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SURVIVAL OF LARGEMOUTH BASS EMBRYOS AT LOW DISSOLVED OXYGEN CONCENTRATIONS

A Thesis
Presented to the Faculty of the Graduate School
of Cornell University for the Degree of
Master of Science

by
Richard George Dudley
June, 1969

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BIOGRAPHICAL SKETCH

Richard George Dudley was born in New Rochelle, New York on March 25, 1945. He was raised in Mamaroneck, New York and received his elementary and high school education in Rye Neck school system. He graduated from Rye Neck High School in June, 1963. In September, 1963 he entered the College of Agriculture at Cornell University and he received the Bachelor of Science degree in June, 1967.

On July 6, 1968 he married Christina Cranston Gillis of Pompano Beach, Florida.

He has held a research assistantship with the New York Cooperative Fishery Research Unit at Cornell University since September, 1967, and is a member of the American Fisheries Society.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (cont.)

INTRODUCTION

The objective of this study was to determine the effects that low dissolved oxygen concentrations have on the embryonic survival of largemouth bass, Micropterus salmoides (Lacépède). Since the year class strength of a fish population is often set by the extent of survival in the early life stages, studies of ecological factors which may be a source of mortality during those stages are of great interest. Kramer and Smith (1962) determined that year class strength in largemouth bass populations was set during the period from egg deposition to two weeks after hatching. They found that embryo survival varied between 0 and 94 percent. From their field studies, Kramer and Smith concluded that dissolved oxygen was not a factor in the early mortality of largemouth bass. The present study was designed to find what oxygen levels would affect survival, and at those levels, what the survival patterns would be at different temperatures.

Many workers have investigated the effects of low oxygen concentrations on the survival of fish embryos, but most of these studies have dealt with salmonids. Coble (1961), Garside (1959 and 1966), Shumway, Warren and Doudoroff (1964), Silver, Warren and Doudoroff (1963) and Wickett (1954), have examined the various effects of relatively constant dissolved oxygen levels on salmonid embryos. Alderice, Wickett and Brett (1958) examined the effects of temporary exposure to low oxygen levels in Pacific salmon embryos.

The above workers found that the oxygen requirements of salmonid embryos were higher for more advanced stages of development (Alderice et al. 1958). They also reported that at constant low oxygen levels as

low as 2.5 parts per million embryos could survive to hatching. All oxygen levels below air saturation were found to decrease the size of larvae at hatching. As the oxygen level was decreased, length of the incubation period was increased (Shumway et al. 1964). Investigations by these workers of the effects of varying water velocities showed that, within each oxygen level tested, the size of larvae produced was smaller at lower water velocities. Silver et al. (1963) reported that increases of a few degrees above 10 C could increase the required oxygen levels by several milligrams per liter.

In view of the above observations on salmonid embryos, this investigation of bass embryos was designed to include variation in the test levels of oxygen and temperature. Original plans also included an investigation into the effects of water movement and its relationship to survival of embryos at low oxygen concentrations. It was hoped that the water movement would imitate the effects of a male bass fanning the embryos. Unfortunately the method of water flow chosen, which incorporated vertical movement of the embryos themselves, produced a nearly complete mortality at hatching in the embryos so treated. This method does not give an accurate insight into the final effects of water flow on survival at low oxygen levels.

METHODS

Oxygen-control Apparatus

Water of desired oxygen content was produced with a degasser of the type described by Mount (1961; 1964). The degasser built for this study was virtually a copy of one built at the Robert A. Taft Sanitary Engineering Center which, in turn, was a slight modification of Mount's 1964 description.

The theoretical basis of this degassing system is the linear relationship, at a given temperature, between the partial pressure of a gas and its solubility. A vacuum is used to lower the partial pressure of oxygen, and the maintenance of a given vacuum level produces a constant partial pressure of oxygen. Water circulated under this partial pressure will acquire the corresponding dissolved oxygen level. In other words, the dissolved oxygen content of the water can be controlled by controlling the vacuum.

In the system used, as in the one described by Mount (1964), the basic design included a large tank two thirds filled with water. In the upper third of the tank the desired vacuum was maintained by a Precision Scientific (Model 75) vacuum pump and regulated by a Gilmont Instrument Cartesian diver type vacuum regulator (Model C-2100). Water in the lower two thirds of the tank was constantly drawn off by a Morse-Fairbanks (Model BR-515) turbine pump. The water not being used for experimentation was reintroduced, through a back pressure relief valve (Cash Valve Corp. Type FR, 3/4 inch), into the upper third of the water tank, either as a diffuse stream (in the previous versions) or as a spray (in this version). Water used for test purposes was controlled by a gate valve located on the outflow side of the back pressure relief valve. (Fig. 1).

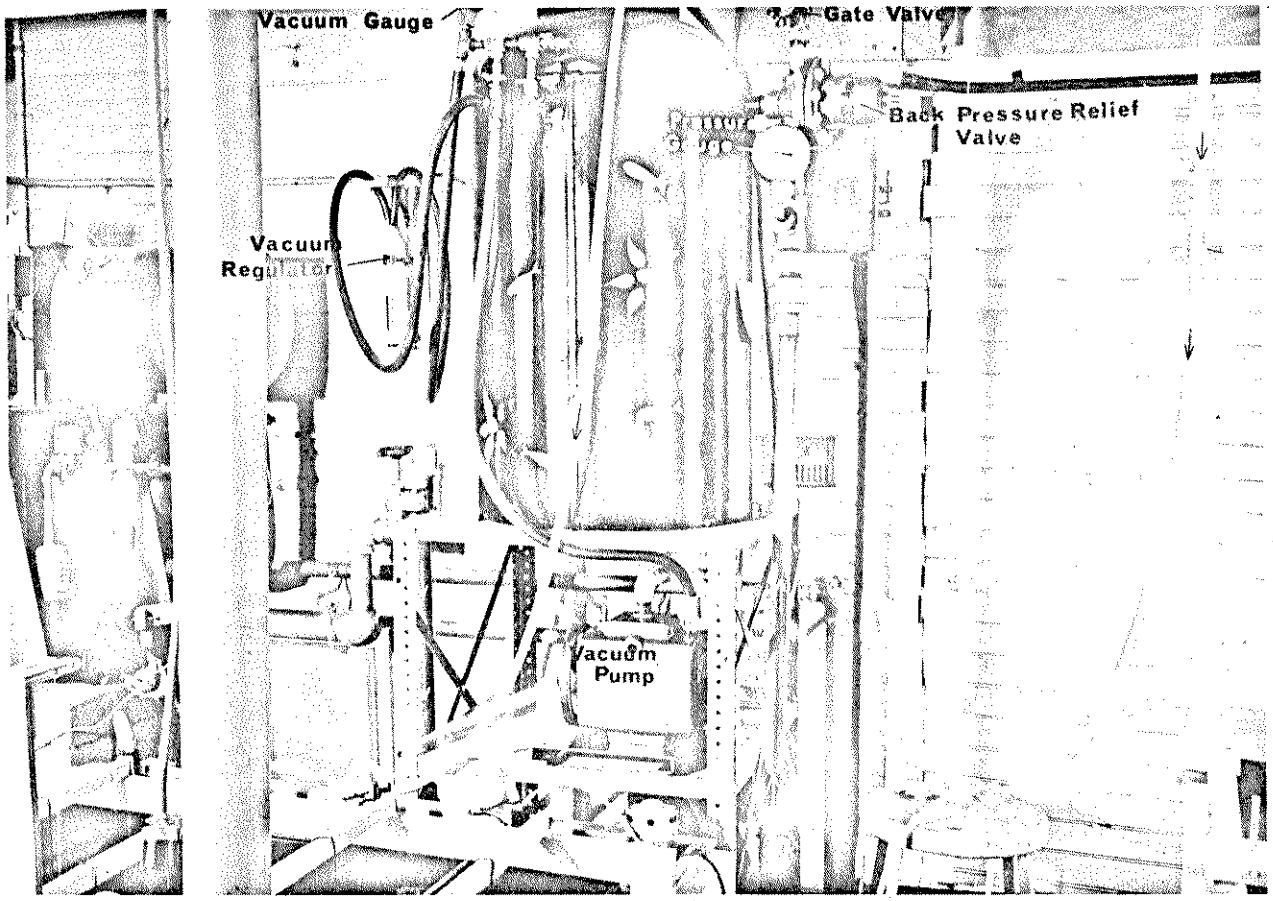

Fig. 1. Degassing equipment of the type used by Mount (1961, 1964). Shown here is the main water tank and the equipment used to circulate water through it as well as the vacuum pump and regulator which maintained a vacuum in the upper third of the tank. The water pump is on a shelf below the vacuum pump and is not visible.

The path of the test water through the degasser was as follows. City water (Ithaca, New York) was led by garden hose to a header tank--a large polyethylene garbage pail fitted with overflow connections. Because the degasser was being operated at high vacuums there was no need to locate this header tank above the degasser; the high vacuum was enough to draw the water into the degasser tank. The water level in the degasser vacuum tank was controlled by a roof tank float valve as described by Mount (1961). Once in the degasser the water was constantly cycled through the water pump and back pressure relief valve. From the back pressure valve most of the water was sprayed back into the degasser vacuum tank while the rest was piped off for use in tests. This test water was led from the degasser through three tygon tubes, each one going to a different temperature bath.

Previous to Mount's 1961 paper the most common means of controlling dissolved oxygen levels was to lower the partial pressure of oxygen by replacing it with nitrogen in the atmosphere that was bubbled through water as it flowed to the test chamber. Since degassing by vacuum was the only method used in my experiments, no comparisons of methods are available, but I can report that the degassing system proved to be highly satisfactory.

Temperature Baths

In order to provide tanks of test water at 12 different oxygen-temperature combinations, three large temperature baths were constructed, each to contain four smaller test tanks.

The temperature baths (Fig. 2) were made of 22-gauge galvanized iron sheet and measured 86.3 X 104.1 X 45.7 cm deep. A plywood control panel

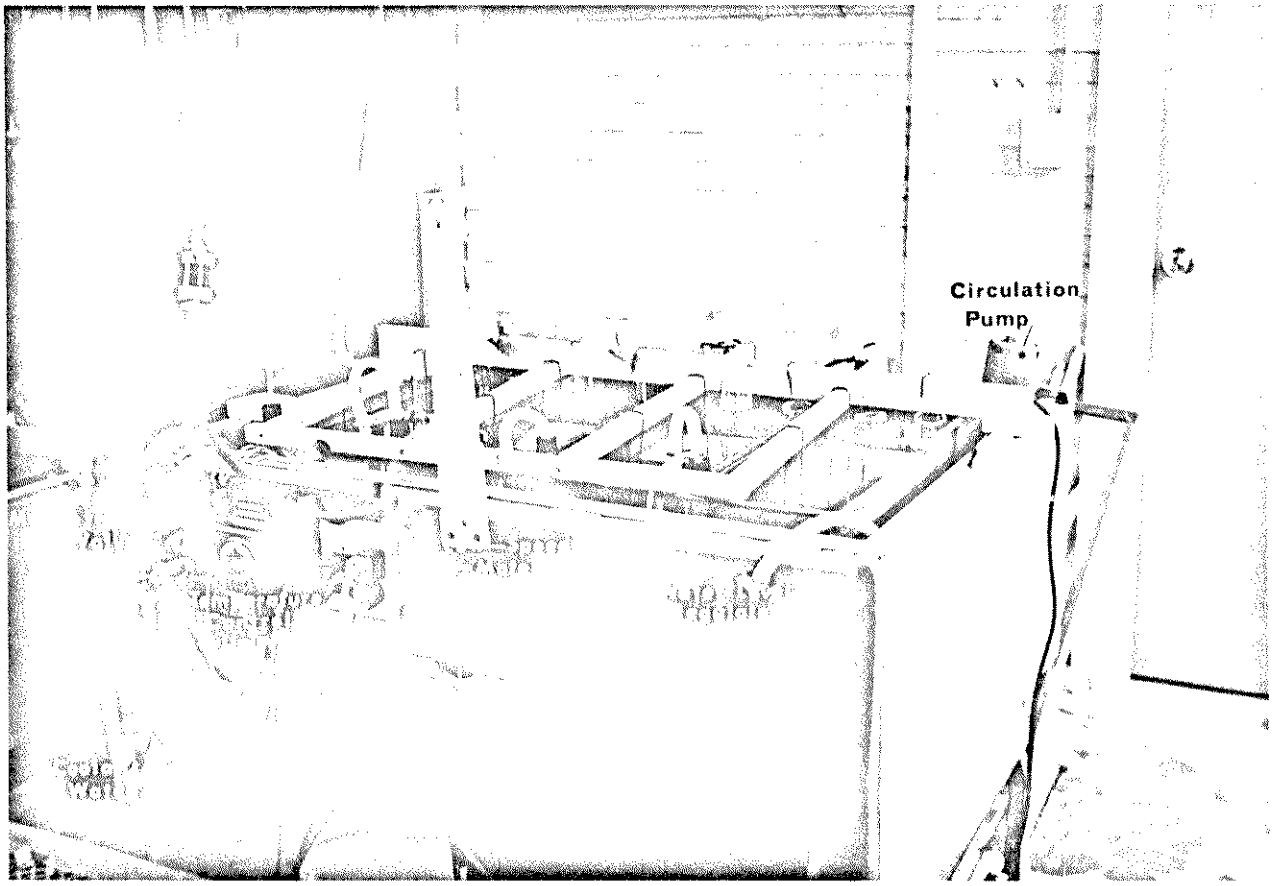

Fig. 2. A temperature bath containing four test tanks. On the plywood control panel from left to right are: a magnetic valve, temperature control unit, electrical connection box, low-speed motor, and an overflow regulator. The tube entering the overflow regulator should lead from the right-hand test tank, not from the temperature bath.

was attached to strap-iron brackets near the top of one of the longer sides on each bath. The sides and bottom of each bath were insulated with 2-inch polyurethane foam. The temperature of each bath was controlled by a Precision Scientific 300-watt heating coil and an aluminum cooling coil (Alcoa Alclad tubing). A thermostat (Model Z-7 of the Partlow Corp.) was connected electrically to both the heating coil and a magnetic valve controlling flow of coolant through the cooling tubes in each bath. The thermostat, magnetic valve, and electrical connections were mounted on the plywood control panel. When the bath temperature deviated from the desired level by .5 C, the heating or cooling coils would correct the temperature. The source of coolant water and operation of the temperature control apparatus described above are well explained by Regier and Swallow (1968).

A small water pump (Teel model P1618) was mounted in a corner of each temperature bath with its outflow lead to the opposite corner of the bath. This pump assured proper circulation of the bath water.

Test Tanks

The four test tanks within each temperature bath (Fig. 3) were set side by side on a rack and were supported about 20 cm above the bottom of the bath to allow room for cooling and heating coils and for water circulation beneath the tanks. The supporting rack was made of four wooden strips and two strap iron rectangles.

The test tanks are made of 24 gauge stainless steel sheet and measured 20.3 X 70.5 X 27.9 cm deep. A U made of two 30.5-cm pieces, two elbows, and one 67-cm piece of one-inch ABS plastic pipe was fitted into each test tank. The 67-cm section lays along the base of the tank side

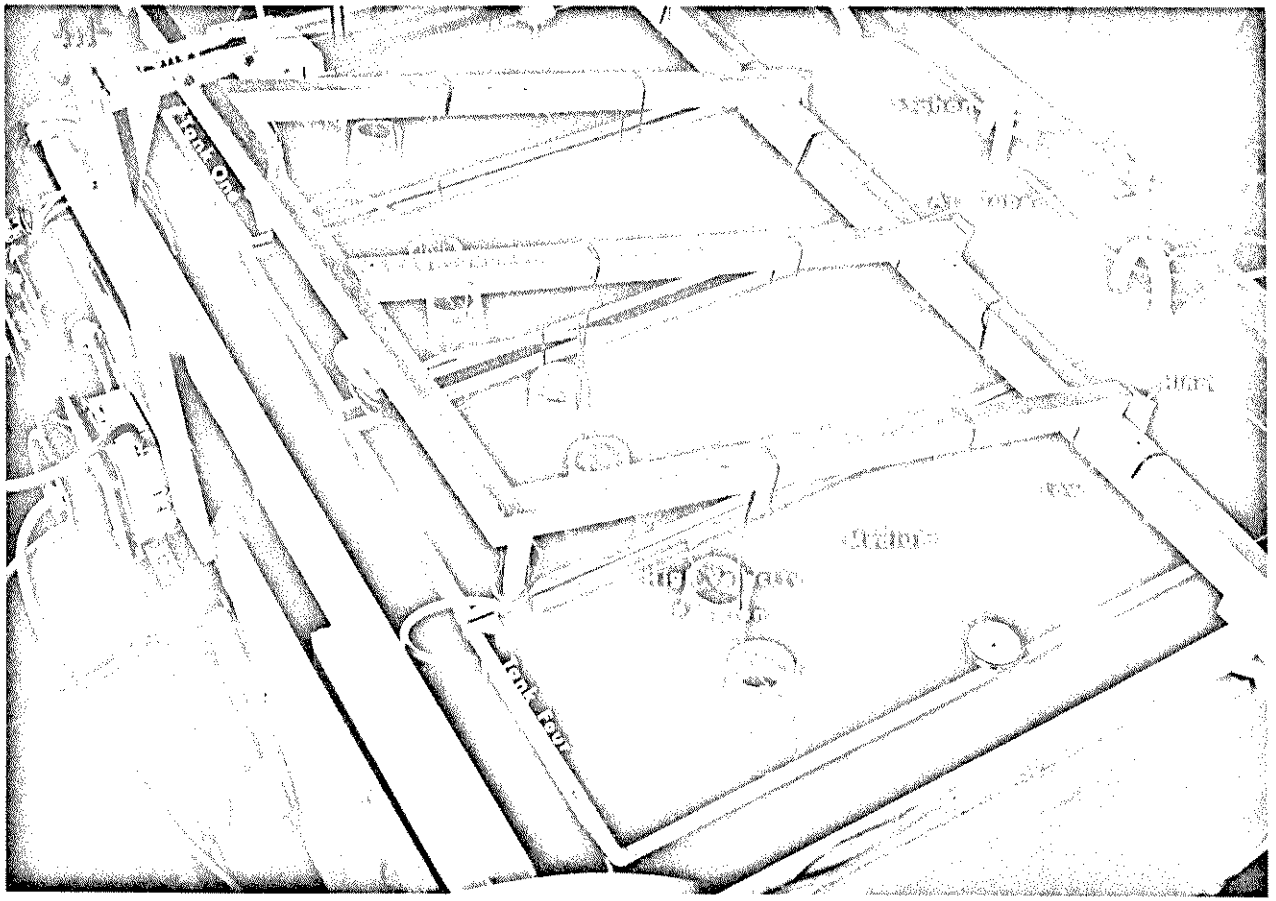

Fig. 3. A temperature bath containing four test tanks of different oxygen levels. Wooden rack was used to produce movement of the embryo containers hanging in the water only in Experiments I and II. Note line coming from left side of rack through pulley system to a wooden arm (not visible) on low-speed motor. Siphons and aeration tubes lead into the distribution pipes described in the text.

and was perforated with 1.27 cm holes along its length. The elbows and 30.5-cm pieces of pipe were fitted to the ends of the 67-piece and extend to the top of the tank. Water introduced into the tank through both upright pipes was distributed in the test tank through holes in the horizontal piece.

Before entering the test tanks, water from the degasser was first run through an aluminum (Alcoa Alclad) tube coil immersed in the bath to bring it to the bath temperature. From the coil the water was introduced into the first test tank via the plastic distribution pipe previously described. Within each temperature bath, water was siphoned from one test tank to the distribution pipe of the next by two Tygon tubing siphons, one at each end of a test tank (Fig. 3). Siphons carried the water from the second tank to the third and from the third to the fourth tank. Water from the fourth test tank siphoned into a water level regulator fashioned from a piece of plastic pipe and a plastic bottle (Fig. 2). The water was then led to a drain.

The oxygen level in the first test tank of each temperature bath was the same as that coming from the degasser. Water in tanks two, three, and four was oxygenated slightly as the water flowed into the plastic distribution pipe of each tank. That is, at the two points of inflow in test tanks two, three, and four a small stream of air from an air pump (Silent Giant) was bubbled into the water. Although this system of slightly aerating the water to raise the oxygen level did not afford delicate control, once the oxygen levels were set they varied little.

The embryos to be subjected to test conditions were placed in mesh bottomed containers made from 50 ml disposable plastic cups (Fig. 4). The bottoms of the cups were removed with a hot soldering gun. A piece of

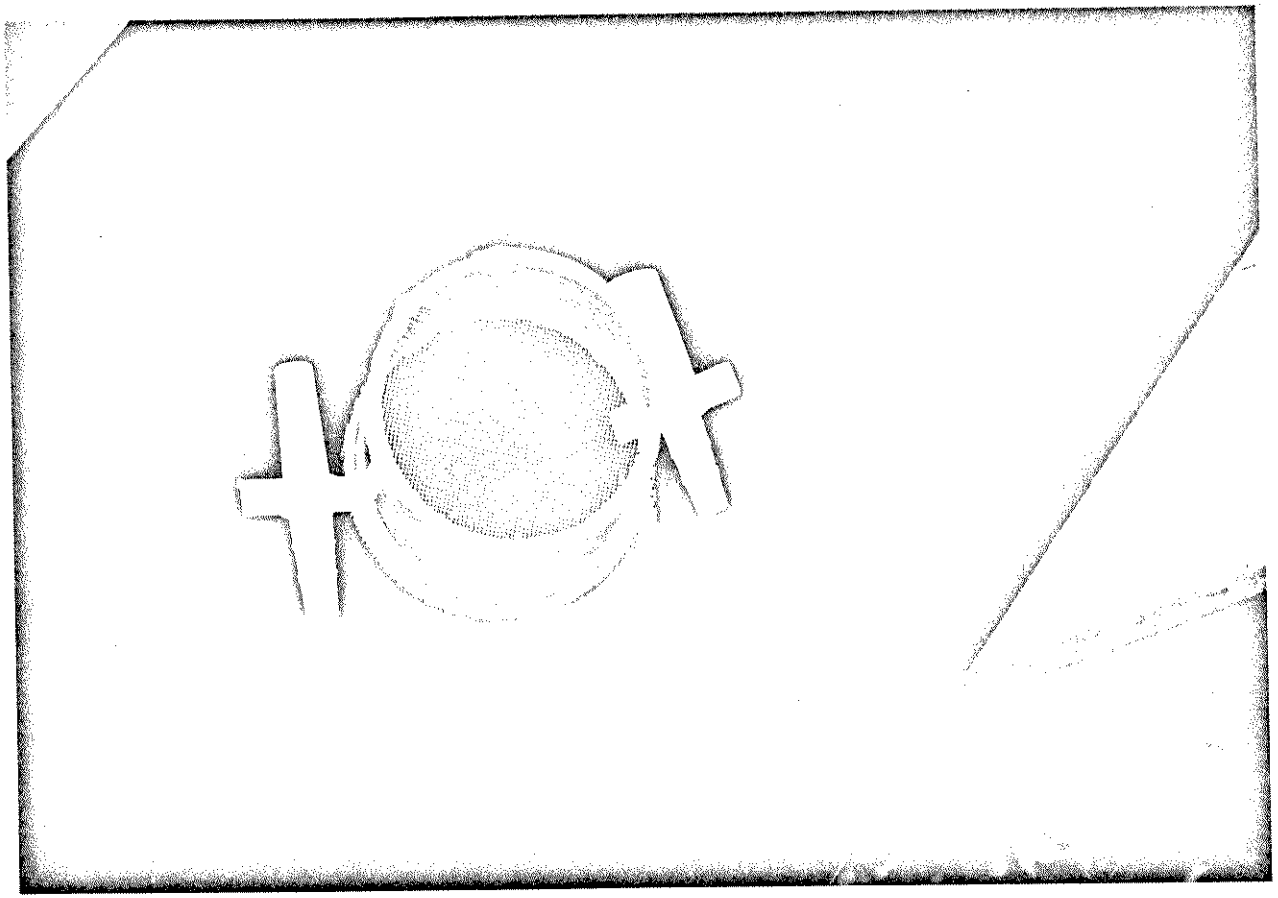

Fig. 4. Embryo container of the type used in Experiments III and IV. Note embryos on the mesh. White objects are supports used to facilitate photography.

fine nylon mesh (bridal veil) glued across the top of each cup formed the new bottom with the cup inverted. These mesh-bottomed cups were suspended in the test tanks of the desired oxygen level. In the first and second experiments conducted, in which motion was given to the embryos, a second layer of mesh was added after the embryos were placed in the containers. In these two experiments the embryos were placed on top of the mesh of the inverted container. A stainless steel ring of a diameter slightly smaller than the cup diameter was placed on top of the mesh of the inverted container. Another piece of mesh was then stretched over the ring and embryos, and was secured by a rubber band (Fig. 5). This put the embryos in a disc-shaped space the diameter of the cup and the thickness of the stainless steel wire. The purpose of enclosing the embryos in this way was to protect them from excessive motion due to water flow, without squeezing them.

In experiments I and II it was hoped that the effect of water movement could be initated by moving the embryos vertically. The movement was accomplished by building a hinged wooden frame over the test tanks. Since the tanks were arranged parallel to each other within a temperature bath, four parallel wooden supports, one over each test tank, were each hinged to a common crossbar (Figs. 2 and 3), that was fixed to the sides of the tank. Another crossbar connected the other ends of the supports and was moveable in a vertical direction. The moveable bar was attached by a monofilament line through a pulley to a wooden arm on a low-speed electric motor (Bodin, Type KYC 22RC, 1 rpm) mounted on the plywood panel of each temperature bath. As the motor turned the moveable crossbar and four parallel supporting arms moved up and down very slowly.

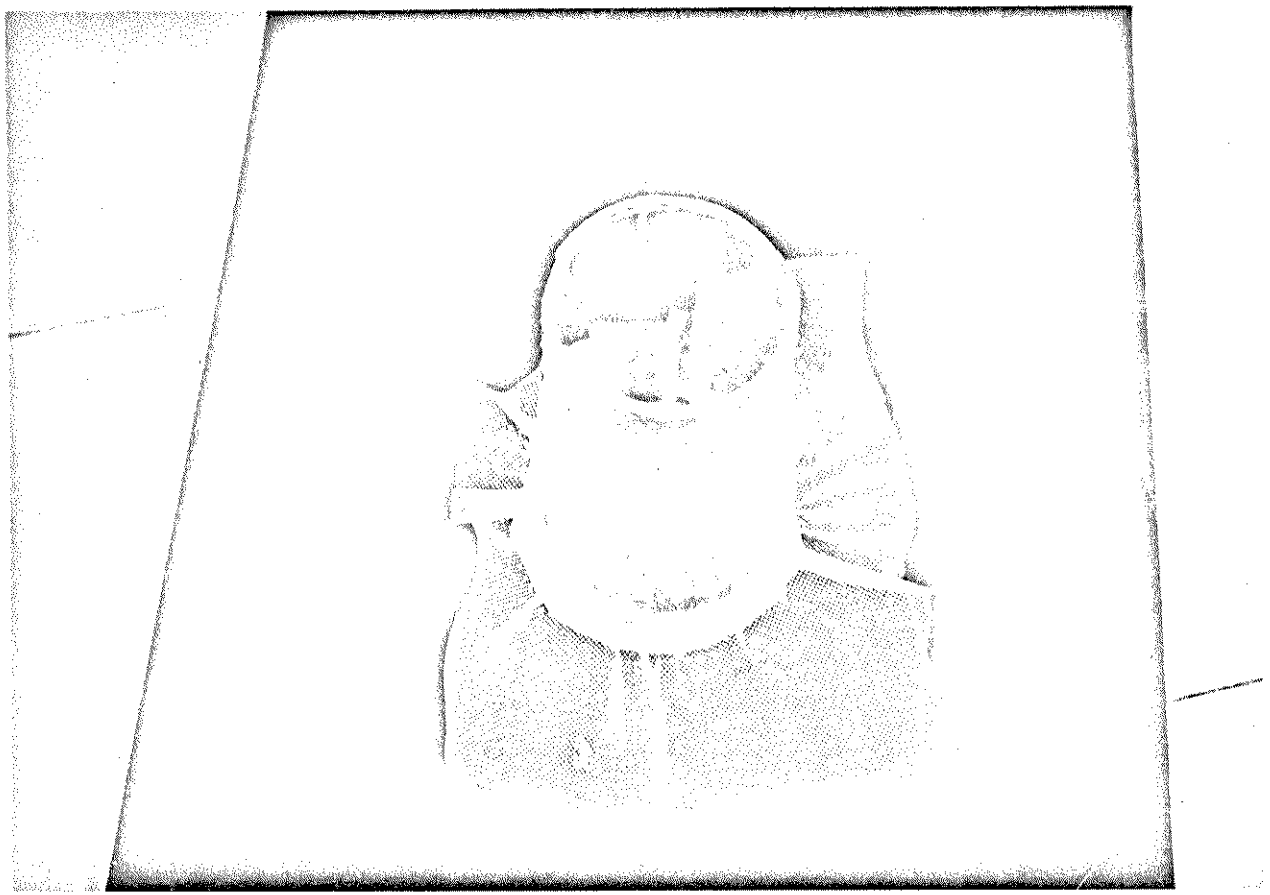

Fig. 5. Embryo container of the type used in Experiments I and II. The second mesh shown here is stretched over a steel ring placed on the bottom of the container.

The hinged ends moved less than the free ends. The radius of the motor arm was 11.3 cm which allowed the free ends of the parallel supports to move about 23 cm vertically.

Embryo containers were hung into each test tank from two points on the appropriate parallel bar and from a position on the stationary cross-bar (Fig. 3). The containers could move a vertical distance of about 7.5 cm in the medium velocity position. The velocity of these two container positions at any given time depended on the position of the motor arm. The maximum velocity occurred when the supports were halfway up or down. The maximum velocities were 23.5 cm per minute for the medium position and 45.5 cm per minute for the high position. Embryos at the low or stationary positions were only subject to the water movement caused by water exchange.

Experimental Procedures

Oxygen determinations were made using the azide modification of the Winkler method as described by the American Public Health Association et al. (1965). For each set of determinations, a 250 ml sample was siphoned from each test tank into a 250 ml reagent bottle. After being treated with the sodium azide and manganous sulfate and acidified, a 203 ml portion was placed over a magnetic stirrer. This was titrated with thio-sulfate solution from a 10 ml burette. The azide modification is described as having a standard deviation of about .05 miligrams of dissolved oxygen per liter for good water conditions.

In Experiment I embryos were carefully pipetted from a bass nest, then transferred to the embryo containers. In Experiments II and IV eggs

and sperm were obtained from fish that were about to spawn or from fish that had initiated spawning. In Experiment III eggs were stripped from a female which previously had been injected with a water solution of a carp pituitary. The general procedure for fertilizing eggs was as follows:

Ponds at the Cornell Fishery Laboratory containing adult largemouth bass were constantly watched for signs of spawning activity. When an increased amount of such activity was observed at a particular nest site, electrodes were placed on each side of the nest. When the male and female bass both returned to the nest site a 110-volt alternating current was applied to the electrodes for about 5 to 15 seconds, stunning the fish just long enough to recover them with a dipnet. The fish were then taken to the laboratory. Eggs could be easily stripped from the female but because of the difficulty in getting sperm from the male, the procedure was to dissect the testes from the male. After the eggs were stripped into a plastic bowl, the testes were wrapped in moist cheesecloth, which was then twisted and squeezed to drip sperm on the eggs. The eggs and sperm were then stirred with the fingertip. Before being counted into the embryo containers the fertilized eggs were left for about two hours to water harden and lose their adhesiveness. This fertilization procedure is well described in the Research Report of the New York Cooperative Fishery Unit for 1967[1].

Twenty (Experiment I and II) or fifty (Experiment IV) embryos were counted into each embryo container. This was done by placing several

[1]Annual research report of the New York Cooperative Fishery Unit for 1967. 56 pp. New York Cooperative Fishery Unit, Fernow Hall, Cornell University, Ithaca, New York.

hundred eggs on a shallow three-sided tray submerged in a basin of water. The tray was supported far enough above the bottom of the basin to allow embryo containers to be placed under the open side of the tray. The embryos were counted as they were pushed over the edge to sink down into the embryo container.

The tray was made of four pieces of window glass held together with silicone rubber cement. The underside of the tray was painted black to facilitate counting the embryos. Embryos were manipulated with a small paddle made from fine mesh stretched over a wire frame.

The general procedure followed, after finding a pair of fish ready to spawn, was as follows:

1) Fish were brought to the laboratory where eggs were stripped from the female, fertilized, and counted as described above.

2) When the desired number of embryo containers had been filled with the planned number of embryos, the containers were hung in the test tanks.

3) Each container was examined periodically (about every 8 to 10 hours) to determine the number of dead embryos.

4) Dissolved oxygen levels were determined periodically (about every 8 or 12 hours).

5) When all embryos in a container had hatched or died, the container was removed from the tank. The living larvae and intact dead embryos were preserved in either formalin, acetic acid or a mixture of both.

Because several problems were encountered during Experiments I and II, the experimental procedure was modified for Experiments III and IV. Each of the experiments is described below:

Experiment I. In this experiment, naturally-fertilized eggs were taken from a nest in Pond H at the Cornell Fishery Laboratory. The exact

time of fertilization of these eggs was not known but a theoretical time was calculated by counting back 73 hours from the approximated mean hatching time at 20 C. Seventy-three hours is the time it took a subsequent group of artificially-fertilized eggs to hatch at 20 C. Since the pond temperature was actually 16 C the eggs were undoubtedly fertilized prior to the calculated time. However, if they had been developing at 20 C, the theoretical fertilization time, 2300 on May 18, would be the time useful in comparisons of times to hatching.

Since only a limited number of embryos was available, embryos were hatched at only one temperature, 20 C. Within this temperature, embryos were allowed to develop at four oxygen levels. Oxygen levels were measured every eight hours. A record of these levels is shown in Fig. 6. The mean dissolved oxygen level and its standard error for each tank were:

Tank	Oxygen level mg/l
1	.78 ± .014
2	1.28 ± .016
3	2.03 ± .023
4	2.42 ± .026

Within each oxygen level there were three water movement levels; high, medium, and low, which have been described earlier. Two containers, that is two lots, each containing 20 embryos, were placed at each movement level within each oxygen level. Thus there was a total of 3 X 2 X 4 = 24 embryo containers in the 20 C temperature bath, each containing 20 embryos (Fig. 3).

The number of dead embryos in each container was recorded every eight hours. When all the embryos in a container had hatched, died, or obviously

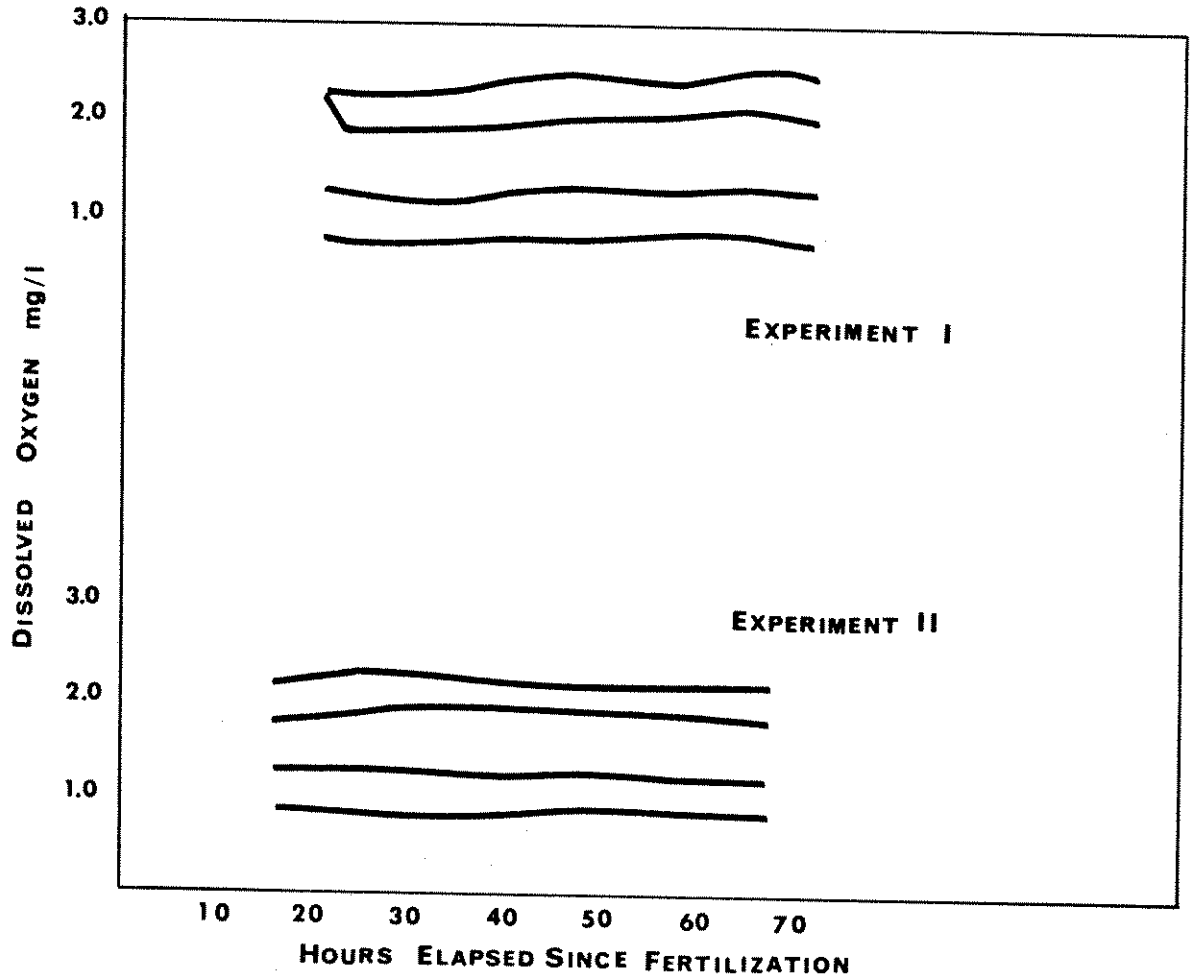

Fig. 6. Dissolved oxygen levels in test tanks during Experiments I and III. Four test tanks in each experiment. 20 C in Experiment I and 25 C in Experiment III.

would not hatch, they were removed, examined, and intact specimens were preserved.

Experiment II. For this experiment a pair of spawning bass was obtained from Pond H at the Cornell Fishery Laboratory. The male of the pair weighed 156 gm and was 24.38 cm long. The female weighed 191 gm and was 25.14 cm long. The eggs were stripped and fertilized as previously described at 1630 on May 26, 1968.

Twenty embryos were then placed in each of 72 embryo containers. Two containers, or lots, were placed at each of 36 oxygen-temperature-movement combinations. The temperatures used were 15, 20, and 25 C. Means of the test tank oxygen levels and the standard errors were:

15 C		20 C		25 C	
Tank	Oxygen mg/l	Tank	Oxygen mg/l	Tank	Oxygen mg/l
1	.76 ± .024	1	.87 ± .022	1	.81 ± .018
2	1.32 ± .022	2	1.14 ± .022	2	1.27 ± .014
3	2.02 ± .013	3	1.87 ± .029	3	1.97 ± .019
4	2.42 ± .014	4	2.42 ± .021	4	2.42 ± .044

A record of the oxygen levels is shown in Fig. 7. The movement levels were described earlier.

The number of dead embryos in each container was recorded every eight hours. When all the embryos in a container had hatched or died or when neither had occurred within a reasonable length of time compared to other embryos under similar conditions, the intact embryos and larvae in that container were examined and preserved in five percent formalin.

Experiment III. This experiment was quite different from the first two in several respects. Because of the high mortality associated with the method of moving embryos the movement factor was eliminated in this

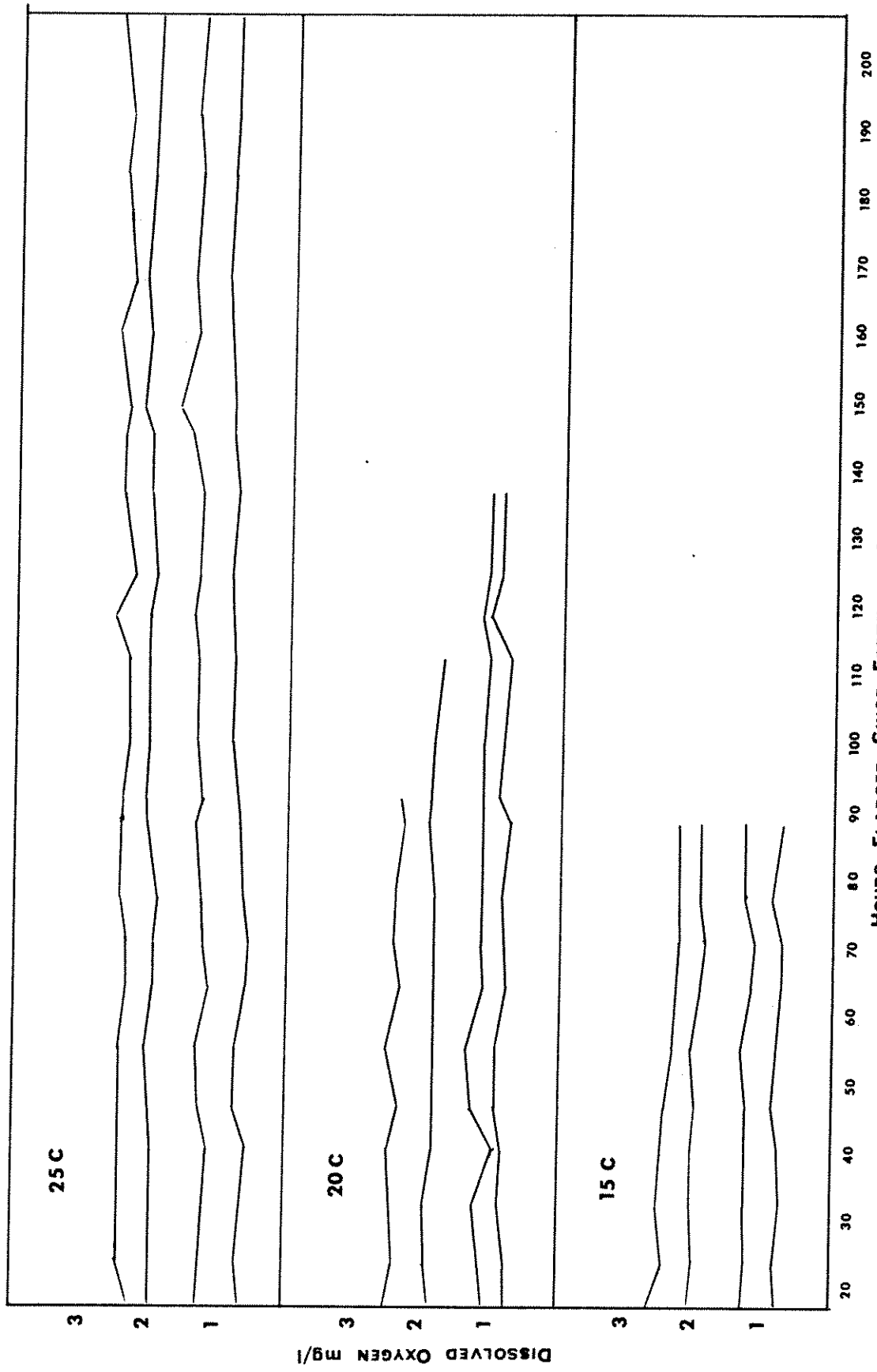

Fig. 7. Dissolved oxygen levels in test tanks during Experiment II. Four tanks in each of three temperature baths.

and the next experiment. The eggs for this experiment were obtained from a bass which had been injected 48 hours earlier with a solution of a carp pituitary dissolved in 5 ml of distilled water. The solution was injected into the peritoneal cavity. The fish was checked periodically until eggs were easily stripped. The sperm source was a male taken from Pond D at the Cornell Fishery Laboratory. The eggs were fertilized as described earlier.

Because this experiment was done while part of Experiment II was still in progress, only the 25 C temperature bath was available. The general purpose was to see what the effect would be when no motion was involved, and to determine what oxygen levels should be tested at 25 C since the levels used in Experiment II were too low.

The embryos were not counted but were merely taken in a small dipper, a bottle cap, and poured into the mesh bottomed containers. During the first inspection period the total number as well as the number of dead embryos was recorded. Numbers of embryos in the 16 containers, four containers in each of four oxygen levels, ranged from 37 to 109. A secondary objective of this experiment was to determine if more than 20 embryos per container could be easily handled and examined.

Containers were examined and dead embryo counts made every 8 to 10 hours. Oxygen levels were checked every 4 to 12 hours. The record of oxygen levels is shown in Fig. 6. The mean oxygen level and its standard deviation for each tank were:

Tank	Oxygen mg/l
1	.81 ± .018
2	1.22 ± .014
3	1.83 ± .016
4	2.16 ± .024

No embryos were preserved.

Experiment IV. A spawning pair of fish for this experiment was taken from Pond A at the Cornell Fishery Laboratory. The male fish weighed 202 gm and was 37.7 cm long. The female weighed 206 gm and was 32.2 cm long.

During the previous experiment (III) it was found that groups of 50 embryos could be handled with only slight difficulty. In Experiment IV lots of 50 embryos were placed in each embryo container. Three containers were placed at four different oxygen levels in each of three temperature baths. The temperatures were 15, 20, and 25 C. A record of the oxygen levels is shown in Fig. 8. Mean oxygen levels and their standard errors were:

15 C	
Tank	Oxygen mg/l
1	1.04 ± .013
2	1.27 ± .046
3	1.96 ± .037
4	2.11 ± .048

20 C	
Tank	Oxygen mg/l
1	1.12 ± .023
2	1.47 ± .038
3	2.11 ± .059
4	2.46 ± .089

25 C	
Tank	Oxygen mg/l
1	1.30 ± .025
2	1.63 ± .043
3	2.14 ± .043
4	2.72 ± .044

The oxygen levels were determined every 8 to 10 hours. Because the embryos rested on a single layer of mesh and were not moving, as in experiments one and two, it was possible to remove dead embryos with wire loop forceps when the containers were inspected every eight hours. The removal of dead embryos was done with wire loop forceps without removing the containers from their positions in the test tanks. Dead embryos were preserved when they were removed. This allowed preservation of more intact dead embryos. In experiments one, two, and three dead embryos had to be left in the containers until termination of the experiment and by that time were already decomposing.

SUMMARY

The year-class strength of largemouth bass populations is determined by the extent of survival during the early life stages. The objective of this study was to determine in the laboratory what levels of dissolved oxygen could cause high mortalities in largemouth bass embryos incubated at different temperatures. In two experiments an attempt was made to study the effect of water movement on embryonic survival at low oxygen levels. The method of water movement, which employed vertical movement of the embryos, caused a nearly complete mortality at hatching.

Water of low dissolved oxygen content was provided by a degassing apparatus. Test water flowed slowly through three series of four test tanks each. The water temperature in each series of four tanks was either 15, 20, or 25 C. Within each series, the four test tanks formed a dissolved oxygen gradient. Thus four dissolved oxygen levels could be maintained at each temperature.

Artificially fertilized largemouth bass embryos were counted into mesh-bottomed containers and were placed in the test tanks. The embryos were examined every 8 to 10 hours without removing them from the test conditions.

Although living bass larvae were produced at dissolved oxygen levels as low as 1.0, 1.1 and 1.3 mg/l, survival of embryos dropped sharply at oxygen levels below 2.0, 2.0, and 2.8 mg/l in incubation temperatures of 15, 20, and 25 C respectively. Production of normal larvae equal to that at 90 percent oxygen saturation is probably possible only at oxygen levels above 2.0, 2.5 and 3.5 mg/l in incubation temperatures of 15, 20, and 25 C respectively.

Data from all experiments show that the critical point in embryonic development, with regard to dissolved oxygen at the levels tested, is the hatching period. It is suspected that the increased activity of the embryo during the hatching period increases its oxygen requirements above the level that can be supplied from the test water.

Although movement did not appear to affect the survival of embryos before the hatching period, it caused a complete mortality of embryos during the hatching period. This observation may be related to an increased activity, and thus an increased oxygen consumption, of embryos due to movement during the hatching period, but the exact reason for the movement-associated mortalities is unknown.

Some evidence of mortality due to crowding of embryos was apparent in Experiment III. This mortality could have been due to a depletion in dissolved oxygen levels in water surrounding the embryos or to an accumulation of metabolic wastes.

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