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Respiratory Metabolism and Energy Requirements of Embryo, Larval and Juvenile Mountain Whitefish, Prosopium Williamsoni

Pokkavil Karunakara Rajagopal
Utah State University

RESPIRATORY METABOLISM AND ENERGY REQUIREMENTS OF EMBRYO, LARVAL AND JUVENILE MOUNTAIN WHITEFISH, PROSOPIUM WILLIAMSONI

by

Pokkavil Karunakara Rajagopal

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of

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ABSTRACT

Respiratory Metabolism and Energy Requirements of

Embryo, Larval and Juvenile Mountain

Whitefish, Prosopium Williamsoni

by

Pokkavil Karunakara Rajagopal, Doctor of Philosophy

Utah State University, 1975

Major Professor: Dr. C. B. Stalnaker
Department: Wildlife Resources

The upper optimum temperature for embryonic development of mountain whitefish is 6 C, and for the post yolk sac stage is 9 to 12 C. The need to know effects on all stages in the life cycle in cases of thermal pollution is demonstrated. Abnormalities caused by thermal pollution in hatched larvae were agape jaws, coloboma or fissure of the eye, monophthalmia or the presence of only one eye, monomicrophthalmia or the presence of one small eye and one case of twinning. High mortalities of eggs occurred at 9 C and higher.

Study of the energy expenditure, by the dry weight method showed an energy deficit at 6 and 9 C at the time of hatching. The larvae are then fully capable of offsetting these deficits by feeding. The oxygen consumption method showed an energy deficit during hatching at 6 C, but failed to show any at 9 C. The efficiency of yolk conversion generally decreased as development progressed. No group effect in active metabolism was observed in the underyearlings.

The electrochemical method of measuring oxygen consumption of embryos gave a cumulative value which was 6.8% higher than values obtained using the manometric method. This increase is attributed to the increase in activity of embryos caused by the nature of the experimental set up.

Active metabolism and scope for activity were high at 9 and 12 C compared to 6 and 15 C. Standard metabolism steeply increased at 15 C. There was very little scope for activity at 15 C.

The embryonic development was similar to that of other whitefishes. The mountain whitefish requires more thermal units to reach corresponding stages than does the lake whitefish, after the stage when the blastodisc is prominantly raised up on the yolk.

(111 pages)

INTRODUCTION

Hjort (1914) observed that relatively weak year classes of fishes may result from unusually heavy mortality of ova, larval or postlarval fish. Bagenal and Braum (1971) noted that mortality is very high during the early stages of life and a small change in the daily mortality rate could produce a "weak" or "blank" year-class. The causes of this mortality are relatively little known. The period when a fish changes from endogenous yolk to exogenous food material, is referred to in fishery literature as the "critical period" since the fish are highly delicate during this period.

An adequate annual recruitment of individuals to the population is essential to maintain a maximum or optimum sustained yield from sport or commercial fisheries. Recruitment is determined by the survival rate of each year-class from the time the eggs are fertilized to the time that the fish attain a size which makes them vulnerable to fishing gear. The numerical strength of a year-class is determined very early in life between egg fertilization and a few days after hatching. Field studies of marine and freshwater fishes have indicated that some factors are more important than others. However, there is not enough information available to predict the size of future year-classes from data gathered on environmental conditions during the spawning and early larval stages. A great need exists for intensive physiological studies to determine environmental requirements and tolerance limits of fish embryos, larvae and post-larvae under several different environmental situations. Many of these factors can be studied

only in the laboratory where they can be adequately controlled.

Bioenergetics, permits us to evaluate the cost of life under different environmental conditions and provide a means of predicting the effects of environmental changes (Warren, 1971). The fish eggs have reserves of nutrient material and are highly organized systems with all attributes essential for normal development. The fish embryo must maintain a balance between the yolk energy and the energy required to sustain the activities essential for normal development. A favourable environment will permit this balance. However, if any environmental factor, like temperature is changed either naturally or due to the activities of man, this balance will be upset.

The ability of the embryo to survive on the endogenous yolk energy until development is complete at any prevailing temperature will determine the success or failure of hatching. Temperature will affect metabolic rate and one has to determine the particular temperature at which the endogenous material becomes limiting and at what temperature the endogenous supply is at optimum. This of course will vary with different species of fishes and will also be influenced by the amount of yolk energy available.

Unless the larva is successful in the search and capture of food before its remaining yolk supply becomes exhausted it will die of starvation, when it switches from endogenous yolk to exogenous food material. Any extension of the period of search will clearly increase the probability of survival, while a shortening will decrease it. Temperature during development is one of the factors which determine how much yolk is available for this activity.

Oxygen consumption of an organism is an index of its respiration rate and its physiological condition. Basal and active metabolism have long been used in studies of human and mammalian physiology, and fisheries scientists have been studying respiratory metabolism of fishes for over thirty years. Most of the emphasis has been on adult fish. As already mentioned, the survival rate during the early life history stages determines the numerical size of each year-class. More emphasis should be placed on research during the egg, embryo and larval stages. Effects of most environmental factors are reflected in changes in the respiration rate. Research in the area of respiratory metabolism, therefore, should be expected to shed much light on survival mechanisms, and adaptations during the early life history stages. An advantage of rearing young fish in the laboratory is that very often the fishery scientist learns how little is actually known about the early periods of a fish's life.

The fish chosen for the present study was the mountain whitefish Prosopium williamsoni (Girard). It inhabits the cold water streams and lakes of Western United States and Canada. It belongs to the subfamily Coregoninae of the family Salmonidae (Eddy, 1974). The Coregoninae is given the family status by some (Ex. Maitland, 1972). The mountain whitefish is rapidly gaining acceptance as a sportfish as the fishing pressure on trout is increasing. It is also listed, by the Duluth Water Quality Laboratory in Minnesota, as one of the key species on which studies are recommended to be undertaken by the environmental protection agency.

Rocky mountain whitefish is found to be harder to catch by angling than rainbow trout (Salmo gairdneri). One reason for this may be that the latter has become more "domesticated" and if rocky mountain whitefish is successfully reared in hatcheries and stocked as the rainbow trout they too might be easier to catch by angling. Sigler (1951) reported that efforts by Canadian fish culturists to rear mountain whitefish were not successful. But with the recent advance in knowledge of fish culture techniques it may be possible to successfully rear them in hatcheries.

Objectives

The objectives of the present study were:

1. To attempt to define the critical period wherein mountain whitefish (Prosopium williamsoni) larvae must succeed in finding and feeding on exogenous food material to insure survival, within the upper viable temperature range of the egg.

2. To describe the rate at which the yolk supply is depleted within the upper viable temperature range of the eggs.

3. To describe the oxygen consumption of the embryos and larvae at these temperatures.

4. To determine the "scope for activity" of the underyearlings within the range of temperatures at which they are viable.

5. To study the group effect of the underyearlings.

6. To compare the manometric and palarographic methods of measuring oxygen consumption.

7. To describe the general development of the mountain whitefish.

REVIEW OF LITERATURE

There is much confusion in the literature as to whether the energy of yolk of the fish larvae is enough to supply the larval metabolic requirements. Wood (1932) studied the rate of respiration of embryos of Salmo fario and noted that the rate (ml 0_2/gm wet weight per hr.) remained constant at any given temperature until the embryo had reached its maximum growth rate and then it declined. Phillips (1940) studied the oxygen consumption of eggs of Fundulus and various pelagic fish eggs. He noticed that the respiratory rate ($mm^3 . 0_2$/egg/hr.) increased during the first two and a half days of development and attributed the increase in cellular oxidation in early development to increase in cell number and amount of material incorporated into active embryonic mass.

Hayes, Wilmont and Livingstone (1951) studied the oxygen consumption of salmon eggs under ideal conditions of dissolved oxygen, water current and temperature in relation to development and activity. They found that as eggs developed, oxygen demand per egg increased, but that consumption per gram of living embryo was unaltered.

Nakano (1953) studied respiration during maturation and at fertilization of the eggs of Oryzias. He noted that the respiration of oocytes increased as cell growth advanced. Oxygen consumption of oocytes was higher than that of ripe unfertilized eggs. As maturation division proceeded, respiration decreased gradually and reached a low and constant level when the eggs became ripe. There was no decline or increase in oxygen consumption of

unfertilized eggs after removal from the ovary. In the same species of fish, Hishida and Nakano (1954) found that the oxygen consumption of the eggs increased exponentially with development. Kramer and Smith (1965) measured oxygen uptake of rainbow trout embryos while studying sublethal effects of papermill wastes on Minnesota fishes. These studies all indicated that dissolved oxygen requirements of a fish embryo increase with time at a logarithmic rate. Silver, Warren and Doudoroff (1963) controlled dissolved oxygen levels and water velocities during incubation and found that the largest embryos were hatched under conditions of high water velocities and high oxygen levels. Respiratory rates of developing embryos were not measured, however, and no information is available on physiological mechanisms responsible for the larger larvae which were observed. Work by Daykin (1965) indicates that concepts and formulae of mass transfer theory may have application to the study of oxygen uptake by fish eggs. The need for basic studies in this area of fish embryology is great. Fish physiologists are far behind the insect and mammalian physiologists who have long been gathering extensive experimental data which can be expressed in Vant Hoff and Arrhenius equation (Richards, 1964).

After hatching, a larva (or fry) has limited mobility and can move away from less tolerable conditions. In many cases this mobility is limited to swimming to the surface and being supported by the surface film. Young fishes hatch at various stages of embryonic development. Members of the sunfish family (Centrarchidae), for example, hatch during the early embryonic stage and remain in the nest for several days before swimming up in search of food. Walleye

(Stizostedion vitreum) and suckers (Catostomidae) hatch as prolarvae with large yolk sacs and have functional gills and mouth parts. Salmon and trout hatch as alevins and have functional gills and mouth parts, but do not begin to feed for several days after hatching. Some species of the minnow family (Cyprinidae) hatch with small yolk supplies, have well developed mouths, and must begin to search for food immediately. Pike (Esocidae) larvae remain inactive during the yolk sac period, stuck by the head to plants or other substrates, by means of special glands. Trout larvae after hatching are inactive and stay in the gravel moving only from time to time (Bagenal and Braum, 1971). Larval and fingerling mountain whitefish remain near the bottom while feeding, moving laterally or vertically only to capture prey, mostly those organisms drifting in the current. Whitefish larvae begin feeding at a total length of 14-15mm, before complete absorption of the yolk (Stalnaker and Gresswell, 1974).

Environmental requirements of newly-hatched fishes are vastly different. Those that must engage in much physical activity immediately after hatching must, of necessity, have a higher active metabolic rate than those that lie in the nest or on the bottom for several days. Standard or resting metabolic rates probably differ to a lesser extent. The difference between standard and active metabilism, expressed as rates of oxygen uptake, has been designated "scope for activity" by Fry (1947) and is the measure of the amount of activity an organism can engage in without becoming fatigued from a buildup of lactic acid in muscle tissue and blood. Adverse environmental conditions after hatching that cause a newly-hatched larva with a low scope for activity to exert itself severely could

easily cause the death of that individual. Knowledge of the capacity for activity of fish larvae may aid in explaining the mysterious "mass mortalities" of young fish that have been observed in nature or deduced from circumstantial evidence.

There is scant information on the scope for activity of fish larvae. Considerable work on the subject of scope for activity has been done on larger fish. One reason for the pausity of information on respiratory metabolism of fish larvae has been the lack of suitable respirometers for aquatic organisms that could be used to study oxygen consumption during activity, i.e. swimming against a current. The advent of polarographic and galvanic-cell dissolved oxygen analysers has simplified the problems of dissolved oxygen measurements. The problem of quantitatively expressing spontaneous activity of larval fishes in standard metabolism respirometers remains to be solved.

The metabolism of Pacific sardine (Sardinops caerulea) larvae was studied by Lasker (1962). He showed that the energy from the yolk was not enough to supply the requirements of larval catabolic processes before the larvae were capable of feeding. He thus defined a real cause of mortality before yolk absorption. Lasker and Theilacker (1962) found that the active metabolic rate of sardine (Sardinops caerulea) larvae was 3.5 times its standard rate, while Ivlev (1960) found the increase to be 7 to 14 times in the fry of Salmon, Salmo salar.

Energy deficits before complete yolk absorption have also been observed by Gray (1926), Smith (1947) and Toetz (1966) in larvae of brown trout, rainbow trout and blue gill Lepomis macrochirus, respectively. However these deficits

were in the terminal stages of yolk absorption when the larvae were capable of offsetting these deficits by feeding.

Laurence (1969) did not observe any energy deficit in largemouth bass (Micropterus salmoides) larvae prior to complete yolk absorption by the oxygen method. But by the dry weight method he noticed a slight energy deficit at 288 hr. after fertilization, when the larvae were capable of feeding. Laurence (1973) using both the dry weight method and the oxygen method found no deficiency of yolk energy for growth and metabolism prior to exogenous feeding capability at 16 C, 19 C, and 22 C in tautog, Toutoga onitis.

There are also different accounts in the literature regarding the oxygen uptake of fertilized and unfertilized fish eggs. Nakano (1953) and Rothschild (1956) reported that in teleost eggs no respiration increase is observed after fertilization. Boyd (1928) however found that the oxygen consumption of the eggs of Fundulus heteroclitus is greatly increased after fertilization.

In the Atlantic salmon 41% of the yolk contributes to larval tissue (Hollet and Hayes, 1946) while in the planktonic sardine larvae, 77% of the yolk energy is used for growth (Lasker, 1962). In largemouth bass larvae, however, 44.8% of the yolk becomes living tissue (Laurence, 1969).

MATERIAL AND METHODS

Collection of Adults and Fertilization and Incubation of Eggs

Adult whitefish were collected from Logan River, Utah (Dewitt Campground site) during the middle of November in the years 1972, 1973 and 1974. Electrofishing gear and dipnets were used to collect the fish. Males were predominant in the catch at the peak spawning period during all three years.

The eggswere stripped from the females into a spawning pan and were fertilized by milt taken from males using the dry method described by Leitritz (1972). Eggs were immediately transported to the laboratory in ice boxes and kept in egg hatching trays at 6 C in 1972; at 6 C, 9 C, 12 C,and 15 C, in 1973 and at 6 C, 9 C, and 10 C in 1974. All those kept at 15 C died within five days and those kept at 12 C died within 14 days. More eggs were collected from another pair at the end of November and kept at 6 C, 9 C, 10 C, and 11 C in 1973. More eggs were taken on December 3, 1973 and kept at 9 C, 10 C, and 11 C.

Oxygen Consumption Measurements

Oxygen consumption of the eggs were measured at intervals using a Warburg constant volume manometer. The flasks used were about 15ml capacity. Three ml of water was kept in the flask and 0.2ml of 20% KOH was kept in the center well of the flask. The number of eggs or larvae kept in the flask varied from

one to 10 individuals depending on the temperature of the experiment and the stage of development of the eggs. The higher the temperature and further the stage of development the lesser the number of individuals used. The duration of an experiment was generally from 6 to 24 hours, depending on the stage of development and temperature, so as to give a noticeable difference in the manometric readings.

Weighing of eggs and larvae

Samples of eggs and larvae were weighed (correct to 0.1mg) at intervals using a Sartorius electrobalance after removing the adherent water with tissue paper. They were then dried in an oven at 80 C until constant weight was attained. The dry eggs and larvae were also weighed.

Yolk Sac Measurements

The yolk volumes were measured at intervals. The yolk dimensions were measured using a fish scale reader. The volume of the yolk was calculated using the formula $V = \pi d^3/6$ where d is the diameter of the yolk. At later stages the yolk was not spherical, but rather elliptical and for those stages the volume of the yolk was calculated from the formula $V = (\pi/6)\ LH^2$ where L is the length and H is the height of the yolk (Blaxter and Hempel, 1963). At least 10 eggs were picked at random and measured and the mean volume was taken.

Caloric Measurements

Fresh ova, newly fertilized ova and larvae which had no visible yolk in the yolk sac were frozen and later these were freeze dried. The caloric value of these samples were determined using a Parr's bomb calorimeter. Samples used for each analysis averaged about 1gm dry weight.

Comparison of Electrochemical and Manometric Methods of Measuring Oxygen Consumption of Fish Embryos

Edwards (1958) stressed the need for comparative studies of oxygen consumption of animals using different respirometer systems. It was considered desirable to compare the oxygen consumption of fish embryos using the Warburg constant volume manometer and the Beckman oxygen probe. Equal stages of embryos incubated at 6 C were used in both the methods. Oxygen uptake was measured at 9 C, as the rate is higher at 9 C and a better comparison could be made between the two methods.

The Warburg constant volume manometer is based on the principle that when an animal is placed in an enclosed space the carbon dioxide produced during respiration is absorbed by potassium hydroxide solution or some other alkaline solution, and the gas pressure within the space will decrease as oxygen is used (Umbreit et al., 1972). Based on the gas law $PV = RT$, where P = pressure, V = volume, R = constant and T = temperature, in Warburg manometry volume and temperature are held constant and change in pressure is a measure of the amount of oxygen consumed.

The Beckman Oxygen electrode operates on the principle that when a rhodium electrode is made negative with respect to a silver anode, molecular oxygen is reduced electrochemically at the cathode and current flows in proportion to the oxygen. To prevent fouling of the diodes the electrode assembly is covered with a thin teflon membrane which is freely permeable to oxygen. Potassium chloride solution serves as electrolyte covering the electrodes. A potential of 0.53 volt is applied between the cathode and anode in the oxygen sensor. For each molecule of oxygen consumed at the cathode, a current of four electrons flows, hence 1u amp/hr = $2X10^{-4}$ ml 0_2.

In the Warburg flask of about 15ml capacity, 3.0ml water and 30 eggs were kept with 0.2ml of 20% potassium hydroxide in the center well. The flasks were agitated only two minutes prior to taking a reading. The experiments were of five to six hours duration.

For the electrochemical method a flask of about 25ml capacity was used. The flask was filled with water with fifty to sixty eggs inside. A Beckman oxygen probe was tightly fitted into the flask mouth. The whole was kept in a constant temperature bath. A magnetic stirrer kept the water agitated. The magnetic stirring rod was covered by a screen to prevent it from damaging the eggs.

Active Metabolism Measurements

Active metabolism was measured by a method similar to that described by Rajagopal and Kramer (1974). Conical flasks of 25ml capacity were used for active oxygen uptake measurements. A magnetic stirring rod at the bottom of the flask was covered with a wire net to prevent the fish from being injured by the stirrer. A piece of black tape fastened to one side of the flask served as a source for orientation for the swimming fish. The flask was filled with water and closed with a one-holed rubber stopper through which a Beckman oxygen probe was inserted. The entire flask was immersed in a water bath to maintain constant temperature. A magnetic stirrer was placed beneath the water bath and adjusted to a predetermined velocity needed to keep the fish swimming at its maximum speed. The initial dissolved oxygen content of the water was determined with a Beckman oxygen meter, model 1008, and recorded on a strip chart recorder. The stirrer was stopped and a fish was introduced into the flask. After allowing each fish about 30 minutes to become adjusted, the flask was flushed with fresh water, stoppered with the oxygen probe inside the flask, and the stirrer set at the predetermined speed. Changes in the dissolved oxygen were monitored and recorded for 15 to 20 minutes with the fish swimming at its maximum speed. After each experiment the fish was anaesthetized in a solution of quinaldine and MS-222 (Schoetteger and Steucke, 1970). The total length of each fish was measured to the nearest mm., blotted dry and weighed to the nearest tenth of a mg. using a Sartorius electric balance. The fish was then placed in a jar

containing water from the holding tank and after it recovered from the effects of anaesthesia it was returned to the tank.

Standard Metabolism Measurements

For measurements of standard metabolism a 60ml respiration chamber was made of stainless steel. Inlet and outlet tubes were fitted to the chamber. The chamber was kept in a constant temperature bath. Water was pumped from the chamber by a peristaltic pump into a 25ml flask fitted with a inlet and outlet tube. The water from the outlet was recirculated back into the respiration chamber. An oxygen probe was inserted into the flask. A magnetic stirring rod was kept in the flask and a magnetic stirrer was placed beneath the flask, outside the bath to keep the water agitated.

Index of spontaneous activity of the fish inside the chamber was measured with an apparatus devised on the heat-loss principle described by Beamish and Mookherjii (1964) and modified by Mathur and Shrivastava (1970). The respiration chamber was fitted with a glass-flow meter. The flowmeter tube extended down into the chamber to within 5 mm of the bottom and was supported by a close-fitting collar. The flowmeter consisted of a Chemical Rubber Company thermoregulator, and a small electric heater of approximately 5w inserted into a blind tube in a mercury bulb. A relay in the heater circuit was controlled by the thermoregulator. The heater maintained the mercury bulb at a constant temperature, slightly above that of water in the respiration chamber. The contacts were

adjusted so that the heater circuit was closed approximately 50 percent of the experimental period. A constant-voltage transformer prevented line-voltage fluctuations from changing the heater's wattage and affecting the operation of the flowmeter. The length of time the heater was in circuit was measured in seconds by a manual reset electrical timer.

When a larva swam inside the respirometer chamber it produced movements in the water which increased heat loss from the flowmeter bulb, and increased the time the heater was on. This increase in time was equated to the activity of the larvae. The time that the heater was in circuit in the absence of larvae was expressed as a percentage of the experimental period. This was termed the "base line." The activity index was the percentage time increase in heater operation above the base line reading. The procedure for the experiments was similar to that described by Rajagopal and Kramer (1974).

Fixing and Clearing of Embryos and Larvae

Samples of eggs and larvae were taken at intervals and fixed in 10% formalin. After a few days the eggs were washed for about two hours in running tap water to remove traces of formalin, and then taken through an ascending series of glycerine (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% respectively) (Galat, 1972). The eggs were kept for 4 hours each in the first four series and twenty-four hours each in the higher series of glycerine. Sometimes the chorion collapsed slightly in the glycerine solution. Eggs were transferred to the next series only when they returned to the original shape.

Chorion was removed from some eggs so that the embryo might become clear. The egg was held with a forceps and the chorion punctured with a needle and teased out, avoiding pressure on the embryo. The chorion was removed after fixing in formalin but before clearing in glycerine.

Photographs of the embryo and larval stages were made with a polaroid MP3 land camera.

RESULTS

Incubation and Hatching of Eggs

Eggs fertilized on November 15, 1973 (at the peak of the spawning period) were kept at 6 C, 9 C, 12 C and 15 C. At 6 C mortality of the nearly 5000 eggs was only 3.5% throughout the incubation period of 66 to 96 days till hatching (Table 1), and 96.5% completed normal development (Figure 1).

At 9 C there was a total of 56.8% mortality throughout the incubation period of 61 to 70 days and only 43.2% survived until hatching (Figure 2).

At 15 C all the eggs died and turned opaque within five days of incubation. The eggs were clumped together by growths of Saprolegnia, which grew very rapidly at these temperatures. Massive growths of this fungus occurred overnight at these temperatures. At 12 C all the eggs were dead within two weeks of incubation.

More eggs were fertilized during the late spawning periods on November 30, 1973 and December 3, 1973 and kept at 9 C, 10 C and 11 C. Eggs taken during the late spawning periods had a higher mortality rate, 95.2% and 94.8% respectively when incubated at 9 C compared to 56.8% for eggs fertilized during the peak spawning period (Tables 2, 3 and 4). The viability of eggs taken during the peak spawning period was higher than those taken during the late spawning period (Figure 2). At 10 C there was 100% and 96.4% mortality of eggs taken during the late spawning periods (Tables 5 and 6). The viability of eggs taken during the

peak spawning period was higher than those taken during the late spawning period at 10 C also (Figure 3). At 11 C there was 100% and 97.8% mortality of eggs (Table 7, Figure 4).

Eggs fertilized during the peak spawning period and incubated at 9 C took longer to hatch (61 to 71 days) than those fertilized during the lat spawning period (43 to 54 days). The percentage of hatching was higher for eggs from the peak spawning period (43%) compared to only about 5% for those from the late spawning period (Figure 5).

Eggs fertilized during the 1974 spawning season were kept at 6 C, 9 C, 10 C, and 12 C. At 6 C only 9.2% of eggs died throughout the incubation period. At 9 C the percentage mortality of eggs was 34.4% and at 10 C it was 91.5%. All the eggs at 12 C died within two weeks (Table 8 and Figure 4).

Regression lines of days after fertilization versus percent eggs surviving were calculated from about the blastula stage until hatching (Figures 1, 2, 3, and 4). The arithmetic plot of percent eggs surviving gave a better correlation than $\log_e$ of percent eggs surviving.

The hatching at 6 C of eggs during the 1974 spawning season had peaks during 76 to 77th day after fertilization. Hatching tapered off, the last hatch occurring on the 92nd day (Figure 6).

The mortality of eggs (Tables 1 to 7) was expressed as $\underline{i}$, the daily instantaneous mortality rate (Ricker, 1958)

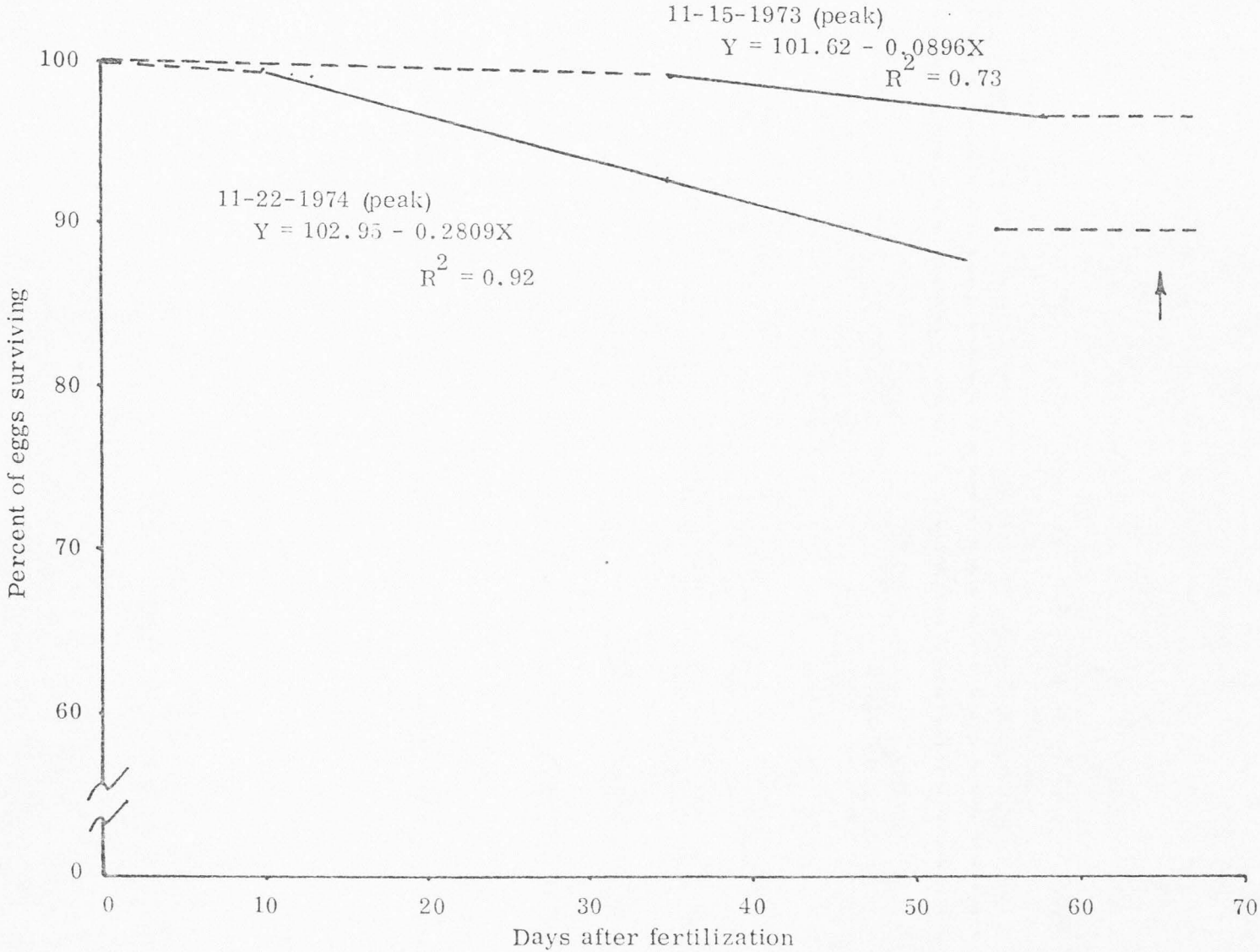


Figure 1. Survival of mountain whitefish eggs at 6 C. The unbroken lines are regression lines fitted to that stanza of development (blastula stage until hatching) where the data points appeared linear. The arrow indicate the time hatching started. Both samples were during the peak spawning period.

Figure 2. Survival of mountain whitefish eggs at 9 C. The unbroken lines are regression lines fitted to that stanza of development (blastula stage until hatching) where the data points appeared linear. The arrows indicate the time hatching started. The upper two lines represent eggs collected during the peak spawning period and the lower two lines represent eggs collected during the late spawning period.

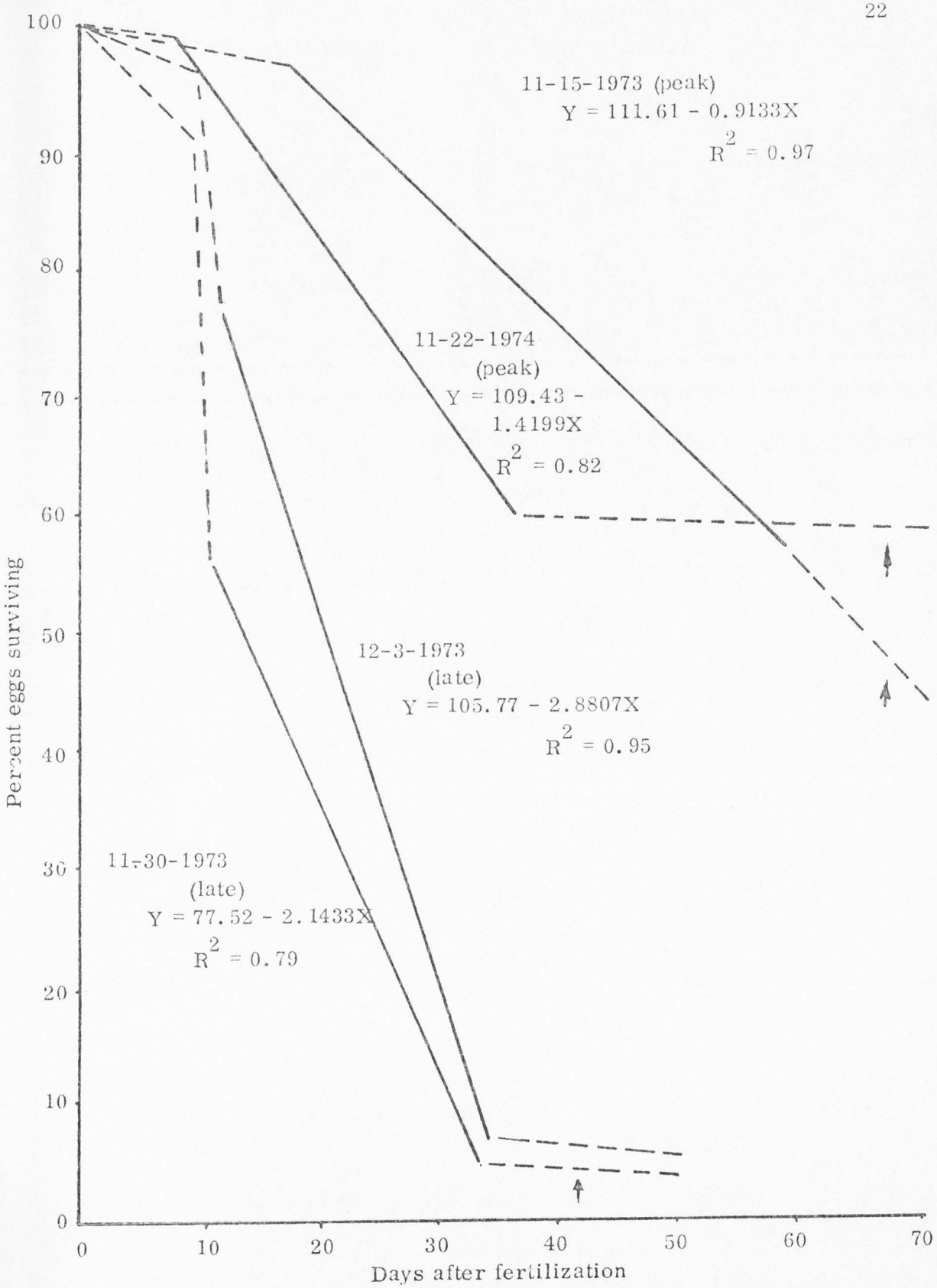

11-15-1973 (peak)
Y = 111.61 - 0.9133X
R2 = 0.97
11-22-1974
(peak)
Y = 109.43 -
1.4199X
R2 = 0.82
12-3-1973
(late)
Y = 105.77 - 2.8807X
R2 = 0.95
11-30-1973
(late)
Y = 77.52 - 2.1433X
R2 = 0.79
Percent eggs surviving
Days after fertilization
0
10
20
30
40
50
60
70
80
90
100

Figure 3. Survival of mountain whitefish eggs at 10 C. The unbroken lines are regression lines fitted to that stanza of development (blastula stage until hatching) where the data points appeared linear. The arrows indicate the time hatching started.

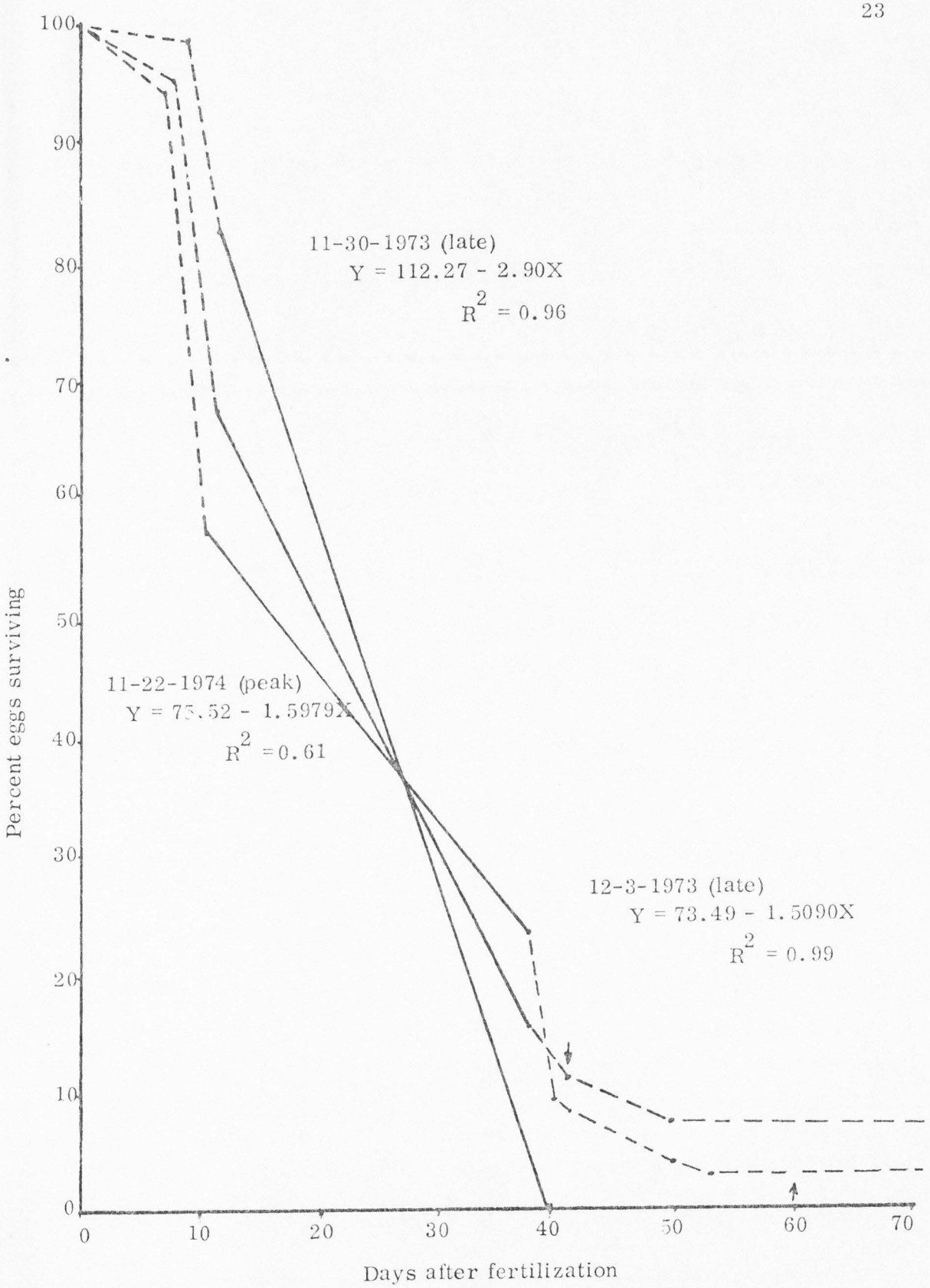

100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Percent eggs surviving
Days after fertilization
11-30-1973 (late)
Y = 112.27 - 2.90X
R2 = 0.96
11-22-1974 (peak)
Y = 75.52 - 1.5979X
R2 = 0.61
12-3-1973 (late)
Y = 73.49 - 1.5090X
R2 = 0.99

Figure 4. Survival of mountain whitefish eggs at 11 and 12 C. The unbroken lines are regression lines fitted to that stanza of development (blastula stage until hatching) where the data points appeared linear. The arrow indicates the time hatching started.

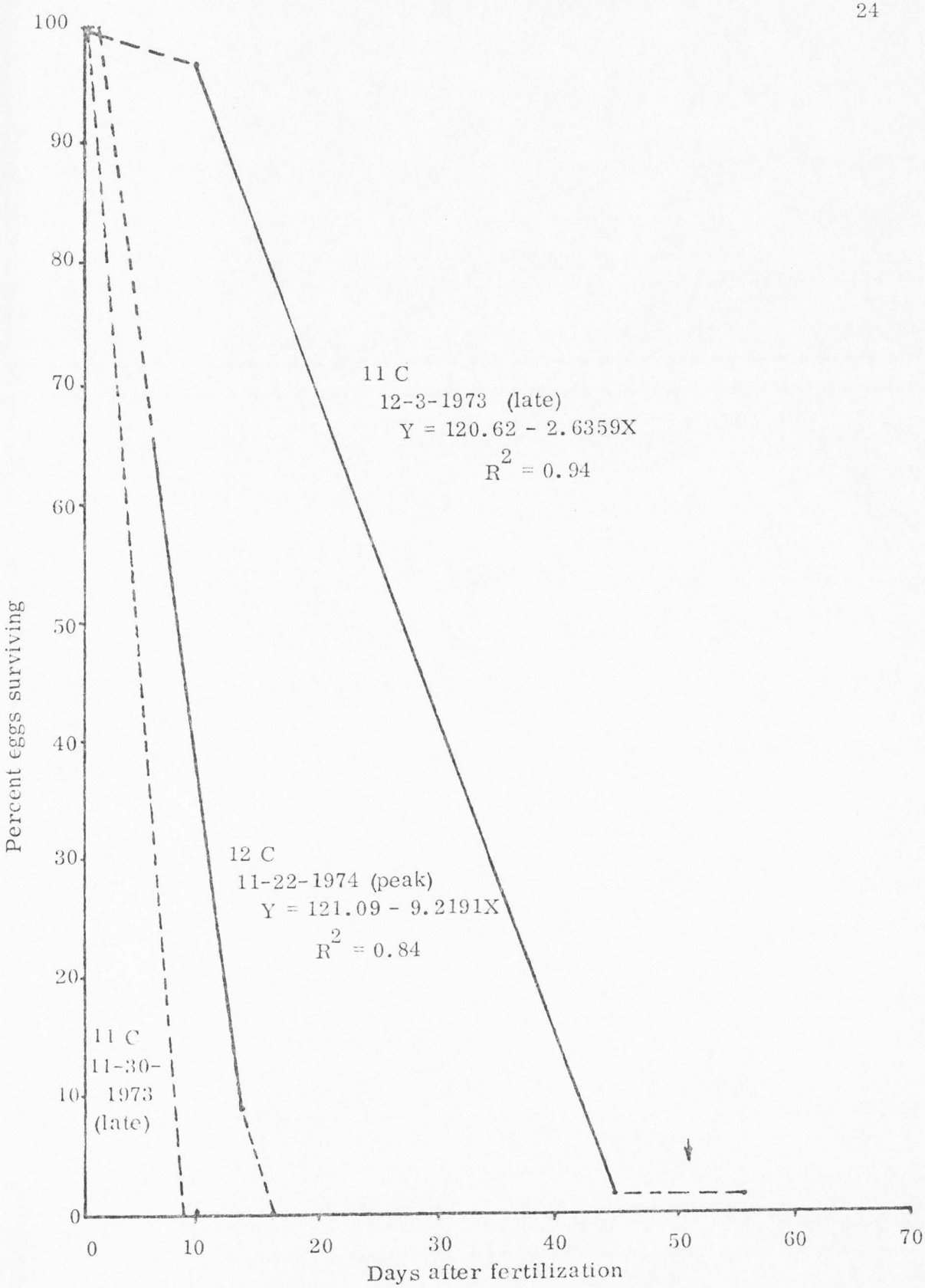

100
90
80
70
60
50
40
30
20
10
0
Percent eggs surviving
0
10
20
30
40
50
60
70
Days after fertilization
11 C
12-3-1973 (late)
Y = 120.62 - 2.6359X
R2 = 0.94
12 C
11-22-1974 (peak)
Y = 121.09 - 9.2191X
R2 = 0.84
11 C
11-30-
1973
(late)

Table 1. Mortality rate of mountain whitefish eggs, collected during the peak spawning season and incubated at 6 C.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
11-15-1973	--	--	--	--
12-20-1973	35	40	0.86	.0002
12-22-1973	37	41	0.87	.0002
12-24-1973	39	83	1.78	.0005
12-26-1973	41	102	2.18	.0005
12-27-1973	42	121	2.59	.0006
1-2-1974	48	146	3.12	.0007
1-3-1974	49	158	3.38	.0007
1-6-1974	52	159	3.40	.0007
1-7-1974	53	162	3.47	.0007
1-12-1974	58	163	3.49	.0006
1-21-1974	67	163	3.49	.0005

Table 2. Mortality rate of mountain whitefish eggs, collected during the peak spawning period and incubated at 9 C.

Date	Age(days)	Cumulative number of dead eggs	Percentage of total	i
11-15-1973	0	--	--	--
12-1-1973	16	20	8.5	.0055
12-7-1973	22	23	9.7	.0040
12-8-1973	23	27	11.4	.0053
12-9-1973	24	28	11.9	.0053
12-11-1973	26	30	12.7	.0052
12-14-1973	29	31	13.1	.0049
12-16-1973	31	35	14.8	.0052
12-17-1973	32	36	15.2	.0052
12-19-1973	34	38	16.1	.0052
12-20-1973	35	43	18.2	.0057
12-21-1973	36	46	19.5	.0060
12-22-1973	37	51	21.6	.0066
12-24-1973	39	55	23.3	.0068
12-25-1973	40	57	24.2	.0069
12-28-1973	43	58	24.6	.0066
12-29-1973	44	65	27.5	.0073
12-31-1973	46	69	29.2	.0075
1-1-1974	47	71	30.1	.0076
1-2-1974	48	78	33.0	.0084
1-5-1974	51	82	34.7	.0084
1-6-1974	52	90	38.1	.0092
1-8-1974	54	96	40.7	.0097
1-10-1974	56	99	41.9	.0097
1-12-1974	58	101	42.8	.0096
1-13-1974	59	102	43.2	.0096
1-15-1974	61	104	44.1	.0095
1-16-1974	62	108	45.8	.0099
1-17-1974	63	108	45.8	.0097
1-18-1974	64	109	46.2	.0097
1-19-1974	65	116	49.2	.0104
1-20-1974	66	116	49.2	.0102
1-21-1974	67	117	49.6	.0102
1-22-1974	68	123	52.1	.0123
1-23-1974	69	123	52.1	.0122
1-24-1974	70	134	56.5	.0120

Table 3. Mortality rate of mountain whitefish eggs collected during the late spawning period and incubated at 9 C.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
11-30-1973	0	--	--	--
12-6-1973	6	31	1.0	.0017
12-7-1973	7	129	4.2	.0060
12-8-1973	8	281	9.0	.0119
12-9-1973	9	389	12.5	.0149
12-10-1973	10	690	22.2	.0252
12-11-1973	11	772	24.9	.0260
12-12-1973	12	1104	35.6	.0366
12-13-1973	13	1266	40.8	.0403
12-14-1973	14	1472	47.4	.0459
12-15-1873	15	1715	55.3	.0536
12-16-1973	16	1739	56.0	.0514
12-17-1973	17	1975	63.6	.0595
12-18-1973	18	2163	69.7	.0663
12-19-1973	19	2244	72.3	.0676
12-20-1973	20	2368	76.3	.0720
12-21-1973	21	2471	79.6	.0758
12-22-1973	22	2524	81.3	.0763
12-23-1973	23	2566	82.7	.0763
12-24-1973	24	2616	84.3	.0772
12-25-1973	25	2672	86.1	.0790
12-26-1973	26	2729	87.9	.0814
12-27-1973	27	2761	89.2	.0817
12-28-1973	28	2784	89.7	.0812
12-29-1973	29	2815	90.7	.0820
12-30-1973	30	2833	91.3	.0814
12-31-1973	31	2849	91.8	.0807
1-1-1974	32	2858	92.1	.0793
1-2-1974	33	2870	92.5	.0784
1-3-1974	34	2876	92.7	.0769
1-4-1974	35	2890	93.1	.0765
1-5-1974	36	2900	93.4	.0757
1-6-1974	37	2906	93.6	.0745
1-7-1974	38	2911	93.8	.0732
1-8-1974	39	2913	93.9	.0716
1-9-1974	40	2917	94.0	.0704
1-11-1974	42	2924	94.2	.0679
1-12-1974	43	2926	94.3	.0666
1-13-1974	44	2930	94.4	.0656

Table 3. Continued.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
1-15-1974	45	2939	94.7	.0653
1-16-1974	46	2941	94.8	.0642
1-17-1974	47	2943	94.8	.0631
1-18-1974	48	2950	95.1	.0627
1-19-1974	49	2950	95.1	.0614
1-21-1974	51	2950	95.1	.0590
1-22-1974	52	2951	95.1	.0580
1-24-1974	54	2953	95.2	.0561

Table 4. Mortality rates of mountain whitefish eggs, collected during the later spawning season and incubated at 9 C.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
12-3-1973	0	-	-	-
12-10-1973	7	5	0.2	.0003
12-11-1973	8	80	3.8	.0049
12-12-1973	9	241	11.6	.0137
12-13-1973	10	301	14.5	.0156
12-14-1973	11	422	20.3	.0206
12-15-1973	13	543	26.1	.0233
12-17-1973	14	678	32.6	.0282
12-18-1973	15	757	36.4	.0302
12-19-1973	16	863	41.5	.0335
12-20-1973	17	896	43.1	.0332
12-21-1973	18	1025	49.3	.0376
12-22-1973	19	1089	52.4	.0391
12-23-1973	20	1183	56.9	.0421
12-24-1973	21	1240	59.7	.0432
12-25-1973	22	1305	62.8	.0450
12-26-1973	23	1371	66.0	.0469
12-27-1973	24	1446	69.6	.0496
12-28-1973	25	1486	71.5	.0502
12-29-1973	26	1605	77.2	.0569
12-30-1973	27	1633	78.6	.0571
12-31-1973	28	1675	80.6	.0586
1-1-1974	29	1715	82.5	.0602
1-2-1974	30	1747	84.1	.0612
1-3-1974	31	1779	85.6	.0625
1-4-1974	32	1812	87.2	.0642

Table 4. Continued.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
1-5-1974	33	1847	88.9	.0666
1-6-1974	34	1858	89.4	.0660
1-7-1974	35	1872	90.1	.0660
1-8-1974	36	1897	91.3	.0581
1-9-1974	37	1903	91.6	.0669
1-11-1974	39	1930	92.9	.0677
1-12-1974	40	1939	93.3	.0676
1-13-1974	41	1945	93.6	.0670
1-14-1974	42	1952	93.9	.0667
1-15-1974	43	1954	94.0	.0614
1-16-1974	44	1948	94.2	.0648
1-17-1974	45	1961	94.4	.0639
1-18-1974	46	1961	94.4	.0625
1-19-1974	47	1963	94.5	.0616
1-20-1974	48	1964	94.6	.0607
1-21-1974	49	1965	94.6	.0594
1-22-1974	50	1966	94.6	.0584
1-23-1974	51	1967	96.7	.0574
1-24-1974	52	1970	94.8	.0569

Table 5. Mortality rate of mountain whitefish eggs, collected during the late spawning season and incubated at 10 C.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
11-30-1973	0	-	-	-
12-10-1973	10	117	4.7	.0048
12-10-1973	12	259	10.3	.0091
12-13-1973	13	523	20.9	.0180
12-14-1973	14	735	29.3	.0248
12-15-1973	15	769	30.7	.0244
12-16-1973	16	909	36.2	.0281
12-17-1973	17	1033	41.2	.0312
12-18-1973	18	1118	44.6	.0328
12-19-1973	19	1213	48.4	.0348
12-20-1973	20	1271	50.7	.0354
12-21-1973	21	1358	54.2	.0372
12-22-1973	22	1439	57.4	.0388
12-23-1973	23	1513	60.4	.0402
12-24-1973	24	1571	62.7	.0410
12-25-1973	25	1619	64.6	.0415
12-26-1973	26	1660	66.2	.0417
12-27-1973	27	1715	68.4	.0427
12-28-1973	28	1757	70.1	.0431
12-29-1973	29	1800	71.8	.0436
12-30-1973	30	1819	72.6	.0431
12-31-1973	31	1849	73.7	.0431
1-1-1974	32	1914	76.3	.0450
1-2-1974	33	1975	78.8	.0470
1-3-1974	34	2057	82.0	.0505
1-4-1974	35	2196	87.6	.0596
1-5-1974	36	2272	90.6	.0302
1-6-1974	37	2375	94.7	.0796
1-7-1974	38	2480	98.9	.1192
1-8-1974	39	2507	100.0	-

Table 6. Mortality rate of mountain whitefish eggs, collected during the later spawning season and incubated at 10 C.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
12-3-1973	0	-	-	-
12-10-1973	7	98	5.8	.0085
12-11-1973	8	310	18.3	.0253
12-12-1973	9	628	37.1	.0516
12-13-1973	10	695	41.1	.0530
12-15-1973	12	769	45.5	.0506
12-16-1973	13	783	46.3	.0479
12-17-1973	14	799	47.3	.0457
12-18-1973	15	829	49.0	.0450
12-19-1973	16	862	51.0	.0446
12-20-1973	17	886	52.4	.0437
12-21-1973	18	911	53.9	.0430
12-22-1973	19	942	55.7	.0429
12-23-1973	20	960	56.8	.0420
12-24-1973	21	983	58.2	.0415
12-25-1973	22	1009	59.7	.0413
12-26-1973	23	1031	61.0	.0409
12-27-1973	24	1061	62.8	.0412
12-28-1973	25	1082	64.0	.0409
12-29-1973	26	1106	65.5	.0409
12-30-1973	27	1123	66.4	.0404
12-31-1973	28	1148	67.9	.0406
1-1-1974	29	1169	69.2	.0406
1-2-1974	30	1210	71.6	.0420
1-3-1974	31	1239	73.3	.0426
1-4-1974	32	1260	74.5	.0428

Table 6. Continued.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
1-5-1974	33	1294	76.6	.0440
1-6-1974	34	1311	77.6	.0440
1-7-1974	35	1378	81.5	.0483
1-9-1974	37	1421	84.1	.0497
1-10-1974	38	1459	86.3	.0524
1-11-1974	39	1475	87.3	.0529
1-12-1974	40	1491	88.2	.0535
1-13-1974	41	1517	89.8	.0556
1-14-1974	42	1529	90.5	.0560
1-15-1974	43	1546	91.5	.0573
1-16-1974	44	1563	92.5	.0588
1-17-1974	45	1573	93.1	.0593
1-18-1974	46	1580	93.5	.0594
1-19-1974	47	1582	93.6	.0585
1-21-1974	49	1598	94.5	.0594
1-22-1974	50	1606	95.0	.0600
1-24-1974	52	1617	95.7	.0604
1-25-1974	53	1629	96.4	.0627

Table 7. Mortality rate of mountain whitefish eggs, collected during the later spawning season and incubated at 11 C.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
12-3-1973	0	-	-	-
12-14-1973	11	30	3.9	.0036
12-15-1973	12	78	10.2	.0090
12-17-1973	14	120	15.7	.0122
12-18-1973	15	196	25.6	.0197
12-19-1973	16	225	29.4	.0218
12-20-1973	17	242	31.6	.0224
12-21-1973	18	253	33.1	.0223
12-22-1973	19	258	33.7	.0216
12-23-1973	20	267	34.9	.0215
12-24-1973	21	278	36.3	.0215
12-27-1973	24	285	37.2	.0194
12-28-1973	25	295	38.6	.0195
12-29-1973	26	302	39.5	.0193
12-30-1973	27	321	42.0	.0201
12-31-1973	28	342	44.7	.0212
1-1-1974	29	363	47.4	.0222
1-2-1974	30	380	49.7	.0229
1-3-1974	31	411	53.7	.0248
1-4-1974	32	435	56.9	.0263
1-5-1974	33	470	61.4	.0289
1-6-1974	34	506	66.1	.0318
1-7-1974	35	570	74.5	.0390
1-8-1974	36	588	76.9	.0407
1-9-1974	37	628	82.1	.0465
1-11-1974	39	693	90.6	.0606

Table 7. Continued.

Date	Age (days)	Cumulative number of dead eggs	Percentage of total	i
1-12-1974	40	707	92.4	.0645
1-13-1974	41	715	93.5	.0665
1-14-1974	42	725	94.8	.0703
1-15-1974	43	733	95.8	.0738
1-16-1974	44	736	96.2	.0744
1-17-1974	45	737	96.3	.0735
1-18-1974	46	745	97.4	.0792
1-22-1974	50	748	97.8	.0761

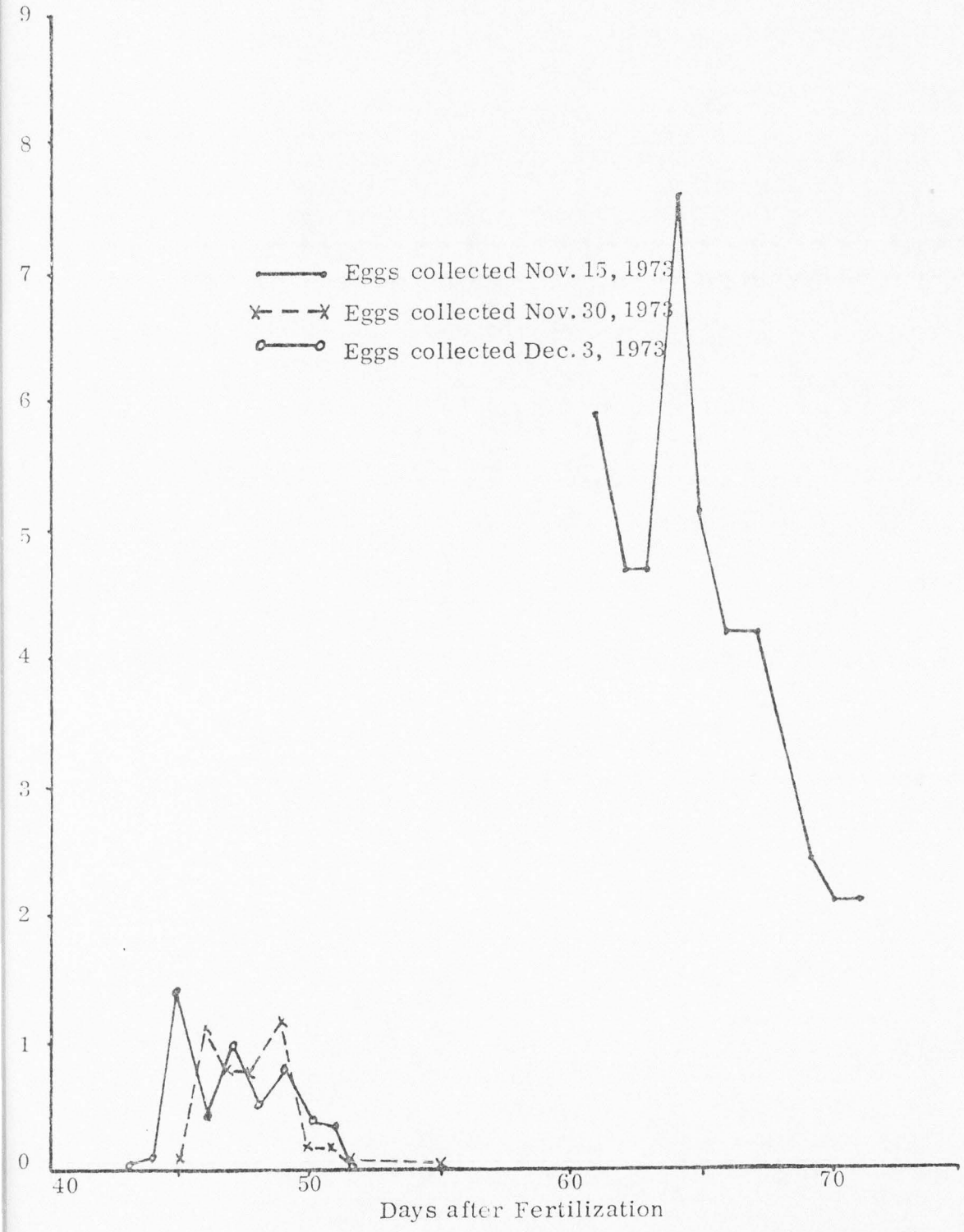


Figure 5. Percentage hatch and duration of hatching period of mountain whitefish larvae from eggs taken during peak and late spawning periods and incubated at 9 C.

Table 8. Mortality of mountain whitefish eggs incubated at different temperatures.

Date	Days after fertilization	12 C			10 C			9 C			6 C		
		Total number of eggs = 890			Total number of eggs = 686			Total number of eggs = 890			Total number of eggs = 928		
		Number of eggs dead	Cumulative total eggs dead	Percentage mortality	Number of eggs dead	Cumulative total eggs dead	Percentage mortality	Number of eggs dead	Cumulate total eggs dead	Percentage mortality	Number of eggs dead	Cumulative total eggs dead	Percentage mortality
11/22/74	1	0	0	0	30	30	4.4	0	0	0	0	0	0
11/27/74	6	200	200	22.5	0	30	4.4	6	6	0.7	0	0	0
11/28/74	7	89	289	32.5	0	30	4.4	0	6	0.7	0	0	0
11/29/76	8	211	500	56.2	10	40	5.8	0	6	0.7	0	0	0
11/30/74	9	109	609	68.4	57	97	14.2	0	6	0.7	0	0	0
12/1/74	10	203	812	91.2	194	291	42.5	6	12	1.3	4	4	0.4
12/3/74	12	67	879	98.8	51	342	49.8	9	21	2.4	3	7	0.8
12/4/74	13	4	883	99.2	73	415	60.7	25	46	5.2	4	11	1.2
12/5/74	14	7	890	100.0	40	455	66.5	18	64	7.2	0	11	1.2
12/6/74	15				18	473	69.2	20	84	9.4	1	12	1.3
12/7/74	16				15	488	71.3	10	94	10.6	1	13	1.4
12/8/74	17				5	493	72.1	12	106	11.9	0	13	1.4
12/9/74	18				4	497	72.7	79	185	20.8	0	13	1.4
12/10/74	19				3	500	73.1	0	185	20.8	1	14	1.5
12/11/74	20				2	502	73.4	74	259	29.1	3	17	1.8
12/13/74	22				12	514	75.1	25	284	31.9	6	23	2.5
12/17/74	26				0	514	75.1	0	284	31.9	8	31	3.3
12/18/74	27				2	516	75.4	17	301	33.8	10	41	4.6
12/20/74	29				10	526	76.9	0	301	33.8	0	41	4.6
12/23/74	32				14	540	78.9	0	301	33.8	17	58	6.2
12/26/74	35				21	561	82.0	0	301	33.8	16	72	7.8
12/27/74	36				21	582	85.1	5	306	34.4	9	81	8.7
12/29/74	38				20	602	88.0				0	81	8.7
12/30/74	39				0	602	88.0				4	85	9.2
1/1/75	41				14	616	90.0				0	85	9.2
1/10/75	50				10	626	91.5				0	85	9.2

gure 6. Percentage of total hatch and duration of hatching of mountain whitefish eggs collected during 1974 peak spawning period and incubated at 6 C.

$$i = \frac{\log_e N_o - \log_e N_t}{t}$$

where N_o = number of eggs at the start of the period; N_t = the number at the end of the period and t = number of days in period.

Abnormalities

None of the larvae which hatched at 11 C survived for more than seven days. Of those which hatched at 10 C and 9 C, 17.2% and 22% respectively, were abnormal. Abnormalities observed in order of decreasing frequency were jaws which were agape (Figure 7a), coloboma or fissure of eye (Figure 7b), monophthalmia or the presence of only one eye and monomicrophthalmia or the smallness of one eye (Figure 7c). One case of twinning was observed at 9 C. The twins had a common yolk sac and a common peduncle and caudal fin (Figure 7d). Total length of the twin larvae was 8 mm compared to the normal larva which was 13 mm in total length (Figure 7c). The larvae which had jaws which were agape were not able to feed. They became thinner by reabsorption of body tissue and finally died. Some had abnormalities like agape jaw and coloboma in one and the same individual (Figure 7f).

Energy Expenditure - Dry Weight Method

The mean of four calorimetric determinations of the energy content of the unfertilized ova taken from ripe females was 5793 $\pm$ 8.6 cal/gm. This included the chorion. In mountain whitefish the chorion is hard and tough and made up

Figure 7. Abnormalities produced by thermal pollution

Figure 7a. Agape jaws in larva incubated at 9 C

Figure 7b. Cologoma or fissure of eye in larva incubated at 9 C

Figure 7c. Monomicrophthalmia in larva incubated at 9 C

Figure 7d. Twinning in larva incubated at 9 C, total length 8 mm

Figure 7e. Larva of normal length incubated at 9 C, total length 13 mm

Figure 7f. Agape jaws and coloboma in larva incubated at 10 C

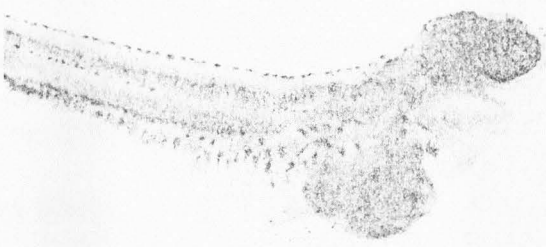
a

b

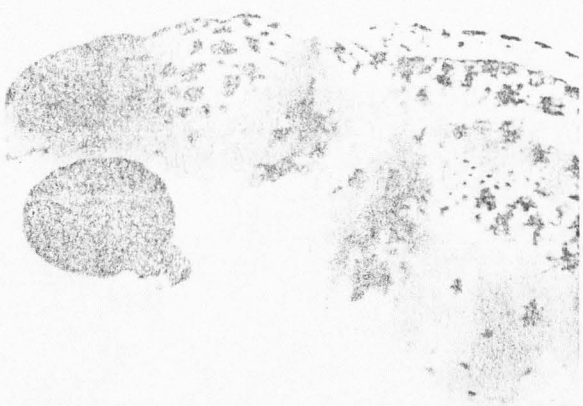
c

d

e



f



14.6% of the total dry weight of the egg. The weight of the chorion is assumed to be constant throughout and has been deducted from the weight of the egg to give the weight of the yolk and the available yolk energy in calories. Energy value of eggs, frozen soon after fertilization and later freeze dried, was also determined. The mean of four calorimetric determinations gave a value of 5787 $\pm$ 44.8 cal/gm. These values were not significantly different. Hence for the determination of energy content available to the developing embryo it will suffice if estimations are made utilizing ripe unfertilized ova. The mean of four determinations of the energy value of larvae after there was no visible yolk in the yolk sac was 5258 $\pm$ 20 cal/gm.

The energy utilization of the developing embryo was approximated by comparing the energy content of the embryo with that in the yolk sac from the time of fertilization till yolk sac absorption, following the procedure employed by Lasker (1962), Toetz (1966) and Laurence (1969, 1973). The average energy budget for a simple mountain whitefish larva, using the dry weight method, at 6 C and 9 C was estimated (Table 9 and 10). From the volume of yolk calculated at different time intervals (Table 9 and 10, Column A), the percent of yolk remaining at the respective time intervals was noted (Tables 9 and 10, Column B). From the weight of the embryo or larva plus yolk, determined at different time intervals during development (Tables 9 and 10, Comumn C), the percentage of embryo or larva plus yolk remaining was determined (Tables 9 and 10, Column D). The weight of the yolk in mg was the product of the percentage yolk remaining at different time intervals and 3.97 mg, the original dry weight of the fertilized egg

less the dry weight of the chorion (Tables 9 and 10, Column E). The percent dry weight of embryo or larval tissue was obtained by subtracting the percent yolk remaining at any particular time from the percent embryo or larva plus yolk remaining at the corresponding period (Tables 9 and 10, Column F). The dry weight of the embryo or larval tissue was the product of the percent embryo or larval tissue at any particular period and 3.97 mg, the original dry weight of the fertilized egg minus the dry weight of the chorion (Tables 9 and 10, Column G). Yolk energy in calories available at any time interval during development was obtained by multiplying the yolk weight during the corresponding period by 5793 calories per gm, the caloric value of the yolk at the start of development (Tables 9 and 10, Column H). Embryo or larval energy was obtained by multiplying the dry weight of embryo or larval tissue by 5298 calories per gm, the caloric value of the larva after there was no visible yolk in the yolk sac (Tables 9 and 10, Column I). Energy sum in calories was obtained by summing the yolk energy and embryo or larval energy (Tables 9 and 10, Column J). Energy loss in calories was the difference of energy sums between successive time intervals (Tables 9 and 10, Column K).

At 6 C the yolk completely disappeared by about 120 days and at 9 C by about 70 days (Figure 8). Though theoretically the larvae could survive up to that period and beyond by reabsorption of tissue, most of the larvae did not feed even when provided with abundant food, if the exogenous food was supplied after 50% of the remaining yolk was absorbed. They reached a stage, which has been termed "point of no return" or "PNR" by Blaxter and Hempel (1963). However,

Table 9. Average energy budget for a single mountain whitefish embryo or larva using the dry weight method at 6 C.

Time interval (days)	A Yolk volume (mm^3)	B Percent yolk Remaining	C Embryo or larva plus yolk dry weight (mg)	D % Initial weight of embryo or larva plus yolk remaining	E Yolk weight (mg) Column B X 3.97 mg	F Percent dry weight larval tissue (Column D - Column B)	G Dry weight larval tissue (mg) Column F X 3.97 mg	H Yolk energy (calories) Column E X 5793 cal/g	I Embryo and Larval energy Column G X 5298 cal/g	J Energy sum (calories)	K Energy loss (calories)
0	16.36	100	3.97	100	3.97	0	0	23.00	0	23.00	0
19	15.25	93.2	3.91	98.5	3.70	5.3	0.21	21.43	1.11	22.54	0.46
30	8.85	54.1	3.86	97.2	2.15	43.1	1.71	12.45	9.06	21.51	1.03
40	7.67	46.9	3.85	97.0	1.86	50.1	1.99	10.77	10.54	21.31	0.20
57	5.21	31.8	3.66	92.2	1.26	60.4	2.40	7.30	12.72	20.02	1.29
77 (hatched)	1.76	10.8	2.35	59.2	0.43	48.4	1.92	2.84	10.17	13.01	7.01
81	1.61	9.8	2.21	55.7	0.39	45.9	1.82	2.26	9.64	11.90	1.11
92	1.50	9.2	1.70	43.0	0.36	33.8	1.34	2.08	7.10	9.18	2.72

Table 10. Average energy budget for a single mountain whitefish embryo or larva using the dry weight method at 9 C.

Time interval (days)	A Yolk volume (mm^3)	B Percent Yolk	C Embryo plus yolk or larva plus yolk dry weight (mg)	D Percent embryo or larva plus yolk	E Yolk weight (mg) Column B X 3.97	F Percent dry weight embryo or larva tissue (Column D-Column B)	G Dry weight embryo or larval tissue (mg) Column F X 3.97 mg	H Yolk energy (calories) Column E X 5793 cal/g	I Embryo or larval energy Column 6 X 5298 cal/g	J Energy sum (calories) Column H X Column I	K Energy loss (calories)
0	16.36	100	3.97	100	3.97	0	0	23.00	0	23.00	0
7	11.99	73.3	3.95	99.5	2.91	26.2	1.04	16.86	5.51	22.37	0.63
16	10.88	66.5	3.91	98.5	2.64	32.0	1.27	15.29	6.73	22.02	0.35
26	6.98	42.7	3.83	96.5	1.70	53.8	2.14	9.85	11.34	21.19	0.83
33	5.99	36.6	3.75	94.4	1.45	57.8	2.29	8.40	12.13	20.53	0.66
42	4.23	25.9	3.49	87.9	1.03	62.0	2.46	5.97	13.03	19.00	1.53
49 (hatched)	2.72	16.6	2.77	69.8	0.66	53.2	2.11	3.82	11.18	15.00	4.00
75	0.05	0 .3	2.03	51.1	0.01	50.8	2.01	0.06	10.65	10.71	4.29

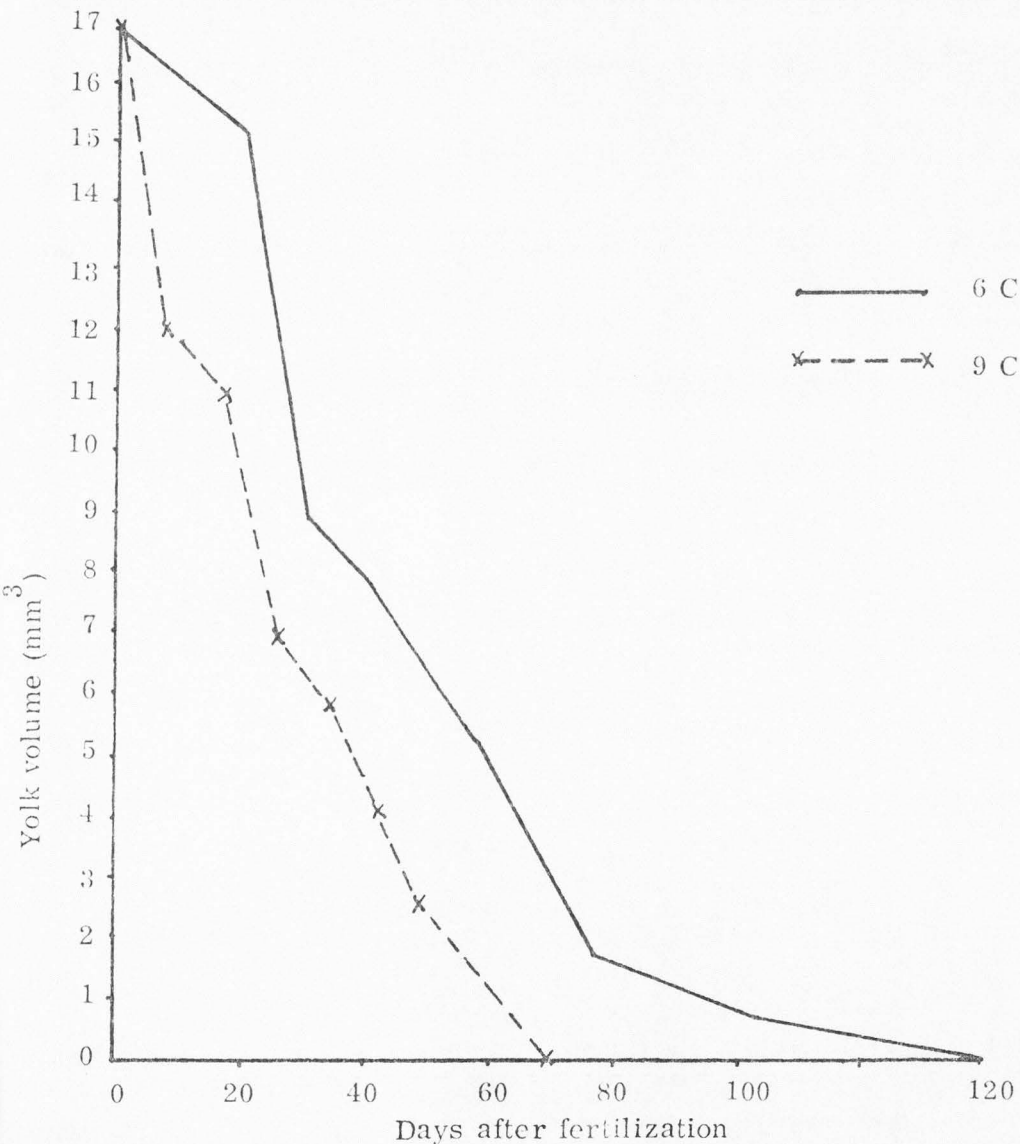


Figure 8. Decrease in yolk volume with development in mountain whitefish eggs.

the larvae were capable of feeding soon after hatching at 6 C, as they had fully formed jaws (see section on development). The sooner the larvae can start feeding on exogenous sources the better are chances of survival. Hatching started about the 77th day at 6 C and the 49th day at 9 C for eggs collected during the 1973 spawning season. High mortality and abnormality occurred at 9 C; only about 75% of those hatched had normal jaws which were capable of feeding.

Both at 6 C and 9 C the larval dry weight decreased at hatching (Tables 9 and 10, Column G). There was an energy deficit at the time of hatching at 6 C and at 9 C (Tables 9 and 10, compare columns H and K). This energy deficit could be reversed if the larvae could immediately start feeding. Otherwise further absorption of larval tissue and remaining yolk takes place until reaching the "point of no return."

The average dry weight loss of mountain whitefish embryo or larval tissue plus yolk sac during absorption (Figure 9) was slow halfway through development and then the loss became steeper during the latter half of development both at 6 C and 9 C.

The tissue dry weight gain at 6 C (Figure 10) was slow until about 20 days of development, then the gain became steeper, corresponding to a steep decline in yolk dry weight. The weight of the embryo was greatest a few days before hatching. At hatching the dry weight of the larva was less than its dry weight a few days before hatching, perhaps due to the greater activity of the embryo in its efforts to break through the chorion. Though there was adequate

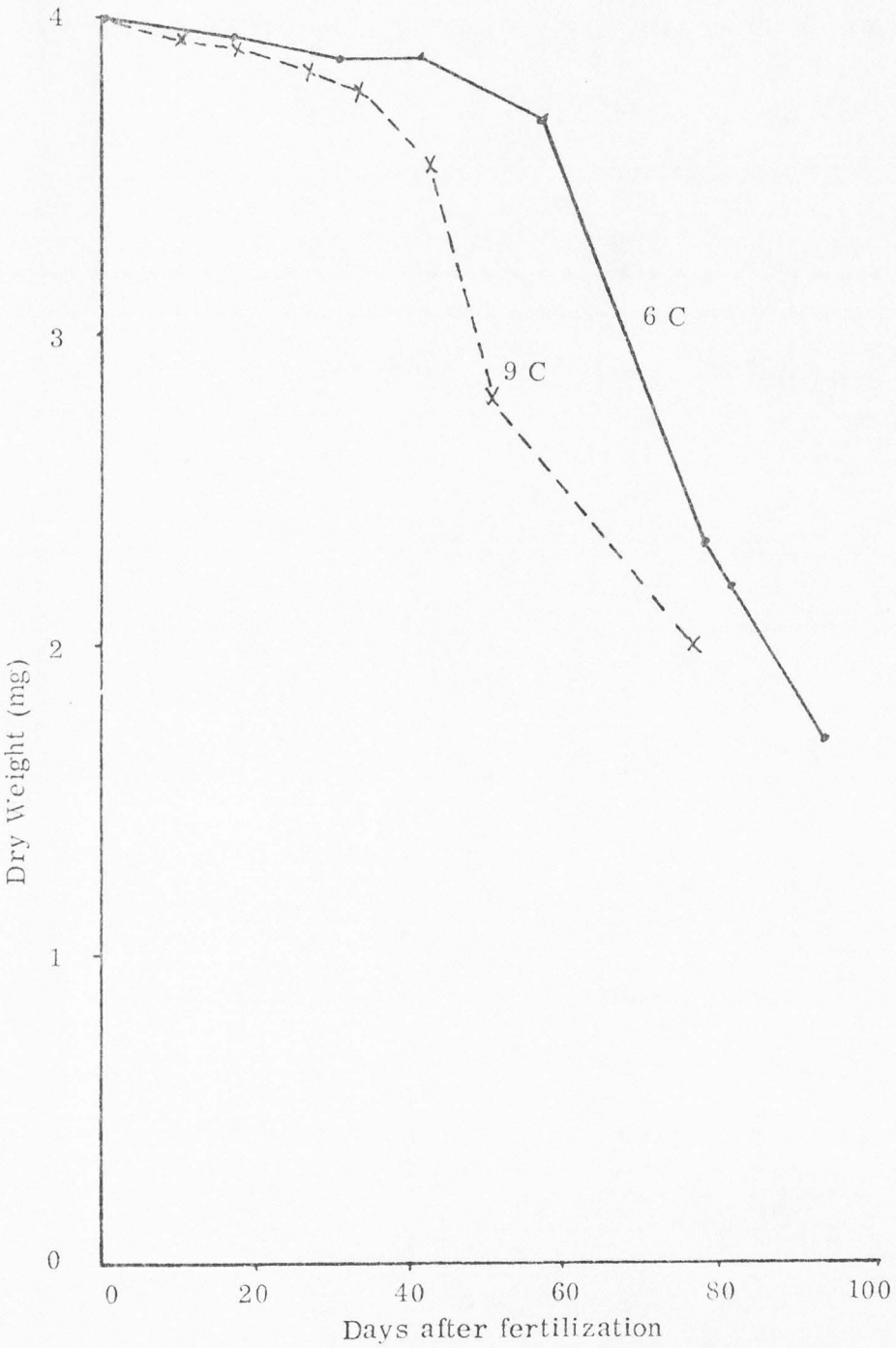


Figure 9. Average dry weight loss of mountain whitefish embryo or larva plus yolk sac during yolk absorption.

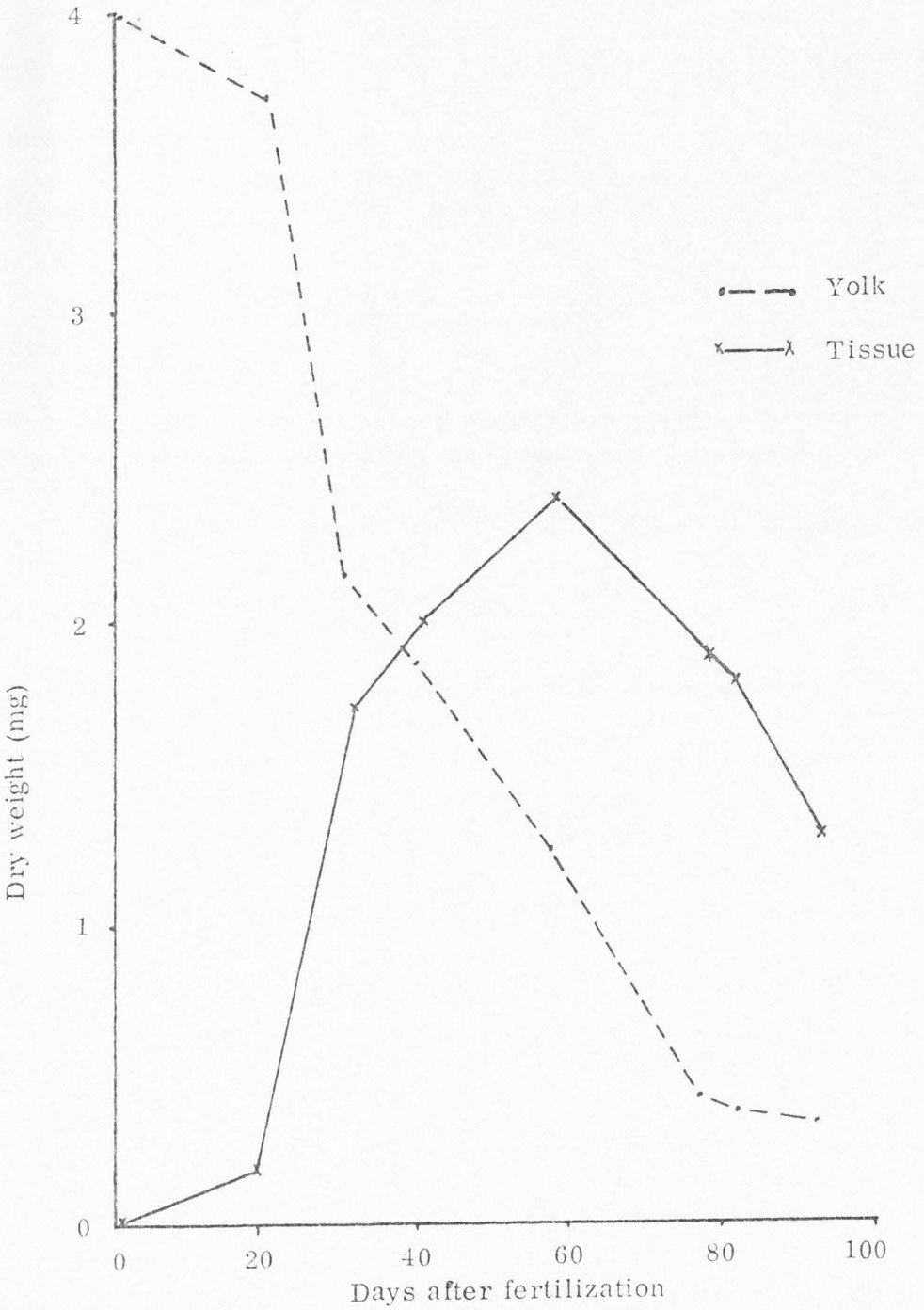


Figure 10. Average tissue dry weight gain and yolk dry weight loss of mountain whitefish embryo or larva during yolk absorption at 6 C.

yolk energy still remaining, the chemical nature of these reserves may preclude their use for this activity. From hatching on, the larval dry weight steadily declined unless feeding commenced.

At 9 C the tissue dry weight gain was more rapid than at 6 C and there was a corresponding rapid decline in yolk weight loss (Figure 11). The embryo dry weight a few days before hatching was greater than the larval dry weight at hatching, as was also observed at 6 C. After hatching the tissue dry weight began to decrease as it was being reabsorbed for catabolic purposes.

The efficiency of yolk conversion which is defined as:

$$\frac{\text{dry weight increment of body}}{\text{dry weight decrement of yolk}} \times 100$$

(Blaxter, 1970) was 54.2% at 6 C and 63.7% at 9 C during hatching (Tables 11 and 12). On the 57th day of incubation when maximum embryo weight was noticed at 6 C the efficiency of yolk conversion was 88.6% (Table 11). At this temperature the efficiency increased until about the 40th day of incubation, then efficiency declined as development proceeded. At 9 C the maximum embryo weight was noticed on the 42nd day and the efficiency of yolk conversion was 83.7% (Table 12). Yolk conversion was 83.7% (Table 12). Yolk conversion efficiency steadily declined at this temperature also as development proceeded.

Energy Expenditure - Oxygen Consumption Method

The average oxygen consumption of whitefish embryo or larva at 6 C and 9 C $\pm$ S.E. were plotted for the duration of development (Figure 12 and 13).

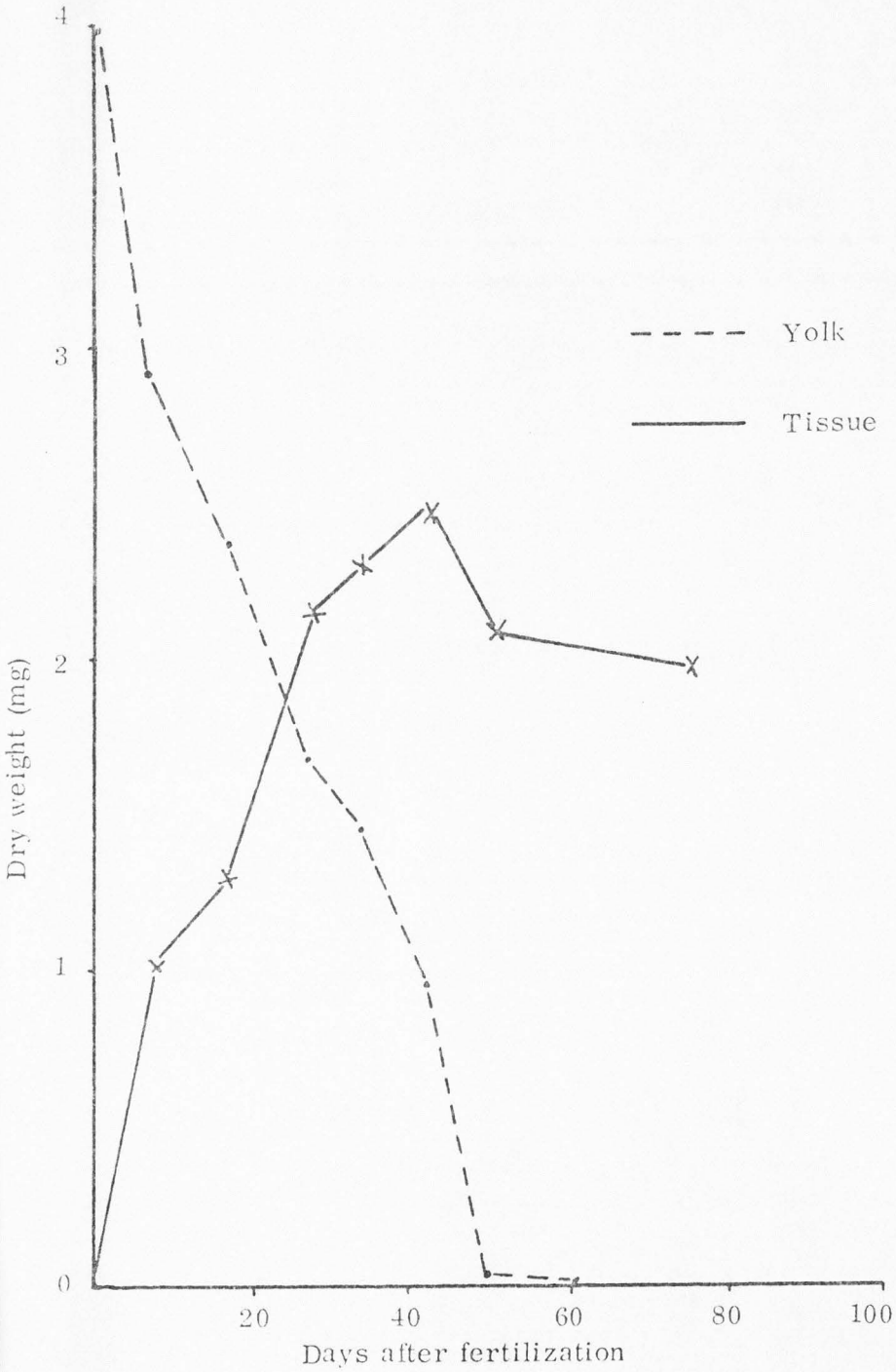


Figure 11. Average tissue dry weight gain and yolk dry weight loss of mountain whitefish embryo or larva during yolk absorption at 9 C.

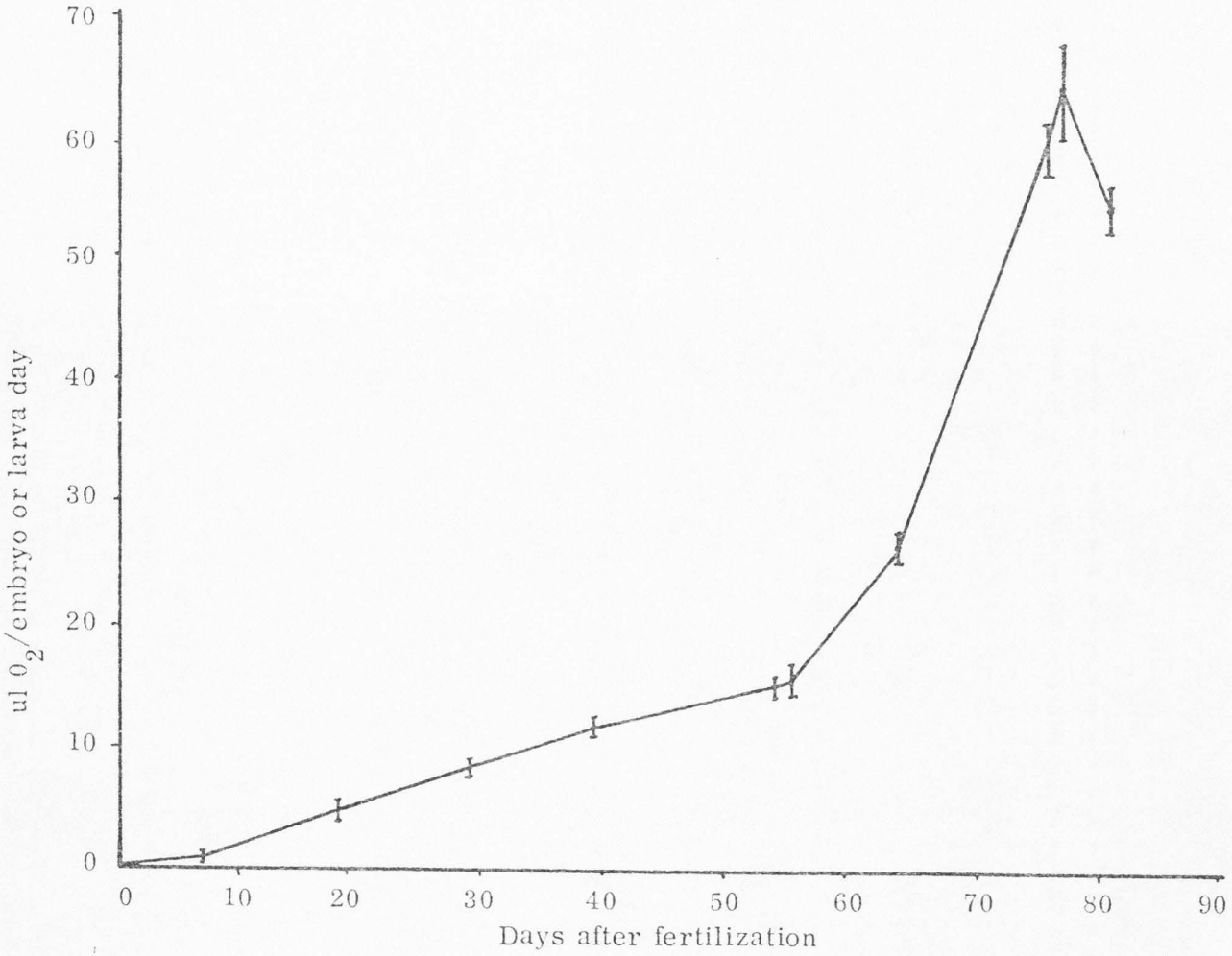


Figure 12. Average oxygen consumption (±SE) of mountain whitefish embryo or larva at 6 C.

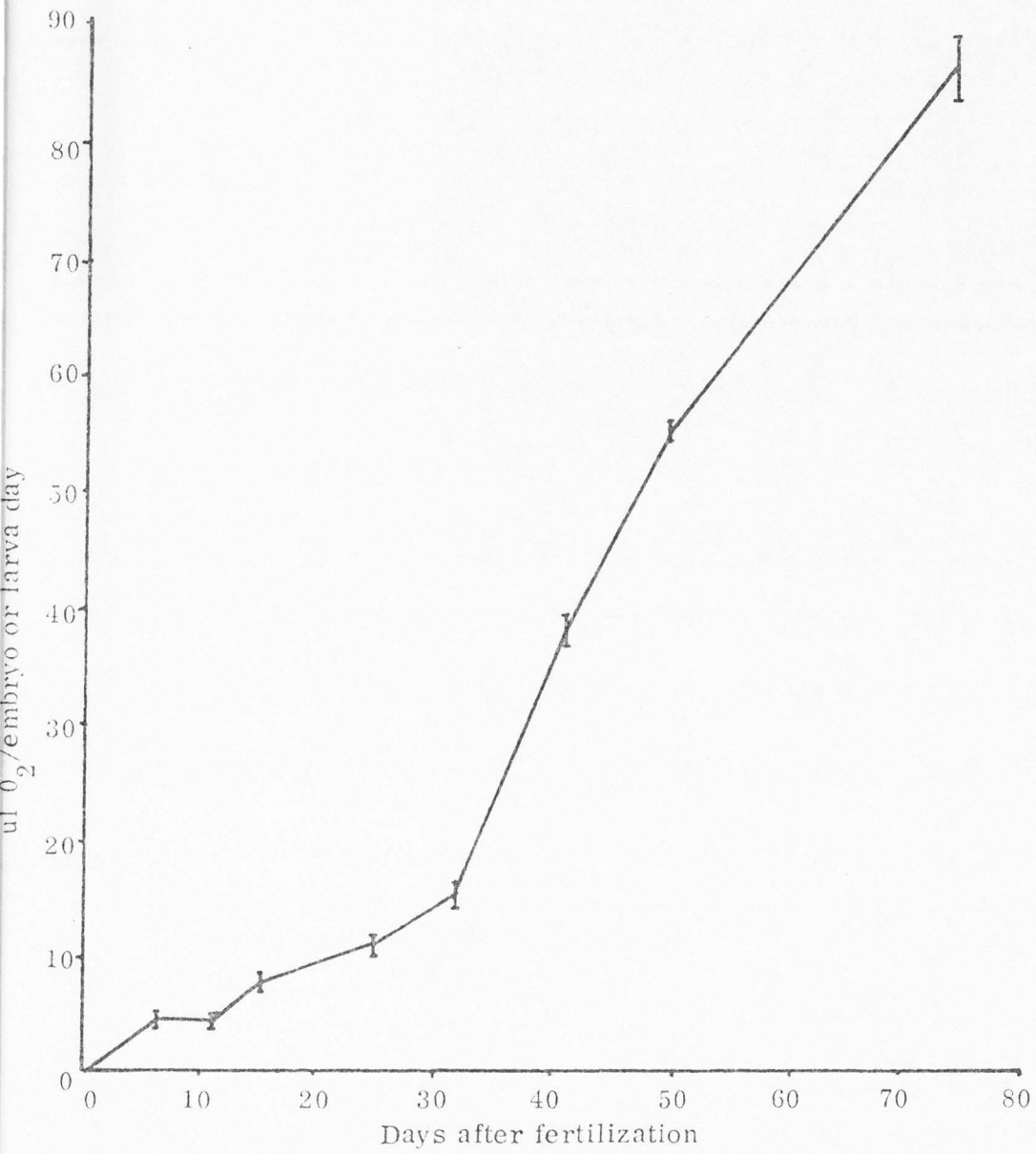


Figure 13. Average oxygen consumption (±SE) of mountain whitefish embryo or larva at 9 C.

able 11. Efficiency of yolk conversion at 6 C.

Time interval (days)	Yolk conversion efficiency
0	0
19	77.8%
30	94.0%
40	94.3%
57	88.6%
77 (hatched)	54.2%
81	50.8%
92	37.1%

able 12. Efficiency of yolk conversion at 9 C.

Time interval (days)	Yolk conversion efficiency
0	0
7	98.1%
16	95.5%
26	94.3%
33	90.9%
42	83.7%
49 (hatched)	63.7%
75	50.8%

Each point represents the mean of 10 to 15 determinations. The mean values were joined by straight lines. From these curves the total amount of oxygen consumed up to any time period was obtained by integration. The oxygen consumption values were converted into calories by multiplying by 0.005, utilizing the oxycaloric coefficient of 4.77 cal/100 ul 0_2 (Blaxter, 1970). The embryo or larval dry weight increment for the corresponding time interval was obtained. Larval energy increment was the product of the embryo or larval dry weight increment and 5298 (calories per gm), the caloric value of larva after all visible yolk in the yolk sac were observed. Sum of embryo or larva energy and oxygen consumption energy was obtained by adding the two values for any time interval (Tables 13 and 14).

At 6 C the oxygen consumption method showed an energy deficit on the day of hatching (77th day). But on the 81st day there was no apparent energy deficit (compare columns A and G, Table 13), and the larvae had already started to utilize energy by reabsorption of its body tissue, as evidenced in the decrease in dry weight.

The oxygen consumption method showed no deficit prior to or during hatching at 9 C (compare columns A and G, Table 14), but the larvae had already started to utilize energy by reabsorption of its tissue.

Average cumulative oxygen consumption until hatching (77th day) at 6 C was 1166 ul (Table 15). Oxygen consumption was at its maximum during hatching then declined as the larva became less active and rested on the bottom with only occasional swimming.

Table 13. Average energy budget for a single mountain whitefish embryo or larva using the oxygen consumption method at 6 C.

Time interval days	A Yolk energy (cal)	B Oxygen Consumption ul/larva time interval	C Oxygen energy (cal) (0.005 cal X Column B)	D Larval dry weight (mg)	E Larval dry weight Increment (mg)	F Larval energy (cal) 5298 cal X Column E)	G Sum larval and oxygen Consumption energy (cal)
0	23.00	0	0	0	0	0	0
19	21.43	29.50	0.1475	0.21	0.21	1.1126	1.2601
30	12.45	66.25	0.3312	1.71	1.50	7.9470	8.2782
40	10.77	103.00	0.5150	1.99	0.28	1.4834	1.9984
57	7.30	240.00	1.2000	2.40	0.41	2.1722	3.3722
77 (hatched)	2.84	727.75	3.6388	1.92			3.6388
81	2.26	233.75	1.1688	1.82			1.1688

Table 14. Average energy budget for a single mountain whitefish embryo larva using the oxygen consumption method at 9 C.

Time interval (days)	A Yolk energy (calories)	B Oxygen Consumption ul/embryo or larva time interval	C Oxygen Energy (calories) 0.005 cal X Column B	D Embryo or larval dry weight (mg)	E Embryo or larval dry weight Increment (mg)	F Embryo or larval energy (calories) 5298 calories X Column E	G Sum Embryo or larval and oxygen consumption energy (calories)
0	23.00	0	0	0	0	0	0
7	16.86	19.00	0.0950	1.04	1.04	5.5099	6.5499
16	15.29	49.75	0.2488	1.27	0.23	1.2185	1.4485
26	9.85	97.00	0.4850	2.14	0.87	4.6093	5.4793
33	8.40	97.25	0.4862	2.29	0.15	0.7947	0.9447
42	5.97	241.00	1.2050	2.46	0.17	0.9007	1.0707
49	3.82	324.50	1.6225	2.11			1.6225
75	0.06	1824.75	9.1238	2.01			9.1238

Table 15. Oxygen consumption of mountain whitefish embryo or larva at 6 C.

Time Interval (days)	Oxygen consumption (ul 0_2/embryo or larva. time interval)	Cumulative oxygen consumption (ul 0_2/embryo or larva. time interval)
0	-	-
19	29.50	29.50
30	66.25	95.75
40	103.00	198.75
57	240.00	438.75
77	727.75	1166.50
81	233.75	1400.25

Table 16. Oxygen consumption of mountain whitefish embryo or larva at 9 C.

Time Interval (days)	Oxygen consumption (ul 0_2/ embryo or larva. time interval)	Cumulative oxygen consumption (ul 0_2/embryo or larva. time interval)
0		
7	19.00	19.00
16	49.75	68.75
26	97.00	165.75
33	97.25	263.00
42	241.00	504.00
49	324.50	828.50
75	1824.75	2653.25

At 9 C mean cumulative oxygen consumption until hatching (49th day) was 828 ul (Table 16). However oxygen consumption did not drop after hatching. Oxygen uptake continued to increase as the larvae were very active and swimming around in search of exogenous food materials, usually within 24 hours after hatching.

Comparison of Electrochemical and Manometric Methods of Measuring Oxygen Consumption of Fish Embryo

The results are given in Table 17 and Figure 14. The r^2 between two methods was 0.9348. The mean using the electrochemical method was 0.3459 ul 02/egg. hr. and using the manometric method the mean was 0.3256 ul 02/egg. hr.

Active metabolism

Ninety mountain whitefish fry were used for active metabolism measurements at the four different temperatures (6 C, 9 C, 12 C, and 15 C) (Table 18). Some preliminary measurements were made using only one fry in the flask and some using three fry in the flask to see if there were any group effect. The results show there was no statistical difference between individuals tested individually and in groups of three ($t=1.32$, d.f=5). So results obtained using individual fry and fry in groups of three have been pooled to obtain the regression of $\log_e$ weight of fish versus $\log_e$ oxygen uptake (mg 0_2/hr.). The slopes varied from 0.6265 to 0.8790 and were not significantly different from their mean of 0.7548

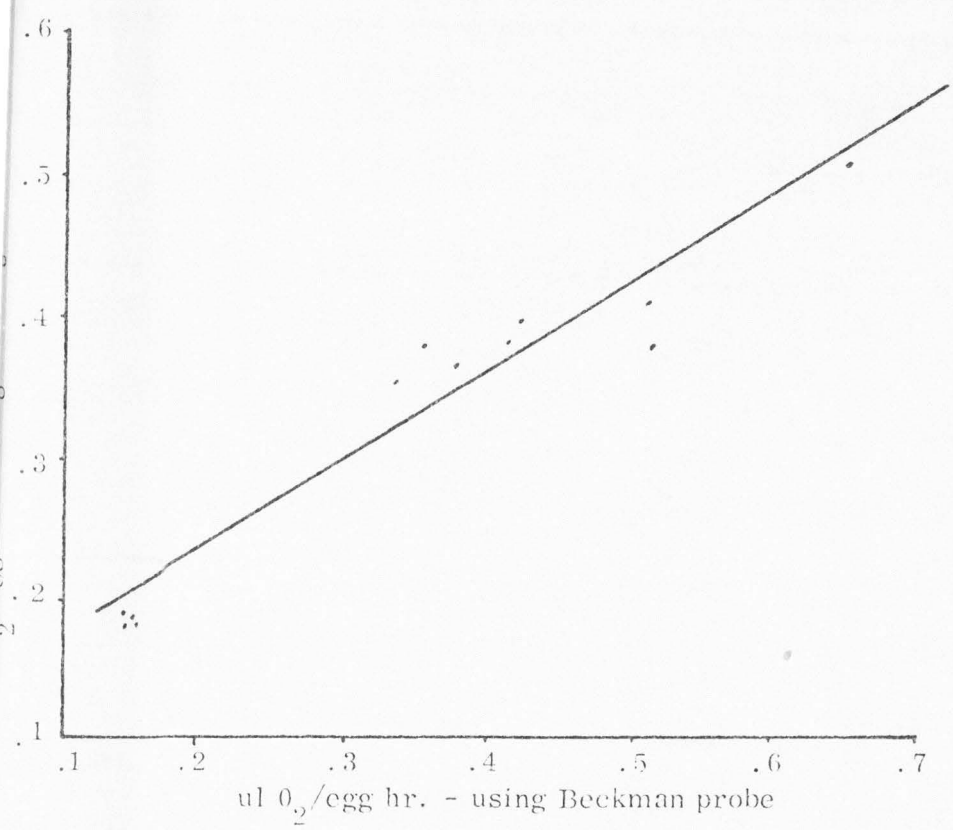


Figure 14. Comparison of electrochemical and manometric methods of measuring oxygen consumption of mountain whitefish embryos.

Table 17. Oxygen consumption of identical stages of embryos measured using manometric and electrochemical methods.

Number	Date	Using Warburg ul 0_2/egg hr	Using Beckman probe ul 0_2/egg hr
1.	12/6/74	0.1845	0.1454
2.	12/7/74	0.1848	0.1454
3.	12/9/74	0.1841	0.1454
4.	12/12/74	0.1831	0.1454
5.	12/16/74	0.3532	0.3331
6.	12/17/74	0.3664	0.3739
7.	12/19/74	0.3843	0.5111
8.	12/20/74	0.3800	0.4121
9.	12/27/74	0.3795	0.3490
10.	12/28/74	0.3946	0.4235
11.	12/30/74	0.4052	0.5122
12.	1/12/75	0.5069	0.6544
		Mean = 0.32555	Mean = 0.34591

(Table 18). A multiple regression of $\log_e$ active metabolic rate, temperature and $\log_e$ wet weight gave the following equation with an r^2 of 0.80

$$\log_e M_A = -1.2269 + 0.0329\ T + 0.8156 \log_e W$$

where M_A = active metabolic rate in mg 0_2/hr, T = temperature C, and W = wet weight of fish in gm.

The active metabolic rates were corrected for a fish 0.1772 gm in weight (Table 18, Figure 15) which is the mean weight for all the fry used in all the active and standard metabolism experiments. At 6 C and 15 C the maximum weight of fry used was slightly less than the grand mean weight and the values were obtained by extrapolation.

Table 18. Active oxygen consumption of mountain whitefish fry.

Temperature (C)	Number of experiments	Total length (mm)	Wet weight (gm)	Oxygen consumption mgO_2/hr (corrected for a 0.1772 gm fish)
6	11	16-29	0.0194-0.1492	0.0781
9	12	19-32	0.0264-0.2052	0.1094
12	9	18-37	0.0323-0.3400	0.1123
15	10	21-30	0.0599-0.1410	0.0985

Standard metabolism

Activity indices and oxygen uptake rate were determined at the four temperatures and the standard metabolism (Table 20) was obtained by extrapolating to zero activity a regression of oxygen uptake versus activity index as described by Dickson (1968). The slopes of the regression lines for $\log_e$ weight

Table 19. Regression equations of active metabolism and body weight of mountain whitefish fry at different temperatures

Temperature (C)	Regression equation
6	$\log_e M_A = -1.2875 + 0.7748 \log_e W$
9	$\log_e M_A = -1.2029 + 0.6265 \log_e W$
12	$\log_e M_A = -0.5764 + 0.8790 \log_e W$
15	$\log_e M_A = -1.0513 + 0.7389 \log_e W$

b values not different from mean: 0.7548

and $\log_e$ mg 0_2/hr varied from 0.8649 at 6 C to 1.3112 at 12C; but all b values were not significantly different from their mean of 1.0699 (Table 20). A multiple regression as described before for the active metabolism rates gave the following equation with an $r^2 = 0.83$

$$\log_e M_S = -3.3471 + 0.1538\ T + 0.8854 \log_e W$$

where M_S = standard metabolic rate in mg0_2/hr, T = temperature ^{o}C, and W = wet weight of fish in gm.

The standard metabolism rates are also corrected for a fish 0.1772 gm weight for purposes of comparison (Table 20, Figure 15). The standard metabolic rate gradually increased from 6 C to 12 C then there was a sharp rise at 15 C.

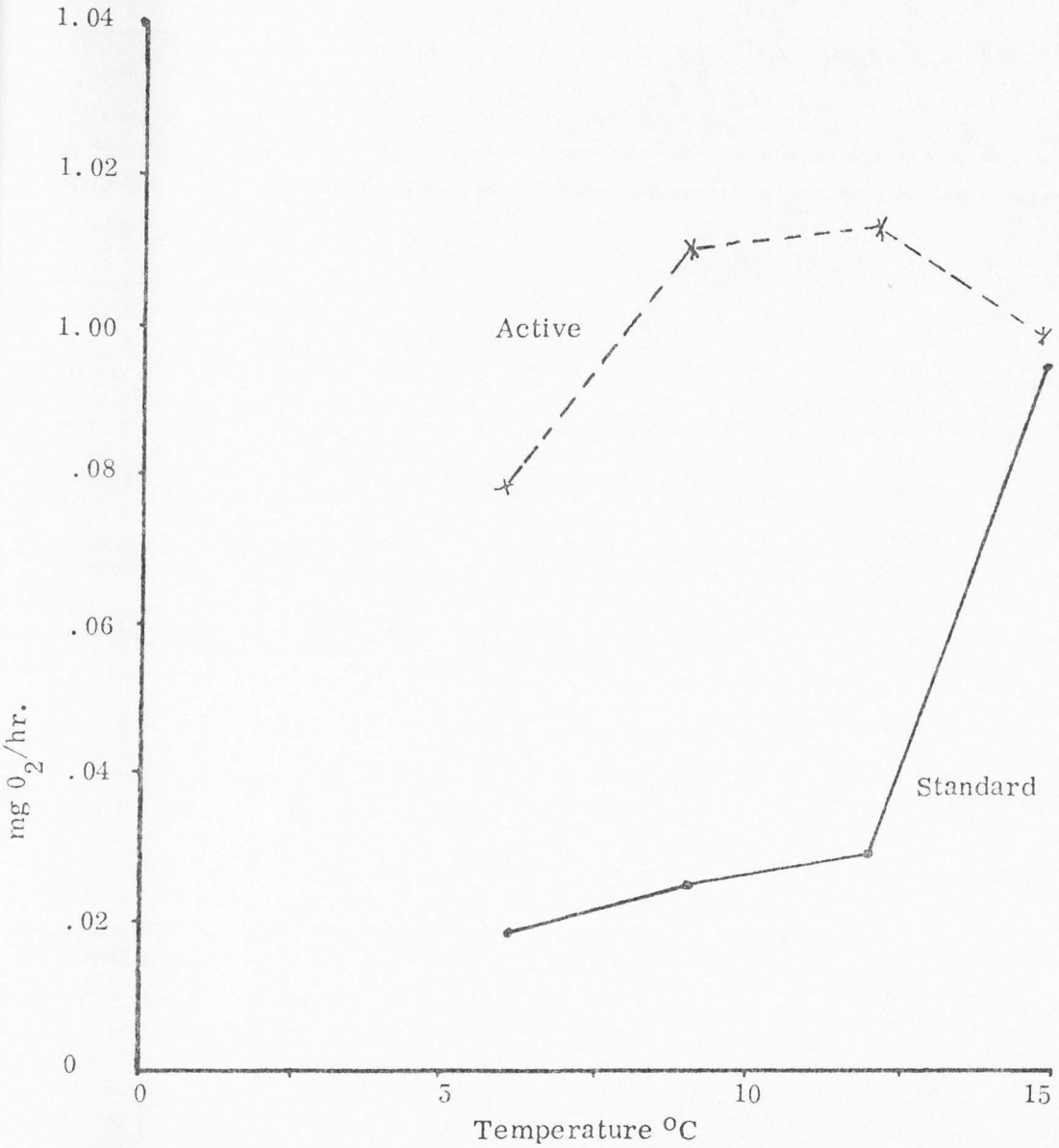


Figure 15. Oxygen consumption of mountain whitefish under yearlings in relation to temperature, based on data corrected for a 0.1772 gm fish.

Table 20. Standard oxygen consumption of mountain whitefish fry.

Temperature	Number of experiments	Total length (mm)	Wet weight (gm)	Oxygen consumption mgO_2/hr corrected for a 0.1772 gm fish
6	7	27-50	0.1025-1.0215	0.0195
9	10	34-60	0.1912-1.2700	0.0254
12	6	28-50	0.1100-1.0243	0.0295
15	11	24-35	0.0745-0.3652	0.0928

Table 21. Regression equations of standard metabolism and body weight of mountain whitefish fry at different temperatures.

Temperature	Regression equation
6	$\log_e M_S = -2.3468 + 0.8649 \log_e W$
9	$\log_e M_S = -1.9400 + 0.9346 \log_e W$
12	$\log_e M_S = -1.5632 + 1.3112 \log_e W$
15	$\log_e M_S = -0.3776 + 1.1688 \log_e W$

b values not different from mean 1.0699

Scope for activity

Scope for activity which is the difference between active and standard metabolic rates (Fry, 1947) increased from 6 C to 9 C then there was a slight decline at 12 C and dropped to a very low level at 15 C (Figure 16).

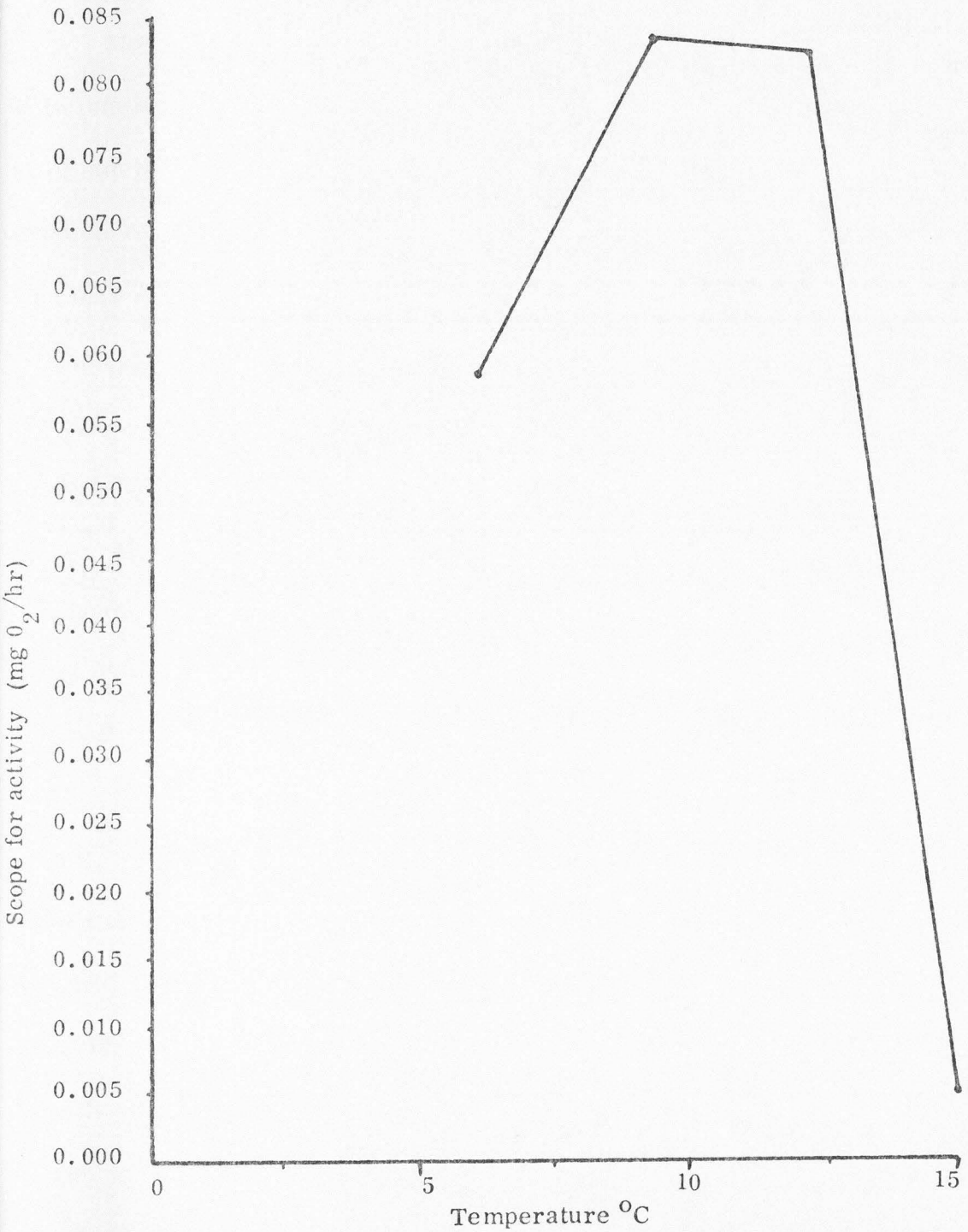


Figure 16. Scope for activity of mountain whitefish underyearlings at different temperatures

Development of embryos

Mountain whitefish eggs were round and dark yellow in color and averaged 3.16 mm in diameter, the range in the sample taken from two females were from 3.10 mm to 3.24 mm. A mature mountain whitefish egg has a cluster of oil droplets at one end which is termed the animal pole. The perivitelline space was less evident in the unfertilized eggs and the chorion was smooth. If unfertilized eggs are placed in water the process of absorption of water in most of the eggs continues up to 24 hours. The chorion became weaker and the eggs almost doubled in size.

The fertilized egg stops the process of abosrption and swelling in about two hours and the chorion becomes tougher. The perivitelline space becomes more evident (Figure 17a). The yolk rotates inside until the animal pole (Figure 17b and 17c) comes to the top.

The stages described below are from samples taken from eggs incubated at 6 C. The embryonic disc forms over the cluster of oil droplets. The blastodisc divides by increase in cell numbers and decrease in their sizes. By 32.4 thermal units or T.U. (one T.U. equals one degree Fahrenheit above freezing or 32 F for a period of 24 hours) (Table 22). The blastodisc is somewhat more prominently raised up on the yolk (Figure 17d). By 64 T.U. the germinal layers ectoderm, endoderm and mesoderm are formed and epiboly or overgrowth of the yolk sac by the embryonic blastoderm occurs (Figure 17e).

This stage is followed by organogenesis. At about 118 T.U. the embryo is about 3.24 mm in length. By about 20 days at 6 C (216 T.U.) the embryo is

Figure 17. Early stages in the embryology of mountain whitefish (incubated at 6c)

Figure 17a. One hour after fertilization

Figure 17b. Animal pole rotates to the top, 6 hours after fertilization

Figure 17c. Six hours after fertilization (dechrionated)

Figure 17d. Blastodisc raised 3 days after fertilization, 32.4 T.U.

Figure 17e. Germinal layers evident 11 days after fertilization, 118.8 T.U.

Figure 17f. Embryo clearly outlined on the surface of the yolk 20 days after fertilization, 216.0 T.U.

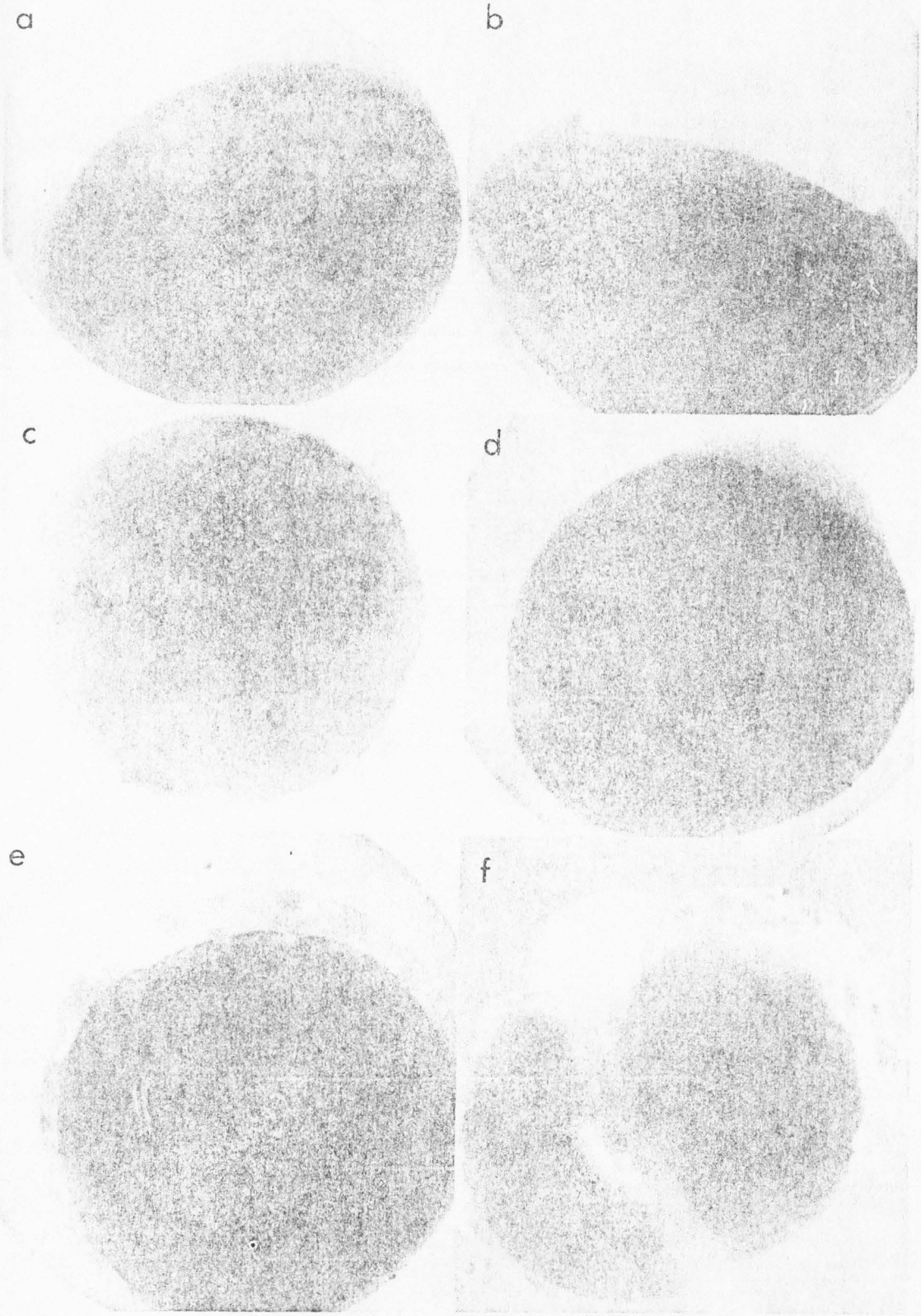

a
b
c
d
e
f

Figure 18. Later stages in the embryology of mountain whitefish (incubated at 6 C)

Figure 18a. Pigment appears in the eye, 25 days, 270.0 T.U.

Figure 18b. Eye fully pigmented and chromatophores appear on the body, 36 day, 388.8 T.U.

Figure 18c. Thirty-six day, dechrionated

Figure 18d. Embryo forms approximately 1 1/2 circle over the yolks, 53 day, 572.4 T.U.

Figure 18e. Fifty-nine day, 637.2 T.U.

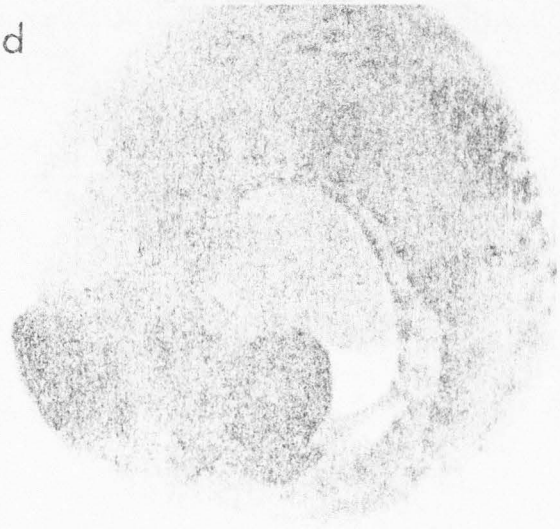
Figure 18f. Seventy-seven day, 831.6 T.U., dechrionated

a

b

c

d



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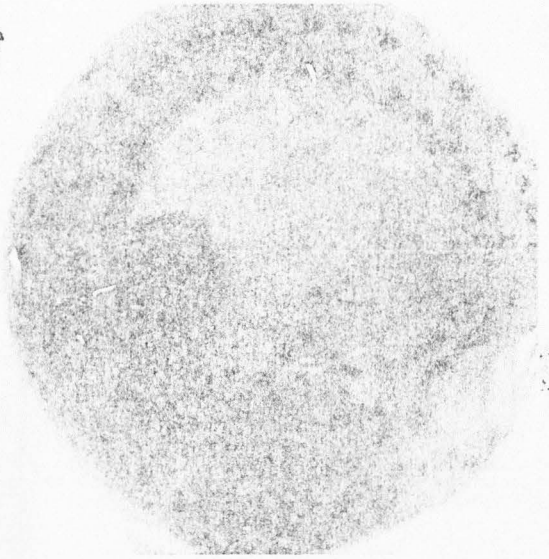

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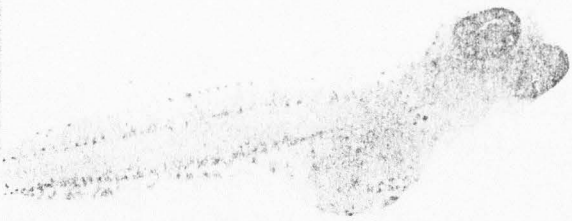

Table 22. Thermal units at different temperatures in relation to days of incubation.

Age (days)	6 C (42.8F)	9 C (48.2F)	10 C (50F)	11 C (51.8)
1	10.8	16.2	18	19.8
2	21.6	32.4	36	39.6
3	32.4	48.6	54	59.4
4	43.2	64.8	72	79.2
5	54.0	81.0	90	99.0
6	64.8	97.2	108	118.8
7	75.6	113.4	126	138.6
8	86.4	129.6	144	158.4
9	97.2	145.8	162	178.2
10	108.0	162.0	180	198.0
11	118.8	178.2	198	217.8
12	129.6	194.4	216	237.6
13	140.4	210.6	234	257.4
14	151.2	226.8	252	277.2
15	162.0	243.0	270	297.0
16	172.8	259.2	288	316.8
17	183.6	275.4	306	336.6
18	194.4	291.6	324	356.4
19	205.2	307.8	342	376.2
20	216.0	324.0	360	396.0
21	226.8	340.2	378	415.8
22	237.6	356.4	396	435.6
23	248.4	372.6	414	455.4
24	259.2	388.8	432	475.2
25	270.0	405.0	450	495.0
26	280.8	421.2	468	514.8
27	291.6	437.4	486	534.6
28	302.4	453.6	504	556.4
29	313.2	469.8	522	576.2
30	324.0	486.0	540	594.0
31	334.8	502.2	558	613.8
32	345.6	518.4	576	633.6
33	356.4	534.6	594	653.4
34	367.2	550.8	612	673.2
35	378.0	567.0	630	693.0
36	388.8	583.2	648	712.8
37	399.6	599.4	666	732.6
38	410.4	615.6	684	752.4
39	421.2	631.8	702	772.2

Table 22 . Continued

Age (days)	6 C (42.8F)	9 C (48.2F)	10 C (50F)	11C (51.8)
40	432.0	648.0	720	792.0
41	442.8	664.2	738	811.8
42	453.6	680.4	756	831.6
43	464.4	696.6	754	851.4
44	475.2	712.8	792	871.2
45	486.0	729.0	810	891.0
46	496.8	745.2	828	910.8
47	507.6	761.4	846	930.6
48	518.4	777.6	864	950.4
49	529.2	793.8	882	970.2
50	540.0	810.0	900	990.0
51	550.8	826.2	918	1009.8
52	561.6	842.6	936	1029.6
53	572.4	858.6	954	1049.4
54	583.2	874.8	972	1069.2
55	594.0	891.0	990	1089.0
56	604.8	907.2	1008	1108.8
57	615.6	923.4	1026	1128.6
58	626.4	939.6	1044	1148.4
59	637.2	955.8	1062	1168.2
60	648.0	972.0	1080	1188.0
70	756.0	1134.0	1260	1386.0
80	864.0	1296.0	1440	1584.0

about 4.28 mm in length and the optic lobes are starting to develop (Figure 17f). The head region is imbedded in the yolk and the tail region is free.

By 270 T.U. the optic lobes are distinct. The optic vesicles are compressed and cup like, and the pigment begins to appear in the eye (Figure 18a). The embryonic circulating system is well developed. The embryo lies prominently on the yolk sac which is constantly decreasing in size.

By 388 T. U. the chromatophores have appeared dorsally in a row on either side of the median fold and also laterally. The eyes become fully pigmented (Figures 18b and 18c). Beneath the notochord the dorsal aorta can be traced throughout the length of the body into the tail region. The caudal vein runs beneath it. The vent is not open at this stage.

By about 432 T. U. gill slits are apparent. A mouth depression is found on the underside of the head and the rudiments of the lower jaw appear.

Movements of the pectoral fins of the embryo are noticed at 475 T.U. The pectoral fin is about 1 mm in length at this stage. Movements of the pectoral fin and tail and rotation of the embryo within the egg becomes more pronounced around 572 T. U. These movements produce more intensive mixing of the perivitelline fluid and increases the respiratory activity as evidenced by the increase in oxygen consumption. There are a lot of chromatophores over the entire yolk sac membrane (Figure 18d). There is a median fin fold which is divided only into the anterior and posterior parts on the dorsal surface. The viteline blood vessels are fully formed and can be observed on either side of the now almost elliptical yolk at 637 T.U. (Figure 18e).

By about 750 T.U. the movement of the jaws and gills could be observed.

Hatching occurs by about 800 T. U. (Figure 18g). In normal hatch, the tail region comes out first. Length of the pectoral fin increases. Most of the embryos become active soon after hatching. Some rest at the bottom of the hatching trays from a few hours to about four or five days. The lake whitefish hatches at about 355 T. U. (Price, 1940).

The larva which hatched at 6 C had a total length of 13 to 14 mm.

DISCUSSION

Preponderance of Males in the Catches

Males were predominant in the catches during the peak spawning season in mid-November during 1972 and 1973. There was only one female per about 20 males caught. It is probable that the females move into the spawning area only when they are actually ready to spawn and may be staying upstream or in deeper waters. Maitland (1969) noted a preponderance of males of powan Coregonus clupeoides on the spawning grounds, even when other evidence suggested a more even ratio of sexes in the population. A similar preponderance of males during spawning has also been noted for the schelly, Coregonus lavaretus (L) by Bagenal (1970). He suggests that this is probably due to males remaining near the spawning grounds longer than the females who only move in when ready to shed their eggs.

Chernyoyev (1973) observed that males of the Baikal Lake whitefish, Coregonus lavaretus are the first to move to the spawning grounds during the spawning season which lasts from three to four months, compared to only three to four weeks for the mountain whitefish.

However, during the middle of November in 1974 the catches of whitefish showed a more equal ratio of sexes. Most of the females were not ripe, probably due to the warmer fall weather during 1974, compared to the previous two years.

In late November which was the peak spawning period during 1974, the males were predominant. This suggests that the sex ratio is more equal at times other than the peak spawning period.

Temperature Optima for the Development of Mountain Whitefish

The results of the present study show that until about 5 to 10 days (about the blastula stage) at 9 and 10 C the effect of high temperature is not shown by the embryo (Figures 2 and 3). Gorodilov (1969) studying the sensitivity to high temperature effects of developing eggs of Salmo Salar and Coregonous peled found that there is a large lag period between fertilization and blastula stage before detrimental effects are manifested. This agrees with the present observations. The mortality was steeper at later stages. From mid-blastula to the eyed stage Gorodilov (1969) also found that the embryo is very sensitive and death was immediate at later stages.

The results indicated that 6 C is about the upper optimum temperature for the successful development of mountain whitefish eggs. The development process is greatly disrupted if the temperature is raised to 9 C. The functions of living organisms often change quite abruptly when subjected to small temperature changes and are due to complex reactions occurring within living organisms (Drost-Hansen, 1956). A certain amount of discreteness in these phenomena may be caused by anomalies in the temperature dependence of the properties of liquid water and these anomalies represent some kind of structural changes in

water, for example the thermal degradation of polymers of the type $(H_2 0)_n$ (Drost-Hansen, 1973). He reported temperatures at which these anomalies are found (to within $\pm$ 2 C) 15 C, 30 C, 45 C, and 60 C. Optimum temperature for biological processes are found to occur at midpoints between these thermal anomalies. The optimum temperature of 6 C for normal embryonic development of mountain whitefish is midway between 0 C, the freezing point of water which is the physiological zero for most processes, and 15 C where thermal anomalies are reported to occur. Mountain whitefish is precisely adjusted during its embryonic development to a very narrow range of temperature. The low temperature range limits this species for its embryonic development to waters which can maintain a wintertime temperature. In the lake whitefish Coregonus clupeaformis, Price (1940) found that its range of optimum development was between 0.5 C and 6 C. Some development was reported at 10 C, but none hatched at 0 C or 12 C. Thus the mountain whitefish and the lake whitefish appear to have similar temperature optima for development. The thermal pollution limit for the successful development of mountain whitefish is 6 C. Any increase in temperature will have a noticeable effect on its development and thus affect the entire population development.

The eggs incubated at 9 C, taken during the peak spawning period, and two weeks later during the late spawning period show that the late spawning period eggs had reduced their incubation period by about two weeks (Figure 5). This may be due to the ultimate factors which exert a selective pressure (Lofts, 1970) ensuring that populations hatch at the optimal season.

However 6 C is not the optimum temperature for development and growth after yolk absorption. Fry which were maintained for over a year at 6 C became stunted and did not survive when they were slowly acclimated to 9 C. The growth of mountain whitefish fry have been found to be greater at 9 C and 12 C than at 6 C (Stalnaker and Gresswell, 1973).

Since the temperature optima for development of embryos and larvae are 6 C and for the fry about 12 C, it is probable that the embryo and larva have metabolic pathways quite different from that of the fry, based on the concept of different metabolic pathways at different temperature intervals (Hochachka and Somero, 1970). This also demonstrates that the study of the effects of thermal pollution should be concerned with the effects of temperature on all stages of the life cycles ranging from the egg and sperm stages through the developed mature individuals. The ecological significance may be great, even if only one life stages is sensitive to the temperature changes around the thermal anomalies (Drost-Hansen, 1969).

In the lake herring, Coregonus artedii the optimum temperature for normal development was 2 C to 8 C, and mortality was high at 0.5 C and 10 C (Colby and Brooke, 1969).

Lawler (1965) demonstrated that year classes of whitefish in Lake Erie were strong only when suitable temperatures prevailed. Fall temperature must drop early to 6.1 C and the decrease to the optimum temperature must be steady and spring temperature must increase slowly and late to provide prolonged incubation near the optimum developmental temperature. It would be worth

investigating how the year classes of mountain whitefish fluctuate when there are warmer fall and spring seasons.

In the mountain whitefish abnormalities of the lower jaw were observed at 9 C and 10 C. In the Pacific herring Clupea pallasi abnormalities of the lower jaw occur at 4 C and a salinity of 25‰, while maximum viable hatch occurred at 6.8 C and a salinity of 16.98o (Alderdice and Velsen, 1971). In the sardine larvae no functional jaws developed at temperatures below 13 C, while another clupeoid inhabiting the same ecological niche developed normally (Lasker, 1964).

Energy Expenditure

Eggs obtained for these experiments were taken from two females with total lengths 393 mm and 401 mm. The eggs were uniform in size being $3.16 \pm .048$ mm in diameter. Since the chorion formed 14.6% of the total dry weight, its weight was deducted from all the calculations until hatching. After hatching the weights determined were that of the larvae alone. Toetz (1966) working with blue gill larvae included the weight of the chorion since he assumed that the chorion weight is only 1.33% of the dry weight, as has been reported for pike and carp (Konig and Grossfeld, 1913). Lasker (1962) and Laurence (1969, 1973) have also included the chorion weight in their calculations. Blaxter and Hempel (1966) however reported the chorion weight to range from 11% of the total egg weight in the Kiel herring Clupea harengus and 15 to 21% in other herrings. They deducted the chorion weight from the total egg weight to give an estimate of the original yolk weight, as was also done in the present study.

Mountain whitefish is a relatively highly fecund fish. Sigler (1951) reported 5,500 to 14,000 eggs per fish ranging in weight from 11 to 25 ounces (338 gm to 768 gm). It also has a long embryonic period lasting from about mid-November to early March (Daily, 1971). As noted by Laurence (1973) fish which exhibited possible energy deficits were from relatively high fecund fish which produce pelagic embryos or prolarvae like tautog and Pacific sardine larvae. The possible energy deficit is also seen in the mountain whitefish which is a highly fecund fish. Species which were reported having no energy deficits were fish with low fecundity.

The energy deficit reported in the results are those from calculations. But an actual energy deficit will occur only when the larvae are not capable of capturing prey. Many of the larvae at 9 C had jaws which were agape, and they will have an actual energy deficit. A potential energy deficit can occur only when the prey organisms are made scarce by the activities of man or due to natural catastrophic causes.

The tissue reabsorption noticed at hatching was during a time when there was still energy present in the form of yolk. As mentioned earlier, the chemical nature of these reserves might preclude their use in the muscular and enzymatic activity necessary to break the chorion during the time of hatching. Laurence (1969) found that in largemouth bass larvae tissue absorption coincided with the initiation of free-swimming when there was still more than enough yolk energy left at the time to meet the calculated catabolic loss. He attributed this tissue

reabsorption to increased energy expenditure due to swimming. In the bluegill Sunfish (Toetz, 1966), in the largemouth bass (Laurence, 1969) and in the tautog (Laurence, 1973) the tissue weight increases after hatching, while in the mountain whitefish the tissue dry weight starts decreasing before hatching.

There was a general tendency for the efficiency, of yolk conversion to become lower as development proceeded both at 6 C and 9 C, even though at the former temperature there was an initial increase. Gray (1928) found a drop in efficiency from about 74% to 56% in the trout from hatching until the yolk was completely absorbed. Smith (1958) also reported such a decrease. Similar tendency for the efficiency of yolk conversion to become lower as development proceeded was reported for herring larvae (Blaxter and Hempel, 1966).

In the mountain whitefish larvae oil globule persisted for a few days even after all the yolk was apparently absorbed. Oil globules are probably triglyceric fat (Toetz, 1966) and are the last reserves to be metabolised (Smith, 1957).

Efficiency of yolk conversion was 54.2% at 6 C and 63.7% at 9 C at the time of hatching, and as more yolk was absorbed the efficiency dropped to 37.1% and 50.8% respectively. Lasker (1962) reported 77% yolk efficiency for growth in the planktonic Pacific sardine larvae. Ryland and

Nichols (1957) reported 35% efficiency for the European plaice. Laurence (1969) observed in the largemouth bass that 44.8% of yolk became living embryo and 55.2% of yolk was used for metabolic purposes. In the tautog the efficiency of conversion was 36.3%, 25.5% and 25.8% at 16 C, 19 C and 22 C (Laurence, 1973). In the tautog the conversion efficiency decreased with temperature increase. However for the mountain whitefish the efficiency of yolk conversion was greater at 9 C than at 6 C. Hayes and Pelluet (1945) also found an increase in efficiency of yolk conversion with increasing temperature for the salmon embryo.

Most of the yolk conversion efficiency values reported in the literature are for the entire yolk sac period. For larvae which feed only several days after hatching, (the jaws and alimentary tracts are formed towards the end of the yolk sac period) the percentage efficiency until the end of the yolk sac period is most meaningful. But for the larvae such as the mountain whitefish which are ready to feed soon after hatching, percentage efficiency at the time of hatching is a more useful index for comparison.

At hatching the larva has more spare yolk energy at 9 C than at 6 C (Figure 19) though the former is not the optimum temperature for development.

In the laboratory cultures newly hatched fry were fed brine shrimp nauplii. It was observed that after initiation of feeding, during the first 12 months, when exogenous food supply became scarce for two days or more of the larvae became too weak to feed and died. The first year of life is very critical and the "point of no return" could occur at any time during this period even after the larva had absorbed all yolk and had switched to an exogenous food supply. The term

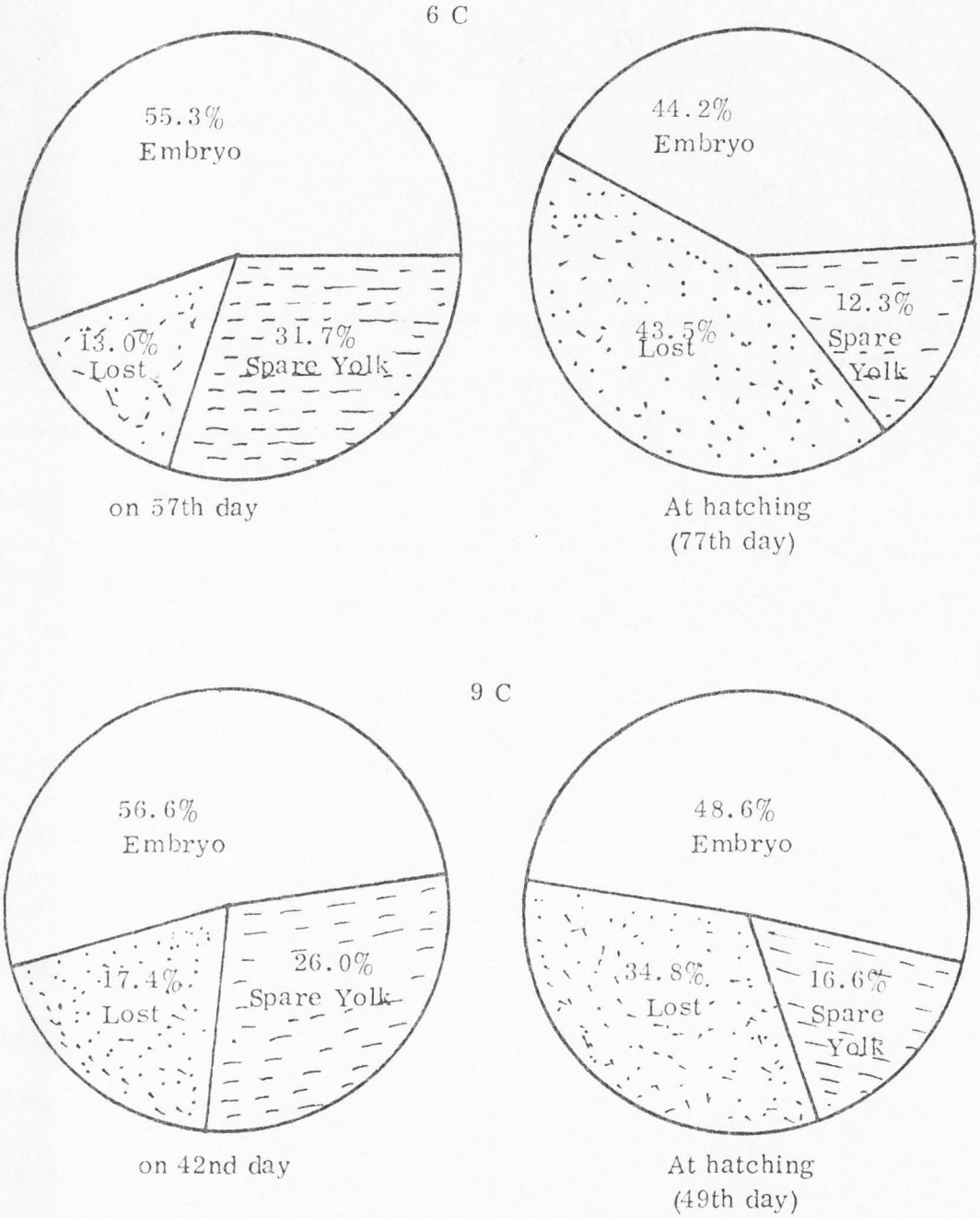

Figure 19. Distribution of calories in mountain whitefish eggs at 6 C and 9 C.

"PNR" was used only for the stage during yolk absorption by Blaxter (1970).

Group Effect

Animals in groups are known to exhibit peculiar forms of physiological response in contradistinction to the characteristic response of an isolated individual of the same species to certain diverse physical or chemical agencies. "The group effect" or the phenomena of mass physiology may be displayed in one or more of the following ways.

1. Differential growth rates
2. Percentages of survival under favourable conditions
3. Reproductive rates
4. Differential sex ratios
5. Conditioned learning times
6. Oxygen consumption rates

and other like quantitative criteria (see Escobar, Minahan and Shaw, 1936 for references)

In the present study no statistical difference in active oxygen consumption of mountain whitefish fry tested individually and in groups of three were found for the size range studied (16-37mm total length). The few published reports suggest that fish in schools have a lower locomotor activity and lower respiration rates compared to individual fishes (Escobar, Minahan and Shaw, 1936; Shlaifer, 1938, 1939; Malyukina et al, 1964; and Parker, 1973). In most of the published

work on fish respiratory metabolism this aspect has not been taken into account. The objective of most of the laboratory experiments is ultimately to relate these results to the phenomena occurring in nature. It is of significance therefore to find if there is any difference when individuals are tested singly or in groups. Contrary to the findings reported for the adult fishes, there is no decrease in respiratory metabolism for the underyearling mountain whitefish when tested in groups. Osborne (1961) reported the ability of salmon to extract energy from the turbulent velocity. The decrease in metabolism when adult fishes are tested in groups may be partially due to the turbulent velocity caused by the swimming of the other fishes, which is absent when a fish is tested individually. The underyearlings are too small to cause any significant turbulent velocity of water and this may explain the lack of any difference in the oxygen uptake when they were tested individually and in groups of three. Escobar, Minahan and Shaw (1936) suggested that two types of behavior may be a clue to possible explanation of the decreased activity of fishes in groups. "First it appears that the free path or direction of movement of a grouped fish is being continually obstructed by the nearby presence of his fellows. A solitary fish describes a path or trajectory that is more smooth and much more free than the path of a member of the group. Members of a group are continually making small adjustments in their orientation or movements as they react to the presence of their nearby fellows. The total movement performed during a given time interval is always less than when the separate movements are short and interrupted by pauses than when they are more continually prolonged as in the case of solitary fishes." Probably the

swimming of fellow underyearling mountain whitefish is not influencing the others. This influence may occur only in adult stages. Perception of form rather than color or movement of the fishes was suggested as responsible for decreased metabolism of goldfish in groups (Shlaifer, 1939). Goldfish when in heterotypic groups with other fishes have increased activity exceeding even that in the isolated condition (Escobar, Minahan and Shaw, 1936).

However Konchin (1971) studying the rate of oxygen consumption in the early ontogeny of the summer Bakhtak (Salmo ischchan) when placed in respirometers in groups and singly, found that the group effect is absent from the initial stages of development, in the embryo and fingerling stages. This agrees with the present observations with the mountain whitefish underyearlings.

Comparison of Electrochemical and Manometric Methods of Measuring Oxygen Consumption of Fish Embryo

The electrochemical method generally gave a higher rate than the manometric method. The mean for the whole series of experiments was 6.2% higher using the Beckman oxygen probe. The reason for the higher value may be due to the fact that in the electrochemical method the water was kept agitated all the time which in turn may have caused more activity and more oxygen uptake of the embryo.

Edwards and Learner (1960) using the isopod Asellus aquaticus measured oxygen uptake using both the Warburg manometer and the polarographic method (dropping mercury electrode). They found that the oxygen uptake was 35 to 75%

higher in the flow through polographic respirometer than in the constant-volume respirometer. This was attributed to the differences in the intensities of activity associated with rather different experimental conditions.

Reuger, Olsen and Scofield (1969) working with benthic insects and using both polarographic and manometric methods found the mean rate of the electrochemical method to be about twice that of the Warburg method.

The much higher rate of increase observed by the above authors may be due to the nature of the organism. The isopod and benthic insects are likely to be made more active by agitation of water than a fish embryo which is protected by the chorion.

Active metabolism

Active metabolism of mountain whitefish underyearlings increased from 6 C to 12 C. Then it dropped sharply at 15 C (Figure 16). There is no published account on the relation between temperature and active metabolism in young of the year fishes and so it is not possible to make any comparisons. The rate of active oxygen consumption varied from 440.7 mg 0_2/kg hr at 6 C to a maximum of 633.7 mg 0_2/kg hr at 12 C. The range of active metabolic rates reported for adult rainbow trout was 366 mg 0_2/kg hr at 5 C to 588 mg 0_2/kg hr at 25 C (Dickson, 1968).

Standard metabolism

Standard metabolism of mountain whitefish yearlings increased from 110 mg 0_2/kg hr at 6 C to 166.5 mg 0_2/kg hr at 12 C, when corrected for a fish of mean weight of 0.1772 gm. There was a sharp rise to 523.7 mg 0_2/kg hr at 15 C. There is no published account on the standard metabolism of young of the year fish. Hence no comparison could be made. One of the temperatures at which the occurrence of thermal abnormalities in various properties of (aqueous) physicochemical, biochemical, and biological systems are observed is 15 C (Drosh-Hansen, 1969). The system obviously has to do more work to maintain equilibrium at this temperature, and, this may account for the steep rise in standard metabolic rates at 15 C.

Scope for activity

High scope for activity was noticed at 9 and 12 C. Growth of the under-yearlings was also higher at 9 and 12 C, compared to 6 C (Stalnaker and Gresswell, 1974). One generally thinks of the manifestations of activity as movements. But growth, excretion etc are also manifestations of activity (Fry, 1970). The high scopes for activity at 9 and 12 C can be utilized by the underyearlings for maximum growth at the temperatures when abundant food is available.

Development of embryos

In the lake whitefish Coregonus clupeaformis (Price, 1940), the embryo becomes distinctly fish like by 101 T.U. But the 216 T.U. stage of mountain whitefish is only comparable to the 125 T.U. stage of the lake whitefish. The

mountain whitefish embryo takes longer time to reach the early stages of organogenesis (Table 23).

The general development of the mountain whitefish is similar to that of the lake whitefish (Price, 1940) but the mountain whitefish requires more thermal units to reach the corresponding stage of the lake whitefish, after the stage when the blastodisc is prominently raised upon the yolk (Table 22). Chernyayev (1973) described the development of the Baikal Lake whitefish. He reported that the larvae hatched 200 to 220 days after fertilization. The temperature experience of the larvae during the period of incubation is not given and hence the corresponding stages could not be compared.

Table 23. Thermal units required by lake whitefish and mountain whitefish to reach corresponding stages during embryogenesis.

Stage	Lake whitefish (Price, 1940)	Mountain whitefish
Blastodisc prominently raised up on the yolk	30 TU	30 TU
Embryo clearly outlined on the surface of the yolk	125.8 TU	216 TU
Embryo forms an almost complete circle on yolk	155 TU	432 TU
Embryo forms approximately 1 1/2 circle over the yolk	324.5 TU	572.4 TU
Hatching	355 TU	800 TU

SUMMARY AND CONCLUSIONS

1. Mountain whitefish eggs were incubated at 6, 9, 10, 11, 12 and 15 C. The results indicated that 6 C is about the upper optimum temperature for successful development. The development process was greatly disrupted and heavy mortality of eggs and abnormalities of hatched larvae occurred, when the temperature was raised to 9 C or higher.

2. The optimum temperature for growth of the post-yolk sac larvae was observed to be at 9 and 12 C. A need is demonstrated to undertake studies on all stages of the life cycle in determining the effects of thermal pollution on a population of fish.

3. Abnormalities observed in hatched larvae by increasing the optimum incubation temperature of the eggs were in the order of frequency agape jaws, coloboma or fissure of the eye, monophthalmia or the presence of only one eye, monomicrophthalmia or the smallness of one eye and one case of twinning.

4. The caloric value of the mountain whitefish eggs were 5793 $\pm$ 8.6 cal/gm and that of the larvae after absorption of all visible yolk in the yolk sac were 5258 $\pm$ 20 cal/gm. The dry weight method of determining energy expenditure showed an energy deficit in mountain whitefish larvae. At hatching the deficit occurs at a time when the larvae can offset these deficits by feeding. If exogenous food becomes available only after about 50% of the remaining

yolk was absorbed the larvae reached the "point of no return." The first twelve months of life was very critical in that the larvae stopped feeding when the exogenous food became scarce for two days or more and died.

5. The oxygen consumption method show an energy deficit during hatching at 6C. It did not show a deficit at 9 C.

6. At 6C the efficiency of yolk conversion was 88.6% on the 57th day of incubation when the embryo was at its maximum weight. The efficiency declined as development proceeded further. At 9C the maximum weight of the embryo was noticed on the 42nd day of incubation and the efficiency of yolk conversion was 83.7% which steadily declined as development proceeded.

7. No group effect in active metabolism was observed in the underyearlings.

8. The electrochemical method of measuring oxygen uptake of the embryos gave a cumulative value which was 6.2% higher than the values using the manometric method. This increase was apparently due to the increased activity induced in the embryos by the former method because of the nature of the experimental set up.

9. Active metabolism of underyearlings increased from 440.7 mg 0_2/Kg hr (0.0781 mg 0_2/hr) at 6C to a maximum of 633.7 mg 0_2/Kg hr (0.1123 mg 0_2/hr) at 9C for an underyearling 0.1772 gm weight and decreased significantly to 555.9 mg 0_2/ Kg hr (0.0985 mg 0_2/hr) at 15 C.

10. Standard metabolism of an underyearling 0.1772 gm/weight increased from 110.0 mg 0_2/Kg hr (0.0195 mg 0_2/hr) at 6C gradually to 166.5 mg 0_2/Kg hr

(0.0295 mg 0_2/hr) at 12 C then there was a sharp rise to 523.7 mg 0_2/Kg hr (0.0928 mg 0_2/hr) at 15 C, for an underyearling 0.1772 gm weight.

11. Scope for activity of an underyearling 0.1772 gm weight increased from 0.0586 mg 0_2/hr to a maximum of 0.0840 mg 0_2/hr at 9 C and 0.0828 mg 0_2 at 12 C which is the temperature at which maximum growth of the underyearling is reported, to a low of 0.0067 mg 0_2/hr at 15 C.

12. The general development of the mountain whitefish is similar to other whitefishes. Mountain whitefish requires more thermal units to reach corresponding stages than does the lake whitefish, after the stage when the blastodisc is prominently raised up on the yolk.

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VITA

Pokkavil Karunakara Rajagopal

Candidate for the Degree of

Doctor of Philosophy

Dissertation: Respiratory Metabolism and Energy Requirements of Embryo, Larval and Juvenile Mountain Whitefish, Prosopium Williamsoni

Major Field: Fishery Biology

Biographical Information:

Personal Data: Born June 18, 1936, Mahe, South India, son of the late Dr. M. K. Menon and the late Bharathi Menon; married Remani, March 21, 1966; one child, Rajiv.

Education: Matriculated from LaBourdonnais College, Mahe, in 1952; attended Zamorins College, Calicut from 1952 to 1954; attended Annamalai University from 1954 to 1958 and received B.Sc (Hons), M.A., and M.Sc degrees; entered Graduate School at Utah State University as Ph.D. candidate in 1972.

Professional Experience: April 1972 to present, graduate research assistant, Utah Cooperative Fishery Unit, Utah State University; March 1971 to March 1972, research technologist, US/IBP Desert Biome Program, Utah Cooperative Fishery Unit; 1966 to 1971, senior research officer, Ghana Academy of Sciences; Summer 1968, research associate, Department of Biology, University of Nevada, Las Vegas; 1962 to 1965, commonwealth research fellow, University College of Rhodesia, Salisbury; 1958 to 1962, senior research scholar, University of Madras.

Honorary and Professional Membership: Freshwater Biological Association of United Kingdom, American Fisheries Society, Bonneville Chapter of American Fisheries Society; certified Fisheries Scientist of American Fisheries Society; member American Institute of Fishery Research Biologists; member American Institute of Biological Sciences.

Personal Publications:

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