Oxygen Content (c.c.)	.4	4.64	.8	.4	.4	4.64	.8	.4
Time from fert. to:								
1st cleavage	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Early blastula	.....	24	.....	.....	.....	.....	24	24
Late blastula	24	56	24	24	24	24	.....	56
Blastopore lip	56	.....	56	56	60	56	.....	.....
Yolk plug large	.....	.....	.....	.....	73	.....	.....	73
Yolk plug medium	.....	.....	73	.....	.....	.....	.....	.....
Early neural folds	.....	.....	.....	73	.....	.....	.....	.....
Late neural folds	73	.....	.....	95	95	73	.....	.....
Elongation	.....	73	.....	123	123	95	.....	.....
Tail differ	95	95	.....	.....	143	125	.....	.....
Tail 1/4 body	123	123	.....	.....	.....	143	.....	.....
Tail 1/3 body	143	.....	.....	.....	.....	.....	.....	.....
Tail 1/2 body	168	143	.....	.....	.....	168	.....	.....
Tail 2/3 body	192	168	.....	.....	.....	.....	.....	.....
Tail = body	.....	192	.....	.....	.....	.....	.....	.....

In the first type of experiment eggs were put into the experimental dishes at different stages of development, and the effects as shown by retardation, acceleration or death noted. These results were tabulated in Table I, while the *A* series and the same results are shown graphically in figures 2 and 3. In making the graphs, normal development was taken arbitrarily as a straight line from fertilization at 0 hours to the internal gill stage at 196 hours. Time in hours was plotted as ordinates, and the stages of development, or abscissae, were obtained by dropping perpendiculars from the normal development line to the x axis according to the number of hours required to

reach that development. All experimental curves were then drawn with both time and period of development fixed by the normal curve. When the experimental curve rises above the normal line retardation is indicated; when it drops toward the normal or below it, acceleration is indicated. Curves parallel to the normal denote normal rate of growth.

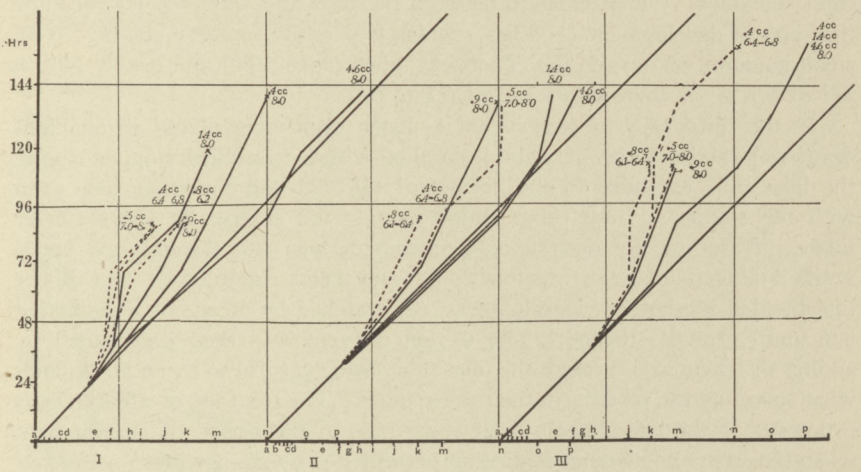

FIG. 3. Toad eggs put into experimental conditions: I at late blastula, II at yolk plug, and III at blastopore lip stages. Notation same as Fig. 2.

Using a pH of 8.0 and four concentrations of oxygen, .4 cc., .9 cc., 1.41 cc., and 4.64 cc. per liter, we find that a certain degree of development was attained regardless of the concentration, death not occurring in any of the experiments short of the yolk plug stage even when the oxygen was as low as .4 cc. per liter. Eggs put in before the first cleavage (Fig. 2–I) show marked retardation up to the late blastula stage then some acceleration so that further development approaches normal. Eggs exposed to the experimental conditions between the first cleavage and the late blastula stage (Figs. 2 and 3) show a period of eighteen or twenty hours with little or no retardation and then a period of quite marked retardation occurring in the blastopore or yolk plug stages. Later development for the higher concentrations of oxygen again approaches normal. Eggs put in after the late blastula stage (Fig. 3) show general retardation for the lower oxygen concentrations but fairly normal development for the higher concentrations.

The results of this type of experiments show that where the oxygen was as low as .4 cc. to .9 cc. per liter death occurred in part of the experiments, but at higher concentrations only retardation showed the effects of exposure. At the late blastula and blastopore lip stages the higher concentrations even caused acceleration of growth. The most sensitive stages appeared to be from fertilization to the first cleavage, and the gastrulation stages.

The second type of experiment was where the oxygen was low (.4 to .8 cc. per liter) and the pH varied, 6.1, 6.4, and 7.0 to 8.0. The stages reached in development are shown in the graphs of Figs. 2 and 3. Exposures to these conditions beginning before the four cell stage gave death with retardation in 24 to 120 hours, *c* to *k*. The same effect is seen in eggs put in after the eight cell stage. Those exposed between the four and eight cell stages show the greatest development (198 hrs.) which is past the sensitive stages. With small amounts of oxygen the effect was much more rapid and deadly with a pH of 6.1 to 6.4 than with a pH of 6.4 to 6.8.

In the third type of experiment a dozen eggs were placed in an eight dram vial (about 26 cc. of water) supplied with a two-holed stopper having the inlet tube extended to the bottom of the vial and the oulet tube even with the stopper. Both tubes ended outside the stopper in short rubber tubes. Water of the desired oxygen content was run through the bottle until all oxygen due to air was flushed out and then a clamp on the two rubber tubes sealed the bottle. Development was watched by means of a dissecting lens until growth stopped. The oxygen content was then determined by adding the chemicals through the inlet tube, being careful to avoid air bubbles when inserting the pipettes in the rubber tubes. The cleavage of a dozen eggs proceeded to the late blastula stage regardless of the period of development when put in or the amount of oxygen present (.1–5.7 cc. per liter). Further development was roughly proportional to the amount of oxygen present, as was also the time to death.

B. Reaction of Eggs to Hydrogen Peroxide as Development Progresses

A universal property of protoplasm (both plants and animals) is the abitilty to liberate oxygen from hydrogen peroxide. What this property is due to is a debated question at present, but in general it is ascribed to an enzyme called catalase. Several theories have been advanced as to its significance in animal life. References are given to these in the literature cited (Becht '19, Burge and Burge '21, Zieger '15). As a measure of the sensitivity of the developing egg at different stages in the early life cycle the reactions to oxygen and carbon dioxide (acidity) have been discussed. It was thought that a study of the amounts of oxygen released from hydrogen peroxide at various periods of development might be of significance. Accordingly a series of determinations was made with the toad egg using the method outlined by Burge ('16), Fig. 4A. A second series was made for the toad eggs the following spring using eight dozen eggs instead of one dozen and shaking the material by machine instead of by hand (Fig. 4B). A comparison of **A** and **B** shows a striking parallelism and the two curves seem to confirm each other.

From a study of these curves (Fig. 4) it may be seen that there was a decrease in the power of liberating oxygen from hydrogen peroxide from the

unfertilized egg to the fertilized, an increase from fertilization to the early blastula, a decrease from early blastula to elongation of the embryo, and from this point to twenty-one days a steady increase. The stages most sensitive to low oxygen and high acidity were (1) fertilization to first cleavage, and (2) the gastrulation stages. These were the low points on this curve. Is there a correlation between sensitivity and a lowered power of liberating oxygen from hydrogen peroxide?

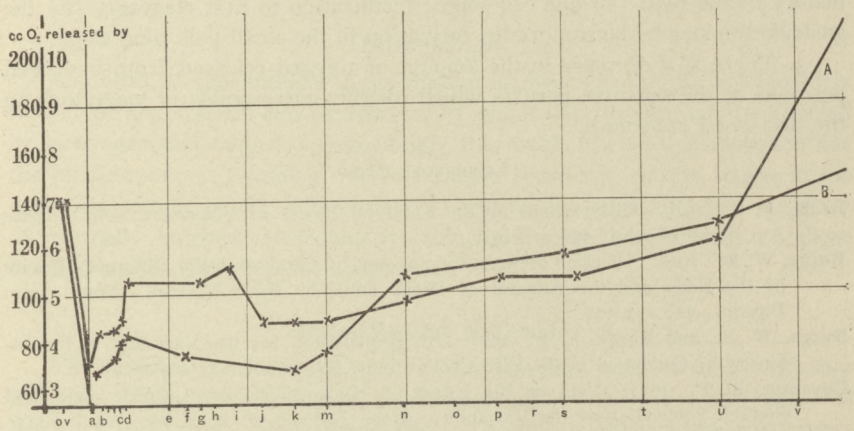

FIG. 4. Amounts of oxygen released from hydrogen peroxide at different stages of development of toad eggs. Series **A** determinations were made with one dozen eggs in 1920. Series **B** was made using eight dozen individuals in 1921.

Winternitz and Rogers ('10) have shown a definite increase in what they call catalase for the different stages of the hen's egg, as development proceeds. Burge and Burge ('21) have shown an increase in this power from egg to adult for the Colorado potato beetle. Zieger ('15) has found a definite rythm for catalase in the insect life history, reporting that the power is high where rapid growth or metamorphosis is going on (early larval stages, pupal stages) and low during resting stages. Child in his book " Senescence and Rejuvenescence " has shown that metabolism is high during the more sensitive stages. Burge believes that increase in catalase brings about an increase in metabolism. This curve for the toad egg seems to be a contradiction of one statement or the other since it shows the power of splitting oxygen from hydrogen peroxide to be low at the sensitive stages. More work is needed on complete life histories before definite conclusions can be drawn about this point.

I wish to thank Dr. V. E. Shelford, at whose suggestion this work was undertaken, for his helpful advice and kindly criticism during the course of the investigation. I am indebted to Dr. W. E. Burge and Dr. H. B. Lewis for suggestions in the enzyme work.

Summary and Conclusions

1. Conditions of high carbon dioxide or low oxygen content such as may occur in the normal environment and which are tolerated by the other periods of development, cause great retardation and even death at sensitive stages. The effects of low oxygen are much more detrimental in acid than in alkaline water.

2. There are two definitely sensitive stages in the early physiological life history of the toad (*a*) one cell stage; fertilization to first cleavage, (*b*) the gastrulation stages; blastopore lip formation to the small yolk plug stage.

3. There is a decrease in the amount of oxygen released from hydrogen peroxide at the sensitive periods which roughly corresponds in magnitude to the decreased resistance.

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EFFECTS OF OXYGEN AND CARBON DIOXIDE ON THE DEVELOPMENT OF THE WHITEFISH[1]

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Introduction

A subject of vital interest is the rhythm of events in the physiological life history of any species, and the manner in which this rhythm may be affected by environmental factors. Accordingly this work has been undertaken for the purpose of: (1) finding out the relative sensitivity of the stages in the early life history of the Whitefish; (2) testing the resistance and reactions of normally hatched individuals as compared with the reactions of those hatched under experimental conditions.

Materials and Methods

The material for this work was the lake whitefish, *Coregonus clupeiformis* Mitchill. Some work was done on fertilization and early cleavage stages at the U. S. Hatchery, Put-in-Bay, Ohio. The major part of the work, however, was done at the Vivarium, University of Illinois, on material shipped from the hatchery in ice. The temperature of the lake water was about 8° C. at the beginning of the season. It had a pH of 7.0 and an oxygen content of 4.08 cc. per liter. At the Vivarium the stock was kept in water from the University wells aerated to about 2.6–3.3 cc. per liter, and with a pH of 7.8. It was cooled to 10° C. by means of brine coils. The water (pH 8.0–9.0; carbonates 232 ± parts per million) for all the experiments was boiled free of all dissolved gases, and part of the salts precipitated (Shelford '18).

The apparatus used for varying the pH and oxygen content is shown in figure 1. Bottle **1** contained approximately N/4 sulphuric acid which siphoned over into the mixing bottle **4**. The stopcock **2** controlled the flow which could be measured by counting the drops through the glass bulb **3**. Thus a known amount of the acid was added to a known flow of the boiled water which entered at *W*. This water was also 10° C. The water leaving **4** had a known pH, and was oxygen free. Since it contained carbonates, acids set free carbon dioxide which changed the pH. The oxygen was controlled by adding compressed air (see fig. 1 and explanation). Three such sets of apparatus were used giving three hydrogen ion concentrations, each with three oxygen concentrations.

[1] Contributions from the Zoological Laboratory of the University of Illinois, No. 256.

The pH was determined by the use of Hynson, Westcott, and Dunning indicator sets with additional tubes for high and low values (Clark '20). Brom cresol purple (5.8–6.6), brom thymol blue (6.6–7.6) phenol red (6.6–8.0), and thymol blue, alkaline range (8.0–9.2) were used. The colorimetric

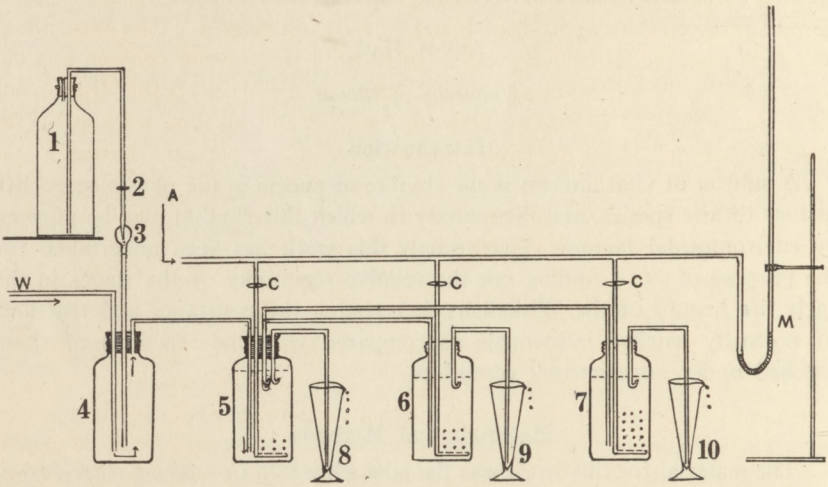

FIG. 1. Apparatus for the control of experimental conditions. Method of varying hydrogen-ion concentration and oxygen content. **1**, N/4 sulphuric acid; **2**, cock for control of acid by drops at **3**; **4**, mixing bottle for acid and boiled water from **W**; **5**, **6**, and **7**, jars, to which air from **A** is added in varying amounts; **C**, cocks for control of air flow; **8**, **9**, and **10**, half-pint sedimentation glasses for eggs; **M**, mercury manometer for keeping air flow constant.

standards were checked electrometrically by Dr. R. E. Greenfield. Oxygen determinations were made by the Winkler method with an apparatus which used only 26 cc. of water for a determination (Hall '23).

Experimental Data

Experiments at the Hatchery

All the experimental work was done at 10° to 11° C. at the U. S. Hatchery. The length of life of eggs and sperm was tested. Three sets were run (*a*) dry eggs and sperm, (*b*) dry eggs and sperm mixed thoroughly with water to uniform milky fluid, and (*c*) dry sperm and eggs standing in lake water. With wet eggs and dry sperm eight minutes was the latest time at which fertilization was possible. With wet sperm and dry eggs nine minutes showed a small number of fertile eggs. But with both eggs and sperm dry fertilization took place at seven and a half hours. It may have been possible after longer time as such an experiment was destroyed and could not be verified.

In early development the eggs were exposed to constant oxygen (4.08 cc. per liter) but with varying pH. Normal lake water had a pH of 7.0. Acidity (pH 6.2–6.6) was produced by adding sulphuric acid, and alkalinity (pH 8.4–8.6) by adding sodium hydroxide. Dishes of standing water were used and changed frequently. Eggs fertilized directly in the solutions were normal at the end of twenty-four hours (32–64 cell stage). This experiment was then repeated using lake water boiled until the oxygen content was 2.9 cc. per liter. Fertilization and development occurred in all the dishes, but there was a marked difference between the acid and alkaline waters. With a pH of 6.2 and 6.6, respectively, 80 per cent and 25 per cent fertilized and developed; with a pH of 7.0 only 3 per cent, and at a pH of 8.4 but 1 per cent. A hydrogen ion concentration which is too great to favor later development appears best for fertilization (Cohn '18).

Experiments with Shipped Eggs

In the work on later stages with treated running water at the University of Illinois, special attention was paid: (1) to keeping conditions as near constant as possible from day to day; (2) to watching the stages of development reached in each concentration and comparing these with each other and the control stock; (3) to working out the death rate and the percentage hatching for each concentration; and (4) to testing the vitality and reactions of the larvae hatching from the different stocks.

In this work apparatus was set up in triplicate (fig. 1). Values of pH 6.4, 7.0, and 8.0–9.0 respectively were used, each pH with an oxygen content of 1 cc., 3 cc., and 4.5 cc. per liter; pH was taken in all the dishes each day, and oxygen was determined every two or three days.

The eggs were obtained from the hatchery in four lots. The first lot, spawned December 2, was in the thirty-two cell to early germinal cap stage when received December 3. The second lot, spawned December 5 and received December 24, had the tail just elongating and the fin buds starting. The eye vesicles had formed but no pigmentation had occurred. The third lot, spawned December 7 and received January 31, were only slightly farther along in development than lot two. They had just begun to show the pigment in the eyes. The fourth lot, received March 12, were fully developed and started to hatch immediately.

A comparison of the chemical analyses of Lake Erie water (the average of Huron at Port Huron and Erie at Buffalo, Clarke '20, p. 70) and the boiled University water is important in connection with the experimental results obtained with the whitefish, and is given in Table I.

The four stocks differed in: (*a*) the length of time in these two kinds of water, (*b*) temperature at hatchery and in experiments, and (*c*) the stage at which shipment was made.

TABLE I. *Comparison of Lake Erie and boiled University of Illinois water*

	Lake Erie Water	Boiled U. I. Well Water
pH (CO_2)	7.0	9.0
CO_3	54 ppm.	250 ppm.
SO_4	10 ppm.	trace
Cl	5 ppm.	1 ppm.
Ca	27 ppm.	33 ppm.
Mg	7 ppm.	27 ppm.
Na, K	5 ppm.	16 ppm.
Total solids	112 ppm.	248 ppm.

TABLE II. *Showing the development of whitefish eggs under different conditions*

Figures are days to stage indicated on left. Dates of spawning, receipt at Urbana, and beginning of experiment, respectively, follow each series number. Series II and III are stock 1; series VIII is stock 3. The entries in italics indicate that embryos died soon afterward. Oxygen all given in cc. per liter.

	A–1	B–1	C–1	A–2	B–2	C–2	A–3	B–3
Series II; 12/2; 12/3; 12/6.								
pH range	6.2–7.5	5.8–8.9	6.4–8.1	6.4–8.0	6.2–8.9	7.9–8.5	6.6–8.3	6.8–9.2
pH mean	6.2	6.2	6.4	7.0	7.2	7.2	7.8	8.0
Oxygen range	0–.9	0–1.04	0–2.08	0–.9	0–1.24	.01–3.7	0–.9	0–1.7
Oxygen average	.1	.2	.6	.1	.2	.7	.1	.2
Cap small cells	4	4	4	4	4	4	4	4
Embryo forming	*5*	5	5	*5*	5	5	*5*	5
Post ring large		*6*	6		6	6		
Post ring small	7	7	7	7	7	7	7	10
Eye vesicle								
Tail flat	*10*	10	9.5	*10*	10	9	*10*	10.8
Tail starting		12	11		11	10		11
Tail elongating		13	13		13	11		
Tail ¼ body						13		
Series III; 12/2; 12/3; 12/17.								
pH range	5.9–6.9	6.8–7.0	6.8-9.0	5.9–6.9	6.8-7.0	6.8–9.0	4.9–6.9	6.8–7.0
pH mean	6.4		8.5	6.4		8.5	6.4	
Oxygen range	.4–2.4	.4–2.2	.2–1.7	1.3–3.1	1.9–3.7	1.7–3.2	3.2–5.3	3.3–4.9
Oxygen average	1.64	.88	1.07	2.32	2.56	2.49	4.37	3.96
Eye pigmented	15	15	15	15	15	15	15	15
Fin rays short	19	19	17	*19*	*17*	17	19	17
Fin rays ½ fin			21				21	21
Tail near head	*26–27*	*26*	26			26		26
Tail to head			*27*			*27*	*27*	*27*
Tail to eye								
Hatching								
Series VIII; 12/7; 1/31; 2/21.								
pH range	6.1–7.2	6.2–9.0	9.0	6.1–7.2	6.2–9.0	9.0	6.1–7.2	6.2–9.0
pH mean	6.4	7.6	9.0	6.4	7.6	9.0	6.4	7.6
Oxygen range	.2–1.1	.1–.5	.8–2.4	2.0–3.2	.7–3.5	2.6–3.0	3.3–3.8	2.4–5.3
Oxygen average	.61	.4	1.6	2.6	2.12	3.0	3.5	3.8
Ready to hatch	76	76	76	76	76	76	76	76
Hatching—1st	78, 7%	78, 13%	78, 18%	78, 18%	78, 16%	78, 15%	78, 8%	78, 17%
Max. hatching	79–82	79–82	79	81–83	79–82	79–81	81–83	81–83

Effect of Conditions on the Different Stocks

Table II shows the number of days required to reach the different stages in the various concentrations. The numbers and letters used below will be found in this table.

Stock 1. In series II, conditions varied materially in all dishes. With a pH around 6.2–6.4 we find that a set of eggs put into the dishes *A, B* and *C*–1 on the seventh day varied in both amount of development and length of life roughly in proportion to the amount of oxygen supplied. This was .1 cc., .2 cc., .6 cc. per liter respectively. This amount was not enough to carry development beyond the elongation of the tail bud, and was lethal in every case. This same oxygen effect seemed to hold with higher pH, and was evidently below the threshold of whitefish development.

In series III, oxygen content was raised so that dishes No. 1 had about 1 cc. per liter, dishes No. 2 about 2.5 cc., and dishes No. 3 about 4 cc. per liter. With this increase in oxygen concentration the more alkaline water showed the farthest development. The eggs lived as long in the acid and neutral waters of high and low oxygen but development was retarded. It is significant that those eggs in neutral waters were more retarded than those in either the acid or alkaline waters. Where neutrality was maintained as in this set a detrimental effect is noticed in development equal to that of higher hydrogen ion concentrations. The results suggest that pH of 7.8 is optimum and 7.0 too high a concentration of hydrogen ions. This lot of eggs, put in at the early pigmentation stage of the eyes lived nearly to hatching.

Stock 2. The experiments of series IV and V were started three days apart and run simultaneously so that conditions were identical except for the time of entering the experimental waters. The average oxygen for the different dishes was slightly higher than in series III. The eggs of series IV were put in when the fin buds were just starting. A distinct oxygen differential was established: the low oxygen eggs developed to the point where the tail reached the mendian line of the head; the medium oxygen eggs to the point where the tail reached past the head and around to the eye; while the high oxygen eggs developed just to hatching in the acid and neutral waters. The alkaline water did not seem enough lower in oxygen to account for the retardation which occurred, and this retardation was probably due to the hydrogen ion concentration.

In series V more difference in development is apparent between the different dishes. The greatest amount of retardation occurred in the low oxygen jars and in the high oxygen of the neutral series (pH fluctuating). Two eggs hatched in the medium oxygen of the neutral series, but all others died just before hatching time. These were the first individuals to

hatch in the experimental dishes, though a few hatched in the stock (pH 7.8 and oxygen 3.3 cc. per liter).

Stock 3. Hatching occurred in the experiments run with both the third and fourth stocks. As these stocks were older, the time of exposure to the experimental waters was later, and a different reaction resulted. Series VI and VII were started two days apart but were run simultaneously. Hatching occurred in several of the dishes and extended over several days, the beginning and duration of the process being recorded in Table II. The per cent hatching is given as the second member opposite "Hatching-1st."

Series VIII was started just as the eggs of stock 3 were ready to hatch, to test the effect of different concentrations on this process and on the life of the larvae. Hatching occurred in all the dishes beginning on the same day. Maximum hatching was 2 days later in the acid "medium" oxygen and in the acid and neutral high oxygen, showing some retardation for these concentrations.

Stock 4. Series IX is the result of the exposure of eggs reared for the entire time of development at the hatchery in lake water. It shows the relative sensitivity of the hatching period and the newly emerged larvae. The first hatching occurred in the acid and neutral low oxygen an hour or two after putting the eggs in the water. A few also hatched in alkaline "low" and "medium" oxygen later the same day.

Differences in the Stocks at Hatching

The normal time for the development of these eggs at the hatcheries was four to five months. Due to higher temperature my first stock began hatching at thirty-two days. The newly hatched larvae from each of my four stocks were measured for body length, and for size and shape of yolk, and the time from hatching to death recorded.

Only a few individuals were measured in stocks 1 and 2. In stock 1 the fry were 8 to 11 mm. in length with round yolk sacs 2 mm. in diameter. These lived only two days. In stock 2 the fry were 11 mm. long, also with round yolk sacs. These lived sixteen days with no food. Stock 3 averaged 11.1 mm. with similar yolks and lived at least ten days. Stock 4, raised in lake water, gave fry of a distinctly different type. They were 13.4 mm. to 15 mm. long and were extremely active. The yolk sacs were oval but of the same volume as the earlier stocks. These lived for twenty-five days without food, and at the time of death measured 15 mm. This shows that eggs reared for a long period at low temperature attain a larger size and have more energy than they would have if forced at higher temperature (an argument against forcing conditions in hatchery work).

Mortality

Due to Fungus. One factor in mortality was fungus growth. This was in direct proportion to the amount of oxygen present, therefore was most detrimental in the high oxygen concentrations. The pH seemed to have no differential effect. The fungus grew very rapdily on dead eggs but also entangled live ones and killed them. Infected eggs left in the dishes would mat dozens of eggs together and kill them in a day or two. Since higher temperatures were more favorable to the fungus it grew more abundantly in my dishes than at the hatchery.

Due to Experimental Conditions. The eggs of the different series (II to IX) were counted and the dead recorded from day to day. From this data the per cent of deaths was calculated for each dish of the series. The stages from early formation of the embryo and ring seemed very sensitive, as in all concentrations the deaths quickly amounted to 100 per cent. From the time of closure of the posterior ring and beginning of elongation of the tail and fin buds sensitivity decreased, as shown by the following figures:

Series II: Early gastrulation. At the end of three days the per cent which had died was as follows: A-1, 100 per cent; B-1 100 per cent; C-1, 66 per cent; A-2, 100 per cent; B-2, 75 per cent; C-2, 50 per cent; A-3, 100 per cent; B-3, 95 per cent; C-3, 50 per cent.

Series III: Eye pigmented, lens showing. At the end of nine days the per cent which had died was as follows: A-1, 20 per cent; B-1, 20 per cent; C-1, 10 per cent; A-2, 50 per cent; B-2, 100 per cent; C-2, 5 per cent; A-3, 10 per cent; B-3, 50 per cent; C-3, 5 per cent.

Series IV: Tail 1/4 body length. At end of sixteen days the per cent which had died was as follows: A-1, 91 per cent; B-1, 26 per cent; C-1, 49 per cent; A-2, 64 per cent; B-2, 96 per cent; C-2, 98 per cent; A-3, 70 per cent; B-3, 66 per cent; C-3, 98 per cent.

The deaths due to fungus were in most cases small compared to total deaths. Total mortality is the index to the effect of varying conditions of oxygen and carbon dioxide, and shows the relative sensitivity of the different ages of eggs.

Effect of Other Acids

As sulphuric acid liberated the carbon dioxide in the above experiments, it was thought desirable to run a check with other acids. A series of finger bowls was prepared such that a pH of 6.3, 7.0, and 9.0 was obtained for each, sulphuric, hydrochloric, and acetic. Salts such as sodium chloride antagonize the acid which may be present in water (Loeb, '12, Osterhout, '14), and therefore a duplicate series was made up in which one third of the volume of water was substituted by M/6 sodium chloride. The same amount of

mixing was given each solution as it was made up to insure a uniform oxygen content of 1.5 cc. per liter. The finger bowls were filled full and covered. Readings were taken without uncovering. Table III shows the time to death in the different concentrations. In the acetic and sulphuric acid (no salt) death occurred in pH 7.0 first, 9.0 second and 6.3 last, but for hydrochloric, death in 6.3 preceded 7.0. Neutrality was most quickly fatal, and alkalinity more toxic than acidity. The sulphuric plus salt was least toxic of all with a pH of 6.3, as these larvae were still alive at the end of 23 days. The differ-

TABLE III. *Resistance of whitefish larvae to acidity, neutrality, and alkalinity in 500 cc. fresh boiled water and M/6 NaCl water; acid, acetic 0.529N sulphuric 0.485N, hydrochloric 0.442N, CO_2 gas added directly*

No. Fish	Chemical Used	O_2 in cc. per L.	pH	Av. Time to Death	Remarks
4	.8 cc. acetic + boiled H_2O	1.5	6.3	47 hrs.	
4	.8 cc. " + M/6 NaCl	"	"	84 "	
4	.18 cc. " + boiled H_2O	"	7.0	22 "	
4	.18 cc. " + M/6 NaCl	"	"	84 "	
4	.6 cc. HCl + boiled H_2O	"	6.3	20 "	
4	.6 cc. " + M/6 NaCl	"	"	180 "	
4	.26 cc. " + boiled H_2O	"	7.0	51 "	
4	.26 cc. " + M/6 NaCl	"	"	61 "	
4	.6 cc. H_2SO_4 + boiled H_2O	"	6.3	52 "	
4	.6 cc. " + M/6 NaCl	"	"	216 "	1 alive 24 days
4	.16 cc. " + boiled H_2O	"	7.0	27 "	
4	.16 cc. " + M/6 NaCl	"	"	500 "	1 alive 24 days
4	control boiled water	"	9.0	47 "	
4	" M/6 NaCl.....	"	"	87 "	
24	CO_2 + boiled water	.1	6.3	2 hrs. 25 min.	Stock 3
45	" + " "	.1	6.3	4 "	" 4
15	boiled water	.1	9.0	3 " 45 "	" 3
9	" "	.1	9.0	5 "	
15	CO_2 + boiled water	2.8	6.4	5 " (not dead)	" 3
18	" + " "	2.8	6.4	12 " " "	" 3

ence in the reactions to the three acids was small. The variation in the hydrogen ions was probably influencing growth rather than the anions, sulphate, acetate, and chloride. This is in keeping with the work of Loeb ('04, '12) on the chemicals most influencing growth. He has studied the effect of salt solutions on growth and regeneration and finds that the cations or substituted hydrogen ions are most influential in changing growth, calcium, sodium, and potassium being necessary at some time during the life cycle for normal individuals to develop.

The effect of direct addition of carbon dioxide to water of high and low oxygen was studied in relation to length of life in whitefish larvae. In low oxygen the larvae died more rapidly in acid water (6.3) than in alkaline

(9.0). With high oxygen (3 cc. per liter) a pH of 6.3 did not kill, though the exposure was for the greater part of two days. This is in accordance with Well's statement that large amounts of oxygen antagonize the detrimental effects of high concentrations of carbon dioxide. Statements of the effect of the different combinations of oxygen and carbon dioxide in this paper seem contradictory. The experiments were all started with different stocks so the number of items that can be compared is unavoidably small. However when the time to "fin ray short" (series III, table II) is plotted on cross section paper with oxygen and pH scales, and the points showing equal time connected in a manner suggested by Huntington's ('19) plots of human death rate in relation to temperature and humidity, the results appear orderly (Fig. 2). The time for "tail flat" in series II shows a similar

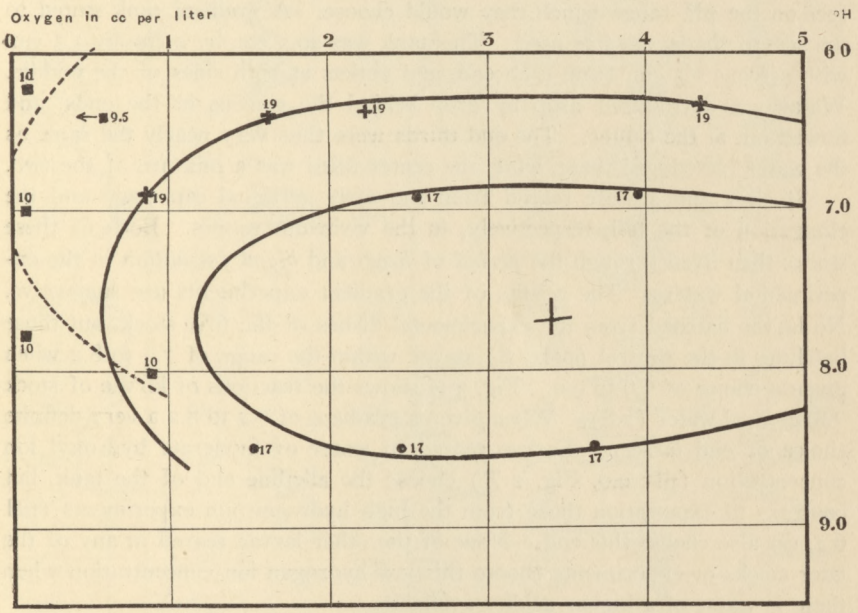

FIG. 2. Showing equal time lines on an oxygen-hydrogen-ion chart. The broken line passes approximately through combinations of oxygen and hydrogen-ion concentration in which the embryos developed a flat tail in ten days, beginning four days after spawning. The solid lines pass through combinations of oxygen and hydrogen-ion concentration in which the embryos developed short fin rays in 17 and 19 days respectively. The general trend of these lines suggests that development may be expected to be most rapid at about 3.4 c.c. of oxygen and pH 7.6 to 7.7, where the large cross is placed. This cross is in the center of the ellipse. The center of the ellipse suggested by the flat tail curve would fall at a higher H-ion concentration.

relation. Observations were not made often enough to bring out the necessary details in any but series III, as noted above; accordingly this interpretation can be suggested only as a basis for further investigation.

Under experimental conditions eggs develop into normal embryos with a pH of 7.0 and a much lower oxygen content than in the hatchery. Much greater variations were produced in per cent hatching and in length of time for development by varying the pH toward the acid or alkaline end of the scale than by changing the oxygen. This is in accord with the idea of Powers ('20). He concluded from his reconnaissance of the pH of Puget Sound waters under varying conditions of tides, weather, *etc.*, that pH has more to do with compatibility of habitat than oxygen.

Effect of Conditions on Reactions of Fry from Different Stocks

As each of the stocks hatched, gradient experiments were run with the larvae to see if the environment during the early embryonic life had any effect on the pH range which they would choose. A gradient tank suited to the size of the larvae was used. This tank was 20.5 cm. by 2 cm. by 1.5 cm. with screens 1.5 cm. from each end, and outlets at both sides of the middle. Water was introduced drop by drop behind the screens at the ends, and flowed out at the center. The end thirds were thus very nearly the same as the water introduced there, while the center third was a mixture of the two.

Stock 1 and 2 were reared from the early germinal cap stage and the elongation of the tail, respectively, in the vivarium waters. Both of these stocks then lived through the *period* of *heart* and *blood formation* in the experimental waters. The results of the gradient experiments are suggestive. No larvae hatched from the experimental dishes of the first stock, but those hatching in the control (pH 7.8) stayed within the range of 7.6 to 8.2 when given a choice of 6.6 to 9.0. Fig. 3 *A* shows the reactions of larvae of stock 2 to control water (7.8). When given a gradient of 6.4 to 8.2 a very definite choice of end is seen. Larvae reared in water of moderate hydroxyl ion concentration (pH 9.0, Fig. 2 *B*) choose the alkaline end of the tank, but contrary to expectation those from the high hydrogen ion experiments (pH 6.4, *C*) also choose this end. None of the other larvae reared in any of the later stocks or experiments choose this low hydrogen ion concentration when there is water of a higher acidity available.

In stock 4 the eggs were raised to hatching at Put-in-Bay and so began hatching a few hours after they were put into the experimental waters. These therefore *passed the time of heart and blood formation in the lake water.* Animals hatched on the 14th and 16th of March were kept in the experimental waters, and gradients run with them on the 17th and 22d. Nearly all of these showed the characteristic exploration of the whole tank for from three to five minutes then a preference for the acid end of the tank. The turning back occurred at a very definite pH and the avoidance was from either end, for often when an animal entered the unfavorable water it stayed there turning back from the changing point to again enter the unfavorable

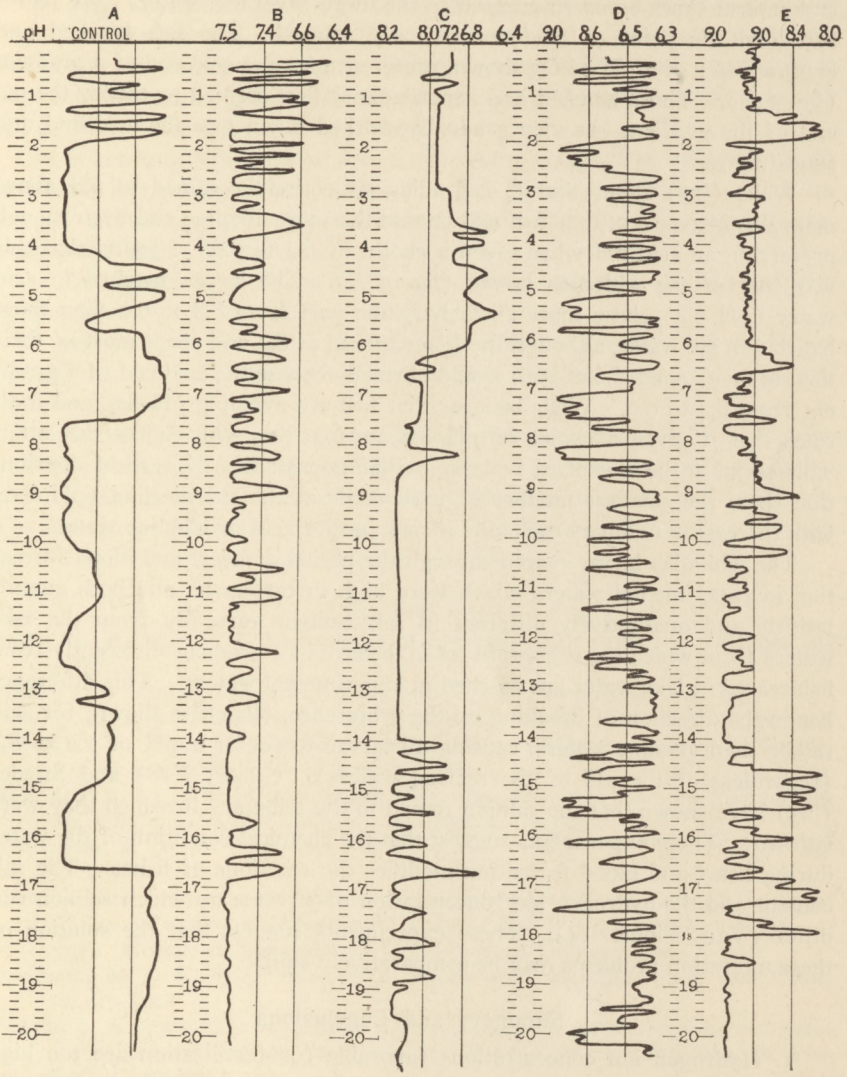

FIG. 3. Reaction of larvae of the four stocks to a permanent gradient. A long, narrow tank was used with water of the desired pH entering at each end, making a permanent gradient for the entire period of the experiment. The fish swam freely in the tank for the time recorded in the marginal figures. **A,** control larva from stock 2. **B,** larva from stock 2 raised through the period of heart and blood formation in a pH of 8.6 to 9.0. **C,** larva from stock 2 raised through the period of heart and blood formation in a pH of 6.4. **D,** larva of stock 4 raised in lake water, but hatched in pH of 6.4. **E,** larva of stock 4 hatched in a pH of 6.4 and given a choice of 8.0 to 9.0.

end several times before it escaped to the more favorable water. We therefore find the point of preference clearly marked. The fish hatched and kept in water of a pH of 6.3 show a preference for the acid end of a gradient (fig. 3 *D*). Those hatched and kept at 9.0 at first seemed to choose the 9.0 end of the gradient but they gradually worked down into the acid end and stayed there.

Wells ('15) states that if a gradient is entirely confined to pH above neutrality, 8.0 to 9.0, that fish will choose the more alkaline end even though preferring an acid pH when given a choice of 6.4 to 9.0. I found that this was true of the whitefish larvae (fig. 3 *E*). The larvae hatched in acid water (pH 6.4) chose more exclusively the part from 8.8 to 9.0 than those hatched in 9.0 water, although the latter turned at 8.4 and spent most of their time in the 9.0 end. Yet both would normally choose the acid end of a gradient from 6.4 to 9.0. Wells believes that fish are avoiding pH 8.0, and indicates that if given a choice of pH 8.0 and 9.0 they will choose the latter. This seems to be a general reaction. Plankton studies of vertical distribution show the smallest number of individuals at the thermocline (pH 8.0) with increasing numbers each side of it in either acid or alkaline waters.

The whitefish larvae reared through the period of heart and blood formation in experimental waters which were high in carbonate, and both quantitatively and qualitatively different in salt content generally from the lake waters from which the eggs were taken, behave in a gradient differently from fish reared in lake water but hatched in experimental waters. This difference lies in the direction of breaking up the preference, otherwise shown, for acid rather than alkaline waters, causing all larvae to prefer a pH of 7.2 to 8.2 (regardless of the pH of the rearing waters). Gilbert ('18) and Snyder ('23) have shown that the salmon return to the tributary in which they were *hatched*. These experiments suggest that the chemical condition of the water during heart and blood formation modifies the reactions of fishes. The salt content and the hydrogen ion concentration of streams up which salmon run differ (Van Winkle '14). These experiments suggest that the solution of these migration problems may be comparatively simple.

Summary and Conclusions

1. Hydrogen ion concentrations favorable for fertilization are too high for later development. Optimum hydrogen ion concentration is gradually lowered as the embryo becomes older.

2. The most sensitive stages in whitefish development seem to be the first cleavages and early gastrulation.

3. Fry hatching at one, two, and four months after spawning differ in size of body but not in size of yolk; those hatching at four months are 4 to 6 mm. longer than those hatching earlier. The later fry also live longer than those hatching earlier, in spite of having the same amount of yolk available.

4. Eggs raised through the period of heart and blood formation in water high in carbonates and differing from their normal environment show a different type of gradient reaction to hydrogen ion concentrations. Such larvae raised in both acid (6.3) and alkaline water (9.0) choose the alkaline end of the gradient when 6.3 to 9.0 is offered.

5. When exposed to water with a pH of 6.3 obtained by adding CO_2 directly, the larvae died earlier in the acid water than in the alkaline with a low oxygen content. A high oxygen content antagonizes the CO_2 present, prolonging the life of the larvae.

6. In a gradient of 8.0 to 9.0 larvae of both acid and alkaline hatching environment choose the alkaline end (8.8 to 9.0).

I wish to thank Dr. V. E. Shelford, at whose suggestion this work was undertaken, for his helpful advice and kindly criticism during the course of the investigation.

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VITA

Ada Roberta Hall was born in Florida in 1890. She received her preparatory training in the Lincoln High School, Portland, Oregon, graduating in 1908. She taught in country grade schools until February, 1911, when she entered the University of Oregon. During the period of her undergraduate work she taught the primary grades at Fern Ridge and Wendling, Oregon, for two years and a half, and was senior Playground Director in the City of Portland Parks for three summers. She was laboratory assistant in Zoology at the University of Oregon during her senior year, and was a member of Scroll and Script, Senior Honorary Society. She received her A.B. degree with Highest Honors in Zoology in 1917. She continued at the University of Oregon for two years as graduate assistant in Zoology and received her A.M. degree in 1919. The summer of 1918 was spent at the Puget Sound Biological Station, Friday Harbor, Washington. In the fall of 1919 she went to the University of Illinois as Fellow in Zoology. During the year 1920-21 she was elected to Sigma Xi and in the fall of 1920 to Iota Sigma Pi. The summer of 1920 was spent at the University of Michigan Biological Station at Douglas Lake. She completed her work for the degree of Doctor of Philosophy in June, 1921. During the period of graduate study she published the following papers: 1. Some Experiments on the Resistance of Sea Urchin Eggs to Sulphurous Acid. Pub. Puget Sd. Biol. Sta. 2:113-119. 2. Regeneration in the Annelid Nerve Cord. Jour. Comp. Neur. vol. 33, No. 2:163-191.

THE EFFECTS OF OXYGEN AND CARBON DIOXIDE ON THE DEVELOPMENT OF CERTAIN COLD BLOODED VERTEBRATES.

BY

ADA ROBERTA HALL
A. B. University of Oregon, 1917
A. M. University of Oregon, 1919

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

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IN ZOOLOGY

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1921

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May 12, 1921 191

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Ada Roberta Hall

ENTITLED The Effects of Oxygen and Carbon Dioxide on the Development of Certain Cold Blooded Vertebrates.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy in Zoology.

Victor E. Shelford
In Charge of Thesis

Henry B. Ward
Head of Department

Recommendation concurred in*

J. Kingsley A. Vanderbeck
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Charles Zeleny
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Committee on Final Examination*

*Required for doctor's degree but not for master's

THE EFFECTS OF OXYGEN AND CARBON DIOXIDE ON THE DEVELOPMENT OF CERTAIN COLD BLOODED VERTEBRATES

Contents

I. INTRODUCTION

A subject of vital interest to the ecologist is the rhythm of events in the physiological life history of any species and the manner in which this rhythm may be affected by environmental factors. Accordingly this work has been undertaken for the purpose of:

(1) Finding out the relative sensitivity of the stages in the early life history of some cold blooded vertebrates. The whitefish, a delicate organism, was chosen and a comparative study made of the toad, a rather resistant form.

(2) Testing the resistance and reactions of the normally hatched individuals of a species as compared with the reactions of individuals hatched under experimentally controlled conditions.

With the increase of mills and cities along our lakes and waterways the effect of pollution on the plant and animal life therein has become an important problem. Not only do dissolved substances take their toll of adult forms but decaying matter covering the former clean breeding grounds may prevent development of the eggs, thereby wiping out a species. To estimate the harm which may be done any given species one must know the most sensitive stage in the entire physiological life history. At the present time comparatively few complete physiological life histories are known. Much work has been done on morphological life histories and a number of interesting theories advanced in regard to the cell, gastrulation, and the form of cleavage pattern. There are few theories as yet in connection with physiological life histories, though enzyme action and the rate of reaction of the chemical substances in different parts of the developing embryo in relation to external factors such as light,

temperature, acidity, etc., promises to be a very fruitful field.

The student of environment is also interested in both the evolutionary and hereditary aspect of the case. If animals have a certain form of development and type of reaction when raised under the conditions of the normal habitat, can a permanent change be effected in these by varying the environmental conditions? May this new type of reaction be transmitted to the offspring, that is, may environment affect all the cells of the body germ as well as soma, or will the succeeding generations revert to the earlier type? This paper does not attempt to answer this last question but some interesting suggestions have arisen from a study of the reactions of whitefish larvae reared under varying conditions.

II. MATERIALS AND METHODS

The water breeding amphibia are represented in central Illinois by salamanders, frogs and toads. The former breed early but the adults are hard to capture. The toads breed in a small artificial lily pond on the campus and also in several temporary puddles in an adjoining pasture. The adults are easily captured with a long handled net. They begin laying early in the morning and will continue until noon or after when brought into the laboratory. This affords a continuous supply of freshly fertilized eggs throughout the morning and is more satisfactory than artificial insemination. A string of eggs six to twelve inches long will be laid and fertilized in five minutes so that the time of fertilization is as definite as it could be under artificial insemination. A further advantage that these eggs possess for such a study as this is the fact that the time from fertilization to the functioning of the internal gills at 16°C. is very short, about twenty days. This makes it possible to watch their growth from hour to hour to see the effect of changing the conditions. The eggs are large enough to show the stages well under a hand lens.

The toad, although ordinarily repulsive, becomes quite attractive on closer acquaintance. The skin partly loses its rough warty appearance in the water and shows brighter markings. The breeding call is a musical trill. Individuals will pair under laboratory conditions but rarely will they lay their eggs. If laying starts under natural conditions it will continue during the first day after they are captured.

The other source of material for this work was the lake whitefish, Coregonus clupeiformis Mitchill. Some work was done on

fertilization and early stages at the U.S. Hatchery, Put-in-Bay, Ohio. The major part of the work, however, was done at the Vivarium, University of Illinois, on material shipped from the hatchery in ice. The temperature of the lake water was about 8°C. at the beginning of the season. It had a pH of 7.0 and an oxygen content of 4.08 cc. per liter. At the Vivarium the stock was kept in water from the University wells aerated to about 2.6 cc. to 3.3 cc. per liter and with much of the precipitated sludge removed. When this water was exposed to the air much of its carbon dioxide passed off thereby precipitating some of the iron and part of the bicarbonates as calcium carbonate. With extreme aeration some magnesium hydroxide might also be formed. A stringy matted appearance was given this sludge by the growth of iron bacteria Chrenothrix. This aerated water had a pH of 7.8. It was cooled to 10°C. by means of brine coils.

The water for all the experiments was treated by the method described by Shelford ('18). After this boiling the water was free of all dissolved gases and had part of the salts precipitated. It therefore had a higher alkalinity (pH of 8.0 - 9.0) and a lower buffer action than the untreated water. The total carbonates were 232 parts per million expressed as calcium carbonate.

The type of apparatus used for varying the pH and oxygen content is shown in Fig.1. Bottle 1 contained approximately N/4 sulphuric acid which siphoned over into the mixing bottle 4. The stopcock 2 controlled the flow which could be measured by counting the drops through the glass bulb 3. Thus, a known amount of the acid was added to a known flow of the boiled water which entered at W. This water was also 10°C. The water leaving 4 had a known pH

and was oxygen free. Since it contained a certain amount of carbonates the addition of small amounts of sulphuric acid merely set free small amounts of carbon dioxide and this free carbonic acid was what gave the pH. We are therefore dealing with differing amounts of carbon dioxide when we vary the pH.

The oxygen was controlled by means of the airline A which had a mercury manometer of bent glass tubing at the far end. The tubes which entered the experimental jars 5, 6, and 7 terminated in pieces of straight rubber tubing which had several holes burned in them with a hot needle. These broke the air current and distributed it more evenly through the jar. The stopcocks C controlled the amount of air going to each jar. When the cocks were all set the mercury could be set at a mark on the tube and a practically uniform flow of air maintained.

Water pressure forced the flow from the mixing jar to number 5 but it was siphoned from 5 into 6 and 7. It was also siphoned from 5, 6 and 7 into 8, 9 and 10 respectively. These were half pint sedimentation glasses such as are used in urine analysis and made very good jars for whitefish eggs as they worked on the same principle as the large jars in use at the hatcheries. (For the toad egg development finger bowls were substituted for these sedimentation glasses). Because air bubbles tend to go into the straight tubes and stop the siphons the ends of all outlets on the siphon tubes were bent upward. A second set of dishes could be run by siphoning the water from 8, 9 and 10 into other dishes. The pH was fairly constant for all the jars of one set, not varying more than .2 on the pH scale. This amount of variation was due to the increased aeration in 6 and 7.

Such a set-up as the one described above gave three concentrations of oxygen with one pH. Three such sets of apparatus have been used thereby getting three different hydrogen ion values each with three oxygen concentrations.

The pH has been determined by the use of indicator sets made up by Hynson, Westcott, and Dunning, with additional tubes for high and low pH values made up at this laboratory from the buffer solutions of Clark and Lubs ('17) and checked against the Hynson, Westcott and Dunning sets. Four indicators were used, brom cresol purple (5.8 - 6.6), brom thymol blue (6.6 - 7.6), phenol red (6.6 - 8.0), and thymol blue, alkaline range (8.0 - 9.2). During the course of the experiments the colorimetric standards were checked electrometrically by R.E. Greenfield.

The oxygen determinations were made by the Winkler method. As the amounts of water in the experimental dishes were small, 200 to 400 c.c., the use of 250 cc. bottles for this determination was impracticable. A piece of apparatus was therefore devised that would handle small quantities of water. This is shown in Fig.2. A second rod X was attached to a ring stand in such a manner that it could readily be slipped in and out of the clamps. To this was firmly bound the tube A which was furnished with a glass stopcock at 1. A piece of cork y on the rod gave support to the stopcock. The tube A was furnished at the upper end with a heavy rubber tube which had been boiled and paraffined, and had rings of glass tubing (B, C and D) inserted in it at intervals with just room for screw clamps (2,3,4 and 5) between. This rubber tube was ligatured with copper wire to the tube A and also at each glass ring. The lower end of A was connected by smaller rubber tubing to the mercury cup

which was supported by a clamp and could be raised and lowered on the stand.

In making a determination the mercury cup was raised to position I, such that the mercury just rose to the top of the opposite tube above 5 (all cocks from 1 to 5 being open). A siphon of water from the experimental dish was then inserted in the top of A tube, being careful not to include any air. When the mercury cup was lowered to position II, A filled with the water. Cocks 1 and 3 were next closed and 26 cc. of water were ready for the oxygen determination. The water was emptied from the upper part of the tube (B to D). B was filled with the first solution (manganous chloride), a glass bead added for a mixer, and clamp 3 closed. Any surplus solution was rinsed out with distilled water. The second solution (potassium hydroxide and potassium iodide) was added to C and clamp 4 closed. The tube was emptied, rinsed, and D filled with concentrated hydrochloric acid. Clamp 5 was closed. Then clamp 2 was removed and the first solution allowed to mix with the contents of A. Clamp 3 was opened and the second solution mixed with A. The glass bead was useful here in breaking up the precipitate and mixing the liquids. If the solutions were slow in leaving B and C, pinching the rubber between acted as a pump and forced the mixing of the liquids. Clamp 4 was removed and the acid dissolved the precipitate, freeing the iodine. The contents of A was emptied and titrated against approximately N/40 sodium thiosulphate which was frequently standardized with N/40 potassium dichromate. The amount of oxygen was calculated in cc. per liter according to the formulas given in Birge and Juday ('11).

A. Control of Conditions.

A number of workers in the last twenty five years have tried varying the acidity and bacisity of water and studying its effect on organisms. They have used distilled, fresh and sea water. Coventry ('11), Sumner ('97) and Wells ('15) used distilled water but failed to state the total solids present. Jenkinson used tap water, the salts of which are an unknown quantity. Moore, Roaf, and Whitley ('05) added acids and alkalis to sea water figuring molecular solutions without correcting for the carbonates present. In working with small amounts of acid of low normality there may be enough carbonates or bicarbonates in the water to neutralize all the acid added so that the end result is merely a lessened alkalinity. In this way animals may appear to tolerate larger amounts of acid than is really possible. With this in view I have worked out the data in Table IX. Definite amounts of acid of known normality have been added to 250 cc. of the boiled water used in all these experiments. At that time the water had a pH of 9.0 and an alkali reserve of 11.66 cc. of N/44 hydrochloric acid or an alkalinity of 132.46 parts per million calcium carbonate. The normality of the water in calcium carbonate was calculated and also the normality of each concentration of acid used for acetic, hydrochloric and sulphuric. The pH was determined immediately for each concentration. From the normalities of the acids used and of the carbonates in the water the residual normality of the bicarbonates has been determined and stated in parts per million calcium carbonate. For hydrogen ion values below 8.0 this is all bicarbonates. The amount of carbon dioxide liberated in parts per million was determined for the sulphuric acid series by titration with sodium carbonate.

From the mass law equations the amount of carbon dioxide

which might be liberated by the acid added was calculated as a check on the titration method. Using this calculated carbon dioxide the pH was then determined by the formula of Greenfield and Baker ('30) to see how the theoretical pH would compare with the determined pH.

$$\text{"Hydrogen ion (pH) concentration} = \frac{4.0 \times 10^{-7} \times CO_2}{HCO_3} + 1 \times 10^{-8}$$

where the free carbonic acid is stated in parts per million of CO_2 and the bicarbonates in parts per million of calcium carbonate."
Or when both free carbonic acid and bicarbonates are expressed in either parts per million CO_2 or cc. per liter of CO_2 the equation becomes:

$$\text{Hydrogen ion (pH) concentration} = \frac{3.5 \times 10^{-7} \times CO_2}{HCO_3} + 1 \times 10^{-8}.$$

For acid values from 5.8 to 6.5 both pH values and carbon dioxide volumes check but as we approach neutrality and the buffer action disappears the results do not check. More work is needed to find out why values do not check at this point. It is interesting to note that the series of jars used for the whitefish experiments which were run on a neutrality basis show wide fluctuations for pH in most cases but where neutrality was maintained for a period of time quite unfavorable development resulted.

III. EXPERIMENTAL DATA

A. Toad Eggs.

In the work on toad eggs several types of experiments were performed. (1) Keeping the pH constant (8.0) the oxygen content was varied, four concentrations, .4 cc., .9 cc., 1.41 cc., and 4.64 cc. per liter being used. (2) Keeping the oxygen about the same in all dishes, .4 cc. to .8 cc. per liter, the pH was varied from 6.1 to 8.0. (3) Eggs were sealed into known quantities of water with differing oxygen content and the time and extent of development noted.

In the first type of experiment eggs were put into the experimental dishes at different stages of development and the effects as shown by retardation, acceleration or death noted. These results were tabulated in Tables I to III, the A series and the same results shown graphically in Figs. 3 to 6. In making the graphs normal development was taken arbitrarily as a straight line from fertilization at 0 hours to the internal gill stage at 196 hours. Time in hours was plotted as ordinates and the stages of development, or abscissae, were obtained by dropping perpendiculars from the normal development line to the x axis according to the number of hours required to reach that development. All experimental curves were then drawn with both time and period of development fixed by the normal curve. When the experimental curve rises above the normal line this denotes retardation, when it drops toward the normal or below it, acceleration. Curves parallel to the normal denote normal rate of growth.

Using a pH of 8.0 and four concentrations of oxygen, .4 cc., .9 cc., 1.41 cc., and 4.64 cc. per liter, we find that there was a certain degree of development attained regardless of the concentration,

death not occurring in any of the experiments short of the yolk plug stage even when the oxygen was as low as .4 cc. per liter. Eggs put in before the first cleavage (Fig.3-I) show marked retardation up to the late blastula stage then some acceleration so that further development approaches normal. Eggs exposed to the experimental conditions between the first cleavage and the late blastula stage (Fig. 3 and 4) show a period of eighteen or twenty hours with little or no retardation and then a period of quite marked retardation occurring in the blastopore or yolk plug stages. Later development for the higher concentrations of oxygen again approaches normal. Eggs put in after the late blastula stage (Fig.5) show general retardation for the lower oxygen concentrations but fairly normal development for the higher concentrations.

The results of this type of experiments show that where the oxygen was as low as .4 cc. to .9 cc. per liter death occurred in part of the experiments but at higher concentrations only retardation showed the effects of exposure. At the late blastula and blastopore lip stages the higher concentrations even caused acceleration of growth. The most sensitive stages appeared to be fertilization to the first cleavage, and the gastrulation stages.

The second type of experiment was where the oxygen was low (.4 to .8 cc. per liter) and the pH varied, 6.1, 6.4, and 7.0 to 8.0. The stages reached in development are shown in Tables II and III (C series) and graphs in comparison with the normal in Figs. 3 to 6.

Exposure made before the four cell stage gave death with retardation in all cases. The same effect is seen in eggs put in after the eight cell stage. Those exposed between the four and eight cell stages show the greatest development. With small amounts

of oxygen the effect was much more rapid and deadly with a pH of 6.1 to 6.4 than with a pH of 6.4 to 6.8 (compare C-1 with C-2, Tables II and III).

In the third type of experiment a dozen eggs were placed in an eight dram vial (about 26 cc. of water) supplied with a two-holed stopper having the inlet tube extended to the bottom of the vial and the outlet tube even with the stopper. Both tubes ended outside the stopper in short rubber tubes. Water of the desired oxygen content was run through the bottle until all oxygen due to air was flushed out and then a clamp on the two rubber tubes sealed the bottle. Development was watched by means of a dissecting lens until growth stopped. The oxygen content was then determined by adding the chemicals through the inlet tube being careful to avoid air bubbles when inserting the pipettes in the rubber tube. Table IVa shows the results of these experiments; the stage at which the eggs were put in, the number of hours to death, and the stage at which death occurred. Fig.7 shows the same graphically. The number of hours on each line was obtained by subtracting the initial age of the eggs from the time to death, giving the number of hours they were confined in the bottles. The cleavage of a dozen eggs proceeded to the late blastula stage regardless of the period of development when put in or the amount of oxygen present (.1 - 5.7 cc. per liter). Further development was roughly proportional to the amount of oxygen present, as was also the time to death.

B. Whitefish Eggs.

In the work on whitefish eggs more apparatus was set up so as to get a wider range of conditions. Three sets of apparatus (Fig.1) with a pH of 6.4, 7.0, and 8.0 - 9.0 respectively were used.

The oxygen content of the three jars in each was approximately 1 cc., 3 cc., and 4.5 cc. per liter. In such a set of conditions one could compare the effects of differing pH at a constant oxygen content and also the effects of varying the oxygen content at a constant pH.

The eggs were obtained from the hatchery in four lots. The first lot, spawned December 2nd, was in the thirty two cell to early germinal cap stage when received at the Vivarium, December 3rd. The second lot, spawned December 5th and received December 24th, had the tail just elongating and the fin buds starting. The eye vesicles had formed but no pigmentation had occurred. The third lot, spawned December 7th and received January 31st, were only slightly farther along in development than lot two. They had just begun to show the pigment in the eyes. The fourth lot received March 12th were fully developed and started to hatch immediately.

All the experimental work was done at 10°C to 11°C. The first experiments were conducted at the U.S. Fish Hatchery at Put-in-Bay, Ohio. First an attempt was made to test the length of life of the eggs and sperm. For this three sets were run, one with dry eggs and sperm, a second with dry eggs and sperm mixed thoroughly with lake water to a uniform milky fluid, and a third using dry sperm and eggs standing in lake water. Table V shows the time during which fertilization would take place. With wet eggs and dry sperm eight minutes was the latest time at which fertilization was positive. With wet sperm and dry eggs nine minutes showed a very small number of fertile eggs, about 1%. But with both eggs and sperm dry fertilization took place at seven and a half hours and I feel may have been possible after longer times. The experiment using a longer time was accidentally destroyed and there was no opportunity to try again.

In the early development experiments (Table VI) the eggs were exposed to water of constant oxygen content (4.08 cc. per liter) but with varying pH. Normal lake water had a pH of 7.0. Acidity (pH of 6.2 - 6.6) was produced by adding sulphuric acid and a bacisity (pH of 8.4 - 8.6) by adding sodium hydroxide. As running water was not available dishes of standing water were used and the solutions changed frequently. Eggs fertilized for thirty minutes dry and then put into the solutions were still developing normally at four days. Eggs fertilized directly in the solutions were normal at the end of the first twenty four hours (32 to 64 cell stage). This experiment was then repeated using lake water which had been boiled until the oxygen content, after running it into the dishes, was 2.9 cc. per liter. Table VII shows the results of this treatment. Fertilization and development occurred in all the dishes but there was a marked difference between the acid and alkaline waters. With a pH of 6.2 and 6.6 80% and 25% fertilized and developed, with pH of 7.0 only 3%, and at a pH of 8.4 but 1%.

In the work on later stages with treated running water at the University of Illinois special attention was paid (1) to keeping conditions as near constant as possible from day to day, (2) to watching the stage of development reached in each concentration and comparing these with each other and the control stock, (3) to working out the death rate and percentage hatching for each concentration, and (4) to testing the vitality and reactions of the larvae hatching from the different stocks.

Table X shows the conditions of pH and oxygen content in the individual dishes for the entire period of the experiments. These are shown graphically in the lower half of Figs. 8 to 16.

From December 6 to 15 the set-up was different from the rest of the period in that it was attempted to keep the oxygen constant and vary the pH by adding a little alkaline water to jars 6 and 7 (Fig.1). It was impossible to keep conditions constant so December 16 the apparatus was remodeled according to Fig.1. This gave quite constant results.

Table VIII shows the number of days required to reach the different stages of development in the various concentrations. Four series of experiments were run using the first two shipments of eggs. Series II was run with the first type of apparatus and consequently conditions varied within rather wide limits in all the dishes. With a pH around 6.3 - 6.4 we find that a set of eggs put into the three dishes, A, B, and C-1 on the seventh day varied in both amount of development and length of life roughly in proportion to the amount of oxygen supplied. This was .1 cc., .2 cc., .6 cc. per liter respectively. This amount was not enough to carry development beyond the elongation of the tail bud to one fourth of the body length, and proved lethal in every case. This same oxygen effect seemed to hold with higher pH as is seen from a study of dishes A, B, and C-2 and C-3. This amount of oxygen was evidently below the threshold for the whitefish.

In series III therefore the oxygen content was raised somewhat so that the dishes 1 had about 1 cc. per liter, dishes 2 about 2.5 cc. per liter, and dishes 3 about 4 cc. per liter. With this increase in oxygen concentration the more alkaline water favored the farthest development. The eggs lived as many days in the acid and neutral waters of high and low oxygen content as they did in the alkaline water but development was retarded. It is interesting to

note that those eggs in neutral waters were more retarded than in either the acid or alkaline medium. This set (Series III) was the only one in which true neutrality was approximated as the pH fluctuated greatly in all later experiments. Where neutrality was maintained, as in this set, a detrimental effect is noticed in development. This set of eggs put in at the early pigmentation stage of the eyes lived nearly to the time of hatching, the period where the tail has grown past the median line of the head and around to the eye.

Series IV and V were run with eggs from the second stock. The experiments were started three days apart and run simultaneously so that conditions were identical except for the time of entering the experimental waters. The average oxygen for the different dishes was slightly higher than in Series III, but the relative values of the dishes were the same. The eggs of Series IV were put in at the time the tail was one fifth to one fourth of the body length and the fin buds were just starting. A distinct oxygen differential was established; the low oxygen eggs (1.4 - 1.8 cc. per liter) developing to the point where the tail reached the median line of the head, the medium oxygen eggs (3.3 - 3.9 cc. per liter) having the tail developed past the head and around to the eye, while the high oxygen (4.6 - 4.7 cc. per liter) had the tail reaching to the ear in the acid and neutral pH. This alkaline content did not seem enough lower in oxygen (4.13 cc. per liter) to account for the retardation which occurred and I believe it was due rather to the pH.

In Series V (eggs put in three days later at the early pigmentation of the eye stage) more difference in development is apparent between the different dishes. The greatest amount of retardation occurred in the low oxygen jars and in the high oxygen of the

neutral series (pH fluctuating). The eggs lived longer in the low oxygen of the neutral series but were retarded in development. Two eggs hatched in the medium oxygen of the neutral series but all others died when the tail was around to the eye. These were the first individuals to hatch in the experimental dishes though a few hatched in the stock (pH 7.8 and oxygen 3.3 cc. per liter).

Hatching occurred in the experiments run with both the third and fourth stocks. As these stocks were older when they arrived the time of exposure to the experimental waters was later and a different physiological reaction resulted. Series VI and VII were started two days apart but were run simultaneously. There was no hatching in the low oxygen (.6 - 1.4 cc. per liter) of acid or alkaline sets and but four individuals in the low oxygen of the neutral series. In the medium oxygen (1.7 - 2.5 cc. per liter) there was hatching in the acid and neutral sets, and in high oxygen (3.8 - 4.7 cc. per liter) in the neutral and alkaline sets. The hatching extended over several days, the beginning and duration of the process being recorded in Table VIII. The percent hatching and the corresponding pH and oxygen averages are shown in Table XII.

Series VIII was started just as the eggs of stock three were ready to hatch, to test the effect of different concentrations on this process and on the life of the larvae. Hatching occurred in all dishes beginning on the same day. Maximum hatching was two days later in the acid 'medium' oxygen and in the acid and neutral high oxygen, showing some retardation for these concentrations. The apparent earlier maximum for alkaline high oxygen was due to the small number hatching and early death of the eggs. The percent hatching and the average pH and oxygen concentration for these is shown in

Table XII.

Series IX is the result of the exposure of eggs reared for the entire time of development at the hatchery in lake water. It shows the relative sensitivity of the hatching period and the newly emerged larvae. The first hatching occurred in the acid and neutral low oxygen an hour or two after putting the eggs in the water. A few also hatched in alkaline 'low' and 'medium' oxygen the same day. Tables VIII and XI show maximum hatching time and percent hatched.

The eggs of these different series (II to IX) were counted and the dead recorded from day to day in the course of the experiments. From this data the percent of deaths day by day was calculated for each dish of each series. This is given in Table XI. Tables X and XI are combined graphically in Figs. 8 to 16. Here the death rate may be compared with the pH and oxygen content of the jars and the effect of these concentrations on the steepness of the death rate curve observed. The sensitivity of the different stages of the early life history may also be compared as each series was for eggs put into experimental conditions at a little later stage of development. The stages from early formation of embryo and ring seemed very sensitive as in all concentrations the death rate was 100% and the curve very steep. From the time of closure of the posterior ring and beginning of elongation of the tail and fin buds sensitivity decreased, as conditions which were quickly fatal at earlier stages here were less toxic. The curves from this point on become flatter and concave showing more resistance. As the eggs of the earlier stocks approached hatching a quick rise of the curves shows a heightened sensitivity. The amount that these fell short of the 100% mark shows the amount of hatching. The last series put into the experimental dishes just at

hatching time show very short and flatly concave graphs since the number dying was much less than the number hatching. The fact that the last stock was less sensitive at hatching was caused, I believe, by the greater vitality of the eggs due to their normal development. The eggs exposed to the different concentrations for a period of time before hatching lost their vitality and therefore their resistance was lowered at that time. Another measure of the resistance of eggs at different stages was shown by the mortality ensuing when the water accidentally stopped running through the dishes. From the time of early elongation to nearly hatching time a stoppage of a few hours was not fatal but the last day or two before hatching a very short stoppage would kill the whole set of eggs. Immediately after hatching, however, the young larvae could be put into standing water with no ill results. They would live as long there as a similar set in running water.

Another factor which entered into the problem with the whitefish eggs was fungus growth. This fungus growth was in direct proportion to the amount of oxygen present (Figs. 8 to 16), therefore, was most detrimental in the high oxygen concentrations. The pH seemed to have no differential effect. An attempt was made to sterilize eggs to see if this could be eradicated. Some were treated with corrosive sublimate, one part in a thousand, applied for one, two, and four minutes, others with seventy percent alcohol for one and two minutes. These were all fatal to the fish rather than the fungus, so the attempt was abandoned. The fungus grew very rapidly on dead eggs but also entangled live ones and seemed to smother them to death. Fungused eggs left in the dishes would mat dozens of eggs together and kill them in a day or two. A small rise in temperature

was highly favorable to the fungus growth which was probably the reason that it grew worse in my experiments than at the hatchery. The experimental temperature was about two degrees centigrade higher than that at the hatchery.

As all these experiments were run using sulphuric acid to liberate the carbon dioxide and give the desired pH, it was thought desirable to run a check experiment with other acids to see if the effect would be the same regardless of the type of anion present, sulphate, chloride, or acetate. Accordingly a series of finger bowls was prepared such that a pH of 6.3, 7.0 and 9.0 was obtained for each acid. It is a well known fact that salts such as sodium chloride antagonize the acid which may be present in water (Loeb '13, Sumner '07, Osterhout '14), and therefore a duplicate series was made up in which one third of the volume of water was substituted by M/2 sodium chloride. The solutions were all made up using the proportions of acid and water for each pH as given in Table IX. The same amount of mixing was given each solution as it was made up to insure a uniform oxygen content for the series. This was 1.5 cc. per liter. The finger bowls were filled full and a cover put on. The readings were taken without removing this. Table XIII shows the time to death in the different concentrations. In the acetic and sulphuric acid (no salt) death occurred in pH of 7.0 first, 9.0 second, and 6.3 last, but for hydrochloric death in 6.3 preceded 7.0. Neutrality was most quickly fatal and alkalinity more toxic than acidity. In the series to which salt was added we find a very much lengthened life, though here we find 7.0 more toxic than 9.0 or 6.3. The sulphuric plus salt was least toxic of all with a pH of 6.3 as these larvae were still alive at the end of twenty three days. The difference in the reactions to the three acids was so small that it seemed

to be the variation in amount of the positive ions- the hydrogen ions- which was influencing growth rather than the negative ions- sulphate, chloride, or acetate.

The normal time for the development of these eggs at the hatcheries was four to five months. When my first stock began hatching at thirty two days it raised the question as to whether the vitality of such fry would differ from that of hatchery fry and wherein would lie the difference. Would such individuals be as large as later ones? Would the length of life of early hatched fry be longer or shorter than later stock? Would their reactions be different? Accordingly the newly hatched larvae from each of my four stocks were measured for body length and size and shape of yolk, the length of time from hatching to death recorded, a comparative study of the length of life in high carbon dioxide content made, and gradient experiments run.

Only a few individuals were measured in stocks one and two. In stock one the fry were eight to eleven millimeters in length with round yolk sacs two millimeters in diameter. These lived only two days. In stock two the fry hatched were eleven to eleven and a half millimeters long and had round yolk sacs two millimeters in diameter. These lived sixteen days with no food but the yolk. The last individual to die measured fifteen and two tenths millimeters and the yolk was nearly absorbed leaving only the large clear globule of the anterior part which measured about five tenths of a millimeter. The third stock averaged eleven and one tenth millimeters in length. These yolks were also round and two millimeters in diameter. These lived at least ten days and I believe several days longer. Stock four, raised in the lake water gave fry of a distinctly different

type. They were thirteen and four tenths to fifteen millimeters long on hatching. Although the yolk sacs were oval they were about the same in volume as the earlier stocks being two and a half by one and a half millimeters in the two axes. These were extremely active. This stock lived without food for 25 days and at the time of death measured fifteen millimeters in length. Some of these fry were placed in an aquarium which had become balanced and had algae and small crustaceans growing in it. The larvae began eating and seemed to thrive there. Growth was rapid, the larvae after 44 days measuring thirty four millimeters in length.

Shelford ('21 unpublished) has studied the effect of carbon dioxide in both high and low oxygen water and made a comparison of the time to death in the different concentrations. It was thought advisable to try such experiments on the whitefish larvae from different stocks and see if the same laws held true for larval as for adult forms. Table XIV shows the results for newly hatched larvae of stocks three and four. When carbon dioxide was added to the practically oxygen free water until a pH of 6.3 was reached larvae of stock three lived for two hours and twenty five minutes and two hours and thirty minutes, while larvae of stock four lived four hours and twenty minutes, three hours and forty minutes, and four hours. Larvae of stock three, put into the same water but with no carbon dioxide added (pH 9.0), lived for three hours and forty five minutes while those of stock four lived five hours. This would seem to indicate a difference in the vitality of the two stocks. When larvae of stock three were put into water with some aeration (2.8 - 3.0 cc. per liter) with a pH of 6.4 one set was still alive and active at the end of five hours, another at twelve hours, and a third at eighteen hours. This

last set was interrupted, having nine hours at 6.3, eleven hours at 9.0, and then nine hours at 6.3. The larvae in aerated water of pH 9.0 lived a week.

As each of the stocks hatched gradient experiments were run with the larvae to see if the environment during the early embryonic life had any effect on the pH range which they would choose. A gradient tank suited to the size of the animals was used. This tank was twenty and five tenths centimeters long two centimeters wide and one and five tenths centimeters deep with screens one and five tenths centimeters from each end and outlets at both sides of the middle. Water was introduced drop by drop behind the screens at the ends and flowed out at the center. The end thirds were thus very nearly the same as the water introduced there while the center third was a gradient of the two. The experiments were run using as light a forty watt mazda lamp thirty inches directly above the tank. Light is an important factor with these fish as their first reaction on hatching is positive to light and negative to gravity, a reaction which brings them to the surface where their food supply of microscopic plankton is found.

Stock one and two were reared from the early germ cap stage and the beginning of the tail elongation, respectively in the vivarium waters. Both of these stocks then lived through the period of heart and blood formation in the experimental waters. Would this relatively constant experimental pH exert any influence on the blood and body fluids thereby changing the pH which the animals would naturally choose in a gradient of waters? Probably the pH chosen by the gravid adults for the eggs to develop in is about 7.0 as this was the pH of the waters in November at the hatchery and in the lake

nearby. The eggs were laid in the shallows among the islands where the hatchery is situated. Of course the pH at the bottom on the shoals may be different from that of the water nearer shore from which the hatchery supply was pumped. To draw definite conclusions one should know the pH on the spawning grounds throughout the winter season when the eggs are developing.

The results of the gradient experiments are suggestive, however. The graphs in Fig. 17 show the choice made by the larvae from these stocks. No larvae hatched from the experimental dishes of the first stock, but those hatching in the control (pH 7.8) gave such a graph as Fig. 17a when given a choice of 6.6 to 9.0. The animals were fairly active but they stayed within the central third of the tank, a range of 7.6 to 8.8. Fig. 17b and c show the reactions of larvae of the second stock to control water (pH 7.8) both with running water and standing water. Some effect of current is noticeable in the running water but no preference for one end is established. When given a gradient of 6.4 to 8.2 a very definite choice of one end is seen. Larvae reared in water of low hydrogen ion concentration (pH of 9.0) choose the alkaline end of the tank but contrary to expectation those from the high hydrogen ion experiments (6.4) also choose this end. None of the other larvae reared in any of the later stocks or experiments choose this low hydrogen ion concentration if there is water of higher acidity available.

The third stock of eggs were received when the larvae were in the stage of early eye formation. In these the heart and blood system was formed and the heart beating. Fig.18 and 19 show the reactions of these larvae hatched both in the controls and experimental dishes. All of these fishes whether reared in high or low pH had a

tendency to choose waters on the acid rather than on the alkaline side of neutrality. There were individual variations. Some such as Fig. 19b and d showed this preference immediately. Others such as Fig.18a and b and Fig.19c took a period of ten to fifteen minutes of wandering before the preference was established. Animals hatched in water with a pH around neutrality gave the least decisive graph. Such larvae chose first one end and then the other, seemingly finding it hard to make a choice (Fig.18e and f, Fig.19a). In graph 19d during the nineteenth and twentieth minutes the larva was put from the acid end where it had been active into the alkaline end of the tank. Here it lay motionless and would not even contract on stimulation. Two minutes after replacement in the acid end it again responded to stimulation and became active. This seems to be the type of response to acid and alkali, acid waters stimulate and make active while alkaline waters cause sluggish movements or quiescence.

In stock four the eggs were raised to hatching at Put-in-Bay and so began hatching a few hours after they were put in the experimental waters. These therefore passed the time of blood and body fluid formation in the lake water. Animals hatched on the 14th and 16th of March were kept in the experimental waters and gradients run with them on the 17th and the 22nd (Fig.20 and 21). Nearly all of these showed the characteristic exploration of the whole tank for from three to five minutes, then preference for one end. The turning back occurred at a very definite place and the avoidance was from either side, for often when an animal entered the unfavorable water it stayed there turning back from the changing point to again enter the unfavorable end several times before it escaped to the more favorable water. We therefore find the point of preference clearly marked.

The fish hatched and kept in water of a pH of 6.3 show a preference for the acid end of the gradient (Fig.20a, b, c, and d). Those hatched and kept at 9.0 at first seemed to choose this end of the gradient but they gradually worked down into the acid end (Fig. 20f and g).

Wells ('15) states that if a gradient is entirely confined to pH above neutrality, 8.0 to 9.0, that fish will choose the more alkaline end even though preferring an acid pH when given a choice of 6.4 to 9.0. I found that this was true of the whitefish larvae (Fig. 21b, c, d and e). The larvae from acid water (b and c chose more exclusively the part from 8.8 to 9.0 than those hatched in 9.0 water, although these turned at 8.4 and spent most of their time in the 9.0 end.

D. Reaction of eggs to Hydrogen Peroxide as development progresses.

A quite universal property of protoplasm (both plants and animals) is the ability to liberate oxygen from hydrogen peroxide. What this property is due to is a debated question at present but in general it is ascribed to an enzyme called catalase. Several theories have been advanced as to its significance in animal life. References are given to these in the bibliography (Becht, Burge and Burge, Levine and Morgulis, Loew, and Stehle). As a measure of the sensitivity of the developing egg at different stages in the early life cycle the reactions to oxygen and carbon dioxide (acidity) have been discussed. It was thought that a study of the amounts of oxygen liberated from hydrogen peroxide at various periods of development might be of significance. Accordingly a series of determinations was made with the toad egg using the method of procedure outlined by Burge ('16), Table XIVa and Fig.22a. A second series was made for

the toad eggs the following spring using eight dozen eggs instead of one dozen and shaking the material by machine instead of by hand (Table XIVb and Fig. 22b). A comparison of A and B shows a striking parallelism and the two curves seem to confirm each other. Determinations were also made for various stages in whitefish development and these show a similar type of reaction (Table XIVc).

From a study of the curves (Fig.22) it may be seen that there was an increase in the power of liberating oxygen from hydrogen peroxide for the stages from fertilization to the early blastula, a decrease from the early blastula to elongation of embryo, and from this point to twenty one days a steady increase. The stages most sensitive to low oxygen and high acidity were (1) fertilization to first cleavage, and (2) the gastrulation stages. These were the low points on this curve. Is there a correlation between sensitivity and a lowered power of liberating oxygen from hydrogen peroxide?

Winternitz and Rogers ('10) have shown a definite increase in what they call catalase for the different stages of the hen's egg as development proceeds. Burge and Burge ('21) have shown an increase in this power from egg to adult for the Colorado potato beetle. Zieger ('15) has found a definite rythm for catalase in the insect life history, reporting that the power is high where rapid growth or metamorphosis is going on (early larval stages, pupal stages) and low during resting stages. Child ('15) has shown that metabolism is high during the more sensitive stages. Burge believes that increase in metabolism brings about an increase in catalase or the power of liberating oxygen from hydrogen peroxide. This curve for the toad egg seems to be a contradiction of one statement or the other since it shows the power of splitting oxygen from hydrogen peroxide to be low

at the sensitive stages. More work is needed on complete life histories before definite conclusions can be drawn.

IV. GENERAL DISCUSSION

The length of life of eggs and sperm as tested by the power of fertilization accords with the work of Quatrefages (53), Coste ('59), and Reighard ('93). Quatrefages found the time during which fertilization is possible to be 8 minutes 10 seconds for the Pike, 3 minutes 10 seconds for the Carp, and 2 minutes 40 seconds for the Perch. Coste found it impossible to fertilize Trout eggs with sperm which had been eight minutes in water. Reighard attributes this long time of Quatrefages (8 minutes) as due to improper mixing of the milt with the water thus leaving sperm in the middle of the pieces untouched by water. He gives the time for the walleyed Pike as between 1 and 2 minutes, and suggests that since the sperm of the whitefish are motionless at the end of two minutes in water their time for fertilization is also extremely short. He did not check this experimentally, however. In my work on fertilization where the milt was mixed with lake water a thin suspension of uniform consistency was prepared and great care taken that no lumps be present. Fertilization took place at the end of eight minutes indicating that though the time for this process is extremely short when compared with some marine forms such as the sea urchin, still it is quite long as compared with other lake fish such as the walleyed Pike. It indicates a wide range of variability for the fishes in similar habitats.

In studying the effects of environment on development the results of exposure to acidity, neutrality, and alkalinity have been noted and the sensitive stages in the early life history determined. In some respects these results agree with those of other workers but in some ways they differ.

The most sensitive stages in both toad and whitefish are the first early cleavages and the gastrulation cleavages. Whitefish eggs raised in the various solutions up to the time of hatching show a drop in resistance at this point, but eggs raised in lake water to the time of hatching show a high percentage of larvae when hatched under experimental conditions. This would seem to show but a slight rise in sensitivity at this point under normal conditions. Sollman ('06) in his study of the effect of the alkaloid poisons, barium chloride, sodium fluoride, and potassium cyanide on the developing fundulus eggs found that the period from the one cell stage to early embryo formation on the shield was very sensitive. The period from heart formation to hatching was much more resistant but there was a sharp rise in sensitivity at hatching time. His embryos were in the solutions throughout the whole period of development. Stockard ('21) working on fundulus development from the standpoint of temperature and lack of oxygen reports similar results as to sensitivity. He exposed eggs at different stages and found that those which were retarded during the first cleavage and gastrulation stages form a high percentage of abnormal individuals. Eggs exposed after gastrulation suffer little. He reports no drop in resistance at the time of hatching. The production of twins must take place at the time of gastrulation, or budding of the germ shield. This is a very common result in developing trout eggs. In normal development of the embryo from the germinal shield the first growing point is dominant over all other points which might grow much as the terminal bud of a twig is the dominant one. Adverse conditions may destroy this dominance thus allowing other points to start at the same time. The extent of this twinning is dependent on the distance apart these

points may be on the shield. If 180° apart two perfect individuals are formed joined by the yolk sac but as the points approach, more and more of the two buds appear as a single individual thus forming double headed monsters. According to his theory such twins cannot be formed after gastrulation. The fact that I found no double monsters or twins in my experiments may be due to the high sensitivity of the gastrulation period, all the eggs started at that time having died rapidly. Twins and double embryos are reported for the whitefish eggs at the hatchery and are thought to be due to overcrowding, the same reason that Stockard reports.

As to the effects of acidity, neutrality and alkalinity on animals different workers disagree. J. Loeb was one of the earliest workers on the chemical environment in development and regeneration. In his work on Arbacia ('98) he added hydrochloric acid and sodium hydroxide to the sea water in which the eggs were developing and from a study of their subsequent behavior decided that acid delayed development through lowering the oxidations, but that alkali hastened growth by aiding oxidations. He considered sea water neutral, a result of the fact that at that time neutrality was determined as the end point of phenolphthalein. In his later work ('04-'13) he has studied the effect of various salt solutions, isotonic as well as hypo- and hypertonic with sea water, on development and regeneration and finds that the cations or substituted hydrogen ions are most influential in changing growth, calcium, sodium, and potassium being necessary in definite concentrations sometime during the life cycle for normal individuals to result. He believes that any substance such as alkali or alkali salts (carbonates, phosphates) hasten or aid the growth process by neutralizing acid products formed in the

organism; that is, by their buffer action.

Osterhout's idea of salt action and antagonism between salts and acids is that of a change of permeability in the cell wall. He believes that if alkali aids development by hastening oxidations it is because the alkali reacts with the cell wall increasing its permeability and allowing more oxygen to enter. His theory rests on a series of experiments with Laminaria saccharina, a marine alga. He has found that the electrical resistance of living tissues is a measure of their permeability, an increase in permeability being shown by a decrease in resistance and vice versa. By this method he has shown that NaOH and NaCl increase permeability but $CaCl_2$ and HCl decrease it. Antagonism is due to the presence of two substances having opposite reactions on the protoplasm, NaCl and $CaCl_2$ or NaCl and HCl. The amount of antagonism between two substances may be predicted if we know the degree of increase in permeability which may be induced by each separately. (Osterhout '14, '14a, '14b).

A comparison of the chemical analyses of the lake water and the boiled University water is quite interesting in the light of this discussion and the experimental results on the whitefish.

	lake water	boiled water
pH(CO_2)	7.0	9.0
CO_3	54 ppm	250 ppm
SO_4	10 ppm	trace
Cl	5 ppm	1 ppm
Ca	27 ppm	33 ppm
Mg	7 ppm	27 ppm
Na, K	5 ppm	16 ppm
Total Solids	112 ppm	248 ppm

Addition of acid would have as its first effect a reaction with part of the carbonates to form free CO_2 and raise the pH. Then according to Osterhout's theory the acid present (as mineral acid or CO_2) would tend to antagonize the higher concentration of salts in the boiled water.

Jenkinson ('10) and Coventry ('11) have worked on the variability induced by sodium chloride on frog tadpoles and the effects of hydrochloric acid, acetic acid and sodium hydroxide on toad tadpoles. Their method has been to make up solutions in distilled or tap water, put in the eggs and leave them a certain length of time. The material was then preserved and studied. They do not take into consideration the composition of the water employed nor the fact that such solutions may change markedly in the course of animal growth both in chemical composition and amount of dissolved gases so that the final result may be due to several factors. Jenkinson finds that the mortality increases and body length decreases as the strength of sodium chloride increases. Coventry found that of the three reagents used tadpoles in sodium hydroxide solution were the only ones to hatch in any number and here the mortality was high (32% - 54%). A few hatched in the .0016% hydrochloric acid but these were smaller than the individuals in the alkaline solution.

Moore, Roaf, and Whitley ('05) in working with marine eggs, Echinus esculentus, also report acids as more deadly than alkalis. They state the limits of acidity and alkalinity in which growth may take place to be small, .0015 M sodium hydroxide or .001 M hydrochloric acid causing death. Whitley ('05) has extended this work to include the plaice eggs. He suggests that the less harmful effects of adding alkali may be due to its being thrown out as

insoluble hydrates and carbonates. He notes that there is a direct increase in the power of resistance with the advance of development without a drop at the time of hatching. These workers all believe that an excess of OH ion or of some basic salt that will absorb carbon dioxide (that is, lower the hydrogen ion concentration) is most beneficial to growth and development in marine forms.

Other workers, Wells, Shelford, and Allee believe that a certain amount of dissolved carbon dioxide is beneficial to the development of fresh water forms. Wells ('15) states that fishes when given a choice will choose the acid side of neutrality rather than the alkaline. In all his work he regards neutrality as the end point of phenolphthalein which we now know to be a pH of 8.0 or one unit on the pH scale on the alkaline side of neutrality. Accordingly his experimental fish show a preference for waters between 7.0 and 8.0 or really slightly alkaline waters. These waters however contained free carbon dioxide to the extent of 18 to 36 parts per million. He concludes that different species have a definite carbon dioxide optimum and this may vary from a very slight concentration, pH near 8.0, to 18 parts per million, pH of 7.3. If this definite concentration is necessary for normal development to occur how could whitefish eggs develop, hatch, and live for several weeks in water with a pH of 9.0, strongly alkaline with carbonates and with no free carbon dioxide? Wells believes that these fishes are avoiding neutrality (pH of 8.0) and states further that if given a choice of neutrality and alkalinity (pH 9.0) they will choose the latter. This seems to be a quite general reaction. Plankton studies of vertical distribution show the smallest number of individuals at the thermocline (pH 8.0) with increasing numbers each side of it, in either

acid or alkaline waters. The whitefish larvae hatched either in pH 6.4 or 9.0 (both normally choosing the more acid end of a gradient when it varies from 6.4 to 9.0) all choose a pH of 8.6 to 9.0 when the gradient is 8.0 to 9.0. In low oxygen waters acidity was more favorable to fertilization in the whitefish eggs than alkalinity. These facts confirm Wells' observations.

However, the question as to which of the other pH concentrations is best for development should be considered further. Development and hatching occurred in all the solutions, acid, neutral, and alkaline. With low oxygen content (.4 - 1.6 cc. per liter) a pH fluctuating around 7.0 shows the highest percent hatching (Table XII) as well as the flattest death rate curves (Fig.8-16). With a medium oxygen supply (1.7 - 3.7 cc. per liter) the acid series pH 6.4, shows the highest percent hatching and the lowest death rate. With high oxygen (4-5 cc. per liter) the highest hatching rate is in the alkaline and neutral dishes. The best hatching occurs where the oxygen is of medium concentration rather than high probably due to the rapid fungus growth which tends to smother the eggs. The growth of this fungus is in direct relation to the amount of oxygen present.

The question is then raised as to which is the more effective in development, variations in oxygen content or in hydrogen ion concentration. From these experiments I believe that pH is more effective though oxygen changes do also cause variations. Eggs develop normally at the hatchery with an oxygen content of 4.08 cc. per liter and a pH of 7.0. Under experimental conditions they develop into normal embryos with a much lower oxygen content and pH of 7.0. Much greater variations were produced in percent hatching and in length of time for development by varying the pH toward the

acid or alkaline end of the scale than by changing the oxygen. This is in accordance with the idea of Powers ('20). He concluded from his reconnoissance of the pH of the Puget Sound waters under varying conditions of tides, weather, etc. that pH has more to do with the compatibility of habitat than oxygen content. He cites barnacle distribution in support of his view. Shelford and Powers ('15) worked out the pH reactions of the herring. They found that this fish chooses a pH of about 8.0 regardless of whether this was offered them in fresh or salt water. Shelford ('18) showed that herring recognize the difference between 8.0 and 8.1 (uncorrected for salt error which would make it approximately 7.75 and 7.95). Powers ('21) found that herring chose a pH of 7.6 - 7.7 (corrected for salt error) and in following up the schools in Puget sound observed that they continually chose this same pH, a fact which is at least very suggestive. McClendon also believes that pH is an important factor in animal life. He has done a great deal of careful work on the determination of pH in sea water both colorimetrically and electrometrically. He has then varied the pH of the water with which he perfused the heart of the conch and found that it stopped in systole with a pH of 9.7 and in diastole with a pH of 5.6.

Fishes from different habitats are affected by change of pH as is shown by the time to death of several marine species, Shelford ('18). The following table shows the results of his work.

Species	pH	Time to death
Herring	7.25	Lives indefinitely
	6.85	480 minutes
	6.3	60 minutes
Viviparous perch	6.85	Lives indefinitely
	6.3	300 minutes
	5.5	40 minutes
Flat fish	6.3	Lives one day
	4.35	127 minutes

These pH values are not corrected for salt error of .15 or less, but this makes little difference in their comparative value.

The effect of direct addition of carbon dioxide to water of high and low oxygen content was studied in relation to length of life of whitefish larvae. In low oxygen the larvae died more rapidly in acid water (pH 6.3) than in alkaline (pH 9.0). With high oxygen content (3 cc. per liter) a pH of 6.3 did not kill though the exposure was for the greater part of two days. This is in accordance with Well's statement that large amounts of oxygen antagonize the detrimental effects of high concentrations of carbon dioxide. He found that with low oxygen content fish lived longer in slightly acid water than in alkaline. My results seem at first glance to contradict this. However, his slightly acid water had a pH around 7.5 and would in all probability show a much lower toxicity than 6.3. Shelford ('21) has also done some experiments on adult forms. These die more rapidly in pH of 6.4 - 6.6 than in a pH of 8.6. when the oxygen content is low. Rock bass in a pH of 6.5 lived 350 minutes and in a pH of 6.2 lived but 60 minutes. The oxygen was low for

both being about .97 parts per million. When oxygen is high acidity is not so rapidly fatal.

Can one then change the pH requirement of an organism by changing the environment during the early formative period of heart and blood development? Shelford believes that each species has an optimum pH which it will choose so definitely that we can use this as a mark of identification as to its habitat relations. All the work on post embryonic fishes seems to uphold this theory. The pH requirement of the herring and its relation to habitat choice shows this. The difference in the power of resistance of fishes from ponds and from clear swift streams is marked. But experiments with embryonic forms are very suggestive. Allee ('12) working with isopods has been able to change the rheotactic response of young stream forms by keeping them in high oxygen. C.R. Griffith*, in subjecting white rats to a revolving environment during the period from gestation to adulthood has so changed their equilibratory reactions that many can not live normally in what we consider a normal world but go to pieces nervously if deprived of their revolving world. The whitefish larvae, reared through the period of heart and blood formation in experimental waters (pH of 6.4, 7.0 and 9.0) behave differently in a gradient than do fish reared in lake water but hatched in experimental waters. This difference lies in the direction of breaking up the preference, otherwise shown, for acid rather than alkaline waters, causing all larvae to prefer a pH of 7.2 to 8.2. If the hydrogen ion concentration of an animal's blood determines the pH which will be most tolerable in the adult environment, may not the

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*Unpublished results from the Psychology Laboratory, University of Illinois.

pH of the embryo's environment at the time of blood formation determine what the pH of the blood will be? Larvae whose blood system was formed while in lake water showed a preference for the acid end of the gradient when the pH varied from 6.4 to 9.0. Larvae hatched in experimental waters, however, showed a choice for the alkaline end.

V. SUMMARY AND CONCLUSIONS

1. The length of life of egg and sperm is very short, eight minutes after entering the water being the limit of viability for both.
2. Fertilization and early cleavage occur normally in acid neutral and alkaline waters if the oxygen is high (4 cc. per liter) but the acid waters (6.2 - 6.6) are much more favorable than neutral and alkaline water (7.0 and 8.4) when the oxygen is lower than 2.9 cc. per liter.
3. The most sensitive stages seem to be first cleavage and early gastrulation with somewhat of a drop at the time of hatching when the eggs have been in the unfavorable solution for a long period. Eggs raised at the hatchery to full development show a high percentage of hatching for all concentrations except alkaline low oxygen.
4. Fungus, which thrives best with high oxygen content, is a detrimental factor for whitefish development in the high oxygen concentrations of these series, as it materially raises the mortality. The most favorable oxygen content then for 11° Centigrade is 2.5 - 3 cc. per liter.
5. There is a definite increase in the power of liberating oxygen from hydrogen peroxide as development proceeds. There are two low points in the series coincident with the sensitive stages of development.
6. Fry hatching at one, two, and four months after spawning differ in size of body but not in size of yolk, those hatching at four months being four to six millimeters longer than those hatching earlier. They also differ in vitality, the later fry living longer than the earlier in spite of having the same amount of

yolk available.

7. Eggs raised through the period of heart and blood formation at a pH differing from their natural environment show a different type of gradient reaction. Such larvae raised in both acid (6.3) and alkaline water (9.0) choose the alkaline end of the gradient when 6.3 to 9.0 is offered. Larvae raised to hatching in lake water and then hatched in acid or alkaline water all prefer the acid end of the gradient.

8. When exposed to water with a pH of 6.3 obtained by adding carbon dioxide directly, the larvae died earlier in the acid water than in the alkaline (9.0) with a low oxygen content. A high oxygen content antagonizes the carbon dioxide present prolonging the life of the larvae.

9. In a gradient of 8.0 to 9.0 larvae of both acid (6.3) and alkaline (9.0) hatching environment choose the alkaline end, 8.8 to 9.0 in most cases (8.4 - 9.0 in others).

10. When compared with the development in the toad eggs we find the sensitive stages corresponding, first cleavage and gastrulation, but the whole level for toad development is lower for oxygen and higher for acidity a result one might expect from the difference in habitat, the whitefish requiring a clean aerated bottom free of decaying organic matter and with a low constant temperature, while the toad develops in small stagnant pools free of growing vegetation, often with decaying material present and subject to wide limits of variation in temperature.

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TABLE I Time for normal development of toad eggs at 16° centigrade.

Fertilization	0 hrs	
First cleavage (2 cell)	3 "	20 min.
4 cell	4 "	30 "
8 cell	6 "	
12 to 16 cell	8 "	
Early blastula	10 "	30 "
Late blastula	23 "	30" "
Blastopore lip	28 "	
Yolk plug - large	31 "	30 "
Yolk plug - medium	41 "	
Yolk plug - small	48 "	
Early neural folds	51 "	
Late neural folds	69 "	
Elongation	72 "	
Out of jelly.		
Tail differentiating	95 "	
Tail 1/4 length of body	114 "	
" 1/3 " " "	123 "	
" 2/3 " " "	143 "	
Tail equal to body	168 "	
Tail longer than body (gills-three)	192 "	

TABLE II Time in hours for reaching various stages of development in the Toad experiments.

Series I. Put in at one cell stage, 25 minutes before I. cleavage.

	A-1	A-2	A-3	A-4	C-1	C-2	C-3
pH	8.0	8.0	8.0	8.0	5.8-6.6	6.0-6.4	8.0-6-8
Oxygen content	.4	.9	1.41	4.64	.8	.4	.5
Time from fert. to:							
I.st cleavage	3.5	3.5	3.5	3.5	3.5	3.5	35
4 cell							
8 cell							
12-16 cell							
Early blastula		23	24	24			
Late blastula	24	54	56	56	24	24	23
Blastapore lip	56				56	56	51
Yolk plug large							
" " med.					73		
" " small							
Early neur.fold		73				73	69
Late neur.folds	73		73			95	90
Elongation			95	73		123	123
Tail differ.	95		(most)	95			
Tail 1/4 body	123			123			
" 1/3 "	143						
" 1/2 "	168			143			
" 2/3 "	192			168			
" = "				192			

Series II. Put in at the 2 cell stage.

A-1	A-2	A-3	A-4	C-1	C-2	C-3
8.0	8.0	8.0	8.0	5.8-6.6	6.0-6.4	8.0-6.8
.4	.9	1.41	4.64	.8	.4	.5
3.5	3.5	3.5	3.5	3.5	3.5	3.5
				24	24	23
24	24	24	24		56	56
60	56	60	56			
73					73	73
	73	73				
95	95	95	73			
123	123	123	95			
143	143	143	123			
	192	168	143			
		192	168-192			

Series III. Put in at the 4 cell stage just after II. cleavage.

Time from fert. to:							
4 cell	5	5	5	5	5	5	5
8 cell							
12-16 cell							
Early blastula							
Late blastula	27	27	27	27	27	27	27
Blastopore lip				58	62	62	58
Yolk plug large	58	58	58				
" " med.					75		
" " small							75
Early neur.fold		75				75	97
Late neur.folds	75		75			97	
Elongation	97	97	97	75		126	126
Tail differ.	126	171				171	195
Tail 1/4 body	146		126			195	
" 1/3 "	171		146	126			
" 1/2 "	195		195	146			
" 2/3 "				195			

Series IV. Put in at 8 cell stage, just after III. cleavage

6	6	6	6	6	6	6
28	28	28	28	28	28	28
					61	59
	59		59			
59		59		98		
						76
	76	76			76	98
76	98				98	127
98	147	98	76		127	
127		127			147	
147		147	127			
172		173	147			
		196	172			
			196			

TABLE III Time in hours for reaching various stages of development in the Toad experiments.

Series V. Put in at the early blastula stage.

	A-1	A-2	A-3	A-4	C-1	C-2	C-3
pH	8.0	8.0	8.0	8.0	5.8 -6.6	6.0-6.4	8.0-6.8
Oxygen content	.4	.9	1.41	4.64	.8	.4	.5
Time from fert.to:							
Early blastula	14	14	14	14	14	14	
Late blastula							
Blastopore lip	28	29	28	28		28	
Yolk plug large					28		
" " med.	59	59	59	59			
" " small		76			59	59	
Early neur.fold					76		
Late neur.folds			76		98	76-98	
Elongation	76		98	76		127	
Tail differ.	98		127	98		147	
Tail 1/4 body	127		147	127			
" 1/3 "	147		172	147			
" 1/2 "	172		196				
" 2/3 "	196			172			
" = "				196			

Series VI. Put in at the late blastula stage.

	A-1	A-2	A-3	A-4	C-1	C-2	C-3
pH	8.0	8.0	8.0	8.0	5.8-6.6	6.0-6.4	8.0-6.8
Oxygen content	.4	.9	1.41	4.64	.8	.4	.5
Time from fert.to:							
Early blastula	14						
Late blastula		18	18	18	18	18	18
Blastopore lip	28					40	40
Yolk plug large			40	40		40	68
" " med.	40			69	40	69	
" " small	59				69		
Early neur.fold			65			89	89
Late neur.folds	76-98	67	89	89		89	
Elongation	127	89		114	69		
Tail differ.		138			89		
Tail 1/4 body					114		
" 1/3 "							
" 1/2 "					138		
" 2/3 "							
" = "							

Series VII. Put in at the blastopore lip stage.

	A-1	A-2	A-3	A-4	C-1	C-2	C-3
pH	8.0	8.0	8.0	8.0	5.8 -6.6	6.0-6.4	8.0-6.8
Oxygen content	.4	.9	1.41	4.64	.8	.4	.5
Time from fert.to:							
Blastopore lip	24	24	24	24	24	24	24
Yolk plug large							
" " med.	38	41			38	38	41
" " small	(gone)		41	41			
Early neur.fold					69	69	
Late neur.folds		69			89	89	69
Elongation		89	69	69			89
Tail differ.		138	89	89			114-138
Tail 1/4 body			114-138	114			
" 1/3 "				138			
" 1/2 "							
" 2/3 "							
" = "							

Series VIII. Put in at the medium yolk plug stage.

	A-1	A-2	A-3	A-4	C-1	C-2	C-3
pH	8.0	8.0	8.0	8.0	5.8-6.6	6.0-6.4	8.0-6.8
Oxygen content	.4	.9	1.41	4.64	.8	.4	.5
Time from fert.to:							
Blastopore lip							
Yolk plug large							
" " med.	41	41	41	41	41	41	41
" " small							
Early neur.fold			61		61-89	61	61
Late neur.folds	69	61	89	61	61	109	89-109	89
Elongation	89	89	109	89	89		134	109
Tail differ.	114-138	109		109	109		158	
Tail 1/4 body		134		134	134			
" 1/3 "		158		158	158			
" 1/2 "								
" 2/3 "								
" = "								

TABLE IV. Time for the first cleavage of Toad eggs under varying conditions of pH and Oxygen, in open dishes and sealed bottles.

No. indiv.	dish bottle	pH	initial O_2	final O_2	O_2 used	Time for 1st cleavage
6 doz.	dish	6.8	.64			4hrs. 30 min.
		6.8	.64			3 " 30 "
		6.8	1.18			4 " 30 "
		6.8	1.18			3 " 30 "
		6.9	3.75			4 " 30 "
		7.1	3.75			3 " 30 "
		7.6	1.20			3 " 20 "
		7.6	5.79			3 " 30 "
6 doz.	bottle	8.0	.41	.09	.32	2 " 25 "
		7.8	1.02	.36	.66	2 " 20 "
		7.6	1.23	.43	.80	2 " 10 "
		7.6	2.05	.98	1.07	1 " 55 "

TABLE IVa. Amount of development possible when a number of Toad eggs are sealed in a restricted amount of Oxygen.

No. indiv.	stage put in	age put in	pH	initial O_2	final O_2	O_2 used	Time to: early blast	late blast	yolk plug	early N.folds	late N.folds	elong.	tail differ.	tail 1/4 body
2 doz.	fert.	0 hrs.	8.0	.4	.1	.3		19 D						
				.8	.1	.7		19 D						
				1.61	.2	1.41		19	48 D					
				6.15	.2	5.9		10	36	54	71	89 D		
	I.Cleav	3.3 "		.42	0	.42	20	27 D						
				1.41	0	1.41	18	27	46 D					
				2.12	0	2.12	18	27	46 D					
				5.95	0	5.95	20	27-46	76 D					
	4 cell	4.5 "		.3	.2	.1		27D						
				.81	.3	.51		27 D						
				1.61	.1	1.51		27	56 D					
				6.15	?	6.		20	56		79 D			
	early	10.5 "		.42	0	.42		27-34D						
	blast			1.41	0	1.41		27	53 D					
				2.12	0	2.12			34	53 D				
				5.95	0	5.95		27	53		86	115 D		
	early			.4	?	?						95 D		
	elong.	72 "		.3	?	?						95-135 D		
				.81	.4	.4						95-135 D		
				1.61	.4	1.21						95-135 D		
				6.15	.3	5.75						95		135 D

TABLE V. Length of life of Whitefish eggs and sperm when kept 'dry' and when exposed to water.

Time	Dry ♀ Dry ♂	Dry ♀ Wet ♂	Wet ♀ Dry ♂
1 min,	fert. +	fert. +	fert.
2 "	" +	"	" +
3 "	" +	" +	"
4 "	" +	"	" +
5 "	" +	" +	"
6 "	" +	"	" +
7 "	" +	" +	
8 "	" +		" +
9 "	" +	" 1% +	
10 "	" +	" —	" —
1 hour	" +	" —	" —
1.5 "	" +		
2.5 "	" +		
4 "	" +		
7.5 "	" +		

TABLE VI Whitefish eggs exposed to waters of varying pH before first cleavage. O_2 same as lake water.

Stage exposed	pH	O_2	Development
Fert. dry 30 min.	6.4	4.08	Normal at 4 days.
" " " "	6.7		" " 4 "
" " " "	7.0		" " 4 "
" " " "	7.8		" " 4 "
" " " "	8.0		" " 4 "
Fert. in dishes	6.2	4.08	Normal at 64 cell
" " "	6.6		" " " "
" " "	7.0		" " " "
" " "	8.1		" " " "
" " "	8.4		" " " "

TABLE VII Whitefish eggs exposed to waters of varying pH before first cleavage. Boiled lake water.

Stage exposed	pH	O_2	Development
Fert. in dishes	6.2	2.9	80% fert. and devel.
" " "	6.6		25% " " "
" " "	7.0		5% " " "
" " "	8.4		1% " " "

TABLE VII. Time in days for reaching various stages of development in Whitefish stock and experiments.

Series II.	A-1	B-1	C-1	A-2	B-2	C-2	A-3	B-3	C-3	Stock
pH range	6.2-7.5	5.8-8.9	6.4-8.1	6.4-8.0	6.2-8.9	7.0-8.5	6.6-8.3	6.8-9.2	7.0-8.9	7.8
pH mean	6.2	6.2	6.4	7.0	7.2	7.2	7.8	8.0	8.0	
Oxygen range	0-.9	0-1.04	0-2.08	0-.9	0-1.24	.01-3.7	0-.9	0-1.7	.01-1.7	1.8-3.3
Oxygen average	.1	.2	.6	.1	.2	.7	.1	.2	.9	2.5
Spawned 12/2										
Rec.at Vivarium 12/3										
Exp,started 12/6										
Cap small cells	4	4		4	4		4	4		4
Embryo forming	5	5	5	5	5	5	5	5	5	5
Post ring large		6	6		6	6			6	6
" " small	7	7	7	7	7	7	7	10		
Eye vesicle forming										8
Tail flat	10		9.5	10			10		10	
Tail starting		12	11		11	10		11		9
Tail elongating 1/5		13	13		13	11				11
Tail 1/4 body						13				13

Series III.										
pH range	5.9-6.9	6.8-7.0	6.8-9.0	5.9-6.9	6.8-7.0	6.8-9.0	5.9-6.9	6.8-7.0	6.8-9.0	
pH mean	6.4		8.5	6.4		8.5	6.4		8.5	
Oxygen range	.4-2.4	.4-2.2	.2-1.7	1.3-3.1	1.9-3.7	1.7-3.2	3.2-5.3	3.3-4.9	3.0-4.8	
Oxygen average	1.65	.88	1.07	2.32	2.56	2.49	4.37	3.96	3.7	
Spawned 12/2										
Rec.at Vivarium 12/3										
Exp.started 12/17										
Eye pigmented-lens	15	15	15	15	15	15	15	15	15	15
Fin rays short	19	19	17	19	17	17	19	17	17	17
Fin rays 1/2 fin			21				21	21	21	21
Tail almost to head	26-27	26	26			26		26		26
Tail to head			27			27	27	27	27	27
Tail to eye									29	
Tail to ear										
Hatching										32

Series IV.										
pH range	6.0-8.6	6.8-9.0	8.6-9.2	6.0-8.6	6.8-9.0	8.6-9.2	6.0-8.6	6.8-9.0	8.6-9.2	7.8
pH mean	6.4		9.0	6.4		9.0	6.4			
Oxygen range	.4-1.9	.8-2.7	1.0-2.7	1.8-4.2	2.4-4.9	1.4-4.4	3.5-5.6	3.5-5.8	3.8-4.8	1.8-3.3
Oxygen average	1.43	1.67	1.82	3.27	3.92	3.27	4.7	4.6	4.1	2.5
Spawned 12/5										
Rec.at Vivarium 12/24										
Experiment started 27										
Tail 1/5-1/4,fin buds	22	22	22	22	22	22	22	22	22	22
Eye pigmented ring	23		23							
Eye lightly pigmented		24		23	24	23	23	23	23	23
Fin rays-short										29
Fin rays 1/2 fin										32
Tail almost to head	32	32	32	32	32		32			
Tail to head	36	36	36			32		32	32	
Tail to eye				36	36	36	36	36	36	
Tail to ear										43
Tail past ear										45
Hatching										

Continued

TABLE VIII Continued.

Series V.	A-1	B-1	C-1	A-2	B-2	C-2	A-3	B-3	C-3
pH range	6.3-6.5	6.1-9.0	8.7-9.2	6.3-6.5	6.1-9.0	8.7-9.2	6.3-6.5	6.1-9.0	8.7-9.0
pH mean	6.4	6.8	9.0	6.4	6.8	9.0	6.4	6.8	9.0
Oxygen range	1.5-1.9	1.9-2.7	1.5-2.7	3.3-4.2	2.4-4.9	1.5-4.4	3.5-5.6	4.3-5.8	3.9-4.5
Oxygen mean	1.76	2.22	2.08	3.74	3.98	3.29	4.7	4.9	4.2
Spawned 12/5									
Rec. Vivarium 12/24									
Exp.started 12/31									
Eye pigmented-lens	26	26	26	26	26	26	26	26	26
Tail almost to head	36								
Tail to head	39	36	36					35	
Tail to eye		41	39	36	36	34			36
Tail to ear				39	39	39	36-39		39
Hatching					35(2)				
Series VI.									
pH range	6.0-6.6	6.4-9.0	7.2-9.0	6.0-6.6	6.4-9.0	7.2-9.0	6.0-6.6	6.4-9.0	7.2-9.0
pH mean	6.4	7.0	9.0	6.4	7.0	9.0	6.4	7.0	9.0
Oxygen range	1.0-1.4	.2-1.2	.8-2.4	1.6-3.3	.7-3.2	.8-3.4	3.4-4.4	4.2-5.3	4.1-4.3
Oxygen average	1.22	.64	1.4	2.46	1.7	2.5	3.5	4.7	4.09
Spawned 12/7									
Rec. Vivarium 1/31									
Exp.started 2/1									
Eye pigmented-lens	56	56	56	56	56	56	56	56	56
Tail to ear	68	68-77	68			lost	71	lost	68
Hatching-1st		69		68	68				69
Max. hatching				72-75	69-72				
SeriesVII.									
Exp.started 2/3									
Fin rays short	58	58	58	58	58	58	58	58	58
Tail to ear	71	71-79	72			72	72		
Hatching-1st		71		69	68			68	68
Max.hatching				72-79	69-76			71-74	71-74
Series VIII.									
pH range	6.1-7.2	6.2-9.0	9.0	6.1-7.2	6.2-9.0	9.0	6.1-7.2	6.2-9.0	9.0
pH mean	6.4	7.6	9.0	6.4	7.6	9.0	6.4	7.6	9.0
Oxygen range	.2-1.1	.1- .5	.8-2.4	2.0-3.2	.7-3.5	2.6-3	3.3-3.8	2.4-5.3	4.28
Oxygen average	.61	.4	1.6	2.6	2.12	3.0	3.5	3.82	4.28
Exp. started 2/21									
Ready to hatch	76	76	76	76	76	76	76	76	76
Hatching-1st	78	78	78	78	78	78	78	78	78
Max.hatching	79-82	79-82	79	81-83	79-82	79-81	81-83	81-83	79
Series IX.									
pH range	6.2-8.0	6.0-9.0	6.8-9.0	6.2-8.0	6.0-9.0	6.8-9.	6.2-8.0	6.0-9.0	6.8-9.0
pH mean	6.3	7.0	9.0	6.3	7.0	9.0	6.3	7.0	9.0
Oxygen average	1.9	1.6	.6	3.8	3.7	3.8	4.0	5.0	3.0
Spawned 12/7									
Rec.Vivarium 3/12									
Exp. started 3/12									
Ready to hatch	95	95	95	95	95	95	95	95	95
Matching-1st	95	95	95	96	96	95	96	96	96
Max.hatching	97-101	97-98	96	98-101	97-98	97-98	98	101	97-98

TABLE IX Normalities and corresponding pH values for acetic, hydrochloric, and sulphuric acid.

cc acid in 250 cc * boiled water	acetic .529N resultant norm. acid	pH	HCl .442N resultant norm. acid	pH	H_2SO_4 .485N resultant norm. acid	pH
.9 cc	.00189	5.8	.00167	5.9		
.8 "	.00168	5.9	.00158	6.0	.00155	5.8
.7 "	.00147	6.0	.00123	6.1	.00136	5.9
.6 "	.00126	6.1	.00105	6.2	.00116	6.0
.5 "	.00105	6.2			.00097	6.1
.4 "	.00084	6.3			.00077	6.2
.3 "	.00063	6.4	.00053	6.3	.00058	6.3
.2 "	.00042	6.6	.00035	6.4	.00039	6.4
.19 "			.00033	6.5		
.18 "			.00032	6.6		
.16 "			.00028	6.8		
.15					.00029	6.6
.14 "			.00025	6.9		
.13 "			.00023	7.0		
.12 "			.00021	8.0		
.1 "	.00021	6.7			.00019	6.8
.09 "	.00019	6.8				
.08 "	.00017	8.2			.00015	7.0
.07 "					.00014	7.1
.06 "					.00012	8.3

* boiled water had pH of 8.9 and total alkalinity of 132 parts per million calcium carbonate.

cc .485N H_2SO_4 in 100 cc water#	result. norm. (1)	bicarb. alk. in ppm $CaCO_3$ after total acid react. (2)	CO_2 calc. from react. H_2SO_4 and bicarb. (3)	CO_2 by titrat. with Na_2CO_3 (4)	pH observ. (5)	pH calc. from CO_2 (3) and bicarb. (2) (6)
.35 cc	.001697	37.6 ppm	60.1 ppm	66.8ppm	5.9	6.16
.3 "	.001455	57.2 "	49.5 "	54.4 "	6.0	6.45
.25 "	.001212	76.8 "	38.9 "	48.6 "	6.3	6.6
.2 "	.000970	96.4 "	28.2 "	33.8 "	6.5	6.9
.15 "	.000727	116.1 "	17.6 "	27.6 "	6.6	7.15
.09 "	.000436	139.6 "	4.8 "	5.7 "	6.7	7.63
.08 "	.000388	143.5 "	2.6 "	4. "	6.8	7.75
.07 "	.000339	147.4 "	.46 "	3.5 "	7.0	7.9
.05 "	.000243	148.3 "	——	——	8.4	——

water had pH of 9.2 and total alkalinity of 172 parts per million calcium carbonate.

TABLE X. Conditions of pH and Oxygen content in the experimental dishes during the Whitefish experiments.

Dish	A-1		B-1		C-1		A-2		B-2		C-2		A-3		B-3		C-3	
Date	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2
12/6			-5.8						5.9						6.9			
7	9.0	.2	9.0	.3			9.0	.2	9.0	.3			9.0	.2	9.0	.3		
8	6.8	.2	7.9	1.04	6.7	0.0	6.8	.2	8.0	1.24	7.3	3.7	7.0	.2	9.2	1.66	8.9	1.7
	6.6		-5.8		7.0		6.8		-5.8		7.7		6.8		8.9		8.9	
9	7.9		6.5		8.0		7.4		6.8		8.5		7.9		7.6		8.9	
10	7.5		6.0		8.1		8.0		6.2		8.3		8.3		7.3		8.7	
11	6.9	.2	6.5		7.4		6.9	.2	6.8		8.0		7.2	.2	7.8		8.0	
	6.2		6.2		6.6		6.4		6.8		6.6		6.7		7.2		7.2	
12	6.2		6.2		7.2		6.2		6.6		8.0		6.7		6.7		8.0	
13	6.4	.9	8.9		6.4		6.7		8.9		7.1		6.7		8.9		7.1	
14	6.9	1.14	8.9	.2	6.9	1.25	6.9	.62	8.9	.2	8.2	.01	7.2	.62	8.9	.0	7.8	1.46
	6.4		6.2		6.4		6.4		6.4		7.9		6.6		6.9		7.0	
15	6.4	.4	6.7		7.0		6.4	.01	7.2		8.3		6.6		8.4		7.9	
16	Remodeled apparatus																	
17	6.8		6.9		8.0		7.2		7.0		8.0		7.4		7.2		8.0	
	6.1	.82	6.9	1.04	8.0	.3	6.2	1.25	6.9	3.02	8.0	3.2	6.4	4.57	6.9	4.57	8.1	3.43
18	6.1	1.04	6.9	.42	8.6	.52	6.2	2.70	7.0	1.98	8.6	2.08	6.4	3.22	7.2	3.32	8.6	3.02
	6.2		6.9		8.7		6.4		7.1		8.7		6.4		7.2		8.7	
19	6.9		7.0		6.8		6.9		7.1		6.8		6.9		7.5		7.0	
20	5.9	1.76	7.0	2.08	7.5	1.35	5.9	3.12	7.3	3.74	7.5	3.12	5.9	4.57	7.3	4.88	7.6	4.57
	6.4		6.8		6.9		6.5		6.9		6.9		6.5		7.1		6.9	
21	6.4	1.77	7.0	.73	8.4	.7	6.4	1.87	7.0	2.08	8.4	2.71	6.4	5.28	7.0	3.74	8.4	4.26
22	6.3	2.28	6.8	1.46	8.5	.2	6.3	4.37	6.8	3.02	8.5	1.66	6.3	3.95	6.8	3.74	8.5	2.7
23	6.3	.62	6.8	.73	9.0	1.66	6.3	1.66	6.8	3.02	9.0		6.3	4.57	6.8	4.16	9.0	
26	6.4		7.0		9.0		6.4		7.2		9.0		6.4		7.2		9.0	
27	6.4	2.39	7.1	.62	9.2		6.4	2.89	7.3	1.25	9.2		6.4	4.57	7.2	4.16	9.2	
	6.3		6.8		6.6		6.3		6.8		6.6		6.3		6.8		6.6	
	8.6		9.0		9.0		8.6		9.0		9.0		8.6		9.0		9.0	
28	6.0	.42	6.6	.83	8.7	1.04	6.0	1.87	6.8	3.74	8.7	3.20	6.0	4.57	6.6	3.53	8.7	3.8
	6.3		6.8		8.7		6.3		6.8		8.7		6.3		6.8		8.7	
29	6.4		6.6		8.6		6.4		6.6		8.6		6.4		6.8		8.6	
30	6.4		6.8		8.8		6.4		6.8		8.8		6.4		6.8		8.9	
31	6.3		7.0		9.0		6.3		7.0		9.0		6.3		7.0		9.0	
1/1	6.3	1.89	8.2	2.08	9.0	1.46	6.3	3.33	8.2	2.39	9.0	1.46	6.3	3.53	8.2	4.37	9.0	3.9
2	6.4	2.08	8.1		8.7	2.18	6.6	3.95	8.1	2.18	8.7	3.22	6.7	5.2	8.1		8.2	5.2
3	6.3		6.6		9.0		6.3		6.6		9.0		6.3		6.6		9.0	
4	6.5		8.4		9.1		6.6		8.3		9.2		6.6		8.3		9.2	
5	6.3		6.6		9.2		6.3		6.6		9.2		6.3		6.6		9.2	
6	6.3	1.46	9.0	1.87	9.2	2.08	6.3	3.74	9.2	4.57	9.2	4.05	6.4	5.61	9.2	5.82	9.2	4.47
	6.4		8.2		9.2		6.4		8.3		9.2		6.4		8.2		9.2	
7	6.4		6.6		9.2		6.4		6.6		9.2		6.4		6.6		9.2	
8	6.4		9.0		9.2		6.4		9.0		9.2		6.4		9.0		9.2	
9	6.3		6.2		9.2		6.3		6.2		9.2		6.3		6.2		9.2	
	6.4		6.6		9.2		6.4		6.6		9.2		6.4		6.6		9.2	
10	6.3	1.87	6.6	2.71	9.2	2.7	6.3	4.16	6.6	4.99	9.2	4.37	6.3	5.09	6.6	4.78	9.2	4.37
	6.4		7.2		9.2		6.4		7.2		9.2		6.4		7.2		9.2	

continued

TABLE X. Continued.

Dish	A-1		B-1		C-1		A-2		B-2		C-2		A-3		B-3		C-3	
Date	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2	pH	O_2
1/11	8.6		8.6		9.2		8.6		8.6		9.2		8.6		8.6		9.2	
	6.4						6.4						6.4					
12	6.1		6.6		9.2		6.1		6.6		9.2		6.1		6.6		9.2	
13	6.3		6.6		9.2		6.3		6.6		9.2		6.3		6.6		9.2	
14	8.2		7.0		9.2		8.2		7.0		9.2		8.2		7.0		9.2	
	6.3		6.5		9.2		6.3		6.5		9.2		6.3		6.5		9.2	
2/1	6.4	1.02	7.0	1.22	8.4	1.12	6.4	3.06	7.0	3.26	8.4	3.0	6.4	4.48	7.0	4.59	8.4	4.08
2	6.3		7.0		8.6		6.3		7.0		8.6		6.3		7.0		8.6	
3	6.3		7.0		8.6		6.3		7.0		8.6		6.3	3.7	7.0		8.6	4.08
5	6.4		6.8		7.2		6.4		6.8		7.2		6.4		6.8		7.2	
6	6.4		6.8		7.2		6.4		6.8		7.2		6.4		6.8		7.2	
7	6.3		6.8		8.6		6.3		6.8		8.6		6.3		6.8		8.6	
8	6.4		6.8		7.2		6.4		6.8		7.2		6.4		6.8		7.2	
9	6.0		6.4		8.6		6.0		6.4		8.6		6.0		6.4		8.6	
11	6.6	1.43	8.2	.2	9.0	1.64	6.6	1.84	8.2	1.43	9.0	.8	6.6	3.47	8.3	4.28	9.0	4.08
12	6.0		8.2		9.0		6.0		8.2		9.0		6.0		8.2		9.0	
	6.0		6.4		9.0		6.0		6.4		9.0		6.0		6.4		9.0	
	6.7		6.2		9.0		6.7		6.2		9.0		6.7		6.2		9.0	
13	6.3		8.6		9.0		6.3		8.6		9.0		6.3		8.6		9.0	
14	6.3	.0	7.2		9.0		6.3	2.45	7.2		9.0		6.3	2.44	7.2		9.0	
	6.6		6.6		9.0		6.6		6.6		9.0		6.6		6.6		9.0	
15	6.3		6.8		9.0		6.3		6.8		9.0		6.3		6.8		9.0	
	6.9		6.5		9.0		6.9		6.5		9.0		6.9		6.5		9.0	
16	6.3		9.0		9.0		6.3		9.0		9.0		6.3		9.0		9.0	
	6.2		7.0		9.0		6.2		7.0		9.0		6.2		7.0		9.0	
17	6.3		9.0		9.0		6.3		9.0		9.0		6.3		9.0		9.0	
	6.3		6.9		9.0		6.3		6.9		9.0		6.3		6.9		9.0	
18	6.3		9.0		9.0		6.3		9.0		9.0		6.3		9.0		9.0	
19	6.3		7.0		9.0		6.3		7.0		9.0		6.3		7.0		9.0	
20	6.2		8.0		9.0		6.2		8.0		9.0		6.2		8.0		9.0	
21	9.0	1.02	9.0	.5	9.0	2.41	9.0	3.26	9.0	.7	9.0	3.41	9.0	3.3	9.0	5.3	9.0	4.28
	6.4		7.0		9.0		6.4		7.0		9.0		6.4		7.0		9.0	
22	7.2		8.0		9.0		7.2		8.0		9.0		7.2		8.0		9.0	
23	6.1		6.2		9.0		6.1		6.2		9.0		6.1		6.2		9.0	
24	7.0	.2	9.0	.01	9.0	.82	7.0	2.04	9.0	3.55	9.0	2.6	7.0	3.88	9.0	2.35	9.0	4.28
	6.4		7.0		9.0		6.4		7.0		9.0		6.4		7.0		9.0	
26	6.6		9.0		9.0		6.6		9.0		9.0		6.6		9.0		9.0	
28	7.2		9.0		9.0		7.2		9.0		9.0		7.2		9.0		9.0	
	6.3		9.0		9.0		6.3		9.0		9.0		6.3		9.0		9.0	
3/1	7.2		9.0		9.0		7.2		9.0		9.0		7.2		9.0		9.0	
2	7.2		9.0		9.0		7.2		9.0		9.0		7.2		9.0		9.0	
12	6.3	2.04	7.0	1.73	9.0	1.22	6.3	4.08	7.0	4.28	9.0	3.46	6.3	4.69	7.0	6.52	9.0	3.16
13	7.0		7.0		9.0		7.0		7.0		9.0		7.0		7.0		9.0	
14	8.0		8.9		8.9		8.0		8.9		8.9		8.0		8.9		8.9	
	6.3		6.0		8.9		6.3		6.0		8.9		6.3		6.0		8.9	
15	6.6		8.0		9.0		6.6		8.0		9.0		6.6		8.0		8.9	
16	6.6		6.6		6.8		6.6		6.6		6.8		6.6		6.6		6.8	
	6.3		6.6		9.0		6.3		6.6		9.0		6.3		6.6		9.0	
17	6.3		6.6		9.0		6.3		6.6		9.0		6.3		6.6		9.0	
19	6.3		6.6		9.0		6.3	.41	6.6	3.26	9.0	.41	6.3		6.6		9.0	
												4.28						

TABLE XI. Comparison of the death rates in Whitefish eggs exposed to varying pH and O_2 contents at different stages of development.

Series II. Early gastrulation.

Date	A-1	B-1	C-1	A-2	B-2	C-2	A-3	B-3	C-3
12/6	0%	0%		0%	0%		0%	0%	
7		100%	0%		100%	0%			0%
8	100%	40%	66%	100%	75%	50%	100%	95%	50%
		100%			100%				
9	0%	84%	75%	0%	50%	66%	0%		55%
13	100%			100%			100%		
15								100%	100%
16		100%	100%		100%	100%			

Series III. Eye pigmented, lens showing.

Date	A-1	B-1	C-1	A-2	B-2	C-2	A-3	B-3	C-3
12/17	0%	0%	0%	0%	0%	0%	0%	0%	0%
23				10%	10%			10%	
26	20%	20%	10%	50%	100%	5%	10%	50%	5%
28				100%			50%		
29	100%	100%	100%			100%	100%	100%	95%
31									100%

Series IV. Tail 1/4 body length.

27	0%	0%	0%	0%	0%	0%	0%	0%	0%
1/2	5%	5%	5%	5%	50%	5%	3%	10%	5%
3	10%	10%	10%	10%	75%	10%	8%	18%	10%
4	15%	15%	15%	15%	92%	15%	15%	29%	15%
	75% (acid)								
9	85%	20%	30%	30%	94%	40%	40%	40%	48%
10	87%	22%	33%	33%	95%	52%	44%	50%	68%
12	91%	26%	49%	64%	96%	98%	70%	66%	98%
15	100%	97%	100%	100%	100%	100%	100%	100%	100%
17		100%							

Series V. Eye pigmented, lens showing.

31	0%	0%	0%	0%	0%	0%	0%	0%	0%
1/3	3%	3%	3%	3%	3%	3%	3%	3%	3%
5	5%	5%	5%	5%	5%	5%	5%	5%	5%
6	15%	10%	8%	10%	12%	10%	9%	12%	9%
9	45%	15%	15%	24%	27%	90%	35%	100%	37%
12	81%	31%	37%	67%	87%	98%	86%		98%
15	100%	90%	100%	100%	100%	100%	100%		100%
17		100%							

Series VI. Eye pigmented, lens showing.

Date	A-1		B-1		C-1		A-2		B-2		C-2		A-3		B-3		C-3	
	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.
2/1	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%			0%	0%			0%	0%
4	15%	.2%	12%		15%	.2%	11%	.4%	9%	.3%			18%	.5%			11%	
8	45%	2.0%	14%	.5%	20%	.8%	18%	1.4%	11%	.5%			52%	15.0%			15%	1.3%
12	100%	4.4%	24%	1.0%	100%	2.0%	21%		20%	3.0%			91%	36.0%			29%	4.7%
13			26%	1.0%			22%		28%	4.5%			92%	36.5%			38%	8.0%
15			32%	2.0%			27%	4.8%	54%	17.5%			100%	41.0%			85%	40.0%
16			41%	3.0%			35%	6.8%	72%	23.3%							97%	46.0%
18			65%	8.2%			75%	9.8%	97%	25.9%								
21			100%	22.4%			94%	30.0%	99%	26.7%								

Series VII. Fin rays short.

3	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
8	92%	.6%	5%	1.0%	5%	1.5%	5%	.7%	5%	1.0%	12%	7.8%	23%	2.0%	6%	2.7%	5%	2.0%
12	98%	.9%	9%	1.4%	17%	2.1%	7%		7%	1.5%	20%	9.0%	59%	20.0%	11%	3.6%	9%	3.5%
13	99%		12%		23%		7%		11%	2.0%	99%		89%	23.0%	20%	5.2%	16%	
15	100%		21%	2.8%	57%	5.8%	16%	2.0%	23%	10.0%			100%	29.0%	37%	21.0%	46%	21.0%
16			28%	3.4%	100%	11.8%	22%	2.5%	43%	20.0%					51%	34.0%	57%	27.4%
18			56%	5.2%			39%	4.0%	70%	38.0%					69%	49.0%	70%	35.4%
21			88%	13.1%			69%	26.0%	95%	48.0%					87%	68.0%	77%	43.0%
23			100%	16.8%			79%								89%	69.0%	84%	45.0%
24							81%	26.5%										
26							86%	28.0%										
28							88%	28.5%										

TABLE XI.Continued.

Series VIII. Fish ready to hatch(stock three).

Date	A-1		B-1		C-1		A-2		B-2		C-2		A-3		B-3		C-3	
	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.	dead	fung.
2/21	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
23	13%		17%		36%		16%		13%		30%		13%		20%		21%	
24	27%	1.0%	37%		64%		23%	2.0%	28%	4.0%	49%		32%	6.0%	34%	5.7%	50%	
26	61%	6.0%	65%		73%		43%	11.5%	56%	24.0%	85%	13.0%	55%	17.0%	58%	21.0%	82%	
28	81%	9.0%	77%	1.6%	82%	4.0%	60%	16.2%	74%	34.0%			78%	28.0%	72%	30.0%		
3/ 2	93%						82%		84%				92%		83%			

Series IX. Fish ready to hatch (stock four).

3/12	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
14	3%		1%		98%		1%		2%				2%		3%			
15	7%		3%				2%		5%		2%							
18	25%		10%				9%		22%		17%		20%		6%		17%	
19	33%		16%				17%				23%				10%			

TABLE XII Percentage of eggs hatching in the Whitefish experiments with the corresponding pH and oxygen content.

	Series VI.			Series VII.			Series VIII.			Series IX.		
Dish	pH	O_2	%hatch	pH	O_2	%hatch	pH	O_2	%hatch	pH	O_2	%hatch
A-1	6.4	1.2	0%	6.4	1.2	0%	6.4	.6	7%	6.3	1.9	67%
B-1	7.0	.6	.2%	7.0	.6	.6%	7.6	.4	13%	7.0	1.6	84%
C-1	9.0	1.4	0%	9.0	1.4	0%	9.0	1.6	18%	9.0	.6	2%
A-2	6.4	2.5	6%	6.4	2.5	12%	6.4	2.6	18%	6.3	3.8	83%
B-2	7.0	1.7	6%	7.0	1.7	5%	7.6	2.1	16%	7.0	3.7	78%
C-2	9.0	2.5		9.0	2.5	1%	9.0	3.0	15%	9.0	3.8	77%
A-3	6.4	3.8	0%	6.4	3.8	0%	6.4	3.5	8%	6.3	4.0	80%
B-3	7.0	4.7		7.0	4.7	11%	7.6	3.8	17%	7.0	5.0	90%
C-3	9.0	4.1	3%	9.0	4.1	16%	9.0	4.3	12%	9.0	3.0	83%

TABLE XIII Resistance of Whitefish larvae to acidity, neutrality and alkalinity in fresh and M/6 salt boiled water.

Exp.	Composition of medium	pH	O_2	Time to death
1	.8 cc .529 N acetic in 500 cc boiled H_2O	6.3	1.5	2 fish 29 hrs. 30 min. 1 " 45 " 30 " 1 " 87 "
2	.8 cc .529 N acetic in 500 cc M/6 NaCl sol.	6.3	1.5	1 " 75 " 3 " 87 "
3	.18 cc .529 N acetic 500 cc boiled H_2O	7.0	1.5	3 " 20 " 30 " 1 " 25 " 30 "
4	.18cc .529 N acetic in 500 cc M/6 NaCl sol.	7.0	1.5	1 " 75 " 3 " 87 "
5	no acid 500 cc boiled H_2O	9.0	1.5	1 " 20 " 30 " 1 " 29 " 30 " 2 " 69 "
6	no acid 500 cc M/6 NaCl sol.	9.0	1.5	4 " 87 "
7	.6 cc .442 N HCl in 500 cc boiled H_2O	6.3	1.5	4 " 20 " 30 "
8	.6 cc .442 N HCl in 500 cc M/6 NaCl sol.	6.3	1.5	1 " 75 " 3 " 9 days
9	.26 cc .442 N HCl in 500 cc boiled H_2O	7.0	1.5	3 " 45 hrs 30 min. 1 " 69 "
10	.26 cc .442 N HCl in 500 cc M/6 NaCl sol.	7.0	1.5	2 " 54 " 2 " 69 "
11	.6 cc .485 N H_2SO_4 in 500 cc boiled H_2O	6.3	1.5	2 " 20 " 30 " 1 " 75 " 1 " 93 "
12	.6 cc .485 N H_2SO_4 in 500 cc M/6 NaCl sol.	6.3	1.5	1 " 54 " 2 " 9 days 1 " alive at 24 days
13	.16 cc .485 N H_2SO_4 in 500 cc boiled H_2O	7.0	1.5	2 " 20 hrs 30min 2 " 34 "
14	.16 cc .485 N H_2SO_4 in 500 cc M/6 NACl sol	7.0	1.5	2 " 18 days 1 " 24 " 1 " alive at 24 days

TABLE XIV Length of "time to death" of newly hatched Whitefish in waters whose pH is varied by adding CO_2.

No.ind.	O_2	pH	Time exposed	Time to death	Stock
9	.1	6.3	2 hrs.25 min.	2 hrs.25 min.	3
15			2 " 30 "	2 " 30 "	
15		9.0	3 " 45 "	3 " 45 "	
15	2.8	6.4	5 "	Not dead	
18			12 "	" "	
15	3.0		18 " (11 hr 9.0)"	"	
		9.0	2 da.to a week "	"	
15	.1	6.3	3 hrs.40 min.	3 hrs. 40 min.	4
16			4 "	4 "	
14			4 " 20 "	4 " 20 "	
9	.1	9.0	5 "	5 "	

TABLE XV Amount of oxygen released by toad eggs at different stages of development. a six individuals, hand shaken 1920; b eight dozen individuals, machine shaken 1921.

Stage of development	a cc O_2 released	b cc O_2 released
Ovarian eggs	7.1 cc	136 cc
1 cell	3.34 "	84 "
8 cell	3.6 "	85 "
12-16 cell	4.04 "	
32 cell	4.03 "	89 "
Early blastula	4.2 "	105 "
Blastopore lip	3.69 "	
Yolk plug large		103 "
" " small		112 "
Early neur. folds	3.4 "	89 "
Late neur. folds	3.8 "	90 "
Elongation		
Tail differen.	5.57 "	98 "
Tail 1/4 body		108 "
" 2/3 "	5.86 "	108 "
" 1-1/4 "(8da)	6.3 "	121 "
12 days		354 "
21 "	16.03 "	

c Amount of oxygen released by whitefish eggs at different stages of development.

Stage of development	c cc O_2 released.
Unfert. eggs	18 cc
Eye lightly pigmented	41 "
Hatching	112 "

Figure 1. Apparatus for the control of experimental conditions. Method of varying hydrogen ion concentration and oxygen content. 1 N/4 sulphuric acid; 2 cock for control of acid by drops at 3; 4 mixing bottle for acid and boiled water from W; 5,6,and7 jars to which air from A is added in varying amounts;C cocks for control of air flow; 8,9,and 10 half pint sedimentation glasses for eggs; M mercury manometer for keeping air flow constant.

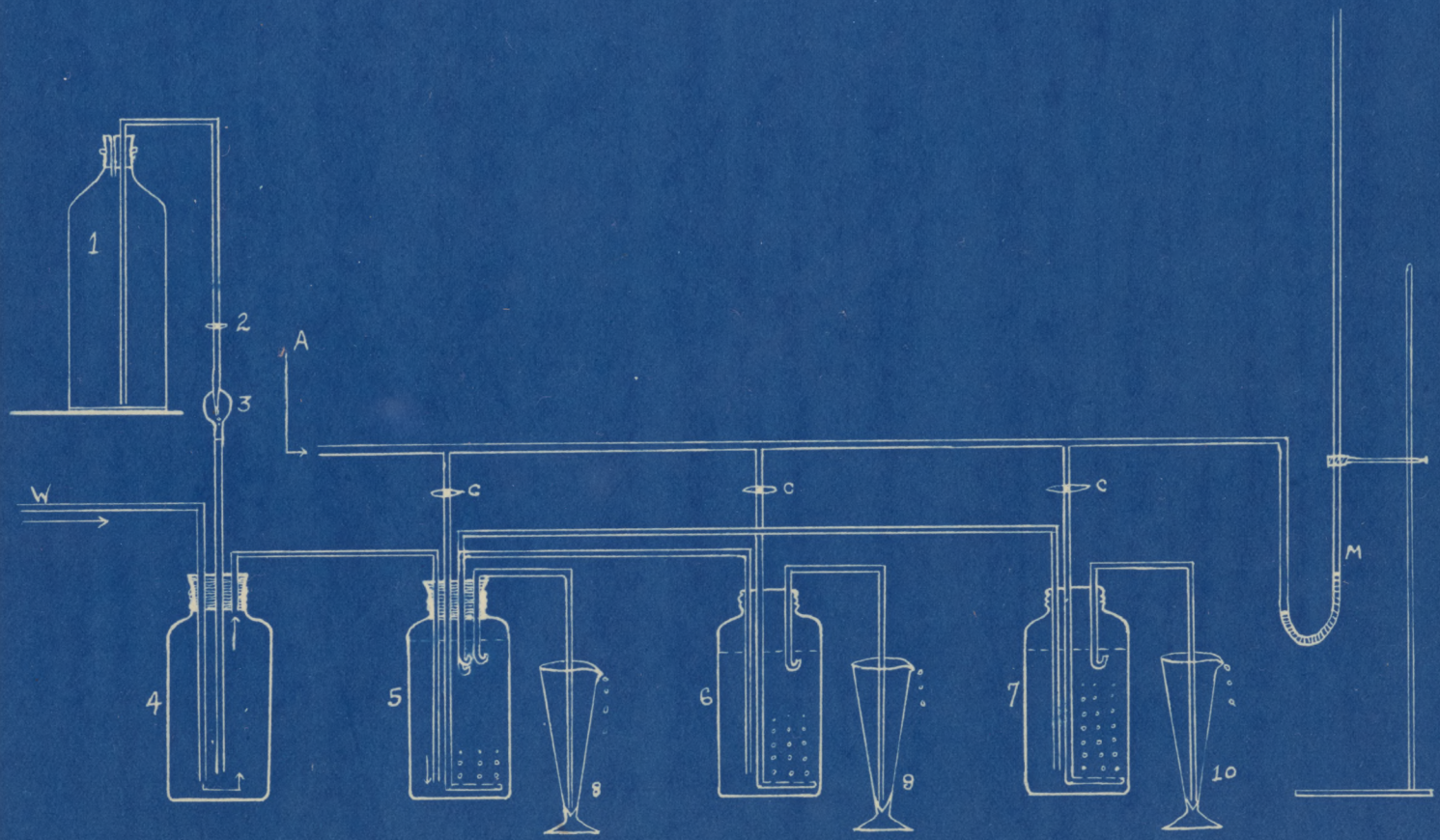


Fig 1.

Figure 2. Apparatus for measuring the oxygen content of small amounts of water by the winkler method. Dimensions of parts

A large tube 2.2 cm diameter by 6.5 cm length.

small tube .8 mm diameter.

Total length from 1 to 2 ---17 cm.

Capacity 26 cc.

B glass ring 1 cm outside diameter, .6 cm inside diameter.

length 1 cm.

C same as B.

D same diameter as B length 3 cm.

R to R heavy black rubber tubing enclosing glass rings B C and D diameter 1 cm outside, .6 cm inside.

2 to 5 screw clamps.

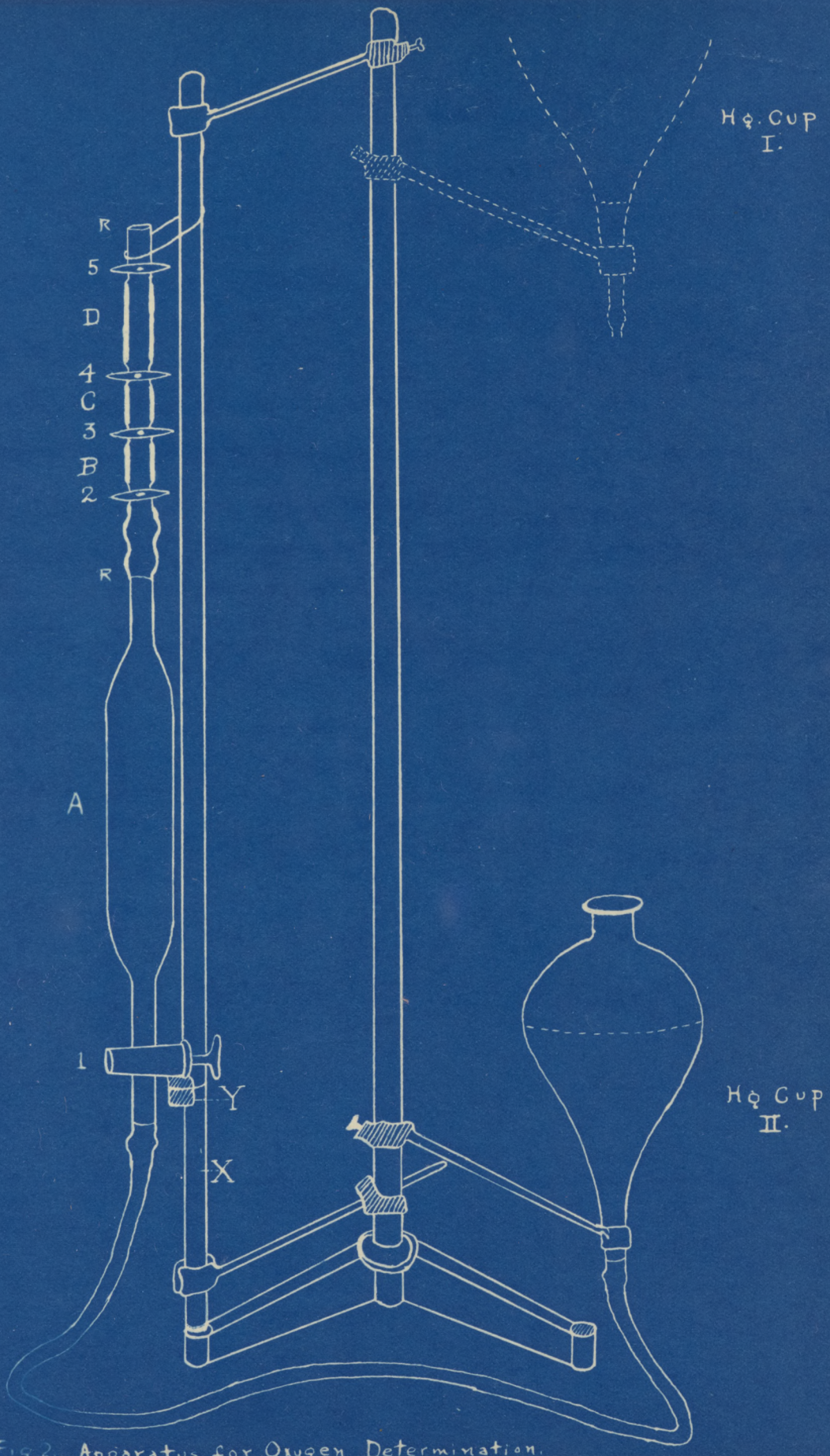


Fig 2. Apparatus for Oxygen Determination.

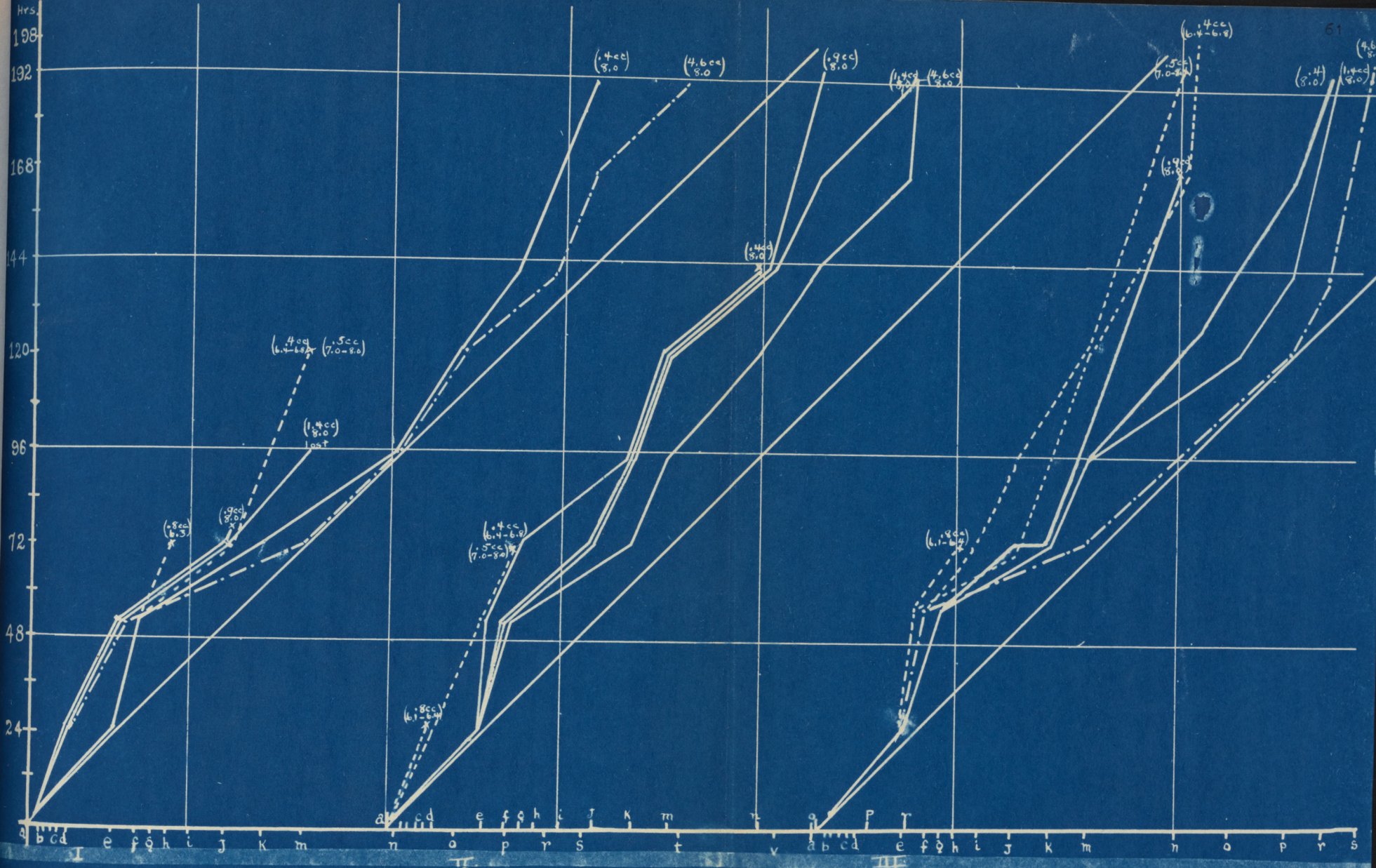


Fig.3.Toad eggs put into experimental conditions at 1 cell (I), 2 cell (II),and 4 cell(III) stages.a fertilization,b 2 cell, c 12-16 cell,d early blastula,e late blastula,f blastopore lip,g yolk plug large,h yolk plug medium,i yolk plug small, j early neural folds,k late neural folds,m elongation,n tail differentiating,o tail 1/4 body,p tail 1/3 body,r tail 1/2 body s tail 2/3 body,t tail equal to body, x eggs dead.

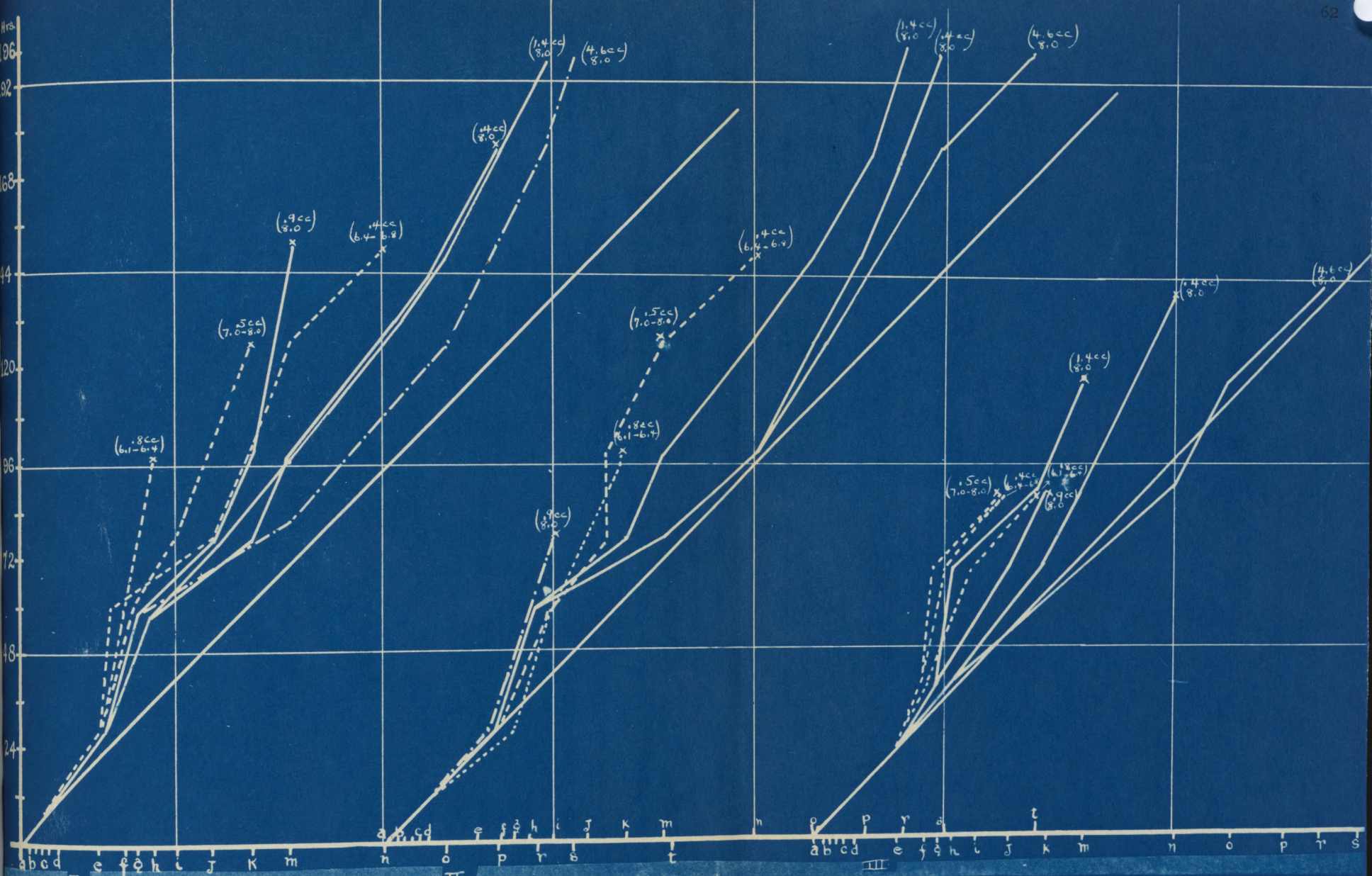


Fig.4. Toad eggs put into experimental conditions at 8 cell (I), early blastula (II), and late blastula (III) stages. Lettering same as fig.3.

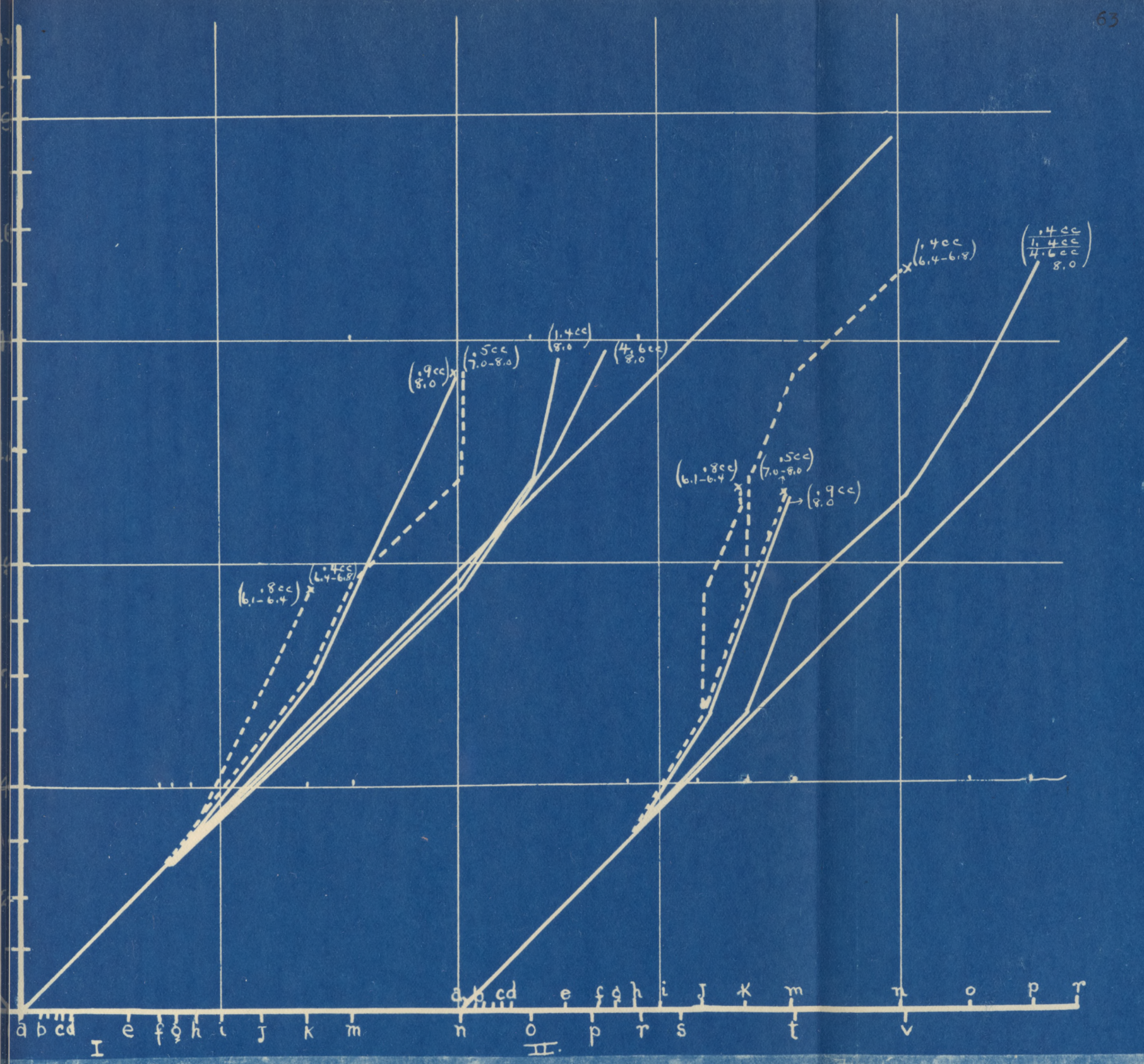


Fig.5. Toad eggs put into experimental conditions at yolk plug (I), and blastopore lip (II) stages.

Lettering same as fig.3.

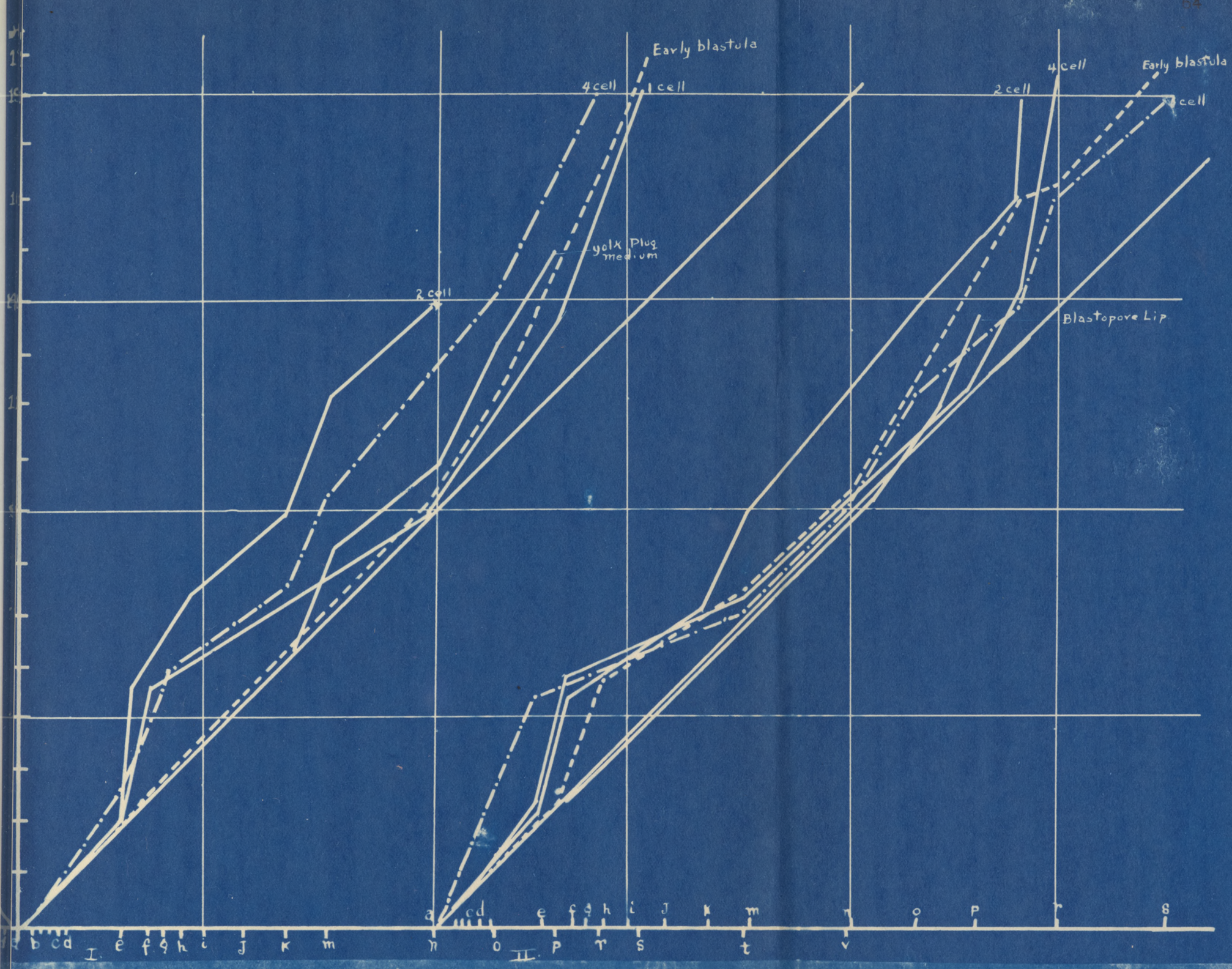


Fig.6.Comparison of the effects of pH 8.0 and O_2 .4 cc per liter (I),and pH 8.0 and O_2 4.64 cc per liter (II) on different stages of development in the Toad egg.Lettering same as fig.3.

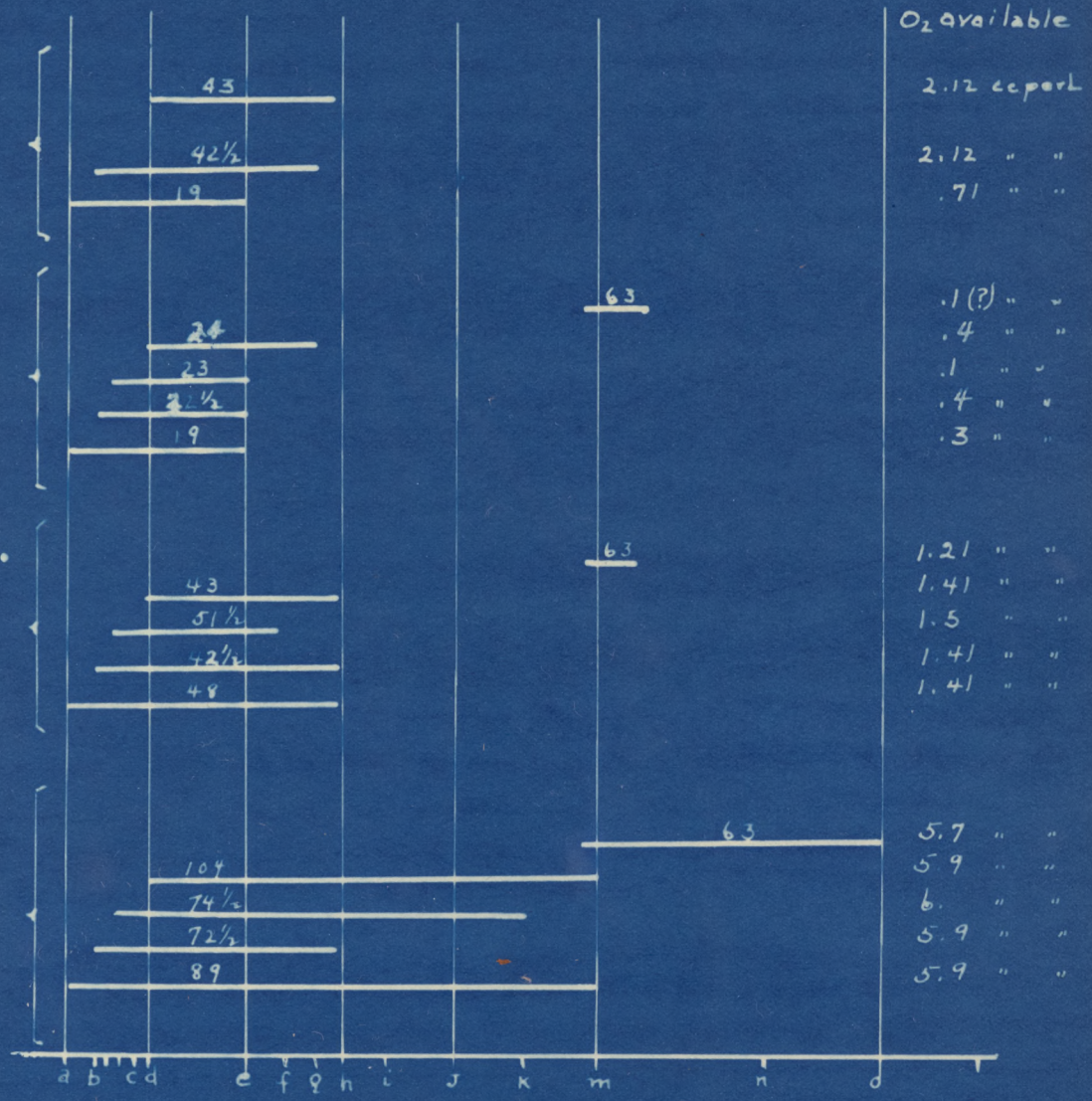


Fig. 7. Amount of Development in Toad Eggs Sealed in Known amounts of Oxygen at different Stages of Development. (pH 8.0) Data of Table 4a. Lettering same as Table 3.

Figure 8 to 16. Rate at which whitefish died under experimental conditions. Lower half, pH and oxygen content plotted for entire period of experiments. Upper half, number dead each day plotted as percent of the total number of eggs in the dish at beginning of experiment.

II eggs of stock 1 put in at early germinal cap stage.

III eggs of stock 1 put in at the stage of eye pigmentation with lens formed.

IV eggs of stock 2 put in when tail was 1/4 length of body.

V eggs of stock 2 put in at the stage of eye pigmentation with lens showing.

VI eggs of stock 3 put in at the stage of eye pigmentation with lens showing.

VII eggs of stock 3 put in two days later than VI.

VIII eggs of stock 3 put in just at hatching time.

IX eggs of stock 4 developed to hatching at the hatchery and then put into experimental condition

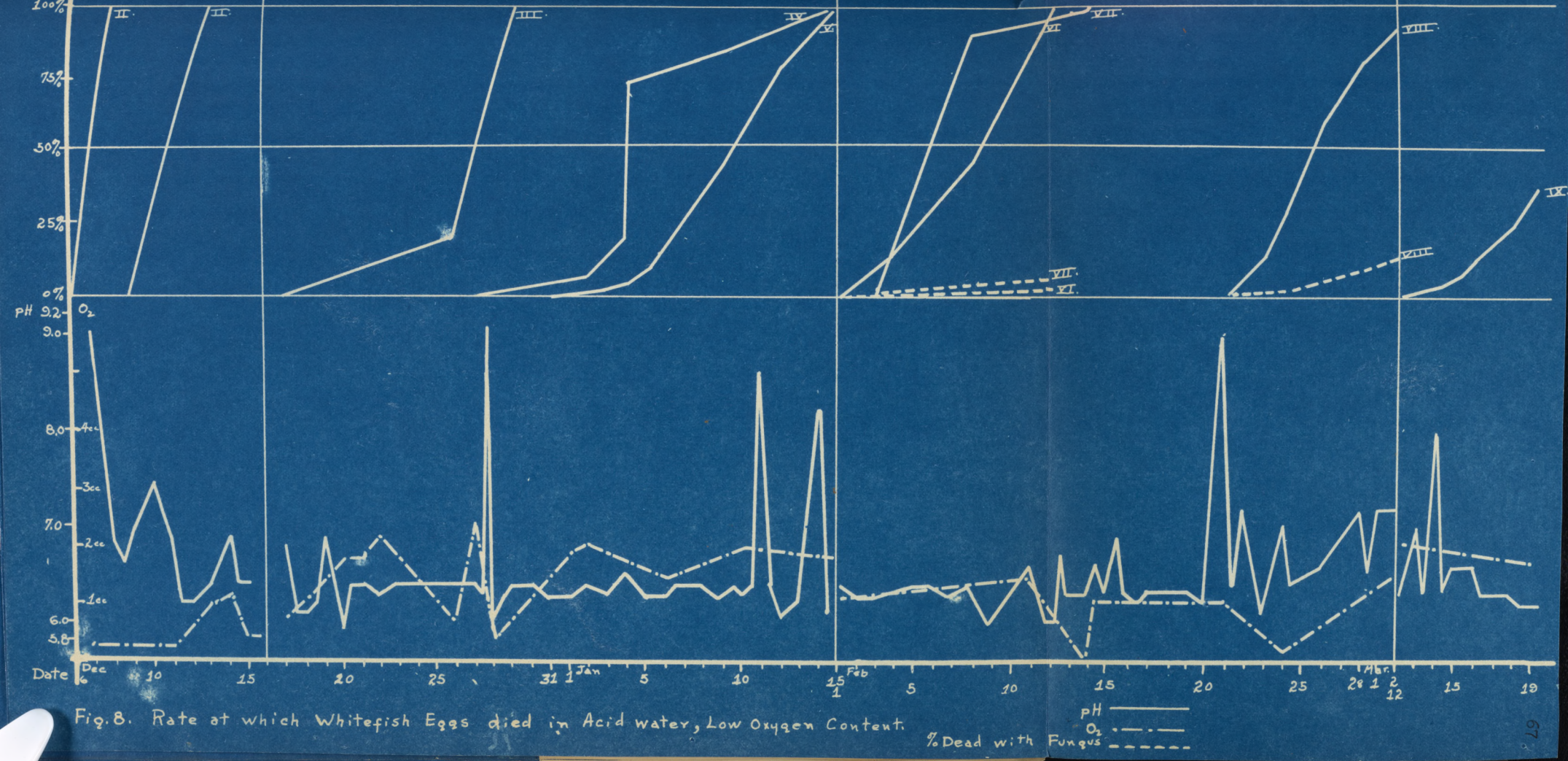


Fig. 8. Rate at which Whitefish Eggs died in Acid water, Low Oxygen Content.

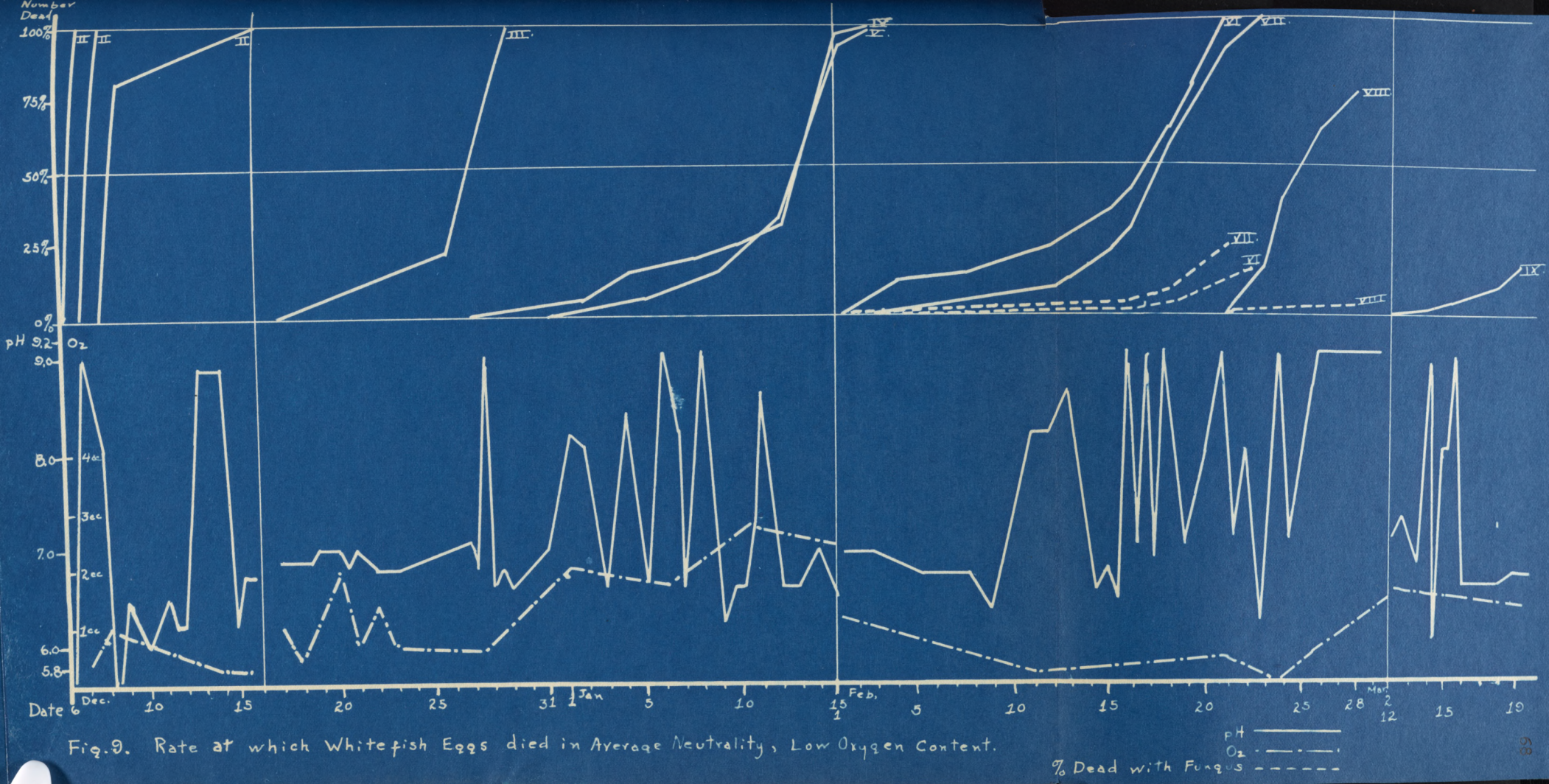


Fig. 9. Rate at which Whitefish Eggs died in Average Neutrality, Low Oxygen Content.

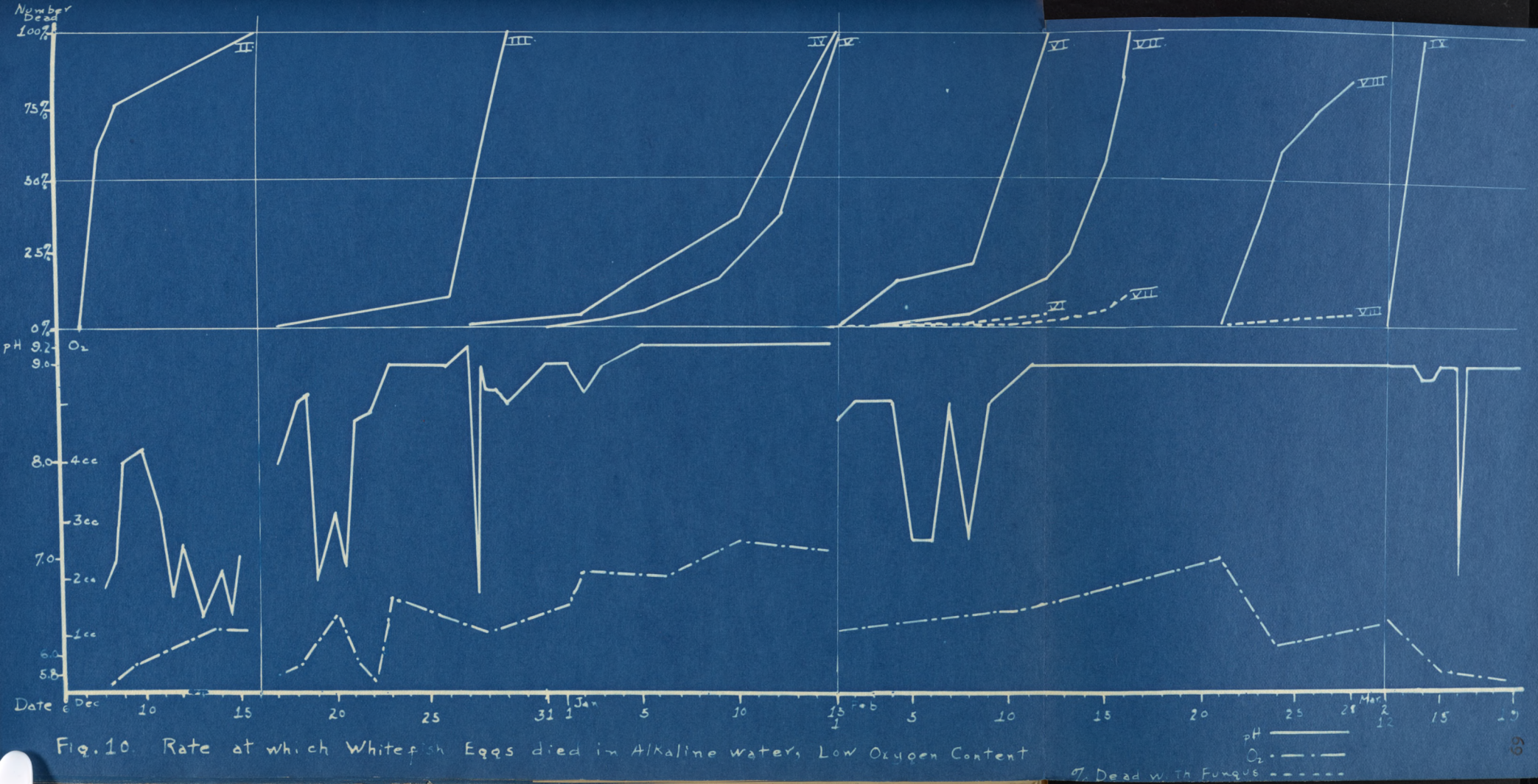


Fig. 10 Rate at which Whitefish Eggs died in Alkaline water, Low Oxygen Content

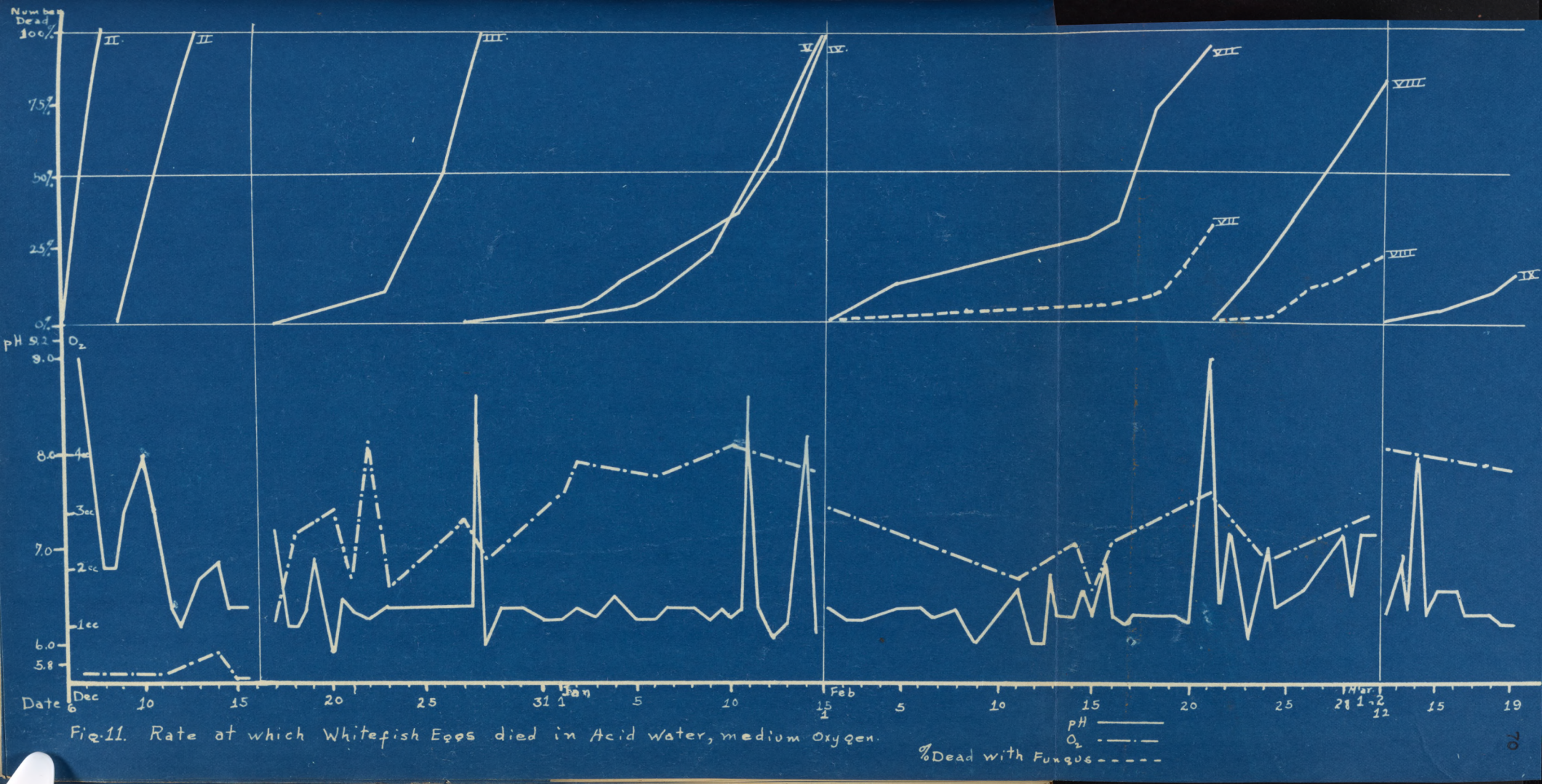


Fig. 11. Rate at which Whitefish Eggs died in Acid water, medium Oxygen.

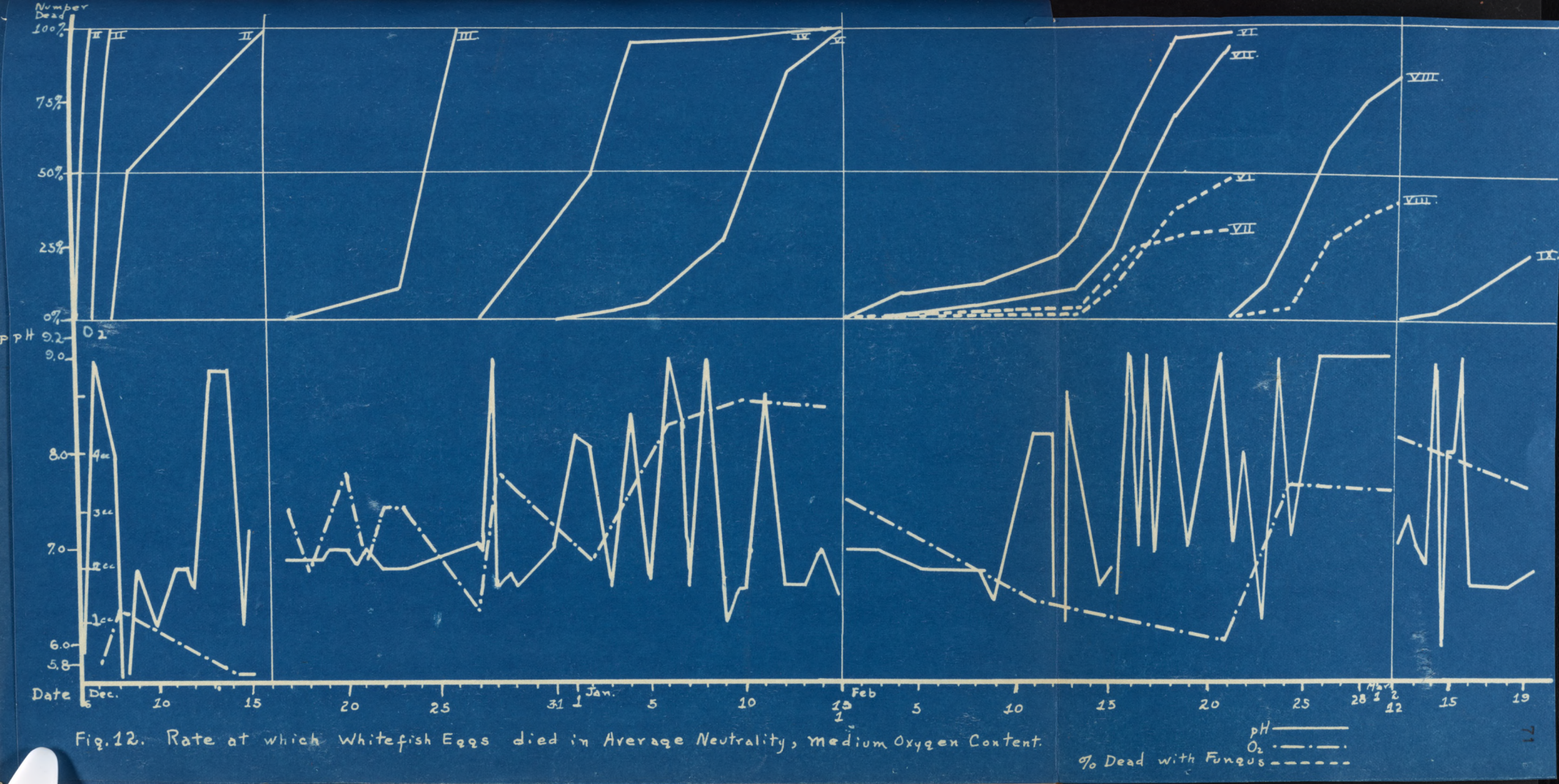


Fig. 12. Rate at which Whitefish Eggs died in Average Neutrality, Medium Oxygen Content.

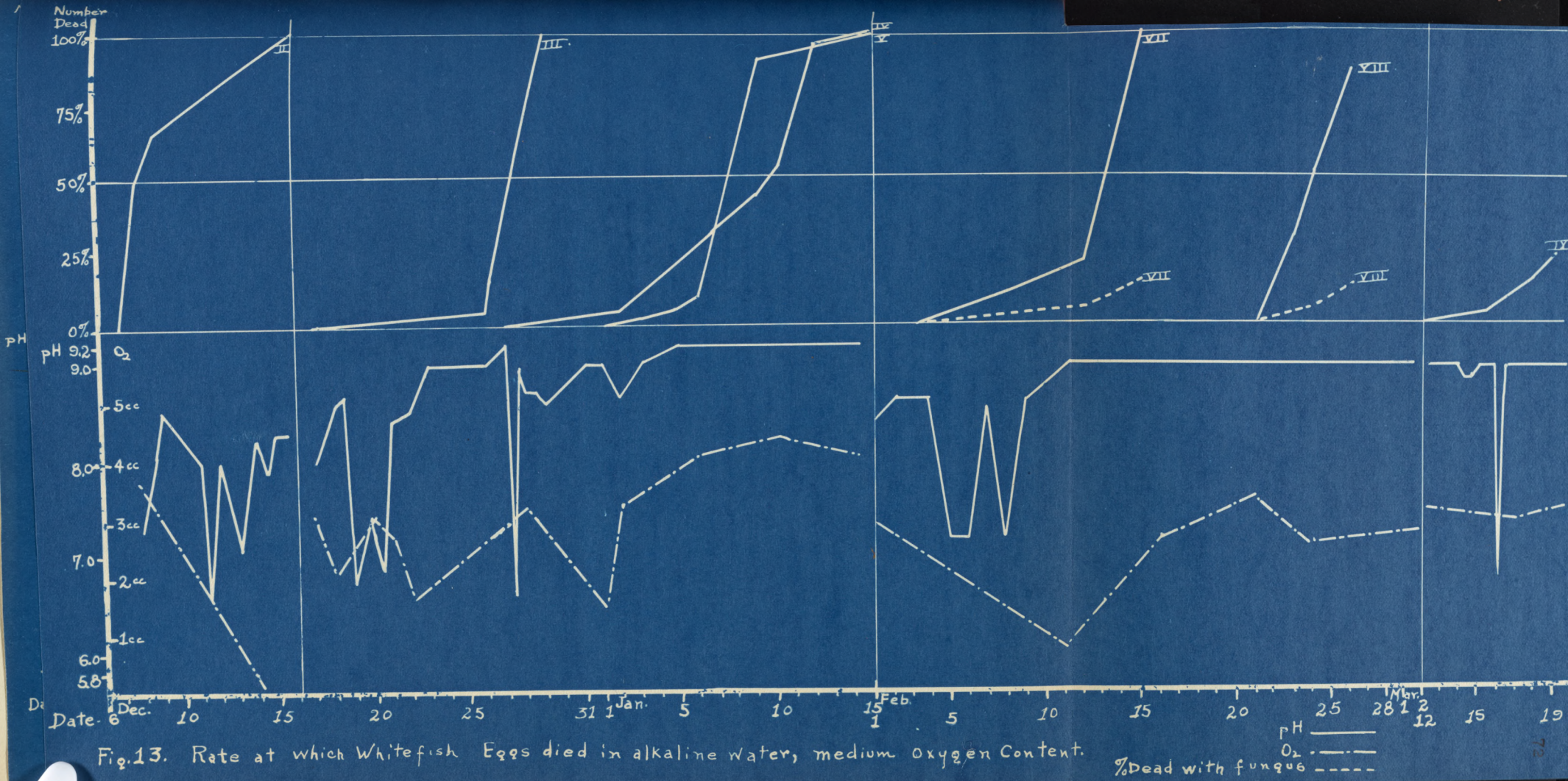


Fig. 13. Rate at which Whitefish Eggs died in alkaline water, medium Oxygen Content.

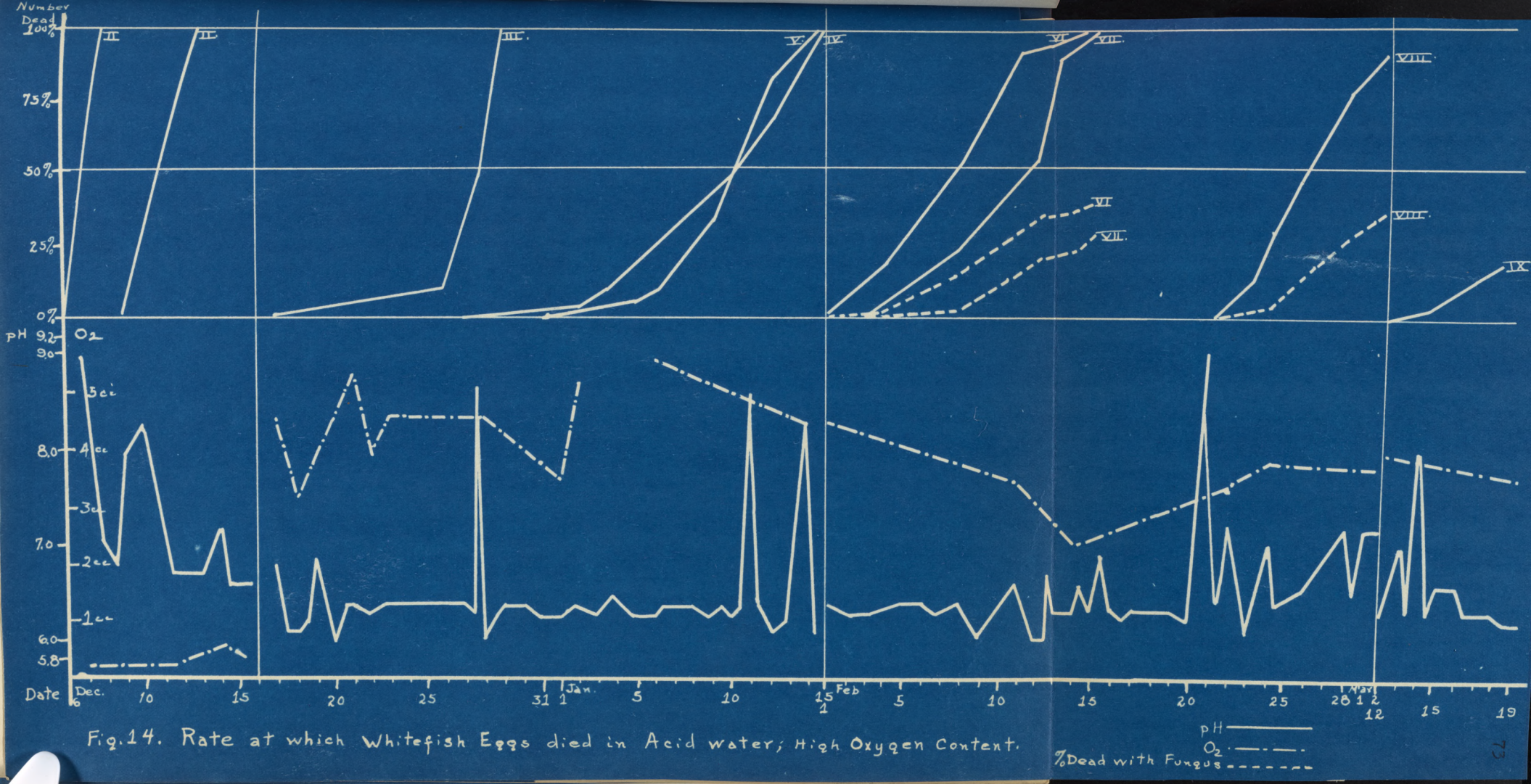


Fig. 14. Rate at which Whitefish Eggs died in Acid water; High Oxygen Content.

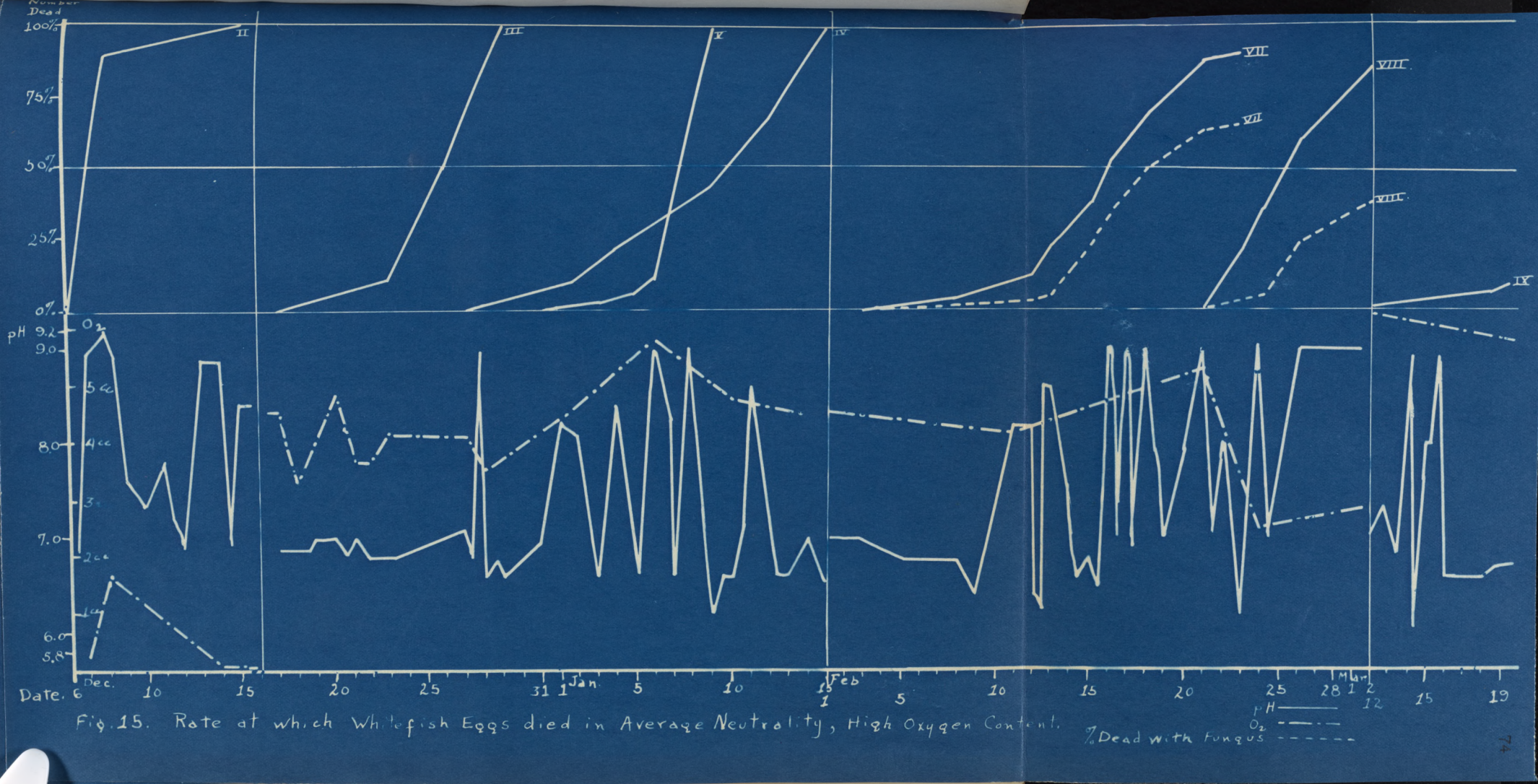
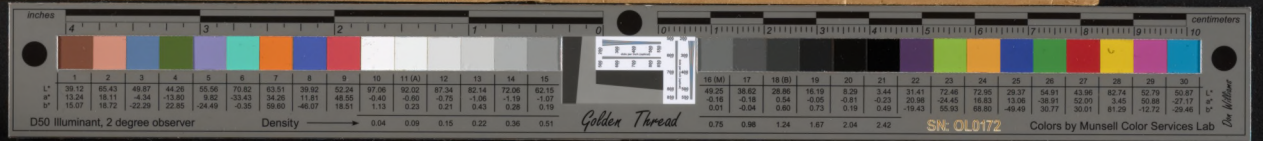


Fig. 15. Rate at which Whitefish Eggs died in Average Neutrality, High Oxygen Content.



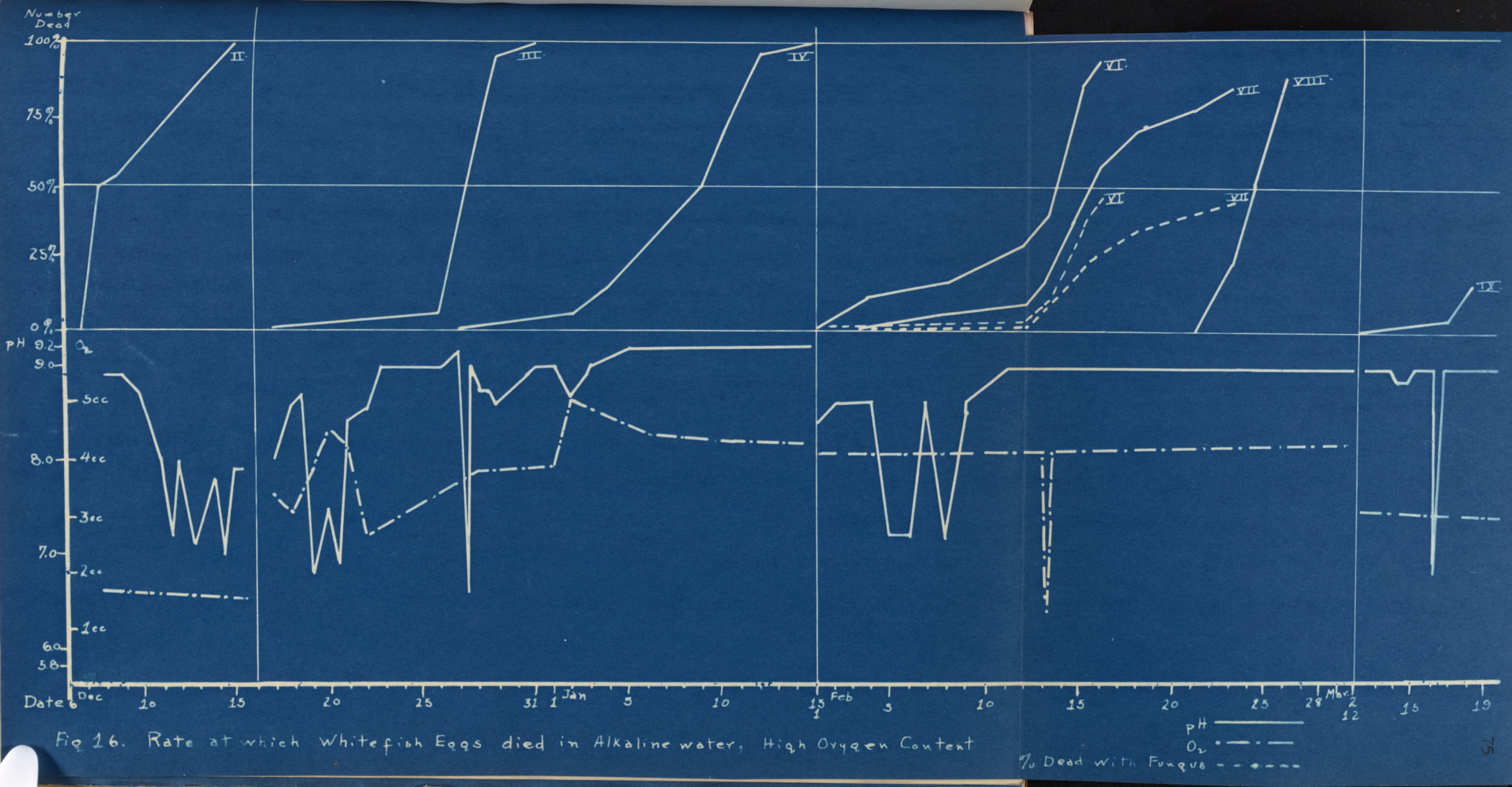


Fig 16. Rate at which Whitefish Eggs died in Alkaline water, High Oxygen Content

ure 17. Gradient reactions of larvae of stock 1 and 2.

a larvae hatched in pH of 7.8 from stock 1.

b and c gradient controls, running and standing water, from stock 2.

d to g gradient reactions of larvae of stock 2 raised through the period of heart and blood formation in experimental waters. A-3 had a pH of 6.4, C-3 had a pH of 8.6-9.0.

A long narrow tank was used with water of the desired pH entering at each end, making a permanent gradient for the entire period of the experiment. The fish swam freely in the tank for the time recorded in the marginal figures.

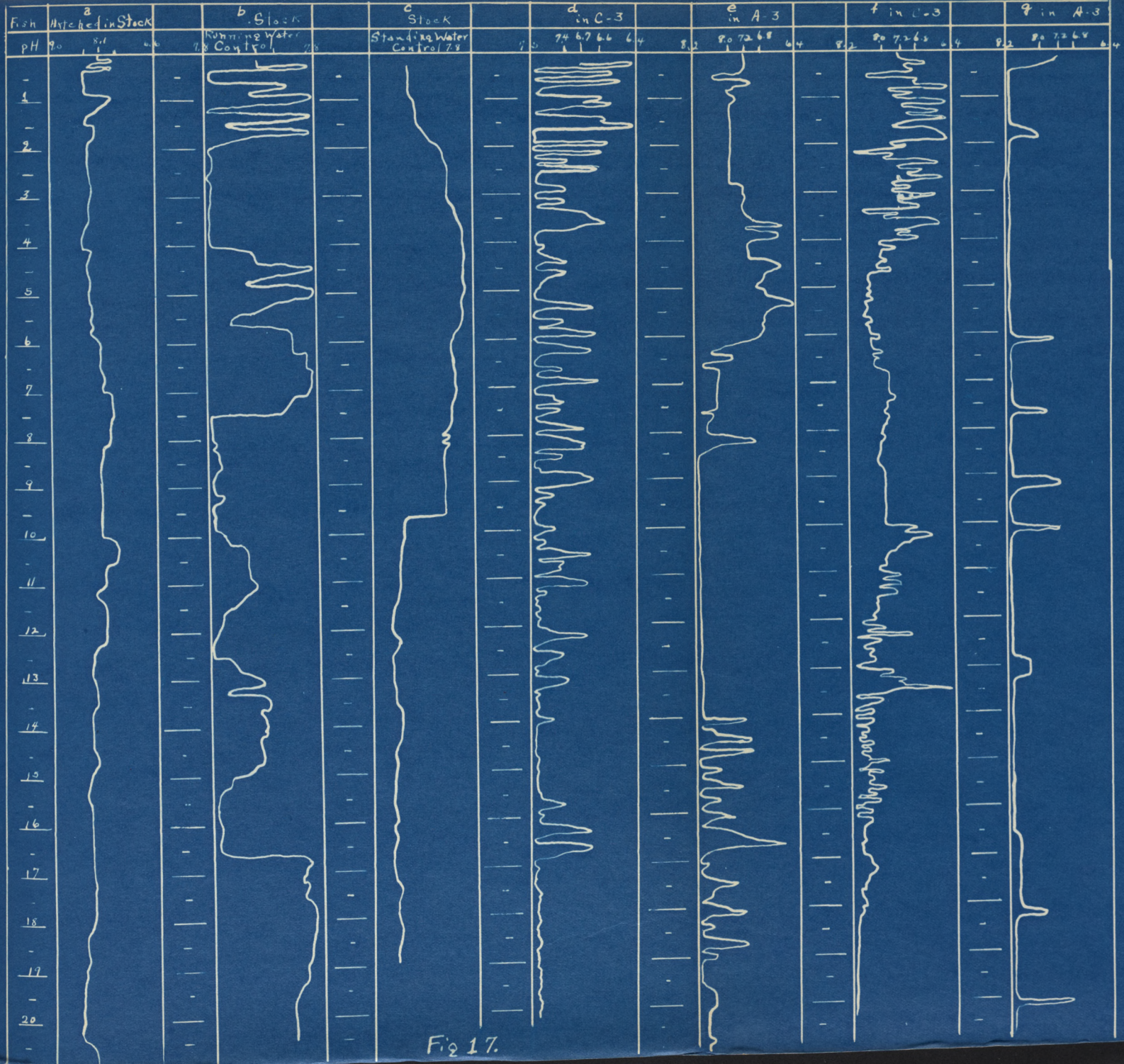

Fish
pH
a
Hatched in Stock
9.0
8.1
b Stock
Running Water
Control
7.8
c
Stock
Standing Water
Control 7.8
d in C-3
7.4 6.7 6.6 6.4
e in A-3
8.0 7.2 6.8 6.4
f in C-3
8.0 7.2 6.2 6.4
g in A-3
8.0 7.2 6.4

Fig 17.

Figure 18 and 19 Gradient reactions of larvae of stock 3. A-2 had a pH of 6.4; B-2 around 7.0; and C-2 of 8.6-9.2. The stock had a pH of 7.8.

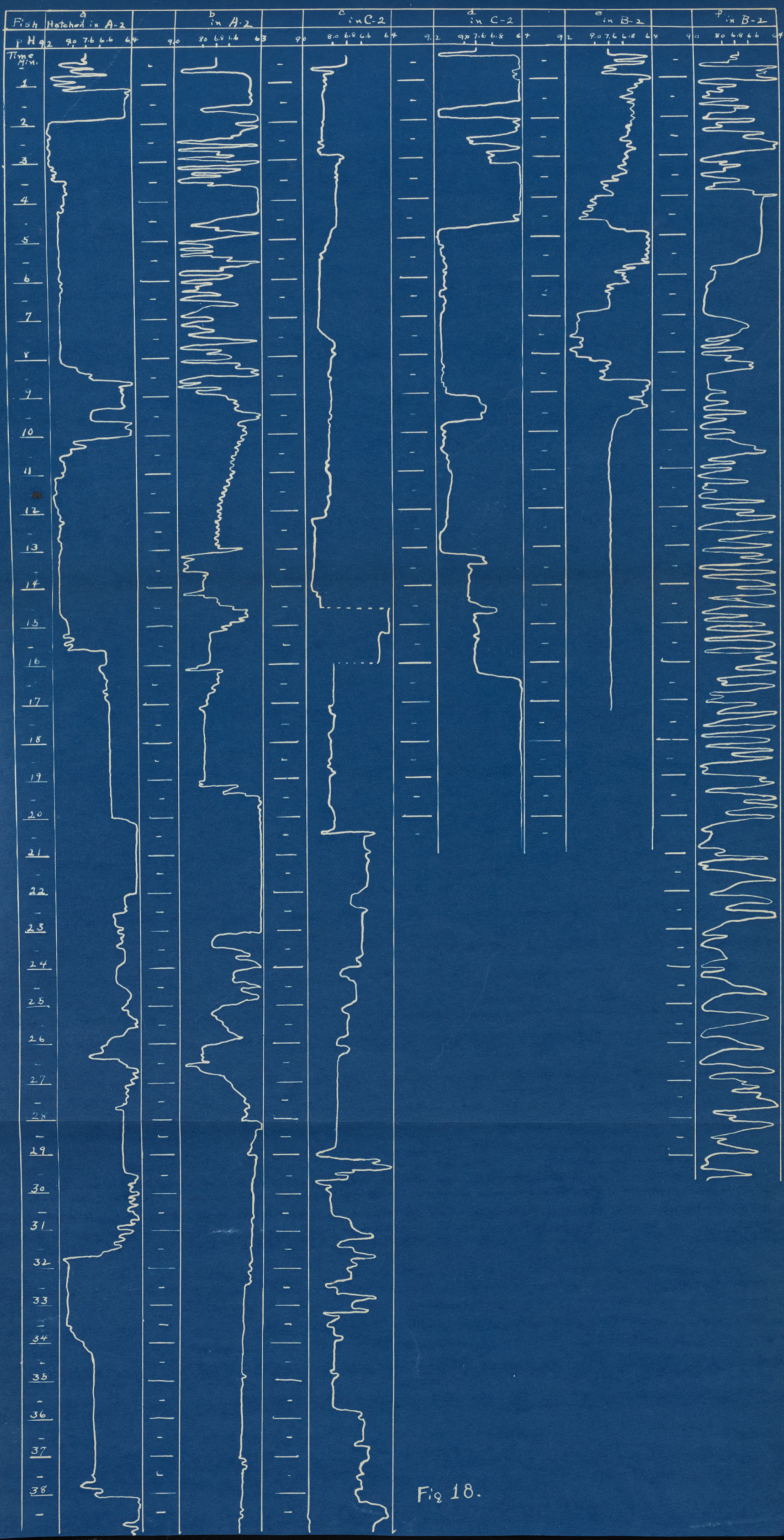

Fish
a
Hatched in A-2
b
in A-2
c
in C-2
d
in C-2
e
in B-2
f
in B-2
pH
Time
Min.

Fig 18.

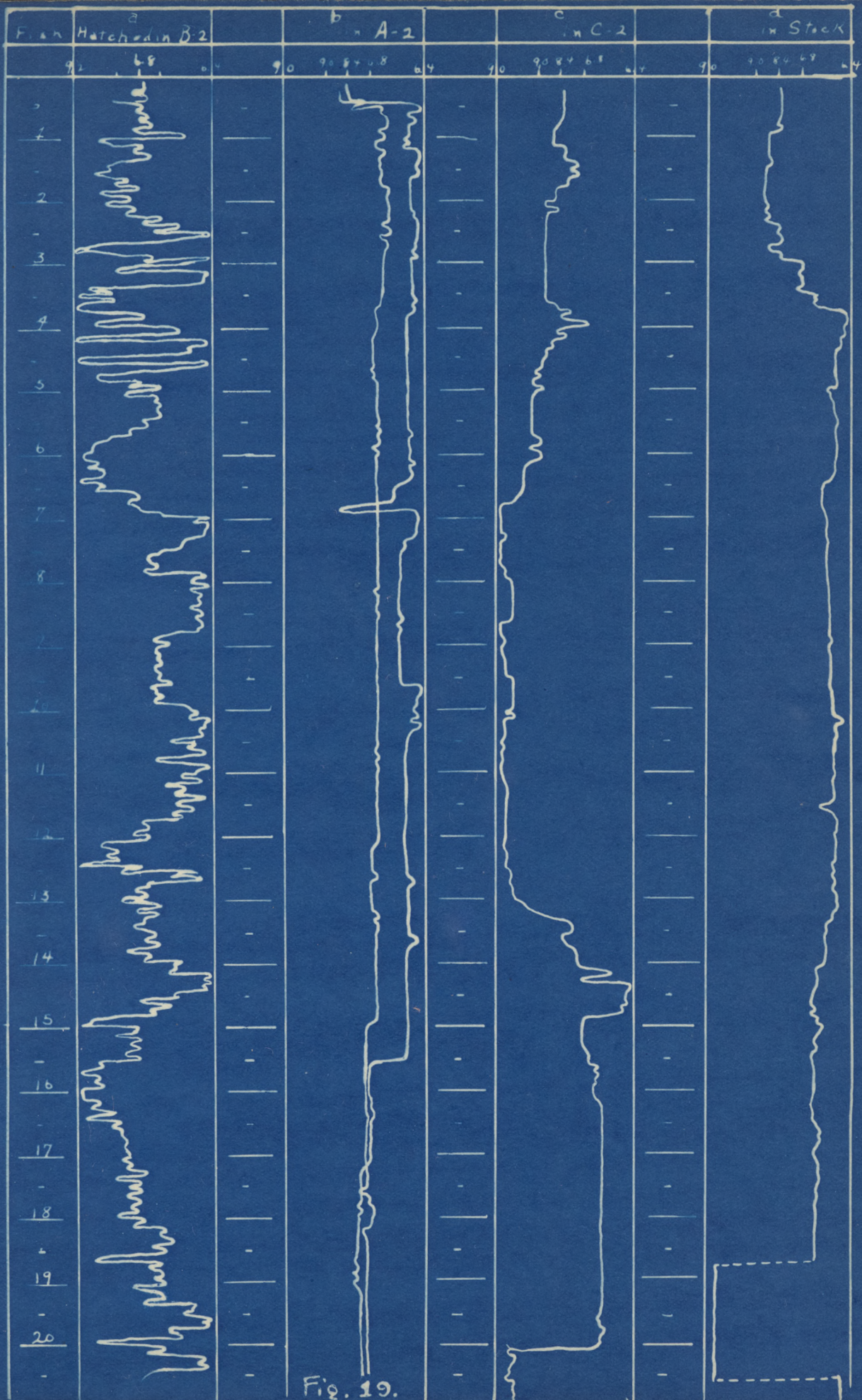


Fig. 19.

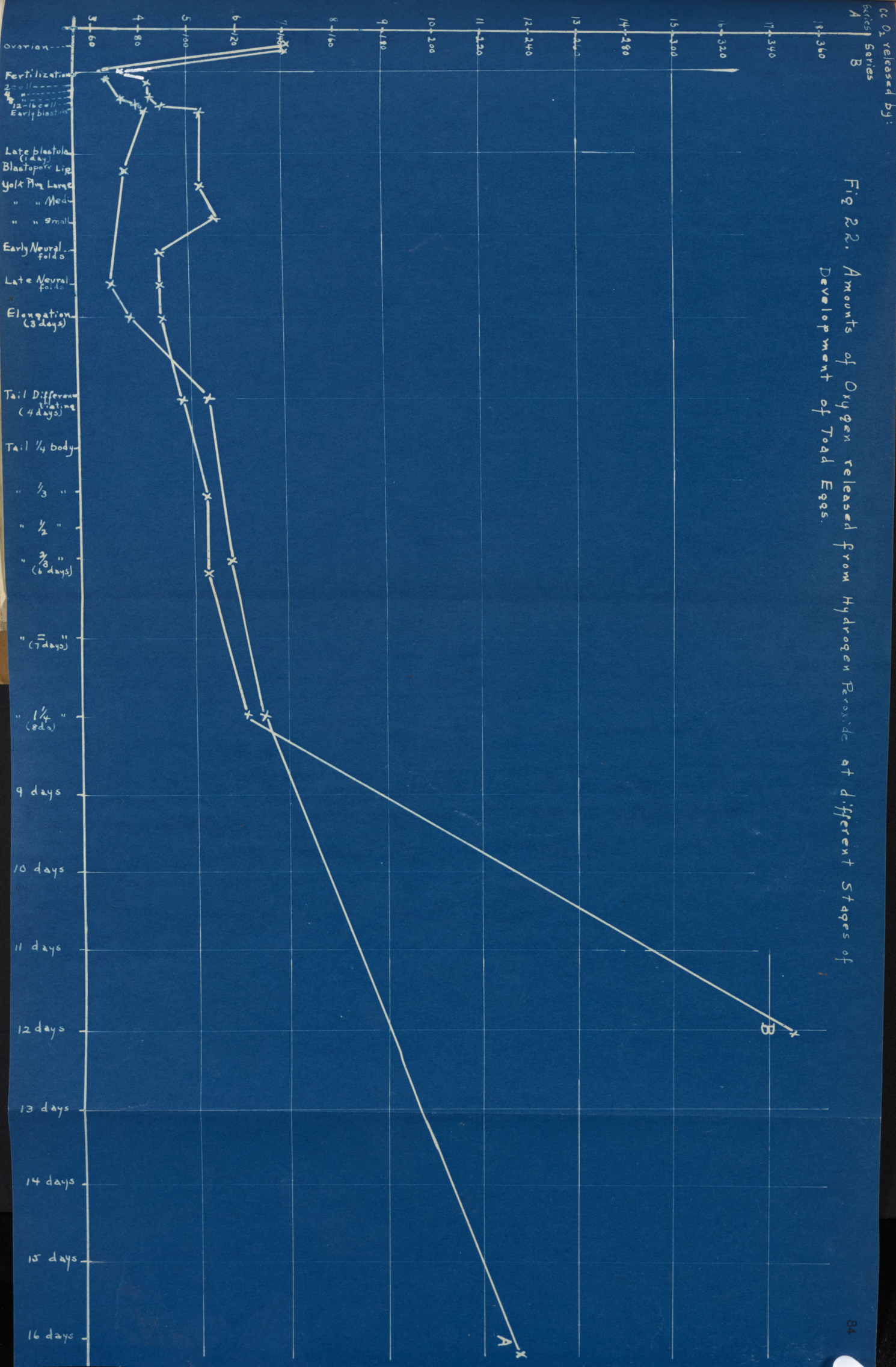


Fig 22. Amounts of Oxygen released from Hydrogen Peroxide at different Stages of Development of Toad Eggs.

Figure 20 and 21. Gradient reactions of larvae of stock 4. A-2 had a pH of 6.4; B-2 around 7.0; andC-2 of 8.6-9.2. The stock had apH of 7.8.

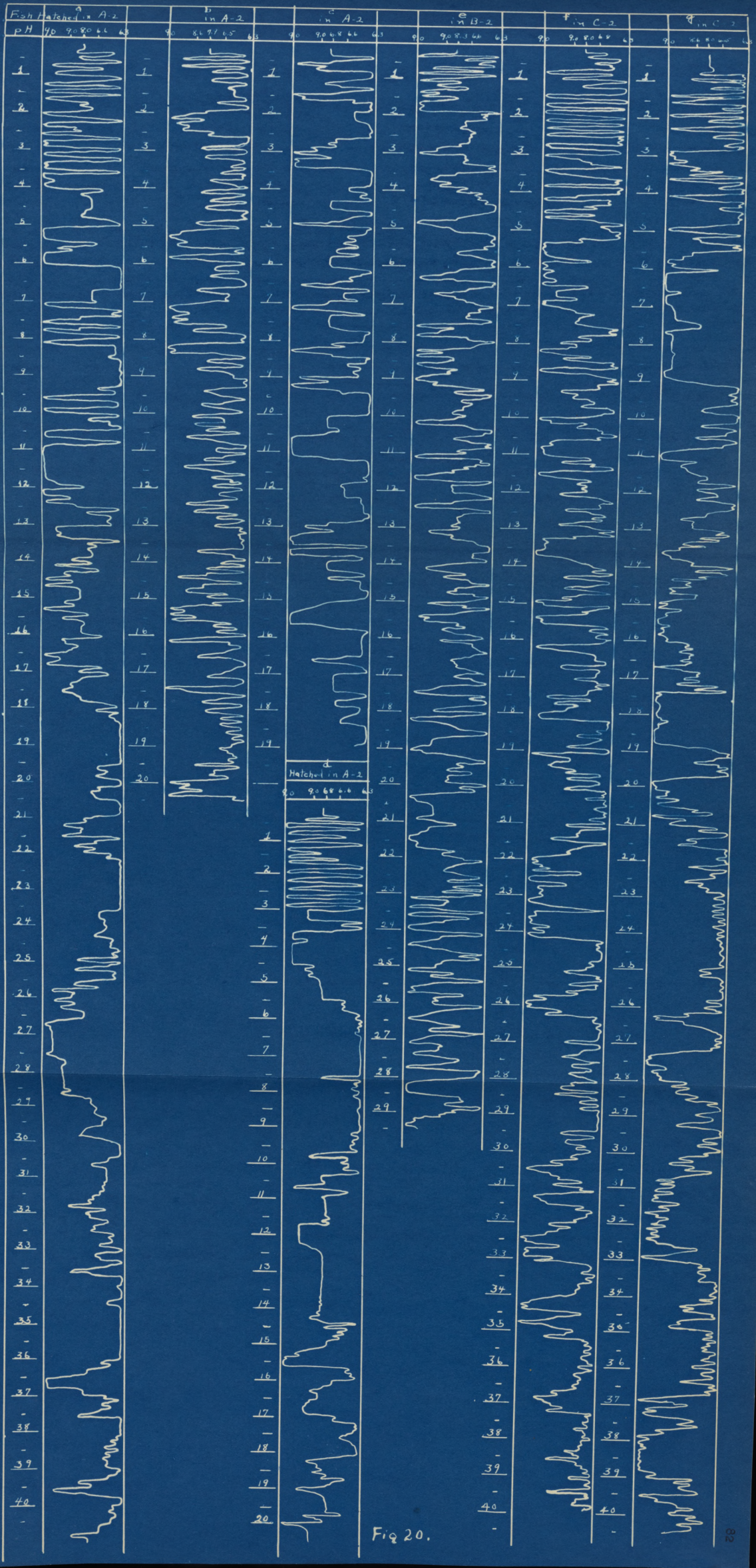

a Fish Hatched in A-2
pH 9.0 9.0 8.0 6.6 6.3
b in A-2
9.0 8.6 7.7 6.5 6.3
c in A-2
9.0 9.0 6.8 6.6 6.3
d Hatched in A-2
9.0 9.0 6.8 6.6 6.3
e in B-2
9.0 9.0 8.3 6.6 6.3
f in C-2
9.0 8.0 6.8 6.3
g in C-2
9.0 8.6 8.0 6.3

Fig 20.

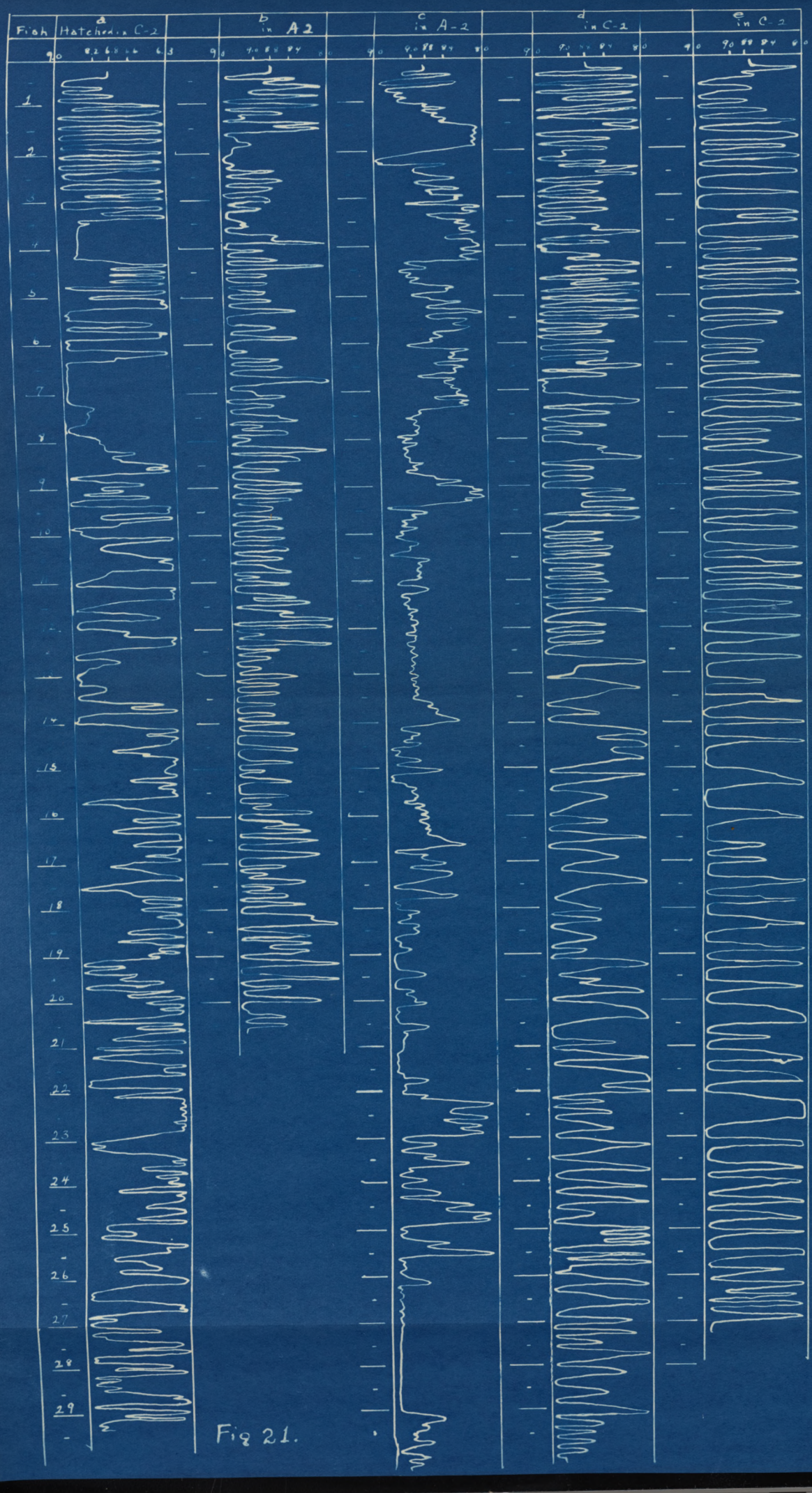


Fig 21.

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