ENVIRONMENTAL REGULATION OF DEVELOPMENTAL METABOLISM OF EMBRYOS OF ATLANTIC SALMON

Salmo salar L.

by

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ABSTRACT

Atlantic salmon ova were incubated from fertilization to hatching, or to total yolk absorption, at various temperatures and levels of dissolved oxygen. Chemical, structural, gravimetric and respiratory analyses were performed. Development proceeded more slowly at lower temperature but was complemented by greater utilization of yolk lipids, particularly phospholipids and cholesterol, for energy, and greater diversion of yolk proteins to tissue synthesis so that at hatching these embryos were heavier and longer than those incubated at higher temperature. This effect was modified by the level of dissolved oxygen which produced heavier and longer embryos at air-saturation than at 30% of air-saturation, with corresponding trends in the differential utilization of lipids and proteins. Estimates of total oxygen consumed from fertilization to hatching ranged from 16.6 to 412.8 mg/g, varying inversely both with temperature and dissolved oxygen. Caloric efficiency values inferred from depletion of lipid and protein fractions of yolk (yolk contains only a negligible amount of carbohydrates) vary directly with temperature and inversely with oxygen supply from 14 to 35%.

The controlling and limiting effects of temperature and oxygen supply also determine the initial levels of such "intrinsic" control mechanisms as RNA and DNA and the cyclic nucleotides 3'5' cAMP and 2'3' cAMP. Then, as development proceeds, a feedback system evolves in which the levels of these control mechanisms influence the oxygen consumption and this affects lipid utilization and protein synthesis. Thus, higher RNA/DNA ratios are associated with increased

embryonal growth. The number of vertebrae differentiated is also correlated positively with the value of the RNA/DNA ratio of advanced embryos.

The synthesis of lipids within the more advanced embryos is probably also an important energy source to supplement yolk lipids. In advanced embryos the quantity of 3'5' cAMP changes directly with temperature and oxygen consumption. Lipid synthesis in these embryos is, however, inversely related to amounts of 3'5' cAMP so that embryos developing in a lower oxygen supply have more 3'5' cAMP/unit weight and less lipid synthesis to supplement yolk lipids. Conditions which promote greater total oxygen consumption also produce an increase of the nucleotide 2'3' cAMP which is thought to control protein synthesis. Protein levels in embryos varied in direct proportion to the amount of the nucleotide.

Smaller studies on the role of the zona radiata (capsule) and the metabolic effects created by light, salinity and extraordinary magnetic fields are appended.

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TABLE OF CONTENTS

GENERAL INTRODUCTION

The central aspect of the research to be reported here is the role of metabolic performance in the determination of developmental rate, embryonal growth, and utilization of energy stores in the yolk of the Atlantic salmon Salmo salar Linnaeus. Temperatures and the supply of dissolved oxygen have been selected as the variables of modify metabolic performance in these embryos. The literature on the controlling effects of temperature and the limiting effects of reduced oxygen supply as designated by Fry (1947, 1971), on embryogenesis of fishes, has been summarized by Brett (1970 b), Garside (1966 a, 1970), Kinne and Kinne (1962 a,b), and Silver et al. (1963).

The point was made by Garside (1966 a), that retardation of development (differentiation) by lowered temperature creates a larger embryo at hatching than that produced by accelerated development at higher temperature in salmonid embryos and that an opposite effect is created when retarded or accelerated development produced by reduced or increased oxygen supply. The central thesis is that body size and differentiation are consequences of environmentally regulated states of metabolic efficiency. The extent to which the energy source must be utilized for maintenance of basal function and for the differential demands of growth and organ differentiation will be offered as evidence in support of this thesis. Literature is considerable on changing metabolic efficiencies through altered enzyme activities and shifts in metabolic pathways. These will be examined in relation to the environmental regulation of respiration. Fluchter and Pandian (1968) have summarized the literature on thermally controlled shifts in metabolic efficiency in fish embryos. No literature is available on the influence of oxygen supply on metabolic efficency in fish embryos.

I have included in this study measures of developmental rates, size relations, meristic variations, respirometry, qualitative and quantitative composition and their changes during embryogenesis together with suggested intrinsic control mechanisms. Additionally the Appendix contains peripherally associated reports on studies of the structure and composition of the zona radiata and the regulation of respiratory rate by incident light, salinity electromagnetic field and circadian cycle in oxygen consumption.

GENERAL MATERIALS AND METHODS

Freshly fertilized ova from a single pair of Atlantic salmon (Salmo salar L.), were obtained from Cobequid Fish Culture Station, Canada Department of Environment, RR# 2, Collingwood, Nova Scotia, in November, 1970, 1971 and 1972. The general incubation procedures were similar to those described by Garside (1959, 1960). The ova were incubated on aluminium-screen trays in 150 x 150 mm acrylic cylindrical chambers supplied with running water at 1-litre/min. During the progress of incubation at various relatively constant levels of dissolved oxygen, 30, 50 and 100% of air-saturation, at 5 and 10 C, subsamples were removed for developmental respirometry and chemical analyses. Additionally small lots of ova were incubated in the coolant water of the two temperature baths at slightly variable temperatures of approximately 3.6 and 6.8° C, the data for which have been used only in a few calculations of the later developmental processes, hatching time, and change in weight.

Some ova were kept continuously in simple continuous-flow respirometers for periodic determination of oxygen uptake (see Appendix 1 for method). Incident illumination in these experiments was 2 lux for a daily 12-hr cycle. The water supplying the ova was dechlorinated tap water with total hardness equivalent to 18.4 mg/l $CaCO_3$. The chemical analysis of the water is presented in Table 1. Chemical analysis of tap water for inorganic constituents and heavy metals was done by the laboratory of Environment Canada, Water Quality Division at Moncton, New Brunswick.

The dissolved oxygen content of water was controlled by differential extraction with a system described by Mount (1961, 1964), utilizing regulated partial vacuum in a partially recirculating system (Figure 1).

All groups were incubated until they reached developmental stage 18 (Garside 1959) but some of the subsequent alevins were kept until

Figure 1

Vacuum deoxygenation system modified from Mount (1961, 1964). Reduced constant atmospheric pressure in the chamber lowers the solubility of the gases in solution to selected stable levels. Efficiency is enhanced by recirculation of surplus partially degassed water. Vacuum chamber 1.0 x 0.5 m in diameter is made of fiberglass with removable cover. Pipes and valves are polyvinylchloride. Partially deoxygenated water passed through sealed cooling system for temperature adjustment before delivery to respirometers.

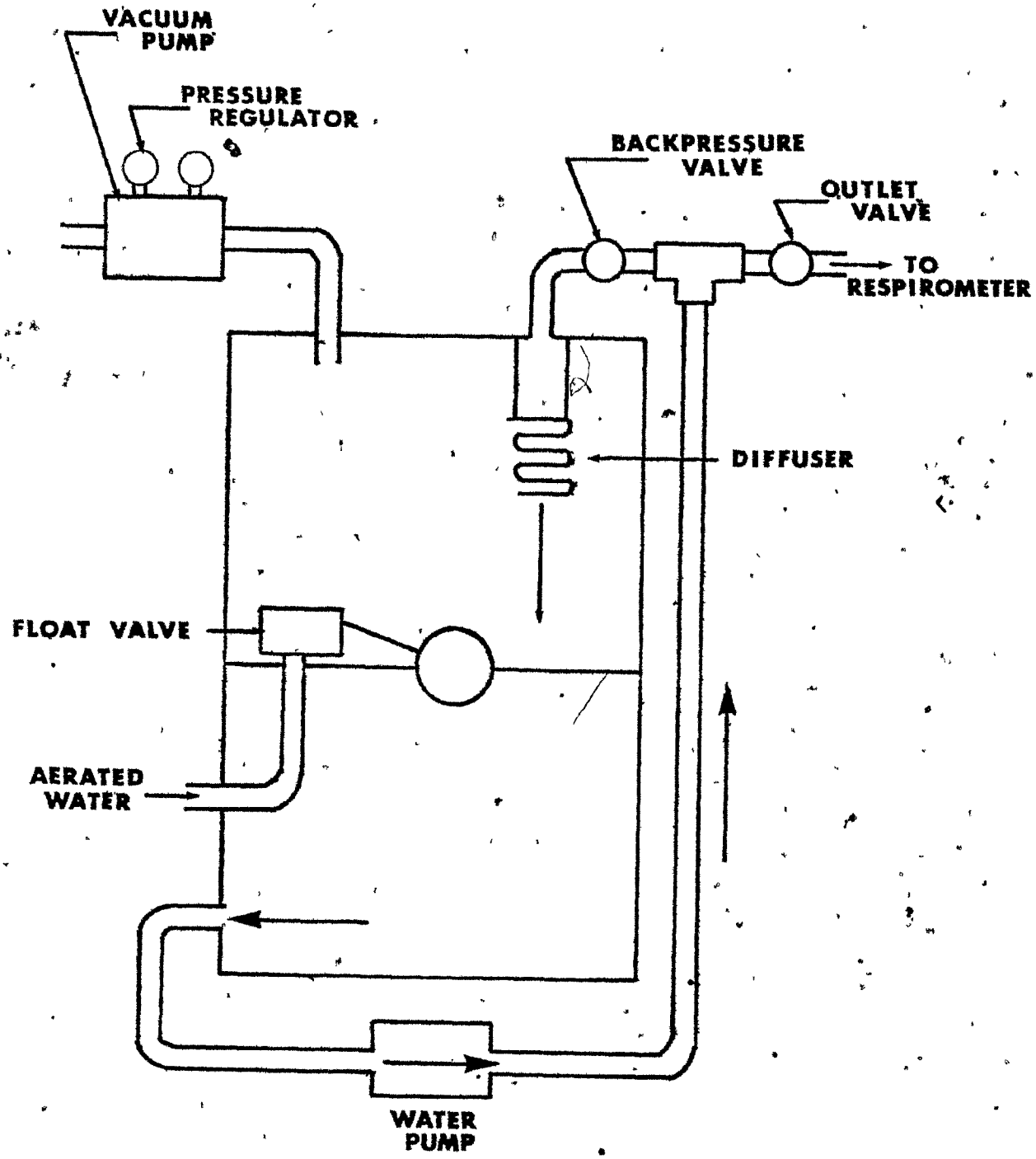

VACUUM PUMP
PRESSURE REGULATOR
BACKPRESSURE VALVE
OUTLET VALVE
TO RESPIROMETER
DIFFUSER
FLOAT VALVE
AERATED WATER
WATER PUMP

TABLE 1

Average chemical composition of dechlorinated tap water in Halifax, Nova Scotia, used for incubation of Atlantic salmon embryos 1971-1973.

Temperature at testing	21.7	Alkalinity total ($CaCO_3$)	13.9
Turbidity	1.3	Hardness total ($CaCO_3$)	18.4
Colour	5.0	Calcium dissolved (Ca)	4.9
pH	6.8	Sulphate dissolved	5.0
Chloride dissolved (Cl)	15.0	Silica reactive (SiO_3)	1.5
Copper extractable (Cu)	0.008	Sodium dissolved (Na)	7.1
Fluoride dissolved (F)	0.12	Zinc extractable (Zn)	0.95
Iron extractable (Fe)	0.07	Cadmium: extractable	0.002
Lead extractable (Pb)	0.07	Non. Carb. Hardness	4.51
Magnesium dissolved	1.5	Sum Const. PPM	46.53
Manganese extractable	0.04	S A R	0.72
Nitrogen Nitrate, Nitrite	0.06	Dissolved Ammonia (N)	0.60
% Na (Sodium)	41.27	Phosphorus dissolved, inorg.	--
Potassium dissolved (K)	2.8	Phosphate (P)	0.05
Saturation index	3.3	Stability index	12.9
Spec. Cond. (μ mho/cm)	92.9		
	(45-110)		

yolk was absorbed (approximately 25 mm TL).

Samples of ova were taken, mortality recorded, and dead ova were removed daily. Each experiment (1970, 1971 and 1972), required depending on developmental rate from 80 - 150 days to carry remaining alevins to the completion of yolk absorption.

CHAPTER I

DEVELOPMENTAL RATES OF EMBRYOS OF ATLANTIC SALMON INCUBATED AT VARIOUS LEVELS OF TEMPERATURE AND DISSOLVED OXYGEN

INTRODUCTION

The development of fish embryos can be described by morphological, physiological or chemical changes. Such changes have a certain pattern of events which can be described as "developmental stages". Several series of embryonal stages have been described for salmonids (Battle 1944, Pelluet 1944, Garside 1959, Devillers 1965, Vernier 1969, Ballard 1973 a, b and c).

The times to achieve developmental stages differ in different environmental conditions. Increased temperature causes a shorter developmental time, at lower temperature the embryo develops more slowly, a consequence at reduced metabolic rate. When temperature controlled developmental times are transformed to rates there are typically three phases, a sigmoidal relation, in which the long central segment is virtually linear, while the tails reflect slow progress at low and high temperatures (Kinne and Kinne 1962 a, and b, Garside 1966 a).

The control of developmental rate by temperature in fish embryos can be greatly modified by the level of dissolved oxygen. Reduced levels of dissolved oxygen retard the rate of development. This has been demonstrated by several investigators, including Johansen and Krogh (1914), Alderdice et al. (1958), and Garside (1959, 1966 a).

On the other hand, a richly oxygenated environment accelerates the development (Kinne and Kinne 1962 b, Ostroumova 1969). However, if development is already at an advanced stage a decrease in the concentration of oxygen can induce earlier hatching (Alderdice et al. 1958, Kotlyarevskaya 1967).

As a preliminary aspect of the study of metabolic performance during development, the occurrence in time of structural stages was recorded for embryos of Atlantic salmon incubated in various combinations of temperature and levels of dissolved oxygen. The data were then analyzed

by multiple regression to produce a predictive model for developmental time based on temperature, level of oxygen, and flow-rate of the water supply.

Most environmental variables are known to have ranges of concentration beyond which a lethal effect occurs in fish embryos as in nearly all aquatic organisms. Interference with developmental processes by temperature and oxygen supply beyond certain levels, to the point of causing death, has been reported for fish embryos by several authors (Alderdice et al. 1958, Combs 1965, Alabaster 1967, Duodoroff and Shumway 1970, Eipper 1963, Garside 1959, 1966 a, Kotlyarevskaya 1967, Platt 1974, Saksena and Joseph 1972, Wickett 1954).

A comparative survey of mortality was also performed in this study to measure the viability of salmon embryos during their development at various levels of temperature and dissolved oxygen.

MATERIALS AND METHODS

During the winters of 1971-1974 collections of ova of Atlantic salmon were incubated at 5 to 10 C (and small lots at 3.6 and 6.8 C) and from 30, 50 and 100% air-saturation in water having flows from 0.2 to 15 ml/min. Samples of 5 to 50 ova were taken every day and stored in aqueous alcohol-glycerin-formalin solution, for reference in the determination of developmental rates. Stages of embryogenesis were identified from the schedule given by Garside (1959) and the average developmental times were recorded. The 50% hatching time during the hatching interval was calculated with BMDO5-polynomial regression programme of the State University of New York at Buffalo in which

$$X = \frac{b \pm \sqrt{b^2 - 4ac}}{2a}$$

The data for developmental and hatching times were analysed with multiple linear regression, programme BMDO3R. (Dixon 1970).

Survival was determined as the percentage of starting number in each group determined by subtraction of the number of dead ova collected during daily inspections.

RESULTS

The experimental conditions, the developmental times, the 50% hatching times, the percentage survival at stage 18 and completion of hatching are presented in Table 2. The time for development increased with decreased temperature and decreased air-saturation, as shown also in Fig. 2 for groups between 3.9-4.1 ml water-exchange (flow).

The times in days, required by embryos of the various lots of Atlantic salmon ova to attain each of the 18 stages (Garside 1959) have been listed in Table 3. These figures show the typical relation of development to temperature and degree of air-saturation of the water.

All of the calculated median (50%) hatching times were highly significantly different, ($P<0.005$). The number of embryos which hatched each day in these experiments followed the second degree parabolic curve,

$$y = a + bx + cx^2$$

The developmental time in days, the 50% hatching time, and the embryo weight seem to be highly predictable. The calculated equations are:

$$Y_1 = 146.3 - 7.25\ X_1 - 1.72\ X_2 - 2.29\ X_3 \quad \ldots\ldots(1)$$

$$\log Y_2 + 3.12 - \log 1.33\ X_1 - \log 0.72\ X_2 - \log 0.67\ X_3 \quad \ldots\ldots(2)$$

$$Y_3 = 21.4 - 1.2\ X_1 + 4.95\ X_2 + 2.46\ X_3 \quad \ldots\ldots(3)$$

$$Y_4 = 4.1 - 1.32\ X_1 + 5.7\ X_2 + 4.33\ X_3 \quad \ldots\ldots(4)$$

$$\log Y_5 + 0.88 - \log 0.52\ X_1 + \log 0.63\ X_2 + \log 0.18\ X_3 \quad \ldots\ldots(5)$$

Y_1 = developmental time in days

Y_2 = 50% hatching time in days

Y_3 = total survival in percent

Y_4 = hatching survival in percent

Y_5 = embryo weight in mg at stage 18

X_1 = temperature (C)

X_2 = oxygen level mg/l

Figure 2

Relation of a measure of developmental rate (reciprocal of time, in days to reach stage 18) for embryos of Atlantic salmon incubated at two temperatures and three levels of dissolved oxygen.

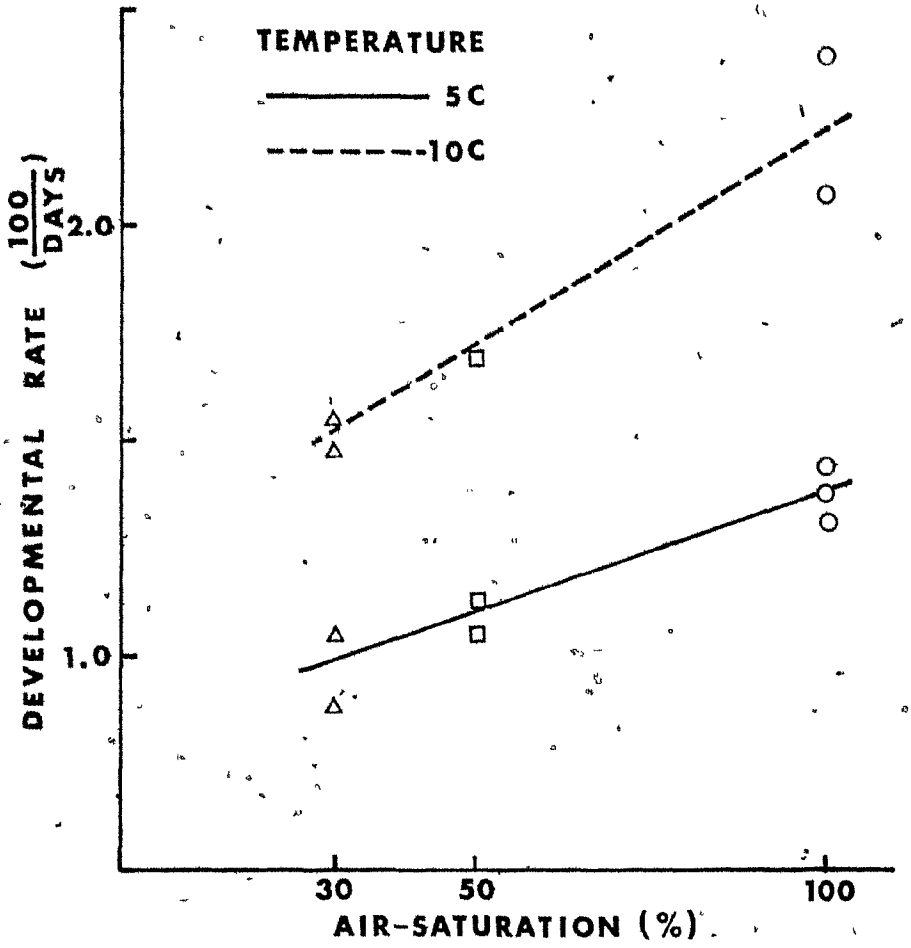

TEMPERATURE
5C
10C
DEVELOPMENTAL RATE (100/DAYS)
2.0
1.0
30
50
100
AIR-SATURATION (%)

TABLE 2

Average environmental conditions, developmental times, median hatching times in hatching period, survival to stage 18, and to hatching (percent of number at stage 18), based on all lots of Atlantic salmon ova incubated in the three years of experiments. Those marked (*) are the lots which were employed in all subsequent procedures.

Temp. C	Oxygen mg/l	Flow ml/sec	Developmental time Days	50% Hatch	Survival Percent	Hatch percent	Weight mg
3.0	12.4	3.7	96	17.6	97	88.2	26.5
3.7	7.8	3.8	97	30.7	72	57.0	26.8
3.7	12.4	3.8	90	18.9	92	88.3	28.4
3.8	10.6	3.9	92	19.1	80	82.0	28.7
4.0	3.0	3.9	125	38.0	41	43.0	9.5
4.3	11.8	4.0	86	14.3	96	96.0	25.7
4.9	3.7	3.9	93	32.6	48	39.0	11.9*
4.9	6.8	3.9	90	10.1	65	69.0	26.6*
4.9	11.9	4.0	83	16.3	85	84.0	22.1*
4.9	12.4	3.8	83	18.3	89	79.0	24.3*
5.0	12.0	4.0	77	15.2	86	87	22.4*
5.1	3.5	4.0	70	25.7	43	35	9.3
5.2	5.4	6.3	85	11.8	66	55.0	13.2
5.4	8.8	1.8	88	23.4	79	18.0	16.0
5.5	8.1	1.9	89	25.0	38	15.0	13.2
5.6	10.2	1.9	84	18.4	77	34.4	15.5
5.8	10.3	4.9	76	16.8	89	76.2	7.6
5.8	10.4	0.16	87	30.0	36	69.0	14.5

continued...

TABLE 2(continued)

Temp. C	Oxygen mg/l	Flow ml/sec	Developmental time Days 50% Hatch	Survival Percent	Hatch percent	Weight mg
6.0	3.6	4.1	86	19.5	42	34.0	9.0
5.9	6.7	4.2	83	12.0	64	72.0	26.6
6.4	10.0	3.8	74	8.7	74	54.4	14.1
6.5	11.9	3.8	70	3.5	88	90.4	14.9
6.6	3.4	4.0	125	4.6	47	18.5	8.1
6.7	7.8	3.6	76	7.3	61	40.6	11.5
6.8	11.8	4.9	69	3.2	87	96.0	18.5
7.5	12.2	4.0	57	7.8	83	81.0	16.6*
8.6	7.8	1.9	66	15.7	51	45.0	8.1
9.0	3.4	4.1	67	13.2	37	15.2	6.8
9.0	6.5	3.9	53	7.6	53	46.0	10.0
9.1	6.0	1.5	67	24.0	20	10.0	5.4
9.3	3.6	3.7	64	10.4	38	14.9	5.4
9.9	5.8	4.0	47	4.3	55	40.3	12.5*
9.9	6.2	6.4	49	5.0	63	54.0	7.6
9.9	8.3	6.7	46	3.6	76	67.0	9.5
9.9	8.9	1.7	57	13.6	42	73.0	8.0
9.9	9.5	8.0	40	2.7	98	80.0	10.9
9.9	11.6	4.0	49	3.0	78	82.6	14.5*
10.0	3.9	4.0	69	10.0	47	34.0	10.0*
10.0	11.9	3.9	43	2.1	95	92.0	14.6*
10.3	9.6	1.8	50	8.6	88	53.0	8.2
10.5	8.1	3.3	49.0	7.2	65.0	50.6	8.9

TABLE 3

Times in days required by embryos of Atlantic salmon to attain 18 developmental stages (Garside, 1959) when incubated at 30, 50 and 100% air saturation at 5 and 10C.

	Temperature					
	5 C			10 C		
	Air saturation					
Stage	30%	50%	100%	30%	50%	100%
1	3	3	3	3	2	2
2	9	9	9	5	5	5
3	14	14	12	6	6	6
4	17	17	14	9	9	7
5	18	18	17	10	10	8
6	19	19	18	11	11	9
7	22	22	20	12	12	10
8	28	28	24	14	14	11
9	35	35	28	17	17	14
10	38	38	31	21	21	16
11	44	41	35	22	22	17
12	49	43	37	25	24	19
13	58	49	39	28	25	21
14	62	58	43	31	28	26
15	66	60	58	38	31	28
16	73	68	65	43	34	32
17	83	80	68	53	36	35
18	93	90	83	69	47	43

TABLE 4

Intercept values of developmental equations 1-5, and regression coefficients (b), in which, Y_1 = developmental time (days); Y_2 = 50% hatching time (days); Y_3 = total survival (per cent); Y_4 = hatching survival (per cent); Y_5 = yolkless embryo weight, stage 18 (mg).

Equation	Intercept	C Temp	Oxygen mg/l	Flow ml/min	St. error	R^2	F	Remarks
Y1	146.3	-7.25	-1.72	-2.19	9.20	0.99	2186.1	arithmetic
Y2	3.12	-1.33	-0.72	-0.67	4.54	0.78	49.6	log/log
Y3	21.41	-1.20	4.95	2.46	5.47	0.47	20.0	arithmetic
Y4	4.07	-1.32	5.71	4.33	3.78	0.55	10.47	arithmetic
Y5	0.88	-0.52	0.63	0.18	4.89	0.68	41.77	log/log

N = 80 F0.001 = 6.17

TABLE 5

"t" values and partial correlation coefficients for the developmental equations. Y1 = developmental time in days; Y2 = 50% hatching time in days; Y3 = total survival in percent; Y4 = hatching survival in percent; Y5 = embryo weight in mg at stage 18.

Equation	Temperature		Oxygen		Flow	
	"t" value	Partial corr. coeff.	"t" value	Partial corr. coeff.	"t" value	Partial corr. coeff.
Y_1	76.1	-0.99	-20.2	-0.92	-30.9	-0.96
Y_2	8.43	-0.80	-4.58	-0.58	-8.90	-0.82
Y_3	1.3	-0.16	6.1	0.60	3.6	0.41
Y_4	0.86	-0.16	4.27	0.64	2.00	0.37
Y_5	-6.22	-0.63	5.59	0.59	5.70	0.60

X_3 = flow (ml/cm^2/min)

Values for r^2, F and standard errors are presented in Table 4.

The calculated "t" values and the coefficients of partial correlation are presented in Table 5. Water temperature is the most important factor which determines the developmental time and the embryo weight. The variables can be ranked in order of the strength of their effects on development, early survival and weight. Such ranking is given in Table 6 along with the degree of statistical significance determined by t-tests.

The oxygen content is the most important factor to interact with development and hatching. The greatest expression of thermal influence occurs in the overall rate of development. The general results of the effects of the monitored variables are presented in Table 6.

DISCUSSION

Developmental Rates

The developmental times for similar flow rates seem to be highly dependent on temperature and somewhat less on the oxygen supply (Table 2), a result which is also described by Alderdice et al. (1958). It is also interesting to note that the retarding effect of the lower levels of oxygen is more pronounced at the higher temperature than at the lower (Table 2 and 3).

The developmental rate was less affected by reduced levels of oxygen at early stages but after the first 10 percent of the development time had passed the retarding effect became progressively stronger. This increasing retardation has been demonstrated in three other salmonids by Garside (1959, 1966a).

The developmental time to stage 18 is accurately predictable if the three important variables, temperatures, degree of air-saturation, and water velocity are known, (Table 4). The same conclusion can be drawn for hatching times, that is, the time required to achieve 50% hatching. However, percentage hatching survival has less agreement with the regressions and the precentage survival to stage 18 was the least predictable process. This may be due in some part to longer period of handling in the sampling procedure. The explanation might also lie partially in the different influences of environmental factors employed in the experiments (Table 6).

The features which are less predictable (survival to stage 18 and hatching survival) seem to be mostly influenced by the available oxygen (Saksena and Joseph 1972). The second ranking factor, the flow rate or water supply, also caused differences in availibility of oxygen. Thus these two variables can either partially offset one another or be additive depending on the quantity of oxygen. In spite of this, correlation

TABLE 6

The effects of temperature, level of dissolved oxygen, and flow-rate of water through incubation chambers on various developmental states or processes in the embryos of Atlantic salmon. Rank order was determined from "t" values in Table 5.

Status or process	Rank of Effect		
	Primary	Secondary	Tertiary
Developmental time (stage 18)	Temp.***	Flow***	Oxygen.***
Hatching time	Flow***	Temp.***	Oxygen**
Total survival %	Oxygen***	Flow**	Temp.*
Hatching survival %	Oxygen**	Flow**	Temp.**
Embryo weight, stage 18	Temp.***	Flow***	Oxygen***

* P<0.05

** P<0.01

*** P<0.001

between these two variables failed to be significant.

Although temperature has a significant effect on all the aspects of development treated here, its superlative effect on the overall developmental rate indicates its fundamental role in metabolic control.

Oxygen supply measured as absolute concentration or indirectly as water exchange rate, has its prime importance during the hatching process which is created in some measure by more immediate demands of locomotor activity which contributes to the rupture of the zona radiata.

The general conclusions from the present experiments (Table 6) are in good agreement with those concerning developmental retardation and mortality which were made by Alderdice et al. (1958), Garside (1959), Cable (1961), Shumway et al. (1964), Kotlyarevskaya (1967), and Brett (1970 b).

However, an important difference with those results was, that in these experiments the waterflow was measured and evaluated as a factor in the manner of temperature or oxygen content of the water. Utilization of this factor tended to provide a more accurate basis for an appreciation of developmental rate and survival.

CHAPTER II

VERTEBRAL NUMBER IN RELATION TO DEVELOPMENTAL RATE IN EMBRYOS OF ATLANTIC SALMON INCUBATED AT VARIOUS LEVELS OF TEMPERATURE AND DISSOLVED OXYGEN

INTRODUCTION

Numerous correlations have been drawn between mean numbers of seriated parts of populations of the same species of fishes and levels of certain environmental variables notably temperature, dissolved oxygen, light, and salinity of their habitats.

Such correlations are most strongly formed when the values are taken from environments during embryonal development. Generally, natural populations of species which develop in conditions such as lower temperature which retard developmental timing have higher average numbers of such structures (Garside 1959, 1966 b, 1970, Kinne and Kinne 1962 a, b, Sweet and Kinne 1964, Tåning 1952). Removal of the study of more precise analytical conditions in laboratories has extended the information but not the interpretation because of the variety of data obtained and the many contradictory correlations offered (Tåning 1952, Garside 1966 b, 1970, Fowler 1970, Lindsey and Harrington 1972, Ali and Lindsey 1974).

In this study vertebral counts have been compared among samples of young Atlantic salmon fry which had been incubated experimentally in combinations of temperature and dissolved oxygen.

MATERIALS AND METHODS

Freshly fertilized ova from a single pair Atlantic salmon (Salmo salar L.) were obtained from the Fish Culture Station, Canada Department of Environment, RR #3 Collingwood, Nova Scotia, November 14th, 1972 were transported to the aquarium at the Life Sciences Centre, Dalhousie University.

The ova were divided into six lots incubated at constant levels of dissolved oxygen, 30, 50, 100% air-saturation from fertilization until absorption of yolk at 5 and 10 C, at a flow-rate of 4 ml/min. Incident illumination during incubation in this experiment was 8 lux for daily 12-hr period.

Samples of 30 from each of the six batches were collected and the fry were stained with Alizarin Red according to the method of Taylor (1967), and cleared to a state of transparency with glycerin. The vertebrae were counted with a stereoscopic dissecting microscope using transmitted light.

The program of analysis of variance University of Alberta (Anova 22) was applied to the distributions to determine the significance of mean vertebral counts.

RESULTS

The results of vertebral counts are presented in Table 7. The vertebral number was higher in fishes held at lower temperature and higher oxygen concentration. According to the analysis of variance, differences appearently caused by temperature and oxygen concentration were highly significant ($P<0.0001$) and the interaction between temperature and oxygen concentration was also significant, $P<0.041$, (Table 8). No complex or anomalous vertebrae were observed in any of these samples.

I attempted to relate the reciprocal values of developmental time lapsed between stages 4 and 16 with the vertebral number, since this has been considered to be the period of vertebral fixation (Garside 1966 b). The correlation coefficients between vertebral number and the reciprocal value for this period of vertebral determination was $r = -0.64$. Also, correlation was calculated between vertebral number and the percentage of time from fertilization to stage 16 that is occupied by the respective period of determination. The correlation coefficient was $r = -0.016$. Neither r-value was significant.

Correlation coefficent between embryo length at stage 16 and the vertebral number was $r = 0.99$. This correlation is highly significant ($P<0.001$).

TABLE 7

Vertebral numbers, period of determination (T_D) (Garside, 1966 b), period of determination as a percent of total time to stage 16 (Tp) and mean total length of embryos at stage 16 (TL_{16}) in six samples (N = 30) of Atlantic salmon fry incubated at two temperatures and three levels of dissolved oxygen.

Temp. C	Oxygen Percent Air-Saturation	T_D	$\frac{100}{T_D}$	Tp	TL_{16}	Vertebrae $\bar{x} \pm$ S.E.
5	100	51	1.96	78.5	8.2	57.4 ± 0.08
5	50	51	1.96	75.0	8.1	56.6 ± 0.11
5	30	56	1.70	76.7	8.0	56.3 ± 0.08
10	100	25	4.0	78.1	7.8	55.2 ± 0.07
10	50	25	4.0	73.5	7.7	53.9 ± 0.07
10	30	34	2.94	79.0	7.5	52.6 ± 0.08

TABLE 8

Summary of analysis of variance of vertebral counts in Atlantic salmon fry after incubation at 5 and 10 C and 100, 50 and 30 % air-saturation.

Source	Sums of squares	Degrees of freedom	Mean square	Variance ratio	Probability
Temperature	372.0	1	372.0	135.29	0.0001 ***
Oxygen	998.0	2	49.9	18.12	0.0001 ***
Temp x Oxygen	17.8	2	8.93	3.24	0.041 *
Error	479.0	174	2.75		

DISCUSSION

The alteration of vertebral number by genetic mechanisms or the morphogenetic influences of environmental variables is a very complex problem which has largely eluded interpretation.

Effect of temperatures

The general effect of temperature is that fish embryos which develop at lower temperatures have more vertebrae than those which develop at higher temperatures (Fowler 1970, Garside 1970, Lindsey 1962, a, b, Lindsey and Ali 1965). However, exceptions have occurred (Garside 1966, b, Fowler 1970, Lindsey and Harrington 1972). Examples include an increase of vertebral number at higher temperatures in sockeye salmon Oncorhynchus nerka (Canagaratnam, 1959). Other results have been reported by Lindsey (1954). According to other reports the lowest number of vertebrae occurs at an intermediate temperature often called an "optimum" temperature (Schmidt 1921, Tåning 1944, 1946, Marckmann 1954, Seymour 1959, Love 1970).

Unfortunately, the possible effect of an "optimum" temperature cannot be determined from experiments conducted as this was, only at two temperatures. However, it seems to be definite that the vertebral number of Atlantic salmon embryos at 5 C is higher than at 10 C.

Effect of Oxygen Concentration

Oxygen concentration also has an effect on meristic differentiation as shown by works of Kinne and Kinne 1962 a, b), Sweet and Kinne (1964), Garside (1959, 1966 b). Greater vertebral numbers were observed in salmonids incubated at retarding levels of dissolved oxygen by Tåning 1952, Seymour 1959, Garside 1966 b.

In spite of expectation of an increased number of vertebrae the effect of lowered air-saturation had the reverse effect on the number of vertebrae of salmon embryos. This experiment does not provide enough information to explain the cause, but some part can be understood from the view of Fowler (1970).

> "In general, the effect of salinity and oxygen concentration on meristics is not a simple one. It seems probable that metabolic rate and energy balances are involved, but it has not been possible to separate metabolic influences from any others."

Interaction of Temperature and Oxygen Level

According to the analysis of variance the interaction of temperature and oxygen concentration on vertebral number of these embryos was significant. Interactions of this kind have not been reported in the literature. However, it is clear that several environmental and probably genetic factors might be interacting with each other. The scarcity of information on this subject is explained mostly by the lack of homogeneous genetic material (Fowler 1970). Other difficulties arise because of possible differential effects at different times of development. For example, Orska (1962) found that embryos of rainbow trout exposed at different times of their development to higher temperatures responded by forming different vertebral numbers, depending on which period of development that the treatment was applied, (including trends opposite to those observed by Tåning). Experimental results might be affected by interactions of still other environmental variables. For example illumination has effects on bone development as discussed by Fowler 1970, and demonstrated by Korobina et al. 1965. However, no information concerning the interaction

of different intensities and lengths of illumination, temperatures, oxygen concentrations and salinity on vertebral number or other meristic changes has been published.

The failure of these data to fit a regression which would relate the number of vertebrae to some aspect of developmental rate and hence some measure of rate of differentiation as proposed by Garside (1966 b) suggests that if the original hypothesis of Hubbs (1926) applies, then Garside's limits for the period of vertebral determination are not appropriate. However, the strong positive correlation between body length at stage 16 (in ova) and subsequent mean vertebral number does concur with Hubbs' hypothesis that differentiation terminates more gradually in retarding conditions thereby allowing for the differentiation of more somites and vertebrae than in a more abruptly terminating system such as that which is controlled by accelerating developmental conditions.

In a later chapter, metabolic efficiency will be shown to be greater at lower temperature and higher oxygen supply and although in absolute terms there is retardation in these conditions relative to that in higher temperature, this additional efficiency results in the formation of more embryonal tissue generally as seen in enhanced prehatching length and weight. An increase in the number of vertebrae differentiated appears to be part of this additional synthesis.

CHAPTER III

GRAVIMETRIC AND MORPHOMETRIC CHANGES IN OVA AND EMBRYOS OF ATLANTIC SALMON INCUBATED AT VARIOUS LEVELS OF TEMPERATURE AND DISSOLVED OXYGEN

INTRODUCTION

The acellular yolk of teleost ova generally is the obvious supply of nutrients for most or all the prehatching differentiation and growth. Additionally, water and solutes including oxygen may be acquired and these will tend to increase the weight of the ovum. Offsetting losses will occur in varying degrees by respiratory and excretory elimination of carbon and nitrogen compounds.

The available studies on the changes in weight which occur in teleost ova during embryogenesis are those of Hayes (1949), Lasker (1962) Blaxter and Hempel (1966), Williams (1967), Thomas (1968), Fluchter and Pandian (1968), Gulidor (1969), Laurence (1969), Blaxter (1970a), Love (1970).

Differential utilization of yolk during embryogenesis is a reasonable expectation arising from differential respiratory performance. Such differentials could be anticipated in the present experiment in which wide differences in developmental environments with respect to temperature and oxygen supply have created marked differences in developmental rates determined from times required to reach structural stages and hatching. To this end, weights and linear measures for computation of volumes were recorded periodically of samples of intact ova and also the weights of their excised embryos and yolks. Trends in weight changes will be related to aspects of metabolism in a later chapter.

MATERIALS AND METHODS

Ova of Atlantic salmon were incubated at 5 and 10 C from 30 to 100% air-saturation in water flows from 4 to 6 ml/min. Subsamples of embryos were transferred at different times with 1-day step-by-step adaptation from 5 to 10 C and vice versa. Several control batches were left intact during the total time of the experiment. Measurements of weight were taken from fresh and fixed samples after drying the material for 2 min. on filter paper. A Mettler electric semimicrobalance was used for all weighing. The same procedure was followed for the separated embryo, zona radiata and fixed yolk. The fresh yolk weight was obtained by dissection on a plastic weighing tray following removal of embryo and zona radiata. Weights were compared among freshly fixed and dried specimens and multiplying factors were calculated based on these values to calculate changes of weight.

Change in size of embryo, yolk, and embryo with yolk, was followed photographically at different developmental stages. Weight and volumetric measures were then compared with computed volumes generated from planimetry of photographs to measure change in yolk size. Results of these measurements of change in size were then utilized to follow the development of several recently hatched individuals without the necessity to killing them, thereby eliminating the difficulties caused by individual variation in initial yolk-size.

The volume of yolk was calculated as a sphere according to the formula: $4/3\pi r^3$. The value of r was obtained from the planimeter reading which converted the ellipsoid yolk to a circle of equal area. Then r = (circumference /2π) X (constant multiplier) was generated from the data calculated.

Measurement of ovum weights was begun two days after fertilization. The mean ovum weight of a sample of 100 drawn unselectively in the 1972/73 experiment was 138.9 mg, but only 38 of these weighed between 136-140 mg.

At the same time, to avoid the possibility of the effects of weighing errors, the weights of three samples of 10 dead (white) ova were also taken. There was a significant difference between the mean weights for the two sample sizes, but not among the mean weights of the three 10-ovum samples.

The t-tests among the series of measurements showed that the weighing error is not sufficiently great that it would interfere with the ovum weights ($\pm$1.11%). However, the group weight and individual weight of ova were highly significant when the data were evaluated with analysis of variance.

Mean daily changes in various weights, such as daily yolk depletion, daily embryo and alevin increments were determined by the method of weighted means (Selby 1970). As a final observation on growth and size under various developmental conditions, the weighted mean embryo or yolk weight between developmental stages 1 to 18 was calculated from the mean weight of an unselectively drawn sample of 30 from each of these stages in each of the six lots. The mean weight of the 30-sample was then multiplied by the number of days within the corresponding developmental stage. The sum of the 18 products was then divided by the total number of developmental days to stage 18, in the appropriate lot.

RESULTS

Calculations of relations among ovum weight, surface ($4\pi r^2$), and volume ($4/3\pi r^3$) are presented in Table 9. These relations are,

$$S = \frac{\log V^2}{3}$$

$$\text{Volume} = \frac{\text{weight}}{1.2}$$

$$\text{Surface} = 1.21 \log W$$

Combined results of 2500 weighing measurements during development showing the percentage changes during development (stage 1 to 18) in the several environments, are given in Table 10. The weighted mean weights for each developmental group of ova can be graded from heaviest to lightest as, 5C, 100%S > 5C, 30%S > 10C, 30%S > 5C, 50%S, > 10C, 50%S >10C, 100%. According to "t"-tests all weighted means are significantly different ($P<0.001$), except between the groups at 30% air-saturation in 5 and 10C. Interactions of temperature ($P<0.0001$), stage of development ($P<0.0001$); air-saturation ($P<0.0001$); temperature X stage ($P<0.001$); temperature X air-saturation ($P<0.0001$) are very significant. Effects of developmental stage X air-saturation have masked each other ($P<0.69$), so the interaction is not significant on the ovum weight.

The embryo weights at the two experimental temperatures and three levels of air-saturation are presented in Table 11 and Fig. 3, and 4. The absolute and percentage weights of structural components of these salmon ova (embryo, yolk, perivitelline fluid, and zona radiata) incubated at two temperatures and three levels of air-saturation are presented at Table 12 and the "t"-tests to compare these measurements are given in Table 13.

The mean yolk weight at stage 18 is consistently significantly affected by the experimental conditions, while weight of zona radiata and perivitelline fluid is not. The results of measurements of yolk and embryo weights during development from stage 1-18 are presented in Table 14.

Figure 3

Log mean weight at each of 17 developmental stages for embryos of Atlantic salmon incubated at 30, 50 and 100% air-saturation, at 5 C.

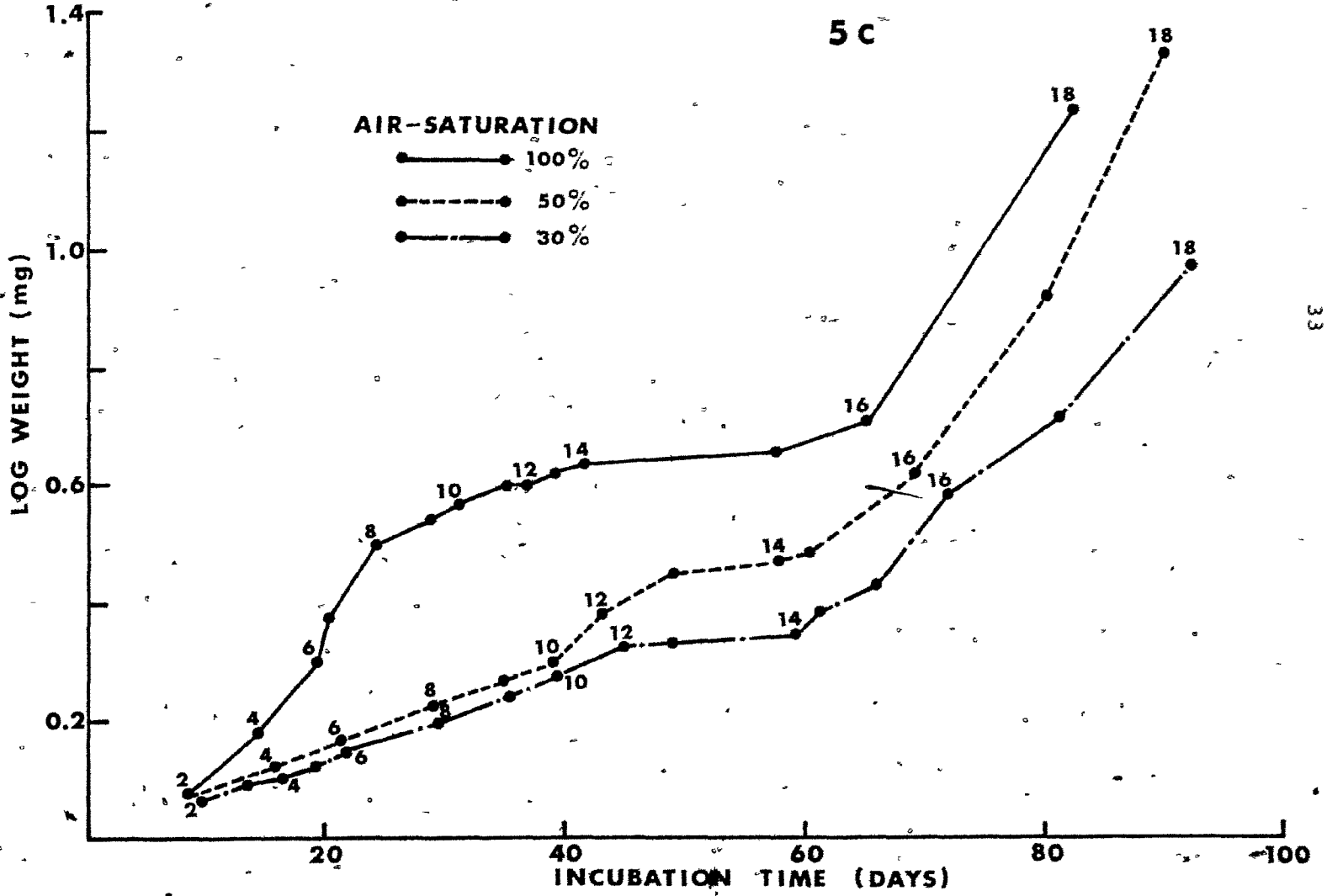

5 C
AIR-SATURATION
100%
50%
30%
LOG WEIGHT (mg)
1.4
1.0
0.6
0.2
20
40
60
80
100
INCUBATION TIME (DAYS)

Figure 4

Log mean weight at each of 17 developmental stages for embryos of Atlantic salmon incubated at 30, 50 and 100% air-saturation at 10 C.

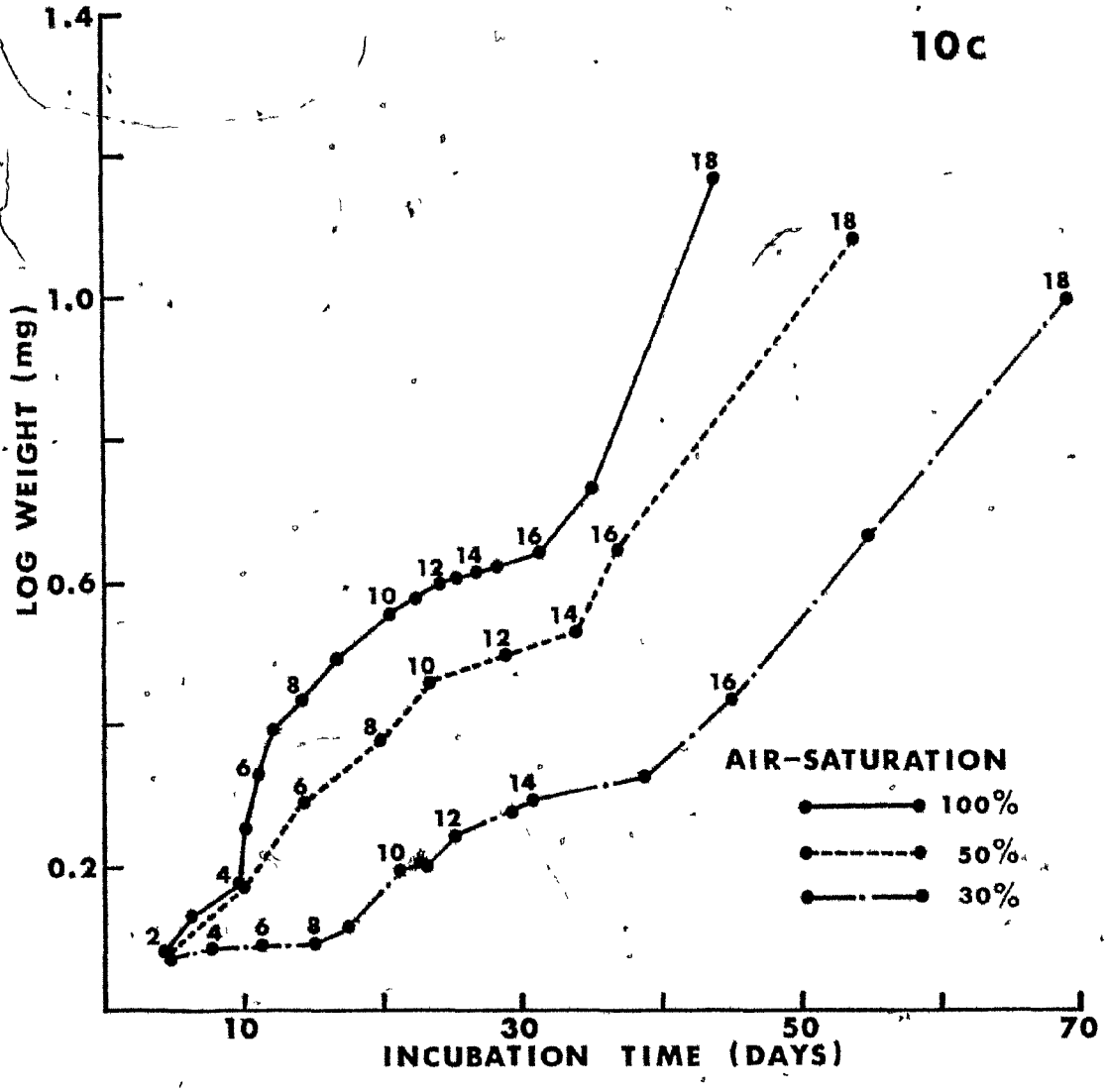

10c
LOG WEIGHT (mg)
1.4
1.0
0.6
0.2
10
30
50
70
INCUBATION TIME (DAYS)
AIR-SATURATION
100%
50%
30%

TABLE 9

Diameter, surface area, volume, weight and surface/volume ratios of freshly fertilized Atlantic salmon ova.

Diameter mm	Surface mm^2	Volume mm^3	Weight mg	Surface / volume
5.0	78.5	65.4	79.8	1.20
5.1	81.7	69.4	84.7	1.17
5.2	84.9	73.6	89.8	1.15
5.3	88.2	77.9	95.1	1.13
5.4	91.6	82.4	100.5	1.11
5.5	95.0	87.1	106.2	1.09
5.6	98.5	91.9	112.1	1.07
5.7	102.0	96.9	118.2	1.05
5.8	105.6	102.1	124.6	1.03
5.9	109.3	107.5	131.1	1.01
6.0	113.0	113.0	137.9	1.00
6.1	116.8	118.8	144.9	0.98
6.2	120.7	124.7	152.2	0.96
6.3	124.6	130.9	159.7	0.95
6.4	128.6	137.2	167.4	0.93
6.5	132.7	143.7	175.3	0.92
6.6	136.8	150.5	183.6	0.90
6.7	141.0	157.4	192.0	0.89
6.8	145.2	164.6	200.8	0.88
6.9	149.5	171.9	209.8	0.86
7.0	153.9	179.5	219.0	0.85

TABLE 10

Live weight of Atlantic salmon ova in percent of initial weight during development at several temperatures and air-saturations. Mean weight at stage 1 = 139.0 mg = 100%.

	Temperature					
	5 C			10 C		
	Air-saturation					
Embryonal stage	100%	50%	30%	100%	50%	30%
1	100	100	100	100	100	100
2	99.2	99.6	101.4	100.6	102.6	104.0
3	98.9	100.9	101.9	98.7	100.6	102.0
4	98.8	108.0	110.1	98.9	100.8	102.2
5	98.3	98.0	114.5	93.7	95.6	96.9
6	98.7	98.0	104.5	92.7	94.6	95.9
7	100.2	101.5	102.9	99.0	100.9	102.3
8	99.2	96.8	107.2	102.7	106.3	101.7
9	98.2	99.0	101.8	99.3	101.5	105.6
10	100.8	99.3	104.3	99.3	101.9	103.3
11	108.1	101.6	99.7	99.5	101.4	102.8
12	103.8	105.1	103.8	109.6	109.1	98.3
13	103.8	104.6	108.4	100.4	108.2	98.7
14	111.4	106.6	115.1	99.5	99.6	107.3
15	109.3	110.5	104.3	100.8	102.3	106.6
16	108.2	108.3	106.7	99.8	99.0	106.0
17	107.0	105.5	108.5	98.8	102.5	108.3
18	106.5	112.8	108.5	105.9	103.2	104.9
Mean	105.7	104.3	104.7	101.1	102.1	104.4
S. E.	0.40	0.50	0.49	0.53	0.40	0.38

TABLE 11

Embryo weight in mg living weight of Atlantic salmon from developmental stages 2 to 18 in temperatures 5 and 10 C and air-saturations of 100, 50 and 30 %.

Stage	Temperature 5 C			Temperature 10 C		
	Air-saturation			Air-saturation		
	100%	50%	30%	100%	50%	30%
2	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.1
3	1.4±0.1	1.3±0.1	1.3±0.1	1.4±0.1	1.5±0.1	1.2±0.1
4	1.5±0.1	1.3±0.1	1.3±0.1	1.5±0.1	1.6±0.1	1.2±0.1
5	1.8±0.1	1.4±0.1	1.4±0.1	1.8±0.1	1.7±0.1	1.2±0.1
6	2.0±0.1	1.4±0.1	1.4±0.1	2.2±0.1	1.8±0.1	1.2±0.1
7	2.4±0.1	1.5±0.1	1.5±0.1	2.5±0.1	1.9±0.1	1.2±0.1
8	3.2±0.1	1.7±0.1	1.9±0.1	2.6±0.1	2.0±0.1	1.2±0.1
9	3.5±0.1	1.9±0.1	2.0±0.1	3.1±0.1	2.3±0.1	1.3±0.1
10	3.7±0.1	2.0±0.1	2.1±0.1	3.6±0.1	2.6±0.1	1.6±0.2
11	3.9±0.2	2.2±0.1	2.1±0.1	3.7±0.1	3.1±0.2	1.6±0.3
12	4.0±0.2	2.5±0.1	2.1±0.1	4.0±0.2	3.2±0.3	1.8±0.4
13	4.1±0.2	2.7±0.2	2.2±0.2	4.0±0.3	3.2±0.6	1.9±0.4
14	4.2±0.3	2.8±0.2	2.4±0.3	4.1±0.4	3.3±0.7	2.0±0.4
15	4.3±0.4	3.0±0.3	2.6±0.3	4.2±0.7	3.3±0.7	2.1±0.5
16	4.5±0.5	4.2±0.8	3.1±0.8	4.3±0.7	3.3±0.7	2.3±0.5
17	7.5±0.8	8.5±0.8	5.2±0.8	5.5±0.8	5.0±0.8	4.8±0.8
18	22.1±3.2	26.6±4.0	11.9±2.8	14.5±3.2	12.5±2.2	10.0±1.8

TABLE 12

Components of Atlantic salmon ova at stage 18 in absolute and relative weights. Total ovum weight at stage 1 was 139 mg, yolk 105, perivitelline fluid 29, zona radiata 5 mg, and embryo <0.1 mg.

Temperature C	Air sat. %	Unit	Embryo	Yolk	Perivitelline fluid	Zona radiata	Total weight
5	100	mg	22.1	83.4	37.0	5.3	147.8
		%	14.9	56.4	25.0	3.6	100.0
	50	mg	26.6	90.7	34.5	4.8	156.6
		%	17.0	58.0	22.0	3.0	100.0
	30	mg	11.9	94.2	40.1	4.4	150.6
		%	7.9	62.5	26.6	2.9	100.0
10	100	mg	14.5	89.0	39.7	3.8	147.0
		%	9.9	60.6	27.0	2.6	100.0
	50	mg	12.5	92.4	34.9	3.4	143.2
		%	8.7	64.5	24.4	2.4	100.0
	30	mg	10.0	89.5	43.6	2.5	145.6
		%	6.9	61.5	29.9	1.7	100.0

TABLE 13

Probability (P) values for yolk, perivitelline fluid (Pvf) and zona radiata (Zr) weight differences at stage 18, based on individual "t" test results.

Temp C	Air saturation	Material	5 C 100%	5 C 50%	5 C 30%	10 C 100%	10 C 50%	10 C 30%
		yolk	-	0.001	0.001	0.001	0.001	0.001
	100	pvf	-	0.05	0.1	0.05	0.1	0.02
		zr	-	0.4	0.4	0.2	0.4	0.4
		yolk	0.001	-	0.001	0.001	0.001	0.001
5	50	pvf	0.05	-	0.2	0.1	0.2	0.05
		zr	0.4	-	0.9	0.4	0.5	0.4
		yolk	0.001	0.001	-	0.001	0.001	0.001
	30	pvf	0.1	0.2	-	0.05	0.4	0.05
		zr	0.4	0.9	-	0.4	0.5	0.4
		yolk	0.001	0.001	0.001	-	0.01	0.1
	100	pvf	0.05	0.1	0.05	-	0.2	0.05
		zr	0.2	0.4	0.4	-	0.4	0.2
		yolk	0.001	0.001	0.001	0.01	-	0.001
10	50	pvf	0.1	0.2	0.4	0.2	-	0.1
		zr	0.4	0.5	0.4		-	0.4
		yolk	0.001	0.001	0.001	0.1	0.001	-
	30	pvf	0.02	0.05	0.05	0.05	0.01	-
		zr	0.4	0.4	0.4	0.2	0.4	-

TABLE 14

Yolk utilization, depletion, conversion, and embryonal growth of Atlantic salmon from stages 1-18, based on initial yolk weight of 105 g. Abbrevations are Predicted (P), Measured (M), Percentage Difference (D).

Temp C	Air-Sat %	Daily Mean Yolk Depletion (mg)	Total Yolk Depletion	Yolk Conversion	Remaining Yolk Weight			Embryo Weight		
					P (mg)	M (mg)	D (%)	P (mg)	M (mg)	D (%)
	100	0.26	21.6	1.02	83.4	83.4	100	22.03	22.1	99.7
5	50	0.16	14.3	1.86	90.6	90.7	99.9	26.6	26.6	100.0
	30	0.11	10.8	1.10	94.8	94.2	100.6	11.88	11.9	99.8
	100	0.37	16.0	0.91	79.5	89.0	89.3	14.56	14.5	100.4
10	50	0.27	12.6	0.99	92.3	92.4	99.9	12.47	12.5	99.8
	30	0.22	15.5	0.65	95.5	89.5	106.7	10.8	10.0	100.8

Also listed are the mean yolk depletion/day, total yolk depletion, yolk utilization (embryo mg/yolk mg), the weight of remaining yolk in stage 18, the calculated ovum weight as a percent of the measured weight at stage 18 as a check on the accuracy of procedure, and the yolk depletion time for the remaining yolk after stage 18.

Mean yolk depletion was greater at higher temperature and at higher oxygen level. Observations of hatching were performed daily in each chamber. Wet weights of hatched embryos with and without yolk are presented in Table 15. To overcome the difficulty in analysis of variance created by different sample size, a matrix of sampling cells was designed to compare the weights on several days during the hatching periods as shown in Table 16.

The analysis of variance with corrected sampling matrix for embryo weights indicates that temperature and dissolved oxygen generally control the weight of embryos newly hatched but the weight at hatching is not related to the point in the hatching period when hatching occurs. By calculating the mean weight values to equalize the sample size the analysis of variance with equal size resulted again in the same form of results (Table 17). The weights at hatching of embryos from which yolk was excised, at two temperatures and three oxygen levels, are presented in Figure 5. Heaviest embryos appear to hatch first at 5C, 50 and 30% air-saturation, and at 10C and 100% air-saturation. However, at 10C 30% air-saturation this trend is reversed with heaviest embryos at end of the hatch, and at 5C and 100% air-saturation heaviest embryos hatch in the middle of the period.

The analysis of variance with unequal sample size (University of Alberta Div. of Educ. Serv. ANOVA 22) reveals that weight of embryos

Figure 5

Relation of yolkless embyro weight to day of hatching during hatching period in Atlantic salmon following continuous incubation at 5 and 10 C, and 30, 50 and 100% air-saturation.

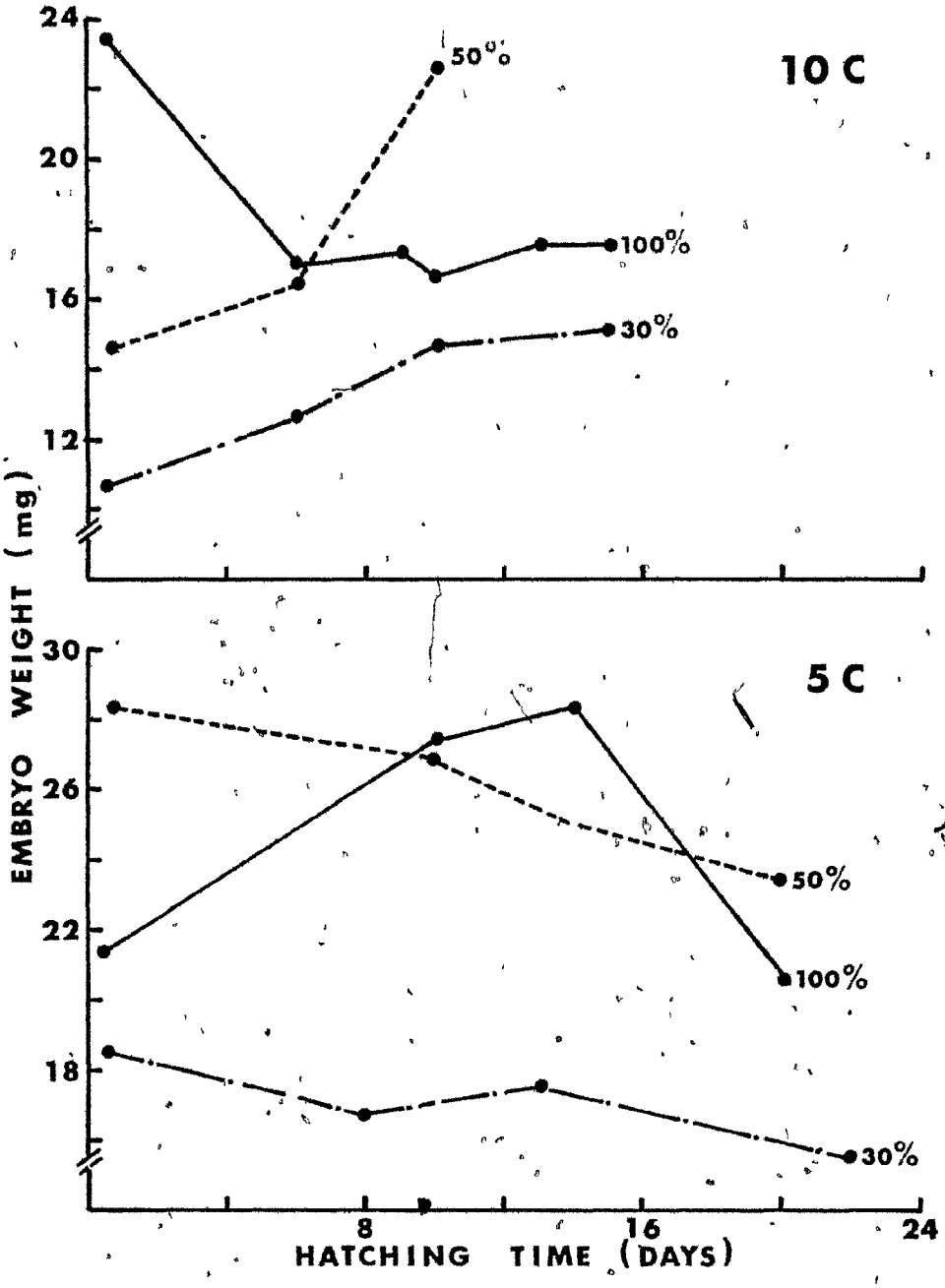

24
20
16
12
50%
10 C
100%
30%
EMBRYO WEIGHT (mg)
30
26
22
18
5 C
50%
100%
30%
8
16
24
HATCHING TIME (DAYS)

TABLE 15

Weights of alevins and yolkless elevins of Atlantic salmon at subsequent days in hatching period.

Temp. °C	Air. sat.	Hatch (days)	Alevin weight (mg) Max	Min	Mean±S.E.	95% limits Lower	Upper	Yolkless Alevins (mg) Mean±S.E.
5	100	1	132.5	105.1	119.5±4.92	109.8	129.1	21.4±0.14
		10	142.3	124.5	133.9±2.90	128.1	139.6	27.5±0.14
		15	156.2	126.2	137.0±6.41	124.4	149.6	28.2±0.14
		20	120.2	115.3	117.9±1.45	115.1	120.7	20.5±0.06
	50	1	136.1	104.3	122.9±5.43	112.3	133.6	28.2±0.38
		10	155.0	118.7	138.9±6.92	125.4	152.5	27.2±0.32
		15	163.2	125.1	149.3±7.27	135.1	163.6	25.0±0.26
		20	152.0	136.2	143.6±4.34	135.0	152.0	23.8±0.24
	30	1	141.0	105.2	116.7±5.03	106.9	126.6	18.4±0.14
		6	140.8	100.9	115.7±4.29	107.3	124.1	17.8±0.12
		8	148.5	124.6	136.0±6.29	123.7	148.4	16.8±0.13
		10	141.3	104.0	124.3±2.55	119.3	129.4	17.2±0.13
		13	157.6	108.5	124.6±4.38	120.1	138.2	17.2±0.14
		23	134.0	127.0	130.7±1.72	124.3	136.1	15.7±0.10

continued...

TABLE 15 (continued)

Temp. C	Air sat.	Hatch (days)	Alevin weight (mg) Max	Min	Mean±S.E.	95% limits Lower	Upper	Yolkless Alevins (mg) Mean±S.E.
10	100	1	124.3	109.6	116.4±1.63	113.2	119.6	23.4±0.14
		7	182.9	123.7	154.8±15.2	125.1	184.6	1.70±0.20
		9	172.5	138.3	157.6±8.23	141.5	173.8	17.4±0.20
		10	168.1	139.2	153.3±4.19	145.1	161.6	16.8±0.14
		13	136.2	134.2	135.1±0.47	134.2	136.1	17.6±0.24
		15	184.5	123.1	152.2±1.25	130.2	174.3	16.8±0.27
	50	1	107.8	122.7	114.8±1.79	111.3	118.3	14.8±0.07
		7	129.5	123.6	126.9±2.13	122.7	131.1	16.5±0.06
		10	135.0	126.5	131.2±1.10	122.4	136.3	23.4±0.07
	30	1	121.7	119.1	120.2±0.67	119.0	121.6	10.8±0.14
		7	137.1	120.7	128.9±3.86	121.3	136.5	12.9±0.10
		10	136.2	134.0	135.0±0.47	134.0	137.2	14.7±0.08
		15	146.3	128.3	137.5±2.47	132.7	142.4	15.0±0.60

TABLE 16

Matrix arrangement for calculation of days during the hatching period, of Atlantic salmon at 5 and 10 C and three levels of air-saturation (see text for complete description).

Choice	5 C Air-saturation (%)			10 C Air-saturation (%)		
	100	50	30	100	50	30
	1	1	1	1	1	1
A	10	10	10	7	7	7
	20	20	20	15	10	15
	1	1	1	1	1	1
B	15	15	13	10	7	10
	20	20	23	15	10	15
	1	1	1	1	1	1
C	10	10	6	9	7	7
	15	15	8	13	10	10

TABLE 1

Summary of analysis of variance among hatching weights of intact alevins in relation to developmental variables, temperature, oxygen supply and hatching day. The analysis has been divided according the three matrices in Table 1b to analyse the hatching weights.

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	Probability
Temp	549.5	1	549.0	9.3	0.038*
Day	105.7	2	52.8	0.89	0.477
Oxygen	1109.0	2	554.0	9.39	0.030*
Temp x Day	120.1	2	60.2	1.02	0.438
Temp x Oxygen	172.7	2	86.3	1.46	0.333
Day x Oxygen	182.8	4	45.7	0.77	0.594
Error	236.1	4	59.0		
Temp	681.2	1	681.2	18.06	0.013*
Day	78.2	2	39.0	1.04	0.433
Oxygen	1332.7	2	666.3	17.66	0.010*
Temp x Day	79.0	2	39.5	1.04	0.430
Temp x Oxygen	204.0	2	102.0	2.70	0.180
Day x Oxygen	216.6	4	54.2	1.44	0.367
Error	150.9	4	37.7		
Temp	125.6	1	125.6	1.24	0.327
Day	44.2	2	22.1	0.21	0.813
Oxygen	894.8	2	447.4	4.43	0.096
Temp x Day	161.2	2	80.6	0.798	0.510

continued...

TABLE 17 (continued)

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	Probability
Temp x Oxygen	302.3	2	151.1	1.50	0.327
Day x Oxygen	95.1	4	23.8	0.235	0.905
Temp x Day x Oxygen	310.8	4	77.7	0.77	0.594
Error	637	4	100.9		

changes with number of days in hatching period, ($P<0.0076$), temperature x hatching days ($P<0.0001$). The effect of temperature x hatching days x oxygen level ($P<0.016$) was significant, but temperature x hatching day was not significant (Table 18). However, "t"-tests failed to show significant differences within most embryo weights at 100% air-saturation, on different hatching days, but they were significant at different temperatures and lowered oxygen levels and within the mean hatching time and the start and end of 5C and 100% air saturation and between the very early (1-2 days) and the following period at 10C and 100% air-saturation. The final analysis of variance is given in Table 19 and Fig. 5. In this test, mean weights of the first, middle and final thirds of each hatching period were compared to determine the roles of temperature, level of dissolved oxygen, and day in hatching period. Temperature and hatching day have significant effects while oxygen has a masking effect (Table 20).

Ten alevins from each group in the 1972-73 experiment were photographed five times on average, during the phase of yolk-absorption. Subsamples of this group were removed periodically and preserved for measurement of both embryos and their yolks. The comparison of photographed alevins with dissected and fixed alevins resulted in the following relations based on the following corrected planimeter measurements:

yolk mm^3 x 313 = yolk mg fixed weight ± 19.5%

yolk mm^3 x 426 = yolk mg living weight ± 21.5% and

yolk fixed weight x 1.36 = yolk living weight ± 9.55%

Alevin length x 6.88 was equal to the living weight in mg ± 2.34%, the alevin fixed weight x 1.19 = live alevin weight in mg ± 1.68%, and the fixed yolk (separated) weight plus fixed alevin (separated) weight x 1.25 = the fresh yolk + embryo weight ± 4.63%.

TABLE 18

Summary of factorial analysis of variance among hatching weights of intact alevins in relation to developmental variables, temperature, oxygen supply, and hatching day. The analysis has been divided to illustrate the shifts in significance which occur by the introduction of another variable (oxygen supply).

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	Probability
Temp.	2848.1	1	2848.1	29.1	0.001**
Hatching day	7782.0	2	3891.0	40.0	0.008**
Temp. x Hatch day	643.6	2	321.8	3.29	0.496
Error	4212	4	98.0		
Temp.	2848.1	1	2848.1	29.2	0.001**
Hatching day	7782.0	2	3891.0	39.8	0.008**
Oxygen	237.7	2	118.8	1.21	0.18
Temp. x Hatch d	643.6	2	321.8	3.29	0.50
Temp. x Oxygen	3645.3	2	1822.7	18.7	0.004**
Hatch. d. x Oxygen	571.1	4	142.8	1.46	0.22
Temp. x Hatch. x Oxygen	1453.6	4	363.4	3.72	0.016
Error	7672.8	4	97.6		

TABLE 19

Mean weight of Atlantic salmon embryos (mg) hatched in three successive intervals of equal time at two temperatures and three oxygen levels.

Temperature C	Lapsed Hatching Time (Days in percent)	Air saturation % 100	50	30
5	33	21.4	28.2	18.4
	67	28.2	25.0	17.6
	100	20.5	23.8	15.7
10	33	23.4	14.8	10.8
	67	17.4	16.5	14.7
	100	16.8	23.4	15.0

TABLE 20

Summary of factorial analysis of variance in hatching weight of yolkless alevins in relation to temperature, oxygen supply, and hatching day in period. The analysis has been divided to illustrate the shifts in significance which occur by the introduction of another variable (oxygen supply).

Source	Sum of squares	Degrees of freedom	Mean square	F ratios	Probability
	two way lay-out				
Temperature	117.0	1	117.0	10.95	0.006**
Day	157.0	2	78.8	7.34	0.008**
Temp x Day	12.2	2	6.13	0.57	0.579
Error	128.7	4	10.7		
	Three way lay-out				
Temperature	117.0	1	117.5	6.9	0.058
Day	157.6	2	78.8	4.63	0.09
Oxygen	1.48	2	0.74	0.043	0.957
Temp x Day	12.2	2	6.14	0.36	0.717
Temp x Oxygen	28.5	4	14.0	0.84	0.495
Hatching x Oxygen	30.6	4	7.6	0.449	0.77
Tem. x Day Oxygen	625.6	4	156.4	9.2	0.045
Error	1149.7	4	17.0		

The results for both original and transfer lots were analysed by multiple regression. The calculated regression equations (coefficients given in Table 21) are for embryo weight (mg) at stage 18 (equation 5 in "Developmental rate") chapter, for yolk utilization in mg fish/1 mg yolk, and yolk depletion in mg/day and change in alevin weight in mg/day.

$$\log Y_1 = -1.31 + \log 0.056\ X_1 + \log 0.05\ X_2 + \log 0.82\ X_3 - \log 0.18\ X_4 - \log 0.06\ X_5 \quad \ldots\ldots(6)$$

$$\log Y_2 = -1.43 + \log 0.56\ X_1 + \log 0.37\ X_2 - \log 0.30\ X_3 + \log 0.03\ X_4 + \log 0.62\ X_5 \quad \ldots\ldots(7)$$

$$\log Y_3 = -1.77 + \log 0.71\ X_1 + \log 0.45\ X_2 + \log 0.19\ X_3 - \log 0.06\ X_4 + \log 0.17\ X_5 \quad \ldots\ldots(8)$$

$$\log Y_{1a} = 0.57 - 0.03\ X_1 + 0.002\ X_2 + 0.02\ X_3 \quad \ldots\ldots(9)$$

$$\log Y_{2a} = -1.49 + \log 0.92\ X_1 + \log 0.7\ X_2 + \log 0.14\ X_3 \quad \ldots\ldots(10)$$

$$\log Y_{3a} = -1.86 + \log 0.8\ X_1 + \log 0.5\ X_2 + \log 0.25\ X_3 \quad \ldots\ldots(11)$$

In which

Y_1 = yolk utilization (transfer lot)

Y_2 = yolk depletion (transfer lot)

Y_3 = increasing weight (transfer lot)

Y_{1a} = yolk utilization (original lot)

Y_{2a} = yolk depletion (original lot)

Y_{3a} = increasing weight (original lot)

X_1 = final temperature (C)

X_2 = final oxygen level (mg/l)

X_3 = embryo or alevin weight (mg)

X_4 = original temperature (C)

X_5 = original oxygen level (mg/l)

Partial correlation coefficients (r), multiple regression coefficients (R^2), "t" values and F values for these regressions are given in Table 21. The values of partial correlation coefficients of

TABLE 21

Partial correlation coefficients, multiple regression coefficents, "t" and F values for the development regression (6-11) of Atlantic salmon alevins transferred from original experimental environments to all other conditions (6 x 6 combinations). Y_1 = Yolk utilization (transfer lot), Y_2 = Yolk depletion (transfer lot), Y_3 = Increasing weight (Transfer lot), Y_{1a} = Yolk utilization (original lot). Y_{2a} = Yolk depletion, Y_{3a} = Increasing weight (original lot).

	Correlation coefficents						"t" values					
Gravimetric Variable	Temp. X_1	Oxygen X_2	Weight X_3	Orig. Temp. X_4	Orig. Oxygen X_5	R^2	Temp. X_1	Oxyg. X_2	Weight X_3	Orig. Temp. X_4	Orig. Oxyg. X_5	F
Y_1	-0.03	0.04	0.46	-0.07	0.04	0.29	-0.20	0.28	3.6	-0.48	0.29	3.8
Y_2	0.23	0.23	-0.15	0.01	0.33	0.37	1.62	1.66	-1.0	0.06	2.4	5.5
Y_3	0.36	0.35	0.12	0.02	0.12	0.35	2.6	2.5	0.82	0.18	0.85	4.9
Y_{1a}	-0.5	0.04	0.4	-	-	0.19	-0.4	0.34	3.5	-	-	14.7
Y_{2a}	0.36	0.45	-0.07	-	-	0.28	2.7	3.5	-0.51	-	-	6.1
Y_{3a}	0.42	0.47	0.17	-	-	0.33	3.2	3.7	1.24	-	-	8.0

"t" 0.05 = 1.96, 0.01 = 2.57, 0.001 = 3.29. F 0.01 = 4.62, 0.05 = 3.72. N = 120.

yolk utilization and depletion in response to these variables are low. Since the highest R^2 value is only 0.37 the relative predictability of yolk depletion and utilization in relation to temperature, dissolved oxygen and yolkless alevin weight is weak (Table 22). However, the analysis of variance indicates some highly significant effects from the three variables. The only significant "t" value for yolk utilization was for yolkless alevin weight ($P<0.001$): for yolk depletion. Significant "t" values were determined for the original oxygen level ($P<0.01$) in the transfer group, and temperature ($P<0.001$) in those having constant environment. Temperature ($P<0.01$) and oxygen ($P<0.001$) were significant in both types of experiments on increase in weight of the alevins. Ranking of the effects of these variables, determined by "t" tests, on yolk utilization, yolk depletion, and increase in weight are presented in Table 23.

Multiple regression was also employed to determine the extent to which the embryo weight at stage 18 was determined by total ovum weight and by weight of yolk remaining at stage 18, relative to the regulating influences of temperature and oxygen. The regressions are,

$$Y_4 = 2.43 - 0.95 X_1 + 0.95 X_2 - 0.09 X_3 - 0.001 X_4 \quad \text{.....(12)}$$

$$Y_4 = 34.7 + 0.003 X_1 - 2.09 X_3 - 0.03 X_4 \quad \text{.....(13)}$$

$$Y_4 = 22.0 + 0.07 X_2 - 1.52 X_3 - 0.02 X_4 \quad \text{.....(14)}$$

$$Y_4 = 0.09 + 0.16 X_1 + 0.015 X_4 \quad \text{.....(15)}$$

$$Y_4 = -4.15 + 0.18 X_2 + 0.11 X_4 \quad \text{.....(16)}$$

$$Y_4 = 1.0 - 0.97 X_1 + 0.97 X_2 \quad \text{.....(17)}$$

$$Y_4 = 0.98 + 0.17 X_1 \quad \text{.....(18)}$$

$$Y_4 = -3.5 + 0.18 X_2 \quad \text{.....(19)}$$

TABLE 22

Alevin weights and yolk depletion from stage 18 to total yolk absorption at two temperatures and three levels of dissolved oxygen in the development of Atlantic salmon embryos.

Temp. C	Air sat. %	Measured Weight					Prediced Weight	
		Yolk conversion mg	Yolk depletion mg/day	Alevin weight increment mg/day	Alevin final weight mg	Days to total yolk depletion	Alevin weight final mg	Days to total yolk depletion
5	100	1.60±0.14	2.40±0.20	3.50±0.08	135.2±2.3	32±0.8	145	35
	50	1.58±0.04	1.0 ±0.20	1.60±0.05	153.3±2.4	80±1.2	171	90
	30	0.8 ±0.06	0.90±0.05	0.71±0.02	77.6±1.9	89±1.3	85	105
10	100	1.58±0.02	3.96±0.16	5.0 ±0.15	141.4±2.3	25±0.6	125	22
	50	0.94±0.01	2.06±0.14	2.15±0.13	99.0±1.7	36±1.7	99	46
	30	0.75±0.01	1.14±0.13	1.09±0.01	85.4±1.4	76±0.9	92	82

TABLE 23

Ranked effects of environmental conditions on yolk utilization in the development of Atlantic salmon embryos at two temperatures and three levels of oxygen. Those variables which occur in ranks 1, 2 and 3 were taken from constant environments while those in all five are the transfer lots.

	Degree of Effect				
Process	1	2	3	4	5
Yolk conversion	Embryo weight (X_3)	Original temp. (X_4)	Original oxygen (X_5)	Final oxygen (X_2)	Final temp. (X_1)
	Embryo weight (X_3)	Original temp. (X_1)	Original oxgyen (X_2)	-	-
Yolk depletion	Original oxygen (X_5)	Final oxygen (X_2)	Final temp. (X_1)	Embryo weight (X_3)	Original temp. (X_4)
	Final oxygen (X_2)	Final temp. (X_1)	Embryo weight (X_3)	-	-
Increase in weight	Final temp. (X_1)	Final oxygen (X_2)	Original oxygen (X_5)	Embryo weight (X_3)	Original temp. (X_4)
	Final oxgyen (X_2)	Final temp. (X_1)	Embryo weight (X_3)		

In which

Y_4 = embryo weight in mg

X_1 = yolk weight at stage 18

X_2 = ovum weight at stage 18

X_3 = temperature

X_4 = oxygen level

Partial correlation coefficients (r), multiple regression coefficients (R^2), "t" values and F values for these regression are given in Table 24.

Weighted means weights of the six experimental lots and results of "t" tests are presented in Table 25. All pairs of mean weights are significantly different by "t" test ($P<0.001$), except those from 5 C 30% air-saturation and 10 C 50% air-saturation, and from 10 C, at 30 and 50% air-saturation. Analysis of variance indicated that temperature ($P<0.0002$), level of development ($P<0.00003$) at sampling, and their interaction ($P<0.01$) are highly significant.

TABLE 24

Partial correlation coefficients, multiple regression coefficients, "t" and F values for the development regressions (12-19) for embryo, yolk, and total ovum weights in Atlantic salmon embryos at stage 18.

	Correlation coefficients					"t" values				
	Yolk X_1	Ovum X_2	Temp. X_3	Oxygen X_4	R_2	Yolk X_1	Ovum X_2	Temp. X_3	Oxygen X_4	F
Y_4	-0.98	0.98	-0.16	0.04	0.98	-50.0	52.0	-1.5	0.43	3561.0
Y_4	0.13	-	-0.66	-0.25	0.61	0.12	-	-7.9	-2.37	43.5
Y_4	-	0.27	-0.50	-0.17	0.64	-	2.58	-5.23	-1.6	49.3
Y_4	0.56	-	-	0.10	0.31	6.10	-	-	0.92	19.2
Y_4	-	0.72	-	0.09	0.52	-	9.5	-	0.86	45.6
Y_4	-0.98	0.99	-	-	0.98	-58.1	69.9	-	-	1791.0
Y_4	0.55	-	-	-	0.30	6.13	-	-	-	37.6
Y_4	-	0.72	-	-	0.51	-	9.53	-	-	90.8

Y_4 = embryo weight in mg. "t" 0.05 = 1.96, 0.01 + 2.57, 0.001 = 3.29. FO. 01 + 4.62, 0.05 = 3.72, N = 120

TABLE 25

Probability values from "t" tests for differences between weighted mean weights of Atlantic salmon embryos in six lots incubated at two temperatures and three levels of dissolved oxygen.

			5 C			10 C		
			Air-saturation (%)					
Temp. C	Oxygen	mean+SD weight	100	50	30	100	50	30
5	100	6.8±.3	-	0.001	0.001	0.001	0.001	0.001
	50	5.7±0.3	0.001	-	0.001	0.001	0.001	0.001
	30	3.48±0.6	0.001	0.001	-	0.001	0.1	0.05
10	100	5.43±0.2	0.001	0.001	0.001	-	0.001	0.001
	50	4.94±0.2	0.001	0.001	0.1	0.001	-	0.05
	30	3.9±0.4	0.001	0.001	0.05	0.001	0.05	-

DISCUSSION

Weight has been adopted as the principal measure of size because it provides a more realistic reflection of changes between embryos and yolk than would linear measures which are subject to independent changes in proportionality (Tusnady 1962, Laurence 1969, Thomas et al. 1969, Brett 1970 b.). For instance, the embryos at lower oxygen levels developed a more slender body than those at higher levels according to the measurements from which length-weight relations were calculated in the present experiments.

The salmon ovum has great individual variability in mature diameter and weight. The range of weight for all measurements was 80-220 mg, 1 day after fertilization. The mean weight of ova in stage 1 in this study was 139 mg and 77% were within the range of 132-146 mg. Hayes (1949) gave an average of 108.5 mg for ova from Nova Scotian salmon and Rudy and Potts (1969) reported 83.9 mg average weight for Atlantic salmon ova from British stock. Probably these differences are due mainly to the size of spawning females. The ova in the present study were taken from a 7-kg salmon. The diameter of Atlantic salmon ova ranged between 5-7 mm in these experiments, but most of the ova were between 6.5-6.8 mm diameter.

During the developmental period salmon ova increased in weight. This can be explained by partial permeability of zona radiata toward various ions and water. (Hayes 1930 a, Zotin 1958, Alderdice and Forrester 1968. Blaxter 1969, Potts and Rudy 1969, Rudy and Potts 1969, Eddy 1974). However, in an ancillary experiment in which ova were incubated at 20‰ salinity, the weight declined by a few percent immediately following introduction and remained depressed during the entire developmental period (Appendix 5).

Embryos of salmon and several other species of fishes are known to be consistently of different sizes at hatching and yolk absorption in accordance with their developmental conditions including variation in temperature, dissolved oxygen and ambient water exchange. Higher temperature causes retarded embryonal growth of salmonids and many other fishes (Alderdice and Forrester 1968, Garside 1959, 1966a, 1970, Scott 1972, Fluchter and Pandian 1968, Wadley et al. 1971). Unusually, perhaps, embryonal growth of Pacific sardine embryos was increased at higher temperature (Lasker 1964).

In addition, there are some possible physical explanations, including the temperature effect on circulating plasma (Stitt et al. 1970), the surface to volume relation which becomes increasingly critical as respiratory demand increases (Baravik 1963), and the partial pressure of dissolved oxygen. The principal explanation however, might be differences in enzyme composition in various species (Hochachka and Somero 1971, 1973).

Also, it seems that at higher temperature, differentiation is accelerated, but the weight gain proceeds at a relatively slower rate. At lower oxygen levels the oxygen supply cannot satisfy the increasing requirement, which is inversely related to temperature, and it becomes limiting so that uptake is reduced and weight increment is reduced accordingly. Thus, the amount of yolk used is relatively higher in limiting conditions per unit increment of embryonal weight. This then will alter the relative weights of yolk, embryo and other components of these ova, from stage 1 to 18, as well creating differences among groups at any stage.

However, after stage 18 the rate of yolk absorption, and the growth rate of fry is mainly regulated by the oxygen supply. In experiments in which fry were transferred to different temperatures or levels of oxygen the main regulating factor was the final temperature. (Table 23).

Also, the expected embryo weight to stage 18 can be predicted

with considerable accuracy in a particular constant environment from the remaining yolk weight and the total ovum weight. (Table 22). However, predictions only from yolk weight, air-saturation or temperature are very poor for the expected embryo weight because these factors are in interactions. The different variables can mask each other. For example in the prediction of embryo weight to stage 18, the degree of air-saturation is not significant according to results of analysis of variance, but if the same feature is examined with multiple regression and the temperature is omitted from the calculation the effect of air saturation becomes highly significant (Table 24), Gulidov 1969). Also in a richly oxygenated environment, development and growth accelerate. (Kinne and Kinne 1962b, Ostroumova 1969). The present data and the quoted observations suggest that the embryo size is regulated by temperature and influenced by the oxygen supply, yolk and ovum size.

One explanation for the larger embryos at 5 C 50% air-saturation could be that at 50% air-saturation intraoval body movements of the advanced embryo diminish adaptively and the energy spared can be used for synthesis of tissues and increase in weight. (Brett 1970a, b, Lasker and Theilacker 1962).

Average daily yolk depletion conforms to the general trends for growth of these embryos. These groups of embryos with greater increase in weight also have the greater mean daily yolk depletion. Yolk utilization is the quantity of yolk required to produce some defined unit of embryo and is synonymous then with food conversion efficiency. Yolk utilization drops sharply after hatching, but increases again toward the time of total yolk absorption. These changes can be the result of less efficient transport of yolk materials (Ignat'eva 1970), but also can result from the increased locomotor efficiency which follows the absorption of the

cumbersome yolk. This idea is supported by results of Thomas et al. (1969) who found that the swimming ability of chinook salmon alevins (Oncorhynchus tshawytscha) increased with the reduction in the amount of yolk-sac until an advanced stage of yolk absorption was reached. They also identified a period of reduced swimming ability occurring shortly before complete yolk absorption. Almost the same result was obtained by Laurence (1969) concerning energy expenditure, yolk absorption, and utilization, in larvae of largemouth bass (Micropterus salmoides).

In an attempt to explain extensive larval mortality in largemouth bass, Laurence (1969) examined ovum and yolk size to determine whether there was sufficient nutrient supply to carry larvae to a free-feeding state. He was able to demonstrate that ova and consequently yolk were generally of sufficient size to support these larvae to their feeding state. A similar observation was made for carp Cyprinus carpio by Zonova (1973).

Since the process of development produces a growing embryo and alevin, it is not surprising that yolk utilization depends mostly on the size of the embryo for any stated respiratory rate and amount of yolk remaining, at the designated stage.

A general observation concerning transfer groups is that about a week was necessary for the embryos to adapt a new environment as indicated by stabilization of growth and yolk depletion. (Tables 21-24).

CHAPTER IV

OXYGEN CONSUMPTION OF OVA AND EMBRYOS OF ATLANTIC SALMON AT VARIOUS TEMPERATURES AND LEVELS OF DISSOLVED OXYGEN

INTRODUCTION

Respiration is one of the most important processes the measurement of which gives information about the metabolism of developing embryos. In fish embryos the absolute amount of respired oxygen generally rises with time, but the relative amount respired per unit of weight decreases as development proceeds (Baravik 1963, Brachet 1960, Prosser 1973, Woynarovich 1964).

Examination of data on oxygen consumption by Atlantic salmon ova, presented by Privolnev (1938) and Hayes et al. (1951) indicates somewhat irregular fluctuations although there are rising trends in both studies which are interrupted in each by two pronounced peaks.

These changes in oxygen consumption raise the question, "Is there a certain trend of pattern, or are such changes a matter of chance?". In spite of some contradictions similar results have been described by other authors in teleost fishes and other embryos developing in water. (Bennett 1968, Brachet 1960, Brahma 1966, Laurence 1969, Løvtrup and Iverson 1969, Privolnev 1938, Prosser 1973).

The major purpose of this phase of the study on Atlantic salmon embryos has been to provide further evaluation of oxygen consumption in fish embryos which could be associated with the depletion and the utilization or conversion of the energy sources of the advancing embryo. These processes have been measured in various levels of oxygen supply at temperatures which create pronounced differences in metabolic rate. Equations were subsequently derived for the prediction of oxygen consumption in various conditions.

Ancillary experiments were conducted to determine whether or not there existed cyclic patterns of oxygen consumption, and whether or not the extraembryonal portions of the ovum contribute to the consumption

of oxygen.

The investigation of extraembryonal respiration was prompted by a knowledge of the localization of oxidative enzymes in yolk of the loach Misgurnus fossilis by Abramova et al. (1965) and in the vitelline membrane (synthitial layer) of the ovum of rainbow trout Salmon gairdneri by Porcelli (1969).

MATERIALS AND METHODS

Oxygen consumption in embryonated Atlantic salmon ova was measured in Warburg manometric respirometers and alternatively in continuous-flow chambers. Embryos, yolks, and zona radiata were separated for isolate respirometry in accordance with the procedure of MacKelvie (1971). They were washed in distilled water and then in 0.9% NaCl solution. From zona radiata "ghost shell" was obtained, described by Bell et al. (1969). Oxygen consumption of ova in water, isolated zona radiata, yolk, and embryo in 0.9% saline solution was measured in Warburg manometric respirometers following the procedure of Legname (1968).

The respirometer flask was approximately 17 ml equipped with two sidearms and a centre well. Carbon dioxide was absorbed with 20% KOH in the centre well. The observations were made at 5, 7.5, and 10 C with shaking at 60 cycles/min with an amplitude of 20 mm. The total volume of the system was always maintained at 3.2 ml. Penicillin was administered at the rate of 500 I. U./ml of medium in some of the later tests to estimate potential errors resulting from bacterial respiration (Bell et al. 1971).

An alternative method for the measurement of oxygen utilization of eggs was effected with simple continuous-flow chambers. One consisted of a 4-ml glass tube (100 x 7 mm i.d.) closed at each end by a rubber stopper through which a 3 mm vinyl tube was inserted and sealed. The other type of continuous-flow respiration chamber was a vertical plexiglass cylinder 80 mm x 45 mm diameter in which 25 ova were supported on a horizontal polyvinyl screen having a mesh interval of 2 mm. The inlet was situated below the screen and the outlet passed through a rubber stopper in the upper end.

Mostly the chambers were operated at a constant flow-rate of 4 ml/min but some tests were conducted at higher or lower rates and some

were conducted at variable flow-rates. The incurrent tube was fitted with a T-coupling to establish a bypass flow. Some of the incurrent water was diverted from passage through the chamber via the bypass. Collection in triplicate of 5-ml samples of incurrent and excurrent water were made at the beginning and completion of a test and at some intermediate time variously at 0900, 1200 and 1500 hr daily, from Stage 2 - 18.

The rate of flow was calculated from the time required to fill a 100-ml volumetric flask with the excurrent water. Oxygen extraction was determined from the difference between dissolved levels in the incurrent and excurrent flows. Levels of dissolved oxygen were determined by standard Winkler semimicrotitrimetry (Harper 1953). A blank chamber was also used during each test to confirm results.

The recorded data were processed according to Sokel and Rohl (1969) and by programmes ANOV 80 of University of Alberta Division of Educational Research Services and BMDO3R in CDC6400 computer of Dalhousie University to yield mean oxygen consumption and cumulative oxygen consumption (total consumption at each stage) and to establish an algebraic model from which unit oxygen consumption can be predicted in various combinations of the variables employed.

RESULTS

The oxygen consumption of recently fertilized ova increased from three to five times over that of unfertilized ova in the first 4 hr.

The experimental results in 1972 and 1973 were measured for stage 16 and the values are summarized in µl O/g/hr in Table 26.

Results of respirometry by the two techniques were similar. Oxygen consumption was regularly greater at 10 C than at 5 C for each of the three isolated parts of the ovum as well as in the total embryonated ovum.

Oxygen consumption at stage 16 in water was greatest in yolk and least in zona radiata at 5 C while at 10 C the decreasing sequence was embryo, zona radiata and yolk. Oxygen consumption of the total ovum was only slightly greater than that of isolated yolk which forms about 70% of the total weight of the living ovum at that stage.

In Ringer's solution (Warburg respirometry) at 5, 7.5 and 10 C, oxygen consumption decreased from zona radiata to yolk.

Analysis of variance was applied to the results given in Table 26. The total analysis of all data is presented in Table 27 and reveals that the two most significant factors in the Warburg respirometric experiments were temperature and the medium. Oxygen consumption of the total ovum is significantly influenced by temperature, medium, and their interaction ($P<0.05$). For salmon embryos separately only temperature was significant ($P<0.05$) and for yolk and zona radiata none of these factors caused significantly different consumption.

Other series of Warburg respirometric experiments were conducted on later salmon ova at stage 18. From these experiments, equations were calculated for the respiration of total ovum, embryo, and yolk where log X is the weight of oxygen consuming material in mg and log Y is the oxygen uptake

TABLE 26

Mean oxygen uptake ($mm^3/g/hr$) for intact ova, embryos and corresponding yolks, zona radiata at stage 16 (Garside 1959), measured in 3, 6, and 16 hr. intervals in Warburg respirometers containing either water or Ringer's solution (9g/litre TDS).

Temperature C	Component	Medium	Exposure (hr) 3	6	16
5	Ovum	Water	48.7+4.2	31.4+1.8	18.5+0.8
		Ringer's	48.3+2.2	16.8+3.5	4.4+1.8
	Embryo	Water	63.5+3.5	48.6+4.2	183.4+32.5
		Ringer's	196.8+8.9	160.0+40.0	41.0+5.5
	Yolk	Water	136.0+8.5	104.0+9.3	35.5+2.6
		Ringer's	70.4+20.0	59.1+8.2	60.5+5.5
	Zona radiata	Water	47.0+1.8	36.0+1.9	36.0+1.5
		Ringer's	197.0+30.0	244.0+7.2	110.0+6.3
7.5	Ovum	Ringer's	51.1+1.2	12.9+2.4	6.12+1.2
	Embryo	Ringer's	688.9+13.0	418.1+20.0	418.1+18.0
	Yolk	Ringer's	94.8+1.9	30.1+0.8	73.2+1.9
	Zona radiata	Ringer's	433.0+60.0	313.0+25.0	310.0+35.0
10	Ovum	Water	66.4+2.8	44.5+2.5	34.5+1.2
		Ringer's	42.0+1.9	71.1+3.4	21.5+1.6

continued..

TABLE 26 (continued)

Temperature	Component	Medium	Exposure (hr)		
			3	6	16
10.0	Embryo	Water	289.5±10.2	221.1±25.0	205.8±15.2
		Ringer's	267.0±15.0	320.0±23.0	418.0±22.0
	Yolk	Water	174.0±28.0	38.0±2.4	59.0±2.7
		Ringer's	174.0±31.0	38.0±1.8	59.0±2.1
	Zona radiata	Water	148.0±14.0	172.0±8.8	93.0±3.8
		Ringer's	600.0±75.0	546.0±42.0	250.0±140.0

TABLE 27

Summary of analysis of variance for Warburg respirometric tests of intact ova, embryos and corresponding yolks, zona radiata at stage 16 measured in 3, 6 and 16 hr. intervals in either water or Ringer's solution (9g/l TDS) at 5 and 10 C.

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	Probability
Temperature	298225.0	1	298225.0	6.75	0.041*
Medium	493310.0	1	493310.0	11.15	0.016*
Component	90569.0	3	30190.0	0.68	0.594
Time	80878.0	2	40439.0	0.91	0.45
Temp. x Component	49743.0	3	16581.0	0.37	0.77
Temp. x Medium	241785.0	1	241785.0	5.47	0.058
Temp. x Time	40165.0	2	20083.0	0.45	0.65
Medium x Time	150953.0	2	75476.0	1.70	0.26
Medium x Component	20515.0	3	6838.0	0.15	0.92
Component x Time	493323.0	6	82220.0	1.85	0.23
Temp. x Med. x Comp.	56689.0	3	18896.0	0.43	0.74
Temp. x Med. x Time.	32299.0	2	16150.0	0.36	0.71
Temp. x Comp. x Time	318866	6	53144.0	1.20	0.41
Med. x Comp. x Time	422651.0	6	70442.0	1.59	0.29
Error	265265.0	6	44211.0		

* = 0.05>P>0.01

µ-liter/g/hr (Table 28).

Intact embryonated ova appear to have a circadian rhythm in respiration. The experimental basis for this observation is given in Appendix 4.

The mean oxygen consumption per unit weight and unit time at various developmental stages has been calculated for ova incubated at 5 and 10 C with dissolved oxygen levels of 30, 50 and 100% air-saturation (Figures 6 and 7, Table 29).

The mean oxygen consumption measured daily throughout the embryonal phase, (Table 30) shows that the significant effect by the experimental variables are the interactions of oxygen saturation x flow, and temp x oxygen x flow according to analysis of variance (Table 32).

The total oxygen consumption (Table 31, 32) and its analysis reveals the three most significant experimental variables are oxygen level, flow-rate, and oxygen x flow. In spite of differences in mean hourly oxygen consumption during the developmental time from stage 1 and 18 the total sum of oxygen consumption at every stage does not differ from 5 to 10 C at the same level of oxygen content irrespective of the flow-rate and time required to complete development.

The equations for linear multiple regression which best describe oxygen consumption of salmon ova during embryogeny (Stages 1 - 18) are;

$$\text{Log } Y = -3.62 + 1.1 \text{ Log } T + 1.0 \text{ Log } O_2 + 0.23 \text{ Log } F + 0.39 \text{ Log } D \quad \ldots(26).$$

$$\text{Log } Y = -4.86 + 0.065 \text{ Log } T + 1.11 \text{ Log } O_2 + 0.88 \text{ Log } F + 1.05 \text{ Log } S \quad \ldots(27).$$

Figure 6

Respirometry of samples of Atlantic salmon ova (mg/g/hr) at 18 embryogenic stages during incubation at 5 C and three levels of dissolved oxygen. Oxygen consumption was measured on the day on which each lot of embryos was considered to have just reached the structural stage as defined in text.

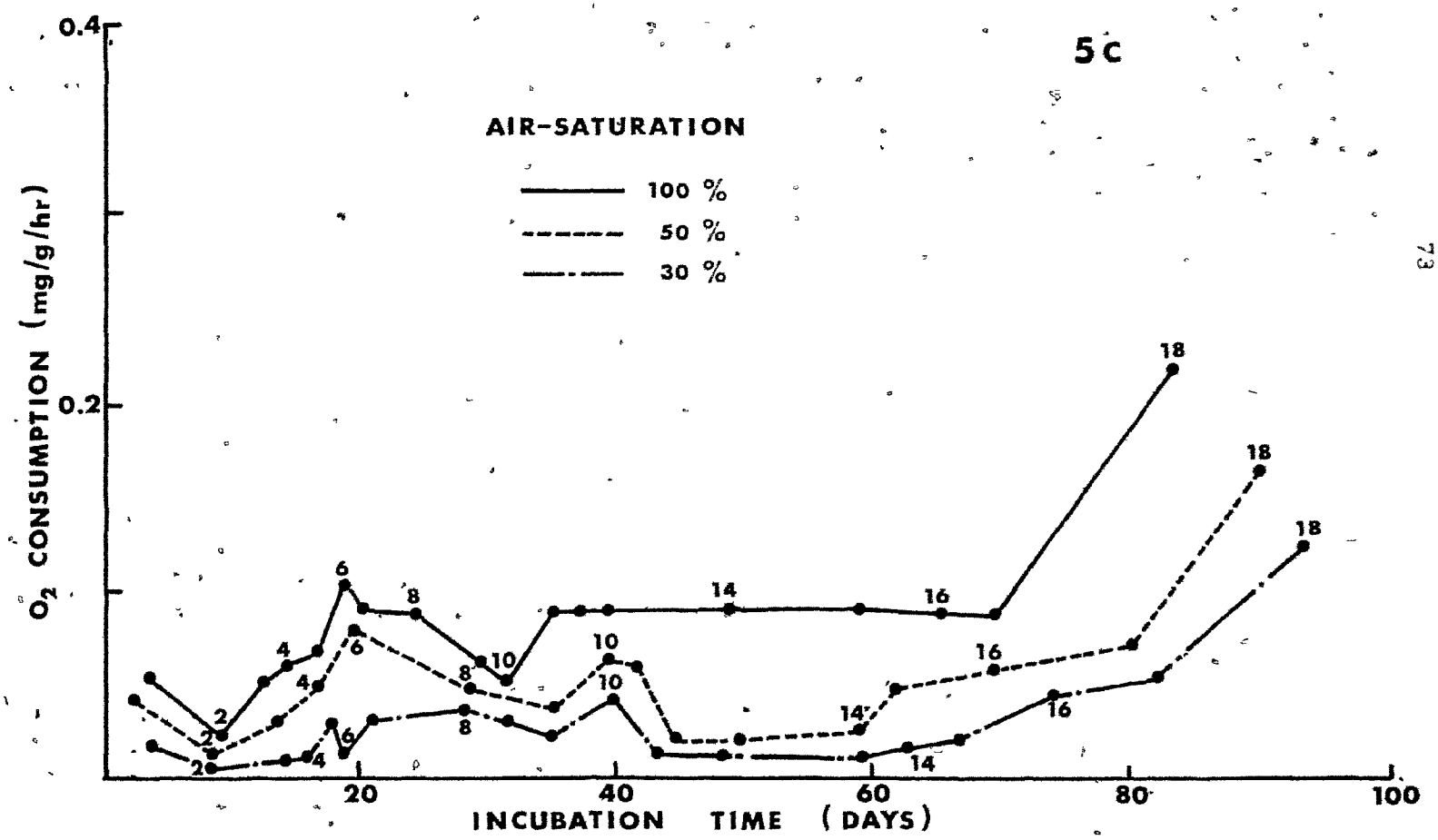
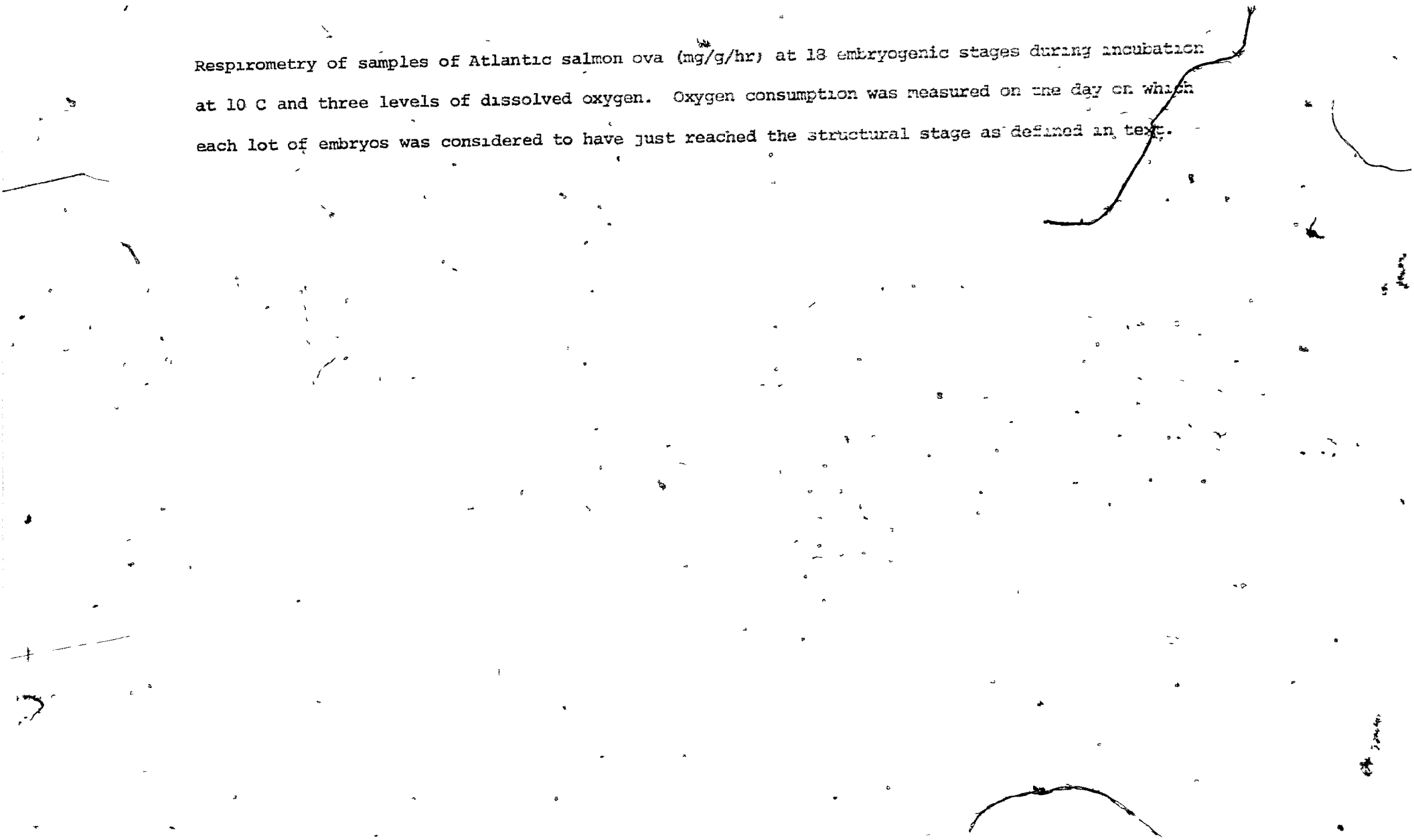

5c
AIR-SATURATION
100 %
50 %
30 %
O_2 CONSUMPTION (mg/g/hr)
0.4
0.2
20
40
60
80
100
INCUBATION TIME (DAYS)

Figure 7



Respirometry of samples of Atlantic salmon ova (mg/g/hr) at 18 embryogenic stages during incubation at 10 C and three levels of dissolved oxygen. Oxygen consumption was measured on the day on which each lot of embryos was considered to have just reached the structural stage as defined in text.

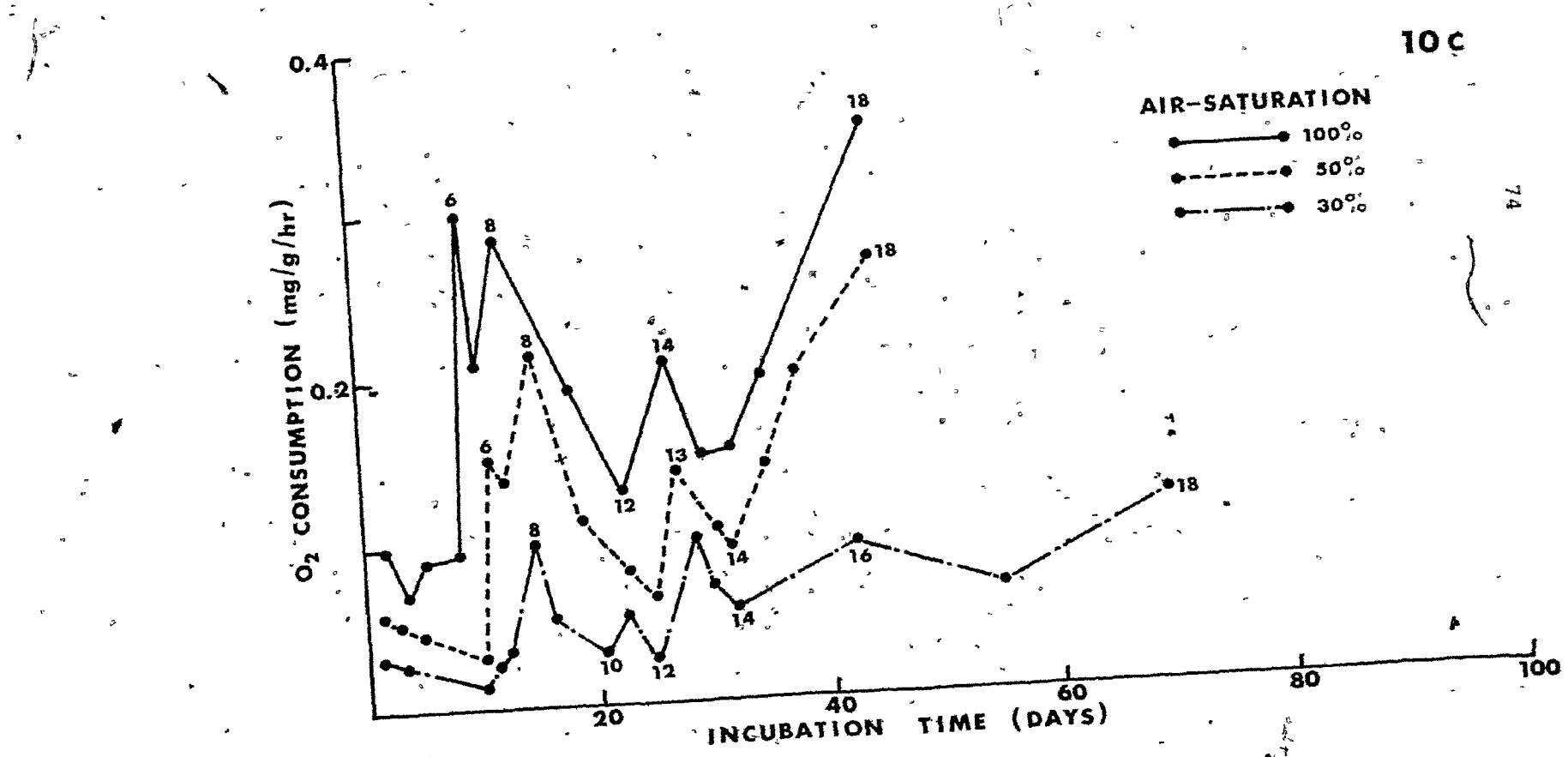

10 C
AIR-SATURATION
100%
50%
30%
0.4
0.2
O_2 CONSUMPTION (mg/g/hr)
INCUBATION TIME (DAYS)
20
40
60
80
100
6
8
10
12
13
14
16
18

TABLE 28

Mean oxygen uptake (mm^3/g/hr) for intact ova, embryos and corresponding yolks, zona radiata at stage 18 measured at 3-hr intervals in Warburg respirometers, containing either water or Ringer's solution (9g/litre TDS) at 5 and 10 C. Coefficents of determination (r^2) and regression equations are also given.

Temperature C	Medium	Component	Mean weight mg	Mean oxygen uptake mm^3/g/hr	r^2	Equation log Y =
5	Water	Ovum	156.0±5.0	41.8±3.2	-0.77	4.75-1.29log X (20)
	Ringer's	Embryo	22.3±1.0	180.0±7.0	-0.90	2.25-0.11log X (21)
	Ringer's	Yolk	73.4±8.5	111.0±8.5	-0.96	3.88-1.05log X (22)
10	Water	Ovum	144.6±4.8	93.0±5.8	-0.70	4.75-1.29log X (23)
	Ringer's	Embryo	18.3±1.1	335.5±22.4	-0.03	2.90-0.28log X (24)
	Ringer's	Yolk	63.2±6.4	130.0±21.0	-0.44	3.91-log X (25)

Y = mean oxygen uptake in mm^3/g/hr

X = weight of component in mg

TABLE 29

Calculated mean oxygen consumption (mg/g/hr) of Atlantic salmon ova at 5 and 10 C and 100, 50 and 30% air-saturated water in stages 1 to 18 at 4 ml/min flow-rate and 2 lux illumination from 800 to 1800 hr in continuous-flow respirometers.

	5 C			10 C		
Stage	100	50	30	100	50	30
1	0.06±0.01	0.03±0.01	0.01±0.01	0.1±0.02	0.05±0.02	0.01±0.01
2	0.02±0.01	0.02±0.01	0.01±0.01	0.05±0.02	0.04±0.01	0.02±0.01
3	0.05±0.01	0.02±0.01	0.01±0.01	0.07±0.01	0.03±0.01	0.02±0.01
4	0.06±0.01	0.04±0.01	0.01±0.01	0.07±0.02	0.02±0.01	0.01±0.01
5	0.06±0.01	0.05±0.01	0.03±0.01	0.08±0.02	0.03±0.01	0.01±0.01
6	0.01±0.01	0.08±0.01	0.01±0.01	0.30±0.01	0.16±0.01	0.03±0.01
7	0.08±0.03	0.08±0.01	0.03±0.01	0.21±0.01	0.14±0.01	0.09±0.05
8	0.08±0.03	0.04±0.02	0.04±0.01	0.30±0.01	0.23±0.01	0.10±0.02
9	0.04±0.01	0.02±0.01	0.02±0.01	0.25±0.01	0.12±0.01	0.05±0.02
10	0.09±0.04	0.06±0.01	0.05±0.01	0.18±0.04	0.09±0.01	0.03±0.01
11	0.08±0.01	0.05±0.02	0.01±0.01	0.16±0.01	0.08±0.01	0.06±0.01

continued...

TABLE 29

Stage	5 C			10 C		
	100	50	30	100	50	30
12	0.08±0.01	0.02±0.01	0.01±0.01	0.14±0.03	0.05±0.01	0.03±0.01
13	0.08±0.01	0.01±0.01	0.01±0.01	0.15±0.01	0.14±0.01	0.10±0.01
14	0.09±0.01	0.02±0.01	0.01±0.01	0.21±0.03	0.11±0.07	0.07±0.02
15	0.09±0.01	0.04±0.01	0.01±0.01	0.16±0.01	0.08±0.06	0.08±0.02
16	0.08±0.03	0.05±0.02	0.04±0.01	0.16±0.04	0.14±0.07	0.09±0.01
17	0.08±0.05	0.07±0.02	0.04±0.01	0.2±0.06	0.20±0.01	0.06±0.01
18	0.21±0.02	0.18±0.03	0.12±0.01	0.35±0.03	0.26±0.01	0.11±0.01

TABLE 30

Calculated mean oxygen consumption (mg/g/hr) for Atlantic salmon ova from stages 1 to 18 at 5 and 10 C, 30, 50 and 100% air-saturation at various flow-rates.

Temp C	O_2 % sat.	Flow-rate ml/min			
		1.0	2.0	4.0	8.0
5C	100	0.02	0.05	0.07	0.18
	50	0.02	0.04	0.06	0.14
	30	0.01	0.02	0.04	0.09
10C	100	0.04	0.08	0.14	0.40
	50	0.02	0.05	0.10	0.20
	30	0.01	0.03	0.06	0.12

TABLE 31

Calculated total oxygen consumption (mg/g) of Atlantic salmon from stage 1 to 18 at 5 and 10 C, 30, 50 and 100% air-saturation. Embryo weight (mg) is taken at stage 18 for group from 4 ml/min flow-rate.

Temp C	O_2 % sat.	Flow-rate ml/min 1.0	2.0	4.0	8.0	Embryo weight
5	100	49.8	99.8	139.4	358.8	22.1
	50	43.2	86.4	129.6	302.0	26.6
	30	22.3	44.6	89.3	200.6	11.9
10	100	41.3	82.5	145.0	412.8	14.5
	50	22.6	56.4	112.8	225.6	12.5
	30	16.6	49.6	99.4	198.8	10.0

TABLE 32

Summary of analysis of variance of mean and total oxygen consumption of Atlantic salmon ova from stages 1 to 18, at 5 and 10 C and 30, 50 and 100% air-saturation various flow rates.

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	Probability
Mean oxygen consumption					
Temperature	0.11	1	0.11	4.51	0.077
Oxygen	0.07	2	0.035	1.44	0.30
Flow	0.013	3	0.04	1.71	0.26
Temp. x Oxygen	0.027	2	0.014	0.56	0.59
Temp. x Flow	0.026	3	0.09	0.36	0.78
Oxygen x Flow	0.12	6	0.02	8.31	0.01*
Temp. x Oxygen x Flow	1.39	6	0.023	9.66	0.001**
Error	1.21	6	0.024		
Total oxygen consumption					
Temperature	438.6	1	438.6	1.03	0.35
Oxygen	7342.0	2	3671.0	8.65	0.017*
Flow	16290.0	3	5430.1	12.80	0.005**
Temp. x Oxygen	1189.3	2	594.7	1.40	0.32
Temp. x Flow	1290.0	3	430.0	1.01	0.45
Oxygen x Flow	236630.0	6	39438.0	93.0	0.001***
Temp. x Oxygen x Flow	20310	6	3385	0.12	0.1
Error	230104	6	424.1		

in which,

Y = oxygen consumption ova (mg/g/hour)

T = temperature of water (C)

O_2 = dissolved oxygen (mg/l)

F = waterflow (ml/min)

D = developmental age of ova (days)

S = development age of ova in embryonal stages

The calculated correlation coefficients are presented in Table 33 with the variables that determine oxygen consumption of salmon ova. Correlation between oxygen consumption and age of embyro, water temperature, oxygen content, and flow-rate are significant in this decreasing order of "t" values (Tables 34). In both Tables variables No. 7, time in days, is stated as the accumulated percentages of time required to reach each stage as a (%) of time required to reach stage 18;

a (%) of time required to reach stage 18;

$$\Sigma \left(\frac{T_1}{T_{18}} \times 100\right) + \left(\frac{T_2}{T_{18}} \times 100\right) + \ldots\ldots \left(\frac{T_{18}}{T_{18}} \times 100\right)$$

in which

T_1 = time in days to stage 1

T_2 = time in days to stage 2

T_{18} = time in days to stage 18

for each of the six environments. Variable No. 8, time, is the accumulated percentage of time required to reach each stage as a (%) of time required to reach stage 18 at 5 C, 100 % air-saturation:

$$\Sigma \left(\frac{T_1}{T_{18}\ C_5} \times 100\right) + \left(\frac{T_2}{T_{18}\ C_5} \times 100\right)$$

TABLE 33

Correlation coefficients of multiple regression analysis of oxygen consumption of salmon ova incubated at 5 and 10°C and at 30, 50 and 100 % air-saturation. In equations 26 and 27, variable No. 7, time, is stated as the accumulated percentages of times required to reach each stage as a (percent) of time required to reach stage 18. Variable No. 8, time as the accumulated percentages of time required to reach each stage as a (percent) of time required to reach stage 18 at 5 C 100 % air-saturation. (See text for expanded explanation).

	* Variable	1	2	3	4	5	6	7	8
1	Temperature	-	-0.12	-0.06	0.77	-0.05	0.22	-0.1	0.13
2	Oxygen mg/l	-0.12	-	0.01	0.60	0.06	-0.16	-0.02	-0.06
3	Flow ml/min	-0.06	0.01	-	0.57	-0.01	-0.02	-0.03	-0.03
4	O_2 cons. mg/g/hr	0.77	0.60	0.57	-	0.93	0.85	0.96	0.95
5	Times (days)	-0.05	-0.06	-0.01	0.93	-	0.85	0.98	0.97
6	Stage	0.22	-0.17	-0.02	0.85	0.85	-	0.87	0.85
7	Time	0.10	-0.02	-0.03	0.96	0.98	0.87	-	0.99
8	Time	0.13	-0.06	-0.03	0.95	0.97	0.86	0.99	-

TABLE 34

"t" values of multiple linear regression for oxygen consumption of Atlantic salmon ova in different combinations of variables. (Equations 26 and 27).

	Variable										
	1	2	3	4	5	6	7	P	r^2	F	P
Combinations											
1.	2.91	4.15	6.7	1.6	-	-	-	0.95	0.9	24.3	0.001
2.	2.56	3.81	6.63	-	0.54	-	-	0.98	0.88	19.9	0.001
3.	2.22	4.05	6.63	-	-	1.41	-	0.95	0.90	24.3	0.001
4.	1.76	4.23	6.71	-	-	-	1.89	0.95	0.90	24.3	0.001
5.	3.57	3.82	6.62	-	-	-	-	0.84	0.71	8.9	0.01
6.	2.95	4.22	-	1.24	-	-	-	0.93	0.86	24.1	0.001
7.	2.17	-	6.75	0.23	-	-	-	0.92	0.85	21.8	0.001
8.	3.50	4.0	-	-	-	-	-	0.78	0.61	9.4	0.01
9.	2.47	-	6.8	-	-	-	-	0.73	0.53	7.0	0.01
10.	4.23	5.56	-	-	-	-	-	0.87	0.75	18.0	0.001
11.	-	4.86	3.82	-	-	-	-	0.83	0.68	13.3	0.001
12.	-	-	5.68	4.22	-	-	-	0.91	0.83	30.7	0.001

continued...

TABLE 34 (continued)

	Variable										
	1	2	3	4	5	6	7	R	R^2	F	P
Combinations											
13.	-	-	-	4.07	3.16	-	-	0.86	0.74	17.7	0.001
14.	2.82	-	-	-	-	-	-	0.36	0.13	2.0	0.05
15.	-	4.35	-	-	-	-	-	0.77	0.59	18.9	0.001
16.	-	-	3.87	-	-	-	-	0.73	0.53	14.9	0.001
17.	-	-	-	2.91	-	-	-	0.62	0.39	8.5	0.01
18.	-	-	-	-	2.76	-	-	0.60	0.37	7.7	0.01
19.	-	-	-	-	-	2.91	-	0.62	0.39	8.5	0.01
20.	-	-	-	-	-	-	2.92	0.63	0.39	8.5	0.01
21.	1.60	3.0	-	-	4.6	-	-	0.93	0.86	24.0	0.001
22.	2.88	-	2.73	-	4.9	-	-	0.92	0.85	21.7	0.001
23.	4.2	-	-	-	5.5	-	-	0.87	0.74	18.8	0.001
24.	-	5.7	-	-	4.2	-	-	0.91	0.83	30.8	0.001
25.	-	-	4.07	-	3.12	-	-	0.86	0.74	17.7	0.001

F 0.05>1.64

continued...

TABLE 34 (continued)

List of variables:

1 = temperature in C

2 = oxygen content mg/l

3 = flow rate of incubation water in ml/min

4 = time of incubation in days

5 = developmental stage of embryo

6 = time in % of stage 18 (st. 18 = 100%) (See table 33, variable No. 7).

7 = time in % of stage 18 (st. 18 of 5 C 100% air saturation = 100%) (See table 33, variable No. 8).

DISCUSSION

One of the main differences between adult and embryonal oxygen consumption in addition to the much greater amount per unit of weight for embryos is that the amount consumed per unit of weight rises almost continuously during embryogeny but after a certain point then gradually decreases somewhat to values which are more or less constant through much of adult life.

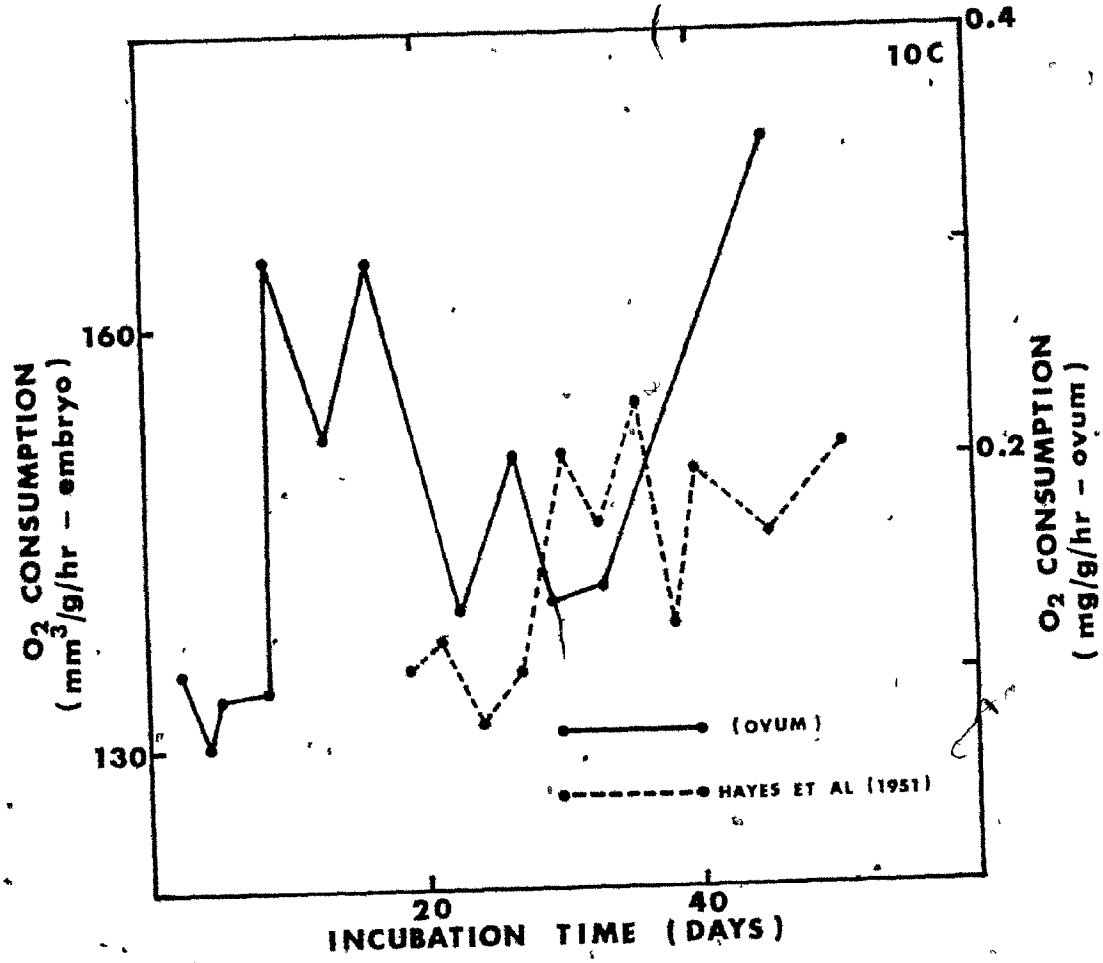
However, the data in Table 29 and Figures 6 and 7 based on oxygen consumption at two levels of temperature and three levels of dissolved oxygen indicate that oxygen consumption is not a simple linear phenomenon in the case of the development of Atlantic salmon embryos. There are substantial peaks in the graphic curves. It is possible perhaps to find several explanations. It is possible that there are measuring errors from different ovum sizes and respirometers, flow rates, and temperatures may create erratic oscillations in oxygen consumption. In spite of such potential sources of error, results collected from three years of experiments totalling 81 series of determinations indicate that such oscillations are indeed real and must be caused by physiological properties of the respiring ova generally, and developing embryos in particular.

Oscillations in level of oxygen consumption have been demonstrated during embyrogenesis in sea urchins (Echinoidea: Echinodermata) by several investigators including Løvtrup and Iverson (1969). Scholander et al. (1958). The oscillations were of sufficient precision as to constitute a cyclic process in these sea urchins. While there is a similarity of pattern among the reported curves of oxygen consumption in salmon ova the fluctuations themselves do not form periodic or cyclic changes.

In Figures 6, 7 and 8 and curves from Hayes et al. (1951) and those of the present study for 10 C are of similar form although not

Figure 8

Comparison of oxygen consumption of Atlantic salmon ova several times during incubation at 10 C and 100% air-saturation in the study by Hayes et al. (1951) and data of the present study (Figure 7). The left Y-axis is graduated in (mm^3) per embryo for the data of Hayes et al. and the right Y-axis is graduated in (mg/g/hr) for mean total ovum. Hayes et al. (1951) measured oxygen consumption in intact ova but converted the unit quantity to unit weight of embryo on the then reasonable expectations that only the embryo per se respired.


10C
0.4
160
130
0.2
20
40
INCUBATION TIME (DAYS)
O2 CONSUMPTION (mm3/g/hr - embryo)
O2 CONSUMPTION (mg/g/hr - ovum)
(OVUM)
HAYES ET AL (1951)

coincident in developmental stages. The curve given by Privolnev (1938, p. 168) coincides rather well with present data. Similar form of the oxygen consumption trend for rainbow trout ova has also been reported by Baravik (1963).

According to Hayes et al. (1951) there is no significant trend in oxygen consumption of Atlantic salmon embryos. In spite of that, their measured range, with considerable fluctuation, is between 131.5 - 156 mm^3 oxygen consumption per gram of fresh embryo per hour at 24 and 35 days of incubation at 10 C and 100 % air-saturation indicating an 18% change in respiration.

There is again a great similarity between the results from constant-flow respirometry (Fig. 6, 7 and those in Fig. 8) and the data of Privolnev (1938), in spite of the different unit measures among the studies. Privolnev (1938) has found a partial correlation between oxygen consumption and the rate of growth, and Ostroumova (1969) reported differences at different embryonal stages.

Structural changes also could be a factor modifying oxygen consumption. Iuchi (1973) observed different types of hemoglobins and erythrocytes in the early embryos and recently hatched alevins of rainbow trout.

Brummett and Vernberg (1972) measured the oxygen consumption in anterior and posterior embryonic shield as development proceeded from early gastrula to closure of the blastopore and the anterior shield exhibited a concomitant decrease. The explanation is probably that at the time of blastopore closure the uptake of oxygen improves because of the completion of the relatively extensive cellular yolk sac which has greatly expanded the respiration surface.

Posterior shield exhibited a significantly higher rate of oxygen uptake than anterior embryonic shield at each of the three stages of

development (stage 12,14,16, Oppenheimer 1937). In general, posterior grafts exhibited an increase in size earlier in the postoperative period than did anterior shield grafts. Therefore, from this information there appears to be a general increase in oxygen consumption until blastopore closure. In an unselectively drawn sample of 10 salmon ova usually six will be in the same stage, but at least one or two will be more advanced and the others will be slightly retarded. The range difference in total can be as much as two or three embryonal stages, being greater in lower temperature and in the later embryonal stages. Naturally, this means that there is some variation in oxygen consumption within and among samples. The presence in these samples of occasional but outwardly undetectable unfertilized living ova, or embryos mysteriously arrested in early blastulation also introduced uncontrolled variation in respirometry, by altering inadvertedly the dividend in the calculation of the mean.

Another factor causing differences in oxygen consumption is ovum size. Although mean sample weights can be similar among samples, the size variations within various samples can differ markedly in composition. Also, within ova of similar size and embyros of the same stage of differentiation, weights of embryos can differ by treatment (light, mechanical agitation, temperature change, pH).

Oxygen consumption can differ according to the type of respiration chamber and the number of ova in the chamber. In a canal-like continuous-flow chamber the fixed linear series of ova could rest in a decreasing gradient of oxygen concentration. The situation is entirely different in a vertical chamber and depends on the horizontal levels of inlet and outlet and the covering effect of one ovum on another. Except in the Warburg respirometer there should be no problem of interference created by accumulated wastes.

Several authors have concluded that the amitotic yolk is a metabolically inactive part of the ovum. However, cytochrome oxidase activity (cytochrome C oxyreductase, 1.9.3.1-I.C.E.) was measured in the yolk of the loach Misgurnus fossilis by Abramova et al. (1965). This localization of the mitochondrial structures is evidently connected with the need for direct utilization of the energy resources of development located in the yolk, according to Abramova. Also, the yolk has been demonstrated to be metabolically active by Thomas (1968) and in this study (Appendix 1).

To consider all these results it might be concluded that oxygen consumption of salmon ova oscillates during embryogenesis. The first peak is located at stages 6-8, and is followed by a general decrease, followed by a second peak at stage 12-14 at 10 C, but not at 5 C, and finally a definite increase at hatching time at both temperatures (Fig. 6 and 7).

To explain these changes in relation to structural changes it is necessary to consider that at stages 6-8 a remarkably high portion of superficial growth occurred, accompanied by sharp rise of embryonal body weight (Table 12 and Figure 3 and 4). The decreasing consumption might be explained by the progressive development of the circulatory system which begins following stage 8. This oxygen transport system efficiently reduces the amount of oxygen required. The relatively rapid increase in oxygen consumption at 10 C in stage 14 might indicate a rapid increase in light sensitivity of the eyes and a rise in stimulation of metabolism. The ensuing decline could then be attributed to the development of considerable iris pigment and a decline in photic stimulation (Grusser and Grusser 1961), (Table 29, Figure 3, 4 and Appendix 3). The effect of light intensity has been shown to have a stimulating effect on oxygen consumption to a certain point after which it is retarded (Appendix 3).

The ova received about 1-3 lux illumination in these experiments, and this is perhaps close to the value affecting them in the river beds if they are not deeply buried. The absence of a peak response at stage 14, 5C, is explainable because at this temperature the embryo is less active and would have lower oxygen demand and the development of light sensitivity would occur more slowly (Table 29 and Fig. 6 and 7).

The rise in oxygen consumption near hatching, particularly pronounced at 10 C, is different from that of Hayes et al. (1951). However, similar increases have been measured near the onset of hatching in other teleost fishes by Laurence (1969), Lasker and Theilacker (1962) and Smith (1952 a). Also, a rising trend can be interpreted from the results of Privolnev (1958) in embryonal development of Atlantic salmon. Apparently salmon embryos are more active before hatching because they are more completely formed and muscular. The enhanced rate of development at 5 C, 50% air-saturation probably is because these embryos are less active as measured by lower heartbeat near hatching than the group at 5 C, 100% air-saturated (Appendix 6). Apparently then, the energy conserved could be used for formation processes.

There is a progressive decline in oxygen consumption per unit weight with increasing weight of embryo, yolk, or both. If the embryo, yolk or both is heavier, the consumption of oxygen/per unit weight is smaller.

The general conclusion from this experiment is that in spite of these modifying influences the total oxygen consumption of ova rises steadily through the developmental phase but the consumption oer unit weight of embryo decreases with increasing weight from embryonal stages 1 to 18.

In spite of the correlation which can be drawn between oxygen consumption and structural changes the explanation of the variations of oxygen consumption must be a complex one.

The level of oxygen consumption by fish ova is determined by three important basic factors; temperature, oxygen content of the water, and water movement or flow about the ova. No doubt there are other factors such as light, pH, and salinity which influence oxygen consumption. However, in this chapter, only the first mentioned three factors are considered, because they are the most important variables in the environment of salmon. Ova in rivers are buried 25-75 mm deep in gravel and illumination then has a very limited effect. Since salmonids spawn in fresh water, there should be no effect ascribable to salinity. All experiments in this study were conducted at pH 6.5-7.5, and no comparative information is available on more extreme levels.

Since it is possible for each of these variables to mask the effects to some extent the effects of any other of these, the data have been subjected to linear multiple regression analysis. The double logarithmic transformation was considered most appropriate after trials with arithmetic and partial logarithmic forms.

According to the results of multiple regression analysis given in Table 34 the most important factor in oxygen consumption of salmon ova is the flow-rate which has "t" values 15% or lower in each combination. The only factor which seems to be almost as important as flow-rate is the oxygen content of water. (See combination 12, Table 34).

Since the decline in the prime variable, temperature, causes lower metabolic rate and since the correlation between oxygen content and metabolism is the same, the two effects tend to mask each other.

By manipulating these variables, it is possible to have the same oxygen consumption at higher temperature while lowering the oxygen content or the flow-rate, or both. In combination 22, Table 34, the temperature is more significant than the flow-rate.

In addition to these major variables (temperature, oxygen saturation, flow-rate) the salmon ovum is affected by several lesser factors. As we can see from the Warburg respirometry, the activity of ovum components is altered not only by temperature, but also the medium, time of exposure, and total weight (Tables 26, 27, 28).

In spite of all the difficulties, simplifying oxygen consumption in relation to several variables to a linear double logarithmic form yielded good approximations to the calculation of absolute values when the varying combinations of environmental factors were known.

Recently, Laurence (1969) measured the oxygen consumption of ova of largemouth bass Micropterus salmoides with standard Warburg manometric respirometers at 20 C. He measured 21.79 ml (15.24 mg) which is about the same as the measurement obtained at 10 C and 4-ml flow at 100% air-saturation 20.3 mg oxygen consumption per ovum from fertilization to hatching. Comparisons with other authors are detailed in Appendix 3.

The effect of low levels of dissolved oxygen and exchange of water (flow-rate) is well known. Both mean difference in the oxygen supply and change in permeability of the zona radiata (Hayes et al. 1958, Garside 1959, Silver et al. 1963) alter oxygen consumption.

The retarding effects of low oxygen supply on development of salmon embryos has been considered previously in the section on development and other authors' experiments as well (Garside 1966, 1970 Eberhardt et al. 1968, Duodoroff and Shumway 1970, Table 12).

The data in this study were applied to the Vant Hoff Q_{10} relationship for comparative purposes. The velocity constants K_5 and K_{10} were calculated from the equation,

$$\log K_1 = \log A + t_1 \cdot \log B$$

given by Belehradek (1930), and Hoar (1966).

These were,

Oxygen level	K_5	K_{10}
100% of air saturation	1.20	1.92
50% of air saturation	0.96	1.20
30% of air saturation	0.47	0.72

The solutions for Q_{10} in the expression, $Q_{10} = \frac{K_{t10}}{K_{t5}} \times \frac{10}{T_1 - T_2}$ are as follows;

Oxygen level	Q_{10}
100% air saturation	3.20
50% air saturation	2.50
30% air saturation	3.10

The value 1.60 for the acceleration in development in air-saturated water is in close agreement with numerous similar calculations in the literature (Belehradek 1930).

We can see then that respiration of salmon ova is directed by intrinsic and extrinsic factors simultaneously. Most of the oscillations in oxygen consumption during the development of salmon ova at constant environmental conditions can be correlated with structural changes. The experimental conditions can alter the metabolic rate so much that the results might be far from what is actually occurring in nature.

With rising temperature, flow, oxygen content, and age, the total consumption of salmon ovum increases. At the same time the specific consumption (oxygen consumption mg/ weight unit) might be decreasing for different tissues or parts of the embryo and ovum generally.

CHAPTER V

QUALITATIVE AND QUANTITATIVE COMPOSITION OF THE FERTILIZED OVUM AND ITS CONSTITUENT PARTS IN THE ATLANTIC SALMON

INTRODUCTION

Yolk is quantitatively the major fraction of unfertilized fish ova, generally, and salmonids in particular. They are, thus, classified as telolecithal ova. Chemically the yolk contains proteins, phospholipids, neutral fats, carbohydrates and minerals. Depending on which component predominates, carbohydrate, protein or fatty yolk can be distinguished.

Yolk has been accorded the most attention in qualitative and quantitative evaluations (Barman et al. 1944, Fujino et al. 1971, Fujino and Momma 1971, Hartmann et al. 1947 Hayes 1949, Hollett and Hayes 1946, Momma et al. 1970, 1971 Smith 1957, Yamagami and Mohri 1962, Yamamoto 1956, Young and Phinney 1947).

There has been considerably less analysis of the encapsulating zona radiata, or the perivitelline fluid, of the water hardened ovum (Bell et al. 1969, Eddy 1974, Fisher 1963, Flüchter and Pandian 1968, Hamor and Garside 1973, Hayes 1949, Hurley and Fisher 1966, Manery 1966, Potts and Rudy 1969, Rudy and Potts 1969, Warren 1947, Young and Smith 1956, Zotin 1958, 1964).

The present study provides both qualitiative and quantitative analyses of the total ovum, yolk, and zona radiata, as a base for a later consideration of progressive changes in composition when yolk is assimilated and metabolized by the advancing embryo and alevin.

MATERIALS AND METHODS

Qualitative and quantitative analyses of organic components were performed on freshly fertilized ova from a single pair of Atlantic salmon (Salmo salar L.) described previously.

Samples of ova, separated yolk, and zona radiata were collected for analytical procedures. The chemical determinations were made in parallel, from living ova and ova frozen at -75 C. Since in preliminary comparisons significant differences were not apparent most of the material used in these analyses was taken from frozen samples.

Quantitative values were determined for phospholipids, phospholipid phosphorus, cholesterol, total lipids, RNA, DNA, total protein, non-protein nitrogen, total carbohydrate, glucose, ash and water from samples collected 24 hr. after fertilization.

Most of the samples were dried at 105 C until weight became constant, but some were dried at 60 C because of some possibility of alteration in the lipid fraction at the higher temperature. (Oser 1965).

Total inorganic solids were determined by gravimetric difference following incineration of previously dried ova and parts in an electric muffle furnace at 600 C for 30 min (Anonymous 1960).

Immediately after weighing, the samples for other analyses were homogenized in a glass homgenizer and extracted with 2.0 ml of a 2:1 chloroform methanol mixture according to Folch-Pi and Sloane-Stanley (1957). The extraction was repeated twice to ensure complete removal of lipids that were soluble in this solvent. After each extraction the suspension was centrifuged, and the clear supernatants were combined (extract A). The residue was set aside for DNA extraction.

Non-lipid contaminants were removed from extract A with 0.2 ml volume of 0.05% $CaCl_2$ solution. After centrifugation, the upper phase

and any material occurring at the interphase were removed and discarded. Washed chloroform-methanol layer was taken to dryness under reduced pressure using a Buchler Rotary Evapo-Mix flash evaporator. The test tubes containing the dry residue were placed in a vacuum desiccator containing KOH at the bottom. The desiccator was evacuated in a refrigerator at 4 C overnight. The dry residue of extract A was redissolved in a small amount of chloroform-methanol 2:1, filtered through glass wool into a volumetric flask and the volume was made up to 2.0 or 5.0 ml. (Solution A). Samples from these solutions were used to determine the phosphorus and cholesterol content and phospholipid composition.

Total phosphorus content was determined by the spectrophotometric method of Fiske Subba and Row as modified by Bartlett (1959). Samples (0.2 or 0.5 ml) were placed in a water bath at 38 C until all the solvent had evaporated. Concentrated sulfuric acid, (10 N) was added in 0.5 ml portions to all the test tubes, including tubes for reagent blanks and inorganic phosphorus standards of 1, 2, 3, and 4 µ g.

The tubes were placed in an oven for 3 hr at 180 C. Following this digestion, the tubes were removed from the oven, allowed to cool, and 2-3 drops of 30% hydrogen peroxide were added. The tubes were then returned to the oven and the contents were digested for an hour at 180 C. To those tubes that still contained undigested material, a further 2-3 drops of 30 percent hydrogen peroxide were added and the contents were digested for an additional hour at 180 C. This step was repeated until all the samples were clear and colorless solutions. To each of these solutions, 4.6 ml ammonium molybdate solution (prepared by mixing 1 ml of ammonium molybdate 5% solution with 22 ml distilled water and 0.2 ml Fiske-Subba-Row Reagent) was added and thoroughly mixed. All tubes were placed in a vigorously boiling water bath for seven minutes. The samples were removed

from the water bath and mixed again. The absorbancy of samples was read at 830 mm wave length in a Beckman D. U. spectophotometer.

The phopholipid composition was determined by thin-layer chromatographic method (TLC), (Mezei and Ambrose 1970, Ambrose and Mezei 1971). These procedures yielded quantities of phospholipids based on the following standards:

		Supplier and catalog number	
LC	= Lysolecithin = Lysophosphatidyl choline	PCC	354601
LA	= L-A-Lecithin = L-A-Phosphatidyl choline	PCC	3-5130
S	= Sphingomyelin	PCC	3-8210
PE	= Phosphatidylethanolamine	K&K	21423
OPE	= o-phosphoethanolamine	K&K	17802
PC	= Lecithin = Phosphatidyl choline	K&K	2462-A
PS	= Phosphatidyl-L-serine	K&K	17816
LE	= Lysophosphatidylethanolamine	Sigma	L 6751

K&K = K&K Laboratories 121 Express Street, Engineers Hills, Plainwiew, New York 11803, USA.

PCC = Pierce Chemical Company, Box 117, Rockford, Illinois, USA.

Sigma = Sigma Chemical Company, P.O. Box 14508, Saint Louis, Missouri 63178, USA.

Cholesterol was also determined spectophotometrically by both the specific procedures of Hanel and Dam (1955) and Zak et al. (1954). Total lipids were determined spectophotometrically according to the procedure of Holland and Gabbott (1971)

The fraction of the material to be used for total carbohydrate determination was hydrolyzed to glucose with trichloroacetic acid.

Total nitrogen was measured by the method of Folin and Wu (Oser 1965). Protein nitrogen was determined by a modified Kjeldahl procedure

described by Holland and Gabbott (1971).. Determination of total protein in bovine albumin was also determined by this technique as a standard (Lowry et al.1951).

The nucleic acids were determined quantitatively by the spectrophotometric technique of Santen and Agranoff (1963). DNA was also measured by the technique of Abraham et al. (1972). In order to correct for interference by substances which absorb ultraviolet radiations an extract of KOH-hydrolyzed "tissue breakdown products" was prepared as described by Santen and Agranoff (1963). The difference in spectophotometric values for unhydrolyzed and hydrolyzed substance gives the total nucleic acid present. These acids were separated by the perchloric acid procedure for spectrophotometry given by Santent and Agranoff (1963).

The corrected absorbancy factor for RNA was estimated to be 0.597 and 0.722 for DNA.

Enzyme activities of 2'3'-cyclic nucleotide-3'-phosphohydrolase and adenosine 3'5'-cyclic monophosphatase, abbreviated and mentioned later as 2'3' cAMP-ase and 3'5'-cAMP-ase respectively, were estimated by the method of Olafson et al. (1969).

RESULTS

The measured dry weight of ovum, yolk, embryo, zona radiata and alevin from fresh samples and those stored at -75 C dried at 60 and 105 C to constant weight are presented comparatively in Table 35.

Analysis of variance revealed that the dry weights of these five entities were significantly different (P = 0.024) and the methods of storage (P = 0.011) also yield significantly different results (Table 36). Significant differences occurred between 60 and 105 C dry weight in all fresh samples, with 't' tests. The differences for zona radiata, yolk, embryo, and alevin were highly significant between frozen samples at 60 and 105 C, but for whole ova it was not significant.

Qualitative and quantitative results of the spectophotometric and chromatographic analyses are presented in Table 37. Protein is the largest component of the dry substance of the ovum and the yolk (50%), but forms only about 20% of the zona radiata. Carbohydrate forms the largest component of the zona radiata, being about 60% of dry substance.

Results for the composition of perivitelline fluid were determined as the difference between total weight and sum of the weights of the partial entities, for each substance identified. These values are given only as fractional wet weights.

TABLE 35

Comparison of dry materials of Atlantic salmon ova (stage 1) dried at 60 C or 105 C from fresh and deeply frozen ova (-75 C). In addition to the ova and constituents in stage 1, hatched alevins were also measured.

Substance	Dry Material (percentage total weight)			
	60 C		105 C	
	Sample			
	Fresh	Frozen	Fresh	Frozen
Ova	28.2±1.6	32.0±1.8	23.5±0.98	31.8±1.41
Yolk	35.3±1.8	35.7±1.2	25.9±0.4	32.6±0.6
Embryo	20.0±1.3	28.0±3.6	18.3±0.5	18.9±0.6
Zona radiata	45.7±1.4	39.1±2.2	8.6±0.8	25.1±0.9
Alevin (Embryo + Yolk)	26.4±0.9	30.8±1.6	24.1±0.8	24.4±3.0

TABLE 36

Analysis of variance of effect of storage method on dry weight of component of Atlantic salmon ova. Components egg, yolk, embryo, zona radiata, alevin. Storage = fresh sample, freezing (-75 c), glycerine-alcohol.

Source	Sum of squares	Degrees of freedom	Mean square	F Ratio	Probability
Components	337.5	4	84.4	5.10	0.024*
Storage	275.2	2	137.6	8.31	0.011*
Error	132.2	8	16.54		

TABLE 37

Chemical composition of Atlantic salmon ova at stage 1.

Component Total	Ovum			Yolk			Zona Radiata			Perivitelline Fluid	
	Mean weight mg	% of total weight	% of dry weight	Mean weight	% of total weight	% of dry weight	Mean weight mg	% of total weight	% of dry weight	Mean weight	% of total weight
Wet weight	138.0	100.0	-	104.0	100.0	-	5.0	100	-	29	100
Water	88.3	64	-	63.4	60.8	-	4.6	92	-	20.6	71.03
Dry material	49.7	36	100	40.8	39.2	100	0.4	8	100	8.4	28.97
Ash	1.42	1.0	2.8	1.2	1.15	2.9	0.09	1.8	22.5	0.13	0.45
Protein	26.0	18.8	52.2	20.0	19.23	49.1	0.08	1.6	20.0	5.9	20.3
Non-protein N	3.6	2.6	7.2	2.8	2.7	6.9	0.004	0.08	1.0	0.8	2.76
LL	0.43	0.31	0.23	0.43	0.41	1.05	-	-	-	-	-
LA	0.03	0.02	0.06	0.03	0.03	0.08	-	-	-	-	-
S	0.7	0.51	1.42	0.7	0.67	1.71	-	-	-	-	-

continued .....

TABLE 37 (continued)

Component Total	Ovum			Yolk			Zona Radiata			Perivitelline Fluid	
	Mean weight mg	% of total weight	% of dry weight	Mean weight	% of total weight	% of dry weight	Mean weight mg	% of total weight	% of dry weight	Mean weight	% of total weight
OPE	0.2	0.15	0.42	0.2	0.19	0.48	0.00015	0.03	0.38	-	-
PE	0.01	0.01	0.03	0.01	0.01	0.03	0.0035	0.07	1.4	-	-
L	5.5	3.98	11.06	5.4	5.2	13.3	-	-	-	0.1	0.35
PS	1.5	1.09	3.03	1.4	1.35	3.44	0.0054	0.11	2.2	0.09	0.30
LE	0.5	0.36	1.00	0.4	0.38	0.97	-	-	-	0.1	0.35
Total Phospholipids	8.87	6.43	17.9	8.57	8.42	21.02	0.009	0.18	3.6	0.29	1.0
Non polar lipids	9.1	6.6	18.3	8.0	7.7	19.6	0.002	0.04	0.5	1.1	3.8
Cholesterol	0.08	0.06	0.17	0.07	0.07	0.18	*	-	-	0.01	0.04
Total lipids	18.0	13.0	36.1	16.6	16.0	40.8	0.011	0.22	2.75	1.39	4.8

continued .....

TABLE 37 (continued)

Component Total	Ovum			Yolk			Zona Radiata			Perivitelline Fluid	
	Mean weight mg	% of total weight	% of dry weight	Mean weight	% of total weight	% of dry weight	Mean weight mg	% of total weight	% of dry weight	Mean weight	% of total weight
RNA	0.8	0.6	1.67	0.7	0.67	1.71	0.016	0.32	4.00	0.08	0.28
DNA	0.2	0.15	0.42	0.2	0.19	0.48	0.008	0.16	2.00	-	-
Glucose	0.17	0.12	0.33	0.15	0.14	0.36	0.01	0.2	2.5	0.01	0.03
Carbohydrate	0.48	0.35	0.97	0.01	0.01	0.3	0.24	4.8	60.0	0.23	0.79

* trace

DISCUSSION

The most frequently analyzed fish ovum is that of the rainbow trout (Salmo gairdneri (Richardson) and most of our knowledge about the composition of Atlantic salmon ovum originates from the pioneer work of F. R. Hayes and coworkers. Most of the presently estimated quantitative values are in good agreement with the results of Hayes (1942, 1949), Hayes and Armstrong (1942), Hayes and Pelluet (1945), and Hayes et al. (1951, 1953). The results of thin-layer chromatography are comparable with the TLC values in the ovum of rainbow trout given by Yamagami et al. (1962). It seems that the only significant difference between rainbow trout and Atlantic salmon ova is that the lipid content of the last is about twice as much as the first.

The composition and weight of ova can vary because of differences in maternal genotype, age, physiological condition and diet during oogenesis. Also, mechanical alteration of ova in preparation for chemical analysis can also create discrepancies in composition. For instance, the difference in weight between freshly dissected and well cleaned zona radiata can be as much as 6 to 1 respectively and this might be an explanation for the results presented here and those described in the literature. Also, there is a serious loss of perivitelline fluid by evaporation and adhesion to the instruments during dissection of the ova.

To measure accurately dry weight is also a difficult task. As it is presented in Table 35 the measured constant weight can vary depending on the temperature used for drying. The weight of the several components more closely total the weight of the intact ovum when drying is performed at 60 C rather than at 105 C. The weight loss at 105 C is due to the decomposition of lipids in the yolk (Oser 1965). Although less chemical alteration would be expected at 60 C than at 105 C, the

reverse has occurred in the zona radiata. The explanation is probably that the water in the zona radiata has cooperative hydrogen bonding (Lehninger 1970) which cannot be broken easily at lower temperatures.

The data in Table 37 have excellent agreement between the weights determined indirectly and the dry weights determined gravimetrically. The discrepancy between the total ovum and the sum of weights of yolk and zona radiata is probably mostly the result of incidental loss of perivitelline fluid during preparations.

Certain components recorded in yolk such as RNA and DNA can not be claimed definitely by the method used, as invariable parts of the yolk, since there is some possibility that they came from the embryonal tissue or the vitelline emembrane. However, this could not be the case in the zona radiata, because the cleaning process makes highly improbable the presence of any embryonal tissue. The presence of nucleic acids in the zona radiata will be considered further in a later section.

The relatively small proportion of carbohydrate in fish ova has been studied in Salmo gairdneri by Smith (1952 b) who showed that depletion of this consitituent is especially noticeable during the establishment of the blood circulation, hatching, and the onset of starvation after the yolk has been absorbed. However, Daniel (1947) was unable to detect a fall in total carbohydrate, but rather progressive increase, during the development of Atlantic salmon embryos, in agreement with the present study. In the zona radiata, carbohydrates are probably in the form of ichthulockeratin and mucopolysaccharides (Bell et al. 1969, Porcelli 1969).

Analyzing directly the perivitelline fluid of Atlantic salmon ova, Eddy (1974) measured 58 % water, 25 % protein, 12 % lipid and 1:7 % carbohydrate. With indirect determination of components, total ovum - (yolk + zona radiata), in the present study, water was 71 %, protein 20 %,

lipid 4.8 % and carbohydrate 0.80 %. The major discrepancy between the two analyses is in the lipid content which can be easily explained by loss of lipid during dissection of yolk (8-10% of total amount).

The composition of phospholipids has indicated that the main component is lecithin (PC = phosphatidyl choline), which corresponds to the determinations in rainbow trout ova by Yamagami et al. (1962).

In a later section the changes in these fractions during development will be considered.

CHAPTER VI

NUTRIENT ENERGETICS AND GROWTH IN EMBRYOS OF ATLANTIC SALMON INCUBATED AT VARIOUS TEMPERATURES AND LEVELS OF DISSOLVED OXYGEN

INTRODUCTION

Embryonal differentiation and growth of a vertebrate occur in a metabolic system under rigorous and constant internal control. The process involves a sequence of coordinated events which must be accomplished in relatively strict sequence and without extended interruption if the animal is to develop normally. The supply of nutrients to the embryo, and the manner in which they are utilized by respiratory and anabolic processes have received relatively little attention in fishes.

The development of a fish embryo is regulated by intrinsic and extrinsic factors which are the genetic materials, and the chemical components of the ovum. The extrinsic factors are the effective environmental conditions.

Extrinsic factors

Extrinsic factors have been categorized by Fry (1947, 1971), in the present context, as being controlling or limiting: a controlling factor exerts a tonic effect on metabolism in the manner of temperature, while a limiting factor is a condition of supply of a metabolite as in the case of dissolved oxygen. There are several observations concerning the effects of temperature on development, metabolism, and growth of fishes (Privolnev 1938, Oya and Kimata 1943, Hayes 1949, Hayes et al. 1951, Einsele 1956, Tanizaki et al. 1957, Fry 1957, 1958 a, b, Baravik 1963, Lasker 1964, Hamor 1967, Alderdice and Forrester 1968, 1970, 1971, Ostroumova 1969, Garside 1970, Carlson and Siefert 1974).

The observations agree that lower temperature causes lower metabolic activity and a relatively longer period of differentiation with larger embryonal size at hatching and at yolk absorption. Higher temperature increases metabolism and the rate of differentiation but

smaller embryos are produced at these stages. There are deviations from this in some fish embryos which are adapted to relatively warmer temperatures. These fishes have thermal optima for maximum growth at a particular stage and both lower and higher temperatures produce smaller embryos. (Lasker 1964, Flüchter and Pandian 1968). Certain differences in embryonal growth reflect differences in efficiency of energy utilization (Fluchter and Pandian 1968). Of course, there are other factors which might affect embryonal growth such as original size of fertilized ova (Garside and Fry 1959), but temperature and oxygen appear to have the major effects on energy utilization.

Intrinsic factors

In teleost fish the nutrient requirements of the embryo are met by components from the yolk, imbibed water, and solutes including dissolved oxygen. The major sources of energy are lipids and proteins; a salmon ovum loses in weight 77% of its original fat and 45% of its original protein in producing an average fry (Hayes 1949, Smith 1957).

Lipids are typically the major dietary energy source in fish (Hochachka and Somero 1973). The aquatic diet is often characterized by a high lipid content, and lipid is an efficient way of storing energy, since it has a higher energy content per gram than either protein or carbohydrate. This latter property is particularly valuable since most fish experience severe depletion of their energy reserve for a part of each year. Hochachka and Hayes (1962), Hochachka and Somero (1971), and Lowenstein (1974) state that fatty acids of lower molecular weight are accumulated by the trout in hypoxia, (Hochachka and Hayes use the word "anoxia"). Fat accumulation also occurs in salmonid embryos, when the oxygen content of water is lowered (Blazka 1958).

There are differences in the utilization of yolk components of

salmonid ova previous to and after hatching. Prior to hatching the main energy supply comes from lipid utilization (Hayes 1930 a, b, 1949) mostly from phosphatidyl choline (PC), which is the main component of yolk lipids (Yamagami and Mohri 1962). After hatching, some protein and other parts of this phospholipid fraction are consumed in Salmo gairdneri (Suyama and Ogino 1958, Yamagami and Mohri 1962), while the non-protein fraction increases about five-fold.

Since cholesterol is an intermediate metabolic product in lipid metabolism it might be a good indicator of growth rate in general, and particularly in the nervous system (Bucher et al. 1960, Howard et al. 1969, Harry et al. 1971, Gaskin and Clayton 1972, Krusky and Naryan 1972, Watanabe and Ando 1972).

The genetic information in the fish embryo determines the basic directions of growth, yolk utilization and later the size reached by the fish in adult age (Balinsky 1968). However, there is very little information available concerning the correlation between the presence and quantities of certain chemical compounds and the quantity of genetic material in fish. An important exception is the significant correlation which has been shown between the growth rate in fish populations and the DNA:RNA ratio by Bulow (1970) and Haines (1973).

Total carbohydrates are quantitatively low (less than 1%) and probably do not contribute much to energy sources. (Hayes and Hollett 1940, Hutchens et al. 1942, Daniel 1947, Zeitoun et al. 1973, Yirowitzky and Milman 1973 a, b).

Although the protein of the yolk is approximately half utilized during embryogenesis to produce an average fry there must also be considerable synthesis in the developing embryo. Since RNA controls protein synthesis and DNA directly controls RNA synthesis, changes

in metabolic regulation by extrinsic factors might be expected to modify this chain of syntheses and create differences in phenotypic characterization. Correlations have been established between oxygen consumption or supply, and DNA synthesis (Brachet 1950, Løvtrup 1953, 1955, 1958, Deuchar 1966, Das 1967, Cameron and Kostberg 1969, Chopra and Simnett 1969, Ivanchik and Kriegsgaber 1969, Løvtrup and Iverson 1969, Abe and Yamana 1970, Dontsova and Ivanchik 1970, Abraham et al. 1972, Haines 1973, Wittliff 1972, Giudice 1973, Gilbert 1974.) In the present study, protein levels have been related to quantities of DNA and RNA and these are referred to the environmental conditions under which they formed.

The best explanation that can be offered on the basis of enzyme activity is that every species of fish or any other animal has a combination of enzymes that produces an optimum energy utilization at a particular temperature and oxygen level, and the effectiveness of substitute isoenzymes in metabolic pathways is generally less than is the series yielding the optimum (Bucher et al. 1960, Brewer and Sing 1970, Deuchar 1966, Dirksen 1971, Hoadley 1938, Harmsen 1969, Hochachka and Hayes 1962, Hochachka and Somero 1973, Lehninger 1970, Somero 1973). Enzyme activity has been demonstrated in various parts of the embryonated teleost embryo, including yolk (Abramova et al. 1965, Hamor and Garside 1973) vitelline membrane (syntitial layer and ectodermal epithelium of the yolk sac (Romanini et al. 1969, Porcelli 1969) and the zona radiata (Hamor and Garside 1973).

There have been implications that 3'5'cAMP is a principal regulator of the activities of other enzymes especially those which are involved in glycogen and lipid metabolism in fish (Brooker et al. 1968, Hardman 1971, Ghosh et al. 1972, Ichii 1972, Ohsawa 1972, Kinght 1973, Laidler and Bunting 1973, Naito and Tsushimo 1973, Tash and Mann 1973,

Yurowitzky and Milman 1973 a, b, Weiss and Stiller 1974). Measures have been made in the present study to determine if there are relations between environmental temperature and oxygen supply, and 3'5'cAMP activity.

MATERIALS AND METHODS

Changes in the composition of the ovum and its constituent parts, embryo yolk and zona radiata were evaluated chemically at several embryonal stages. The analytical techniques were the same as those employed in the initial study of the composition of freshly fertilized ova.

In addition to these analyses the quantities of 2',3'-cyclic nucleotide-3'-phosphohydrolase and 3',5'-cyclic monophosphatase were measured indirectly by spectrophotmetric determination of the hydrolytic liberation of phosphorus (Olafson et al. 1969) from alkaline phosphatase (orthosphosphoric monoester phosphohydrolase E. C. No. 3.1.3.1). Caloric conversions of the estimated quantites of organic compounds have also been calculated in order to make comparisons with values obtained calorimetrically.

The calorie values ascribed to the classes of compounds revealed in this analysis were obtained from Hayes (1949) and Flüchter and Pandian (1968) as follows:

lipid	9400 cal/g.
protein	5650 cal/g.
carbohydrate	4150 cal/g.
nonprotein nitrogen	4280 cal/g.

Conversion of material combusted, to the necessary amount of oxygen was calculated from unit values given by Hayes (1949)

Protein	950 ml/g.
Lipid	2020 ml/g.
Carbohydrate	810 ml/g.

To have the necessary substrate concentration the Michaelis-Menten equation was transformed to the Lineweaver-Burk plot and values of 1/Vmax, $-1/K_m$ and K_m/Vmax were evaluated. (Fig 9, 10 after Lehninger (1970)

Figure 9

Values of 3'5' c AMP-ase activity measured as the amount of phosphorus liberated by alkaline phosphatase, plotted according to the method of Lineweaver-Burk (Lehninger 1970). The Y-intercept is $1/V_{max}$ in which V_{max} is the maximum liberation of phosphorus (g/min). The x-axis is the reciprocal of substrate concentration.

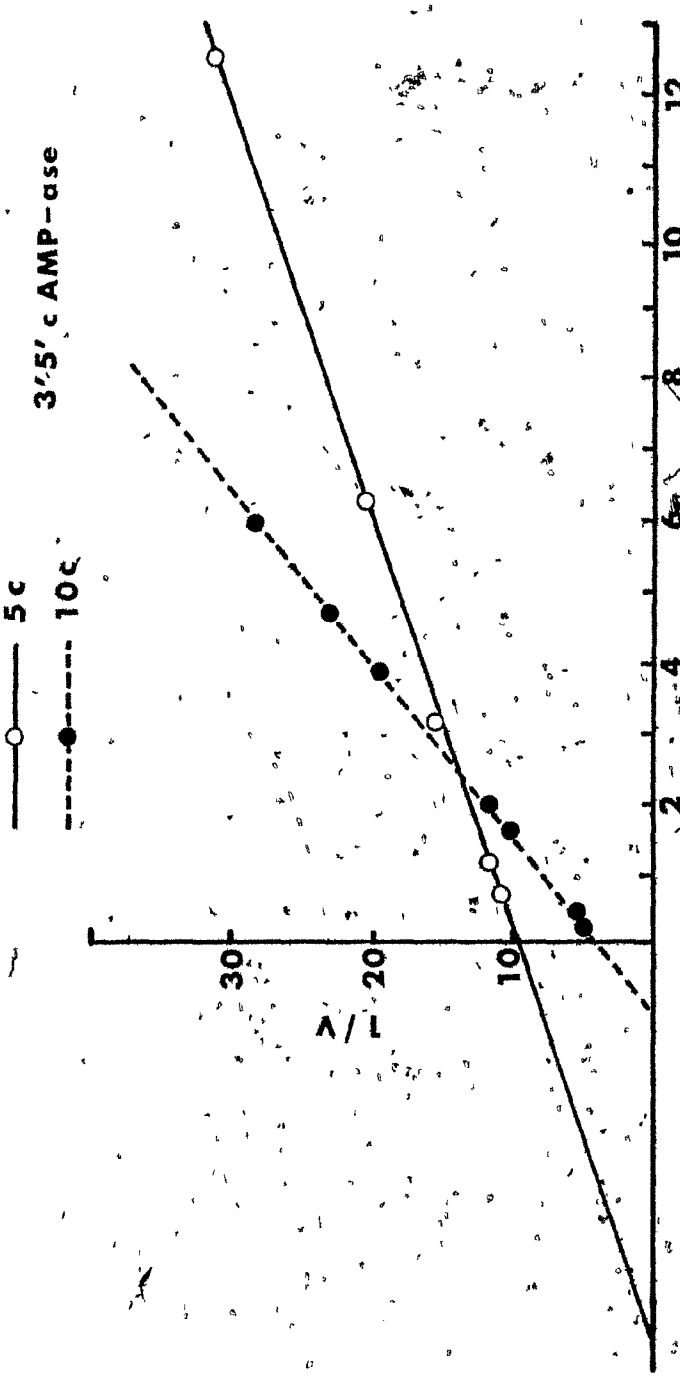
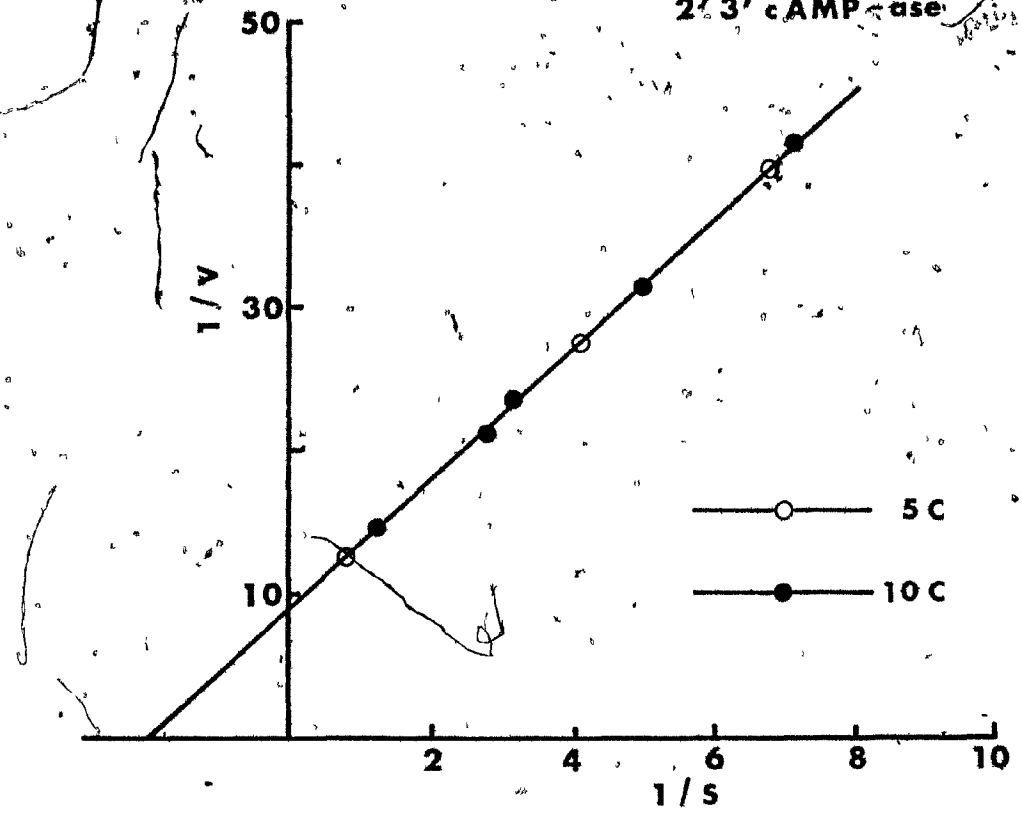

3',5' c AMP-ase
5 c
10 c
1/V
30
20
10
1/S
2
4
6
8
10
12

Figure 10

Values of 2'3' c AMP-ase activity measured as the amount of phosphorus liberated by alkaline phosphatase, plotted according to the method of Lineweaver - Burk (Lehninger 1970). The Y-intercept is $1/V_{max}$, in which V_{max} is the maximum liberation of phosphorus (g/min). The x-axis is the reciprocal of substrate concentration.


2', 3' cAMP-ase
50
30
10
1 / V
2
4
6
8
10
1 / S
5 C
10 C

RESULTS

The dry weight of intact ova measured in late embryonal development (stage 18) from the six developmental environments did not differ from that of stage 1 in the respective groups (Table 38). Neither were there significant differences between temperature treatments ($P>0.40$).

The dry weight of yolk was measured at developmental stage 18 (Garside 1959), from both 5 C and 10 C experiments in samples from all incubation environments (Table 38). There was some increase in dry material at 10 C and higher oxygen level, but according to the analysis of variance dry weight of yolk generally was not affected significantly by temperature or oxygen supply.

The dry weight of the isolated embryos at stage 18 from the six developmental environments differs markedly between temperatures, being regularly lower at 10 C (Table 38). The influence of dissolved oxygen created a progressive decrease in dry weight from 30% to 100% air-saturation at 5 C but no such change was evident at 10 C. The protein content per unit of dry weight of these embryos at stage 18, and the RNA/DNA and protein/DNA phosphorus were in good agreement with each other and with embryo weight at 10 C but not at 5 C. (Fig. 11 and Tables 39, 40, 41)

The total protein diminished slightly in the ova from stage 1 to 18 in all experiments. Decreases were greater at low oxygen levels, and at 10 C. (Table 39).

The amount of RNA and DNA in the total ovum had the same trend as those in the embryo except that the quantities of both components were considerably less per unit weight than in the embryo. The ratio of RNA/DNA in the yolk was generally higher at 5 C than at 10 C, and at 100% than at 30% air-saturation. (Tables 40, 41 and Figures 11, 12, 13). The RNA/DNA ratio increased from development stage 1 to 18 in all

Figure 11

The relation of the RNA/DNA ratio and the quantity of protein (mg/g) to weight of embryo, in Atlantic salmon at stage 18, incubated at two levels of temperature and three levels of dissolved oxygen.

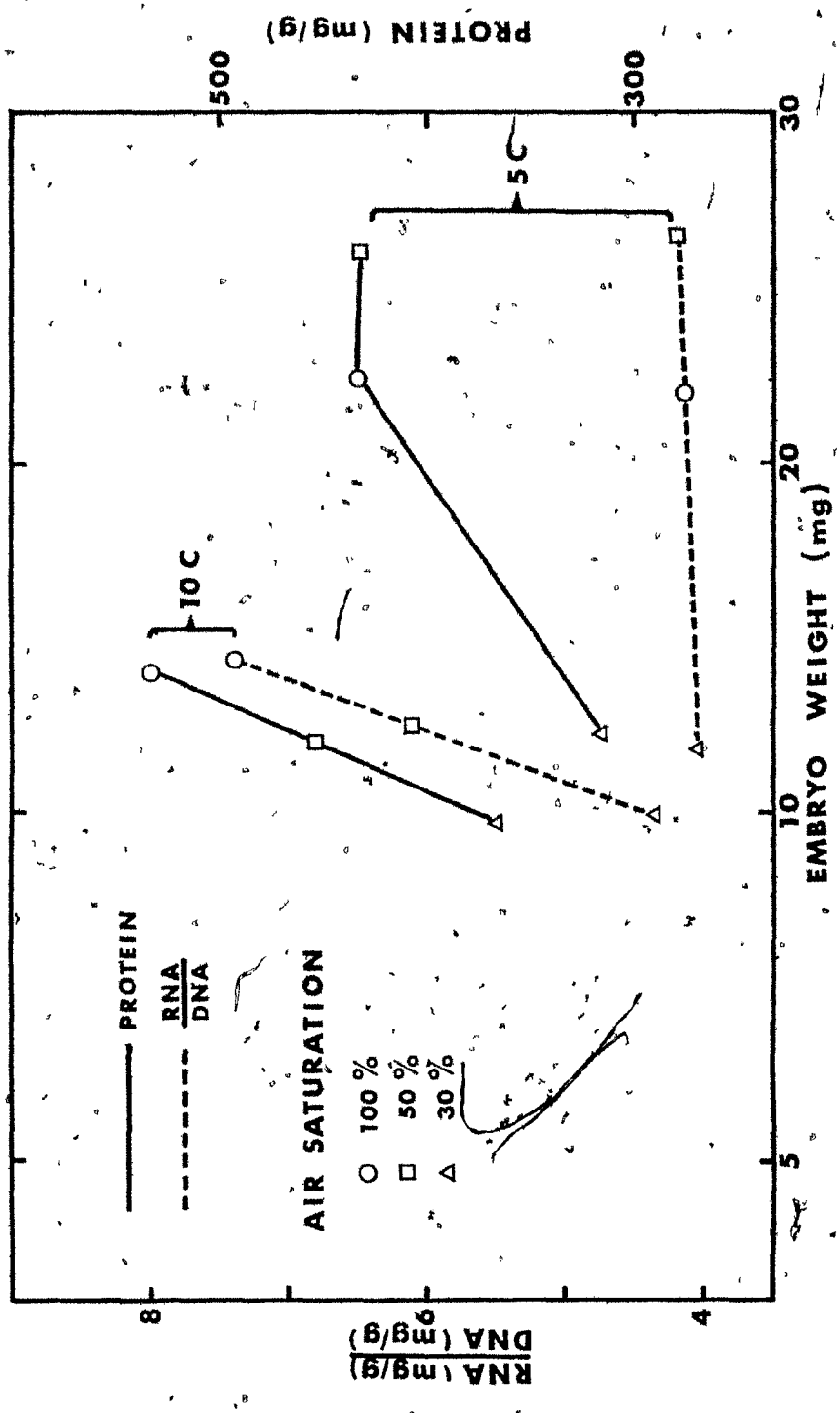

PROTEIN (mg/g)
500
300
5 C
10 C
30
20
10
5
EMBRYO WEIGHT (mg)
PROTEIN
RNA/DNA
AIR SATURATION
100 %
50 %
30 %
RNA (mg/g) / DNA (mg/g)
8
4

Figure 12

Standardized quantities of RNA (mg/g) in Atlantic salmon embryos, at selected developmental stages between 6 and 18, incubated at two temperatures and three levels of dissolved oxygen.

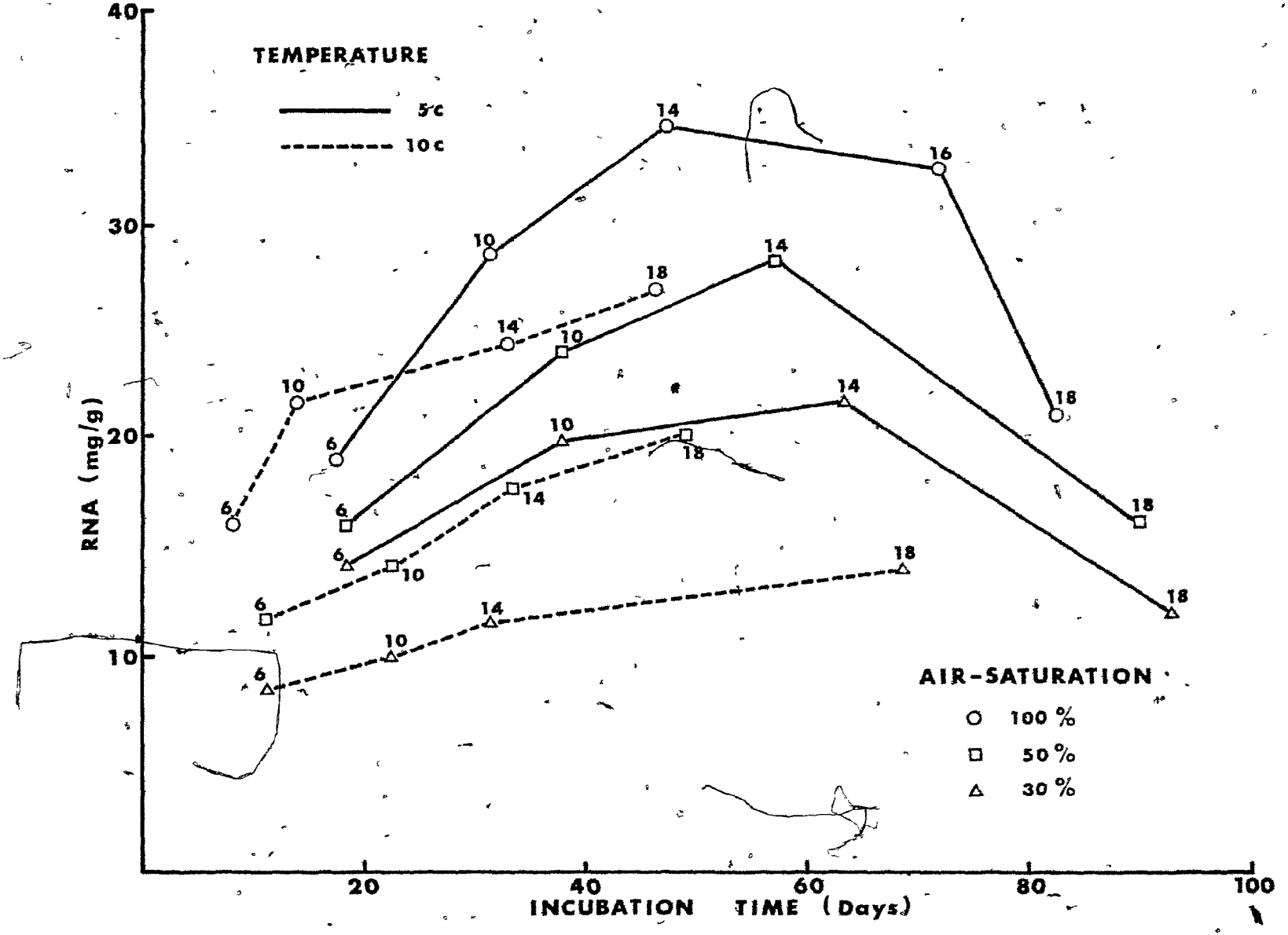

TEMPERATURE
5°c
10c
RNA (mg/g)
40
30
20
10
20
40
60
80
100
INCUBATION TIME (Days)
AIR-SATURATION
100 %
50 %
30 %

Figure 13

Quantity of DNA (mg/g) in Atlantic salmon embyros at stages 6, 10, 14 and 18 (also 16, at 5C, 100% air-saturation) incubated at two temperatures and three levels of dissolved oxygen.

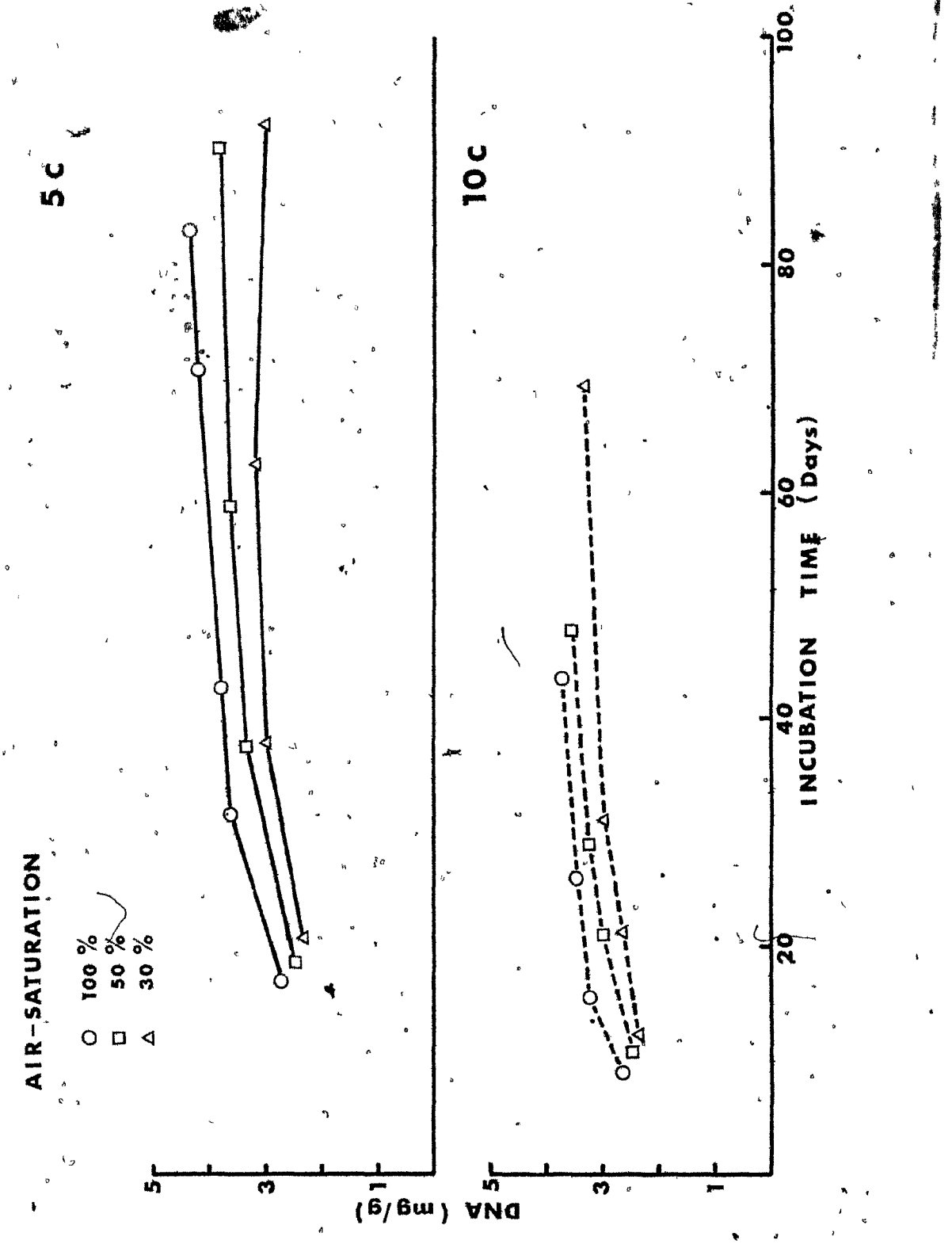

5c
10c
AIR-SATURATION
100 %
50 %
30 %
DNA (mg/g)
INCUBATION TIME (Days)
1
3
5
20
40
60
80
100

TABLE 38

Percent dry material of Atlantic salmon; intact ovum, yolk, and embryo from groups incubated at two temperatures and three levels of dissolved oxygen (% air-saturation), dried to constant weight at 105 C from preserved samples at stage 18.

	5 C			10 C		
Component	100%	50%	30%	100%	50%	30%
Ovum	28.2±1.3	31.6±0.8	30.3±1.3	33.3±1.6	31.7±0.7	28.4±0.9
Yolk	31.3±1.6	33.8±1.0	32.6±1.2	35.8±1.2	33.4±1.4	32.3±1.2
Embryo	22.1±1.2	25.2±1.8	27.0±2.2	19.4±1.4	19.3±1.8	20.2±2.3

TABLE 39

Chemical composition (mg/g) and equivalent caloric values of components of ova and embryos of Atlantic salmon from groups incubated at two temperatures and three levels of dissolved oxygen (% air-saturation) at stages 1 and 18. Upper quantity for each is weight and lower quantity is caloric equivalent.

Temp (c)	Oxygen (%)	Form & Stage	Ash (mg/g)	Protein (mg/g) cal.	Non-Protein N (mg/g) cal.	Lipid (mg/g) cal.	Carbohydrate (mg/g) cal.	Total (mg/g) cal.	Mean Live Weight (mg/g)	Cal/mg Mean Live Weight
					OVUM					
5	100	stage 1	10.3	189 1067	2.6 11	131 1231	3.6 16	337 2326	138.8	323
					OVUM					
5	100	stage 18	14.2	168 949	5.5 24	62.9 591	4.4 18	255 1582	147.8	234
	50	stage 18	12.9	162 915	5.0 21	65.1 612	5.7 23	251 1571	156.6	246
	30	stage 18	11.8	124 701	6.0 26	69.8 656	7.5 30	219 1413	150.6	213
10	100	stage 18	15.8	161 910	7.4 32	69.3 657	4.0 16	257 1615	147.0	237

Temp (C)	Oxygen (%)	Form & Stage	Ash (mg/g)	Protein (mg/g) cal.	Non-Protein N (mg/g) cal.	Lipid (mg/g) cal.	Carbohydrate (mg/g) cal.	Total (mg/g) cal.	Mean Live Weight (mg/g)	Cal/mg Mean Live Weight
					OVUM					
10	50	stage 18	13.3	152	8.0	75.0	4.2	253	143.2	231
				859	34	705	17	1615		
					EMBRYO					
	30	stage 18	8.3	134	8.6	78.4	4.5	234	145.6	226
				757	37	737	18	1549		
5	100	stage 18	10.3	100	6.4	98.9	5.6	221	22.1	34
				565	27	930	23	1535		
	50	stage 18	11.2	113	5.9	115.0	6.4	252	26.6	47
				638	25	1081	27	1771		
	30	stage 18	12.3	84	4.8	160.0	9.1	270	11.9	24
				475	21	1504	38	2037		
10	100	stage 18	10.1	99	5.6	75.2	4.3	194	14.5	19
				560	24	707	18	1308		

Temp (C)	Oxygen (%)	Form & Stage	Ash (mg/g)	Protein (mg/g) cal.	Non-Protein N (mg/g) cal.	Lipid (mg/g) cal.	Carbohydrate (mg/g) cal.	Total (mg/g) cal.	Mean Live Weight (mg/g)	Cal/mg Mean Live Weight
					EMBRYO					
10	50	stage 18	10.8	85 237	5.4 23	86.4 812	5.0 21	193 1337	12.5	17
	30	stage 18	11.4	75 423	5.0 21	105.0 987	6.0 25	207 1456	10.0	15

TABLE 40

Estimated amounts of RNA/DNA of the ovum, yolk and embryo of Atlantic salmon in mg/g, for living weight, at each of 18 developmental stages incubated at two temperatures and three levels of dissolved oxygen (% air-saturation).

	5 C			10 C		
Stage	100%	50%	30%	100%	50%	30%
			Ovum			
1	6.1/1.4	6.1/1.4	6.1/1.4	6.1/1.4	6.1/1.4	6.1/1.4
4	10.3/1.4	9.9/1.8	9.9/1.2	13.2/1.2	13.5/1.1	13.1/1.2
8	10.7/1.5	10.4/1.2	10.6/1.5	15.8/1.3	10.6/1.4	12.3/2.2
11	11.5/1.7	10.7/1.5	11.3/1.5	15.9/1.3	11.6/1.3	12.2/1.9
15	12.1/1.7	9.8/1.6	10.6/1.0	15.1/1.8	11.9/1.4	12.5/1.9
18	16.3/1.8	16.2/1.1	12.0/1.8	15.1/2.2	13.6/1.3	12.5/2.2
			Yolk			
18	12.5/1.4	10.0/1.9	8.8/1.5	8.8/1.7	8.2/1.0	7.6/1.7
			Embryo			
4	6.0/2.6	7.2/2.6	8.9/2.6	15.4/2.4	13.6/2.4	9.7/2.4
6	19.0/2.8	16.0/2.8	14.0/2.8	16.0/2.6	12.0/2.6	8.0/2.6
10	28.8/3.6	24.0/3.5	20.0/3.0	22.0/3.2	14.0/3.0	10.0/2.8
14	34.2/3.8	28.0/3.6	22.0/3.2	24.0/3.4	18.0/3.3	12.0/3.0
16	32.0/4.0	22.0/3.7	17.0/3.1	25.0/3.5	20.0/3.4	13.0/3.1
18	21.1/4.4	16.2/3.8	12.2/3.0	26.6/3.6	21.7/3.5	14.4/3.3

TABLE 41

Content of protein, RNA/DNA ratio, protein/DNA phosphorus, per unit dry weight and embryo weight of Atlantic salmon embryo at stage 18, from incubation at two temperatures and three levels of dissolved oxygen (% air-saturation).

Temperature C	Air-saturation %	Protein mg/g	RNA/DNA	Protein / DNA P	Embryo weight mg
5	100	452	4.11	312	22.1
	50	448	4.26	418	26.6
	30	311	4.07	300	11.9
10	100	510	7.4	380	14.5
	50	440	6.2	340	12.5
	30	371	4.4	326	10.0

experiments. The increase has a broad peak about stages 4 to 8 at 5 and 10 C. The highest ratio at stage 18 occurred at 50% air-saturation at both 5 and 10 C. (Table 40 and Figure 14).

The phosphorus content of phospholipids was estimated from whole ova at six developmental stages.

The estimated amount decreased from stage I to 18 in all experiments. The greatest decrease in total amount of phospholipid P 1108 ug/g, and 43.1 mg/g phospholipid was at 5 C, 100% air-saturation. The decrease was less at higher temperature and also at lower oxygen concentration in both temperatures (Table 42, 43 and Fig. 15). At the same time the daily decrease (total amount decreased/total developmental time in days) was highest at 10 C and less with declining temperature and oxygen concentration.

After comparison with tripalmitin standard the total lipid content was estimated. The decrease of total lipid had the same trend as that for phospholipid. Phospholipid was about half of the total amount of lipid and the decrease of nonpolar lipids was higher at 10 C than at 5 C at 100% air-saturation but was the opposite at lower air saturations. (Table 43, Fig. 15, 16, and 17).

Phospholipids in these embryos generally increased from stage 11 to stage 18 (measurements from earlier stages were omitted to conserve ova). The increase was greatest at low temperature and lower oxygen concentration. (Table 44).

The total lipid content of embryos was estimated only at stage 18 and it seems to follow the pattern of phospholipid change, highest at the lower temperature and lower oxygen levels. (Table 39).

The phospholipid content in the yolk also decreased during development. The decrease was greatest at 5 C and 100% air-saturation

Figure 14

RNA/DNA ratio in embryos of Atlantic salmon at stages 6, 10, 14 and 18 (also 16 at 5 C, 100% air-saturation) incubated at two temperatures and three levels of air-saturation.

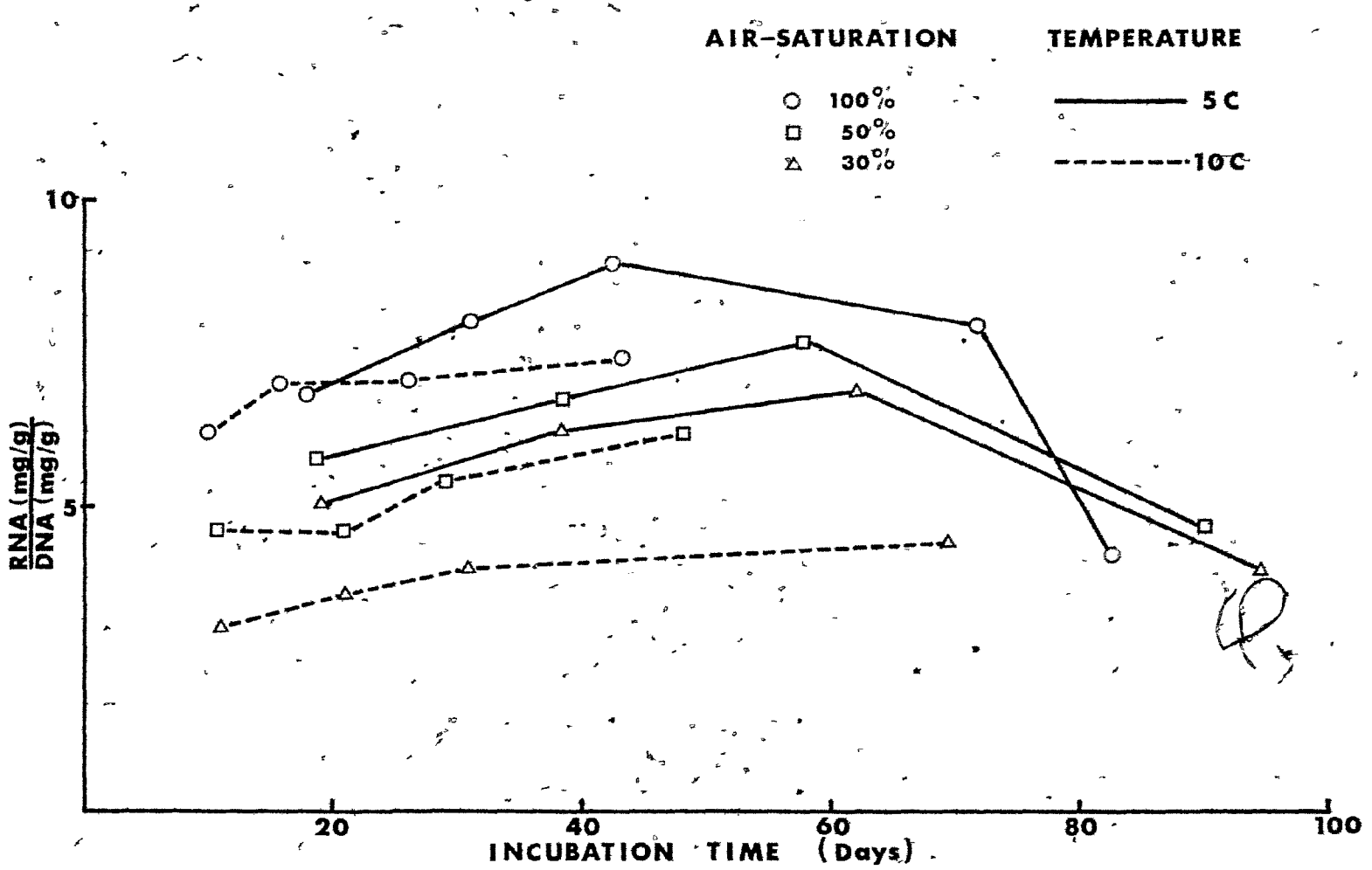

AIR-SATURATION
100%
50%
30%
TEMPERATURE
5C
10C
10
5
RNA (mg/g)
DNA (mg/g)
20
40
60
80
100
INCUBATION TIME (Days)

Figure 15

Phospholipid content (mg/g) of ova of Atlantic salmon from fertilization to stage 18 as measured at the outset and subsequently at stages 6, 10, 14, 16 and 18 in groups incubated at two temperatures and three levels of dissolved oxygen.

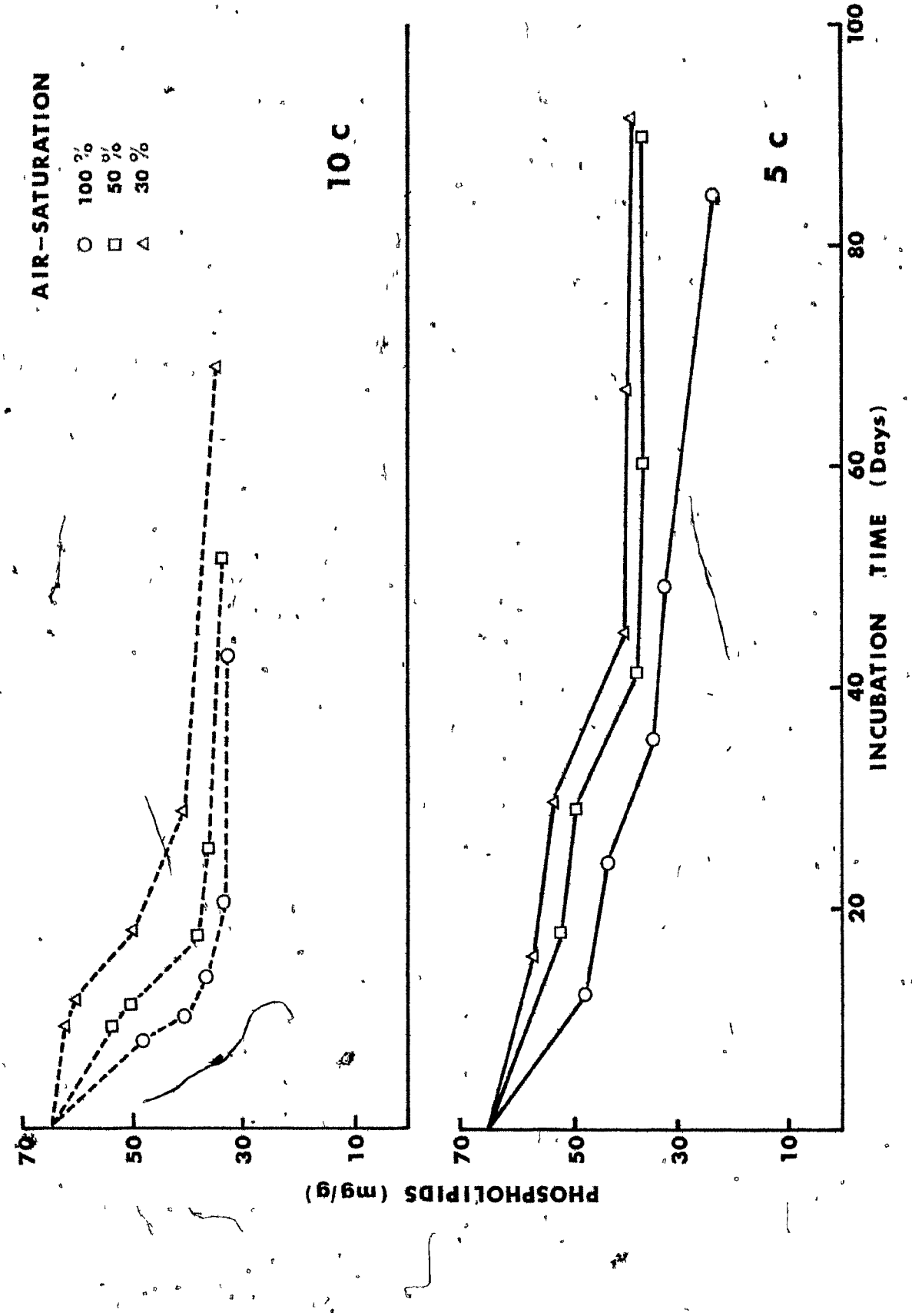

AIR-SATURATION
100 %
50 %
30 %
10 c
5 c
PHOSPHOLIPIDS (mg/g)
INCUBATION TIME (Days)
70
50
30
10
20
40
60
80
100

Figure 16

Non-polar lipid content (mg/g) of ova of Atlantic salmon from fertilization to stage 18 as measured at the outset and subsequently at stages 6, 10, 14, 16, 18 in groups incubated at two temperatures and three levels of dissolved oxygen.

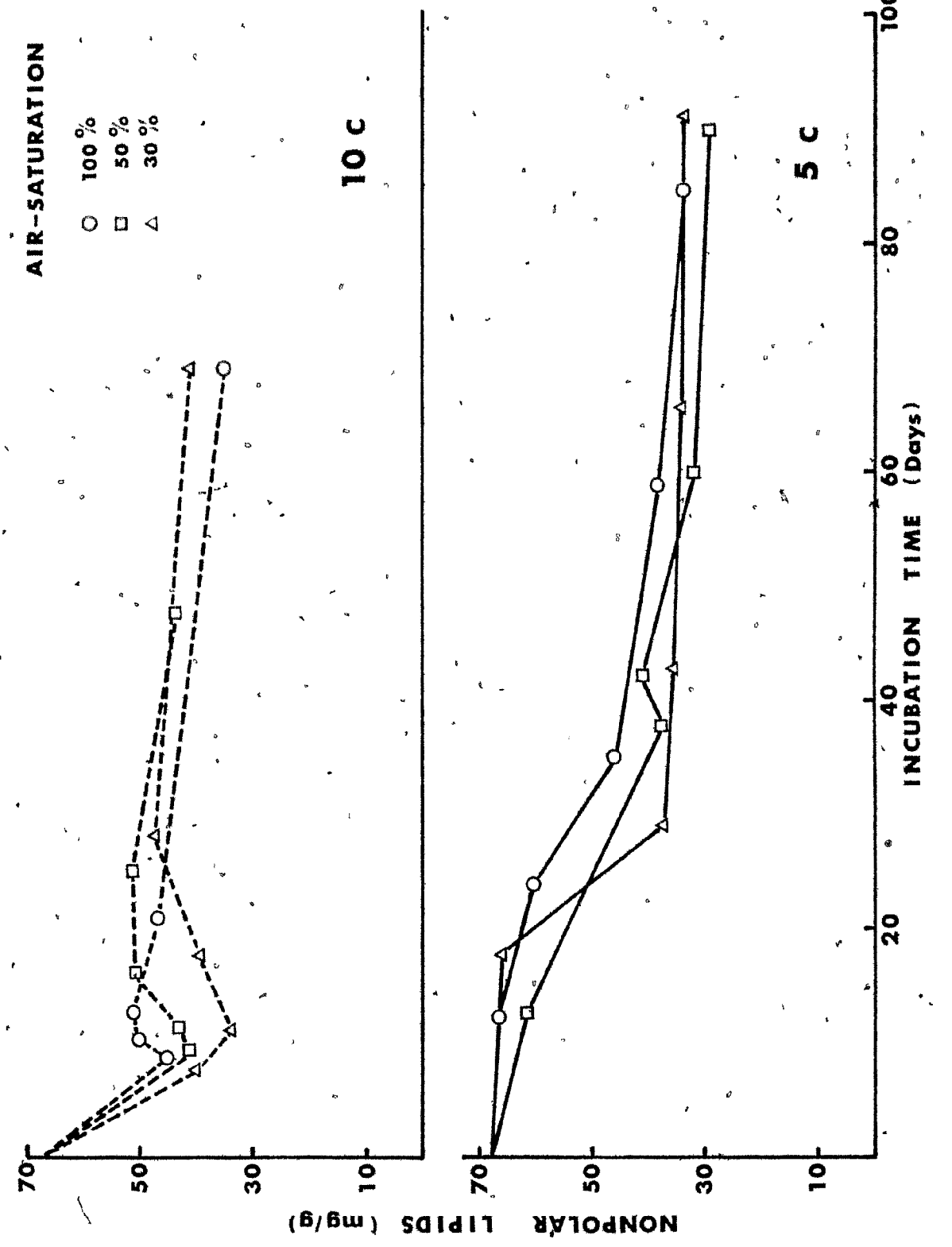

AIR-SATURATION
100 %
50 %
30 %
10 C
5 C
NONPOLAR LIPIDS (mg/g)
70
50
30
10
20
40
60
80
100
INCUBATION TIME (Days)

Figure 17

Total lipid content (mg/g) of ova of Atlantic salmon from fertilization to stage 18 as measured at the outset and subsequently at stages 6, 10, 14, 16 and 18, in groups incubated at two temperatures and three levels of dissolved oxygen.

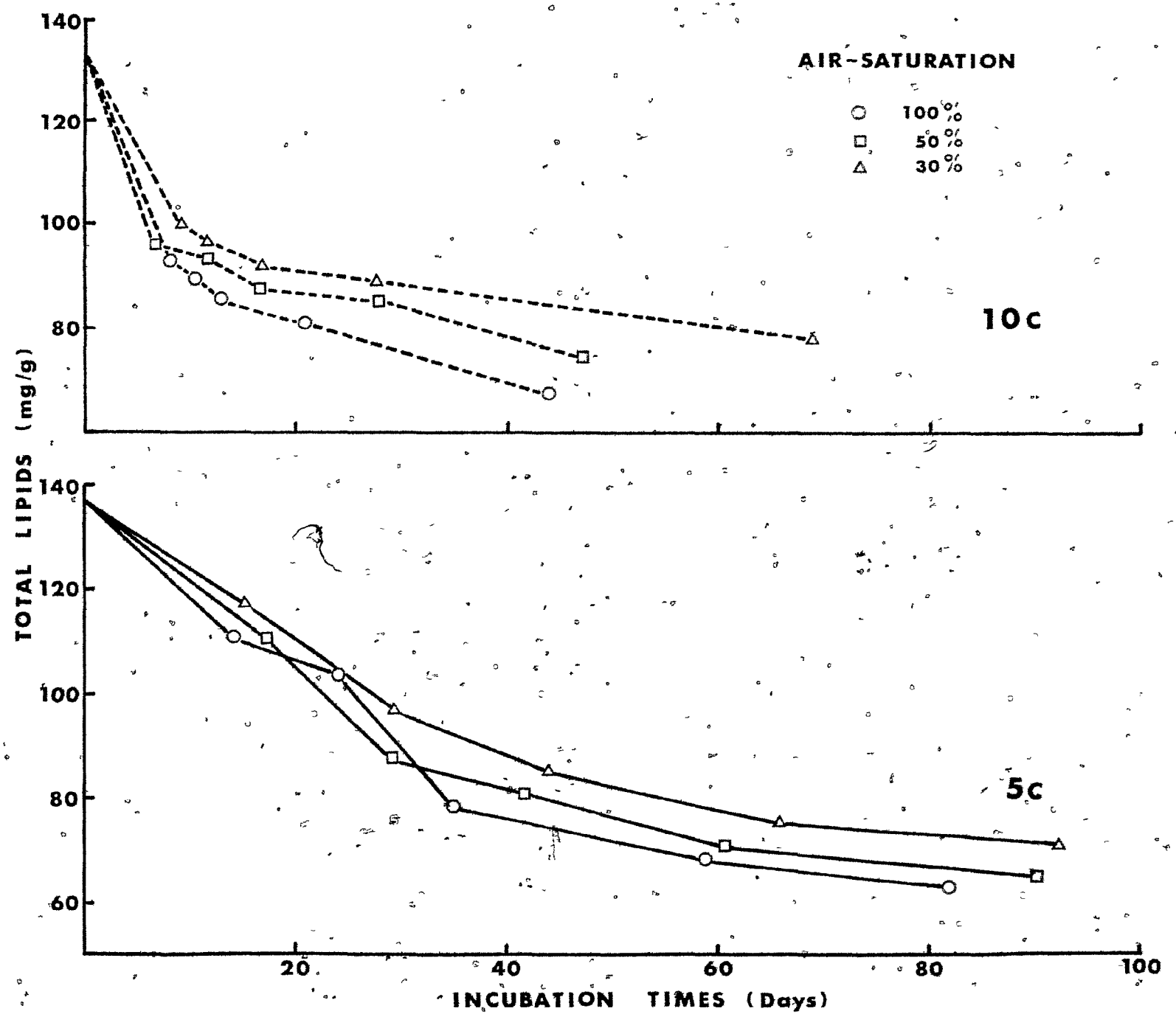

AIR-SATURATION
100%
50%
30%
10c
5c
TOTAL LIPIDS (mg/g)
INCUBATION TIMES (Days)
140
120
100
80
60
20
40
60
80
100

TABLE 42

Total content of phospholipid phosphorus of ovum, yolk, and embryo of Atlantic salmon in µg/g for living weight at some of the 18 developmental stages at two temperatures and three levels of dissolved oxygen (% air-saturation).

	5 C			10 C		
Stage	100%	50%	30%	100%	50%	30%
			Ovum			
1	1659	1659	1659	1659	1659	1659
4	1201	1298	1364	1254	1390	1628
7	-	-	-	1020	1276	1610
8	1135	1251	1276	-	-	-
9	-	-	-	948	970	1293
11	849	946	981	-	-	-
13	-	-	-	871	893	1073
15	792	924	946	-	-	-
18	550	911	928	847	862	927
Total decrease	1108	748	730	812	796	733
Decrease/day	13.3	8.30	7.9	18.8	17.0	10.6
			Yolk			
1	1659	1659	1659	1659	1659	1659
18	631	1030	1276	809	924	990
			Embryo			
11	1628	1716	1980	1496	1540	1760
15	1870	1980	2420	1628	1716	1953
18	2090	2420	3482	1760	2090	2338

TABLE 43

Quantities of phospholipids (PL) non-polar lipids (NPL) and total lipids (TL) of Atlantic salmon ovum and embyro in mg/g, for living weight, at two temperatures and three levels of dissolved oxygen (% air-saturation).

Stage	Temp	100%			50%			30%		
		PL	NPL	TL	PL	NPL	TL	PL	NPL	TL
					Ovum					
1	5	64.5	66.5	131	64.5	66.5	131	64.5	66.5	131
4		46.7	65.3	112	50.5	65.5	116	53.0	65.0	118
8		44.1	60.3	104	48.6	37.6	86	49.6	38.7	88
11		33.0	55.6	79	36.8	43.1	80	38.1	45.0	83
15		31.0	36.6	68	36.0	33.3	69	36.8	38.5	75
18		21.4	41.5	63	35.4	29.7	65	36.0	33.8	70
Total decrease		43.1	25.0	68	29.0	36.8	66	28.4	32.7	61
Decrease mg/day		0.52	0.30	0.8	0.32	0.41	0.73	0.30	0.35	0.65
1	10	64.5	66.5	131	64.5	66.5	131	64.5	66.5	131
4		48.8	45.0	94	54.0	41.2	95	63.3	34.4	98
7		39.7	50.5	90	50.0	44.2	94	62.6	33.5	96
9		36.8	50.7	88	38.0	51.4	89	50.3	40.0	90
13		33.8	47.5	81	35.0	51.0	86	41.7	45.8	88
18		33.0	36.4	69	33.5	41.5	75	36.0	42.4	78
Total decrease		31.5	30.1	62	30.9	25.0	56	28.5	24.1	53
Decrease mg/day		0.73	0.70	1.43	0.66	0.53	1.20	0.41	0.35	0.76
					Embryo					
18	5	81.3	17.7	99	94.0	21.0	115	135.5	45.0	160
18	10	68.4	6.6	75	81.3	4.7	86	91.0	14.0	105

TABLE 44

Absolute amount of phospholipids of yolk and embryo of Atlantic salmon in mg/g, for living weight, at two temperatures and three levels of dissolved oxygen (% air-saturation).

Developmental stage	5 C			10 C		
	100%	50%	30%	100%	50%	30%
			Yolk			
1	64.4	64.5	64.5	64.5	64.5	64.5
18	24.6	40.0	49.6	31.5	36.0	38.5
			Embryo			
11	63.3	66.7	77.0	58.2	61.0	68.4
15	72.7	77.0	94.0	63.3	67.0	76.1
18	81.3	94.0	135.5**	68.4	81.3	91.0

Figure 18

Cholesterol content (mg/g) of ova of Atlantic salmon from fertilization to stage 18 as measured at the outset and subsequently at stages 4, 8, 11, 15 and 18 incubated at 5 C and three levels of temperature and at stages 4, 7, 9, 13, and 18, incubated at 10 C and three levels of temperature. The mean line has been calculated and applied to the figure.

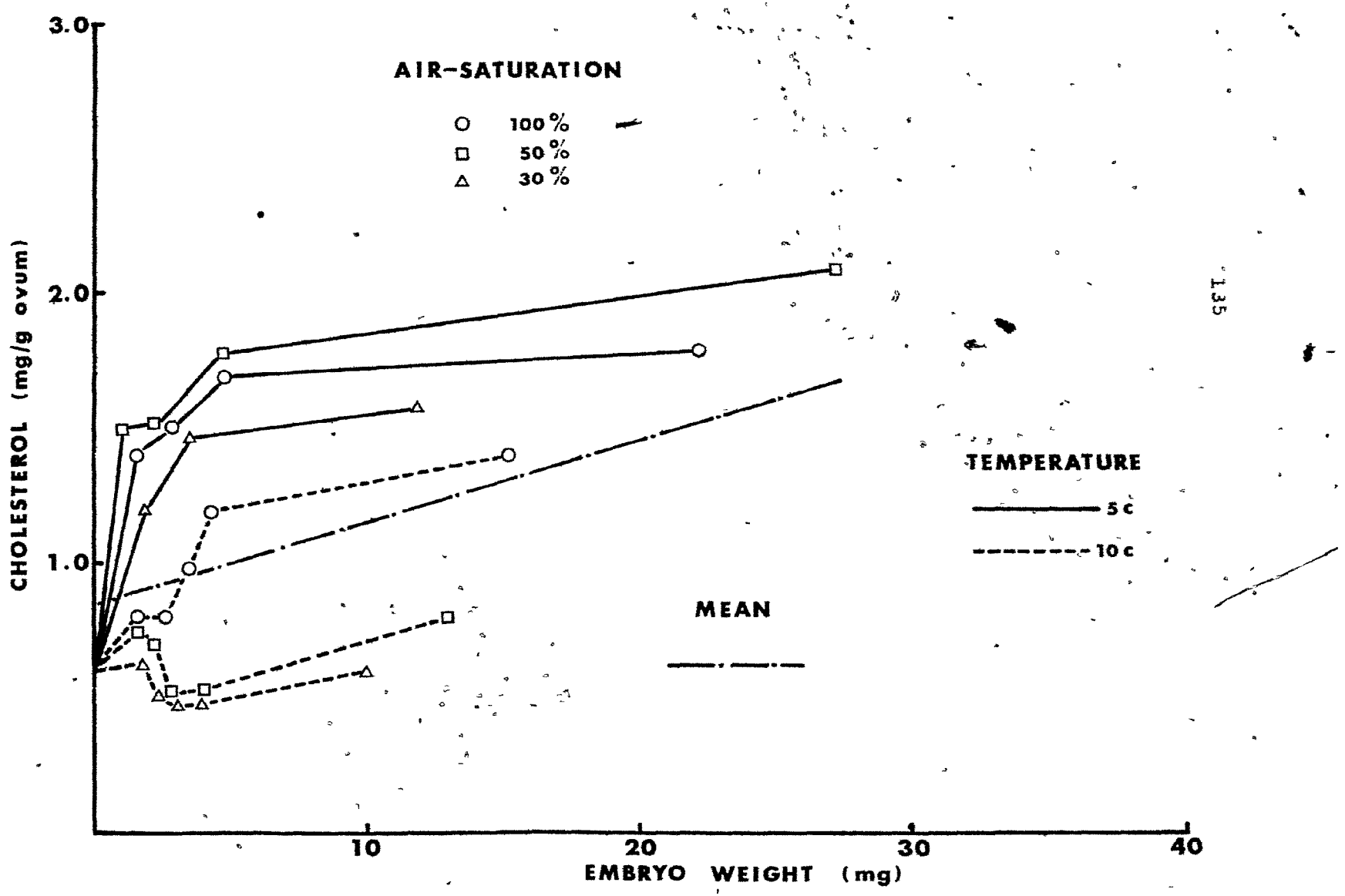

AIR-SATURATION
100 %
50 %
30 %
TEMPERATURE
5 c
10 c
MEAN
CHOLESTEROL (mg/g ovum)
3.0
2.0
1.0
10
20
30
40
EMBRYO WEIGHT (mg)

Figure 19

Spectrophotometric chart record (mg/g) of TLC plate scanning for various phospholipid fraction in the ova of Atlantic salmon, 1 day after fertilization. Abbreviations of fractions are, lysophosphatidyl choline (LC), L-A-phosphatidyl choline (LA), sphingomyelin (S), phosphatidyl ethanolamine (PE), orthophosphoethanolamine (OPE), phosphatidyl choline (PC), phosphatidyl-l-serine (PS), and lysophosphatidyl ethanolamine (LE).

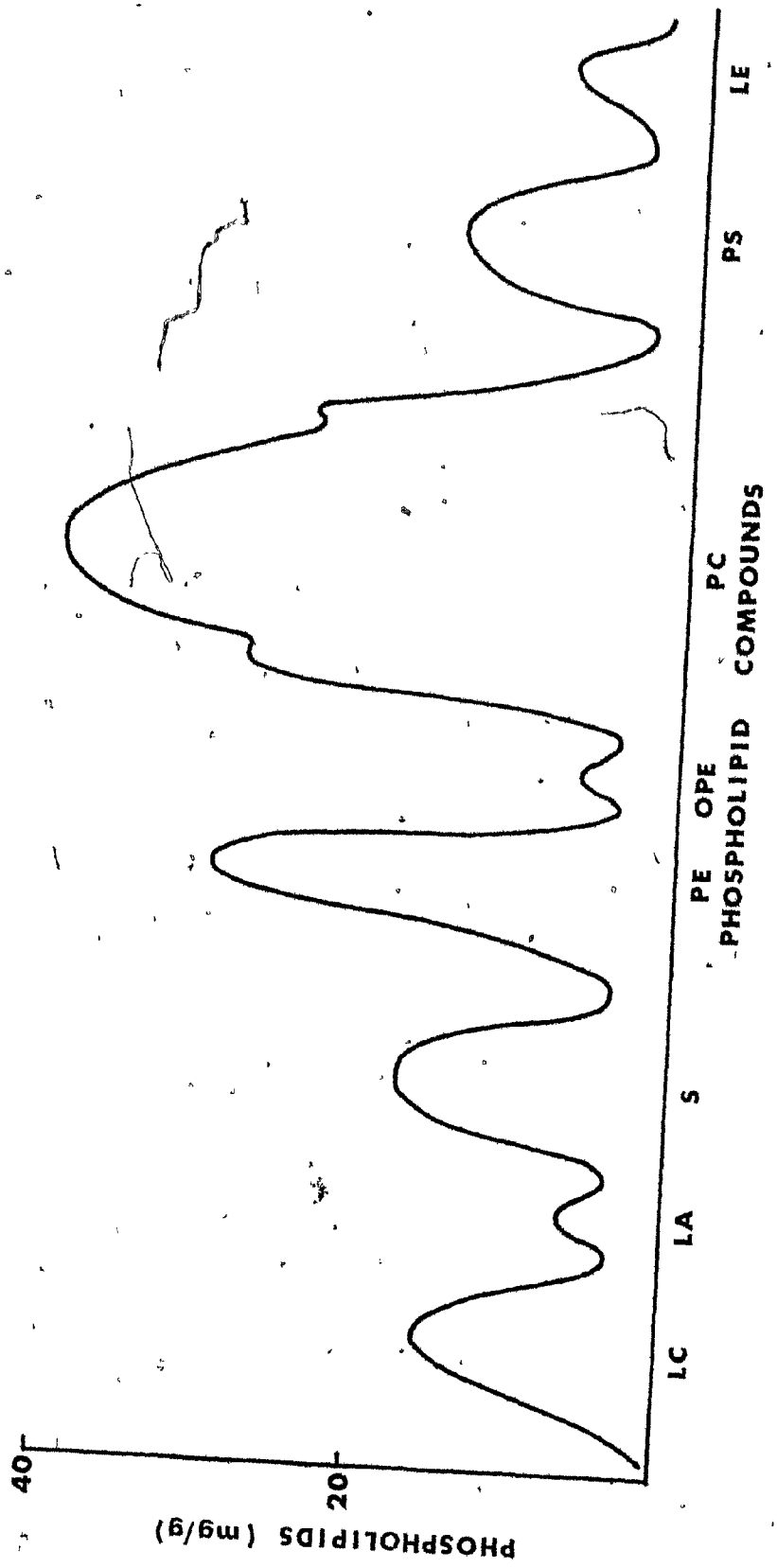

PHOSPHOLIPIDS (mg/g)
40
20
LC
LA
S
PE
OPE
PC
PS
LE
PHOSPHOLIPID COMPOUNDS

Figure 20

Example of distribution of phospholipid compounds on TLC plates (abbreviations given in Figure 19). Phospholipids were extracted from intact ova of Atlantic salmon incubated at two stages of development in various combinations of temperature and dissolved oxygen.

From left to right,

1. Blank (control)
2. Stage 18; 10C and 100% air-saturation
3. Stage 18; 5C and 30% air-saturation
4. Stage 1; (common to all groups)
5. Stage 18; 10C and 50% air-saturation
6. Stage 18; 10C and 30% air-saturation.
7. Stage 18; 5C and 100% air-saturation
8. Stage 18; 5C and 50% air-saturation

and less at 10 C or at lower oxygen concentrations (Table 44).

The relative changes in phospholipids are presented in Tables 45, 46 and 47. At both temperatures, and three levels of air-saturation phosphatidyl choline (PC) seems to be the major component, quantitatively, of metabolic oxidations. The estimation of absolute amounts of phospholipids is presented in Tables 46 and 47. It was also PC which had the greatest decrease from stage 1 to stage 18. The decrease was greatest at low temperature and high oxygen concentration.

In the yolk at stage 18 five components were separated by TLC. These were the same as those identified in stage 18 salmon embryos except for PE which was not detected. At 5 C, 100% air-saturation the order of amounts of phospholipids was, PC>PS>S>LE>LC. In all other experimental conditions the yolk was composed at stage 18 of the same phospholipids, except for the juxtaposed rank of LC; (PC PS>LC>S>LE), as shown in Table 47.

The absolute amounts of phospholipid compounds present in the embryo from the most to least, were: PC>PS>PE>LE>S>LC at 5 C and 100% air-saturation from most to least respectively. At 30% air-saturation this order is different only in the amount of S which is the least in this case. At 10 C and 100% air-saturation this order is, PC>PS>LC>S>PE>LE. At 10 C and 30% air-saturation the order of components is: PC>PS>LE>PE>LC>S, (Table 47).

The quantities of phospholipid P estimated by TLC (Figs. 19, 20) are presented in Table 48. The agreement of the methods of spectrophotometry for phospholipid P and TLC in this example is 96.3%. The difference for the sum of the phospholipids varied in these experiments from 85-98% of that value determined directly from the amount of phosphorus.

The cholesterol content in these salmon ova rose from stage 1

TABLE 45

Relative amount of phospholipids in ova of Atlantic salmon in % of total amount, for living weight, two temperatures and three levels of dissolved oxygen (% air-saturation). Abbreviations are, (LC) Lysophatidyl choline, (S) Sphingomyelin, (PC) Phosphatidyl choline, (PS) Phsophatidyl-L-serine, (LE) Lysophatidylethanolamine, and (PE) Phosphatidylethanolamine.

Stage	5 C 100%						5 C 50%						5 C 30%					
	LC	S	PC	PS	LE	PE	LC	S	PC	PS	LE	PE	LC	S	PC	PS	LE	PE
1	4.9	7.8	62.3	17.3	6.6	1.1	4.9	7.8	62.3	17.3	6.6	1.1	4.9	7.8	62.3	17.3	6.6	1.1
4	5.7	14.0	61.7	13.9	4.7	-	5.8	9.6	62.2	17.1	-	5.0	4.2	6.5	58.0	16.0	3.0	12.2
8	6.3	7.3	41.5	14.1	-	30.4	-	-	-	-	-	-	3.3	4.0	52.5	14.7	1.5	24.0
18	15.8	6.0	30.2	14.0	3.0	31.0	8.3	8.0	34.5	13.9	4.1	31.1	5.5	2.0	42.7	13.2	1.0	35.6
	10 C 100%						10 C 50%						10 C 30%					
1	4.9	7.8	62.3	17.3	6.6	1.1	4.9	7.8	62.3	17.3	6.6	1.1	4.9	7.8	62.3	17.3	6.6	1.1
4	4.7	7.8	65.0	16.4	2.4	3.3	5.8	9.3	62.3	18.4	4.1	-	8.7	6.4	60.7	14.1	6.0	2.1
7	7.4	7.8	60.4	24.3	-	-	-	-	-	-	-	-	6.1	5.3	62.1	22.7	3.6	-
9	6.0	7.9	63.2	17.2	1.9	3.8	5.3	6.1	62.8	17.2	2.2	6.3	-	-	-	-	-	-
13	7.3	13.9	55.1	18.2	1.2	4.2	17.5	35.5	32.6	-	-	14.4	3.1	26.1	44.3	11.5	1.1	13.8
18	9.4	15.0	52.2	18.0	0.8	4.6	4.2	7.8	61.5	18.4	1.8	6.3	2.1	8.6	69.6	8.6	1.0	10.1

TABLE 46

Estimated amounts of phospholipids (µg/g) in ova of Atlantic salmon at stages 1 and 18 and percentage change at two temperatures and three levels of dissolved oxygen (% air-saturation). Abbreviations are, lysophosphatidyl choline (LC), sphingomyelin (S), phosphatidyl choline (PC), phosphatidyl-L-serine (PS), lysophoshatidylethanolamine (LE), and phosphatidylethanolamine (PE).

Component	Stage	5 C			10 C		
		100%	50%	30%	100%	50%	30%
LC	1	3126	3126	3126	3126	3126	3126
	18	3313	2906	1969	3058	1388	744
	%	106	93	63	98	44	24
S	1	5009	5009	5009	5009	5009	5009
	18	1252	2805	700	4884	2585	3058
	%	25	56	14	98	52	61
PC	1	39738	39738	39738	39738	39738	39738
	18	6358	11919	15099	17001	30392	24781
	%	16	30	38	43	51	62

Component	Stage	5 C			10 C		
		100%	50%	30%	100%	50%	30%
PS	1	11000	11000	11000	11000	11000	11000
	18	2970	4840	4730	5863	6101	2058
	%	27	44	43	53	55	28
LE	1	4222	4222	4222	4222	4222	4222
	18	631	1393	337	360	596	354
	%	15	33	8	6	14	8
PE	1	1401	1401	1401	1401	1401	1401
	18	6543	10887	12696	1498	2088	3610
	%	467	777	906	107	149	258

TABLE 47

Amounts of phospholipids of yolk and embryo Atlantic salmon in µg/g upper line and in percent (lower line) of amount at stage 18, for living weight, at two temperatures and three levels of dissolved oxygen (% air-saturation). Abbreviations are, lysophosphatidyl choline (LC), sphingomyelin (S), phosphatidyl choline (PC), phosphatidyl-L-serine (PS), lysophosphatidylethanolamine (LE) and phosphatidylethanolamine (PE).

Compound	Temp C	Oxygen air-sat.	LC	S	PC	PS	LE	PE
Yolk	5	100	1327	2499	14406	4259	1470	-
			5.3	10.1	61.5	17.1	5.9	-
		30	20437	1813	29471	6171	1591	-
			5.5	4.4	7.13	14.9	3.8	-
	10	100	3771	2970	15985	4800	2165	-
			12.2	9.6	55.5	15.6	7.0	-
		30	3249	2281	21839	4649	1540	-
			9.7	6.8	65.1	13.8	4.6	-
Embryo	5	100	3645	6426	32648	14214	9293	14986
			5.7	10.2	47.8	22.2	13.9	19.2
		30	4257	1190	55000	30360	19280	24200
			3.1	0.9	41.3	22.3	14.2	18.2
	10	100	5280	3872	22880	7920	13200	2728
			12.0	8.8	52.0	18.0	3.0	6.2
		30	4365	2182	63466	15866	8140	4840
			4.4	2.2	64.0	16.0	8.2	4.9

TABLE 48

Estimated Rf values (retardation factor), quantities of phospholipid ($\mu g/cm^2$) on TLC plate, phosphorus multiplier (phospholipid/phosphorus), phospholipid (µg)/ovum (g), in stage 1, and phosphorus (µg)/phospholipid (g) in recently fertilized ova of Atlantic salmon. Abbreviations are, lysophosphatidyl choline (LC), L-A-Phosphatidyl choline (LA), sphingomyelin (S), phosphatidylethanolamine (PE), ortho-phosphatidylethanolamine (OPE), phosphatidylcholine (PC), and phosphatidyl-L-serine (PS).

Compound	Rf value	$\mu g/cm^2$	P multiplier	phospholipids (µg/g)	phophorus µg/g
LC	0.30 + 0.05	4.08	36.6	3126	85
LA	0.38 + 0.06	3.8	42.5	182	4.0
S	0.41 + 0.08	9.9	67.2	5009	75
PE	0.44 + 0.07	3.5	25.4	1401	10.8
OPE	0.58 + 0.03	5.0	28.4	95	3.3
PC	0.62 + 0.08	430	48.6	39738	820
PS	0.71 + 0.05	5.0	28.8	11000	380
LE	0.77 + 0.06	5.3	39.2	4222	220
Total					1598.1

to 18 in all experiments. This increase is greater at 5 C than at 10 C but is reduced by lower oxygen levels. (Table 49 and Fig. 18). The cholesterol content of yolk after an initial increase then decreased. The decrease was greater at 10 C than at 5 C and was greater at high oxygen concentration than at low. The cholesterol content of the embryo increased significantly from stage 9 to stage 18 (Table 48). The cholesterol/DNA ratio increased generally from stage 1 to 4 and then levelled and gradually decreased (Tables 40, 49). The cholesterol/DNA ratio was higher at lower temperature and high oxygen concentration at stage 18 in the embryo.

The amount of glucose increased at low temperature and oxygen levels, from early to late stages of development. (Table 50). The amount of carbohydrate increased in all experimental groups with the same trend as that observed in glucose. (Table 50).

The enzyme activity of 3'5'cAMP-ase decreased from stage 4 to 18, but was relatively higher at higher temeprature and higher air-saturation. (Table 51). The enzyme activity of 2'3'cAMP-ase rises from stage 4 to 18 at 10 C but decreases with lower temperature and lower air-saturations. (Table 51).

The chemical composition of 25 mm fry at 5 C and 10 C and 100% air-saturation is presented in Table 52. Amounts of total lipid, protein, non-protein N, total carbohydrate and ash are greater at 5 C. Amounts of RNA, DNA, phospholipid P, phospholipid and glycogen were higher at 10 C. Dry weight was higher at 5 C than at 10 C. Table 53 shows the caloric values of components for comparison of results with other studies derived by other techniques.

The amounts of different organic compounds at certain developmental stages were compared with the respective embryo weights for each experimental

TABLE 49

Estimated amounts of cholesterol in ovum, yolk, and embryo of Atlantic salmon in mg/g, for live weight, at two temperatures and three levels of dissolved oxygen (% air-saturation).

Stage	5 C			10 C		
	100%	50%	30%	100%	50%	30%
			Ovum			
1	0.65	0.65	0.65	0.65	0.65	0.65
4	1.38	0.75	0.60	0.90	0.80	0.70
8	1.50	1.50	1.10	0.92	0.66	0.53
11	1.60	1.50	1.50	1.00	0.53	0.48
15	1.70	1.50	1.30	1.15	0.52	0.50
18	1.80	2.10	1.60	1.20	0.90	0.60
			Yolk			
1	0.68	0.68	0.68	0.68	0.68	0.68
4	0.60	0.83	1.65	0.36	0.76	0.80
9	0.80	1.20	1.80	1.10	-	1.49
18	0.40	0.45	0.50	0.33	0.39	0.54
			Embryo			
4	0.10	0.10	0.10	0.10	0.10	0.10
9	0.30	0.29	0.28	0.45	0.42	0.17
18	4.98	2.70	2.30	1.30	0.85	0.70

TABLE 50

Quantities of glucose and total carbohydrate in ova (mg/g live weight) of Atlantic salmon, at two temperatures and three levels of dissolved oxygen (% air-saturation).

Stages	5 C			10 C		
	100%	50%	30%	100%	50%	30%
			Glucose			
1	1.18	1.18	1.18	1.18	1.18	1.18
6	1.40	1.50	1.60	1.15	1.20	1.20
12	1.65	1.75	2.10	1.10	1.20	1.35
18	2.02	2.30	2.64	1.03	1.25	1.54
			Carbohydrate			
1	3.62	3.62	3.62	3.62	3.62	3.62
6	3.65	3.90	4.10	3.70	3.80	3.90
12	3.70	4.30	4.60	3.80	4.10	4.30
18	3.84	4.70	5.50	4.00	4.25	4.55

TABLE 51

Estimated values of enzyme activity of 3'5' cAMP-ase and 2'3' cAMP-ase of Atlantic salmon ova expressed in amounts of phosphorus liberated by alkaline phosphatase, section (A); pMP/g(ovum) : DNA(mg)/g(ovum); section (B); and, pMP/g(ovum) : cholesterol(mg), section (C); at two temperatures and three levels of dissolved oxygen(% air-saturation).

Stages	5° C			10° C		
	100%	50%	30%	100%	50%	30%
			3'5'cAMP-ase			
			(A)			
4	251.0	251.0	251.0	251.0	251.0	251.0
18	125.0	100.0	33.0	198.0	150.0	110.0
			(B)			
4	96.5	96.5	96.5	104.6	104.6	104.6
18	28.4	31.2	11.0	55.0	42.9	33.3
			(C)			
4	2510	2510	2510	2510	2510	2510
18	25.1	37.0	14.3	152.3	176.4	157.1
			2'3'cAMP-ase			
			(A)			
4	370	-	370	370	-	370
18	173.5	-	34.6	573.0	-	480.0
			(B)			
4	142.0	-	142.0	142.0	-	142.0
18	45.6	-	11.5	159.0	-	33.3
			(C)			
4	3700	-	3700	3700	-	3700
18	34.8	-	15.0	440	-	685

TABLE 52.

Differences in gross chemical composition (mg/g) and calculated calorie values in Atlantic salmon fry at time of total yolk absorption, at 5 and 10°C, 100% air-saturation.

Component	5 C		10 C	
	Weight (mg/g)	Calories	Weight (mg/g)	Calories
RNA	6.1	-	7.9	-
DNA	4.0	-	4.5	-
Cholesterol	1.0	-	0.2	-
Phospholipid P	97.0 µg	-	112.7 µg	-
Phospholipid	3.8	-	4.4	-
Total lipids	11.0	103.4	5.1	47.9
Protein	122.0	689.0	85.0	480.0
Non protein N	10.2	43.6	8.0	34.2
Glycogen	1.1	-	1.9	-
Carbohydrate	3.8	15.8	3.6	14.9
Ash	15.0	-	11.0	-
Total dry matter	162.0	-	112.7	-
Cal/live weight	852	-	577	-
Cal/dry weight	5259	-	5119	-

TABLE 53

Yolk conversion (weight gain mg/yolk depletion mg), total calories/animal, calories consumed, utilization efficiency of calories (calorie/animal 100/ calories consumed), in Atlantic salmon embryos at stage 18, and in fry (at total yolk absorption), at two temperatures and three levels of dissolved oxygen (% air-saturation).

Process	Stage	5 C			10 C		
		100%	50%	30%	100%	50%	30%
Yolk conversion	Embryo	1.08	2.00	1.22	0.97	1.08	0.69
	Fry	1.28	-	-	1.35	-	-
Cal/Animal	Embryo	34	47	24	19	17	15
	Fry	116	-	-	82	-	-
Calories consumed	Embryo	97	85	119	95	100	105
	Fry	208	-	-	241	-	-
Cal/mg gain	Embryo	3.98	2.85	9.15	6.5	7.3	9.6
	Fry	1.54	-	-	1.70	-	-
Utilization efficiency (%)	Embryo	35	55	20	20	17	14
	Fry	55	-	-	34	-	-

group by means of the SPSS multiple regression computer program of Northwestern University. Pearson correlation coefficients of chemically determined compounds (mg/g) with embryo weight (mg) were exceedingly significant for phospholipids ($P<0.0001$), RNA ($P<0.0001$), very significant for total lipid ($P<0.16$), and significant for 3'5'cAMP ase ($P<0.012$) and for RNA/DNA (P 0.029). The coefficient of determination (r^2) was 0.86, the F-value 8.39, and the standard error of estimated embryo weight from the variables was 2.57. Then, if all the variables are computed in order of significance from the most to the least according to this analysis the order is:

Cholesterol

Temperature

3'5' cAMP-ase

RNA

Cholesterol/DNA

Oxygen

Developmental stage

DNA

RNA/DNA

Phospholipid and

Total lipid

In the multiple regression, total lipid ($P<0.00001$), RNA/DNA ($P<0.0001$) and 2'3'cAMP-ase were highly significant ($P<0.0001$) with the 2-tail probability. Variances with the 2 - tailed t-test were highly significant for the amounts of RNA ($P<0.007$), cholesterol ($P<0.0001$) cholesterol/DNA ($P<0.002$), and 2'3'cAMP-ase activity ($P<0.0001$).

The interaction between embryo weight and the chemical components of salmon ova was analysed with BMDO3R computer program. Double logarithmic transformation provided the best least squares

regression. Besides the chemical components, the equation has three other variables, temperature, oxygen and developmental stage as a measure of age of the embryo. The importance of the variables as predictors of embryo weight according to t-tests are, in descending order:

RNA

Temperature

Cholesterol

Oxygen

Developmental stage

Phospholipids

2'3'cAMP-ase

3'5'cAMP-ase

Total lipid

DNA

To reveal significant effects, linear regression of embryo weight as a dependent variable was calculated against each component. According to this analysis the order for prediction of embryo weight using a single factor is:

Phospholipids ($P<0.001$)

Cholesterol ($P<0.1$)

RNA ($P<0.01$)

RNA/DNA ($P<0.1$)

3'5'cAMP-ase ($P<0.005$)

2'3'cAMP-ase ($P<0.1$)

Total lipids ($P<0.2$)

Cholesterol/DNA ($P<0.5$)

The first three components are highly significant and the last four components are nonsignificant according to the tests.

The equations for linear mutiple regression, calculated by the earlier mentioned SPSS and BMD03R computer programs, which best describe the embryo weight calculated from chemical components and environmental factors are:

$$\log Y = 0.61 + 2.0 \log X_1 + 0.52 \log X_2 - 0.31 \log X_3 + 0.12 \log X_4 + 2.75 \log X_5 - 0.12 \log X_6 - 0.83 \log X_9 + 0.34 \log X_{10} - 0.4 \log X_{11} - 0.46 \log X_{12} \quad \ldots\ldots\ldots\ldots (28)$$

$$\log Y = 0.35 - 1.54 \log X_1 + 0.54 \log X_2 - 0.5 \log X_3 + 0.003 \log X_4 + 1.76 \log X_5 + 0.61 \log X_6 + 2.27 \log X_7 - 1.84 \log X_8 + 1.1 \log X_9 + 0.49 \log X_{10} - 1.16 \log X_{11} - 1.76 \log X_{12} \quad \ldots (29)$$

$$\log Y = 5.3 - 3.0 \log X_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (30)$$

The r^2 values for the three equations (28, 29 and 30) are 0.82, 0.86 and 0.63 respectively, and the $P<0.001$ for all the three equations.

The abbreviations are:

Y = Embryo weight in mg

X_1 = Temperature of incubation water in C

X_2 = Dissolved oxygen mg/l

X_3 = Phospholipid content of total in mg/g

X_4 = Total lipid content of total ovum in mg/g

X_5 = RNA content of ovum in mg/g

X_6 = DNA content of ovum in mg/g

X_7 = RNA/DNA ratio in ovum

X_8 = Cholesterol content of ovum in mg/g

X_9 = Cholesterol/DNA ratio in ovum

X_{10} = Developmental stage of embryo

X_{11} = Enzyme activity of 3'5'cAMP-ase of ovum

X_{12} = Enzyme activity of 2'3'cAMP-ase of ovum

DISCUSSION

The development of a salmon embryo is accompanied by changes in the chemical components in the ovum generally. Several components notably lipids and proteins provide the necessary energy for morphogenetic, osmotic, mechanical or structural work (Brett 1962). Components such as minerals, amino acids, saccharides, build the embryonal body.

Because of the high sensitivity of spectrophotometry and TLC analyses it was possible to detect changes in lipids and proteins used in embryogenesis from fertilization to stage 18, changes which are also seen in the weight of ova during embryogenesis.

The composition of the freshly fertilized salmon ovum is rich in protein and lipid which are 56% and 39% of the dry weight respectively. However, from stage 1 to 18 the main energy source appears to have been the lipid fraction while protein seems to have formed the secondary energy source and carbohydrate, non-protein-nitrogen accumulate in very small amounts (Table 39). These results are in agreement with those of Hayes (1949), Løvtrup (1953) and Hayes (1971).

The total lipid content of the ovum decreases somewhat more at 10 C, to 25%, than at 5 C, to 27% (calculated from data in Table 39 and Fig. 14). The quantity per unit of embryo weight at 10 C is almost double that at 5 C, and is to be expected because of the increased energy demand at the higher temperature.

Also, the higher energy demand might be the reason that at higher temperature the utilization of phospholipids takes precedence over the non-polar lipids (Fig. 15 and 16), in agreement with the observation of Smith (1957). At lower air-saturation the tendency for utilization of phospholipids is higher and is also demonstrated in Fig. 15 and 16. The trend seems to be reversed only for non-polar lipids.

Apparently these embryos use more protein at low temperature and low oxygen level. Probably, decomposition and the synthesis of protein are parallel as in the case of Triturus embryos (Imoh and Minamidani 1973) but the synthesis is inhibited somewhat at lower oxygen supply so that the amount of oxidized protein is greater.

The amount of RNA is controlled by the amount of DNA and the DNA content of an embryo is related to the number of viable cells. Since the rate of protein synthesis is dependent on the amount of RNA, then conditions which regulate the quantity of DNA indirectly regulate the protein synthesis. The DNA content of embryo is higher at lower temperature and higher oxygen level but the RNA is almost uniform although it diminishes slightly at the lowest oxygen level (Table 40). Consequently, the RNA/DNA ratio is also higher at low temperature and higher oxygen level. This ratio is in perfect correlation with growth expressed as weight of embryos (Fig. 11). The embryo weight, the RNA/DNA ratio and the amount of protein per unit of dry weight seem to be well stabilized at 5 C, independently of oxygen level, but at 10 C they lose some stability (Table 41 and fig. 4).

The mean ratio from the data available in Table 40 for the RNA/DNA ratio in the embryos reveals that it is higher at 5 C and higher oxygen content. In general, an increase in this ratio indicates an increase in mitotic activity (Balinsky 1968). This suggests that cell proliferation is relatively greater from stages 1 to 18 at the lower temperature and the higher air-saturation. Proceeding to the supraorganismal level there have been two studies which somewhat parallel this relation in salmon embryos. Both studies indicate that mean body-size of age-classes within fish populations increases with increasing ratios of RNA/DNA in their muscle tissue (Bulow 1970, Haines 1973).

The explanation that the smaller embryos at stage 18, at higher temperature, might be that cell proliferation and growth are relatively less at high temperature throughout embryogenesis. A mean ratio of RNA/DNA is used as more relevant information for these earlier stages, because the utilization of yolk protein, either for energy or body growth, is not a steady process, but one in which periods of more rapid utilization alternate with less active periods (Chopra and Simnett 1969, Ignat'eva 1973, Love 1970).

The amount of total lipid consumed from stage 1 to 18 is 68 mg/g. This is 6.8% of the ovum weight while the individual difference within ova due to size variation, discrepancy in water content, shifting of developmental rate, and other factors can be as high as two-fold. Correlation between the total lipid consumption and embryo weight is significant ($r = 0.75$, $P<0.01$, Fig. 21). There is little wonder then that several authors failed to find significant changes in dry weight, lipid or protein of an ovum. Hayes (1949) referred to the high probability of error in these measurements because of normal weight variations among ova.

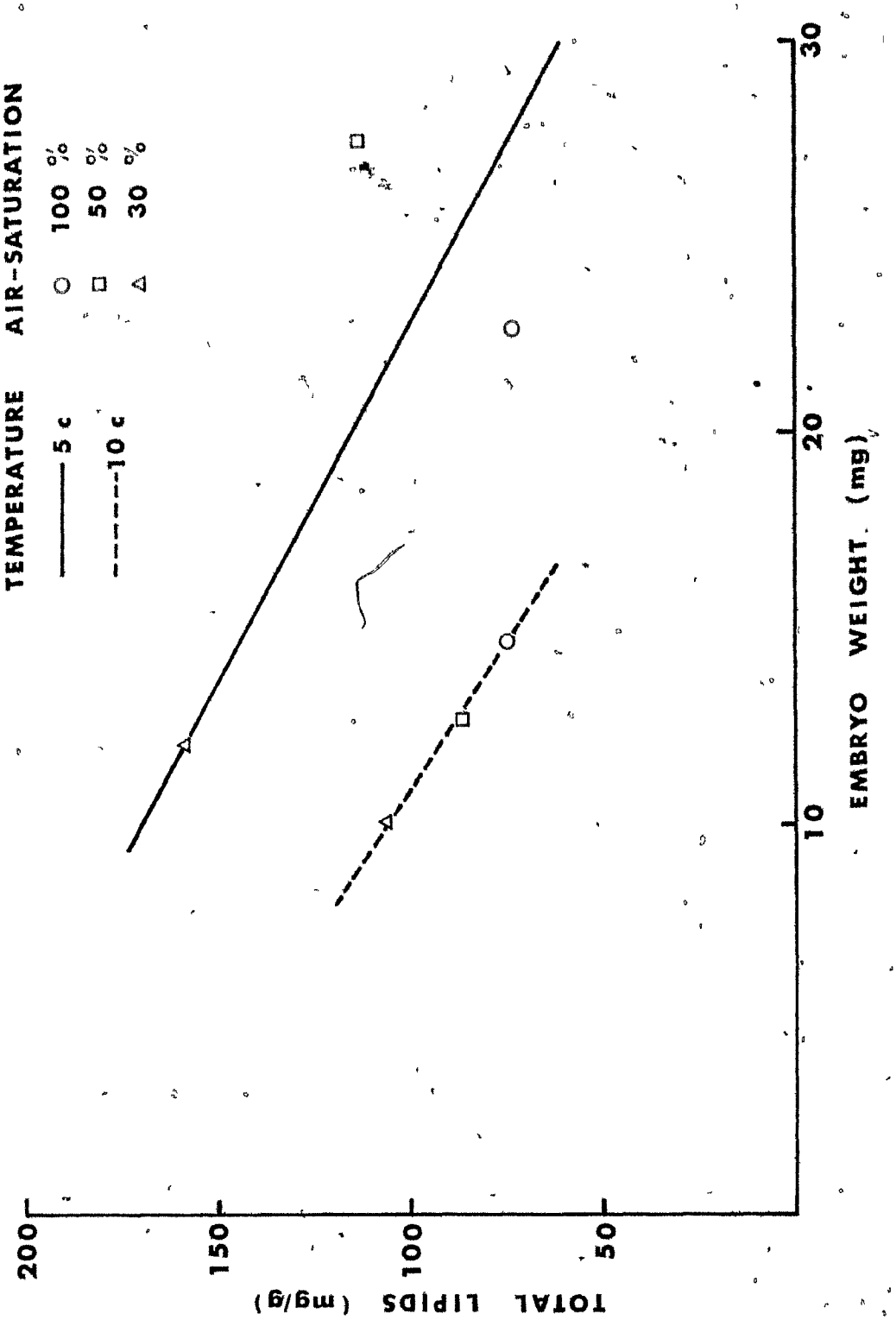
There seems to be a certain change in the relation of lipid components in development at different embryonal stages and environmental conditions (Tables 43, 44, 45, 46 and 47).

Phosphatidyl choline

Phosphatidyl choline (PC) seems to be the compound among the phospholipids of which the highest amount is consumed, an observation that agrees with that of Yamagami and Mohri (1962) for rainbow trout. The relative and absolute amounts of PC decrease with the decreasing order of both temperature and oxygen in the present study while in the other study they noted that a decrease in PC occurred only after hatching. However, they noted a decrease during earlier development in a third compound which

Figure 21

Relation of standardized total lipid content (mg/g) and yolkless embryo weight (mg) at stage 18 incubated at two temperatures and three levels of dissolved oxygen (% air-saturation).


TEMPERATURE
5 c
10 c
AIR-SATURATION
100 %
50 %
30 %
TOTAL LIPIDS (mg/g)
200
150
100
50
EMBRYO WEIGHT (mg)
10
20
30

they claimed was a kind of PC. In the present study, three molecular forms of PC apparently occurred which were not distinctly separable by TLC but which may account for the discrepancy in the two sets of data, since they offer the ratio PC:PE as 91:10, and the ratio PC:lyso-PE is 90:11 for Atlantic salmon embryos. There may also be species-specific differences.

Sphingomyelin

Sphingomyelin (S) was the second-ranking component which was utilized at 5 C and phosphatidyl-L-serine (PS) at 10 C. However, these two components seem to change in their order of utilization with the diminishing level of oxygen so at 30% air-saturation the second component which was utilized was PS at 5 C and S at 10 C. These results support the idea that PS is formed by a metabolic pathway which is different from that of PC or phosphatidyl ethanolamine (PE). (Bloch 1960, Billimoria and Lewis 1968).

Lysophosphatidylethanolamine.

The amount lysophosphatidylethanolamine (LE) decreased in all the experiments considerably during incubation. There was no obvious trend to such decrease which could be associated with developmental environments.

Lysolecithin

The amount of lysolecithin or lysophosphatidylcholine (LC) decreased with rising temperature and lower oxygen content.

Phosphatidylethanolamine

The amount of PE increased with increased air-saturation and was higher at lower temperature.

Amounts of glucose, carbohydrate, and non-protein nitrogen increase during the development of salmon embryos according to results of this experiment and those of Hayes and Hollett (1940), Hayes and Armstrong

(1942), and Hayes (1949), (Tables 30 and 50).

The amount of glucose and other carbohydrates rises with decreasing temperature and oxygen content of the experimental environments. This observation supports the idea that at different temperatures the metabolic pathways are also different. Hochachka and Hayes (1962) postulated that in brook trout, energy from carbohydrate maintained at low and high temperature comes from the Embden-Meyerhof glucose-phosphate-fructose-phosphate-triose, or Dickens direct oxidative hexose-monophosphate pathways, respectively. However, there is little other data on carbohydrate metabolism in fishes except for those of Daniel 1947, Nagai and Ikeda 1971 and 1971a. The first author also observed an increasing tendency for glycogen synthesis from fertilization to late embryonal stages in Atlantic salmon. The other two authors have suggested that fish (carp) have a reversible Embden-Meyerhof pathway so that glycogen is not a principal storage form for energy in carp, and also not in herring (Balbontin 1973).

Cholesterol

Cholesterol is a product of lipid catabolism. It is synthesized in essentially all animal tissues. The estimated values of cholesterol in the whole ovum and the embryo are in accordance with the measured embryonal weight (Table 49 and Fig. 18). At the same time, as the cholesterol content of the whole ovum slowly rises, the content of yolk is decreased but that of the embryo rises sharply (Table 49). The mean absolute amount of cholesterol in mg/g of embryo at stage 18 almost forms a single line ($r=0.98$) from the starting amount at stage 1, except in the embryos from 5 C at 30% and 10 C at 50% and 30% air-saturation. These embryos are probably under certain catabolic stress and this seems to reduce the cholesterol content in the lower levels

of oxygen which are reflected in values of cholesterol somewhat lower than those predicted by the relation in Fig. 15.

Enzymes

Potential hormone and enzyme regulating activity of 3'5'cAMP and 2'3'cAMP has been indirectly estimated through the determination of the corresponding amounts of 3'5'cAMP-ase and 2'3'cAMP-ase activity. When the activity of these two enzymes is high then the amount of 3'5'cAMP and 2'3'cAMP present is also expected to be high and therefore the regulation of other enzymes in certain metabolic pathways should be enhanced.

The comparison and the enzyme activities of 3'5'cAMP-ase and the measured amount of cholesterol and DNA shows that the enzyme activity divided by the amount of cholesterol or DNA gives higher values at higher temperatures and higher air-saturation. The same values for 2'3'cAMP-ase seem to have similar trend (Table 31). The results suggest that higher temperature and lower oxygen levels provide less enzyme of 3'5'cAMP than that of 2'3'cAMP and that this relation might have a role in development. However, possibly the measured 2'3'cAMP-ase is simply RNA-ase activity. The role of 2'3'cAMP might be deduced from the 25-mm alevins. At this time the alevins held at 10 C were heavier than those held at 5 C, being 141.4 and 135.2 mg, respectively. The air-saturation was 100% in both sets. The cal/g of dry weight was estimated as 5119 and 5259 at 10 C and 5 C respectively. Consequently, the lower live weight represents a more efficient developmental rate and lower oxygen consumption. Thus, at this time, those at 10 C showed faster growth than at 5 C. At the same time, enzyme activity of 2'3'cAMP-ase expressed in mM P/DNA mg was estimated as 123.3 and 0.00, respectively, and in the head and the body of alevins from 5 C, and 172.0 and 49.0 respectively,

in the head and the body of alevins from 10 C. Since this activity is higher in the head region in salmon, a parallel to the increase noted in rat brain (Gilbert, 1974), there may also be a protein-synthesis factor interfering with mRNA.

It was found by Weiss and Stiller (1974) that dibutyryl cyclic adenosine 3'5'-monophosphate exerted its greatest suppressive effect upon total lipid synthesis in embryonal rat brain slices aged 7-10 days. They made the suggestion that cAMP, arising from the hydrolysis of dibutyryl cAMP, may control the synthesis of brain lipids by regulating the utilization of acetyl-coenzyme A. Because of the indirect measurement in these experiments the high activity values of 3'5'cAMP-ase might be indicating that lipid synthesis is high in certain experimental conditions. Indeed, these values are low at high temperature and at high air-saturations when at the same time the lipid content of these embryos followed the reversed pattern (Tables 44 and 51).

There appears to be a correlation between these results and those for cell performance, reckoned as duration of sperm motility at various temperatures in brook trout and rainbow trout (Lindroth 1947, Hamor 1966), and the level of adenosine 3'5'cAMP in the sperm at various temperatures (Tash and Mann 1973).

Calorimetry.

The calculated efficiency values in this study were 35% and 20% at 5 and 10 C and normal air-saturation for Salmo salar, and they have a similar trend to those of Flücter and Pandian (1968), which were 68%, 54.5% and 47% for 10, 15 and 20 C for Solea solea, both species measured after hatching.

It seems that the efficiency and chemical changes are the

result of adaptation of Atlantic salmon to a certain environment. During early embryonal development the water temperatures in the natural surroundings of salmon are near 0 C. Later, when they enter the alevin stage the water is warming and indeed the growth relative to temperature and oxygen supply changes in relation to certain chemical components, indicating that the alevin has begun to adapt to warmer temperatures.

The efficiency calculated as calories/unit weight at embryonal stage 18 is greater at 5 C than at 10 C and generally less at lower oxygen concentrations (Table 53). This difference does not correspond fully to weight relations which shift following hatching to favour the 10 C embryos. One possible explanation could be, that the swimming ability of alevins increases until an advanced stage of yolk absorption is reached, as was observed in case of chinook salmon (Oncorhynchus tshawytscha, Thomas et al. 1969). Since the Atlantic salmon embryos at 5 C had relatively smaller yolk per unit embryo weight due to greater embryonal growth the energy required to carry this could cause a substantial difference in energy utilization, as in the case of larvae of largemouth bass (Micropterus salmoides) which was measured by Laurence (1969). Another explanation might involve the changes of surface/volume relations after hatching, and the better oxygen supply created by direct contact of gills with the surrounding water. However, Laurence (1973) had the same low values for embryonal and prolarval teleost tautog (Tautog onitis) when he used ash-free dry weight for determination of efficiency of yolk conversion.

The combusted calories/mg are, in the case of fry, almost the same as those calculated by Hayes and Pelluet (1945) and Hayes (1949). The amounts of required oxygen estimated calorimetrically and the amount of oxygen measured respirometrically are in very good agreement (Table 54).

TABLE 54

Differences in gross chemical composition (mg/g) of Atlantic salmon ova incubated at two temperatures and three levels of dissolved oxygen, between stages 1 and 18. Oxygen (mg) required for combustion is given in parentheses. Negative values indicate decreases in fractional composition.

Temp C	Oxygen % Air-sat.	Protein	Non-Protein Nitrogen	Total Lipid	Carbohydrate	Oxygen Uptake Calculated Total (mg/g)	Oxygen Uptake Calculated Mean Ovum (mg)	Oxygen Uptake Measured Mean Ovum (mg)	Calculated / Measured x100
5	100	-21(14)	+2.9(1.9)	-68(90)	+0.82(0.5)	110.0	15.3	20.6	74
	50	-27(18)	+2.4(1.6)	-66(93)	+2.08(1.1)	111.0	16.4	20.3	81
	30	-65(43)	+3.4(2.3)	-61(86)	+3.87(2.2)	129.0	20.3	13.4	151
10	100	-28(19)	+4.8(3.2)	-62(88)	+0.36(0.3)	107.3	15.7	21.3	74
	50	-37(25)	+5.4(3.6)	-56(79)	+0.58(0.3)	104.0	14.3	16.2	91
	30	-35(37)	+6.0(4.0)	-53(75)	+0.93(0.5)	112.0	16.2	14.5	112

This suggests that the values measured and the trends interpreted give true approximation to what occurs in Atlantic salmon embryos from the effects of environmental conditions in nature.

CHAPTER VII

CONCLUSIONS

CONCLUSIONS

The primary undertaking in this study was to develop an explanation to satisfy the question concerning the causes of growth differentials which create advanced embryos having considerably different lengths and weights while outwardly appearing to have the same degree of structural differentiation.

The primary explahation offered is that this embryonal growth, which is based chemically on the components of yolk both for structural materials and energy, is determined by the control of qualitative and quantitative utilization of these components.

Information from this study indicates that embyros at the moment of hatching are considerably larger and heavier when development has occurred at lower temperature or in greater oxygen supply than those whose development passed in warmer temperature or in a reduced supply of oxygen. The interpretation arising from a study of biochemical changes in the yolk and forming embryos, and caloric conversions, is that efficiency is greater at lower temperature and higher levels of dissolved oxygen. Thus for example, 3980 cal. are required for a gain of 1 g of embryo at 5 C and 5900 are required for the same growth increment at 10 C in air-saturated water. Similarly, in water of 30% air-saturation, respectively 9150 and 9600 cal. are required to produce 1 g of embryonal tissues. Thus, the efficiency of yolk conversion varies from 35 to 14% within the range of developmental environments employed in this study.

These differences are the result of differentials in the proportional quantities of yolk components which are incorporated into structural elements, and oxidized for energy. Since lipids generally have more energy than proteins, on average 9400 and 5650 cal/g respectively,

the consequences of differential utilizations are obvious. It has been demonstrated in this study by monitoring progressive depletions of various yolk fractions, that those embryos developed at lower temperature utilized more yolk lipid, particularly the energy rich phospholipids, and less yolk protein than those developed at a higher temperature and that these trends hold also for yolk utilization in higher and lower levels of oxygen. Concomitantly, the gain through synthesis of protein in the total ovum proceeds in a parallel way to complement the depletion of lipids. Thus, conditions which promote lipid utilization during embryogenesis create more energetic efficiency and produce a larger embryo per unit of depleted yolk, the convertible portion of the ovum.

One of the very frequent conversion products among lipids in synthetic and catabolic processes during embryogenesis is thought to be cholesterol. The higher average levels and the higher levels in late embryogenesis occur in conditions which produced greater oxygen consumption (Table 55, and Fig. 18). This suggests that more lipids were extracted from the yolk and directed into energy production in the embryo. Therefore, the utilization of lipids appears to be the key to growth and availability of energy for physiologic activities.

Although it is commonplace to state that specific conditions produce or cause certain organismic processes of development, including growth, the kinetics are neither direct nor simple and the more so when the organism lives continuously in a relatively unstable environment. In addition to the all pervading tonic influence of environmental temperature on every level or organization in these salmon embryos, they were also regulated in their metabolic performance by various states of

TABLE 55

Average cholesterol content (mg/g) of ova, weighted mean embryo weight (mg) within stages 1-18, cholesterol (mg/g) in embryo, embryo weight (mg) at stage 18, 3'5'cAMP-ase activity/mg, and lipid content of embryo (mg/g) of Atlantic salmon embryos at stage 18 at various temperatures and levels of dissolved oxygen (% air-saturation).

Component	5 C			10 C		
	100%	50%	30%	100%	50%	30%
Cholesterol (mg/g)	1.43	1.33	1.12	0.97	0.68	0.58
Weighted mean weight	6.9	5.8	3.5	5.4	4.9	3.9
Embryo Cholesterol	4.98	2.70	2.30	1.30	0.85	0.70
Embryo weight, stage 18	22.1	26.6	11.9	14.5	12.5	10.0
3'5'cAMP-ase (ovum)	125.0	100.0	33.0	198.0	150.0	110.0
Total lipid (embryo)	98.9	115.0	160.0	75.2	86.4	105.0

respiratory capacity imposed by the degree of availablity of oxygen.

It has been possible to relate these developmental processes to metabolic performance modified by these external conditions on the one hand, and to variations in certain "intrinsic control mechanisms", primarily enzymes, which have also been shown to be influenced in quantity and activity by these same environmental conditions. Thus, it becomes a reasonable notion to see a route in which the metabolic delivery of energy and materials also governs these intrinsic control mechanisms which in turn regulate the mobilization of molecules of structural synthesis and energy production, the gross expression of which is manifest in the form, size, and state of complexity of the developing embryos in equivalent units of time.

Among the well known control mechanisms for which quantitative analyses have been developed with some perfection are, DNA, RNA, and two forms of nucleotides (2'3'cAMP and 3'5'cAMP). The production of DNA which directly controls the production of the protein regulator, RNA, has been demonstrated to be greater in embryos having greater oxygen consumption and these are the embryos which developed in higher levels of oxygen and lower temperature (Table 56, Fig. 22). These are, also, the embryos which attained the largest average length and weight with minimum oxidative utilization of yolk proteins (Table 57).

The cyclic nucleotides which were quantitatively determined only indirectly by the assay of activities of their respective hydrolyzing enzymes (3.1.3 I.E.C.) also increased concomitantly with respiratory performance as shown in Table 55. The higher inferred level of cAMP impeded synthesis of lipids and enhanced the metabolic utilization of existing lipids within a temperature level, with the result that more

TABLE 56

Average DNA content (mg/g) of embryo, total oxygen consumption, mean oxygen consumption quotient (K_{O2}), mean DNA quotient (K_{DNA}) and Q_{10} values for ovum at stages 1-18 of Atlantic salmon embryos at various temperatures and levels of dissolved oxygen (% air-saturation).

	5 C			10 C		
	100%	50%	30%	100%	50%	30%
DNA (mg/g)	3.5	3.3	3.0	3.1	3.0	2.9
Total oxygen (gm)	139.4	129.6	89.3	145.0	112.8	99.4
K_{O2}	1.2	0.96	0.47	1.92	1.20	0.72
K_{DNA}	0.34	0.29	0.15	0.61	0.40	0.24
$Q_{10}{}^{O}{}_{2}$	-	-	-	3.2	2.5	3.1
Q_{10} DNA	-	-	-	3.6	2.6	3.2

Figure 22

Relation of standardized total oxygen consumption (mg/g/ovum) and standardized mean DNA content (mg/g-embryo) in embryos of Atlantic salmon incubated at two temperatures and three levels of dissolved oxygen during the interval from fertilization to stage 18.

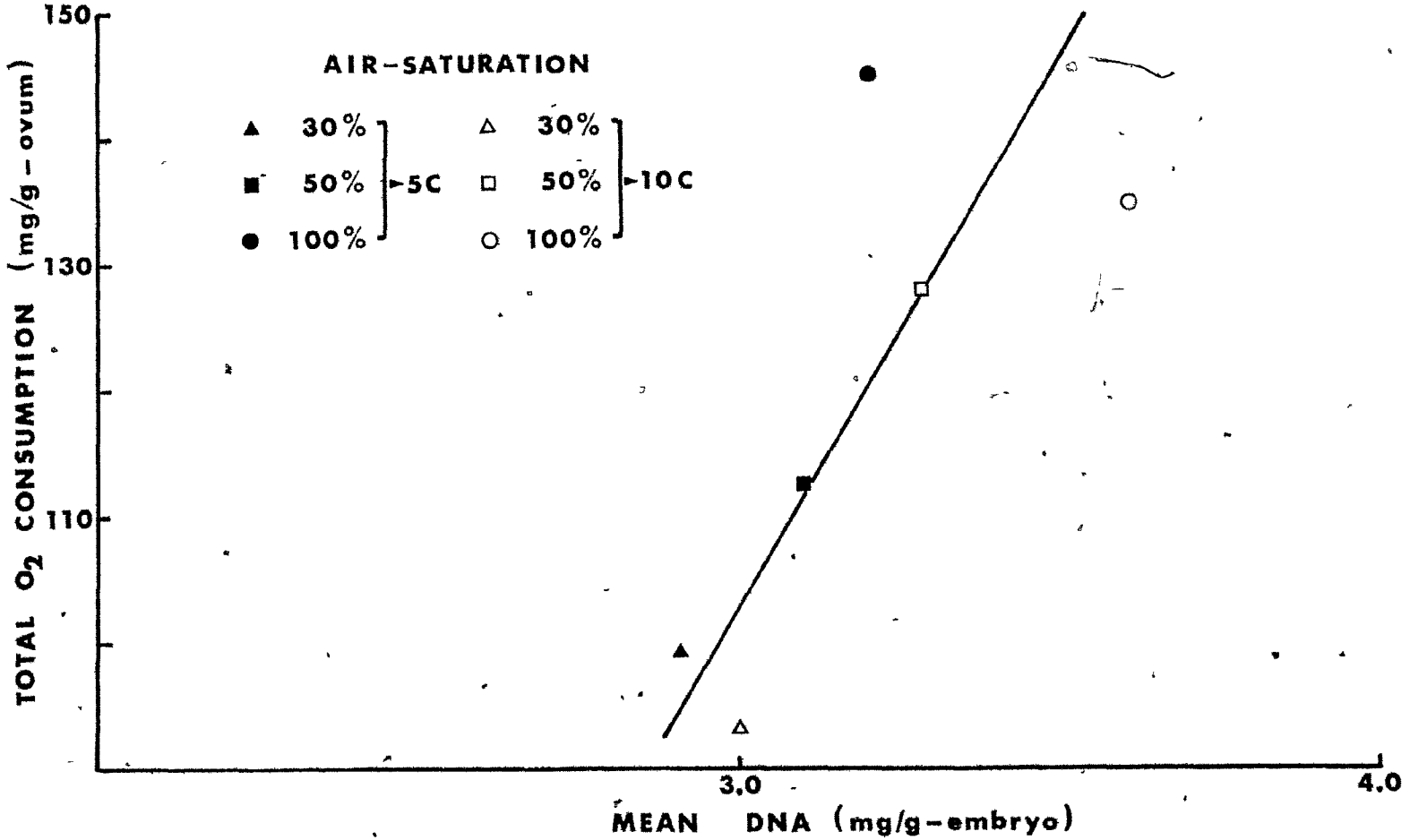

AIR-SATURATION
30%
50%
100%
5C
30%
50%
100%
10C
150
130
110
TOTAL O2 CONSUMPTION (mg/g-ovum)
3.0
4.0
MEAN DNA (mg/g-embryo)

TABLE 57

Energy consumption of Salmo salar embryo from lipid and from protein between stages 1-18 in percent of caloric values and total calorie consumption at various temperatures and levels of dissolved oxygen (% air-saturation).

	5 C			10 C		
	100%	50%	30%	100%	50%	30%
Lipid	86	82	63	80	74	64
Protein	14	18	27	20	26	36
Calories	742	758	910	715	709	774

protein was available for tissue synthesis (Fig. 23).

The nucleotide 2'3' cAMP which is thought to be involved in the regulation of protein synthesis varied directly with total oxygen consumption and therefore, with greater body growth. Indeed, 2'3' cAMP was present in concentrations which were in direct relation to protein levels in advanced embryos.

In addition to growth, and developmental rate measured by the progressive differentiation of observable structures, the number of vertebrae which was differentiated was also directly related to the amount of RNA and indirectly to the amount of DNA (Table 58, Fig. 24). Although it would seem that the basis for the numerical determination of seriated parts in these embryos by temperature and oxygen supply has been demonstrated, it does not account for records in which vertebral number varied directly with temperature (summaries, Garside 1966b, and Lindsey and Harrington 1972). The nature of the relation between RNA and vertebral number in other species, especially non-salmonid species, remains to be demonstrated to determine to what extent the phenomenon might be species-specific.

All of the statistically significant correlations which were determined between various pairs of measures in this study have been assembled in a chart to illustrate the interrelations indicated by the various responses (Figure 25). Control of respiratory performance by temperature, and oxygen consumption as a function of supply, regulates the formation of intrinsic control mechanisms such as nucleic acids and cyclic nucleotides and these mediate the utilization of yolk energy and material supplies so that different conversion efficiencies result.

TABLE 58

Vertebral number, average RNA/DNA ratio between stages 4-16 (time for vertebral differentiation, Garside 1966b) of Atlantic salmon (Salmo salar) at various temperatures and levels of dissolved oxygen (% air-saturation).

	5 C			10 C		
	100%	50%	30%	100%	50%	30%
Vertebral number	57.4	56.6	56.3	55.2	53.9	52.6
RNA/DNA	7.1	6.0	5.6	6.8	5.3	3.8

Figure 23

Relation of standardized total lipids (mg/g) to 3'5' cAMP-ase activity in ova of Atlantic salmon at stage 18, incubated at two levels of temperature and three levels of dissolved oxygen (% air-saturation).

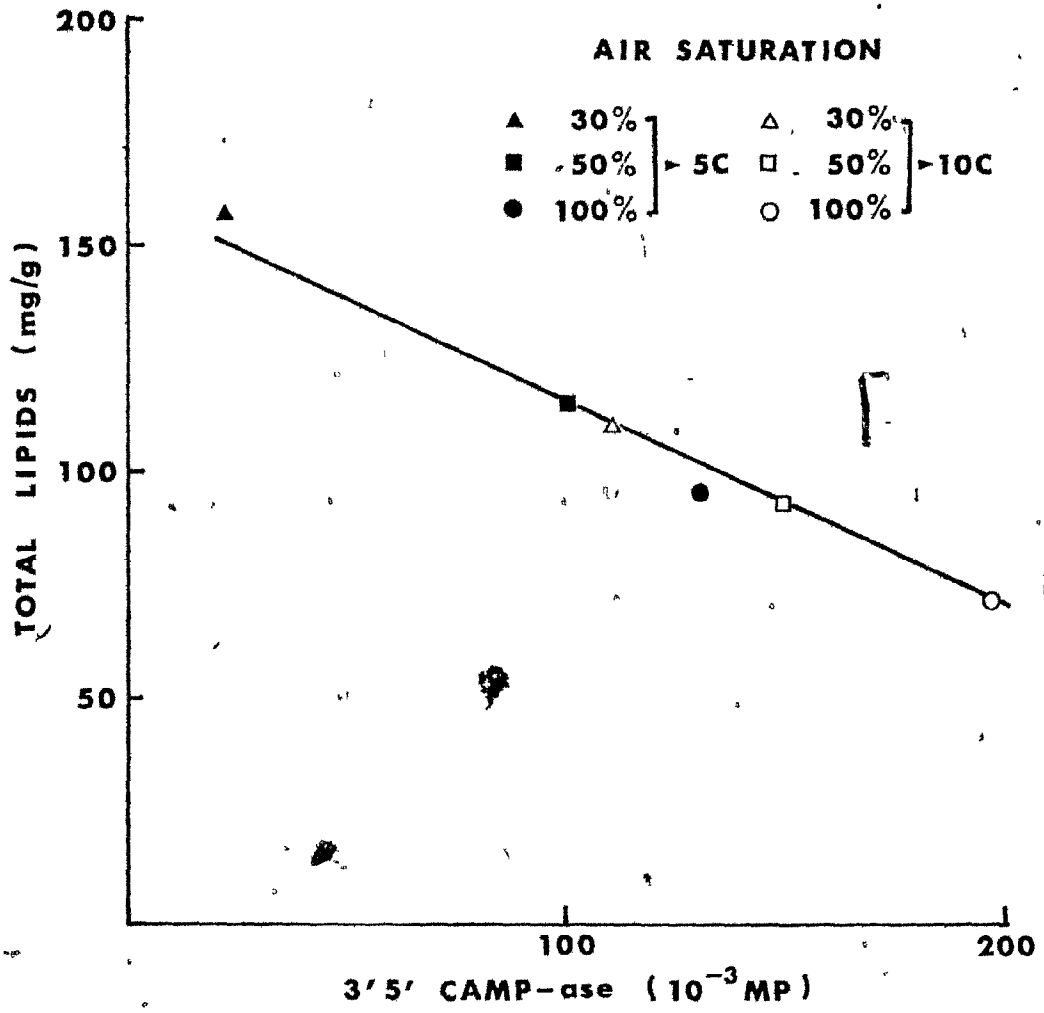

AIR SATURATION
▲ 30%
■ 50%
● 100%
5C
△ 30%
□ 50%
○ 100%
10C
TOTAL LIPIDS (mg/g)
200
150
100
50
100
200
3'5' CAMP-ase (10⁻³MP)

Figure 24

Relation of number of vertebrae to mean value of RNA/DNA ratio during the assumed period of vertebral determination (stages 4-16) in Atlantic salmon embryos incubated at two temperatures and three levels of dissolved oxygen (% air-saturation).

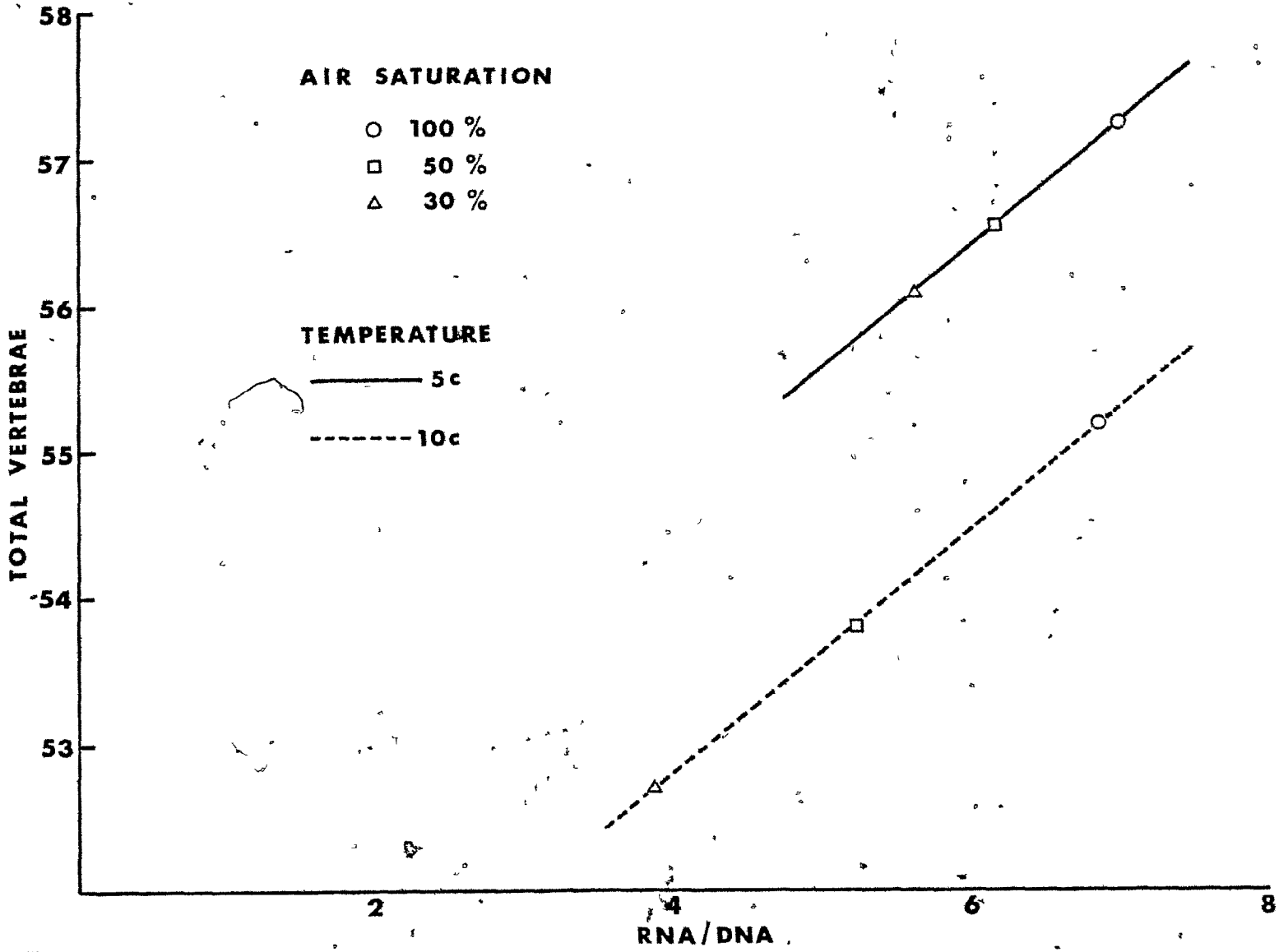
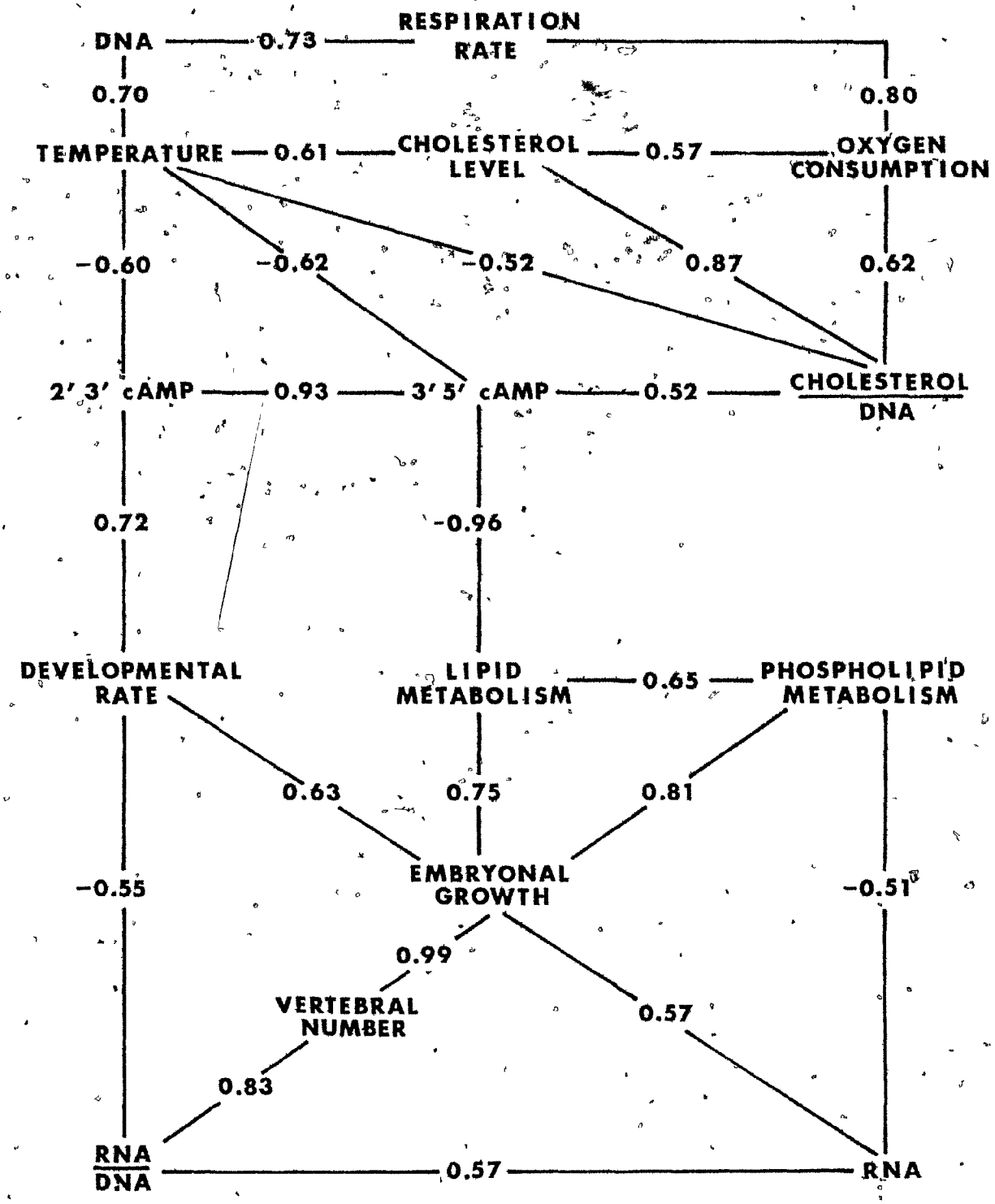

AIR SATURATION
100 %
50 %
30 %
TEMPERATURE
5c
10c
TOTAL VERTEBRAE
58
57
56
55
54
53
2
4
6
8
RNA/DNA

Figure 25

Summary of all significant correlation coefficients calculated between potentially related pairs of data sets from the respiratory, chemical, growth, and differentiational studies on embryogenesis of Atlantic salmon incubated at 5 and 10 C and 30, 50 and 100% air-saturation.


DNA
0.73
RESPIRATION RATE
0.70
0.80
TEMPERATURE
0.61
CHOLESTEROL LEVEL
0.57
OXYGEN CONSUMPTION
−0.60
−0.62
−0.52
0.87
0.62
2′ 3′ cAMP
0.93
3′ 5′ cAMP
0.52
CHOLESTEROL / DNA
0.72
−0.96
DEVELOPMENTAL RATE
LIPID METABOLISM
0.65
PHOSPHOLIPID METABOLISM
0.63
0.75
0.81
EMBRYONAL GROWTH
−0.55
−0.51
0.99
VERTEBRAL NUMBER
0.57
0.83
RNA / DNA
0.57
RNA

These differences result from greater energy cost for physiological maintenance at higher temperature, a stress which is compounded by greater oxidation of the critical protein supply relative to lipid supply, at a phase in the life cycle at which the protein supply is finite and is most urgently required for tissue synthesis.

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APPENDIX I

PEROXISOME-LIKE VESICLES AND OXIDATIVE ACTIVITY IN THE ZONA RADIATA AND YOLK OF THE OVUM OF THE ATLANTIC SALMON

INTRODUCTION

In the course of respirometric procedures on embryos of Atlantic salmon (Salmo salar L.) we were able to detect oxygen utilization by isolated zona radiata and by isolated yolk. The hypothesis that oxidative enzymes were present was tested histochemically. Peroxisome-like microvesicles were demonstrated both in zona radiata and yolk, indicating the presence of such enzymes.

MATERIALS AND METHODS

Freshly fertilized ova of Atlantic salmon were obtained from the Canada Department of Environment fish culture station, Collingwood, Nova Scotia in November 1970 and again in 1971. During the progress of incubation at various relatively constant levels of dissolved oxygen (30, 40, 50 and 100% of air-saturation) at 5 and 10 C, subsamples were removed for respirometry at a later stage of development (stage 16, Garside 1959).

Zona radiata for isolate respirometry were removed from ova in accordance with the procedure of MacKelvie (1971). They were washed in distilled water and then in 0.9% NaCl solution to clean the inner surface and produce the "ghost shell" described by Bell et al. (1969). Oxygen consumption by the radiate membranes was measured in Warburg manometric respirometers following the procedure of Legname (1968). Penicillin was administered at the rate of 500 I. U./ml of medium in some of the later tests to estimate potential errors resulting from bacterial respiration. Oxygen uptake was determined at 5 and 10 C for various intervals from 3 to 16 hr. An alternative method for the measurement of oxygen utilization of radiata membranes was effected with a simple continuous-flow chamber consisting of a glass tube 100 X 7 mm I. D., closed at each end by a rubber stopper through which a 3 mm vinyl tube was inserted and sealed. The incurrent tube was fitted with a T-coupling to establish a bypass flow. Ten complete radiate membranes were placed in a chamber and air-saturated water at 5 C was passed through the chamber for 24 hr. Some of the incurrent water was diverted from passage through the chamber via the bypass. Collections in triplicate of 5-ml samples of incurrent and excurrent water were made at the beginning and completion of a test and at some intermediate time. Rate of flow was calculated from the time required to fill a 50-ml sample bottle

with the excurrent water. Flow was generally about 3 ml/min. Oxygen extraction was determined from the difference between dissolved levels in the incurrent and excurrent flows. Level of dissolved oxygen was determined by standard Winkler semimicrotitrimetry (Harper, 1953). A blank chamber was also used during each test to confirm results. A variant procedure consisted of exposure of four complete membranes for 24 hr in a chamber containing a static volume of initially air-saturated water maintained at 5 C. A blank chamber was also used in this test.

Oxygen uptake by isolated yolk was determined only by the Warburg manometric technique.

Histochemical analyses of parts of these ova were performed on samples collected at Stage 16 (Garside, 1959). Several ova were placed under acetone and stored at -75C for approximately one month. Subsequently, zona radiata were excised and yolks were separated from the embryos. Cryostatic sections, 6 μ thick of zona radiata, yolk, and the kidney region of embryos were mounted on large glass coverslips in the cryostat maintained at 5C. These sections, after air-drying were flooded with ice-chilled acetone for 15 min. and then rinsed in 0.15M NaCl. Then, three sections of each of the three types were flooded with one of the seven incubating media which are listed in Table 1 (Results Section). Incubation in these media was continued for 1 hr at 5C. (Graham and Karnovsky, 1965). Following incubation the preparations were washed in 0.15M NaCl, fixed in 10% formalin, covered with glycerine gel mounting medium and coverslips for microscopical examination of colour development.

RESULTS

Oxygen was utilized by zona radiata in both Warburg and continuous-flow respirometers and by yolk in Warburg respirometers (Table 1). Ranges of values are extensive but obviously much greater than the manometric and titrimetric errors in the blank standards. In those Warburg tests in which penicillin was added as a bacteriostat, oxygen uptake remained within the range of values of the remaining Warburg tests.

Results of the histochemical procedure indicate the presence of vesicles within the zona radiata and yolk which react with certain of the incubation media in an identical manner to the peroxisomes (microbodies) described by Graham and Karnovsky (1965) in the liver of rat and Guinea pig (Table 2). These stained vesicles are illustrated in the cryostatic sections in Fig. 1. Sections of embryonal kidney tissue also shown in Fig. 1 did not develop pink bodies in any of the incubating media. However, kidney tissue did not retain its structural integrity in these treatments.

TABLE 1

Mean values of uptake of dissolved oxygen by isolated zona radiata and isolated yolk of fertilized ova of Atlantic salmon (Salmo salar L.) in Warburg and continuous-flow respirometers. Each value represents three to five trials. Yolks were not tested in continuous-flow chambers.

	Procedure			Zona Radiata	Yolk
Respirometer	Temp. C	Time hr	Flow ml/min	Mean oxygen uptake µg/g/hr	Mean oxygen uptake µg/g/hr
Warburg	5	3	-	33.9	96.7
respirometer	5	6	-	25.3	74.4
	5	16	-	25.4	25.2
	10	3	-	47.9	104.6
	10	6	-	122.5	33.3
	10	16	-	66.6	28.3
Warburg respirometer	10	3	-	97.7	68.5
with 500 I.U. penicillin/ml	10	3	-	77.3	54.5

continued...

TABLE 1 (CONTINUED)

Procedure				Zona Radiata	Yolk
Respirometer	Temp. C	Time hr	Flow ml/min	Mean oxygen uptake µg/g/hr	Mean oxygen uptake µg/g/hr
Continuous-flow respirometer	5.1	8	3.1	130.0	
	5.1	16	3.1	120.0	
	5.1	24	3.1	240.0	
	4.6	24	3.1	15.4	
Continuous-flow blank tube	5.1	8	3.1	2.9	
	5.1	16	3.1	4.2	
	5.1	24	3.1	1.7	
	4.6	24	3.1	0.0	

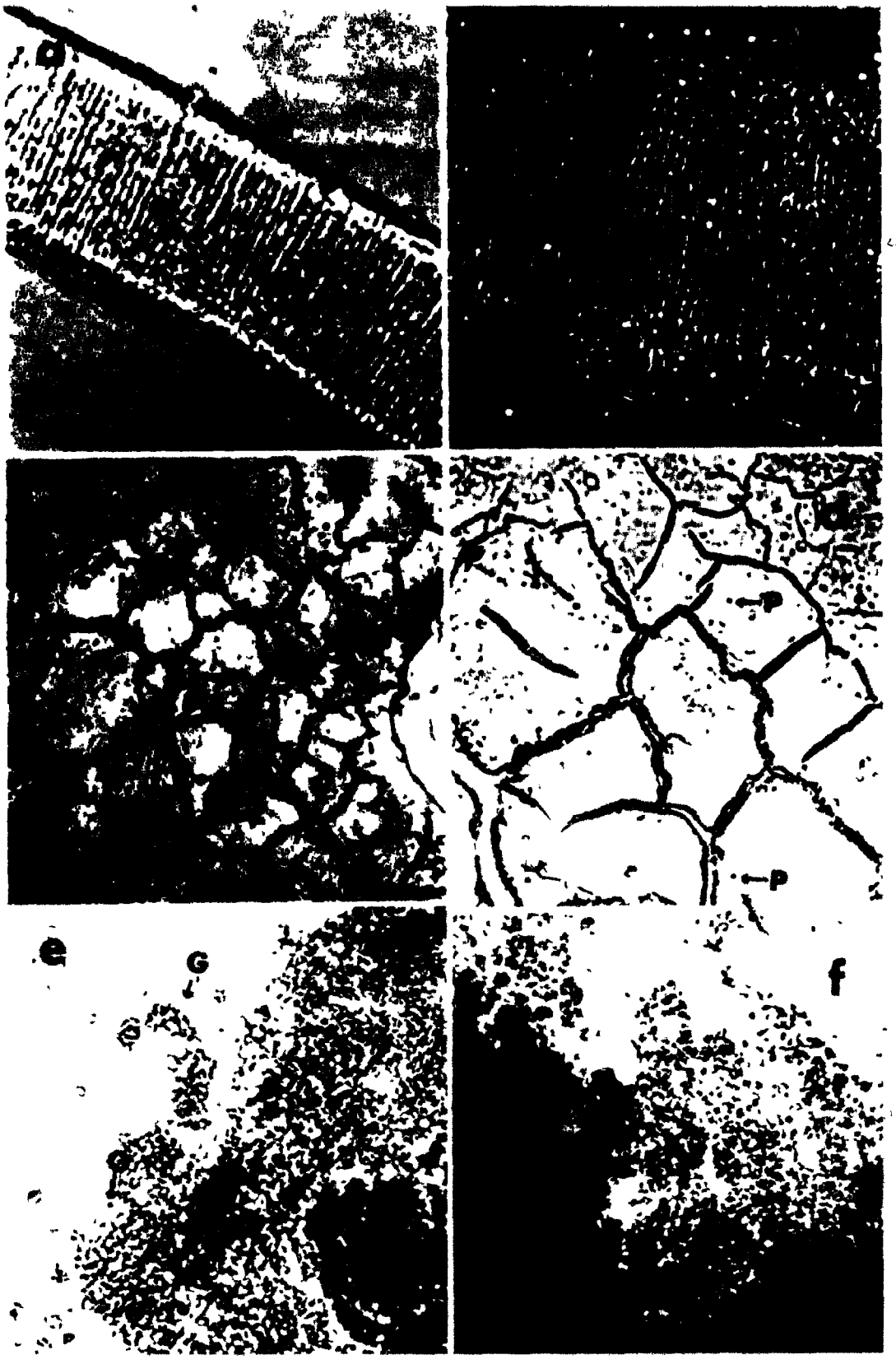
TABLE 2

Summary of results of exposure of zona radiata, yolk and embryonal kidney of ova of Atlantic salmon (Salmo salar L.) to seven histochemically active incubation media (Graham and Karnovsky 1965). Development of pink colour in microvesicles indicates the presence of oxidative enzymes. Asterisks(*) indicate the degree of development of pink colour. Open circles (o) indicate green vesicles and bars (-) indicate absence of colour development,

Constituents of Incubation Media	Composition of Incubation Media and Histochemical Reactions						
	A 1	A 2	A 3	A 4	A 5	A 6	A 7
NaFeCN						+	
2,6,8, trichloropurine							+
Tris - buffer	+	+	+	+	+	+	+
ethylenediaminetetradcetic acid(EDTA)	+	+	+	+	+	+	+
3, amino-9-ethylcarbazole		+	+	+	+	+	+
5,6,7,8, tetrahydro-1-naphtalamine	+		+	+	+	+	+
sodium urate	+	+		+	+	+	+
horseradish peroxidase	+	+	+		+	+	
Structure	o	*	-	**	***	-	-
zona radiata	o	*	-	**	***	-	-
yolk	-	*	-	**	***	-	-
embryonal kidney	o	-	-	-	-	-	-

FIGURE 1

Cryostatic sections of parts of fertilized ovum of Atlantic salmon (Salmo salar L.). (a) Radial section of zona radiata, X500. (b) Radial section of zona radiata, X740. Numerous larger unstained spheroid structures are probably lipid droplets. (c) Yolk, X500. (d) Yolk, X740. (e,f) Embryonal kidney, X740. Legend; A = outer surface of zona radiata, V = pore canal of zona radiata, P = pink microvesicle G = green microvesicle, Y = yolk platelet. Photomicrographs were reproduced from Kodak Tri - X Pan film. Marker is 10 μ on (a) and (c) and 7 μ on (b), (d), (e) and (f).


d
P
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f

DISCUSSION

This is apparently the first documentation of intramembranal oxygen utilization in the zona radiata and yolk of an osteichthyid fish. The presence also of peroxisome-like vesicles leads to the hypothesis that some oxidative metabolism occurs in both zona radiata and yolk.

The interim identification of the vesicles as peroxisomes is based on the description of microbodies given by Graham and Karnovsky (1965). At least some oxidative enzymes such as peroxidase and uricase convert 3 - amino - 9 - ethylcarbazole to an insoluble red compound. Since an insoluble pink compound was generated within the vesicles of radiate membrane and yolk incubated in 3 - amino - 9 - ethylcarbazole in which no enzyme inhibitors were included, we assume that we have observed the same reaction. Colour development by the oxidation product of 3 - amino - 9 - ethylcarbazole was enhanced in those media containing 5, 6, 7, 8, tetrahydro - 1 - napthylamine when inhibitors were not present. Such was also reported by Graham and Karnovsky (1965). Vesicles were stained green in those incubating media in which 3 - amino - 9 - ethylcarbazole was omitted or in which enzyme inhibitors such as NaFeCN both zona radiata and yolk developed a diffuse pale pink colouration except in the vesicles which were stained green. This also parallels colour development in cytoplasm surrounding the microbodies in mammalian liver cells (Graham and Karnovsky 1965).

Heretofore, the zona radiata of osteichthyid ova has been considered to have been composed essentially of protein namely ichthulokeratin (Bell et al. 1969). However, these authors, after failing to degrade completely the zona radiata with proteolytic agents, speculated that mucopolysaccharides were also present. We have analysed the composition of the radiate membrane somewhat more intensively (unpublished data) by thin-layer chromatography and spectrophotometry and we have demonstrated the presence of phospholipids, cholesterol, DNA and RNA in addition to the structural protein and the

enzyme bearing microbodies. Phospholipids and phospholipase have been demonstrated in the stratum corneum of mammalian (bovine) epidermis by Long and Yardley (1972), which may also be indicative of some biodynmic process.

In view of the nature and variety of components in the zona radiata, oxidative enzymes, oxidizable substrate and oxygen uptake in isolation from other parts of the ovum, we hold the opinion that this membrane is more dynamic than previous reporters have allowed. If the zona has such dynamic properties then there can be important consequences in surface to volume ratios for developing embryos particularly in respiratory exchanges and osmoregulation.

APPENDIX II

QUALITATIVE AND QUANTITATIVE COMPOSITION, AND AN INFERRED MODEL, OF THE ZONA RADIATA OF THE OVUM OF THE ATLANTIC SALMON

INTRODUCTION

As a result of the respirometric and histochemical study of the zona radiata of the fertilized ovum of Atlantic salmon Salmo salar L. by Hamor and Garside (1973) a more searching analysis of the qualitative and quantitative composition has been undertaken to extend insight into the biodynamic potential of this encapsulated structure.

MATERIALS AND METHODS

Zonae radiatae were collected from samples of fertilized eva of the Atlantic salmon in early and late stages of embryonal development (Stages 1 and 16, Garside 1959) during incubation in air-saturated water at 5 and 10 C. Quantitative values were determined for phospholipids, phospholipid phosphorus, cholesterol, total lipids, RNA, DNA, total protein, non-protein nitrogen, total carbohydrate, glucose, ash and water. Additionally, the dry weight water, content, quantities of phospholipid phosphorus, and total lipids were determined for zonae from stages 1 and 16, incubated at levels of dissolved oxygen approximating 30 and 50% air-saturation at 5 and 10 C.

The "wet" weight was determined by cleaning and drying a freshly dissected zona radiata on blotting paper before placing on a semimicrobalance according to the procedure of Bell et al. (1969).

Various analytical procedures included dessication, incineration, gravimetry, spectrophotometry and thin-layer chromatography (TLC).

Water content was measured by gravimetric differences following drying at 105 C to constant weight which generally occurred in approximately 24 hours.

Inorganic solids were determined for 25 zonae radiatae by gravimetric differences following incineration of previously dried zonae in an electric muffle furnace at 600 C for 30 min. (Anonymous 1960).

Zonae radiatae were prepared for various lipid evaluations by chloroform-methanal (2:1) extraction following homogenization with an electrically driven glass rod. This mixture was shaken mechanically in a 0.05% aqueous solution of $CaCl_2$ to accumulate particles, and then divided with a separatory funnel. The supernatant lipid-solvent was

reduced in a Buchler rotary evaporator at 28 C for 6 hours. The resultant lipid complex was again dissolved in chloroform-mehtanol solution in a 5-ml volumetric flask. Samples of 0.5 ml of this solution were analyzed spectrophotometrically for phospholipid phosphorus following the procedure of Folch-Pi et al. (1957). Cholesterol was also determined spectrophotometrically by the procedures of both Hanel and Dam (1955) and Zak et al. (1954). Total lipids were determined spectrophotometrically according to the procedure of Holland and Gabbott (1971).

Qualitative and quantitative analyses of phospholipids were performed by TLC according to procedures described by Mezei and Ambrose (1970) and Ambrose and Mezei (1971). These procedures yielded quantities of ortho-phosphoethanolamine (OPE), phosphatidyl ethanolamine (PE) and phosphatidyl-L-serine (PS), based on standards 17802, 21423 and 17816, respectively, of K & K Laboratories Inc. New York, when measured photometrically using the potassium ferricyanide technique described by Folin and Malmros (1929). The fraction of the material to be used for total carbohydrate determination was hydrolyzed to glucose with trichloroacetic acid. Total nitrogen was measured by the method of Folin and Wu (Oser 1965). Protein nitrogen was determined by a modified Kjeldahl procedure described by Holland and Gabbott (1971). Determination of total protein in bovine albumin was also determined by this technique as a standard (Lowry et al. 1951).

The nucleic acids were determined quantitatively by the spectrophotometric technique of Santen and Agranoff (1963). DNA was also measured by the technique of Abraham et al. (1972). In order to make a correction for interference by substances which a-sorb ultraviolet radiations, of KOH-hydrolyzed "tissue breakdown products" extract was pre-

pared as described by Santen Agranoff (1963). The difference in spectrophotometric values for unhydrolyzed and hydrolyzed substance gives the total nucleic acids present. These acids were separated by the perchloric acid procedure for spectrophotometry given by Santen and Agranoff (1963). The correct absorbancy factor for RNA was estimated to be 0.587, and 0.722 for DNA.

The major constituents were converted to their caloric equivalents for comparisons with calorimetric data presented by Fluchter and Pandian (1968). The values used in these conversions were; lipids 9450 cal/g; protein, 5860 cal/g; carbohydrate 4250 cal/g and non protein N, 4280 cal/g (Hayes 1949; Fluchter and Pandian 1968).

RESULTS

The "wet" weight of zonae radiatae of fertilized Atlantic salmon ova averaged 5.3 ± 0.40 mg (Table 1). The water content of the turgid zona radiata is approximately 91% of the total weight (Table 1). The quantity of water is constant in the lots incubated at any of the experimental combinations of dissolved oxygen and temperature at stage 1 (early blastula). There is generally an increase in water content before or during the establishment of stage 10 (dorsal fin-fold evident, Garside 1959). However, within each temperature there is a gradual decrease in water content with decreasing level of dissolved oxygen (Table 1). Since there is considerable opportunity for errors in weighing, as reflected in the relatively large standard errors about the means, the status of statistical significance has been ignored.

Total inorganic solids averaged about 1.7% of the dry weight in the few samples prepared (Table 2). However, there was probably a considerable weighing error with such minute quantities of ash.

The organic constituents of the salmon zona radiata determined spectrophotometrically and chromatographically are grossly itemized in Table 2. Their total weights combined with the ash are within a few mg/g of the dry weight determined gravimetrically.

Protein.

Protein analysis of zona radiata was completed only for ova incubated at 5 C and air-saturation (Table 2). Although there was a minor decline in the weight of dry materials during embryogenesis, the amount of protein in the dry material increased approximately 6%.

Nonprotein Nitrogen

The source of this nitrogen is unknown but it is probably vested in nitrates, nitrites and ammonia (Table 2). Their combined

TABLE 1

Fresh and dry weights (mean ± S.E.) of zona radiata of Atlantic salmon ova at early and late stages of embryogenesis. Ova were divided into six lots and incubated at three levels of dissolved oxygen and two temperatures. Mean ovum weight at beginning was 147 ± 4.0 mg.

Temp C	Embryonal Stage	30% Air-saturation			50% Air-saturation			100% Air-saturation		
		Fresh Weight	Dry Weight	Water	Fresh Weight	Dry Weight	Water	Fresh Weight	Dry Weight	Water
5	1	5.3±0.4	0.45	4.85	5.3±0.4	0.45	4.85	5.3±0.4	0.45	4.85
	16	4.6±0.3	0.35	4.25	5.1±0.3	0.36	4.73	5.2±0.4	0.34	4.85
10	1	5.3±0.4	0.45	4.85	5.3±0.4	0.45	4.85	5.3±0.4	0.45	4.85
	16	3.2±0.5	0.22	2.98	3.6±0.4	0.23	3.37	4.0±0.6	0.24	3.76

TABLE 2

Composition of the zona radiata of Atlantic salmon ova at early and late stages of embryogenesis at 5 C and air-saturation. Values are not directly additive because certain substances (*) are included in weights of other constituents.

Constituent	Stage 1		Stage 16	
	mg/g	Per Cent	mg/g	Per Cent
Water	914.0	91.4	920.0	92.0
Dry material	86.0	8.6	80.0	8.0
	Composition of dry material			
Protein	15.9	18.5	19.3	24.1
Non-protein Nitrogen	0.8	0.9	1.6	2.0
Phospholipid	1.8	2.1	-	-
Non-Polar lipid	0.4	0.5	-	-
Carbohydrate	48.0	55.8	40.1	50.1
Glucose	2.0*	2.3	1.20*	1.5
RNA	3.2*	3.7	3.75*	4.7
DNA	1.6*	1.9	1.48*	1.8
Ash	17.4	20.2	15.40	19.2

contribution constitutes a very minor amount (0.08%) of the solids in the zona radiata.

Lipids

(a) Phospholipid Phosphorus

Changes in level of phospholipid phosphorus from early to late embryogenesis are shown in Table 3. There is a decline in the quantity between stage 1 and stage 16 and between temperatures of 5 and 10 C at stage 16. There are also possible real changes among those zonae radiatae held in various levels of dissolved oxygen, but the number of determinations was insufficient for statistical testing.

(b) Cholesterol

The cholesterol content of the zona radiata increased from 7 to 20 times, between stages 1 and 16, depending on the incubation conditions. There was a decline in cholesterol content from 5 to 10 C and from high to low dissolved oxygen content of the water (Table 4).

(c) Total Lipids

Total lipid content of zona radiata from early and late stages of embryogenesis follows generally the same course as that of phospholipid phosphorus which is an indirect measure of the total phospholipid fraction. Total lipids decline with age, increasing temperature, and decreasing level of dissolved oxygen (Table 5).

(d) Phospholipids

The qualitative yield from TLC determinations contained three components, ortho-phosphoethanolamine (OPE), phosphatidyl ethanolamine (PE) and phosphatidyl-L-serine (PS). The quantities determined from ova in stage 1 incubated at 5 C and 100% air-saturation are indicated in Table 6.

TABLE 3

Phospholipid phosphorus content (µg/g) of zona radiata of Atlantic salmon ova at early and late stages of embryogenesis. Ova were divided into six lots and incubated at three levels of dissolved oxygen and two temperatures.

Temp C	Embryonal Stage	30% Air-saturation	50% Air-saturation	100% Air-saturation
5	1	24.0	24.0	24.0
	16	18.8	20.5	27.5
10	1	24.0	24.0	24.0
	16	16.7	18.5	20.0

TABLE 4

Cholesterol content (µg/g) of zona radiata of Atlantic salmon ova at early and late stages of embryogenesis. Ova were divided into six lots and incubated at three levels of dissolved oxygen and two temperatures.

Temp C	Embryonal Stage	30% Air-saturation	50% Air-saturation	100% Air-saturation
5	1	0.03	0.03	0.03
	16	0.35	0.55	0.85
10	1	0.03	0.03	0.03
	16	0.20	0.40	0.66

TABLE 5

Total lipids (mg/g) of zona radiata of Atlantic salmon ova at early and late stages of embryogenesis. Ova were divided into six lots and incubated at three levels of dissolved oxygen and two temperatures.

Temp C	Embryonal Stage	30% Air-saturation	50% Air-saturation	100% Air-saturation
5	1	2.2	2.2	2.2
	16	1.58	1.68	2.3
10	1	2.2	2.2	2.2
	16	1.40	1.50	1.67

TABLE 6

Phospholipids in zona radiata of Atlantic salmon ovum, following fertilization (Stage 1).

Phospholipid	Dry Weight mg/g	Dry Weight (%)
O-phosphoethanolamine (@PE)	0.70	39
phosphatidylethanolamine (PE)	0.03	1
phosphatidyl-L-serine (PS)	1.08	60
Total	1.81	100

TABLE 7

Caloric equivalents of quantities of major constituents in zona radiata of Atlantic salmon ovum, showing decrease in dry material and increase in caloric value with increasing duration of embryogenesis.

Constituent	Cal/mg	Stage 1		Stage 16	
		mg/g	cal/g	mg/g	cal/g
Protein	5.86	15.9	93	19.3	113
Carbohydrate	4.25	48.0	204	40.1	170
Total lipid	9.45	2.2	21	2.3	22.0
Non-protein N	4.2%	0.8	3	1.6	6.8
Ash	-	17.4	-	15.4	-
Total	-	-	321	-	321
Dry Material	-	84.3	3807	78.7	3964

Nucleic Acids

Measures of RNA and DNA are given in Table 2. RNA and DNA are present in ratios of approximately 2:1 when embryos are in both early and late stages of development.

Caloric Conversion

Caloric values of protein, carbohydrates, lipids and non-protein nitrogenous compounds per gram normal and dry zona radiata during early and late stages of embryogeny are given in Table 7. There is a decline in total caloric value of the older normal zona radiata but there is an increase in the total calories of the dry substance in the older zona radiata.

DISCUSSION

Composition

Previous attempts to define the composition of the zona radiata of a typical fish ovum have not been particularly instructive perhaps because of the state of analytical procedure during a period of earlier interest 25 years ago. Young and Smith (1956) described quantitatively and qualitatively the amino acid composition of the zona radiata of salmon. However, they did not extend their analysis to consider the presence of other substances. Bell et al. (1969), after failing to achieve dissociation of the zona radiata with pepsin, succeeded with trypsin, and concluded that mucopolysaccharides were also present, but again they have not pursued the analysis further. They applied the term ichthylokeratin to this composite structure.

We have extended the scope of information concerning the qualitative and quantitative composition of the zona radiata of the Atlantic salmon which is probably typical for many if not most osteichthyid fishes.

The high water content, 92% of total weight is perhaps somewhat surprising in view of the tensile strength of zona radiata (Zotin 1958). This water is probably largely attached to the proteins as bound water ([illegible] 1962). Since bound water contains no solutes the minerals (ash) must also be linked to protein molecules or be in some other form. The mineralization of the zona radiata has not been reported previously. Hayes et al. (1946) determined the principal cations in the yolk and embryo of Atlantic salmon but apparently ignored the content of zona radiata. The presence of structural carbohydrates in excess of 50% of the dry weight suggests that they are polysaccharides.

Other compounds, lipids, phospholipids, glucose and nucleic acids together with some of the protein almost certainly must be con-

stituents of a membranous lining of the radial canals of the zona radiata, a subject which we shall discuss later.

We have noted changes in the related content of several of the constituents of the zona radiata from early to late stages of embryogenesis. (Table 2-5). In the main, the constituents remain relatively stable but there are certain shifts in lipoids to be noted. Total lipids and the phospholipid fraction decline during embryogenesis (Table 3,5), but the cholesterol level rises. Probably the lipids generally and phospholipids particularly are oxidized in the metabolic processes of the membrane lining the zona radiata. Cholesterol probably increases as a result of progressive leakage from the yolk through the vitelline membrane.

The caloric value of dry zona radiata measured in a calorimeter was 4187 cal/g (Fluchter and Pandian 1968) showing good agreement with our estimation from chemical analysis which is 3807 cal/g. However, methods of dissecting cleaning and chemical analyses might cause alterations in the measured amounts in these studies. Our data (Table 7) indicate a slight increase in caloric content of dry material from early to late stages of development. This appears to result from some loss of low-calorie carbohydrates, probably by oxidation, and a concomitant increase per unit in lipids and proteins.

Structure

The mature salmon ovum when transferred from peritoneal fluid to water absorbs water for several hours until the zona radiata becomes distended spherically by the imbided water, a process termed "hardening" by fish-culturists. The resultant liquid beneath the zona radiata which is said to receive colloidal substances from the yolk is termed "perivitelline fluid". Water increase is approximately 30% of the flaccid weight

(Hayes and Armstrong 1942). Perivitelline fluid is 22% of the hardened weight (Potts and Rudy 1968), a value confirmed elsewhere in the present study. Imbibition is complete in about 8 hr. (Potts and Rudy 1968) and the development of turgidity reaches a maximum in approximately 5 days in salmon and trout (Zotin 1958).

Since the zona radiata is permeable to water for some period it may serve as the prime osmoregulative surface during embryogenesis. Opinions in the literature differ in the extent to which osmoregulation is performed (McCay et al. 1936, Hayes and Armstrong 1942, Hayes 1942, Phillips et al. 1962, Ogino and Yasuda 1962, Zotin 1964, Rudy and Potts 1969, Potts and Rudy 1969).

Heretofore, the mature zona radiata has been described as a tunic of resilient ichthylokeratin perforated by many radially oriented pore canals. We infer from data presented for brook trout Salvelinus fontinalis by Hurley and Fisher (1966) that the diameter of these canals approaches 0.4 μ. Canals of this diameter can pass water particles of considerable size without regulation unless they are contractile, an unlikely process, or unless they contain some form of differentially permeable lining. Membranous microvilli which could conceivably function as such a lining have been demonstrated by Hurley and Fisher (1966).

Electronmicrography of oogenesis in brook trout by Hurley and Fisher (1966) reveals that prior to the formation of the zona radiata the ooplasm becomes extended into numerous tubules many of which grow radially through the forming zona pellucida and into contact with the follicular cells. Subsequently, the substance of the zona radiata unidentified by them as to source, begins to condense over the ooplasm among the radial microvilli until they are encased and canals have been formed in consequence.

Many of the microvilli regress in greater or lesser amounts during oogenesis and the pore canals begin to fill and become obliterated. Hurley and Fisher (1966) estimated an ultimate depletion of about 90% of the maximum number of canals. At ovulation, the remaining microvilli must be sheared from connections with the zona pellucida and if the open distal ends became sealed subsequently, a continuous membrane of the original ooplasm would obtain. When imbibition of water occurs the perivitelline space increases in radius as the zona radiata expands. The result of this separation of the zona radiata and the perivitelline ooplasmic membrane must sever the microvilli from the parent membrane, at their bases. We hypothesize then that the remanents of the microvillar membranes also seal at their proximal ends and remain in the radial canals as elongate sacs which plug the canals. The results of our qualitative analysis of the structural materials amplify this interpretation. In particular, the presence of RNA,DNA, phospholipids, protein, and microsomes containing oxidative enzyme (Hamor and Garside 1973) suggest a biodynamic complex rather than merely an insert shell of keratinoid substance. The microsomes which were observed in the zona radiata appeared to be lodged in pore canals rather than in the intervening canal walls, an indication that microvilli were still present and active even at a late stage of embryogenesis." These membranes would then provide the necessary surface within the pore canals to account for regulated osmotic exchanges and also, of course, a satisfactory surface for diffusion of respiratory gases.

There is no comprehensive hypothesis known to us to unify the foregoing information into a structural model of the zona radiata. Hurley and Fisher (1966) noted a striated pattern on the walls of pore canals which they suggested could have resulted from a spiral ribbing on the

canal surface. Our hypothesis is that this spiral ribbing is composed of chains of α keratin, (Lehninger 1970) necessarily formed as coils about the pre-existing microvilli. The interstitial substance surrounding these coils is largely a matrix of polysaccharides, or perhaps mucopolysaccharides. Indeed, about 56% of the dehydrated substance of the zona radiata is carbohydrate. Loosely bonded water molecules probably saturate the interstices of this complex matrix since water forms 91% of the wet weight of the freshly excised surface-dried zona radiata. This seemingly high level of water suggests that at least some of it has been acquired from the environment following ovulation. The remainder is probably distributed among the zona radiata, microvilli and ooplasmic membrane, vitelline membrane, and yolk.

Calcium ions have been indicated as an essential component in the hardening process (Warren et al. 1947, Zotin 1958, Ohtsuka 1960, and Fisher 1963). Fisher (1963) has postulated a model of protein polymerization in which each protein molecule is linked with two calcium ions. Such bonding of calcium perhaps accounts for the relatively large quantity of ash in our analysis. Fisher, reflecting on the work of Ohtsuka (1960), suggested that the polymerization reaction could be related to an oxidation process as suggested by Ohtsuka.

Since fibrous proteins have been implicated, if not demonstrated, in the structure of the zona radiata, we are intrigued by the analogy which this hardening process poses with the oft reported but incompletely described coagulation of vertebrate blood. Fisher (1963) has described several compounds which inhibit hardening of the zona radiata and these are also recognized anticoagulants of blood. Zotin (1958) postulated the existence of a hardening enzyme supposedly released by platelets (alveoli or granules) of vaguely described origin and apparently inspired by a report

of Yamamoto (1947) of enzyme-producing cortical platelets in an ill-defined region proximal to the zona radiata in the medaka (Oryzias latipes). Ohtsuka (1960) stated that the hardening process is established by oxidative processes in the zona radiata of the medaka. We have not been able to determine whether these particles are considered to reside in the perivitelline space or within the vitelline membrane. Such structures are not evident in either the electromicrographs or the description given by Hurley and Fisher (1966) for brook trout ova and we are not satisfied that they exist generally in salmonid ova unless they are, in fact, the microsomes which we have illustrated within the membraneous microvilli (Hamor and Garside 1973).

Since calcium ions are generally considered to be a cofactor to the activator enzyme which causes the conversion of prothrombin to thrombin in blood clotting (Guyton 1971), possibly then, in a parallel way, these ions acquired mostly from the maternal ovary (Fisher 1963) coact with an enzyme from the microvillar membrane to alter the protein fibers of the zona radiata and create a tough keratinoid tunic.

Recognition that respiratory and osmoregulative exchanges do not occur over the surface of the zona radiata generally but rather, are most probably restricted to the microvillar surfaces within the pore canals suggests that surface/volume relations remain no less critical for the embryo. Although the diameters of pore canals may vary interspecifically and larger ova may have canals of larger diameter it remains, nevertheless, that as the diameter of an ovum increases the absolute numerical increases in pore canals decreases relatively so that even more critical respiratory responses would be elicited as the respiring mass within the zona radiata increased in volume. This would explain the requirement of salmonid embryos for well aerated water during embryogenesis (Alderdice et al. 1958;

Garside 1959, 1966). Salmonid embryos also require relatively cool water, in the main less than 10 C, for a high fraction of normally developed embyros at hatching. The effect of such relatively low temperatures is the suppression of activities which reduces the respiratory and osmoregulatory demands to viable levels. A considerable increase in thermal tolerance occurs following hatching in salmonids. With suitable thermal acclimation and other conditions being equal the biokinetic range of temperature is all but doubled following hatching, a situation which strongly indicts the zona radiata and its ooplasmic canal linings as limiting surfaces for respiration and osmoregulation.

APPENDIX III

REGULATION OF OXYGEN CONSUMPTION BY INCIDENT ILLUMINATION IN EMBRYONATED OVA OF ATLANTIC SALMON

INTRODUCTION

During a study of respirometry in embyros of the Atlantic Salmon salmo salar L. which has been described in part by Hamor and Garside (1973) speculation was aroused concerning the possible effect of incident illumination on respiratory performance. The only respirometric study of fish embryos using light as an experimental variable known to us is that of McCrimmon and Kwain (1969). Other investigations on the effects of light intensity or duration of exposure have been confined to considerations of developmental regulation and structural consequences (Eisler 1957; 1961; McCrimmon and Kwain 1969; Lyubitskaya 1961; Rough 1963; Vernick 1962).

In the present study oxygen consumption was measured in Atlantic salmon embryos incubated at four levels of illumination in 18 factorial combinations of developmental environment (2 temperatures x 3 levels of oxygen x 3 rates of flow) during the second half of the prehatching period.

MATERIALS AND METHODS

During some phases of respirometric study of Atlantic salmon (stages 11-18 Garside 1959) subsamples of 10 ova were placed in continuous-flow respirometers, described by Hamor and Garside (1973), which were supplied with water at various relatively constant flow rates (0.4, 0.8, 1.6 ml/min) and three levels of dissolved oxygen (30, 50, 100% of air saturation) at 5 and 10 C. These respirometers were exposed to various levels of incandescent illumination for tests of approximately 8 hr. Control of intensity was achieved by manipulation of the distances of the lamp from the respirometer. Incident illumination was measured at the surface of the glass respirometers with a sensitive photometer as foot-candles and these were converted to lux units.

Oxygen extraction by the embryos was measured at 2-hr intervals as the difference between incurrent and excurrent flows. Levels were determined by standard Winkler semimicrotitrimetry (Harper, 1953).

Initially, the effects of regulated variables, temperature, dissolved oxygen, flow rate, age and illumination were separated and measured for significance by multiple regression analysis. Subsequently, respirometric values were computed as average values from all ages in each of the experimental combinations and these values were subjected to factorial analysis of variance to separate any significant effect of temperature, dissolved oxygen and light intensity.

RESULTS

A summary of the results and experimental conditions is presented in Table 1. Oxygen consumption is generally greater at 10 C than in comparable environments at 5 C. The level of oxygen consumption also appears to be controlled by the level of dissolved oxygen and the flow rate. Oxygen consumption increased from low to intermediate levels of high intensity and then declined at the highest level. Significant effects and interactions have been extracted from these measures by factorial analysis of variance, a summary of which is given in Table 2. The only primary effect which is significant is that produced by light intensity. The level of dissolved oxygen narrowly failed the test of significant influence. Three interactions, temperature x oxygen level, flow rate x light and temperature x oxygen x light have significant effects. Other potential interactions do not reach the threshold for probable effect.

To interpret the masking effects of these variables, a factorial analysis was prepared for the effects of flow rate and light without reference to temperature and dissolved oxygen (Table 3). Both had highly significant effects.

TABLE 1

Mean oxygen consumption ($mgO_2/g/hr$) by intermediate and advanced embryos of Atlantic salmon (Salmo salar) subjected to 5 C and 10 C at three levels of dissolved oxygen in continuous-flow respirometers regulated to three flow-rates and subjected to four ranges of light intensity.

Temp.	Oxygen	Flow-Rate	Oxygen Consumption (mg/g/hr)			
C	% air-sat.	ml/min	1.2 - 70 lux	80 - 140 lux	150 - 210 lux	220 - 280 lux
5	100	1.6	0.16 + 0.01	0.19 + 0.01	0.19 + 0.01	0.13 + 0.01
		0.8	0.11 + 0.02	0.14 + 0.0	0.07 + 0.02	0.07 + 0.003
		0.4	0.04 + 0.01	0.07 + 0.03	0.03 + 0.01	0.02 + 0.01
	50	1.6	0.10 + 0.03	0.10 + 0.04	0.11 + 0.04	0.06 + 0.01
		0.8	0.05 + 0.03	0.08 + 0.03	0.06 + 0.01	0.03 + 0.01
		0.4	0.02 + 0.01	0.04 + 0.01	0.03 + 0.01	0.01 + 0.006
	30	1.6	0.05 + 0.02	0.08 + 0.04	0.04 + 0.01	0.05 + 0.02
		0.8	0.01 + 0.00	0.04 + 0.01	0.02 + 0.01	0.01 + 0.001
		0.4	0.001	0.02 + 0.01	0.004	0.01
10	100	1.6	0.04 + 0.01	0.33 + 0.01	0.03 + 0.01	0.13 + 0.05
		0.8	0.2 + 0.01	0.15 + 0.01	0.14 + 0.01	0.07 + 0.03
		0.4	0.01	0.06 + 0.01	0.04 + 0.01	0.04 + 0.01
	50	1.6	0.18 + 0.01	0.26 + 0.04	0.12 + 0.04	0.03 + 0.01
		0.8	0.07 + 0.	0.19 + 0.04	0.06 + 0.01	0.02 + 0.006
		0.4	0.04	0.11 + 0.01	0.03 + 0.	0.01 + 0.003

Temp.	Oxygen %	Flow-Rate	Oxygen Consumption (mg/g/hr)			
C	air-sat.	ml/min	1.2 - 70 lux	80 - 140 lux	150 - 210 lux	220 - 280 lux
	30.	1.6	0.08 + 0.03	0.18 + 0.03	0.06 + 0.01	0.01
		0.8	0.04 + 0.01	0.07 + 0.02	0.03 + 0.01	0.004
		0.4	0.02	0.03 + 0.01	0.01	0.004

TABLE 2

Summary of analysis of variance of oxygen consumption ($mgO_2/g/hr$) by intermediate and advanced embryos Atlantic salmon (*Salmo salar*) in response to four variables, temperature, dissolved oxygen, flow-rate and illumination in a 2 x 3 x 3 x 4 factorial experiment.

Variable	Sums of Squares	Degrees of Freedom	Mean Square	Variance Ratio	Probability
Temp.	0.223446	1	0.223446	2.7545	0.1229
Oxygen	0.580594	2	0.290297	3.5786	0.0604
Flow-Rate	0.379144	2	0.189572	2.3370	0.1390
Light	0.188449	3	0.628165	77.4369	0.0000**
Temp/oxygen	0.114015	2	0.570079	70.2763	0.0000**
Temp/flow	0.947335	2	0.473667	0.5839	0.5728
Temp/light	0.386552	3	0.128851	1.5884	0.2437
Oxygen/flow	0.442180	4	0.110545	1.3627	0.3039
Oxygen/light	0.842054	6	0.140342	1.7301	0.1972
Flow/light	0.222193	6	0.370321	4.5651	0.0123**
Temp/oxygen/flow	0.311029	4	0.777573	0.9595	0.4647
Temp/oxygen/light	0.519995	6	0.866658	10.6837	0.0003**
Temp/flow/light	0.480411	6	0.800685	0.9870	0.4753
Oxygen/flow/light	0.217164	12	0.180970	2.2309	0.0895
Error	0.973435	12	0.811196		

** highly significant

TABLE 3

Summary of analysis of variance of oxygen consumption (mg O_2/g/hr) by intermediate and advanced embryos of Atlantic salmon (Salmo salar) in response to respirometer flow-rate and light intensity.

Variable	Sums of Squares	Degrees of Freedom	Mean Square	Variance Ratio	Probability
Flow-rate	0.661402	2	0.330700	23.44015	0.00145**
Light	0.416087	3	0.138696	9.83078	0.00987**
Error	0.846498	6	0.141083		

** highly significant

DISCUSSION

The analyses of variance, between them, establish that all primary variables can modify oxygen consumption independently. Data in Table 1 indicate that rate of water supply to the embryos is highly significant but that the changes in other conditions, principally temperature, tend to obscure the potential for discrete effects so that the primary effects are not indicated in Table 1. The highly significant interactions (Table 2) give witness to this interpretation.

The results of this study are in general agreement with the respirometric analysis of McCrimmon and Kwain (1969) for rainbow trout embryos with respect to trend. Their results show, in most cases, that oxygen consumption increased from 0 to 200 lux. We also have demonstrated an increase generally to a level near 200 lux which is followed by a decline at 230 lux and higher. We have been unable, however, to explain the differential between the rates of oxygen consumption in the two studies. If the calculations of McCrimmon and Kwain (1969) have been recorded accurately, our results are, for the highest flow-rate about 65 times greater and for the lowest flow rate twice as great for similar temperatures, 9.2 and 10 C (Table 4). Unfortunatley, no further interpretations can be applied since their report is void of any details of their respirometric technique.

Table 4 contains a comparative review of all available reports on respirometric values for embryos or total ova of several salmonid species. We have adjusted the oxygen consumption reported by these authors to a common base of mg O_2/g/hr from their information on oxygen uptake and embryo or ovum size. These standardized values range from being about four times as great for Salmo gairdneri (Hamor 1967) to less

TABLE 4

omparative respirometry of the Atlantic salmon (Salmo salar) and other salmonids. Original values are adjusted to standard base (mg O_2/g live weight/hr) using ovum or embryo weights given by authors. Methods and experimental onditions are summarized.

pecies	Age or Stage	Weight	Temp C	Method	Original O_2 uptake	Adjusted O_2 uptake	Author Reference
almo alar	hatching	35 embryos 37.9 mg dry weight	10	Warburg Manometry	1.2 mg/g	0.172	Privolniev (1938)
"	hatching	one ovum 150 mg*	5	Winkler titration	0.0039 mg/hr	0.026	Lindroth (1942)
"	50 days	one embryo 15 mg	2.5	Polarography	1.52 mm^3/g/hr	0.106	Hayes et al. (1951)
"	hatching	one ovum 150 mg	10	Polarography	0.048 mg/g/hr	0.030	Hayes et al. (1951)
"	eyed embryo	one yolk 80 mg	10	Warburg manometry	0.16 mg/g/hr	0.163	Hamor and Garside (1973)
"	eyed embryo	one ovum 160 mg	10	Winkler titration	0.4 mg/g/hr	0.4	Present study
"	eyed embryo	one ovum 160 mg	5	Winkler titration	0.4 mg/g/hr	0.4	Present study
almo airdneri	eyed embryo	1-liter ova**	7	Winkler titration	400 mg/hr	0.7	Einsele (1956)
"	25-day embryo	16.4 mg	10	Winkler titration	0.28 mg/g/hr	0.28	Baravik (1963)

continued...

TABLE 4 (continued)

Species	Age or Stage	Weight	Temp °C	Method	Original value O_2 uptake	Adjusted value O_2 uptake	Author Reference
" "	alevin	100 mg	10	Winkler titration	590 mg/kg/hr	0.59	Hamor (1967)
" "	eyed embryo	one embryo 1.42 mg	9.2	not given	6.58 mg/kg/hr	0.007	McCrimmon and Kwain (1969)
Oncorhynchus keta	10 days to hatch	one ovum 74 mg	6	Winkler titration static	0.0002 mg/ovum	0.003	Wickett (1945)
Oncorhynchus gorbuschka	7 days to hatch	one ovum 120 mg	8	Winkler titration static	0.0007 mg/ovum	0.006	Wickett (1954)
Oncorhynchus kisutch	hatching	one ovum 300 mg	10	Winkler titration static	0.003 mg/ovum	0.01	Wickett (1954)

than 3% of our value for comparable temperature in chum salmon Oncorhynchus keta (Wickett 1954) and less than 2% of our value in rainbow trout (McCrimmon and Kwain 1969). Our data, however, show good agreement with those of Hayes et al. (1951).

Our survey of the literature reveals great lack of regard for standardized procedure in the measurement of oxygen consumption and in some instances even disregard for thorough description of the procedure which was adopted.

The physiological basis for the tonic regulation of oxygen consumption by light is not obvious. Since light intensity is known to modify endocrine functions in post-embryonal fishes through the intemediary of the central nervous system, we can speculate that thyroid function which in turn has been stimulated by the thyrotropic pituitary hormone has caused the observed increase in oxygen consumption. Ruhland (1971) has established a relation between thyroid function and oxygen consumption in the cichlid fish Aequidens latifrons. Johansen and Gomery (1973) have suggested from their study that thyroxine acts directly at the cellular level to control oxygen consumption in the goldfish (Carassius auratus). However, there is some conflict concerning the role of thyroxine in the control of oxygen consumption in fish and some studies have indicated there is none (Matty 1957) or that supplemental thyroxine treatment results in a decrease in oxygen consumption (Sage 1968).

Since the ova of salmonids are buried in gravel generally to depths exceeding 0.1 m it would seem unlikely that respiration or embryogenic processes would be significantly affected by natural light. However, light intensity does have obvious accelerating, and at high intensities, inhibiting effects on respiratory rate in laboratory conditions so that any statement concerning oxygen consumption in salmonid embryos and perhaps

many others is incomplete if it is not accompanied by a qualification concerning light intensity or more importantly, a comparative series of evaluations at several light intensities.

APPENDIX IV

CIRCADIAN RHYTHM IN OXYGEN CONSUMPTION OF ATLANTIC SALMON OVA IN AN ADVANCED STAGE OF EMBRYOGENY

INTRODUCTION

Cycles of several metabolic activities are generally well known in physiology (Hoar 1966, Prosser 1973). Such cycles can interact in ways that yield measurements which are not representative of average performance. Several experiments were conducted at stage 16 to determine whether a rhythm exists in the respiration of the Atlantic salmon ovum and whether it is circadian or not.

MATERIALS AND METHODS

Oxygen consumption of salmon ova was measured in flow-through respirometers at 4-ml/min flow-rate and 100% air-saturations at 5 and 10 C, with the same modified Winkler oxygen analysis and respiration chambers which have been described in the chapter on respirometry. These experiments were conducted in darkness to remove the effect of illumination.

Average measurements from hourly respirometric procedures were subsequently taken to be percentages of the highest measured oxygen consumption at each temperature during the 24-hr period.

RESULTS

The results are presented in Table 1 and Figure 1. These data indicate that respiration of the ova has an early afternoon peak about 1400 hr and a lesser peak about midnight. The curve for 5 C has a lower and broader peak than that at 10 C.

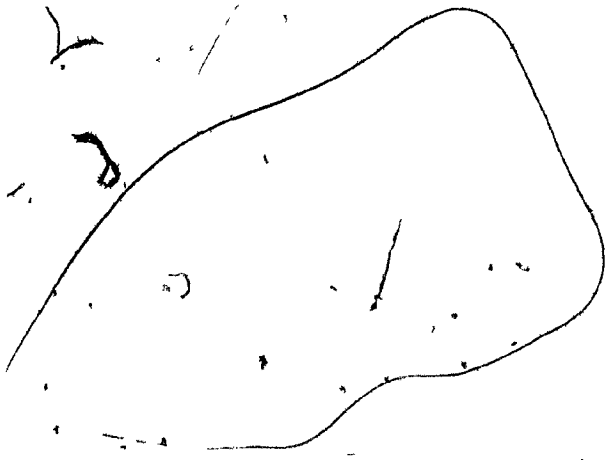
TABLE 1

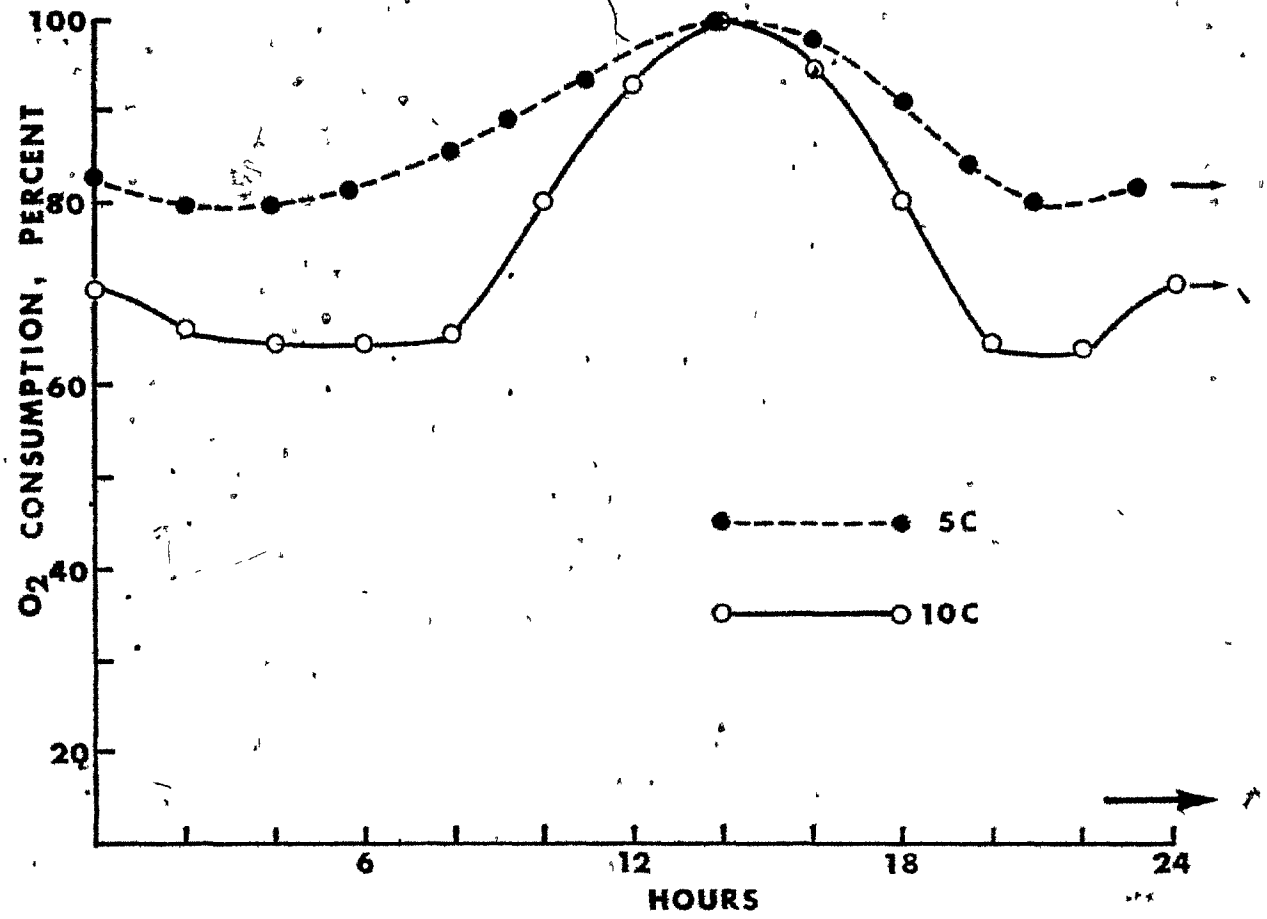
Circadian rhythm in oxygen consumption of embryos of Atlantic salmon. Oxygen uptake is expressed as percent of highest measurement which occurred about 1400 hr in ova in continous darkness at constant flow-rate, in stage 16. Value in 1400 hr consumption taken to be 100%

Time/lapsed	Temperature (C) 5	10	Time/lapsed	Temperature (C) 5	10
0100	70	71	1300	95	98
0200	68	70	1400	100	100
0300	65	70	1500	93	100
0400	65	70	1600	95	98
0500	65	71	1700	90	95
0600	65	72	1800	80	82
0700	65	78	1900	70	78
0800	65	85	2000	65	72
0900	73	88	2100	65	71
1000	80	92	2200	65	70
1100	90	94	2300	72	71
1200	93	96	2400	72	72

Figure 1

Oxygen consumption at 2-hr intervals, from midnight to midnight, expressed as percentages of highest measure during the period in samples of Atlantic salmon ova in stage 16, at 5 and 10 C and 100% air-saturation.




100
80
60
40
20
O_2 CONSUMPTION, PERCENT
5C
10C
6
12
18
24
HOURS

DISCUSSION

These results show that a circadian rhythm exists in a pattern of salmon ovum respiration at stage 16.

The question arises then, as to the comparability of data from other studies since there is generally no information about the exposure time and other pertinent experimental details. In the case of Atlantic salmon embryos, respiratory measurements taken at 0800 and 1700 hr would give a good approximation of mean daily oxygen consumption, particularly at 5 C. At 10 C, the estimated oxygen consumption might be about 10% higher than the actual uptake.

APPENDIX V

INFLUENCE OF AMBIENT SALINITY (20°/oo) ON NUTRIENT ENERGETICS AND GROWTH OF EMBRYOS OF ATLANTIC SALMON

INTRODUCTION

Since temperature and oxygen have effects on aspects of development and these effects were studied extensively in the earlier chapters it is of interest to see some of the differences that salinity can cause in development. The retarding effect of high salinity in development of fish embryos has been observed many times (Alderdice and Forrester 1968, Belyi 1967).

MATERIAL AND METHODS

Experimental materials and methods were the same as these described in the earlier chapters. Atlantic salmon ova from the same origin were kept at 10 C 100% air-saturation, one group in dechlorinated fresh water and the other lot in 20% salinity. The salinity level was achieved by continuous mixing of the dechlorinated water with the filtered sea-water from the supply system of the Life Sciences Centre, Dalhousie University. The flow rate was maintained at 4 ml and the salinity level was checked by hydrometer and with a Buchler-Cotlove chloridometer. Illumination and all the other experimental conditions were similar to those described in the earlier chapters.

RESULTS

The developmental time of salmon embryos in the saline medium from stage 1 to 18, was 60 days, about 40% longer than that of the control lot. Between the appearance of stage 18 and hatching a failure in the sea-water supply occurred and the remaining supply of fresh water was insufficient for about 24 hr and all embryos died. For that reason hatching did not occur. However, developmental survival to stage 18 was very high (95%).

The weight changes of ova from stages 1 to 18 and the significantly smaller amount of perivitelline fluid at stage 18 indicate that the hyperosmotic medium had a dehydrating effect. (Tables 1, 2).

The measured oxygen consumption for the ova in 20 °/ooS was 75% of that in the control group. Activity of 3'5' cAMP-ase was higher but 2'3'cAMP-ase was lower in embryos incubated in saline conditions (Table 3). The RNA, DNA, cholesterol, protein and non-protein nitrogen content were higher in embryos at stage 18 developed in fresh water, than in the group which developed in 20 °/ooS. (Table 4). The utilization efficiency of yolk was higher in the fresh water embryos than it was in the group in saline medium (Table 5).

TABEE 1

Live weight of Atlantic salmon ova in percent of original weight during development, at 10 C and 100% air-saturation in fresh water and in 20 °/ooS. Original weight at stage 1 was 139.0 mg.

Developmental stage	Fresh water (<0.5°/oo)	Saline water (20°/ooS)
1	100	100
2	100.6	99.0
3	98.7	98.4
4	98.9	96.5
5	93.7	95.2
6	92.7	94.8
7	99.0	94.3
8	102.7	95.6
9	99.3	96.2
10	99.3	95.6
11	99.5	95.7
12	109.6	95.8
13	100.4	95.6
14	99.5	95,0
15	100.8	92.0
16	99.8	91.2
17	98.8	92.3
18	105.9	90.2
Mean	101.1	94.1
S.E.	0.53	0.46

TABLE 2

Mean weight (mg) of Atlantic salmon ovum, yolk, embryo, uncleaned zona radiata and perivitelline fluid at 10 C and 100% air-saturation in dechlorinated fresh water and at 20°/ooS at stage 18.

Component	Fresh water	20°/ooS	$\frac{20°/ooS}{\text{Fresh water}} \times 100$
Ovum	151 ± 1.2	125.2 ± 1.6	82.9
Yolk	89.0 ± 0.8	84.0 ± 1.8	94.4
Embryo	14.5 ± 0.3	12.9 ± 0.6	90.0
Zona radiata	11.5 ± 0.4	20.4 ± 0.9	177.4
Perivitelline fluid	36.0 ± 1.5	7.7 ± 2.6	21.4

TABLE 3

Estimated values of enzyme activity of 3'5cAMP-ase and 2'3'cAMP-ase of Atlantic salmon ova expressed in amounts of phosphorus liberated by alkaline phosphatase µMP/g, µMP/g: during incubation in dechlorinated fresh water and at 20°/ooS at 10 C and 100% air-saturation.

Stage	Fresh water	20°/ooS
	3'5'cAMP-ase	
	µMP/g	
4	251	200
18	198	80
	µMP/DNA(mg)	
4	105	84
18	55	25
	µMP/Cholesterol(mg)	
4	2510	2000
18	152	40
	2'3'cAMP-ase	
	µMP/g	
4	370	411
18	573	944

continued

TABLE 3 continued

Stage	Fresh water	20°/ooS
	µMP/DNA (mg)	
4	142	158
18	159	295
	µMP/Cholesterol (mg)	
4	3700	4107
18	440	1062

TABLE 4

Differences in gross chemical composition (mg/g) of Atlantic salmon embryos at stage 18, in dechlorinated fresh water (see Table 1) and at 20°/ooS at 10°C and 100% air-saturation.

Component	Fresh-water		20°/ooS	
	Amount (mg/g)	Amount calories	Amount (mg/g)	Amount calories
RNA	26.6	-	21.2	-
DNA	3.6	-	3.4	-
Cholesterol	1.3	-	1.1	-
Phospholipid P	812×10^{-3}	-	902×10^{-3}	-
Phosphoplipid	68.4	-	78	-
Total lipid	75.2	707	92	865
Protein	99	559	80	452
Non-protein N	5.6	24	4.5	19
Glycogen	2.6	-	3.8	-
Carbohydrate	4.3	18	6.0	25
Ash	10.1	-	26.5	-
Dry material	194	-	203	-
Cal/live weight	1308	-	1361	-
Cal/dry weight	6742	-	6704	-

TABLE 5

Yolk conversion (weight gain mg/yolk depletion mg), total calorie value/animal, consumed calories, utilization efficiency in calories (calorie/animal x 100/calories consumed), of Atlantic salmon embryos at stage 18, in dechlorinated fresh water and at 20°/ooS at 10 C and 100% air-saturation.

Process	Freshwater	20°/ooS
Yolk conversion	0.91	0.58
Cal/embryo	19	.16
Calories consumed	95	108
Cal/mg tissue gain	6.5	8.4
Utilization efficiency %	20	15.6

DISCUSSION

The changes in weight and in chemical composition indicate that the hyperosmotic medium has a retarding effect on the development of Atlantic salmon embryos. However, very few data are available about levels of 3'5'cAMP-ase in fish embryos. Oide (1970) measured the alkaline phosphatase activity from freshwater and seawater adapted eels. He noted an increase in activity in the freshwater group. The same feature was observed in this experiment for 3'5'cAMP-ase using alkaline phosphatase as substrate. According to earlier observations in this study with higher 3'5'cAMP-ase activity lower lipid content can be expected, and actually the lipid content is higher in freshwater than in seawater adapted salmon embryos. However, the 2'3'cAMP-ase did not coincide with the earlier observations, because the higher amount in seawater adapted lot does not correspond with higher protein content. Observations indicate that in seawater rainbow trout fingerlings demand more protein for metabolism (Zeitoun et al. 1973). If this observation is true for Atlantic salmon embryos, the effect of higher protein consumption can offset the higher production, and so the difference in this result from that of the others can be explained.

The calculated efficiency for calorie utilization indicates that development in the 20 °/oo S medium is less efficient (78%) for Atlantic salmon embryos than is development in fresh water to an advanced stage, near hatching.

APPENDIX VI

HEART-BEAT FREQUENCY AS A MEASURE OF ACTIVITY IN ADVANCED EMBRYOS OF ATLANTIC SALMON IN RELATION TO VARIOUS LEVELS OF TEMPERATURE AND DISSOLVED OXYGEN

INTRODUCTION

Activity of Atlantic salmon embryos can significantly interfere with their development (Lasker and Theilacker 1962, Laurence 1973). Since the heartbeat is significantly correlated in fish with the other muscular activities (Szeky 1967) an experiment was made to account for the frequency of heart beats of advanced salmon embryos in various developmental environments.

MATERIALS AND METHODS

Experimental materials and methods were the same as those described generally in the earlier chapters. The frequency of heartbeats was measured at stage 16 using a stereomicroscope fitted with a red filter (7700 Angstrome), for transmitted light. Embryos (in ova) were maintained in a continous-flow respiration tube at 4 ml/min water exchange.

RESULTS

The number of heartbeats per minute using the mean value from 10 individuals at 5 and 10 C and 100, 50 and 30% air-saturation were;

Temperature	Air saturation		
	100%	50%	30%
5	21.5±0.6	17.4±0.6	10.6±0.6
10	40.1±0.5	25.1±0.2	20.5±0.1

According to the analysis of variance the frequency of heartbeats was highly significantly affected by temperature ($P<0.05$). However, interaction of temperature/air-saturation had an extremely significant effect ($P<0.001$). The t-tests indicated significant differences between each combination which was tested.

DISCUSSION

The frequency of heartbeats showed significant differences at stage 16 among different lots of Atlantic salmon embryos adapted to 5 and 10 C and three levels of dissolved oxygen. The possible stimulating effect of incident light was removed by examining the embryos in dark save only for the transmitted red light of a wavelength to which fish are generally insensitive (Blaxter 1970).

The influence of dissolved oxygen on reduced frequency of heartbeat is considerably greater at 10 C than at 5 C, for 50% air-saturation, but the change coincides at 30%. The Q_{10} values for heartbeats for the three different oxygen supplies in decreasing order of air-saturation are: 3.73, 2.88, 3.86.

These values are in agreement with the other Q_{10} values in earlier chapters. The relatively increased Q_{10} values for 30% air-saturation are perhaps explained by the highly adaptive capacities of cardiac muscle (Prosser 1973).

APPENDIX VII

INFLUENCE OF AN EXTRAORDINARY ELECTROMAGNETIC FIELD ON NUTRIENT ENERGETICS AND GROWTH OF EMBRYOS OF ATLANTIC SALMON

INTRODUCTION

The strange stimulating effect of an electromagnetic field in the development of plants was noted by Faraday in the fourth decade of the 19th Century. However, effects on animals were noted much later (Barnothy 1964). The possible results of an electromagnetic field on the development of Atlantic salmon embryos were examined in this experiment and it is the first of its kind according to an exhaustive search of the literature.

MATERIALS AND METHODS

All the experimental materials and developmemtal methods were the same as those described in the earlier chapters. Additionally a sample of freshly fertilized ova was subjected to an electromagnetic field force of 1.9×10^{-3} Oersted. This field was generated by an AlNiCo cylindrical bar-magnet (Aluminium-Nickel-Cobalt-Iron) with X 464 q, cylinder shaped with 2 cm diameter and 1.9 cm height (Edmund Scientific Company, 300 Edscorp Building, Barrington, N. J. 08007, U. S. A.) were covered with thin nylon to avoid metal contamination in the water, and placed beside the salmon ova in the 150-mm cylindrical respiration chamber.

RESULTS

The developmental time of Atlantic salmon embryos in a magnetic field was about 20% shorter than that of the control group. Survival was about 10% higher in development (Stage 18) and in hatching. The oxygen consumption approximately 15% lower than it was for the control group. When the weight measurements were analyzed with analyses of variance ANOVA 80, University of Alberta, from 90 individuals of the experimental and the control groups of ova, magnetism had a highly signficant effect ($P<0.001$) on embryo weight increase at stage 18, yolk weight was barely significant ($P<0.048$), interactions between magnetism and yolk weight was highly significant ($P<0.01$). (Tables 1, 2 and 3). The multiple correlation within different components of ova resulted in two equations for the control and the electromagneticly effected lot, to predict embryo weight:

$$Y_a = 20.2 + 0.18\ X_1 + 0.14\ X_2 - 0.07\ X_3$$

$$Y_b = 19.8 + 0.1\ X_1 + 0.13\ X_2 - 0.11\ X_3$$

where is:

Y_a = embryo weight, stage 18, control group (5 C 100% air-saturation).

Y_b = embryo weight, stage 18, (under 1.9×10^{-3} Oersted)

X_1 = ovum weight at stage 18

X_2 = weight of zona radiata at stage 18

X_3 = yolk weight at stage 18

The coefficient of determination (1-2) for the first equation was 0.6 and 0.55 for the second. Both equations were signifance according to the F test. The rank of partial correletion coefficents was $X_3>X_2>X_1$ in the control and $X_1>X_2>X_3$ in the experimental group.

The ovum and all parts of the ovum were heavier in the experiment lot than in the control at stage 18 (Table 1, and 2). Activity of

TABLE 1

Mean weight (mg) of Atlantic salmon ovum, yolk, embryo, uncleaned zona radiata, and perivitelline fluid (stage 18), at 5 C and 100% air-saturation (control), and in the same conditions but with an electromagnetic field of 1.9×10^{-3} Oersted.

Component	Control	Electromagnetic field
Ovum	141.0 ± 1.4	151.6 ± 1.3
Yolk	85.4 ± 0.76	89.6 ± 0.87
Embryo	24.6 ± 0.60	29.0 ± 0.60
Zona radiata	10.2 ± 0.52	10.3 ± 0.43
Perivitelline fluid	20.8 ± 0.8	22.7 ± 0.9

TABLE 2

Embryo weights (mg) of Atlantic salmon ranked by weight intervals of total ovum (rows) and weight intervals of (columns) for embryos in stage 18 incubated in an electromagnetic field of 1.9×10^{-3} Oersted and in a control also at 5 C, 100°/oo air-saturation.

Group	Ovum weight-class	Yolk weight-class					
		70-72.9	73-75.9	76-78.9	79-81.9	82-84.9	85-88
Control group	100-109.9	24.4±0.1	24.8±0.1	25.2±0.2	23.2±0.1	24.0±0.1	23.8±0.1
	110-119.9	25.0±0.1	25.8±0.2	26.0±0.2	24.0±0.2	23.8±0.2	23.8±0.2
	120-130	26.6±0.1	27.0±0.1	28.6±0.2	25.0±0.2	22.6±0.1	22.6±0.1
Electromagnetic field	100-109.9	26.0±0.1	21.0±0.1	26.0±0.2	24.0±0.2	19.0±0.1	30.0±0.3
	110-119.9	27.2±0.1	27.6±0.2	28.0±0.3	26.0±0.2	31.0±0.3	32.0±0.3
	120-130	27.2±0.2	28.0±0.2	30.0±0.2	29.6±0.2	33.0±0.3	34.0±0.3

TABLE 3

Factorial analysis of variance of the effect of electromagnetic field (ELM) (1.9×10^{-3} Oersted). Ovum weight (mg), and yolk weight (mg) on embryo weight of Atlantic salmon at embryo (mg) stage 18, at 5 C and 100% air-saturation.

Source	Sum of squares	Degrees	Mean square	F. ratios	Probability
ELM	107.2	1	107.2	41.2	0.001 **
Ovum weight	21.7	2	10.8	4.2	0.048 *
Yolk weight	54.4	5	15.0	4.2	0.026 *
ELM X Ovum	11.2	2	5.6	2.1	0.170
ELM X Yolk	74.8	5	15.0	5.7	0.009 **
Ovum X Yolk	18.8	10	1.9	0.7	0.700
Error	26.0	10	2.6		

TABLE 4

Estimated values of enzyme activity of 3'5'cAMP-ase and 2'3cAMP-ase of Atlantic salmon ova expressed in amounts of phosphorus liberated by alkaline phosphatase (A), µMP/g: DNA mg (B), and µMP/g: cholesterol mg (C), for living weight in dechlorinated fresh water under normal and 1.9 (10^{-3}) Oersted electromagnetic field at 5 C and 100% air-saturation.

Stages	Control	Electromagnetic field
	3'5'cAMP-ase	
	(µMP/g)	
4	251	251
18	125	80
	µMP/DNA (mg)	
4	96	96
18	28	44
	µMP/Cholesterol (mg)	
4	2510	2510
18	25.1	75
	2'3'cAMP-ase	
	(µMP/g)	
4	370	370
18	142	940
	µMP/DNA (mg)	
4	142	142
18	46	517

continued .....

TABLE 4 continued

Stages	Control	Electromagnetic field
	2'3'cAMP-ase	
	µMP/Cholesterol (mg)	
4	3700	3700
18	35	880

TABLE 5

Gross chemical composition (mg/g) of Atlantic salmon embryos at stage 18, in dechlorinated fresh water under normal incubation and in an electromagnetic field of 1.9×10^{-3} Oersted.

Component	Control		Electromagnetic field	
	Amount (mg/g)	Amount (calories)	Amount (mg/g)	Amount (calories)
RNA	6.1	-	8.0	-
DNA	4.0	-	3.8	-
Cholesterol	1.0	-	1.5	-
Phospholipid P	97×10^{-3}	-	80×10^{-3}	-
Phospholipid	3.8	-	3.1	-
Total lipid	11.0	103	8.0	75.0
Protein	122.0	689	150	848
Non protein N	10.2	44	13	56
Glycogen	1.1	-	1.0	-
Carbohydrate	3.8	16	2.6	11.0
Ash	15.0	-	10.0	-
Dry material	162	-	184	-
total Cal/liv weight	1308	-	1361	-
Cal/dry weight	6742	-	6704	-

TABLE 6

Yolk conversion (weight gain/mg yolk depletion mg), total caloric value/animal, consumed calories, utilization efficiency in calories (calories/animal x 100 calorie consumed), of Atlantic salmon embryos at stage 18, under normal incubation and in an electromagnetic field of 1.9×10^{-3} Oersted electromagnetic field, at 5C and 100% air-saturation.

	Control	Electromagnetic field
Yolk conversion	1.23	1.93
Cal/animal	21	39
Calories consumed	60	98
Cal/mg gain	4.0	3.4
Utilization efficiency in %	35	40

3'5'cAMP-ase was less, but 2'3'cAMP-ase higher in the experiment group. Amounts of RNA, cholesterol, protein, non-protein N and dry material were higher in the experimental group. (Table 4 and 5). The calculated utilization efficiency was 15% higher in the electromagnetic field than in the control group. (Table 6).

DISCUSSION

The experiments indicated that the electromagnetic field had a stimulating effect on the development of Atlantic salmon embryos. All the measurements gravimetric and chemical support this idea and are in good agreement with the other measurements in the principal experiments, and with other authors. (Barnothy 1964, Barnothy and Sumegi 1964, Cook et al. 1964, D'Souze et al 1964). However, a reasonable explanation at the current level of our knowledge can not be offered. the hypothesis for the effect of electromagnetic field, that it has a polarizing effect on the weak electric bonds and that can alter chemical bonds more efficiently and in this way the field acts as a chemical catalizator for the metabolic reactions. (Szent-Gyorgyi 1960, Neurath 1964).

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