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Some Effects of Temporary Exposure to Low Dissolved Oxygen Levels on Pacific Salmon Eggs[1]

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ABSTRACT

Eggs of the chum salmon (*Oncorhynchus keta*) were exposed to various constant levels of dissolved oxygen for a period of seven days. The procedure was repeated with fresh egg samples at various developmental stages. Temperatures were constant at 10°C. from fertilization to hatching. Estimates of oxygen consumption uninhibited by low dissolved oxygen levels were obtained at various stages of egg development for whole eggs and also on the basis of the weight of larvae, excluding the yolk. Eggs were most sensitive to hypoxia between 100–200 Centigrade degree-days and compensated for reduced oxygen availability by reducing the oxygen demand and rate of development. Very low oxygen levels at early incubation stages resulted in the production of monstrosities. At about the time the circulatory system becomes functional the compensatory reduction in rate of growth under hypoxial conditions is reduced, but eggs no longer survive extreme hypoxial conditions. Eggs subjected to low dissolved oxygen levels just prior to hatching hatch prematurely at a rate dependent on the degree of hypoxia. The maximum premature hatching rate corresponded approximately with the median lethal oxygen level. Estimated median lethal levels rose slowly from fertilization to hatching. Oxygen consumption per egg rose from fertilization to hatching while the consumption per gram of larval tissue declined from a high to a low level at about the time of blastopore closure. Subsequently, a slight rise in the rate occurred up to a level which was more or less constant to hatching. "Critical" dissolved oxygen levels were calculated and they appear to define the oxygen level above which respiratory rate is unmodified by oxygen availability. Critical levels ranged from about 1 p.p.m. in early stages to over 7 p.p.m. shortly before hatching.

INTRODUCTION

Laboratory experiments have been conducted on eggs of the chum salmon (*Oncorhynchus keta*) in order to investigate effects of a low dissolved oxygen

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environment on their development and survival. During recent years a program of field studies at the Fisheries Research Board of Canada's Nanaimo Station has investigated factors which influence the success of incubation and emergence of free-swimming pink (*O. gorbuscha*) and chum fry (Neave, 1947; Wickett, 1954). Wickett further attempted to define the reasons for high pre-eyed egg loss observed in nature on the basis of the relations between the velocity and oxygen content of sub-surface water in the gravel. Following and in conjunction with the latter, this investigation was begun in order to determine the responses of the eggs themselves to variations in oxygen availability and to assess the manner in which such variations might produce mortality.

The availability of oxygen to salmon eggs may be considered to be dependent primarily on the oxygen pressure in the microenvironment surrounding each egg. Whereas temperature may effect the oxygen pressure in the microenvironment, it will also influence the physiological state of the egg and its potential rate of oxygen utilization. Thus, Lindroth (1942) demonstrated that for Atlantic salmon (*Salmo salar*) eggs just prior to hatching, a temperature of 17°C. resulted in oxygen consumption at the rate of about 29 cc./kg./hr. (sustainable by a dissolved oxygen content of not less than about 8.7 p.p.m. or 89% of saturation or greater). By comparison, at 5°C. the rate of consumption was about 16 cc./kg./hr. (sustainable by a dissolved oxygen content of not less than about 5.7 p.p.m., or 45% of saturation or greater). The utilization rate itself may influence the oxygen content of the water if the rate of replacement of the microenvironment is insufficient to provide for the full utilization potential. Investigations by Wickett (loc. cit.), for example, indicated that to satisfy the potential oxygen demand of pre-eyed chum salmon eggs at 8°C., the oxygen content may vary between the equivalent of air saturation and 1.67 p.p.m., depending on the velocity of the perfusing water. Again, under the same conditions not even air-saturated water will sustain the egg if the apparent[2] velocity of the water drops below approximately 5 mm./hr.

Comprehensive reviews concerning the metabolism and development of, and the influence of the environment on teleost eggs have been published by Hayes (1949) for salmonids and by Smith (1957).

In studying the response of salmon eggs to variations in their environment, it is important to recognize that one is dealing with an organism which is changing day by day. Not only are growth and differentiation progressive, but the rates governing these attributes also may be modified by the environment. As anatomical differentiation proceeds, the response of the animal to its environment may vary not only progressively but also by periods of susceptibility associated with variable sensitivity. In order to evaluate the effects of a low dissolved oxygen environment, a proper procedure was considered to be that of exposing eggs to various temporary low dissolved oxygen levels to determine the sensitivity of the egg, in terms of susceptibility, at various stages of development between fertilization and hatching.

[2]See Wickett (1954). The true velocity of a fluid flowing through granular material is impossible to determine, and an estimate of the true value is equal to volume discharge per unit time divided by the cross-sectional area of the gravel bed.

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MATERIALS AND METHODS

The apparatus consisted of a device for providing continuous supplies of air-saturated and deoxygenated water. The two supplies were combined in fixed proportions to provide a series of six constant levels of dissolved oxygen at constant temperature.

Deoxygenated water was provided by a "stripping column", a modification of apparatus described by Fry (1951). Water was allowed to fall through a column of glass chips providing high water-gas surface interfaces. An atmosphere of nitrogen passing up the column permitted the water to equilibrate at the interfaces with the high nitrogen gas content, removing the oxygen. Air-saturated water was provided in the same manner by leading compressed air through a similar column (Fig. 1).

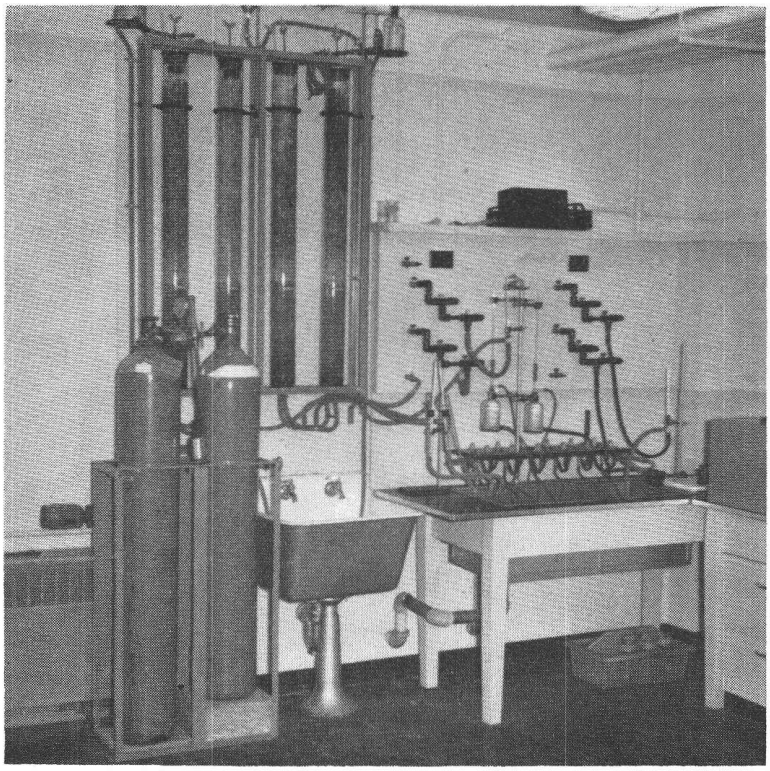

FIG. 1. Equilibration columns used for supplying deoxygenated and air-saturated water. Water is piped to the tops of the columns, falls through the glass chips and is collected at the bottom. Nitrogen gas is passed up through the two left-hand columns, and compressed air through the right-hand pair.

The two water supplies were then led into a constant temperature bath where temperature equilibration was effected in plastic-lined aluminum tubing. From this point the two water supplies were led to mixing valves adjusted to provide prescribed oxygen levels, thence back to the egg chambers housed in the same constant temperature bath (Fig. 2).

Eggs were fertilized and cultured at the Nanaimo Station (Series B–E, Table II), and at Nile Creek Field Station (Series A), for later transfer. The

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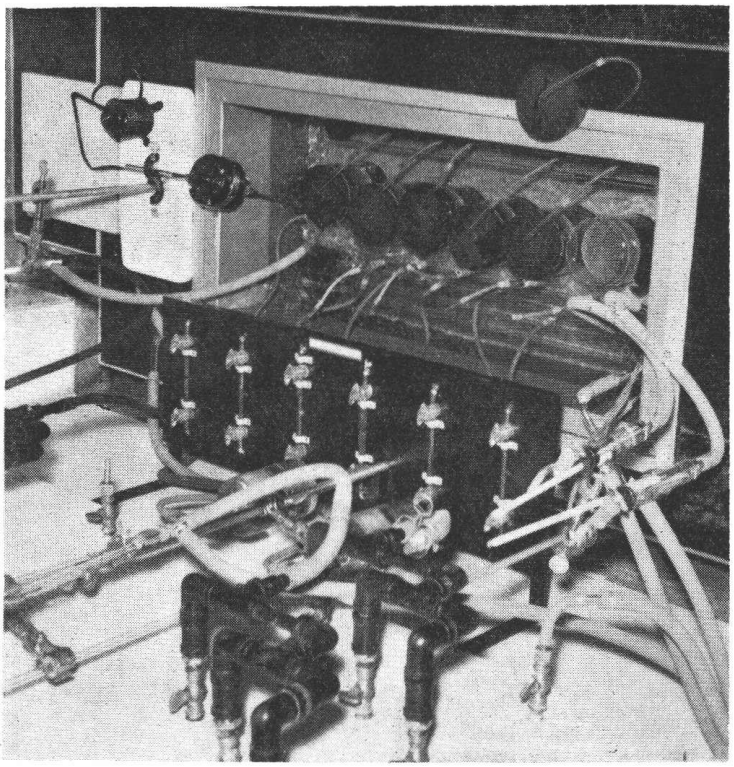

FIG. 2. The two water supplies from the columns are led through bubble traps and into temperature equilibration coils in the constant temperature bath. From there they are led into two manifolds under the bank of mixing valves, through the mixing valves where they are re-combined in prerequisite proportions and delivered to the bottom of the egg chambers where the water passes up through a single layer of eggs to the overflow. The head bottles are attached to the manifolds and provide a constant head at the egg chambers.

first series was conducted in the winter of 1953–54; the remaining four series were completed in the winter of 1954–55.

At the start of an experiment, samples of eggs of known developmental age were removed from incubation trays and carefully inserted into the six experimental chambers, each chamber being maintained at a fixed oxygen level for a period of seven days. The water passing through the single layer of eggs had an apparent velocity of about 850 mm./hr. Egg samples were then returned to individual incubation baskets and held in one hatchery trough until hatching was complete. The temperature to which the eggs were exposed was held constant throughout the total incubation period including the interval of exposure to the low dissolved oxygen conditions.

Dissolved oxygen determinations were carried out in the usual manner, employing the unmodified Winkler titration method. Determinations were made on a 50-ml. aliquot, titrating with 0.01N thiosulphate at those levels of 5 p.p.m. dissolved oxygen or greater. Below 5 p.p.m. dissolved oxygen, titrations were conducted with 0.005N thiosulphate from a 5-ml. microburette.

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In each of four tests on oxygen consumption, 50 eggs were set out on a nylon net platform expanded within an aspirator bottle of about 600 ml. capacity. Water of known oxygen content was allowed to overflow through the bottle until at least two volumes had been exchanged. The lower orifice was closed off and a mineral oil seal of approximately 2 cm. depth was poured into the neck of the bottle. The bottle was then immersed in a constant temperature bath and the contents were slowly circulated using a rheostat-controlled electric stirrer with a length of straight glass rod projecting through the oil seal. Eggs treated in this fashion were allowed to respire until an estimated 2 p.p.m. dissolved oxygen had been removed from the closed bottle. The contents were sampled for dissolved oxygen and the volume of water used in the closed bottle was measured.

For convenience, several terms have been used to describe the young fish throughout development which require explanation. Whereas "embryo" is used to denote the unhatched fish, "larva" is used to denote the young fish separated from its yolk. "Pre-hatching stage" refers to the period of ten days prior to the mean hatching date of a sample of eggs.

Embryo weights were obtained by slitting the egg capsule and dissecting each larva away from the yolk. The larvae were hardened in 10% formalin, dried of external moisture on filter paper, and weighed on an analytical balance. A comparison of fresh and preserved larvae was made on pre-hatching stage embryos and the percent loss in weight through dehydration was applied to the earlier stages as a correction factor. Average larval weights were calculated on the basis of the weights of samples of 7 to 10 larvae.

RESULTS

Exposure of eggs at various developmental stages to low oxygen produced three gross responses: production of monstrosities, delay in the rate of development, and mortality. The distribution of hatching times in each sub-sample of eggs was found to be skewed. The best measure of central tendency was that provided by the median hatching time. Where approximately 50% or more of a sample of treated eggs survived to hatch, the net delay in days to the median hatching date has been calculated using the control groups as a standard. In the remaining cases complete or nearly complete mortality prohibited such estimates. Temperatures associated with the experiments and the incubation periods for control samples are listed in Table I.

"Degree-days" have been employed to express the relationship of temperature and incubation time where one degree-day is equivalent to exposure to a

TABLE I. Temperatures and incubation periods for the experimental controls.

Series	A	B	C	D	E
Av. rearing temperature, fertilization to hatching, °C.	5	9.8	9.9	9.7	9.7
Av. experimental temperature, °C.	4.8	10.7	10.4	10.2	10.1
Interval from fertilization to hatching, *degree-days*	...	510.5	512.7	563.3	588.9
Time to median hatching date, *days*	...	52.1	52.0	57.5	60.8

temperature of one Centigrade degree (above 0°C.) for one day (see Hayes, 1949; Seymour, 1956). Measures of the response of eggs to the various levels of dissolved oxygen imposed at several stages of development are listed in Table II.

It may be seen from examination of Table II that developing eggs are not

TABLE II. Results of exposure of samples of chum salmon eggs to various levels of oxygen saturation. Series A was carried out at 5°C. on sub-samples of 10 eggs, the remaining series were conducted at 10°C. on sub-samples of 20 eggs. In each series sub-sample 6 is a control at oxygen levels approximating air-saturation at the temperatures involved. The alevins marked with an asterisk (*) showed abnormal development.

SERIES A. Development stage—just prior to and during hatching.						
Sub-sample	1	2	3	4	5	6
Av. experimental O_2 level, *p.p.m.*	0.26	0.29	1.41	1.97	4.20	12.47
No. of dead eggs at end of experiment	7	8	1	0	0	0
No. prematurely or unsuccessfully hatched	3	1	0	0	0	0
No. hatched in test period	3	2	7	6	4	3
No. of dead alevins at end of the hatch	3	1	1	0	0	0
Total live alevins hatched	0	1	7	10	10	10
SERIES B. Developmental stage—121.2 degree-days (24% of control incubation period).						
Sub-sample	1	2	3	4	5	6
Av. experimental O_2 level, *p.p.m.*	0.25	0.29	0.72	0.92	2.15	10.17
No. of dead eggs at end of experiment	0	0	1	2	0	0
No. prematurely or unsuccessfully hatched	2	0	0	2	0	0
No. of dead alevins at end of hatch	2*	0	0	3	0	0
Live alevins hatched	17*	20*	17	14	20	20
Median hatching date, January	12.5	12.0	12.9	9.8	7.4	2.1
Delay in hatching, *days*	10.4	9.9	10.8	7.7	5.3	0
Time from fertilization to median hatching date, *days*	62.5	62.0	62.9	59.8	57.4	52.1
SERIES C. Developmental stage—205.8 degree-days (40% of control incubation period).						
Sub-sample	1	2	3	4	5	6
Av. experimental O_2 level, *p.p.m.*	0.20	0.32	0.61	0.87	1.67	10.20
No. of dead eggs at end of experiment	20	20	10	0	0	0
No. prematurely or unsuccessfully hatched	0	0	0	0	0	0
No. of dead alevins at end of hatch	0	0	0	0	0	0
Total live alevins hatched	0	0	8	20	20	20
Median hatching date, January	...	...	12.3	11.1	8.5	2.0
Delay in hatching, *days*	...	...	10.3	9.1	6.5	0
Time from fertilization to median hatching date, *days*	...	...	62.3	61.1	57.5	52.0
SERIES D. Developmental stage—296.1 degree-days (53% of control incubation period).						
Sub-sample	1	2	3	4	5	6
Av. experimental O_2 level, *p.p.m.*	0.37	0.52	0.81	1.94	3.04	10.28
No. of dead eggs, end of experiment	18	11	1	0	0	0
No. prematurely or unsuccessfully hatched	0	0	0	0	0	0
No. of dead alevins at end of hatch	0	0	0	0	0	0
Total live alevins hatched	2	8	19	20	20	20
Median hatching date, January	...	13.3	12.3	9.8	8.6	7.5
Delay in hatching, *days*	...	5.8	4.8	2.3	1.1	0
Time from fertilization to median hatching date, *days*	...	63.3	62.3	59.3	58.6	57.6
SERIES E. Developmental stage—452.4 degree-days (77% of control incubation period).						
Sub-sample	1	2	3	4	5	6
Av. experimental O_2 level, *p.p.m.*	0.36	0.52	0.81	1.81	4.05	10.14
No. of dead eggs at end of experiment	19	16	10	0	0	0
No. prematurely or unsuccessfully hatched	1	1	6	2	1	0
No. hatched during the test period	1	1	5	2	1	0
No. of dead alevins at end of hatch	1	2	6	0	0	0
Total live alevins hatched	0	1	0	20	20	20
Median hatching date, January	...	...	5.0	12.3	11.6	10.8
Delay in hatching, *days*	...	...	[a]	1.5	0.8	0
Time from fertilization to median hatching date, *days*	...	...	55.0	62.3	61.6	60.8

[a]These eggs hatched prematurely, 5.8 days *earlier* than the controls.

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equally affected at different stages by similar hypoxial environments. The three major indications of stress, production of monstrosities, variation in hatching rate, and mortality are treated separately in the following sections.

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Production of Monstrosities

In Series B, undertaken 12 days following fertilization, moderate to low mortality occurred in the sub-samples from 0.25 to 0.92 p.p.m. dissolved oxygen. Examination of the eggs in this series was carried out on the 27th day after fertilization, following the period of low-oxygen exposure between the 12th and 19th days. Eggs from the control (sub-sample 6) corresponded in development with those in the balance of the egg stock, characterized by the heart formed and beating, the eyes pigmented, and the vitelline vessels well established. Development in sub-sample 5 was only slightly less than that in the control. However, in the remaining sub-samples, the vitelline circulation indicated considerable retardation in development with the vitelline vein in sub-sample 4 enclosing an area devoid of vascularization equivalent to a solid angle of about 45°. A progression in retardation was evident right through to sub-sample 1 in which the position of the vitelline vein was slightly above the equator of the yolk and the enclosed area free of vascularization. A progression in degree of eye pigmentation was also evident in sub-samples 4 to 1, no pigmentation being discernible in the latter.

It has been well substantiated that the period of development during which the blastoderm is overgrowing the yolk is associated with marked fragility of the egg (Hayes, 1949). It is quite possible that the mortality which occurred in Series B, sub-samples 1 to 4, could be ascribed in part or entirely to such fragility.

However, in the two sub-samples in Series B subjected to less than 0.3 p.p.m. dissolved oxygen, of 40 eggs involved, 39 of the alevins which hatched were abnormal in form. From gross observation these were characterized by a shortening of the vertebral column posteriorly (Fig. 3). The final form suggests that the low-oxygen environment was imposed upon the embryo at a time when somite formation had proceeded posteriorly to the region of the insertion of the dorsal fin and interruption of somite formation left the embryo blocked at that stage of segmentation, with subsequent appendages being laid down on the incompleted vertebral structure (for an extensive treatment of the experimental production of deformities see Stockard, 1921).

The plasticity and survival apparent in the egg samples at this early stage of development were not maintained in the next experimental stage. Oxygen levels slightly higher than 0.3 p.p.m. (0.32 p.p.m., sub-sample 2, Series C), 22 days after fertilization, produced complete mortality in contrast to successful but abnormal development in Series B.

Variation in Hatching Rate

In Series B to E, variations in the rate of hatching have been calculated as the difference in days between the median hatching date of the controls and that of the other sub-samples. If air-saturation in the controls may be regarded as the condition affording best opportunity for development and survival, then

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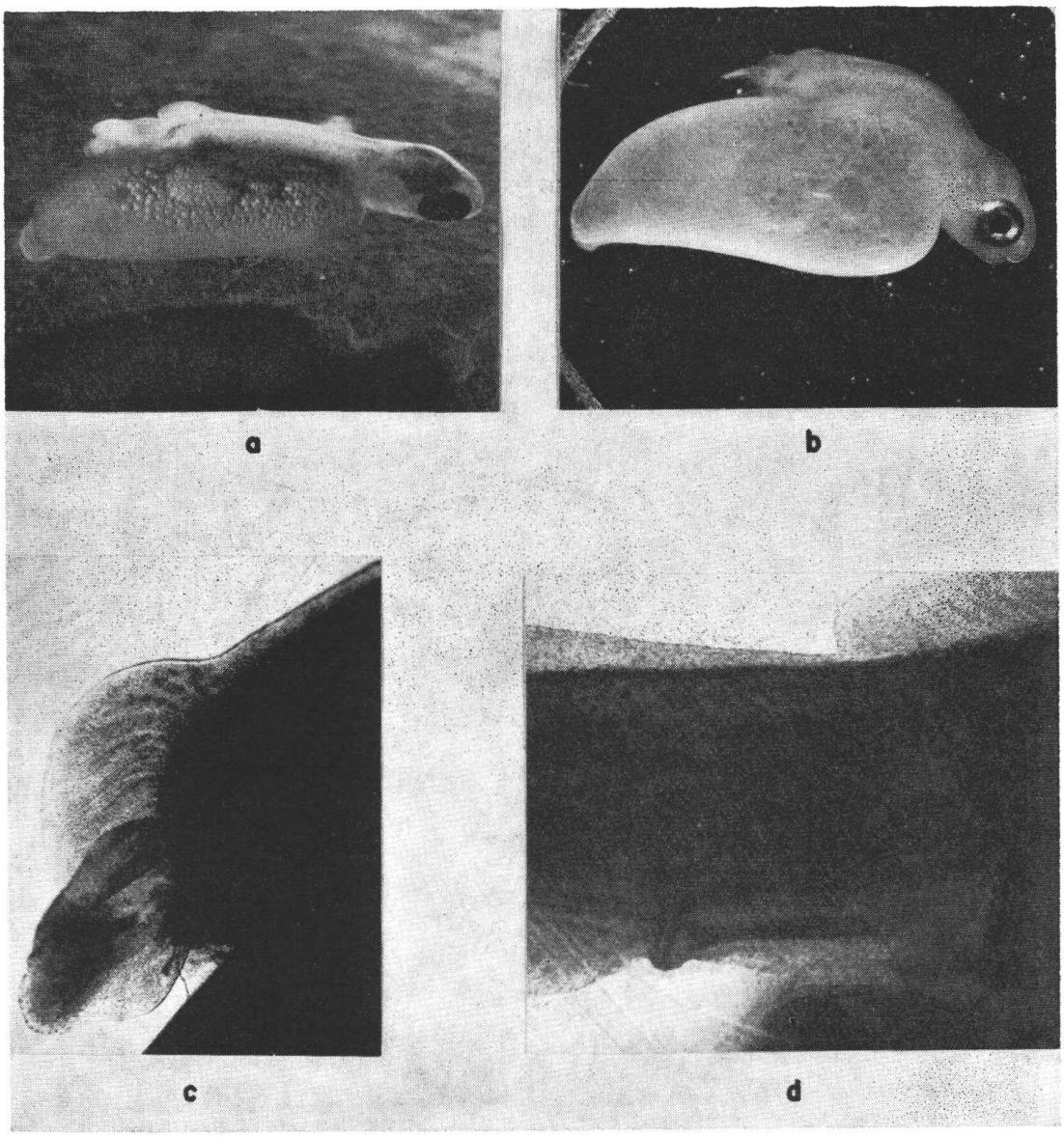

FIG. 3. Deformity resulting from exposure of chum salmon eggs to hypoxia at an early incubation stage: (a) and (b) hatched alevins showing posterior truncation; (c) alevin showing the location of the caudal and pelvic fins immediately beneath the dorsal fin—there is also an oblique displacement of the caudal fin from the dorso-ventral axis; (d) a normal alevin photographed in the same area as (c) illustrating the extreme truncation of the posterior portion of (c).

variations in hatching rate may be regarded as a measure of sub-lethal stress on the developing embryo. The diverse and complex nature of the relationship between oxygen level and developmental stress is illustrated in Figure 4. At the first experimental stage (12 days or 121.2 degree-days), sub-lethal stress reaches a maximum at about 0.3 p.p.m. dissolved oxygen, resulting in a delay in hatching of about 10 to 11 days after an exposure of one week to the hypoxial conditions.

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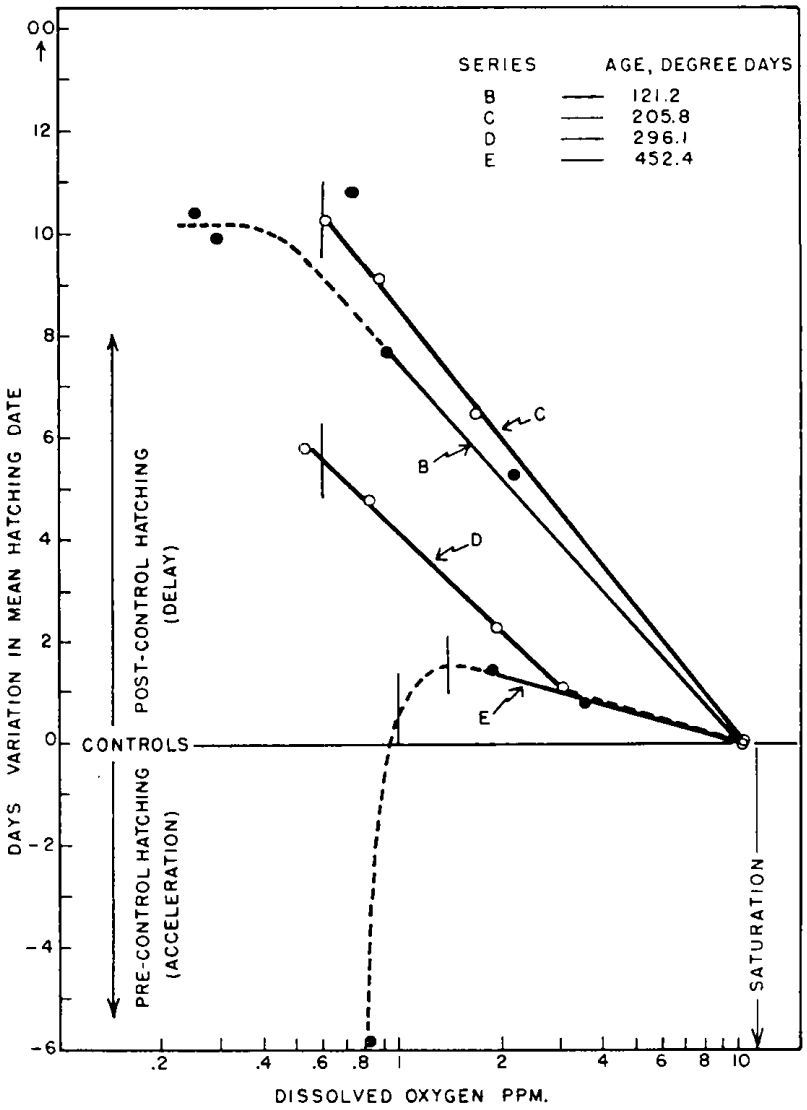

FIG. 4. The relationship between dissolved oxygen level to which developing chum salmon eggs were exposed for a period of 7 days and the variation from the normal or control incubation period at 10°C. The differences in hatching rate are regarded as a measure of the stress applied to the eggs by low-oxygen conditions at the four developmental stages examined. Not all eggs hatched at every point; the approximate position of the median lethal low oxygen level is indicated on each line by a vertical bar. Although almost all eggs hatched at the two lowest oxygen levels in Series B, the resulting alevins were deformed and considered inviable.

It is within this range that abnormal development occurred. In the next series (22 days or 205.8 degree-days) the slope of the line is increased, a unit decrease in oxygen level placing a greater stress on the embryo than at an earlier developmental stage. In Series D (32 days or 296.1 degree-days) the relationship de-

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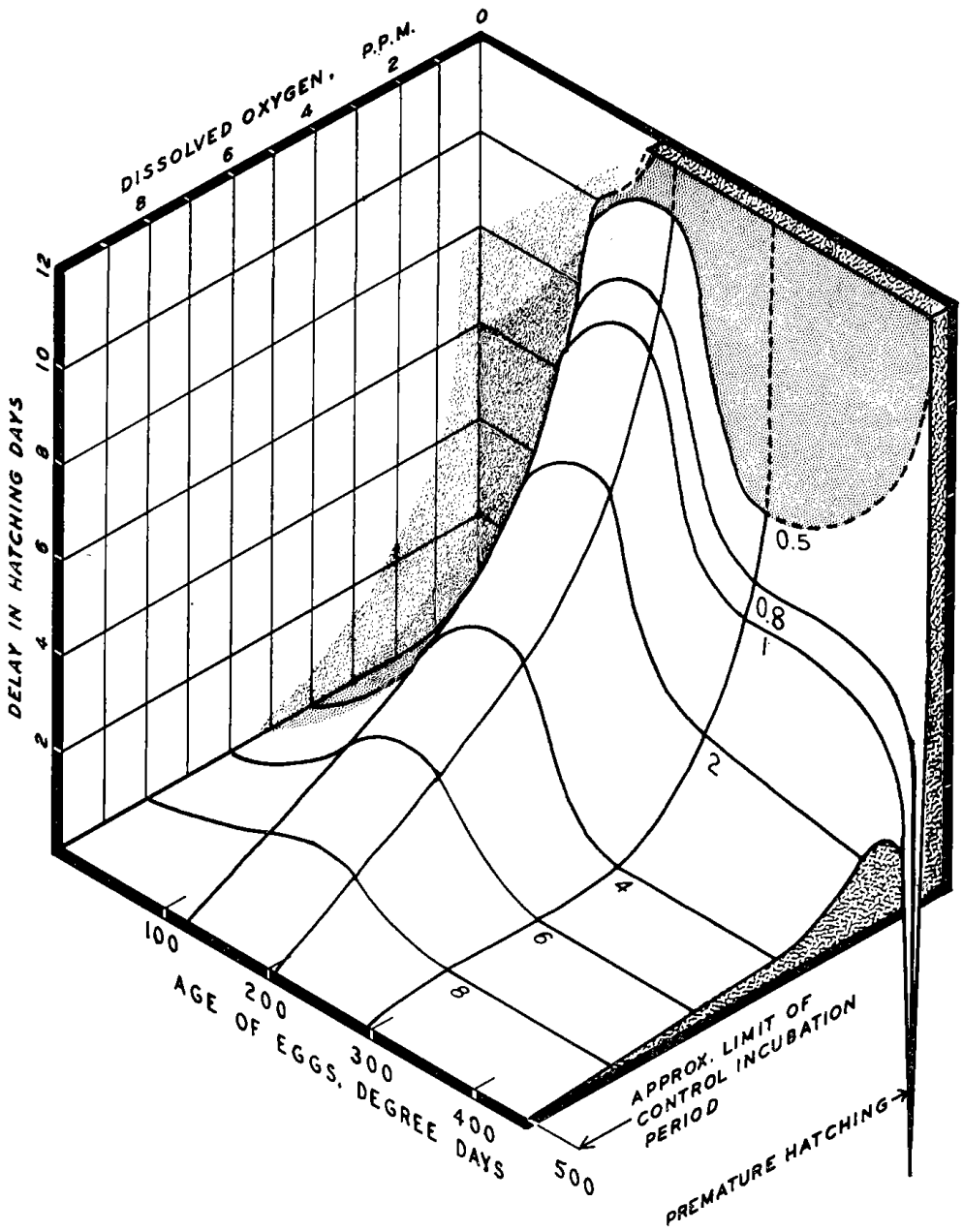

FIG. 5. Variation in hatching rate of samples of chum salmon eggs reared at 10°C. resulting from a 7-day exposure to prescribed oxygen levels at intervals throughout the incubation period. Variation from the control hatching rate, represented by the extent of the "hump", is regarded as a measure of stress (see text). (Drawn by D. Denbigh.)

scribes a break in linearity at about 3 p.p.m. dissolved oxygen, with the stress at all levels considerably reduced from that of the two earlier series. In the final series (48 days or 452.4 degree-days), the change in slope is complete with the stress tending toward a minimum. However, the degree of hypoxia which the

developing embryo may tolerate is considerably reduced and a new condition is produced in which there is a tendency for eggs to hatch prematurely when subjected to low oxygen levels.

To provide a visual indication of the complex variations in response to low oxygen as measured by delayed hatching rate, a three-dimensional diagram has been constructed in Figure 5 by isometric projection of surfaces from Figure 4. The diagram may be interpreted as follows: comparing the hatching rates of control egg samples subjected to oxygen levels approximating air-saturation (10 p.p.m.) with hatching rates at lower oxygen levels (Series B–E, Table II), surfaces have been constructed by scaling off the variations in hatching rate (mostly delay) from Figure 4 at the oxygen levels listed in that figure and at the four incubation stages examined (Series B–E). Dashed lines infer that stress is becoming infinite, in other words, mortality is complete or nearly complete. Increases in the vertical dimension of the solid correspond to increases in the stress resulting from exposure to hypoxial conditions. A period of maximum stress occurs between 100 and 200 degree-days of development. Stress also increases at all levels below air-saturation. Acceleration of hatching results in premature hatches when pre-hatching stage eggs are exposed to oxygen levels below air-saturation. This effect is maximized at about 1 p.p.m. dissolved oxygen at 10°C. The incubation period of the controls is equivalent to about 500 degree-days.

Other work with salmonid eggs reared at various levels of dissolved oxygen has demonstrated no significant variation in hatching rates (E. T. Garside, personal communication). It is possible that eggs developing at oxygen levels below air-saturation may acclimate to a range of those levels by proliferation of accessory respiratory surfaces in the manner described by Smirnov (1953). It is suggested that eggs acclimated to low oxygen conditions would not respond to hypoxial conditions in the same manner as eggs reared at oxygen levels approximating air-saturation by virtue of the ability of the former eggs to extract oxygen with greater efficiency at lower environmental oxygen levels.

If pre-hatching stage eggs are exposed to low-oxygen conditions, premature hatching occurs, illustrated by examination of Series A and E. Figure 6 illustrates that this rate of hatching is greatest at dissolved oxygen levels of about 1 p.p.m. In both series premature hatching is accompanied by mortality. The incidence of mortality at the lowest dissolved oxygen levels diminishes to zero between 1–2 p.p.m. dissolved oxygen.

Mortality

Since a limited number of experimental oxygen levels were set up to examine the effects of a large range of hypoxial conditions from very low levels to air-saturation, the data do not lend themselves to calculation of values describing median lethal effects. However, several general observations may be made by examination of Table II.

Series B, the earliest developmental stage examined, did not sustain 50% mortality in any of the sub-samples. However, as all alevins hatching successfully after exposure to the two lowest dissolved oxygen levels (0.25, 0.29 p.p.m.) were

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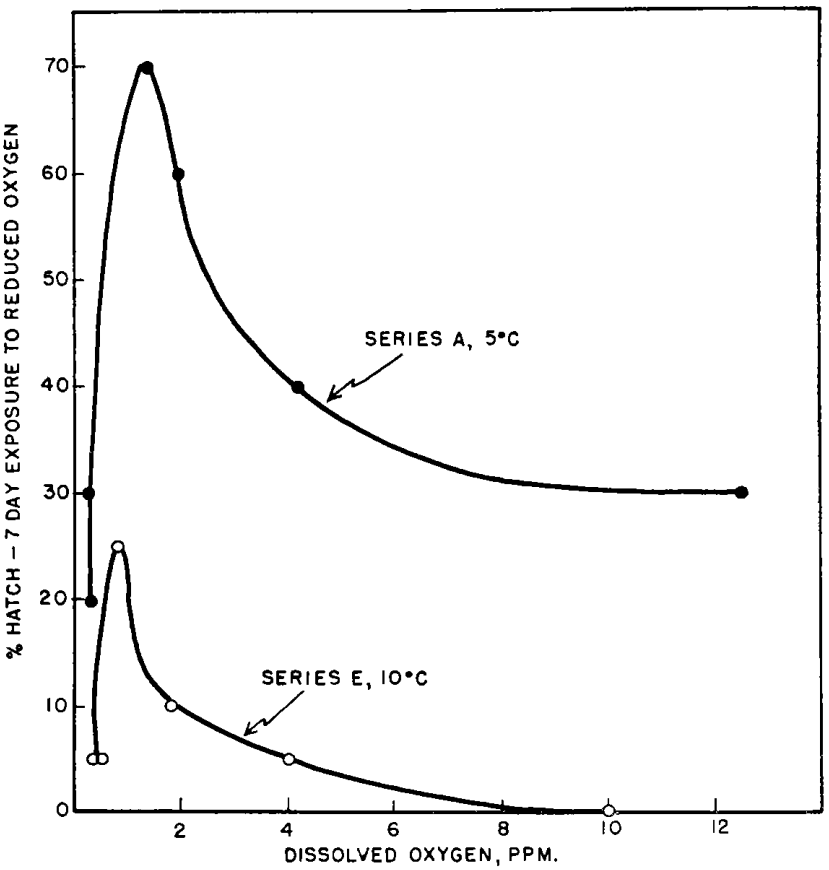

FIG. 6. A comparison of the rates of hatching of eggs subjected to dissolved oxygen levels below saturation. The right-hand points on each curve correspond to the controls. Premature hatching is maximized in both series at about 1 p.p.m. dissolved oxygen. The difference in the magnitude of the hatches between the two series may be concerned with temperature differences but is more probably a result of the eggs in Series A being of a more advanced stage when the experiment started.

deformed and unable to move, even 53 days after their median hatching date, they may be considered to have been inviable. A median lethal dissolved oxygen level could be assumed to be at a level greater than 0.29 p.p.m. dissolved oxygen. Estimates of median lethal levels by inspection are presented in Table III for the Series B to E. (See Fig. 8.)

TABLE III. Median lethal dissolved oxygen levels for chum salmon eggs exposed to hypoxial conditions for a period of 7 days. The values are estimated by inspection from Table II.

Series	Age of eggs after fertilization	Age of eggs	Approx. range of oxygen levels	Estimated median lethal oxygen level
	days	*degree-days*	*p.p.m.*	*p.p.m.*
B	12	121.2	0.3–0.7	0.4
C	22	205.8	0.5–0.9	0.6
D	32	296.1	0.5–0.8	0.6
E	48	452.4	0.8–1.8	1.0–1.4

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Changes in Oxygen Consumption throughout Development

For Series B to E, estimates of oxygen consumption were obtained by allowing samples of 50 eggs to respire in a closed volume of water. The change in oxygen content was used to calculate rates of oxygen uptake listed in Table IV.

Rates of consumption are illustrated in Figure 7.

Table IV. Rate of oxygen uptake of chum salmon eggs (av. radius 0.37 cm.) at four developmental stages at 10°C. The oxygen consumption values in mm³./egg/hr. are for comparison with other literature; values in mm.³/g./hr. are based on the weight of living material, not including the yolk.

Age of eggs	Oxygen in closed system at times: t_0	t_1	Mean larval weight	Oxygen consumption per hour		
degree-days	*p.p.m.*	*p.p.m.*	*g.*	*mg./egg*	*mm.³/egg*	*mm.³/g.*
121.2	10.12	8.70	0.0023	0.00093	0.68	295.1
268.2	9.91	5.84	0.0155	0.00219	1.60	103.1
353.0	10.41	8.11	0.0231	0.00381	2.78	120.4
452.4	10.25	8.23	0.0290	0.00521	3.80	131.2

DISCUSSION

Hayes, Wilmot and Livingstone (1951) present evidence illustrating that oxygen consumption of incubating eggs of *S. salar* at 10°C. may be fitted to the equation

$$\log y = k_0 + k_1 \log (x - 9)$$

where y = oxygen consumption
x = number of days after fertilization.

The constant, 9 days, is equivalent to the time of establishment of the embryonic axis. From inspection, the present consumption values follow the same type of relationship as those of Hayes *et al*; however, the k_0 value of the present series appears to be greater and the last determination tends to inflect. To effect a comparison, a correction may be made to bring egg weights into agreement. Eggs in the present series averaged 7.4 mm. in diameter as compared to a probable value of 6.0 mm. for those of Hayes and his co-workers. Relating the rate of metabolism at comparable ages to the surface areas (Krogh, 1941), consumption rates are between two to three times those of Hayes in the early stages, and are consistently higher throughout development. By comparison with data of Privolnev (see Hayes *et al.*, 1951) Hayes concludes that the oxygen consumption per gram of living tissue in early stages is not constant but declines with development. This conclusion is borne out by the present results (see Fig. 7) where consumption per gram of larval tissue declines from 295 to 103 mm.³/g./hr. between 121 and 268 degree-days development. Data of Hayes *et al.* are presented in Figure 7 for comparison. Whereas Hayes suggests that their data are apparently linear and constant (from 19 days until hatching, at 10°C.), in view of the evidence for curvilinearity and the decline in oxygen consumption from early stages among chum salmon eggs, a liberal re-interpretation of their results (Fig. 7) would suggest a trend similar to our own findings. It must be acknowledged, however, that a smaller number of observations are represented over the same range of ages in our own data. In arriving at

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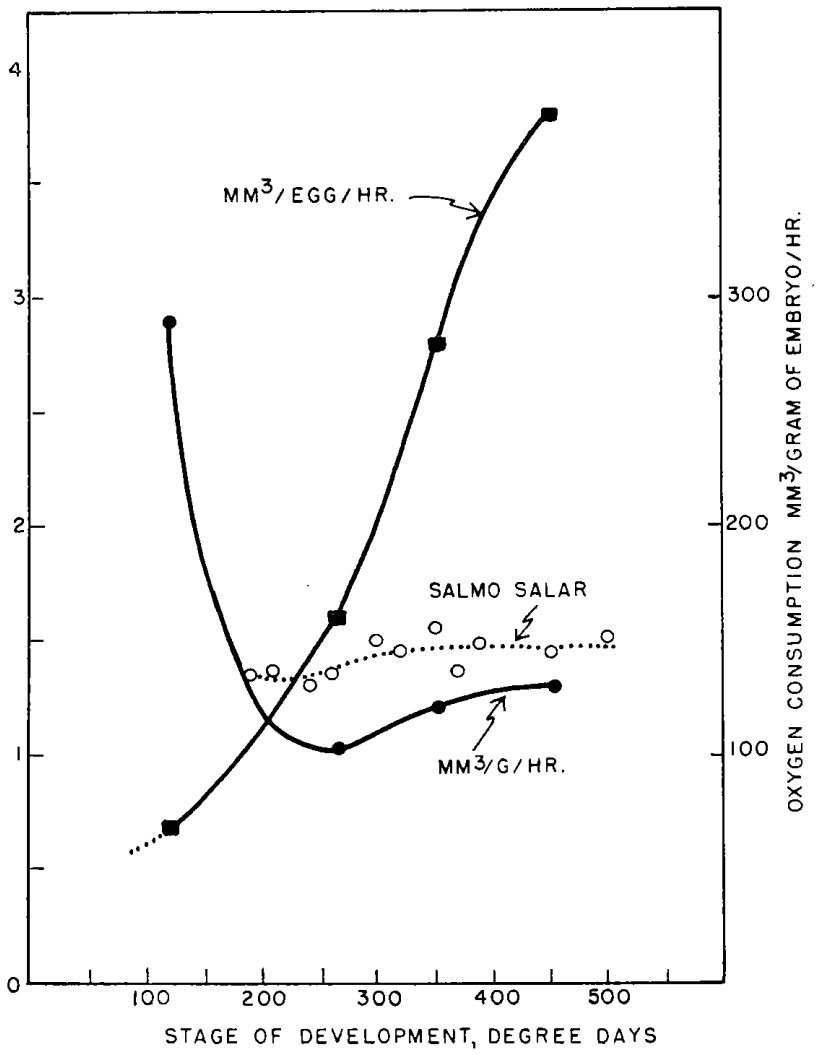

FIG. 7. Oxygen uptake of chum salmon eggs based on total egg weight and embryo weight less yolk. Compared with the latter are data for *Salmo salar* from Hayes, Wilmot and Livingstone (1951), and the interpretation of the trend in these data is our own.

estimates of the oxygen consumption per gram of larval tissue, the greatest potential source of error is found in weighing the embryos. If the high consumption value found for embryos at 121.2 degree-days development were attributed to error in this regard, it would be necessary to assume that the actual larval weight would be approximately 2.3 times greater than the measured weight to reduce the consumption value calculated for this stage to the approximate level of the remaining stages. Such a high weighing error is not considered plausible and the high consumption value for the early embryo is therefore not considered to be spurious. Oxygen consumption per egg for the chum salmon eggs is higher at all ages than comparable ages of Atlantic salmon, whereas the consumption per gram of larval tissue is lower than that of Atlantic salmon (from about 200 degree-days onward). The apparent contradiction suggests that the

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metabolism and growth of the former in earlier stages is greater than in Atlantic salmon. Support for this assumption is suggested by the differences in consumption per gram of larval tissue which may reflect greater embryo weights at comparable ages in the chum salmon.

Variation in Hatching Rate

Within the range of temperatures employed in Series B to E, it may be assumed that the development rate as measured by *temperature* times *time* in days is a constant. However, the incubation periods of the controls in Series B to E exhibit increasing values which are disproportionate to the effects which would be expected from such slight average temperature differences throughout incubation. An uncontrolled variable associated with the development of the eggs was the length of time in which the egg stock was confined to the hatchery tray prior to the withdrawal of samples of eggs for each of the experimental series from B to E. Thus, Series B to E were incubated for 12, 22, 32 and 48 days in the hatchery tray prior to experimentation. Since essentially there is no difference between the mean hatching dates of the controls in Series B and C, the delay in hatching rate in the egg stock is assumed to have manifested itself between the 22nd and 32nd day of development, the latter being the age of Series C at which departure is first evident. In view of the fact that delay in hatching may be caused by exposure to low-oxygen conditions probably at any level below air-saturation at 10°C. (see Fig. 4), it appears reasonable to imply that the variations in rate of development might be explained by a progressively increasing competition for oxygen in the incubating egg stock. Evidence toward this conclusion could be based on a comparison of the period to 50% hatch in Series E with that of the stock which it should approximate. Although an exact record of the latter was not obtained, there is sufficient evidence to indicate that it was within one day of the period for the Series E control.

A variable sensitivity to external stimuli in developing salmon eggs has been noted by numerous investigators (Stockard, 1921; Devillers and Rosenberg, 1953). Battle (1944a) summarizes some of these responses including those from light, pressure, heat, cold, mechanical and electric shock and chemical action. Responses of eggs to such stimuli vary according to developmental stage. In general, it appears that susceptibility rises after fertilization, reaches a peak and begins to decline at a stage coincident with the closure of the blastopore. A second period of increased susceptibility has been found by others to be initiated in the latter part of the incubation period, reaching a peak at about the time of hatching.

In the present study, the effects of temporary low oxygen environments imposed for a period approximating $\frac{1}{7}$ of the incubation period were also indicative of a variable response to an external stress, dependent for its magnitude on the age of development. It is probable that the early period of maximum susceptibility is coincident with the growth of blastoderm over the yolk and closure of the blastopore. No increase in susceptibility of pre-hatching stage eggs, as measured by delay in hatching rate, is indicated in the present tests. However, it is conceivable that the higher oxygen requirements per egg at

pre-hatching stages could easily create a local hypoxial environment providing conditions which would have been quite innocuous at earlier stages.

Hayes *et al.* (1951) considered the possibility that eggs exposed to lowered oxygen levels may incur an oxygen debt. Their evidence indicates that no debt was repaid after exposure of eggs to hypoxial conditions and led them to suggest that lowered oxygen supply may retard the rate of metabolism, or that an anaerobic mechanism may function under such conditions. The present evidence supports the former consideration. Furthermore, the reduction in metabolic rate which must be coincident with the observed deceleration in growth is greatest not only at the lower oxygen levels but also at the period of maximum egg fragility corresponding approximately with the period of blastoderm overgrowth and closure of the blastopore. Smith (1957) cites evidence that the glycolytic system, tricarboxylic acid cycle and cytochrome system are functional in intermediary metabolism of developing *Oryzias* eggs. Other evidence is presented indicating that carbohydrate is utilized during short periods immediately following gastrulation and during the hatching period for eggs of *Salmo irideus*. The capacity for anaerobic glycolysis is also stated to rise steadily throughout development. In view of this evidence the possibility that eggs subjected to low oxygen conditions may develop an oxygen debt can not be denied.

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Mortality

In general, the results tend to indicate a slow but steady increase in the incipient low oxygen lethal level throughout development. Early stages exhibit a plasticity in which development may decelerate virtually to zero under extreme hypoxial conditions. In later stages this plasticity is lost and oxygen levels which would produce no more than a cessation of development at earlier stages become rapidly lethal.

Field sampling of eggs in natural redds by Wickett (1954) has indicated that high losses may be experienced in the pre-eyed stage. Wickett has developed methods of sampling for dissolved oxygen in sub-surface gravel and his work provides evidence that conditions recorded for the water flowing over spawning gravel may have no bearing whatsoever on conditions influencing eggs within the gravel. From consideration of the several variables influencing oxygen uptake in the gravel, Wickett arrived at a relationship providing a measure of sufficiency of oxygen supply. When the number of eggs normal to the direction of flow is considered, the following equation may be applied:

$$v\,(\mathrm{DO} - \mathrm{C}) = \mathrm{K}$$

where v = apparent velocity of the perfusing water
DO = dissolved oxygen present
C = critical dissolved oxygen level

For a single chum egg at 8°C. with an uptake of 0.0003 mg./egg/hr., one of the asymptotes of the relationship is 1.67 p.p.m. dissolved oxygen, equal to the critical dissolved oxygen level calculated after Krogh (1941). Wickett's eggs were at a stage of development equivalent to 48 degree-days. The value presented, 1.67 p.p.m., represents the amount of dissolved oxygen present in the perfusing

water which is just sufficient to provide one egg, normal to the direction of flow, with sufficient oxygen at excess water velocity so that it may respire at an unmodified rate.

With Wickett's evidence, the following assumption is made. Early developmental (pre-eyed) mortality is undoubtedly associated with hypoxial conditions in the gravel, in the majority of cases brought about through a low apparent velocity or low dissolved oxygen content of water percolating through the gravel. Early developmental stages have a plasticity which compensates for hypoxial conditions by a marked reduction in rate of development. Such hypoxial conditions might continue for an extended period at low temperatures in nature (e.g. two months at 0–5°C.). This compensatory cessation in development could ultimately be lethal, and eggs which may have lived for a period longer than that suggested by their developmental stage would be found dead in the pre-eyed stage. In essence, the suggestion made is that the compensatory ability of early stage eggs will allow the egg to sustain a stress for a limited time and if the stress is not removed, eventually the egg will die. The delay factor is reduced after a period of approximately 200 degree-days. This is probably synchronous with the establishment of a functioning circulatory system. The limitation imposed by hypoxia on earlier developmental stages tends to fix the embryo in a stage where the response of the embryo is not only greatest in terms of delay in development, but this very response keeps the egg from overcoming its respiratory deficit by not allowing it to get past that critical stage prior to the establishment of a functional circulatory system.

The other main period of mortality, found just prior to hatching (Hayes, 1949), appears to be related to a greater unit-demand of oxygen per egg at a time when the critical demand is high, or where diffusion through the capsule may be limiting the oxygen consumption of the embryo. Pre-hatching stage eggs subjected to decreasing amounts of dissolved oxygen show a progressively earlier hatch from saturation to lower dissolved oxygen levels, culminating in a maximum premature hatch at about 1 p.p.m. dissolved oxygen. If premature hatching is a response to stress from hypoxial conditions then this stress may be present at all levels of oxygen below air-saturation. It would appear, therefore, that pre-hatching eggs reared at oxygen levels approximating air-saturation are limited in their ability to bring sufficient oxygen through the capsule to sustain their normal requirements probably at all levels below air-saturation. It is concluded that any condition in pre-hatching stage eggs tending to limit dissolved oxygen availability will place a stress on eggs which is partially compensated for by premature hatching and escape from the gas exchange limitations imposed by the capsule.

Critical Dissolved Oxygen Levels

At all stages of development of the salmon egg, the oxygen respired by the embryo must diffuse through a thin enclosing spherical capsule of specified diameter and thickness. Krogh (1941) considers the mechanics of respiration in a spherical body enclosed in a capsule from evidence presented by Harvey. If an homogenous spherical body uses oxygen at a constant rate, and if the

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oxygen tension may be assumed to be maintained at zero in the centre of the body:

$$C_o = \frac{A\, r^2}{6\, D}$$

where C_o = the concentration of oxygen at the surface of the sphere in atmospheres,

A = oxygen consumption of the sphere, in millilitres per gram of total mass per minute (ml./g./min.),

r = total radius of the sphere, in centimetres,

D = diffusion coefficient of oxygen through the capsule, in millilitres per centimetre thickness of capsule per square centimetre of capsule area per minute (ml./cm./cm.^{2}/min.).

The formula may be applied to the egg prior to the establishment of a functional circulatory system in order to provide an estimate of the ambient oxygen level required to maintain respiration at a rate independent of the environmental supply. Although the required conditions are not completely fulfilled: viz., the respiring mass is not located in the centre but on the surface of the yolk, the use of this model is considered justified in the absence of better experimental evidence.

When an egg has reached the stage of possessing a functional circulatory system, oxygen diffusing through the capsule is transported to the embryonic tissue with greater efficiency. Again, according to Krogh's models, the tension difference necessary for diffusion of oxygen where its transport is accomplished by blood circulation under the capsule is given by:

$$C_o = \frac{A\, r\, T}{3\, D}$$

where T = thickness of the capsule in cm.

A = oxygen consumption of the sphere, in millilitres per gram of larval tissue per minute.

In this case, it is assumed that the oxygen pressure in the perivitelline fluid is zero when the ambient oxygen pressure is at the critical level (see Krogh, 1941, p. 24, for further discussion).

In order to arrive at estimates of critical oxygen levels by these models, a composite has been constructed of the data (see Table IV) with certain data of Wickett and of Hayes. Assuming a value for the diffusion coefficient, D, of 0.0000123 ml./cm./cm.^{2}/min. as found for eggs of the Atlantic salmon at 10°C. (Hayes *et al.*, 1951), thickness of the capsule, T, for chum eggs as 0.006 cm. (Wickett, unpubl.), 0.29 g. as the weight of chum salmon eggs (Wickett, 1954) and actual egg radii of 0.37 cm. (this paper), estimated critical levels have been calculated by the above formulae. Calculated critical levels are listed in Table V and illustrated in Figure 8.

Values of A, the oxygen consumption in ml./g./min., have been calculated on the basis of egg weight (0.29 g.) for the period prior to establishment of

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TABLE V. Calculation of critical oxygen levels for chum salmon eggs at various stages of development.

Age	O_2 consumption	Computational model	Calculated critical oxygen level	Source
degree-days	*ml./g./min.*		*p.p.m.*	
4.0	0.0000055[a]	$Ar^2/6D$	0.72	Wickett (1954)
4.8	0.0000078[a]	"	1.14	"
48.0	0.0000138[a]	"	1.67	"
121.2	0.000039[a]	"	3.96	This paper
162.1	0.0000317[a, c]	"	3.7	Wickett (1954)
268.2	0.001719[b]	ArT/3D	5.66	This paper
353.0	0.002006[b]	"	6.60	"
452.4	0.002185[b]	"	7.19	"

[a]Based on egg weights
[b]Based on larval weights
[c]Calculated from Wickett (1954), Table I, entry No. 6.

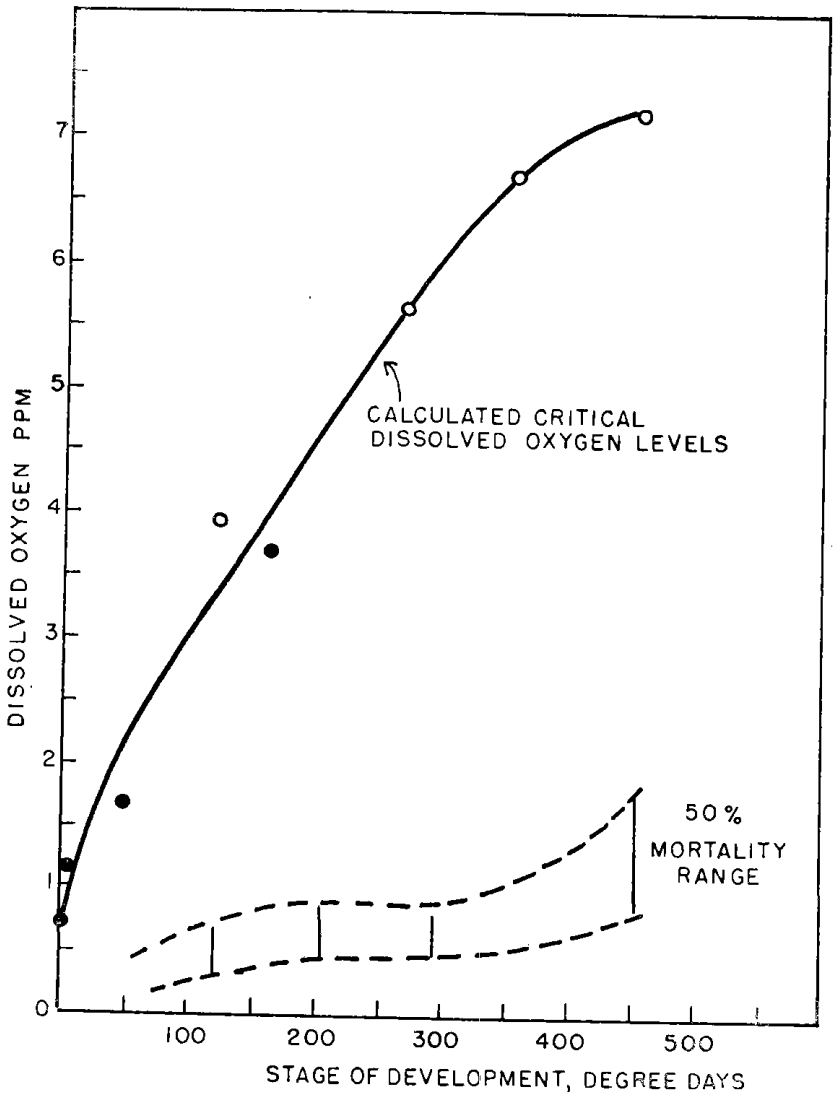

FIG. 8. Estimated median lethal levels of low oxygen and critical dissolved oxygen levels for chum salmon eggs at 10°C. The solid circles represent data for early developmental stages taken from Wickett (1954).

circulation beneath the capsule (estimated at about 200 degree-days) and on the basis of larval weights thereafter (see Table IV).

To illustrate the calculation of critical oxygen levels, the value for eggs at 121.2 degree-days in Table V is obtained as follows. Given $r = 0.37$ cm.; $D = 0.0000123$ ml./cm./cm.^{2}/min.; and $A = 0.00093 \times 0.73/0.29 \times 60 = 0.000039$ ml./g./min. where 0.00093 is the oxygen consumption in mg./egg/hr. (Table IV), 0.29 is egg weight in grams and 0.73 is a factor for converting mg. of oxygen/l. to ml. (at 10°C. and 760 mm. Hg.) of oxygen/l.:

$$C_o = \frac{A\,r^2}{6\,D} = \frac{0.000039 \times (0.37)^2}{6 \times 0.0000123} = 0.0723 \text{ atmospheres}$$

Given further that 11.3 mg. of oxygen will dissolve in fresh water at a partial oxygen pressure of 157 mm. Hg. at a total pressure of 760 mm. Hg., the above is equivalent to:

$$C_o = \frac{0.0723 \times 760 \times 11.3}{157} = 3.96 \text{ p.p.m. dissolved oxygen.}$$

Critical oxygen levels are those at which respiratory demand is just satisfied. If these are equivalent to levels below which oxygen uptake is reduced and metabolism and growth decelerated, critical levels are synonymous with limiting oxygen levels (for a discussion of limiting levels, see Fry, 1947, p. 41 *et seq.*). However, as calculated critical levels are partly theoretical in derivation whereas limiting levels may be obtained experimentally, a comparison is necessary to evaluate properly the biological meaning of the former. Unfortunately, the present authors have no information on limiting levels of oxygen for chum salmon eggs capable of comparison. Such information is highly desirable in order to evaluate the significance of critical oxygen levels.

Because of the recognized high oxygen requirements of pre-hatching stage eggs, their susceptibility to mortality and their tendency to hatch prematurely in water of oxygen content below air-saturation, it would appear that critical dissolved oxygen levels may form a basis to denote minimum permissible oxygen levels throughout incubation until more complete information on limiting levels is acquired.

On the basis of experiences encountered with delay in hatching rate caused by imperfect circulation in incubation trays, it is considered appropriate to advocate the use of the recent "vertical" hatchery techniques involving the use of water upwelling through the incubating eggs (see Burrows and Palmer, 1955; Lindroth, 1956).

SUMMARY AND CONCLUSIONS

Experiments have been conducted on chum salmon eggs (*Oncorhynchus keta*) in order to investigate some aspects of their response to low oxygen conditions. Eggs at various developmental stages were exposed to a series of low oxygen levels in a constant temperature bath for a period of seven days. Subse-

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quently, eggs were cultured under normal conditions until hatching was complete. Observed responses are listed as follows:

1. Oxygen levels below air-saturation at 10°C. produced delay in the mean rate of hatching of eggs tested at four developmental stages. This delay was greatest during early development (between 100–200 Centigrade degree-days) and dropped to lower levels at about the time circulation within the egg was established.

2. In early developmental stages of chum salmon eggs, oxygen levels of 0.3 p.p.m. or less, although not lethal, may result in the production of monstrosities.

3. Eggs of advanced developmental stage are stimulated by low oxygen conditions to hatch prematurely. The peak of premature hatching at 10°C. occurred at an oxygen level approximately equivalent to the estimated median lethal level for dissolved oxygen.

4. The oxygen uptake per gram of larval tissue at 10°C. is high in early development, corresponding approximately to the time of maximum fragility, and falls to a lower value at the end of that period. Subsequently, uptake per gram of larval tissue rises slightly to a level which is more or less constant.

5. Under the experimental conditions, the incipient median lethal level for dissolved oxygen rose with development from approximately 0.4 p.p.m. in early development to 1.0–1.4 p.p.m. previous to hatching.

6. Critical levels of dissolved oxygen were calculated for the present series and compared with values for chum salmon eggs from Wickett (1954). A continuing rise in critical levels is suggested from fertilization at least to a stage shortly before hatching. Until further information is available to the contrary, it is suggested that critical levels of dissolved oxygen may be regarded as a measure of oxygen requirements for successful incubation.

REFERENCES

BATTLE, H. I. 1944a. The embryology of the Atlantic salmon (*Salmo salar* Linnaeus). *Canadian J. Res.*, D, **22**(5): 105–125.

1944b. Effects of dropping on the subsequent hatching of teleostean ova. *J. Fish. Res. Bd. Canada*, **6**(3): 252–256.

BURROWS, R. E., AND D. D. PALMER. 1955. A vertical egg and fry incubator. *Progressive Fish-Culturist*, **17**(4): 147–155.

DEVILLERS, C., AND J. ROSENBERG. 1953. Les premières phases du développement de l'œuf de *Salmo irideus* en anerobiose. *Compte. rend. Acad. Sci. Paris*, **237**: 1561–1562 (cited by Smith, 1957).

FRY, F. E. J. 1947. Effects of the environment on animal activity. *Univ. Toronto Stud., Biol. Ser.*, No. 55; *Pub. Ontario Fish. Res. Lab.*, No. 68, 62 pp.

1951. A fractionating column to provide water of various dissolved oxygen content. *Canadian J. Tech.*, **29**(3): 144–146.

HAYES, F. R. 1949. The growth, general chemistry and temperature relations of salmonid eggs. *Quart. Rev. Biol.*, **24**(4): 281–308.

HAYES, F. R., I. R. WILMOT AND D. A. LIVINGSTONE. 1951. The oxygen consumption of the salmon egg in relation to development and activity. *J. Exper. Zool.*, **116**(3): 377–395.

KROGH, A. 1941. The comparative physiology of respiratory mechanisms. Univ. Pennsylvania Press, Philadelphia. 172 pp.

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LINDROTH, A. 1942. Sauerstoffverbrauch der Fische. II. Verschiedene Entwicklungs- und Altersstadien vom Lachs und Hecht. *Z. vergl. Physiol.*, **29**(4): 583–594.

1956. Salmon stripper, egg counter, and incubation. *Progressive Fish-Culturist*, **18**(4): 165–170.

NEAVE, F. 1947. Natural propagation of chum salmon in a coastal stream. *Fish. Res. Bd. Canada, Pac. Prog. Rept.*, No. 70, pp. 20–21.

SEYMOUR, A. H. MS, 1956. Effects of temperature upon young chinook salmon. Doctoral Thesis, University of Washington.

SMIRNOV, A. I. 1953. [Development of a respiratory vascular system in the skin of embryonic salmonoid fish.] *Zool. Zhurnal*, **32**(4): 787–789.

SMITH, S. 1957. Early development and hatching. *In*, The physiology of fishes. Vol. I—Metabolism. Acad. Press, New York. 447 pp.

STOCKARD, C. R. 1921. Development rate and structural expression: an experimental study of twins, "double monsters" and single deformities, and the interaction among embryonic organs during their origin and development. *Amer. J. Anat.*, **28**(2): 115–277.

WICKETT, W. P. 1954. The oxygen supply to salmon eggs in spawning beds. *J. Fish. Res. Bd. Canada*, **11**(6): 933–953.

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