Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*

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Indirect calorimetry was used to estimate metabolic rates and critical dissolved oxygen concentrations for steelhead (*Salmo gairdneri*) at intervals between fertilization and complete yolk exhaustion at 6, 9, 12, and 15°C. As well, tissue growth and yolk utilization were monitored. Energy budgets were constructed and the total amount of energy expended on respiration at each temperature estimated. On average, respiration accounted for 43.2% of the energy mobilized up to 90% yolk utilization. Absolute values agreed well with those estimated by integrating measured metabolic rates over the same period, confirming that measured metabolic rates and critical levels were representative of routine metabolism. Equations predicting metabolic rate and tissue mass as functions of time and temperature are given. Metabolic rate increased in virtually direct proportion to increases in tissue mass as indicated by a mean metabolic mass exponent of 1.05. Metabolic intensities averaged 200, 311, 405, and 548 $\mu g\ O_2 \cdot g$ wet tissue$^{-1} \cdot h^{-1}$ at 6, 9, 12, and 15°C, respectively. Critical oxygen concentrations increased continuously during embryonic development. Levels rose from $< 1\ mg \cdot L^{-1}$ shortly after fertilization to $7.5-9.7\ mg \cdot L^{-1}$, depending on temperature, just before hatch. Levels abruptly dropped by $2-3\ mg \cdot L^{-1}$ at hatch and then declined gradually to reach stable levels of $2.3-4.8\ mg \cdot L^{-1}$, depending on temperature, midway through the alevin period. Critical levels at all stages increased with increasing temperature as a direct reflection of increased metabolic demand. It is proposed that maximum critical levels (i.e., those just before hatch) be used to define dissolved oxygen criteria for steelhead in natural waters.

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La calorimétrie indirecte a permis d'estimer les taux de métabolisme et les concentrations critiques d'oxygène dissous à divers intervalles chez la truite arc-en-ciel anadrome (*Salmo gairdneri*) après fécondation et disparition totale du vitellus, à 6, 9, 12 et 15°C. La croissance tissulaire et l'utilisation du vitellus ont également été suivies. De plus, les budgets énergétiques et la quantité totale d'énergie dépensée par respiration à chaque température ont été mesurés. De façon générale, jusqu'à ce que 90% du vitellus soit utilisé, 43,2% de la dépense énergétique est consacré à la respiration. Les valeurs absolues concordent avec celles qui ont été estimées après intégration des taux de métabolisme au cours de la même période, ce qui prouve que les taux de métabolisme et les concentrations critiques sont les paramètres représentatifs du métabolisme ordinaire. Les équations qui permettent de prédire le taux de métabolisme et la masse tissulaire en fonction du temps et de la température sont présentées ici. L'augmentation du taux de métabolisme est pratiquement directement proportionnelle à l'augmentation de la masse tissulaire, tel qu'indiqué par la valeur de 1,05 de l'exposant de la masse métabolique moyenne. L'intensité métabolique moyenne a été évaluée à 200 $\mu g\ O_2 \cdot g$ de tissu frais$^{-1} \cdot h^{-1}$ à 6°C, 311 à 9°C, 405 à 12°C et 548 à 15°C. Les concentrations critiques d'oxygène augmentent continuellement au cours du développement embryonnaire, de $< 1\ mg \cdot L^{-1}$ juste après la fécondation à $7,5-9,7\ mg \cdot L^{-1}$ juste avant l'éclosion, selon la température. Les concentrations ont baissé brusquement de $2-3\ mg \cdot L^{-1}$ à l'éclosion, puis ont diminué encore graduellement jusqu'à des concentrations stables de $2,3-4,8\ mg \cdot L^{-1}$, selon la température, vers le milieu du stade alevin vésiculé. Les concentrations critiques à tous les stades augmentent en fonction directe de la température et cette augmentation est le reflet de l'augmentation de la demande métabolique. Les concentrations critiques maximales (c.-à.-d., immédiatement avant l'éclosion) peuvent probablement servir à déterminer les critères relatifs à l'oxygène dissous en nature chez cette truite.

[Traduit par la revue]

Introduction

It is widely recognized that salmonids are extremely sensitive to hypoxia during early life (Doudoroff and Shumway 1970; European Inland Fisheries Advisory Commission (EIFAC) 1973; Davis 1975; Alabaster and Lloyd 1980). Incipient limiting oxygen levels, however, generally remain poorly defined. There are several reasons for this, the major one being that response thresholds change during the course of development (Alderdice et al. 1958). In addition, environmental factors such as temperature can have a significant effect on both the course of development and the incipient limiting level at any given stage of development (Davis 1975). Defining response thresholds would thus appear to be a formidable task, especially if one considers attempting to do so using standard bioassay procedures. There is, however, a relatively simple way to approach the problem. Salmonid embryos and alevins behave as metabolic regulators (Hayes et al. 1951; Wickett 1954, 1975; Alderdice et al. 1958). This means that at high oxygen concentrations their metabolic rate is independent of the ambient oxygen level. At low oxygen concentrations, though, their metabolic rate is directly dependent on available oxygen. The concentration at which the transition from oxygen dependence to oxygen independence occurs, usually referred to as either the incipient limiting level (Fry 1957) or the critical oxygen tension (Prosser and Brown 1962), effectively defines the minimum dissolved oxygen requirement of that particular stage. Oxygen will not be limiting if its concentration is equal to or greater than the critical level, providing of course that other variables do not interfere with its delivery or utilization.

A potential problem with this approach is that critical levels are dependent on metabolic rate and metabolic rate can vary considerably in response to a variety of factors, two of the

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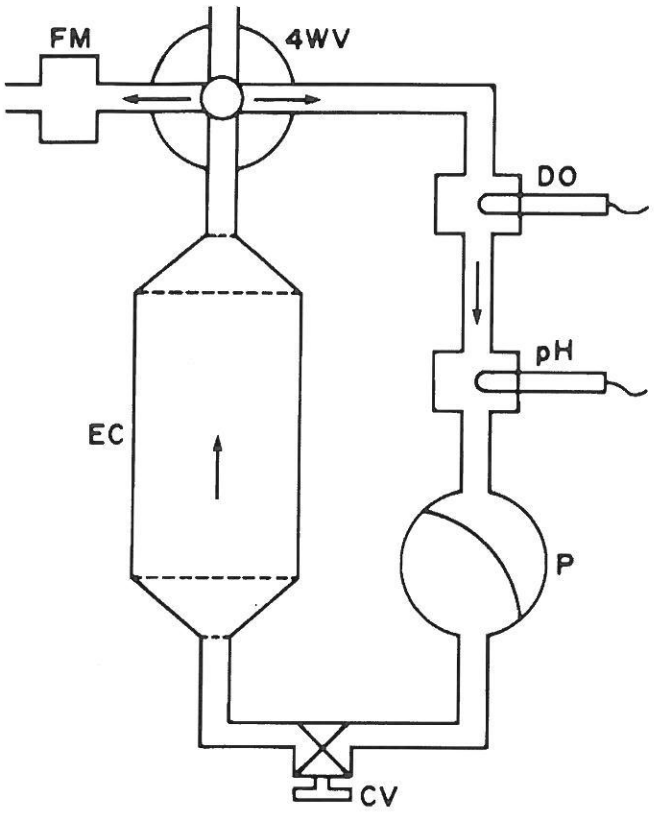

FIG. 1. Schematic diagram of the respirometer used to determine metabolic rates of steelhead embryos and alevins. EC, egg or alevin chamber; FM, flow meter; 4WV, four-way valve; DO, dissolved oxygen probe; pH, pH electrode; P, gear pump; CV, control valve.

most important of which are activity and stress (Fry 1957, 1971). These variables, therefore, must be closely controlled if critical levels are to be representative. Unfortunately, this is not a simple task. Indeed, for very young fish it is difficult to even monitor activity or stress levels, let alone actively control them. There is, however, an *a posteriori* method that can be used to check whether metabolic rates measured in a respirometer approximate those under more normal conditions. Gruber and Wieser (1983) point out that before the start of exogenous feeding, developing fish approximate closed systems. Thus during a given period, the energy equivalent of the amount of oxygen utilized (R) should equal the energy equivalent of the amount of yolk consumed (C) minus the amounts of energy expended on growth (G) and lost through excretion (U). If energy budgets balance (i.e., $R = C - G - U$), measured metabolic rates can be considered representative of routine metabolism. In turn, critical dissolved oxygen levels calculated on the basis of these rates also should be representative of those during normal development. This approach was followed in the current study. Indirect calorimetry was used to estimate metabolic rates and critical oxygen levels for steelhead (*Salmo gairdneri*) at intervals between fertilization and the onset of exogenous feeding at 6, 9, 12, and 15°C. Tissue growth and yolk utilization were monitored and energy budgets determined to confirm that measured metabolic rates and critical oxygen levels were in fact representative. Critical levels were then used to develop dissolved oxygen criteria for developing steelhead in natural waters.

Materials and methods

Gametes were obtained from steelhead returning to spawn in the Big Qualicum River, Vancouver Island, B.C. Eggs were pooled from two females and fertilized by the dry method with the pooled milt of two males. Water-hardened eggs had a mean (± SD) wet weight of 142 ± 7 mg (n = 20), an average diameter of 6.15 mm, and an average volume of 135 mm^3. At fertilization, the mean dry weight of the yolk was 44.3 ± 3.3 mg (n = 19).

Eggs and alevins were incubated in virtually total darkness at nominal temperatures of 6, 9, 12, and 15°C in vertically stacked incubation trays (Heath Techna Corp.). Initial loading densities were approximately 2500 eggs per tray. Water temperatures were monitored daily and averaged (± SD) 6.0 ± 0.4, 9.1 ± 0.4, 12.0 ± 0.4, and 15.1 ± 0.3°C during the incubation period. Water flows were maintained at 12 $L \cdot min^{-1}$ using dechlorinated water from the Nanaimo municipal supply (total hardness 12 $mg \cdot L^{-1}$ as $CaCO_3$, pH 7.2). Dissolved oxygen levels of the outflow water were monitored periodically and always exceeded 90% of the air-saturated value (ASV).

Estimates of routine metabolic rate and critical dissolved oxygen level were obtained by monitoring rates of oxygen consumption of embryos and alevins in three continuous-flow respirometers. A fourth respirometer, run simultaneously without animals, served as a control. Each respirometer consisted of a test chamber, a four-way valve with one port connected to a flow meter, a dissolved oxygen probe holder with magnetic stirrer, a pH electrode holder, a variable speed gear pump, and a microcontrol valve (Fig. 1). The test chamber and probe holders were constructed of acrylic and coated with water-soluble silicone (Clay Adams Co.) to inhibit bubble formation. The balance of the system was made from stainless steel. Respirometer volumes ranged between 70.25 and 71.88 mL. All four respirometers were immersed in a common 40-L constant temperature bath regulated to ±0.1°C by an attached temperature control unit. The dissolved oxygen concentration of the bath water could be varied by regulating the oxygen to nitrogen ratio of the gas supplying an aerator in the temperature control unit. Oxygen levels in individual respirometers were monitored continuously using polarographic oxygen electrodes (Orbisphere model 2104.01) and a multichannel oxygen meter (Orbisphere model 2710). Electrodes were calibrated by the azide modification of the Winkler method. Levels of pH were monitored continuously using a Fisher Accumet model 320 pH meter and combination electrode (Cole-Parmer model 5992-20).

Routine metabolic rates and critical dissolved oxygen levels were determined at 10–12 approximately evenly spaced intervals between fertilization and complete yolk absorption at each of the four incubation temperatures. Tests were conducted using respirometers in the "closed" mode. Initial oxygen concentrations were close to 100% ASV except for the tests involving late-eyed embryos and newly hatched alevins in which oxygen levels were elevated to between 110–160% ASV to ensure the initial level was well above the critical level for that particular stage. Depending on the stage of development, between 30 and 300 individuals were placed in each respirometer and the bulk water velocity past the eggs or alevins was adjusted to 500 $cm \cdot h^{-1}$ (this corresponds to a flow rate of 12 $L \cdot min^{-1}$ in a Heath tray). Respirometers were covered with heavy black plastic sheeting and the animals allowed 30 min in the dark to adapt to the system. Respirometers were then closed and the subsequent decline in oxygen concentrations was monitored. Chambers were reopened when ambient concentrations reached 1–2 $mg \cdot L^{-1}$. Eggs or alevins were removed and fixed in 5% neutral formalin for at least 21 d. Subsamples of five individuals were taken at random from each sample and dissected into tissue and yolk components. These components were gently blotted to remove excess fluid and weighed to the nearest 0.1 mg. Tissue and yolk dry weights were determined after oven drying at 60°C for 48 h.

When respirometers were operated in the closed mode, metabolic

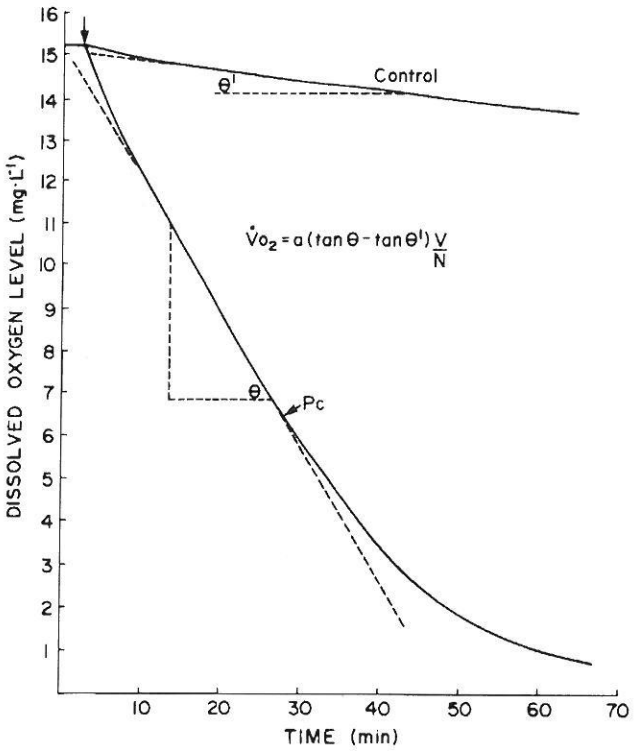

FIG. 2. Representative example of a polarographic record of oxygen utilization by steelhead embryos. Metabolic rate ($\dot{V}O_2$) was determined by applying an appropriate scaling factor (a) to tan (experimental chamber) − tan (control chamber) and multiplying this value by the respirometer volume (V) divided by the number of individuals (N). The critical oxygen level (P_c) was taken as the dissolved oxygen level at which the polarographic record began to deviate perceptibly from a straight line. The vertical arrow indicates the start of the test.

rates were estimated by taking the tangent to the polarographic record relating oxygen concentration to time and applying the appropriate scaling factor (scaling factors varied depending on the sensitivity of the chart recorder and chart speed). Oxygen concentration initially declined in a curvilinear fashion with time in both experimental and control respirometers, apparently as a result of pressure changes associated with closing the system. However, electrodes stabilized within 5−15 min and oxygen levels declined at a more or less constant rate for about 15−30 min. The rate of oxygen uptake over this linear portion of the polarographic record was assumed to approximate routine metabolic rate. Eventually the polarographic record began to flatten, reflecting a decline in oxygen uptake as oxygen became limiting. The point at which the curve first deviated perceptibly from a straight line was taken as an estimate of the critical oxygen level (P_c, Fig. 2). The oxygen concentration at which the rate of uptake fell to one-half the routine rate, here termed the P_{50}, was estimated from the polarographic record.

A frequent criticism of closed system respirometry is that rates of oxygen consumption determined in such systems may not be representative because of the presence of relatively high concentrations of metabolic by-products. The possibility that this was a problem in the current study was examined. Although the respirometers were normally operated in a closed mode, they could be converted to an open configuration by disconnecting the system between the control valve and the egg chamber and attaching the flow meter to the end of the control valve. This was done and metabolic rates were estimated for late-eyed embryos (320−330 accumulated thermal units; degree-days) at 10°C using the difference in the oxygen content of the inflowing and outflowing water and the flow rate. Outflow oxygen levels were taken after they had stabilized to avoid the problem of lag (Fry 1957). These estimates were then compared with those obtained for the same embryos with the respirometers in the closed configuration.

Estimates of routine metabolic rate and mean tissue weights at the various test intervals were fitted to parabolic models of the type $y = a \cdot d^b$, where y is routine metabolic rate or tissue weight, d is days postfertilization, and a and b are constants. Following the recommendation of Hayes et al. (1951), data for the test intervals before completion of epiboly (blastopore closure) were not included. Slopes of the regression lines for each of the four incubation temperatures were compared by analysis of covariance using the BMDP computer program (Dixon et al. 1981). If slopes were not significantly different, a temperature term (T) was incorporated into the model, now $y = a \cdot d^b \cdot T^c$, where c is a constant, and the best fit was determined using the Minitab computer program for multiple linear regression (Ryan et al. 1976).

Absolute growth rates were calculated as $(W_2 - W_1) \cdot (t_2 - t_1)^{-1}$, where W_1 and W_2 represent body weight at times t_1 and t_2, respectively. Gross conversion efficiencies were calculated as the gain in tissue dry weight divided by the loss in yolk dry weight multiplied by 100 (Hayes and Pelluet 1945). The length of time it took alevins to consume 90% of the dry weight of the yolk initially present in the egg, and tissue wet and dry weights at 90% yolk utilization were estimated by interpolation of plots of time, tissue wet weight, and tissue dry weight, respectively, versus the percentage of the yolk consumed. Energy budgets were calculated using caloric values of 6.4642 cal · mg dry wt.$^{-1}$ for yolk and 5.6164 cal · mg dry wt.$^{-1}$ for embryonic tissue (1 cal = 4.184 J). The estimate of the caloric value for yolk was determined by averaging the heat of combustion of fertilized eggs of sockeye, *Oncorhynchus nerka* (6.474 cal · mg dry wt.$^{-1}$; C. E. Duenas, Pacific Biological Station, Nanaimo, B.C., unpublished data), and rainbow trout, *Salmo gairdneri* (6.246 cal · mg dry wt.$^{-1}$; Smith 1947), determined by bomb calorimetry with caloric values based on the proximate analyses of salmonid yolk (an average of 30.4% lipid, 65.2% protein, and 4.4% ash) reported by Hartmann et al. (1947), Suyama and Ogino (1958), Ando (1962), Hayes et al. (1973), and Hamor and Garside (1975). The caloric value for tissue was determined by averaging bomb calorimetry data for embryonic tissue of rainbow trout (5.471 cal · mg dry wt.$^{-1}$; Smith 1947) with caloric values based on the proximate analyses of alevin tissue (an average of 11.8% lipid, 80.6% protein, and 7.6% ash; Hollett and Hayes 1946; Smith 1952; Hayes et al. 1973). Total oxygen consumptions were estimated from graphs and converted to energy equivalents using an oxycaloric value of 3.24 cal · mg O_2^{-1}. Oxycaloric equivalents and heats of combustion were taken from Brett and Groves (1979).

Results

Mortality

Embryonic mortality was less than 4% at 6, 9, and 12°C but increased to 15% at 15°C (Table 1). Most deaths at 15°C occurred shortly before or during hatch. Alevin mortality was low (<5%) at all temperatures.

Growth

Growth curves for steelhead embryos and alevins are displayed in Fig. 3. There was little difference in estimated size at hatch for embryos incubated at 6, 9, and 12°C but at 15°C, newly hatched alevins were considerably smaller and appeared less well developed than the newly hatched alevins incubated at lower temperatures (Table 1). Experiments were terminated too early at 6 and 12°C to allow comparisons of the maximum sizes attained by alevins at the various temperatures. However, there was little difference in estimated tissue weights at 90% yolk utilization (Table 1). Gross conversion efficiencies varied little with temperature, ranging from 70.5% at 6°C to 66.2% at 15°C (mean 68.3%) for the period between fertilization and 90% yolk utilization (Table 1). The following multiple linear regressions were calculated:

TABLE 1. Time and size relationships for steelhead embryos and alevins incubated at 6, 9, 12, and 15°C

	6°C	9°C	12°C	15°C
Survival to hatch (%)	96.0	97.4	96.6	85.0
Time to 50% hatch (d)	61.7	40.0	27.9	20.0
Time to maximum tissue wet wt. (d)	>115	72.7	>50	39.7
Time to 90% yolk utilization (d)	107.1	67.9	47.5	41.6
Tissue wt. at hatch (mg)				
Wet	44.7	45.4	45.3	30.3
Dry	7.3	6.9	6.8	4.8
Yolk wt. at hatch (mg)				
Wet	81.0	77.5	78.6	88.2
Dry	37.1	36.8	36.2	41.4
Tissue wt. at 90% yolk utilization (mg)				
Wet	204.6	196.2	195.7	192.6
Dry	28.1	27.1	27.3	26.4
Gross efficiency of yolk utilization (%)				
Embryo	101	92	84	166
Alevin	61.6	60.4	62.4	56.7
Total	70.5	68.0	68.5	66.2
Total oxygen consumed (mg)				
Embryo	3.34	3.43	3.32	1.99
Alevin	27.67	28.37	27.39	41.61
Total	31.01	31.80	30.71	43.6

NOTE: Gross efficiency of yolk utilization and the total amount of oxygen consumed by alevins was calculated for the period between 50% hatch and the time when 90% of the initial yolk had been consumed.

$$[1]\quad \ln \text{TWW} = -12.718 + 2.685 \ln d + 3.050 \ln T \qquad (R^2 = 98.5\%, n = 35)$$

$$[2]\quad \ln \text{TDW} = -11.726 + 2.222 \ln d + 2.562 \ln T \qquad (R^2 = 96.1\%, n = 35)$$

where TWW is tissue wet weight (mg), TDW is tissue dry weight (mg), d is number of days postfertilization, and T is mean incubation temperature (°C); they provided good predictions of tissue wet and dry weights during the period between completion of epiboly (5 d at 15°C, 7 d at 12°C, 9 d at 9°C, and 15 d at 6°C) and maximum tissue weight.

Metabolism

The mode in which the respirometers were operated did not significantly affect estimates of routine metabolic rate. The routine rate (mean ± SD) for the late-eyed embryos used for this comparison was estimated as 9.31 ± 0.59 $\mu g \cdot h^{-1}$ with the respirometers in the closed mode and 9.17 ± 0.35 $\mu g \cdot h^{-1}$ with the respirometers in the open mode.

Routine metabolic rates increased 200- to 500-fold, depending on temperature, during the experimental period (Fig. 4). The regression

$$[3]\quad \ln \dot{V}O_2 = -16.082 + 2.592 \ln d + 4.220 \ln T \qquad (R^2 = 97.5\%, n = 35)$$

where $\dot{V}O_2$ is routine metabolic rate ($\mu g\ O_2 \cdot h^{-1}$), d is number of days postfertilization, and T is mean incubation temperature (°C), provides good estimates of routine metabolic rate between completion of epiboly and maximum metabolic rate prior to exogenous feeding. The following regression equations describe the relationships between routine metabolic rate and tissue wet (eq. 4) and dry weights (eq. 5):

$$[4]\quad \ln \dot{V}O_2 = -3.734 + 1.054 \ln \text{TWW} + 1.085 \ln T \qquad (R^2 = 99.0\%, n = 35)$$

$$[5]\quad \ln \dot{V}O_2 = -2.107 + 1.246 \ln \text{TDW} + 1.017 \ln T \qquad (R^2 = 97.5\%, n = 35)$$

where $\dot{V}O_2$ is routine metabolic rate ($\mu g\ O_2 \cdot h^{-1}$), TWW is tissue wet weight (mg), TDW is tissue dry weight (mg), and T is mean incubation temperature (°C).

Mass-specific metabolic rates (metabolic intensities), calculated on a wet-weight basis, did not vary significantly with tissue weight at 6, 9, and 12°C (Table 2). Metabolic intensity showed a slight but significant increase ($p < 0.01$) with increasing tissue wet weight at 15°C. The Q_{10} for metabolic intensity calculated on the basis of wet weight averaged 2.99 between 6 and 15°C. In contrast, mass-specific metabolic rates expressed on a dry-weight basis increased significantly ($p < 0.01$) with tissue weight at all temepratures and can be predicted using the following equation:

$$[6]\quad \ln \dot{V}O_2/\text{TDW} = -0.885 + 0.248 \ln \text{TDW} + 0.106 T \qquad (R^2 = 75.3\%, n = 36)$$

where $\dot{V}O_2$/TDW is routine metabolic intensity ($\mu g\ O_2 \cdot$ mg dry wt.$^{-1} \cdot h^{-1}$), TDW is tissue dry weight (mg), and T is mean incubation temperature (°C).

Energy utilization

Total daily rates of energy consumption ($cal \cdot d^{-1}$) by embryos and alevins were estimated by summing the energy equivalents of the absolute growth rate and the metabolic rate at each sample period. Rates of energy consumption peaked at about 18.7 $cal \cdot d^{-1}$ on day 33 at 15°C, 11.6 $cal \cdot d^{-1}$ on day 43 at 12°C, 8.0 $cal \cdot d^{-1}$ on day 60 at 9°C, and 6.0 $cal \cdot d^{-1}$ on day 92 at 6°C (Fig. 5). The proportion of total energy expended on growth ($G \cdot (G + R)^{-1}$) declined steadily throughout development while, conversely, the proportion expended on metabolism ($R \cdot (G + R)^{-1}$) increased (Fig. 6). Energy budgets were calculated for the period between fertili-

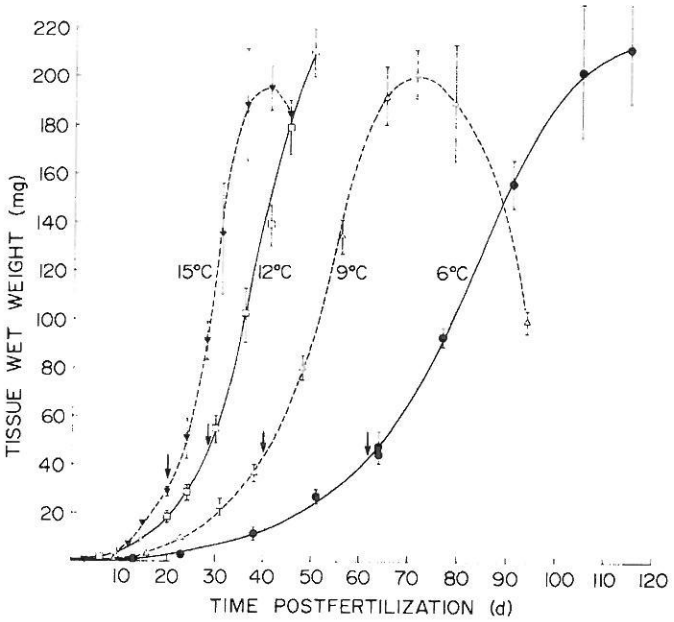

FIG. 3. Relationships between tissue wet weight (mg) and time (days postfertilization) for steelhead embryos and alevins incubated at 6, 9, 12, and 15°C. Vertical arrows indicate median hatching times. Error bars give 95% confidence limits for tissue weight.

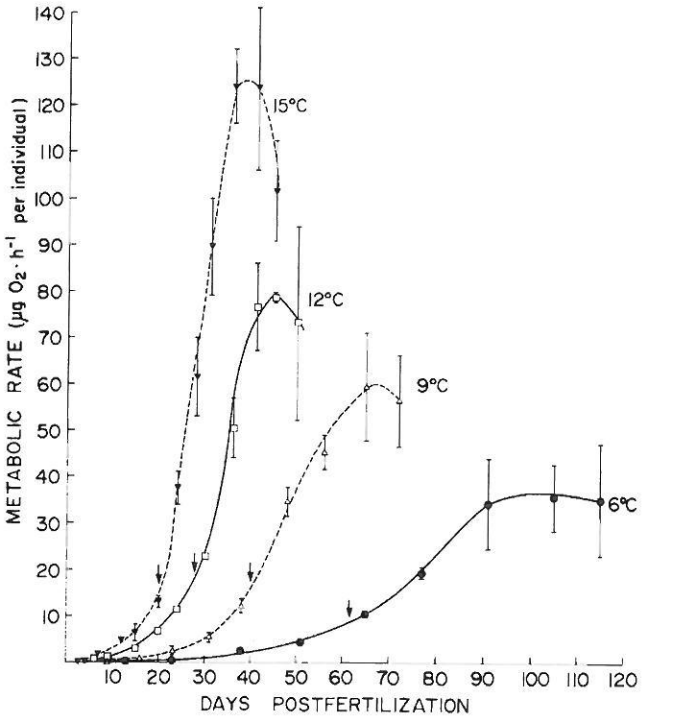

FIG. 4. Relationships between routine metabolic rates and time (days postfertilization) for steelhead embryos and alevins incubated at 6, 9, 12, and 15°C. Vertical arrows indicate median hatching times. Error bars give 95% confidence limits for metabolic rates.

zation and 90% yolk utilization. Budgets balanced extremely well at 6, 9, and 12°C with the energy expended on growth and metabolism accounting for 99.3% on average of the energy content of the yolk consumed during that period ($(G + R) \cdot C^{-1}$; Table 3). At 15°C, the estimated amount of energy expended on growth and metabolism was 12.5% greater than

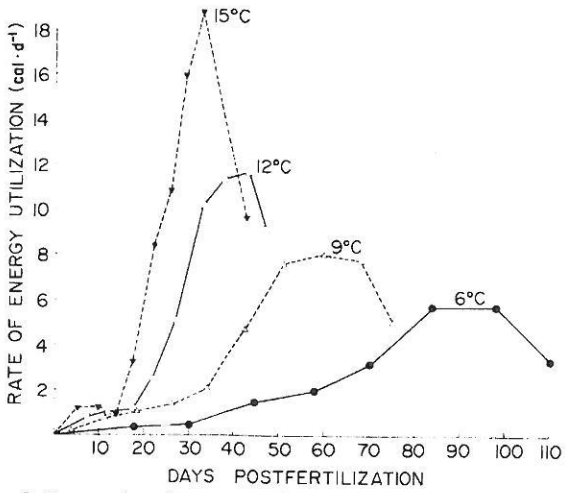

FIG. 5. Rates of total energy utilization as a function of time (days postfertilization) for steelhead embryos and alevins incubated at 6, 9, 12, and 15°C. 1 cal = 4.184 J.

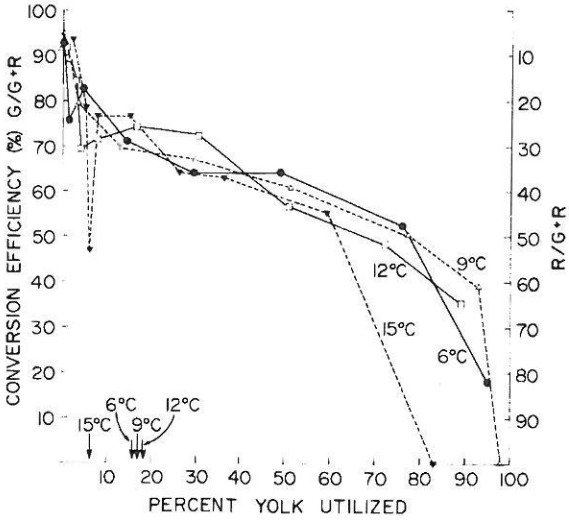

FIG. 6. Yolk conversion efficiency of steelhead embryos and alevins as a function of the amount of yolk remaining. Conversion efficiency is expressed as the percentge of total energy expended on either growth (G) or metabolism (R). Arrows indicate percent yolk at 50% hatch.

could be accounted for by the amount of yolk consumed. Incubation temperature had little effect on growth efficiency. Growth accounted for an average of 59.3% of the total amount of energy consumed between fertilization and 90% yolk absorption ($G \cdot C^{-1}$; Table 3).

Critical dissolved oxygen levels

Critical dissolved oxygen levels (P_c) varied significantly with stage of development and with temperature (Fig. 7). P_c values for embryos increased from <1 mg · L^{-1} shortly after fertilization to estimated maxima of 7.5 (60% ASV), 8.9 (77% ASV), 9.6 (89% ASV), and 9.7 (96% ASV) mg · L^{-1} shortly before hatch at 6, 9, 12, and 15°C, respectively (Fig. 7). Critical oxygen levels during embryonic development can be predicted using the following equation:

$$[7] \quad \ln P_c = -4.50 + 0.949 \ln d + 1.45 \ln T \qquad (R^2 = 97.1\%, n = 20)$$

TABLE 2. Regression analysis of metabolic intensity ($\dot{V}O_2$/TWW; $\mu g\ O_2 \cdot mg$ tissue wet $wt.^{-1} \cdot h^{-1}$) as a function of tissue wet weight (TWW; mg) and incubation temperature for steelhead embryos and alevins incubated at 6, 9, 12, and 15°C (a parabolic relationship, $\ln \dot{V}O_2/TWW = a + b \ln TWW$, gave the best fit to the data)

Incubation temperature	n	a	b	r	Mean $\dot{V}O_2$/TWW ($\mu g \cdot mg^{-1} \cdot h^{-1}$)	Mean TWW (mg)
6°C	9	−1.5213	−0.03141	−0.5929	0.200	23.2
9°C	10	−1.2678	0.03275	0.5722	0.311	27.8
12°C	9	−1.1054	0.05455	0.6435	0.405	24.9
15°C	11	−0.8059	0.0677	0.7782	0.548	21.6

TABLE 3. Energy budgets for steelhead embryos and alevins incubated at 6, 9, 12, and 15°C

Incubation temperature	C, total yolk consumed (cal)	G, total tissue produced (cal)	R, total O_2 consumed (cal)	$G \cdot C^{-1}$ (%)	$G \cdot (G + R)^{-1}$ (%)	$R \cdot C^{-1}$ (%)	$R \cdot (G + R)^{-1}$ (%)	$(G + R) \cdot C^{-1}$ (%)	$R \cdot (C - G)^{-1}$ (%)
6°C	257.7	157.8	100.7	61.2	61.0	39.1	39.0	100.3	100.8
9°C	257.7	152.2	103.6	59.1	59.5	40.2	40.5	99.3	98.2
12°C	257.7	153.3	99.7	59.5	60.6	38.7	39.4	98.2	95.5
15°C	257.7	148.3	141.6	57.5	51.2	54.9	44.8	112.5	129.4

NOTE: Budgets were calculated for the period between fertilization and 90% yolk utilization. Consumed yolk (C) was converted to energy equivalents assuming a heat of combustion of 6.4642 cal · mg dry $wt.^{-1}$. Tissue produced (G) was converted assuming a heat of combustion of 5.6164 cal · mg dry $wt.^{-1}$. The total amount of oxygen consumed (R) was converted to energy equivalents assuming an oxycaloric value of 3.24 cal · mg O_2^{-1}. Excretory loss of energy was ignored.

where P_c is the critical oxygen level ($mg \cdot L^{-1}$), d is number of days postfertilization, and T is the mean incubation temperature (°C). Critical oxygen levels and P_{50} levels were directly dependent on metabolic rate at rates of oxygen uptake above about 1 $\mu g \cdot h^{-1}$ (Fig. 8). These relationships were independent of temperature. At rates of oxygen uptake below about 1 $\mu g \cdot h^{-1}$, the relationships between P_c and $\dot{V}O_2$ and between P_{50} and $\dot{V}O_2$ were curvilinear.

Critical levels dropped 2−3 $mg \cdot L^{-1}$ at hatch. They then declined gradually to reach relatively stable levels about midway through the alevin stage (Fig. 7). P_c values during the first half of the larval period can be estimated using the following regression:

$$[8] \quad P_c = 5.56 - 1.04 \ln d + 0.131T \ (R^2 = 79.5\%, n = 20)$$

where P_c is in milligrams per litre, d is number of days posthatch, and T is incubation temperature (°C). Mean P_c values (± SD, $n = 3$) during the latter half of the alevin stage were 2.27 ± 0.46, 3.35 ± 0.27, 3.39 ± 0.32, and 4.75 ± 0.22 $mg \cdot L^{-1}$ at 6, 9, 12, and 15°C, respectively. P_{50} values for alevins, unlike P_c values, did not vary significantly once hatch was completed. Mean values for P_{50} (± SD, $n = 5$) were 1.20 ± 0.05, 1.77 ± 0.12, 1.88 ± 0.20, and 2.44 ± 0.25 $mg \cdot L^{-1}$ at 6, 9, 12, and 15°C, respectively.

Discussion

The empirical estimates of routine metabolic rates and critical oxygen levels obtained in the current study would appear to be representative of those under normal rearing conditions. As pointed out in the Introduction, measured metabolic rates and critical oxygen levels can be considered representative if energy budgets balance (i.e., $R = C - G - U$). In the current study, the amount of energy expended on respiration between fertilization and 90% yolk utilization at 6, 9, and 12°C averaged 98.1% of the energy content of the yolk mobilized during this period that did not go into tissue growth ($R \cdot (C - G)^{-1}$; Table 3). Excretory loss of energy was not taken into account when energy budgets were calculated but this is unlikely to have seriously biased the results. Data presented by Smith (1947) and by Rice and Stokes (1972) indicate that nitrogenous wastes account for only about 1−2.5% of the energy mobilized before exogenous feeding. Energy budgets did not balance quite so well at 15°C, where total measured oxygen consumption was about 30% greater than that predicted. This discrepancy probably is a reflection of the fact that 15°C is close to the incipient upper lethal temperature for developing steelhead (F. P. Velsen, Pacific Biological Station, Nanaimo, B.C., personal communication). At such a supraoptimal temperature, it would not be surprising if the fish required longer to recover from the stress of being placed in the respirometer than was allowed (30 min).

The accumulation of metabolic wastes in the "closed" respirometers did not significantly influence estimates of routine metabolic rate. This is not surprising given the relative insensitivity of the early life stages to metabolic by-products such as CO_2, low pH, and ammonia. Alderdice and Wickett (1958) reported that oxygen uptake by embryos of chum salmon, *Oncorhynchus keta*, was independent of ambient carbon dioxide levels below 125 $mg \cdot L^{-1}$. Salmon embryos are similarly relatively insensitive to low pH; in chum salmon oxygen uptake is not affected until pH drops below 4.0 (P. J. Rombough, unpublished data). The lowest pH recorded in the current experiment was 6.2. Rice and Stokes (1972) reported that ammonia concentrations up to 100 $mg \cdot L^{-1}$ had little or no effect on survival of rainbow trout until near the end of yolk absorption. The relative insensitivity of the early stages compared with older fish is probably a reflection of major differences in the characteristics of their respective hemoglobins. In particular, embryonic−larval hemoglobin displays much weaker Bohr and Root effects than do juvenile hemoglobins

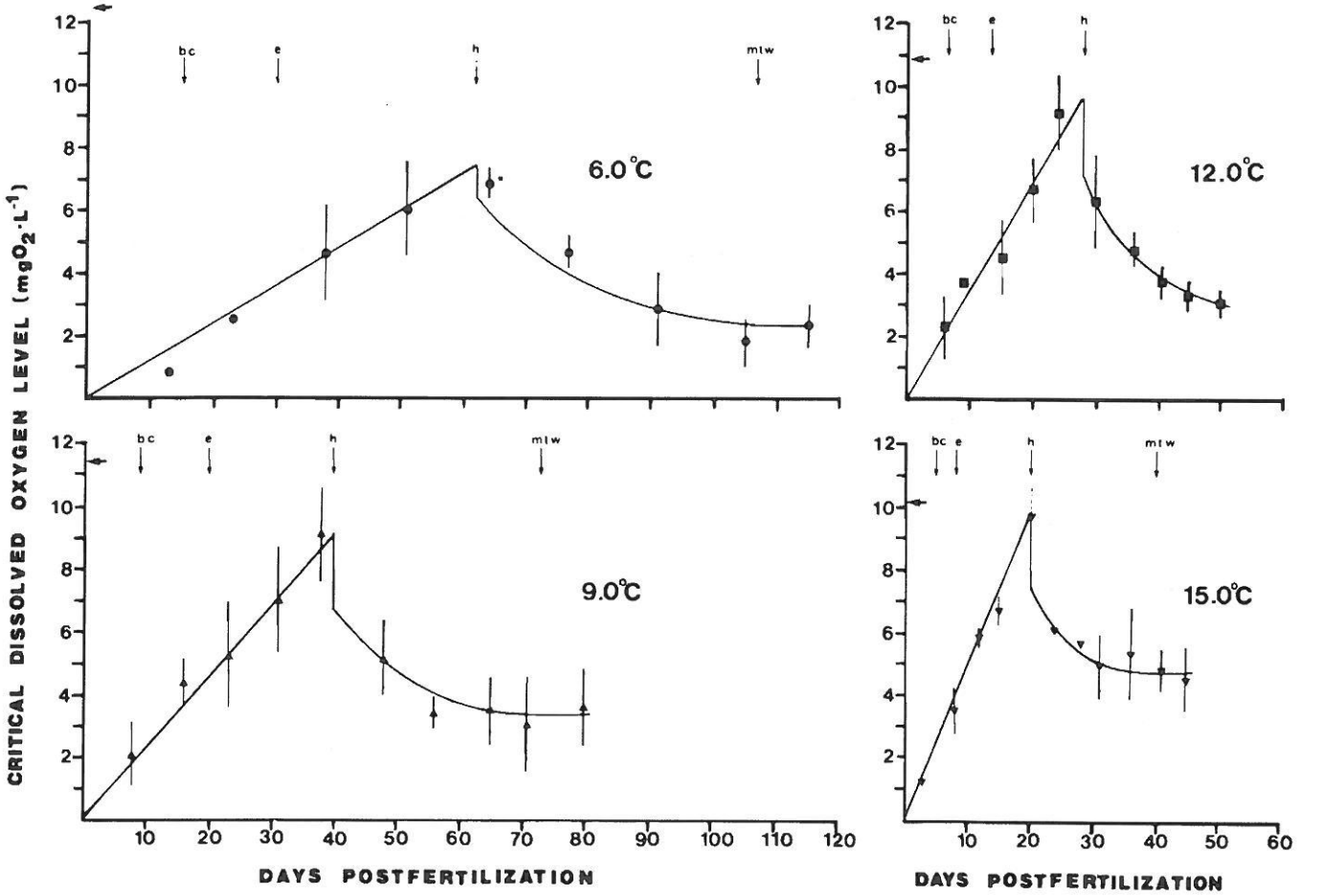

FIG. 7. Critical dissolved oxygen levels (P_c) for steelhead embryos and alevins incubated at 6, 9, 12, and 15°C. *bc*, blastopore closure; *e*, eyed; *h*, hatch; *mtw*, maximum tissue wet weight. Error bars give 95% confidence limits for P_c. Horizontal arrows indicate oxygen concentrations at 100% ASV.

(Iuchi 1973).

Routine metabolic rate increased in virtually direct proportion to the increase in tissue mass in young steelhead as indicated by a mean metabolic mass exponent (b) of 1.05 (eq. 4). Until recently, it was generally assumed that metabolic mass exponents during early life were not appreciably different from those of juveniles and adults (i.e., $b \approx 0.8$; Winberg 1956). Some authors still accept this assumption (e.g., Oikawa and Itazawa 1985), but several recent studies have suggested that high mass exponents ($b \geq 1.0$) during early life are not unusual for fish (Kamler 1976; Forstner et al. 1983; Khakimullin 1985; Wieser 1985). Indeed, it now appears that ontogenetic variation in the value of the mass exponent, characterized by high values during early life, is widespread throughout all vertebrate classes (Adolph 1983; Wieser 1984). The reasons for this are not clear although Pauly (1981) has suggested that, at least for fish, high mass exponents during early life may be a reflection of favorable surface:volume ratios for respiratory gas exchange. At present, the evidence supporting this hypothesis is largely circumstantial but given the theoretical significance of metabolism−mass relationships the proposal would seem to be worthy of further investigation.

It would be useful to be able to compare metabolic intensities of different species. Unfortunately, this is easier said than done. Four problems in particular make such comparisons difficult. The first is the fact that there are stage-specific variations in metabolic intensity (Smith 1947). Thus, to be strictly valid only equivalent stages should be compared. This is difficult to do since many investigators give only the vaguest description of the stage tested. One way to minimize the impact of stage-specific variation is to compare average intensities for the complete embryonic−larval period. Some investigators have attempted to do so by making use of the metabolic rate − mass relationship. Here again there can be problems because, as Konstantinov (1980) points out, levels of metabolism derived from such relationships are only valid if the slopes of the relationships are the same. The second major problem encountered in attempting to compare metabolic intensities is that some investigators include the mass of the metabolically inactive yolk along with the mass of the embryo in their calculations. This results in considerable underestimation of true metabolic intensities early in development when the relative amount of yolk is large. The third major problem is related to measurement technique. As pointed out previously, activity has a very significant effect on the rate at which oxygen is consumed. Thus if metabolic rates are to be comparable, activity levels should be similar. It is difficult to control activity under the best of circumstances but some techniques, especially those involving manometric determination of oxygen consumption, are particularly inappropriate. Manometry requires that the respirometer be shaken. This undoubtedly places considerable stress on the organism and it is not surprising that metabolic intensities determined for young salmonids in such a manner tend to be extremely high (e.g., Wood's

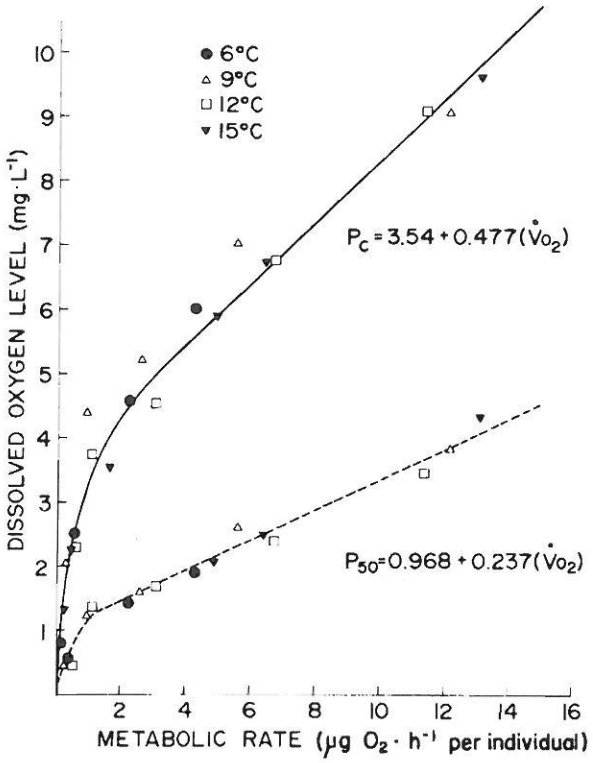

FIG. 8. Relationships between critical dissolved oxygen level (P_c) and routine metabolic rate and between P_{50} and routine metabolic rate for steelhead embryos. Note that equations describe only the linear portion of the curves.

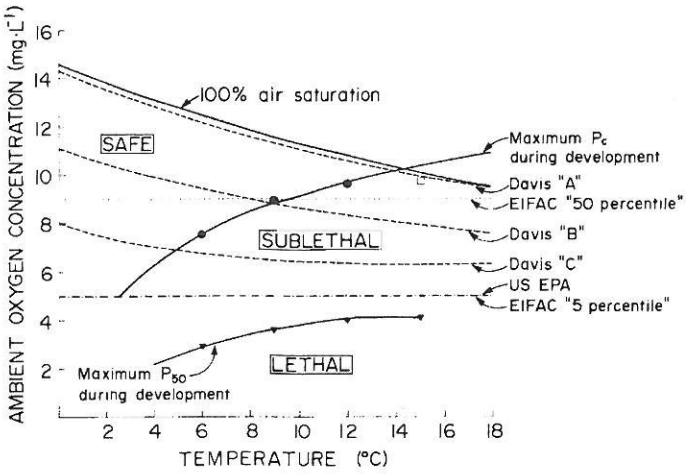

FIG. 9. Proposed dissolved oxygen criteria for steelhead embryos and alevins as a function of temperature (T). Proposed criteria are based on the maximum critical oxygen levels (P_c(max)) observed at 6, 9, and 12°C and are described by the equation $P_c(\text{max}) = 2.07 + 3.06 \ln T$. Maximum P_{50} values approximate oxygen levels at which significant mortality can be expected. Proposed criteria are compared with those of other authors (European Inland Fisheries Advisory Commission 1973; U.S. Environmental Protection Agency 1977; Davis 1975). The open square is the maximum P_c observed immediately before the premature hatch of embryos incubated at 15°C.

(1932) value for the average metabolic intensity of *Salmo trutta* embryos and alevins at 12°C (836 $\mu g \cdot g^{-1} \cdot h^{-1}$) is more than twice that recorded here for steelhead (405 $\mu g \cdot g^{-1} \cdot h^{-1}$)). The fourth major difficulty in comparing literature values relates to the effect of temperature on metabolic rate. Although juveniles and adults have been well studied in this respect, too little is known of metabolism–temperature relationships during early life to be able to adjust measured metabolic rates to a standard temperature with any degree of precision.

It is known that temperature has a more pronounced effect on the metabolic rates of embryos and larvae than on those of juveniles and adults. Values of Q_{10} usually approximate 2.0 for both standard and active metabolism in older fish (Fry 1971). In contrast, Q_{10} values for salmonid embryos and alevins are in the range of 3.0 (this study) to 5.0 (Gruber and Wieser 1983). High Q_{10} values are also typical of teleost developmental rates (Fry 1971). The more pronounced effect of temperature during early life may reflect narrower ranges of thermal tolerance. Gruber and Wieser (1983) point out that large temperature coefficients are characteristic of stenothermal organisms. It is interesting that the Q_{10} for respiration and development are similar. Johns and Howell (1980) suggested that this may explain why, in many species, early growth efficiency varies remarkably little with temperature.

Critical oxygen levels (P_c) varied significantly with temperature and stage of development. This is a reflection of changes in both metabolic rate and overall resistance to respiratory gas exchange. Critical levels tend to increase with increasing metabolic rate during the embryonic period and, as discussed previously, metabolic rate increases with temperature and tissue mass. Overall resistance to respiratory gas exchange obviously changes as well but at present few details are known. One event that appears to be particularly important is the development of a functioning neuromuscular system and the concomitant ability of the embryo to stir the perivitelline fluid. Microelectrode studies indicate that during early embryonic development the major resistance to gas exchange is the unstirred perivitelline fluid (Berezovsky et al. 1979). Embryos begin to move spontaneously shortly after blastopore closure. According to Reznichenko et al. (1977), who used a modified polarographic electrode to model gas exchange in fish eggs, the resulting stirring of the perivitelline fluid should dramatically decrease overall resistance. The rather abrupt decrease shortly after blastopore closure in the instantaneous slope of the line relating embryonic P_c and $\dot{V}o_2$ in steelhead (Fig. 8) suggests that this is, in fact, exactly what occurs. As indicated by the subsequent linear relationship between P_c and $\dot{V}o_2$, overall resistance to gas exchange remains relatively constant once the embryo begins to stir the perivitelline fluid. Overall resistance drops abruptly at hatch as the embryo escapes the confines of the egg capsule (zona radiata). In both steelhead (this study) and Atlantic salmon (Hayes et al. 1951) this is manifested by a drop of 2–3 $mg \cdot L^{-1}$ in P_c. Critical levels declined gradually during the first half of the alevin stage even though metabolic rate continued to increase. This appears to be possible because of a rapid expansion in gill surface area. Morphometric data are not available for salmonids but studies of other families have shown that gill area – mass exponents are considerably greater than unity during the early larval period (DeSilva 1974; Oikawa and Itazawa 1985). In those species studied, gill area – mass exponents decline toward the end of the larval period. A similar decline in the rate at which the gills expand may explain why P_c values remained relatively constant in steelhead during the latter half of the alevin period.

The results of this study, in addition to contributing to an understanding of the ontogeny of respiratory gas exchange in fish, have practical application. It was pointed out in the Intro-

duction that, although it is generally recognized that salmonids are extremely sensitive to low oxygen levels during early life, it has proved difficult to define sublethal response thresholds. A few studies, for example those of Silver et al. (1963) and Shumway et al. (1964), have provided estimates for selected stages at single temperatures but the current study is the first to provide continuous estimates of incipient limiting oxygen levels at a broad range of temperatures. Now that this information is available, it becomes possible to consider developing comprehensive dissolved oxygen criteria for young steelhead.

The minimum dissolved oxygen concentrations required for optimal growth and survival of steelhead embryos and alevins can be predicted relatively precisely for controlled conditions (e.g., laboratory, hatchery) using the relationships between critical oxygen level and time and temperature given in eqs. 7 and 8 (see Rombough (1986) for information as to how these relationships can be modified for application to hatchery situations). More general guidelines are required for natural waters where exact spawning times and temperature regimes are seldom known. Bearing in mind that criteria suitable for general application must be relatively simple as well as provide adequate protection, the most appropriate basis for such criteria would seem to be incipient limiting oxygen concentrations just before hatch. Critical levels are maximal at this time, thus providing adequate protection for all stages, and vary in a simple way with temperature (Fig. 9). Values can be predicted for any temperature using the following equation:

$$[9] \quad P_c(\max) = 2.07 + 3.06 \ln T$$

where $P_c(\max)$ is the critical level just before hatch (in $mg \cdot L^{-1}$) and T is the current ambient temperature (°C). Equation 9 is based on data from the 6, 9, and 12°C tests only. Data from the 15°C test were not included because of poor survival and premature hatching at that temperature, for which insufficient oxygen may have been at least partially responsible. Equation 9 predicts critical oxygen levels in excess of 100% ASV during the later stages of embryonic development at 15°C. It is well documented that embryos respond to hypoxia by hatching prematurely (Ishida 1985).

Oxygen concentrations below the critical level have a detrimental impact of increasing significance as levels decline. Sublethal effects include retarded development (Garside 1959, 1966), reduced growth (Silver et al. 1963; Shumway et al. 1964), premature hatch (Garside 1966), and premature emergence (Bailey et al. 1980). If oxygen levels continue to drop, death eventually ensues. For steelhead embryos, 100% mortality occurs at oxygen concentrations between 2.6 and 1.6 $mg \cdot L^{-1}$ at 9.5°C (Silver et al. 1963). Lethal levels have not been determined for steelhead embryos at other temperatures or for alevins. Until this information is available, it is proposed that maximum P_{50} values be used as rough estimates of incipient lethal levels (Fig. 9).

The criteria proposed here for natural waters (eq. 9) differ significantly from those advanced in the past by others (Fig. 9). The European Inland Fisheries Advisory Commission (1973), the U.S. Environmental Protection Agency (1977) and Alabaster and Lloyd (1980) proposed simple temperature-independent criteria, with the same standard applied to all life stages. The current study, however, indicates that the levels selected (5–9 $mg \cdot L^{-1}$) are not high enough to completely protect steelhead during embryonic development, especially at higher temperatures. Davis (1975) proposed more complicated standards that take into account stage sensitivity, temperature, and the level of protection desired. He suggested that for complete protection (level A) ambient oxygen levels should not fall below 98–100% of air saturation during early life at any temperature (Fig. 9). This would certainly protect developing steelhead but the proposed criteria are somewhat unrealistic both biologically and economically. Under Davis' (1975) scheme, acceptable oxygen concentrations would decline as temperatures increase. However, as pointed out previously, biological demands for oxygen increase, not decrease, as temperatures rise. Economically, it might be difficult to justify maintaining oxygen concentrations near 100% ASV at low temperatures when considerably lower concentrations would be perfectly adequate.

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