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Dissolved Oxygen Requirements of Developing Steelhead Trout and Chinook Salmon Embryos at Different Water Velocities[1,2]

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ABSTRACT

Embryos of steelhead trout, *Salmo gairdneri gairdneri* Richardson, and chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), were reared from fertilization of the eggs to hatching at different constant oxygen concentrations and water velocities. For this purpose, an apparatus was developed that makes it possible to control oxygen concentration independently of water velocity, which was maintained at levels ranging from 6 to 1,350 centimeters per hour. Measurements of the embryos and hatched fry indicate that water velocities must be high enough not only to transport enough oxygen to the redd for supplying the total requirement of all embryos, but also to deliver sufficient oxygen to the surface of the chorion enveloping the individual embryo. Steelhead embryos held at 9.5° C. and chinook salmon embryos held at 11° C. all died at an oxygen concentration of 1.6 mg/l. Survival of large percentages of embryos reared at concentration as low as 2.5 mg/l was apparently made possible by reduction of respiration rates and consequent reduction of growth and development rates. Sac fry from embryos reared at low and intermediate oxygen concentrations were smaller and weaker than sac fry from embryos reared at high concentrations. Although weak sac fry may survive under laboratory conditions, they cannot be expected to do so in nature. The size of steelhead trout and chinook salmon fry at hatching probably was dependent on water velocity even at velocities as high as 740 and 1,350 cm/hr, respectively, and on oxygen concentration even at concentrations near saturation levels. Mean size differences among embryos reared under different conditions at the higher velocity and oxygen-concentration levels were not great, particularly in the case of the steelhead trout.

INTRODUCTION

Salmonid embryos and yolk-sac fry depend on water percolation through redds in streambed gravels to supply the oxygen necessary for their survival and development. In recent years there has been much concern regarding changes in composition of stream bottom materials that tend to decrease the rate of flow or the oxygen content of water in spawning gravels and thus endanger important salmonid populations. This has resulted in an increase of pertinent research effort. The attention of investigators (Shapovalov and Berrian, 1939; Shaw and Maga, 1943) was directed first to demonstrating the adverse effects of stream bottom siltation on developing embryos. Recently, field and laboratory studies have been directed more to the evaluation of oxygen concentrations and water velocities necessary for successful salmonid development. A high mortality of

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salmonid embryos has been found to occur in redds with low oxygen concentrations and associated low water velocities (Wickett, 1954). High mortalities of embryos or of sac fry occurred also when embryos in porous plastic and metal containers were buried in stream-bed gravels where water velocities and oxygen concentrations were low (Coble, 1961; Phillips and Campbell, 1962). In laboratory studies, various low oxygen levels have been shown to cause high embryo mortalities, some delay of hatching, and various developmental abnormalities (Alderdice, Wickett, and Brett, 1958; Garside, 1959), and to influence the growth of sac fry (Nikiforov, 1952).

The experiments presented in this paper were designed to increase knowledge of the environmental requirements of the embryos of steelhead trout, *Salmo gairdneri gairdneri* Richardson, and chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), and particularly to explore the interdependence of the dissolved oxygen concentrations and the water velocities necessary for satisfying the oxygen requirements of the embryos. Water movement has not only the generally recognized function of supplying sufficient oxygen to the redd for meeting the total requirement of all the embryos, but also the function of delivering oxygen to the surface of the chorion enveloping the individual embryo. Water velocities high enough to assure an adequate supply of oxygen to a redd may not always be high enough to insure adequate oxygen concentrations at the chorion surfaces. At a low water velocity, the embryos thus may not develop as expected in the presence of a moderately high mean oxygen concentration in the redd. Under natural conditions, the oxygen concentration in a redd is partly dependent on the velocity of water movement, and the role of water movement in the delivery of oxygen to the chorion enveloping each individual embryo cannot be readily examined. The apparatus employed in the experiments reported in this paper was developed to make it possible to study the influence on developing salmonid embryos of water velocity at independently controlled dissolved oxygen levels.

The effects on salmonid embryos of any restriction in respiration rate are exceedingly complex and difficult to evaluate, not only because the embryo is a continuously changing organism with changing requirements, but also because the embryo is able to reduce drastically its respiration rate and still survive by reducing its growth and development rates. With very severe respiratory restriction the embryo may succumb before hatching, but with slightly less restriction it will hatch as a very small and weak or abnormal sac fry. In the laboratory such sac fry may survive, but they cannot be expected to do so in nature. Thus, in determining the suitability of environmental conditions for the development of salmonid embryos, evaluation of the effects of nonlethal conditions on the development and growth of the embryos may be of far more importance than the determination of lethal conditions. It is for this reason that analysis of data on the growth of embryos at different oxygen concentrations and water velocities has been emphasized in this paper.

EXPERIMENTAL APPARATUS, MATERIALS, AND METHODS

Experimental apparatus

The apparatus shown in Figure 1 was designed to provide developing salmonid embryos with a constant rectilinear flow of water having independently controllable velocities and dissolved oxygen concentrations. Three transparent plastic aquaria were constructed, each of which consisted of two separate 75-liter experimental chambers (Figure 2). Thus six different dissolved oxygen concentrations could be tested during a single experiment.

Each experimental chamber was fitted with supports for holding four 9-inch-square porous plates on which the developing embryos rested. These plates were coarse-grade, ceramic filter plates, composed essentially of silica, and manufactured by Filtros Incorporated, East Rochester, New York. They were 1 inch thick and had an average porosity of 34.5 percent, which permitted rather free passage of water. During the experiments the water beneath the plates was agitated by incoming jets, but the grossly

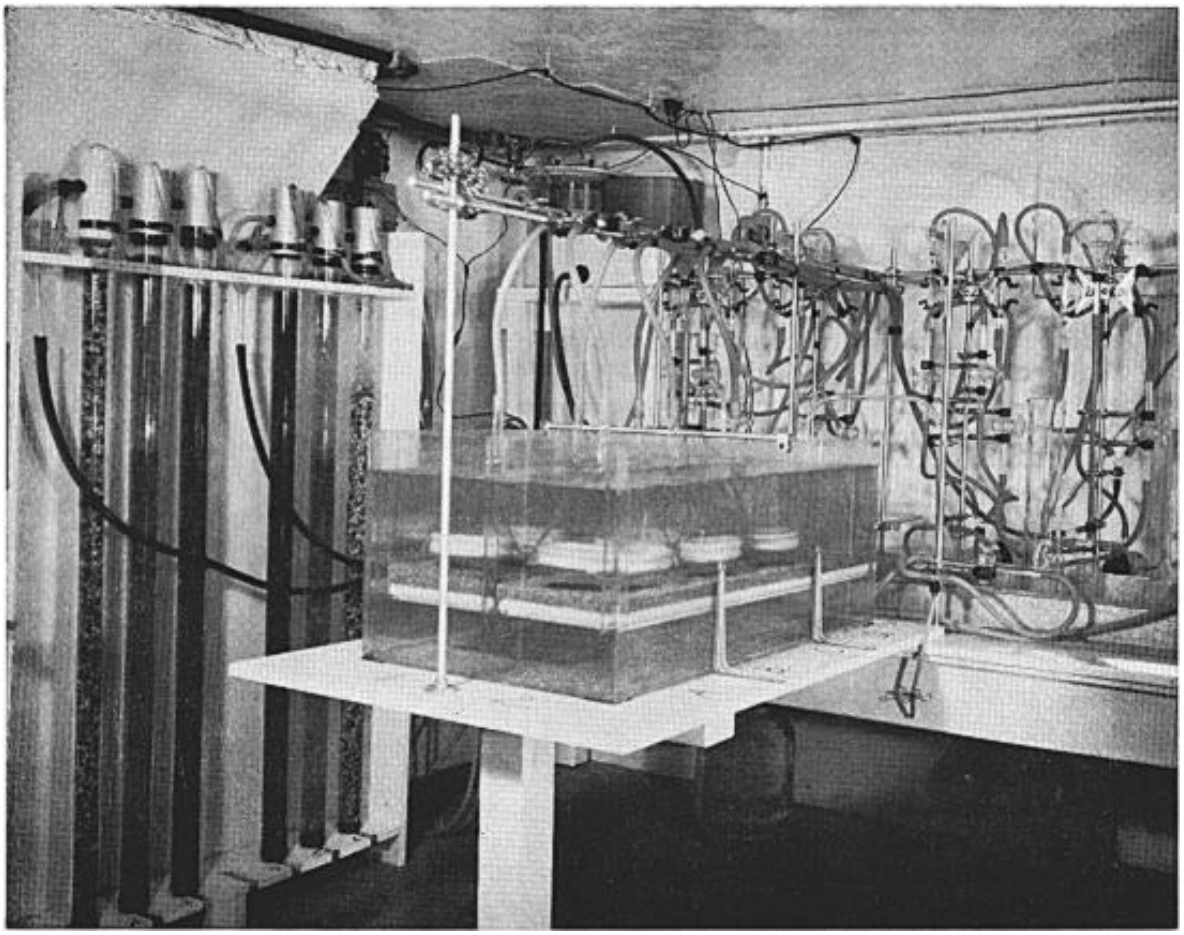

FIGURE 1.—Photograph showing one aquarium with two experimental chambers containing cylinders, and also showing oxygen-stripping columns, the water-heating system, and the flow-control devices.

turbulent water was not in contact with the developing embryos nor with the atmosphere above. The undersides of the porous plates could be reached with an implement for removal of air bubbles through narrow, elongate wells between the chamber walls and vertical baffles.

A glass cylinder 9 centimeters high and with an inside diameter of 13.8 centimeters was sealed to each porous plate. The embryos rested on the plates, inside the cylinders. A polyethylene gasket, held in place by a layer of sand, was used as a seal in the experiment with chinook salmon embryos. A paraffin coating was used to seal the cylinders to the plates for the experiment with steelhead embryos. The seals extended over the surfaces of the plates outside the cylinders for about 1½ inches, and were intended to prevent any of the water above the porous plates from flowing under the edges of the glass cylinders and disrupting the rectilinear upward flow within the cylinders. A foam-rubber gasket was attached around the outside of each cylinder at its upper edge to serve as a seal between the cylinder and an 8-inch inverted glass funnel placed over it.

A description of the course of water through the entire system will give an overall picture of the apparatus. Figure 3 shows the pattern of flow diagramatically for a single experimental chamber. Water from a small, spring-fed stream was supplied through polyethylene pipe to the constant-temperature room containing the apparatus. The water used for the chinook salmon experiment had not been filtered, whereas the water for the steelhead experiment had passed through a filtration system. The flow of water into a glass jar, which provided a constant head

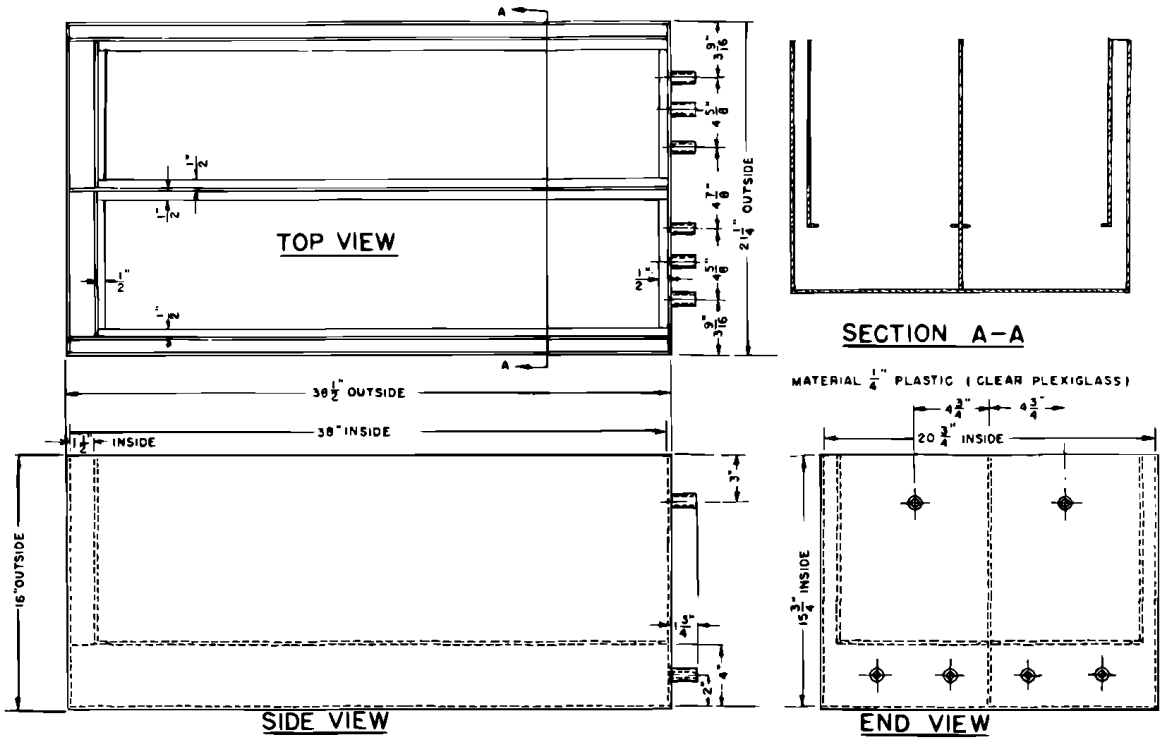

FIGURE 2.—Diagram of one aquarium, showing two experimental chambers, filter-plate supports, baffles, and water inlets and overflows.

for the experiments, was controlled with a gate valve to keep the jar overflowing at all times. The water was siphoned from the overflowing jar by means of U-shaped glass tubes to a second jar in which a thermostatically controlled, stainless-steel, immersion heater was suspended. The water then flowed to a 1-gallon distribution jar containing the heater thermostat and fitted with six outlet tubes. Water passed through these tubes to the tops of six glass columns filled with Raschig rings.

Dissolved oxygen was partially removed from the water passing down five of the columns by counterflows of nitrogen gas, which were closely controlled by two-stage pressure-reducing valves. Air was bubbled through the water in the sixth column to provide a dissolved oxygen concentration near the air-saturation level. After leaving the columns, the water passed through 8-millimeter stopcocks for flow-rate control, then into the experimental aquaria and out through overflow tubes. The water in each aquarium chamber was renewed at a rate of approximately 600 milliliters per minute.

Small centrifugal pumps with synthetic rubber housings and impellers were employed to draw water from beneath the plates past the embryos inside the glass cylinders, through the funnels, through flowmeters, and then through rubber tubing to the pump intakes. The amount of water flowing past the embryos in each cylinder was regulated by means of 8-millimeter stopcocks installed in the pump intake and output lines. After leaving a pump, the water entered a 5-gallon bottle in which air bubbles were trapped and from which the air could be removed periodically by means of relief lines. From the air trap, the water was reintroduced into the experimental chambers beneath the porous plates.

The flowmeters used were of the ball-displacement type and measured the volumes of water passing by the embryos per unit of time. In order to achieve the highest velocities tested in the chinook salmon experiment, it was necessary to install bypass tubes in combination with some of the flowmeters (Figure 3). The flows thus obtained were more than double those obtainable with the flowmeters alone. Each flowmeter so used

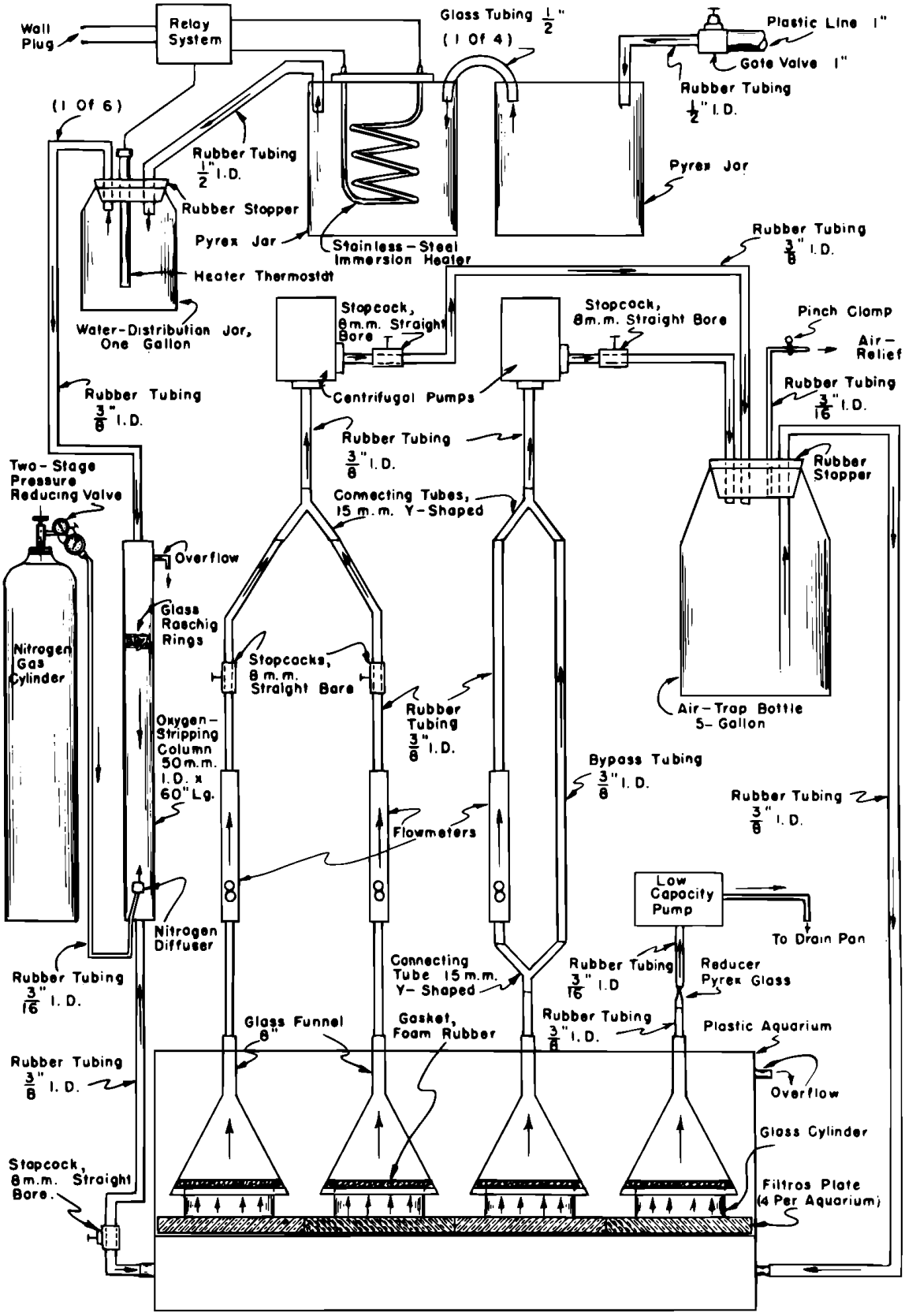

FIGURE 3.—Diagram of apparatus, showing one experimental chamber containing four cylinders for embryos held at different water velocities, flow- and oxygen-control devices, heating system, and pattern of water flow.

was recalibrated with its respective bypass.

Two centrifugal pumps were used for each experimental chamber. One pump was used to maintain the highest experimental velocity, and the two lower velocities were maintained by the second centrifugal pump. Each of two delivery lines to this pump contained a flowmeter and a stopcock for flow-rate regulation. The very low rate of water flow necessary for maintaining the lowest water velocity in the steelhead experiment was produced by means of a low-capacity chemical pump which delivered water from the experimental cylinders either to a graduated cylinder for flow measurement, or to a drain pan. This water was not returned to the experimental aquarium.

Experimenatl material

The chinook salmon eggs were obtained on October 7, 1958, from a single fall-run female at the Oregon State Fish Commission's Oxbow Hatchery on the Columbia River. The steelhead eggs were obtained on February 9, 1959, from a single winter-run female at the Oregon State Game Commission's hatchery on the North Fork of the Alsea River. The eggs were fertilized and allowed to become water-hardened before being transported to the laboratory. The water in which the eggs had been transported was brought to the experimental temperature gradually, over a period of about 6 hours, before transfer of the eggs to the experimental apparatus.

Experimental methods

Renewal and recirculation water flows were started several days before each experiment. This allowed time for trapped air to be removed from the apparatus. The room was kept at a temperature several degrees below the temperature of the incoming fresh water to minimize formation of bubbles in the circulating water. Oxygen concentrations were not brought to the desired levels until after the eggs had been placed in the experimental chambers.

For the chinook salmon experiment, 140 eggs were placed inside each of three cylinders in every experimental chamber. The eggs in each cylinder were divided into two groups, one containing 60 and the other 80 eggs. The group of 60 was used to determine hatching success, and the group of 80 was used for four periodic samples before hatching. For the steelhead experiment, 60 eggs were placed inside each glass cylinder. Each cylinder in this experiment contained an egg separator, which was a ring made of a stainless-steel band strung with nylon filament to form a grid. The nylon strands kept the eggs apart and well distributed. Twenty eggs in each cylinder were designated as a sample to be taken before hatching for examination and measurement, and 40 were used to determine hatching success.

Experimental data were recorded and adjustments of the apparatus were made three times daily: between 7:30 and 9:30 a.m., between 1:00 and 4:00 p.m., and between 9:00 p.m. and midnight. Flowmeter readings and water temperatures were recorded at these times, as well as the numbers of dead embryos and numbers of hatched fry. Water samples also were siphoned from the experimental chambers for dissolved oxygen determinations. After the samples had been taken and data recorded, any necessary adjustments of the apparatus were made. Air that had accumulated at various points in the tubing was forced through the system and then removed from the air-trap bottles by means of relief lines provided. Air that had collected beneath the plates was removed with an L-shaped squeegee, which was inserted between the sides and the baffles of the chambers. After the removal of air, the desired flows of water and oxygen concentrations were obtained by appropriate manipulation of the stopcocks and valves. Water velocities in the cylinders tended to decrease during each period between adjustments. Flowmeter readings made both before and after each adjustment were considered in computing mean water velocities. Water temperatures were taken with carefully calibrated thermometers having 0.1° C. graduations.

Dissolved oxygen determinations were made by the Alsterberg (azide) modification of the Winkler method. Microburettes were used for titrations. Some of the oxygen concentrations reported here have been corrected to adjust for differences between the concentration at the sampling point and the concentra-

tions in the different cylinders. Correction factors were determined through a series of comparative observations. Water samples were at first taken from above the porous plates outside the cylinders; later it was found that the oxygen concentrations in the water beneath the plates, where subsequent samples were taken, were somewhat lower. In the steelhead experiment the dissolved oxygen concentrations in the cylinders with the lowest velocity were slightly lower than those found beneath the plates. Apparently this condition was caused mostly by bacterial respiration within the filter plates, for comparative tests made with and without embryos indicated that oxygen-concentration decreases due to embryo respiration were negligible. No appreciable decreases of oxygen concentration occurred at the higher velocities.

Since the water used for the chinook salmon experiment was not filtered, it was necessary periodically to remove as much deposited silt as possible from the plates and embryos. Silt deposited in the plates caused some progressive reduction and disruption of flow. Silt presented no problem during the steelhead experiment because a water filter was then in operation.

Dead eggs or embryos were not removed until the developing embryos had reached the eyed stage. From this time on, dead embryos were removed as they occurred.

Length measurements of embryos were made at various times after fertilization. Volume as well as length measurements of a sample of fry from each cylinder were made at the time of complete or nearly complete hatching. Inasmuch as complete hatching occurred at different times in the cylinders with different dissolved oxygen concentrations and water velocities, the samples of fry were not all taken simultaneously. In the steelhead experiment, a second sample of fry was taken from each cylinder for length and volume measurements 17 days after complete hatching had occurred in that cylinder. No measurements of chinook salmon fry were made at a corresponding time.

The lengths of embryos and fry were measured to the nearest tenth of a millimeter with a pair of dividers. Mean fry volume was determined by measuring the volume of water displaced in either a 25- or a 50-milliliter graduated cylinder by an entire sample of fry, after removal of yolk sacs. Water was first removed from the fry by placing them on paper towels. All length and volume measurements of fry were made with fresh (not preserved) material.

Samples of 20 embryos each were used to determine mean lengths of chinook salmon embryos at various stages of development, and 10 fry were measured at hatching. Samples of approximately 20 each were used to determine the mean lengths of steelhead embryos 30 days after fertilization and to determine the mean lengths of the newly hatched fry. Volume measurements were made of samples of approximately 30 chinook salmon and 20 steelhead trout fry. Mean lengths and volumes of steelhead fry 17 days after hatching were determined with samples of 9 to 15 fry.

RESULTS

Steelhead trout

In the experiment with steelhead trout embryos, information was obtained on the influence of oxygen concentration and water velocity on the number of days required for hatching, percent survival, incidence of abnormal development, size of embryos 30 days after fertilization, and size of newly hatched and 17-day-old fry. Groups of embryos were reared throughout development at water velocities of about 6, 34, 150, and 740 cm/hr at each of six oxygen concentrations of about 1.6, 2.6, 4.2, 5.7, 7.9, and 11.2 mg/l. Mean temperatures were about 9.5° C. Table 1 gives the conditions in the different chambers and cylinders.

It is believed that the reported mean values of water velocity and dissolved oxygen concentration for the steelhead trout experiment are much more reliable than those for the chinook salmon experiment, in which siltation of the porous plates occurred. The 60 steelhead eggs within each cylinder covered about 13 percent of the cross-sectional area of the cylinder; the true water velocities around the eggs thus were somewhat higher than the reported values. As samples of embryos were taken from each cylinder, the true velocities

TABLE 1.—*Means and ranges of water temperature, velocity, and oxygen concentration for the experiment with steelhead trout embryos*

Experimental chamber number	Temperature (°C.)		Cylinder	Dissolved oxygen (mg/l)		Velocity (cm/hr)	
	Mean	Range		Mean	Range	Mean	Range[1]
1	9.5	7.5–10.0	A	7.9	7.4–9.0	36	23–80
			B	7.9	7.4–9.0	150	100–320
			C	7.9	7.4–9.0	740	700–750
			D	7.7	7.2–8.8	6	6.0–6.8
2	9.5	8.0–10.0	A	2.6	2.1–3.2	34	29–51
			B	2.6	2.1–3.2	150	120–180
			C	2.6	2.1–3.2	750	700–760
			D	2.5	2.0–3.1	6	5.6–6.0
3	9.3	7.8–10.1	A	5.7	5.0–6.9	33	29–39
			B	5.7	5.0–6.9	150	130–190
			C	5.7	5.0–6.9	650	500–750
			D	5.5	4.8–6.7	6	5.6–6.4
4	9.3	7.9–10.0	A	1.6	1.0–3.6	34	31–80
			B	1.6	1.0–3.6	150	94–160
			C	1.6	1.0–3.6	750	720–780
			D	1.4	0.8–3.4	6	0.4–7.6
5	9.7	8.2–10.4	A	4.2	3.6–10.4	36	31–64
			B	4.2	3.6–10.4	140	88–160
			C	4.2	3.6–10.4	740	670–810
			D	4.0	3.4–10.2	6	5.2–6.6
6	9.5	7.6–10.1	A	11.2	10.5–12.0	32	26–48
			B	11.2	10.5–12.0	150	120–160
			C	11.2	10.5–12.0	720	580–760
			D	11.0	10.3–11.8	6	5.9–6.5

[1] Power failure 7 days after experiment began reduced all velocities to zero for 12 hours.

more closely approached the reported values.

Time of hatching, reported as the number of days from fertilization to initial, median (50 percent), and final hatching, and percentages of successful hatching are given in Table 2. Dissolved oxygen concentration is expressed as a mean value for each experimental chamber. Mean values for the cylinders are given in Table 1. The water velocities shown in Table 2 are means for the individual cylinders.

In the experiment with steelhead embryos, hatching success was clearly affected only at the lowest dissolved oxygen concentration tested, and apparently it was not influenced by the water velocities tested. Hatching success at a mean oxygen concentration of 2.6 mg/l ranged from 78.0 to 85.0 percent, and at higher concentrations ranged from 66.7 to 87.5 percent, whereas total mortality occurred at the 1.6 mg/l mean level. Two embryos began to hatch at the latter level, but they were greatly deformed and did not survive to complete the hatching process.

Hatching was delayed at moderately reduced levels of dissolved oxygen and water velocity. The median hatching times of fry reared at various water velocities at oxygen concentrations averaging 2.6 mg/l were 5 to 8 days longer than those of fry reared at concentrations near 11.2 mg/l. A smaller delay of hatching occurred at mean concentrations

TABLE 2.—*Time in days from fertilization to initial, median, and final hatching, and percent of hatching of steelhead trout embryos*

Dissolved oxygen (mg/l)[1]	Velocity (cm/hr)	Time to hatching (days)			Percentage hatching
		Initial	Median	Final	
1.6	6	—	—	—	0
	34	—	—	—	0
	150	—	—	—	0
	750	—	—	—	0
2.6	6	42	44	46	79.5
	34	41	43	44	85.0
	150	41	42	43	78.0
	750	40	41	43	79.5
4.2	6	37	38	40	80.0
	36	37	38	39	82.5
	140	36	37	39	80.0
	740	36	37	39	82.5
5.7	6	37	39	39	87.5
	33	36	37	40	84.2
	150	36	37	38	82.5
	650	36	37	38	85.0
7.9	6	35	36	37	72.5
	36	35	36	36	87.2
	150	35	36	37	85.4
	740	35	35	36	66.7
11.2	6	35	36	37	77.5
	32	35	36	36	85.0
	150	34	36	36	80.1
	720	35	36	36	72.5

[1] Mean dissolved oxygen concentration for each cylinder is given in Table 1.

TABLE 3.—*Mean lengths and volumes of steelhead trout embryos and fry reared at different oxygen concentrations and water velocities and sampled at various times*

Velocity (cm/hr)[1]	Dissolved oxygen concentration (mg/l)[2] 1 6	2.6	4.2	5.7	7.9	11.2
	Length in millimeters 30 days after fertilization					
6	4.2	9.6	12.2	13.2	14.4	15.9
34	5.1	10.3	13.3	14.0	15.1	15.5
150	5.6	10.7	13.5	14.2	15.3	15.9
740[3]	5.6	10.7	13.8	14.4	15.5	15.8
	Length in millimeters at hatching[4]					
6	—	15.2	16.8	17.5	18.5	19.4
34	—	15.3	17.4	18.1	19.1	19.9
150	—	16.0	17.9	18.9	19.3	19.7
740[3]	—	16.5	18.6	19.2	19.6	20.0
	Volume in milliliters at hatching[4, 5]					
6	—	0.010	0.015	0.021	0.024	0.029
34	—	0.015	0.020	0.023	0.025	0.030
150	—	0.015	0.021	0.030	—	0.030
740[3]	—	0.015	0.028	0.028	0.030	0.030
	Volume in milliliters 17 days after hatching[5]					
6	—	—	0.070	0.087	—	—
34	—	0.046	0.082	0.100	0.110	0.118
150	—	0.055	0.100	0.120	0.129	0.122
740[3]	—	0.066	0.110	0.108	—	0.133

[1] Indicated as means calculated from the mean velocities for all experimental chambers.
[2] Mean dissolved oxygen concentration for each cylinder is given in Table 1.
[3] Velocity of 650 cm/hr at the dissolved oxygen concentration of 5.7 mg/l was not included in calculating the mean.
[4] Abnormal fry not included.
[5] Volumes of fry with yolk sacs removed.

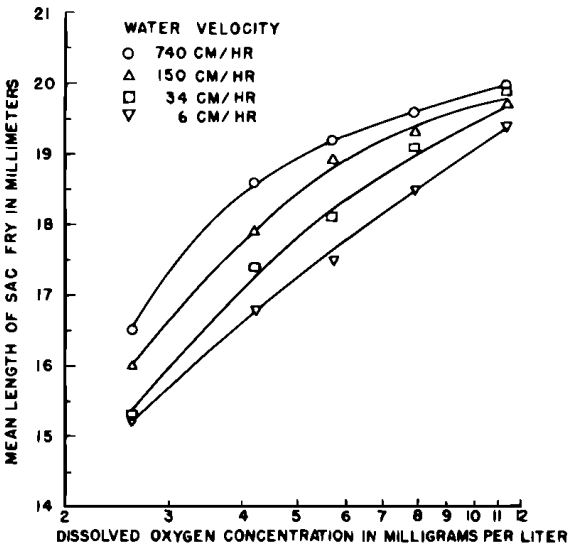

FIGURE 4.—Relationships between mean lengths of steelhead trout sac fry when hatched and dissolved oxygen concentrations at which the embryos were reared, at different water velocities and at 9.5° C.

of 4.2 and 5.7 mg/l, but none was apparent at 7.9 mg/l. The median hatching time of fry reared at the lowest velocity and at about 2.6 mg/l dissolved oxygen was 3 days longer than that of fry reared at the highest velocity and at virtually the same oxygen concentration. At the lower velocities, hatching was delayed at each oxygen concentration up to and including 5.7 mg/l.

Table 3 presents first the mean lengths of steelhead embryos, based on samples of 20 embryos each taken 30 days after fertilization. The size of these embryos increased with increases of oxygen concentration and of water velocity up to 11.2 mg/l and 740 cm/hr, respectively, which were the highest levels tested. Table 3 presents also the mean lengths and volumes of steelhead fry at hatching. Means for each set of conditions are based on samples of 20 fry. Each individual fry was measured for length, whereas only the total volume of all 20 in a sample was measured. The length means can be seen to fall in very orderly sequences, whereas the volume measurements do not, and the latter fact suggests poor sensitivity and low precision of the volume measurements. The ranges of individual lengths of fry in different samples overlapped considerably, but the length means show that the size of steelhead trout fry at hatching is dependent on oxygen concentration even at high concentrations near the air-saturation level. The size is seen to be dependent also on water velocity, even at velocities up to about 740 cm/hr or more, with the possible exception of fry produced at a very high oxygen concentration (11.2 mg/l) near the air-saturation level.

Figures 4 and 5 present sets of curves, fitted by eye, showing the relationships between the mean lengths of steelhead fry at hatching and the dissolved oxygen concentrations and water velocities, respectively. It can be seen that the difference in water velocity between 6 and 740 cm/hr has less influence than the difference in oxygen concentration between 2.6 and 11.2 mg/l, and that the influence of velocity is greatest at intermediate or moderately reduced oxygen concentrations.

The volume measurements in Table 3 show much better than do the length measurements how much more poorly developed are hatching fry at low concentrations and velocities than hatching fry at high concentrations and

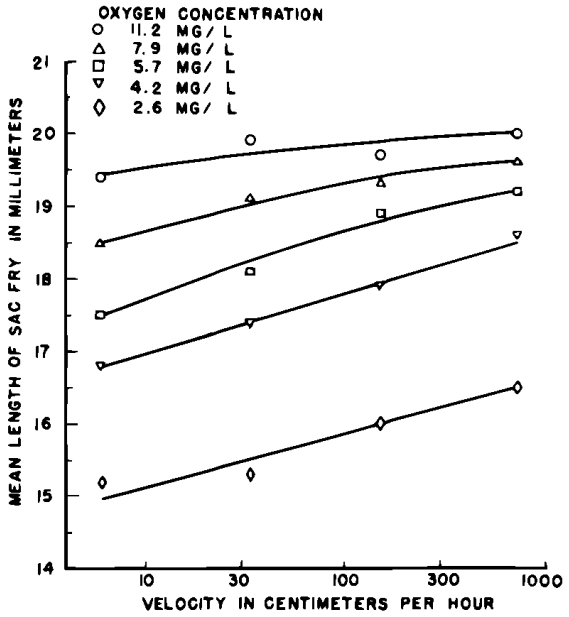

FIGURE 5.—Relationships between mean lengths of steelhead trout sac fry when hatched and water velocities at which the embryos were reared, at different dissolved oxygen concentrations and at 9.5° C.

velocities. The relatively crude volume measurements do not show so well the rather small differences between hatching fry reared under different conditions at the higher oxygen-concentration and velocity levels. The volumetric data, however, again show that the size of fry produced at the intermediate oxygen concentrations was most markedly influenced by water velocity. At oxygen concentrations near the air-saturation level, steelhead trout embryos apparently can attain nearly maximum hatching size at any water velocity above 6 cm/hr.

In each experimental chamber, the mean dissolved oxygen concentration of water having the lowest velocity tested was lower by 0.1 to 0.2 mg/l than that of water having higher velocities (Table 1). A small part of the reduction in size of fry at the low velocity may be due to this. How small this oxygen-concentration effect would be in relation to the influence of velocity differences can be determined from Figure 4.

Abnormal structural development of steelhead trout embryos was observed only at the two lowest levels of dissolved oxygen. At 1.6 mg/l, all embryos exhibited abnormal development, and none survived through the hatching stage. At 2.6 mg/l, the following percentages of abnormal development were observed at the velocities indicated: 9.4 percent at 6 cm/hr, 24.4 percent at 34 cm/hr, 19.1 percent at 150 cm/hr, and 14.9 percent at 750 cm/hr. Abnormal development was evidenced in these cases by twisted or deformed tails or backs and abnormal structure of the nervous system, especially the brain. Most of the deformed embryos reared at the 2.6 mg/l dissolved oxygen level survived through the hatching stage.

Determination of posthatching survival was not satisfactory with steelhead fry. Some of the fry were found to have ruptured yolk sacs. This unusual affliction and consequent deaths could not be ascribed to any environmental conditions being tested, such as reduced oxygen concentrations.

Table 3 presents lastly the volume measurements of fry made 17 days after hatching. The fry were held at the oxygen concentrations and water velocities at which they had been reared as embryos. The samples (9 to 15 fry each) used for these measurements were small, partly because of the aforementioned deaths. Nevertheless, the data clearly show that differences in size of fry due to oxygen-concentration and water-velocity differences persist well beyond the time of hatching. Some of the size difference noted 17 days after hatching was probably due to continued impairment of growth and development at the lower oxygen concentrations.

Chinook salmon

In the experiment with chinook salmon embryos, information was obtained as to the influence of oxygen concentration and water velocity on the number of days to hatching, percent survival, incidence of abnormal development, size of embryos at different stages, size of newly hatched sac fry, and posthatching survival. Groups of embryos were reared throughout development at water velocities of about 92, 580, and 1,350 cm/hr at each of six oxygen concentrations, about 1.6, 2.5, 3.9, 5.6, 8.0, and 11.7 mg/l. Mean temperatures were about 11° C. Table 4 gives the means and ranges of temperatures, dissolved

TABLE 4.—*Means and ranges of water temperature, velocity, and oxygen concentration for the experiment with chinook salmon embryos*

Experimental chamber number	Temperature (°C.)		Cylinder	Dissolved oxygen (mg/l)		Velocity (cm/hr)	
	Mean	Range		Mean	Range	Mean	Range[1]
1	11.3	9.3–13.0	A	8.0	6.8–9.6	97	82–130
			B			580	560–650
			C			1,360	1,130–1,460
2	11.2	9.2–13.0	A	1.6	0.6–5.1	82	20–350
			B			570	330–800
			C			1,310	920–1,600
3	11.1	9.5–13.1	A	5.6	3.4–6.5	97	54–110
			B			580	540–610
			C			1,370	1,000–1,440
4	11.0	9.3–13.5	A	2.5	0.9–4.3	88	20–360
			B			590	260–780
			C			1,330	1,040–1,500
5	11.3	9.9–13.7	A	3.9	2.4–5.9	94	42–350
			B			580	350–680
			C			1,240	850–1,500
6	11.4	10.0–13.8	A	11.7	10.0–13.2	94	30–110
			B			580	440–760
			C			1,360	1,030–1,510

[1] Power failure 8 days after experiment began reduced all velocities to zero for 3 hours.

oxygen concentrations, and water velocities in the different chambers and cylinders in this experiment.

Difficulties encountered during this experiment render questionable the accuracy of the reported mean values of water velocity at which the chinook salmon embryos were reared. Because the water filtration system was not completed in time for use in this experiment, silt was deposited in the porous plates. The occlusion of the pores may not have been uniform, which may have resulted in some embryos being subjected to greater water velocities than others in the same cylinder. The polyethylene gaskets used as seals under and around the cylinders did not prove quite satisfactory. Passage of water under the edges of the cylinders, which became quite evident after hatching of the fry, could have resulted in grossly turbulent flows of water around the embryos and inaccurate determinations of the velocities. The 140 chinook salmon eggs within each cylinder covered about 49 percent of the total area of the porous plate on which the eggs rested; this tended to make the actual initial water velocities around the eggs greater than the reported velocities. Removal of samples of embryos for examination and measurement during the experiment reduced this source of error.

If water from above the plates outside the cylinders did pass under the edges of the cylinders and come in contact with the developing embryos, then the reported dissolved oxygen concentrations may not be quite accurate. Tests indicated that the water above the plates outside the cylinders had a slightly higher dissolved oxygen concentration than the water below the plates. Although the chinook salmon experiment thus was somewhat defective, the observed relationships between oxygen concentration, water velocity, and chinook salmon embryonic development reported in this paper are considered to be generally valid, inasmuch as the results of the steelhead trout experiment presented herein and of subsequent experiments with both steelhead trout and coho salmon indicate generally similar relationships.

Table 5 summarizes the data on hatching success and time of hatching. Time of hatching is reported as the number of days from fertilization to initial, median (50 percent), and final hatching.

Although differences in water velocity had no apparent effect on hatching success of chinook salmon embryos, hatching was affected by the lowest dissolved oxygen concentration tested. Total mortality of the embryos occurred at a dissolved oxygen concentration of 1.6 mg/l at all water veloc-

TABLE 5.—*Time in days from fertilization to initial, median, and final hatching, and percent of hatching of chinook salmon embryos at various dissolved oxygen concentrations and water velocities*

Dissolved oxygen (mg/l)[1]	Velocity (cm/hr)	Time of hatching (days)			Percentage hatching
		Initial	Median	Final	
1.6	82	—	—	—	0
	570	—	—	—	0
	1,310	—	—	—	0
2.5	88	48	51	54	95.1
	590	48	51	52	98.3
	1,330	48	50	52	96.7
3.9	94	45	46	48	100
	580	44	46	48	100
	1,240	45	46	48	100
5.6	97	45	46	47	96.7
	580	45	46	47	98.4
	1,370	45	46	47	93.1
8.0	97	43	44	45	98.3
	580	44	44	45	98.3
	1,360	42	44	45	95.0
11.7	94	42	43	45	100
	580	42	44	45	92.3
	1,360	42	43	45	98.2

[1] Mean dissolved oxygen concentration for each cylinder is given in Table 4.

ities tested. The percentages of hatching were high, ranging from 92.3 to 100 percent, at all tested concentrations from 2.5 to 11.7 mg/l, and at all the water velocities.

Increasing delay of hatching occurred with progressive decreases of dissolved oxygen concentration, but decreases of water velocity caused little or no delay. Embryos reared at all dissolved oxygen concentrations lower than the control level, 11.7 mg/l, exhibited a delay, which was greatest at the concentration of 2.5 mg/l. The embryos reared at this oxygen level, as compared with those reared at the control level, showed a delay of 6 days for initial hatching, 7 to 8 days for median hatching, and 7 to 9 days for final hatching. Water temperatures in all experimental chambers were not exactly equal (Table 4), which could partly account for the difference in time of hatching. However, mean water temperatures at the dissolved oxygen concentrations of 3.9 and 8.0 mg/l were equal, and a difference of about 2 days in hatching time between these two concentrations was evident.

Table 6 presents the mean lengths of chinook salmon embryos sampled 14, 24, 35, and 41 days after fertilization. Embryos reared at the high oxygen concentrations were the largest at all sampling times. Embryos reared

TABLE 6.—*Mean lengths in millimeters of chinook salmon embryos at various times during development and at various dissolved-oxygen concentrations and water velocities*

Velocity (cm/hr)[1]	Days after fertilization	Dissolved oxygen concentration (mg/l)					
		1.6	2.5	3.9	5.6	8.0	11.7
92	14	2.5	5.3	6.0	5.6	6.0	6.3
	24	3.2	10.0	11.7	12.0	12.3	14.3
	35	3.7	13.4	15.5	16.0	17.2	19.6
	41	4.1	14.3	16.7	18.2	19.9	21.3
580	14	3.0	5.3	6.1	5.9	6.2	6.3
	24	3.9	8.9	12.0	11.9	12.0	14.4
	35	4.2	13.6	15.6	15.8	17.8	20.0
	41	4.7	14.9	17.5	18.8	20.3	21.6
1,350[2]	14	3.0	5.5	6.1	5.5	6.2	6.3
	24	4.1	10.4	12.3	12.0	12.2	14.5
	35	4.7	14.0	15.9	16.3	14.8	19.9
	41	5.2	14.9	17.5	18.8	20.2	22.2

[1] Indicated as means calculated from the mean velocities for all experimental chambers.
[2] Velocity of 1,240 cm/hr at the dissolved oxygen concentration of 3.9 mg/l was not included in calculating the mean.

at high water velocities also tended to be larger at all sampling times than those reared at low velocities when oxygen concentration was the same, though the effect of velocity was not as marked as that of oxygen concentration. Although these data are quite variable, particularly those for the 14th day, they suggest that the size of chinook salmon embryos 14 or more days after fertilization is dependent on oxygen concentration even at concentrations near the air-saturation level, and perhaps at higher concentrations.

Table 7 presents mean lengths and volumes of chinook salmon fry at hatching. The volume measurements once more show much

TABLE 7.—*Mean lengths and volumes of chinook salmon fry at hatching at various dissolved oxygen concentrations and water velocities*

Velocity (cm/hr)[1]	Dissolved oxygen concentration (mg/l)					
	1.6	2.5	3.9	5.6	8.0	11.7
	Length in millimeters[2]					
92	—	18.7	20.0	21.6	22.5	23.6
580	—	19.4	21.0	22.4	22.9	23.9
1,350[3]	—	19.7	21.2	22.5	23.2	24.8
	Volume in milliliters[2, 4]					
92	—	0.017	0.033	0.042	0.053	—
580	—	0.020	0.037	0.050	0.066	0.066
1,350	—	0.033	0.043	0.050	0.066	0.073

[1] Indicated as mean velocity calculated from mean velocities for all experimental chambers, and expressed to nearest cm/hr.
[2] Abnormal fry not included.
[3] Velocity of 1,240 cm/hr at the dissolved oxygen concentration of 3.9 mg/l was not included in calculating the mean.
[4] Volumes of fry with yolk sacs removed.

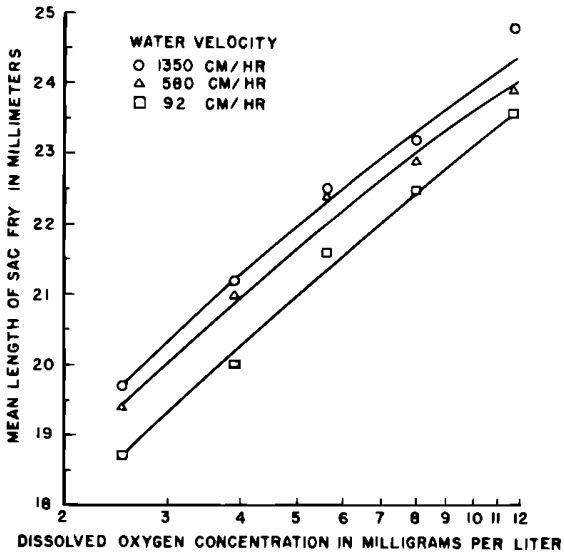

FIGURE 6.—Relationships between mean lengths of chinook salmon sac fry at hatching and dissolved oxygen concentrations at which the embryos were reared, at different water velocities and at 11° C.

better than do length measurements how much more poorly developed are fry reared at low oxygen concentrations and water velocities than fry reared at high concentrations and velocities. However, for reasons discussed in connection with the steelhead data given in Table 3, the length measurements show better than the volume measurements the rather small differences between fry reared under different conditions at the higher oxygen-concentration and water-velocity levels. The mean lengths of chinook salmon fry at hatching (Table 7) show that size is dependent on oxygen concentration at all levels up to a level near 11.7 mg/l, and is dependent on water velocity probably at all the tested velocities in the presence of any tested oxygen concentration.

Figure 6 presents a set of curves showing the relationships between the mean lengths of chinook salmon fry at hatching and dissolved oxygen concentration at water velocities of 92, 580, and 1,350 cm/hr. These curves suggest that an increase of oxygen concentration beyond 11.7 mg/l, which is above the air-saturation level, probably would result in some increase in size of the fry at all water velocities tested. Curves in Figure 7 show the relationships between the mean lengths of the fry and the water velocities at the different oxygen concentrations. These curves indicate that, even at oxygen concentrations near the air-saturation level, increases in water velocity beyond 1,350 cm/hr would probably result in larger embryos and fry.

External examination of chinook salmon embryos revealed abnormal structural development only at the lowest dissolved oxygen concentration tested. Nearly all embryos reared at a concentration of 1.6 mg/l exhibited grossly abnormal development at the three levels of water velocity tested.

Posthatching survival was depressed by reduced oxygen concentration only at the 2.5 mg/l level, the lowest concentration at which hatching occurred. Water velocity also seemed to influence posthatching survival at this concentration. Mortalities within 7 days after hatching at an oxygen concentration of 2.5 mg/l and at velocities of 88, 590, and 1,330 cm/hr were 29.3, 23.7, and 8.5 percent, respectively. The percentages of posthatching mortality at other levels of dissolved oxygen ranged from 6.9 to 0.0 percent. It should be noted that, at the oxygen concentration of 2.5 mg/l, the mean volumes of the fry at hatching were 0.017, 0.020, and 0.033 milliliter at the lowest, intermediate, and highest tested velocities, respectively (Table 7). The higher posthatching mortalities of fry

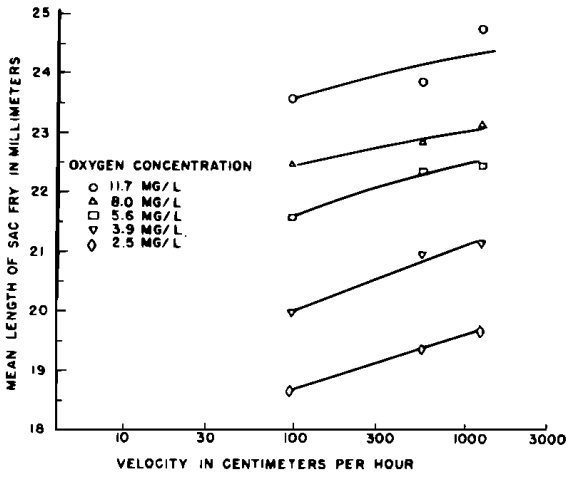

FIGURE 7.—Relationships between mean lengths of chinook salmon sac fry when hatched and water velocities at which the embryos were reared, at different dissolved oxygen concentrations and at 11° C.

that were reared at the lower velocities likely were related to the smaller initial size and weaker condition of the fry.

DISCUSSION

In the studies reported here, steelhead trout and chinook salmon were reared under laboratory conditions from fertilization of the eggs to hatching or longer at different constant oxygen concentrations and water velocities. The apparatus employed was designed to provide nearly rectilinear flows of water with oxygen concentration adjustable independently of flow or water velocity. The effects of oxygen concentration and water velocity, both of which can influence the availability of oxygen at the chorion surfaces, could thus be studied independently. The oxygen concentration in a natural redd is not independent of the velocity of water movement. Likewise, when large numbers of salmonid embryos are reared in glass columns in the laboratory, oxygen reduction occurs due to respiration, the amount of reduction depending in part on the volume of water supplied per unit of time, and hence depending also on the water velocity. In the experiments reported here, oxygen was removed from the water by means of nitrogen, and practically no reduction of dissolved oxygen due to respiration occurred. Reduction in oxygen concentration and in water velocity each resulted in a longer development period to hatching, in smaller embryos at various sampling times and at hatching, in higher prehatching and posthatching mortalities, and in increased occurrence of structurally abnormal embryos.

Alderdice, Wickett, and Brett (1958) exposed embryo chum salmon, *Oncorhynchus keta*, at different developmental stages to various constant levels of dissolved oxygen for 7-day periods, returning the embryos to high levels for the completion of development. In nature, salmonid embryos sometimes must be exposed to low oxygen concentrations only for such short periods of time, but exposures to low oxygen concentrations doubtless can be much more prolonged. The embryo chum salmon at different developmental stages were able to survive 7-day exposures to concentrations ranging from about 0.7 to about 1.8 mg/l and were able thereafter to complete development to hatching. These concentrations are much lower than the concentrations that have been shown in the present paper to permit the development of large and vigorous chinook salmon fry when exposure of the embryos to the reduced concentrations is continuous. Very small and weak sac fry are not likely to survive under natural conditions. The survival of salmonid embryos for a period of time at concentrations that are not suitable for normal growth and development is apparently made possible by a considerable reduction in growth rate and some reduction in development rate, whereby the embryo is kept at a size and stage at which its total oxygen requirement is relatively low.

Unpublished data at Oregon State University show some delay of the attainment of various developmental stages at reduced oxygen concentrations, though developmental rate tends to be much more fixed than growth rate. Alderdice, Wickett, and Brett (1958) demonstrated a delay in hatching time resulting from exposure of embryos at most developmental stages to low concentrations of oxygen, but premature hatching was caused by exposure of almost fully developed embryos to low oxygen concentrations.

For various stages of chum salmon development, Alderdice, Wickett, and Brett (1958) calculated "critical oxygen levels," or theoretically determined levels at which respiratory demand is just satisfied at 10° C. These are carefully distinguished from "limiting levels" determinable experimentally. The critical levels calculated by these workers increased curvilinearly with age throughout development to hatching from about 1 to about 7 mg/l. Hayes, Wilmot, and Livingstone (1951) reported correspondingly low, experimentally determined limiting levels for Atlantic salmon, *Salmo salar*, at 10° C., but their method, involving relatively short-term measurements of oxygen consumption rates, may not be sufficiently precise or sensitive for the detection of very small differences of consumption rates.

In the present paper, data on the length of embryo chinook salmon 14, 24, 35, and

41 days after fertilization (Table 6) show that, with any water velocity tested, the growth of these embryos was restricted before the 24th day at all tested oxygen concentrations below 11.7 mg/l. The data for the 14th day are too variable to be conclusive, but suggest that growth may be restricted before the 14th day at oxygen concentrations below the saturation level. Data presented here on the length of steelhead trout embryos 30 days after fertilization (Table 3) show that growth is restricted before this time at all tested oxygen concentrations below 11.2 mg/l. Unpublished data at Oregon State University show that the growth of embryo coho salmon (*Oncorhynchus kisutch*) at 11° C. is restricted before the 7th day after fertilization at concentrations at least as high as 6 mg/l, and before the 28th day at concentrations slightly below 11.9 mg/l. Other data show that the growth of embryo steelhead trout at 12.5° C. is restricted before the 11th day at concentrations slightly below 10.4 mg/l. It can be concluded that the critical oxygen levels calculated by Alderdice, Wickett, and Brett (1958) are far below the limiting oxygen levels for salmonid embryos throughout most of development at temperatures of 10 to 12.5° C.

The great discrepancies between the calculated values obtained for critical dissolved oxygen levels for chum salmon and our experimentally determined minimal values for corresponding limiting levels for other salmonids perhaps are ascribable mostly to impropriety of the models used in calculating the critical levels, models proposed and used for different purposes by Harvey (1928) and Krogh (1941). Alderdice, Wickett, and Brett (1958) represent the respiring "egg" before establishment of blood circulation as a spherical, homogeneous body in which oxygen is used at the same rate throughout its mass and which has an oxygen concentration of zero at the center. The limiting respiratory surface is taken to be the entire periphery. Before blood circulation develops, a period when an embryo's total oxygen requirement is low, the limiting respiratory surface may be only some overlying portion of the chorion, although it probably is the surface of the embryo itself.

Alderdice, Wickett, and Brett (1958) represent the respiring "egg" after the establishment of blood circulation as a spherical body in which effective respiratory transport occurs just beneath the chorion. This model is perhaps somewhat better than the model employed for the stages of development prior to the establishment of circulation. However, the critical oxygen levels that were calculated with this model supposedly would permit penetration into the perivitelline fluid of sufficient oxygen to satisfy the metabolic requirements of the embryo only when the oxygen concentration in the perivitelline fluid is zero. Such levels, of course, cannot be assumed to be true critical levels, for the embryo could not even survive in the absence of oxygen in the perivitelline fluid. For embryos near hatching, the oxygen concentration in the perivitelline fluid at any realistically determined critical oxygen concentration in the surrounding water cannot be assumed to be very much lower than the critical concentration in the water for newly hatched sac fry.

The growth measurements reported in this paper indicate that water movement promotes embryonic development by delivering oxygen to the surface of the chorion of the individual salmonid embryo as well as by transporting sufficient oxygen to meet the total requirement of all of the embryos in a redd. When the oxygen concentration in a redd is high, the water velocity necessary for satisfying the requirements of the individual embryos evidently is less than that necessary when the oxygen concentration is lower. For the steelhead trout, at oxygen concentrations over 11 mg/l, velocities near 6 cm/hr evidently are as satisfactory as are velocities near 700 cm/hr at oxygen concentrations near 6 mg/l or less. Chinook salmon embryos reared at an oxygen concentration of 11.7 mg/l and a water velocity of 92 cm/hr were larger than those reared at 5.6 and 8.0 mg/l dissolved oxygen, even when the water velocity was as high as 1,350 cm/hr.

The water velocities reported in this paper

for the steelhead trout experiment probably approach the mean velocities of the water actually passing the chorions of the developing embryos, though they may be somewhat lower. In the case of the chinook salmon experiment, errors in reported values are probably greater. Actual water velocities, or pore velocities, in redds have never been measured, and they are difficult to evaluate. Usually the "apparent velocity" (*i.e.*, the discharge in cubic centimeters per hour per square centimeter of cross-sectional area, including both solid particles and voids) is estimated. Actual velocities are higher than the apparent velocities.

In the experiments with steelhead trout and chinook salmon embryos reared throughout development at different constant oxygen concentrations, no embryos survived to hatch successfully at 1.6 mg/l. At tested low oxygen concentrations of 2.5 mg/l and more, prehatching mortalities were low and were similar to those of controls reared at concentrations near the air-saturation level. Embryos reared to hatching at low concentrations were smaller and required more time to reach the hatching stage than embryos reared at high concentrations.

The ability of salmonid embryos to decrease their oxygen consumption rates and still survive at low oxygen concentrations is apparently severely restricted at abnormally high rearing temperatures. Unpublished data at Oregon State University indicate that temperature increases of 2 or 3° C. beyond 10° C. may increase by several milligrams per liter the oxygen concentrations necessary for survival of salmonid embryos to the hatching stage.

It appears that prehatching mortality of salmonid embryos in redds due directly to oxygen lack probably occurs only at very low oxygen concentrations, unless water temperatures are unusually high. Final evaluation of the adequacy of different water velocities and oxygen concentrations in the environment of developing salmonids must be based on knowledge of the posthatching fate of fry coming from embryos reared under these conditions. The abnormal or very small sac fry that result from rearing of embryos at very low oxygen concentrations cannot be expected to survive under natural conditions. The rather small sac fry produced at intermediate oxygen concentrations survived well under laboratory conditions, but may not do so under more rigorous natural conditions. Phillips and Campbell (1962) reared steelhead trout and coho salmon embryos in containers placed around standpipes in stream-bed gravels at different locations. Few or no sac fry were recovered from containers placed where the mean oxygen concentrations recorded were below 8 mg/l, even when the ranges of oxygen concentrations determined at 10-day or 5-day intervals did not indicate that concentrations lethal for the embryos under laboratory conditions had existed. While these results suggested that embryos or newly hatched sac fry produced at the moderately reduced oxygen levels may not survive well in nature, the findings were not conclusive.

Sac fry produced at dissolved oxygen levels only slightly below saturation, though somewhat smaller than those produced at still higher concentrations, probably can survive to emerge from the gravel, but they may not compete well with larger fry. The ecological significance of very small differences in size of newly hatched fry and in hatching time certainly will be difficult to determine. Still, such differences should not be dismissed as unimportant until this matter has been thoroughly investigated through laboratory and field studies.

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LITERATURE CITED

ALDERDICE, D. F., W. P. WICKETT, AND J. R. BRETT. 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. Jour. Fish. Res. Bd. Canada, 15: 229–250.

COBLE, D. W. 1961. Influence of water exchange and dissolved oxygen in redds on survival of steelhead trout embryos. Trans. Am. Fish. Soc., 90: 469–474.

GARSIDE, E. T. 1959. Some effects of oxygen in relation to temperature on the development of lake trout embryos. Canadian Jour. Zool., 37: 689-698.

HARVEY, E. N. 1928. The oxygen consumption of luminous bacteria. Jour. Gen. Physiol., 11: 469–475.

HAYES, F. R., I. R. WILMOT, AND D. A. LIVINGSTONE. 1951. The oxygen consumption of the salmon egg in relation to development and activity. Jour. Exper. Zool., 116: 377-395.

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

NIKIFOROV, N. D. 1952. Growth and respiration of young salmon at various concentrations of oxygen in water. (In Russian.) Doklady Academii Nauk SSSR, 86: 1231–1232.

PHILLIPS, R. W., AND H. J. CAMPBELL. 1962. The embryonic survival of coho salmon and steelhead trout as influenced by some environmental conditions in gravel beds. 14th Ann. Report of the Pacific Marine Fisheries Commission for the Year 1961. Pac. Marine Fish. Comm., Portland, Oregon, pp. 60–73.

SHAPOVALOV, L., AND W. BERRIAN. 1940. An experiment in hatching silver salmon (*Oncorhynchus kisutch*) eggs in gravel. Trans. Am. Fish. Soc., 69: 135–140.

SHAW, P. A., AND J. A. MAGA. 1943. The effect of mining silt on yield of fry from salmon spawning beds. California Fish and Game, 29: 29–41.

WICKETT, W. P. 1954. The oxygen supply to salmon eggs in spawning beds. Jour. Fish. Res. Bd. Canada, 11: 933-953.