



## Growth of swimming muscles and its metabolic cost in larvae of whitefish at different temperatures

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The temperature relationship of routine metabolic rate ( $R_r$ ) of non-feeding, non-growing *Coregonus lavaretus* larvae between 2 and 15° C is characterized by  $Q_{10}$ -values ranging from 1.8–2.45. The rate of growth, based on weight determinations, of first-feeding larvae amounted to 3.5, 7.6 and 9.4% day<sup>-1</sup> at 5, 10 and 12° C respectively, from which  $Q_{10}$ -values between 4.0 and 4.8 can be calculated. The rate of increase of muscle mass between 5 and 10° C, based on the determination of the cross-sectional area of inner muscle fibres, resulted in a  $Q_{10}$ -value of 4.5. Water temperature influenced the pattern of growth of the inner muscle fibres. At hatching, after 360 day degrees, total muscle mass of larvae reared at 4 and 8° C was independent of temperature, but at 4° C the rate of mass increase owed more to *hyperplasia* (increase in fibre number) than to *hypertrophy* (increase in fibre mass), whereas at 8° C the opposite was the case. The calculation of power budgets (including the metabolic cost of growth) of first-feeding larvae yielded net conversion efficiencies ( $K_2$ ) increasing with temperature from 46.3% at 5° C to 54.7% at 12° C. Comparing our data with literature data two general conclusions can be drawn. (1) In first-feeding larvae the *net*, but not the *gross*, conversion efficiency of food energy increases with temperature. This is due to net energy input being characterized by a much higher  $Q_{10}$ -value than energy expenditures. (2) In embryos of freshwater fish so far investigated hyperplasia plays a greater role in the increase of fibre mass than hypertrophy at the *lower* temperature, whereas in embryos of marine fish hyperplasia prevails at the *higher* temperature. It is suggested that this discrepancy correlates with the high concentration of free amino acids in the eggs of marine species which provide an additional, easily available, source of metabolic energy absent in freshwater species.

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Key words: oxygen consumption; growth; metabolic cost of growth; hyperplasia; hypertrophy; conversion efficiency; temperature relationships; embryos; larvae.

### INTRODUCTION

The precise shape of the temperature relationship of complex physiological functions depends on many factors and their interactions. The ensuing heterogeneity of temperature-specific patterns in poikilothermic animals has been discussed by Wieser (1973) and others, but our understanding of its causes, phenotypic and genotypic, is still quite poor. The following two patterns of temperature relationship have been observed frequently. (1)  $Q_{10}$ -values of biological rates may differ greatly over the temperature range of a species; often they are high towards the lower end of the range but low in the zone of thermal preference. (2) In one and the same individual different functions, or components of functions, may be characterized by different temperature relationships.

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This leads to complex relationships of derived properties, like measures of efficiency. Actually, pattern number 1 may be the consequence of pattern number 2, but it is usually difficult to establish the connection between the two.

In this paper we address the problem of the temperature relationship of growth rate and growth efficiency in fish, with particular reference to fast-growing larvae. In reviews on growth and energetics in fish one often finds the generalized statement that the functional relationship between temperature and gross growth efficiency, i.e.  $P_{\text{som}}/C$ , or  $K_1$  according to Ivlev (1945), where  $P_{\text{som}}$  is rate of growth and  $C$  the rate of ingestion, can be represented by an asymmetric bell-shaped curve with the maximum somewhere in the upper half of the biokinetic temperature range of the species (Elliott, 1976; Brett, 1979; Brett & Groves, 1979; Wootton, 1990; Jobling, 1994). The steep increase of this function in the lower temperature range is due to the fact that the maximum rate of energy input,  $C_{\text{max}}$ , rises more steeply with temperature than the maintenance rate of energy expenditure ( $R_m$ ), causing an increase of the scope for growth (Brett, 1976; Kamler, 1992). The decrease of the function in the upper temperature range is due to  $C_{\text{max}}$  reaching a ceiling value at a lower temperature, and dropping more steeply, than  $R_m$ .

It is somewhat unexpected that this textbook generalization about the effects of temperature on energy allocation in fish does not appear to hold for embryos and larvae. In two recent reviews on the energetics of fish larvae (Houde, 1989; Houde & Zastrow, 1993) earlier speculations were confirmed that at this stage *gross* conversion efficiency, i.e.  $K_1$ , is independent of water temperature. On the other hand, since in larvae assimilation efficiency was found to decline with temperature the average *net* conversion efficiency, i.e.  $K_2$ , is a positive function of water temperature.

This is an important finding since it allows us to discern, within the repertoire of metabolic strategies directing the acquisition and allocation of food energy in fish, the blueprint of an ontogenetic switch: in adults the mechanisms concerned with food uptake and resorption are capable of responding to an increase of temperature by accelerating the rate of energy input ( $C$ ) beyond that of maintenance expenditure ( $R_m$ ). In larvae, however, the responses of food uptake to changes in water temperature appear to be more constrained so that the temperature coefficient of energy input does not exceed that of expenditures.

Confirmation of this speculation is highly desirable. Since the efficiency ratios calculated by Houde (1989) and Houde & Zastrow (1993) are based on scattered literature data of different origin and uneven quality it seems imperative to determine the major components of energy budgets of fish larvae at different water temperatures in defined populations and under conditions which allow the simultaneous determination of rates of growth and of energy expenditure (Wieser, 1994, 1995).

Our contribution to this topic arose from the possibility of measuring, at a range of temperatures, rates of oxygen consumption, rates of growth, and rates of muscle differentiation, in larvae of the whitefish *Coregonus lavaretus* (L.). In combination with recent considerations on the cost of growth (Wieser, 1994), we are able to provide new information on the temperature dependence of both energy allocation and muscle growth in small-sized, fast-growing fish. We can

show that in this species somatic production responds much more strongly to an increase of temperature than metabolic expenditures for maintenance. Our conclusions are strengthened by the fact that somatic production was established in two independent ways: by measuring (1) the rate of increase of body mass and (2) the rate of increase of the cross sectional area of the swimming muscles.

## MATERIALS AND METHODS

### FISH

Artificially fertilized eggs from two populations of whitefish were used as the source for the embryos and larvae studied in this investigation. One batch of eggs originated in Lake Pyhäselkä, eastern Finland, the other in Lake Constance at the border between Austria, Germany and Switzerland. The eggs of both populations were transferred to Innsbruck where they were reared in the aquarium of the Institute of Zoology. In none of the subsequent studies did we observe morphological or physiological differences between the two populations. Eggs were incubated in holding tanks at 4 and 8° C until hatching. After hatching the larvae were transferred to aquaria in which the temperature had been raised to 5 and 10° C respectively. A third group, taken from the 8° C holding tank, was transferred to 12° C. The larvae were fed once every hour with *Artemia* nauplii dispensed by an automatic feeding system. Throughout the investigation mortality of embryos and larvae was very low.

### RESPIROMETRY

Rates of oxygen consumption were measured at a series of temperatures with the intermittent respirometer system described previously (Forstner, 1983; Wieser *et al.*, 1988). Two three-chamber systems were available, so that up to six parallel runs on aliquot groups of larvae could be performed per day. Chamber volumes used were 79–89 and 139–167 cm<sup>3</sup> for the measurements of yolk-sac and feeding larvae respectively. The number of larvae per chamber varied from 300 (yolk-sac at 2° C) to 10 (largest feeding larvae at 12° C). Yolk-sac larvae [average body weight 4.8–6.1 mg wet body mass (WBM)] were measured at 2, 8, 10 and 12° C; feeding larvae (11.3–300 mg WBM) at 4, 8, 10 and 12° C. In accordance with previous studies (Wieser & Medgyesy, 1990*a,b*), the mass-specific routine rate of metabolism of the larvae proved to be nearly mass-independent within the mass range studied (e.g. at 10° C:  $b = -0.023 \pm 0.042$  S.E.). In consequence, no correction for differences in body mass were applied. Groups of larvae were taken from the rearing temperatures of 5 and 10° C and transferred to the experimental temperatures in steps not exceeding 3° C day<sup>-1</sup>. We never observed differences in rates between fish acclimated to 5 or 10° C, thus no compensatory metabolic adjustments seem to have played a role in these fish. Before the beginning of the measurements larvae were deprived of food for 8–20 h depending on temperature and size. Experimentally determined evacuation rates were used for the calculation of fasting periods (Karjalainen *et al.*, 1991). In each chamber oxygen consumption was measured intermittently 15 min h<sup>-1</sup> for one whole day. The values reported in Table I represent means and S.D. of the 24-hourly values, each of which resulted from extrapolating the 15-min readings to the full hour. Oxygen consumption of the experimental system without fish was determined at the beginning and at the end of each run. The respirometer systems were cleaned and disinfected every week.

### SAMPLING PROTOCOL

For the determination of total length, wet and dry body mass, random samples of about 10 larvae acclimated to 5, 10 and 12° C were taken at the beginning and the end of the experiment. For the morphometry of swimming muscles, larvae acclimated to 4 and 8° C (from eye stage to hatching) or 5 and 10° C (from hatching to end of experiment)

TABLE I. Routine metabolic rate ( $R_p$ ) in yolk-sac stage (4.8–6.1 mg WBM) and feeding larvae (11.3–300 mg WBM) of *C. lavaretus* measured at a range of temperatures

$T$ ( $^{\circ}$ C)	Yolk-sac stage (g WBM $^{-1}$ h $^{-1}$ )			Feeding larvae (g WBM $^{-1}$ h $^{-1}$ )		
	$\mu$ mol O $_2$	J	$n$	$\mu$ mol O $_2$	J	$n$
2	5.2 (0.9)	2.3 (0.4)	3			
4				10.2 (0.5)	4.6 (0.2)	3
8	11.7 (1.2)	5.3 (0.5)	9	12.3 (0.8)	5.5 (0.4)	12
10	15.3 (0.7)	6.9 (0.3)	9	14.6 (1.4)	6.6 (0.6)	24
12	16.8 (0.5)	7.6 (0.2)	9	17.6 (2.4)	7.9 (1.1)	18
15*				22.7 (2.2)	10.2 (1.0)	3

$n$  refers to number of independent measurements with groups of fish. Mean values and S.D. (in parentheses) are given in  $\mu$ mol O $_2$  and Joule (J).

\*Data from Wieser & Medgyesy (1990b).

were sampled at four dates in such a way that thermal age [expressed in day degrees (DD), after fertilization] was identical in the two acclimation groups, i.e. 360 (hatching), 515, 630 and 690. This corresponds to the following ages after hatching (in days): 5 $^{\circ}$  C: 0, 31, 54, 66; 10 $^{\circ}$  C: 0, 16, 27, 33.

#### HISTOLOGY AND MORPHOMETRY

For light microscopy whole specimens were incubated in a formaldehyde-glutaraldehyde fixative according to Karnovsky (1965) buffered with 0.05 M sodium cacodylate at pH 7.2. From each sample to be studied (four dates at each of two temperatures) three specimens were cut transversely at the level of the anal region. It should be pointed out that in salmon no histological differences between somites were found in this region by Usher *et al.* (1994). The posterior halves of the cut specimens were dehydrated in alcohol series up to 100% and embedded in Kulzer's Technovit 7100 cold-curing resin. Semithin sections (3  $\mu$ m) were cut on a Reichert 2030 rotation microtome and stained with a periodic acid methamine silver (PAMS) method according to the LKB Manual on Preparation Techniques and Plastic Histology. This staining method shows good specificity for the basal lamina and thus led to excellent contrast enhancement of the fibre outline. Digitizing was done by averaging 32 frames per image, followed by linear contrast stretching. Finally the biff-images with a dimension of 768  $\times$  512 pixels and a pixel aspect ratio of 1 : 1 were converted into a suitable format (TIFF, IBM PC Byte Order) with the aid of a graphics file format converter (DeBabelizer, Equilibrium Technologies). Images were processed with an Apple Macintosh Quadra 950 computer using the public domain image processing program NIH Image (written by Wayne Rasband at the National Institutes of Health and available via Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disc from NTIS, 5285 Port Royal Rd, Springfield, VA 22161, part number PB93-504868) and a SCION LG-3 Frame Grabber card. Further processing required measuring cross sectional areas of the inner white fibres of the epaxonic musculature using the image analysis program OPTIMAS (Bio Scan, Incorporated, 170 West Dayton, Suite 204, Edmonds, WA 98020). As the staining method used led to a relatively clear separation of the muscle fibre cross sections from the surrounding material, most of the fibres were identified automatically in the multiple mode of the software package. To separate touching fibres, contrasts were enhanced further manually by drawing around single profiles in the retouch mode of OPTIMAS.

## RESULTS

### METABOLIC RATE

Oxygen consumption was measured in spontaneously swimming yolk-sac and feeding larvae, the latter with guts evacuated. Thus the rates measured correspond to a routine or maintenance rate of energy metabolism ( $R_r$ ,  $R_m$ ; see Wieser, 1995) (Table I). For the feeding larvae the results of previous measurements on the same species at 15° C have been added.

### GROWTH

The variation in size of the fish studied is indicated by the following weight determinations [WBM in mg, means  $\pm$  s.d. ( $n$ ): at hatching (360 DD): 4° C:  $6.0 \pm 0.52$  (15); 8° C:  $6.0 \pm 0.75$  (15); after 690 DD: 5° C:  $52.4 \pm 2.23$  (10); 10° C:  $55.3 \pm 1.49$  (10).

In the larvae acclimated to 5 and 10° C rates of growth were determined during three intervals between 360 and 690 DD (corresponding to 66 and 33 days after hatching at 5 and 10° C respectively). The average relative rates of growth in these two groups were  $3.5 \pm 1.7$  and  $7.6 \pm 2.1\%$  day<sup>-1</sup> at 5 and 10° C respectively. In the larvae acclimated to 12° C the rate of growth was determined only once during the interval between hatching and 648 DD (24 days), yielding a value of 9.4% day<sup>-1</sup>. Since dry body mass (DBM) of the larvae represented a constant proportion of wet body mass (WBM) irrespective of temperature (DBM=0.19 WBM;  $r=0.995$ ,  $n=185$ ), the growth rates determined apply to both expressions of body mass.

### GROWTH OF MUSCLE FIBRES, MORPHOMETRY

As in other species, the larvae of whitefish at hatching possess a single layer of small-diameter muscle fibres which is present beneath almost the entire surface of the skin (superficial muscle fibres: Batty, 1984; red layer: El-Fiky *et al.*, 1987). The fibres of this layer are densely packed with mitochondria, causing the contractile filaments to form a loose, irregular pattern (Hanel, 1995). The space between the red layer and the central organs is filled by the inner muscle fibres (Raamsdonk *et al.*, 1978), packed with contractile filaments, which will develop into the typically glycolytic white fibres of adult fish. In the larvae both the outer and the inner muscle fibres contain more mitochondria than the corresponding fibres of juveniles and adults, attesting to the predominantly aerobic nature of larval metabolism (Wieser, 1995).

In agreement with findings in other species the differentiation of the inner muscle fibres of *C. lavaretus* is clearly influenced by the rearing temperature (Stickland *et al.*, 1988; Vieira & Johnston, 1992; Brooks & Johnston, 1993; Johnston, 1993; Usher *et al.*, 1994). In the course of development the muscle mass increases either by the generation of new fibres from myogenic cells (*hyperplasia*) or by mass increase of the old fibres (*hypertrophy*). The increase in cross sectional area of the swimming muscles during development can be illustrated by plotting fibre area against fibre number. Figures 1 and 2 give an impression of the relationship between hyperplasia and hypertrophy at hatching of embryos of whitefish incubated for 360 DD at 4 and 8° C, and of the larvae after being maintained for another 330 DD at 5 and 10° C. Incubation

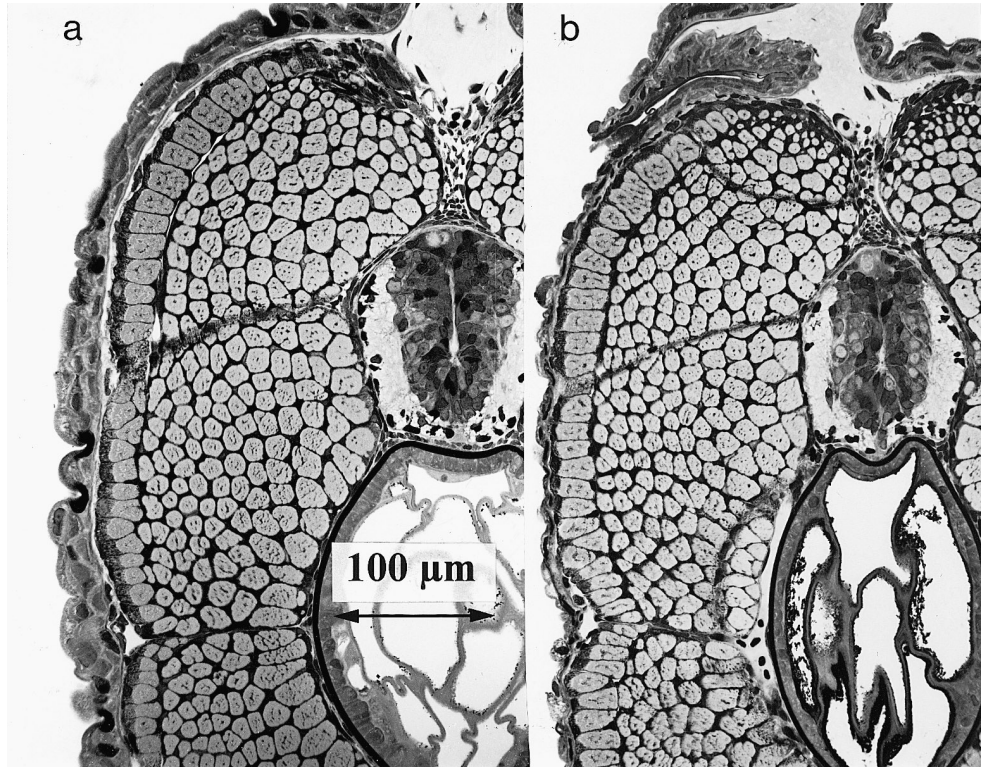


FIG. 1. Transverse sections of one quadrant of epaxial muscles in larvae of *C. lavaretus*, 1 day after hatching, reared at (a) 8°C or (b) 4°C. Fish were fixed in Karnovsky, semithin (3 μm) sections stained with periodic acid methamine silver (PAMS).

temperature had a considerable influence on fibre differentiation up to the end of the yolk-sac period of the embryos. At hatching, average cross-sectional area of the inner muscle fibres was  $92.2 \pm 7.4$  and  $112.8 \pm 4.4 \mu\text{m}^2$  in the 4- and 8°C-acclimated groups respectively. The total number of fibres in the middle of the larval body was  $827 \pm 48$  and  $657 \pm 61$  in the two groups ( $n=3$ ) (Table III). The total cross-sectional area in the two groups of larvae was nearly identical at hatching, i.e. 0.076 and 0.074 mm<sup>2</sup>, but hyperplasia contributed more to the increase of muscle mass in the cold-acclimated than in the warm-acclimated fish.

In contrast to the events during embryonic development, in the free feeding larvae the muscle fibres of the cold-acclimated group experienced a boost in mass increase (hypertrophy), those of the warm-acclimated group a boost in the increase of fibre number (hyperplasia). After 330 DD, that is 66 and 33 days after hatching at 5 and 10°C respectively, the total cross sectional area of muscle fibres increased about tenfold but was similar (0.76 and 0.80 mm<sup>2</sup>) in the fish acclimated to the two experimental temperatures.

#### SOMATIC PRODUCTION AND POWER BUDGETS

On the basis of the determination of body mass of larvae acclimated to 5, 10 and 12°C somatic production at three temperatures can be calculated for larvae between hatching and approximately 690 DD after fertilization. By means of

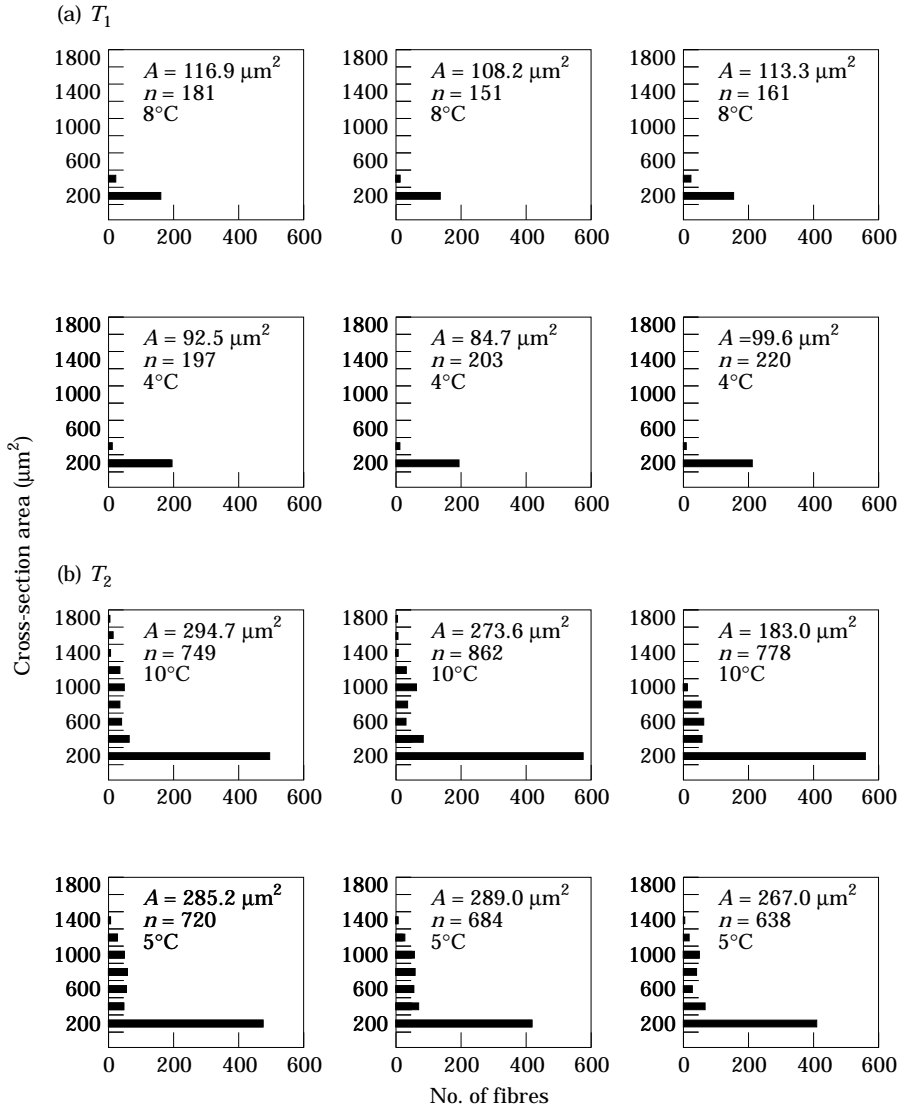


FIG. 2. Cross-sectional area and number per quadrant of inner muscle fibres in larvae of *C. lavaretus* reared at two temperatures. Two developmental stages are compared, each represented by three specimens: (a)  $T_1$ , 1 day after hatching; (b)  $T_2$ , 330 day degrees after hatching (for further details see Table II). Means of cross-sectional area ( $A$ ) and number of fibres per quadrant ( $n$ ) indicated for each specimen.

the two relationships:  $\text{DBM} = 0.19\text{WBM}$ , and  $1 \text{ mgDBM} = 22.7 \text{ J}$  (Wieser & Medgyesy, 1990b), mass production can be converted to rate of energy deposition. A second rate of growth can be calculated by measuring the muscle cross-sectional area at  $T_1$  and  $T_2$  (Table II) and calculating the rate of increase of this variable. If the changes occurring in the body region at which the cross sections were taken are considered to be representative of the changes in the whole body, the rate of increase of muscle cross sectional area corresponds to the rate of increase of total muscle mass. The fact that the two rates of growth

TABLE II. Aspects of the differentiation of the central muscle mass and of growth in early larvae of *C. lavaretus* at three temperatures

Variable	(4) 5° C	(8) 10° C	(8) 12° C
$T_1$			
Day degrees	360	360	c. 360
Fibre cross section ( $\mu\text{m}^2$ ) ( $A$ )	92.2	112.7	
No. of fibres ( $n$ )	827	657	
$A \times n$ ( $\text{mm}^2$ )	0.076	0.074	
WBM (mg)	6	6	5.5
Total length ( $l$ )	11	11	
$T_2$			
Day degrees	690	690	c. 648
Fibre cross section ( $\mu\text{m}^2$ ) ( $A$ )	280	251	
No. of fibres ( $n$ )	2723	3185	
$A \times n$ ( $\text{mm}^2$ )	0.76	0.80	
WBM (mg)	52.4	55.3	47.6
Total length ( $l$ )	23.3	23	
$T_1 \rightarrow T_2$			
Day degrees	330	330	c. 288
Days	66	33	24
$\Delta A$ ( $\text{mm}^2 \text{ day}^{-1}$ )	0.0104	0.022	
$\Delta \text{WBM}$ (mg)	46.4	49.3	42.1
$\Delta \text{DBM}$ (mg)	9.2	9.8	8.4
$\text{J day}^{-1}$ (1 mg DBM=22.7 J)	3.16	6.74	7.9
Average WBM (g)	0.023	0.0245	0.0211
$P_{\text{som}}$ ( $\text{J g WBM}^{-1} \text{ h}^{-1}$ )	5.56	11.1	15.2
$\Delta l_t$ (mm)	12.3	12.0	
$l \cdot \text{day}^{-1}$ (mm)	0.186	0.364	
$R_g$ ( $\text{J g WBM}^{-1} \text{ h}^{-1}$ )	1.70	3.41	4.67

The eggs of the larvae observed at 5 and 10° C had developed at 4 and 8° C respectively and hatched at 360 day degrees (DD) ( $T_1$ ). The eggs of the larvae observed at 12° C had developed at 8° C and were transferred to the new temperature at hatching. In this batch the number of DD after fertilization can only be approximated, but in the other two batches the observation period ended after 690 DD ( $T_2$ ). The histological data are mean values (individual values given in Fig. 2) of three, the morphological data of 10–15, specimens, at each of the two sampling dates.

Cost of growth ( $R_g$ ) calculated on the basis of the calibration curve in Wieser (1994).

calculated ( $\Delta A$  and  $P_{\text{som}}$  in Table II) are very similar proves the validity of this assumption. An estimate of the contribution of production to the total energy budget of the larvae requires an estimate of the metabolic cost of growth. This is possible by using the empirical calibration curve recently published (Wieser, 1994) which yields a metabolic 'work load' of approximately 15  $\mu\text{mol O}_2$  for the deposition of 1 mgDBM with a caloric value of about 22 J. The data required for the calculation of the rates of production processes in larvae of *C. lavaretus* at three temperatures are summarized in Table II,  $T_1$  referring to the time of hatching of all groups of fish at about 360 DD,  $T_2$  to the end of the experiment approximately 288 (12°) and 330 (5, 10° C) DD after hatching.

By converting metabolic expenditures and growth to the same units ( $\text{J g WBM}^{-1} \text{ h}^{-1}$ ) a power budget for small larvae can be constructed (Table III). There are two components of metabolic expenditures: (1) the routine



TABLE III. Power budgets (unit: J g WBM<sup>-1</sup> h<sup>-1</sup>) of larvae of *C. lavaretus* between approximately 360 (hatching) and 690 DD after fertilization (see Table II for details) at three experimental temperatures

Function	5° C	%	10° C	%	12° C	%
$R_r$	4.8	(74)	6.6	(66)	7.9	(63)
$R_g$	1.7	(26)	3.4	(34)	4.7	(37)
$R_{tot}$	6.5	(100)	10.0	(100)	12.6	(100)
		53.7		47.4		45.3
$P_{som}$	5.6	46.3	11.1	52.6	15.2	54.7
$C_m$	12.1	100	21.1	100	27.8	100

Relative values (%) are based either on  $C_m$  or on  $R_{tot}$ , the latter in parentheses. The ratio  $P_{som}/C_m$  represents the net growth efficiency ( $K_2$ ).  $R_r$ , Routine rate of metabolism;  $R_g$ , cost of growth (see Table II);  $R_{tot}$ , total metabolic expenditures;  $P_{som}$ , growth;  $C_m$ , metabolizable food energy, i.e.  $R_{tot} + P_{som}$ ; WBM, wet body mass.

or maintenance rate ( $R_r$ ) of spontaneously swimming, non-feeding, non-growing larvae as summarized in Table I; (2) the cost of growth, i.e. the metabolic expenditures connected with the deposition of body mass in larvae fed *ad libitum* ( $R_g$ ). This component has been calculated on the basis of the rates of growth ( $P_{som}$ ) (Table II) and the calibration curve in Wieser (1994). Between 5 and 12° C both  $P_{som}$  and  $R_r$  increased with temperature (Fig. 3, Table III), but the power invested in the form of body mass increased more steeply than the power expended in maintenance metabolism. This divergence resulted in an increase in net production efficiency, i.e.  $P_{som}/(P_{som} + R_{tot}) \times 100$ , from about 46% at 5° C to about 55% at 12° C.

#### EFFECTS OF TEMPERATURE ON DIFFERENT BIOLOGICAL FUNCTIONS

The data summarized in Tables I–III allow the determination of temperature coefficients ( $Q_{10}$ -values) of the following biological functions: (1) metabolic rates; (2) rate of muscle growth based on the morphometry of muscle fibres as presented in Fig. 2; (3) rate of body growth ( $P_{som}$ ). Somatic production increases with temperature more steeply than rate of metabolism (Table IV)—with the exception, of course of  $R_g$  which is directly proportional to  $P_{som}$ . It is reassuring that two completely different methods for the determination of production, the calculation of muscle cross sectional area from histological sections, and the determination of body weight, led to approximately the same  $Q_{10}$ -value of about 4.0, more than twice the value for the maintenance rate of metabolism.

### DISCUSSION

Our data contribute to two aspects of the temperature relationships of fish: the effect of acclimation temperature on growth efficiency and energy allocation; and the effect of rearing temperature on muscle growth ('tissue cellularity').

The literature suggests that gross growth efficiency ( $K_1$ ) is temperature independent in the larvae of fish (Paloheimo & Dickie, 1966; Houde, 1989; Houde & Zastrow, 1993), whereas in juveniles and adults  $K_1$  increases with temperature up to a critical threshold (Elliott, 1976; Brett, 1979; Brett & Groves,

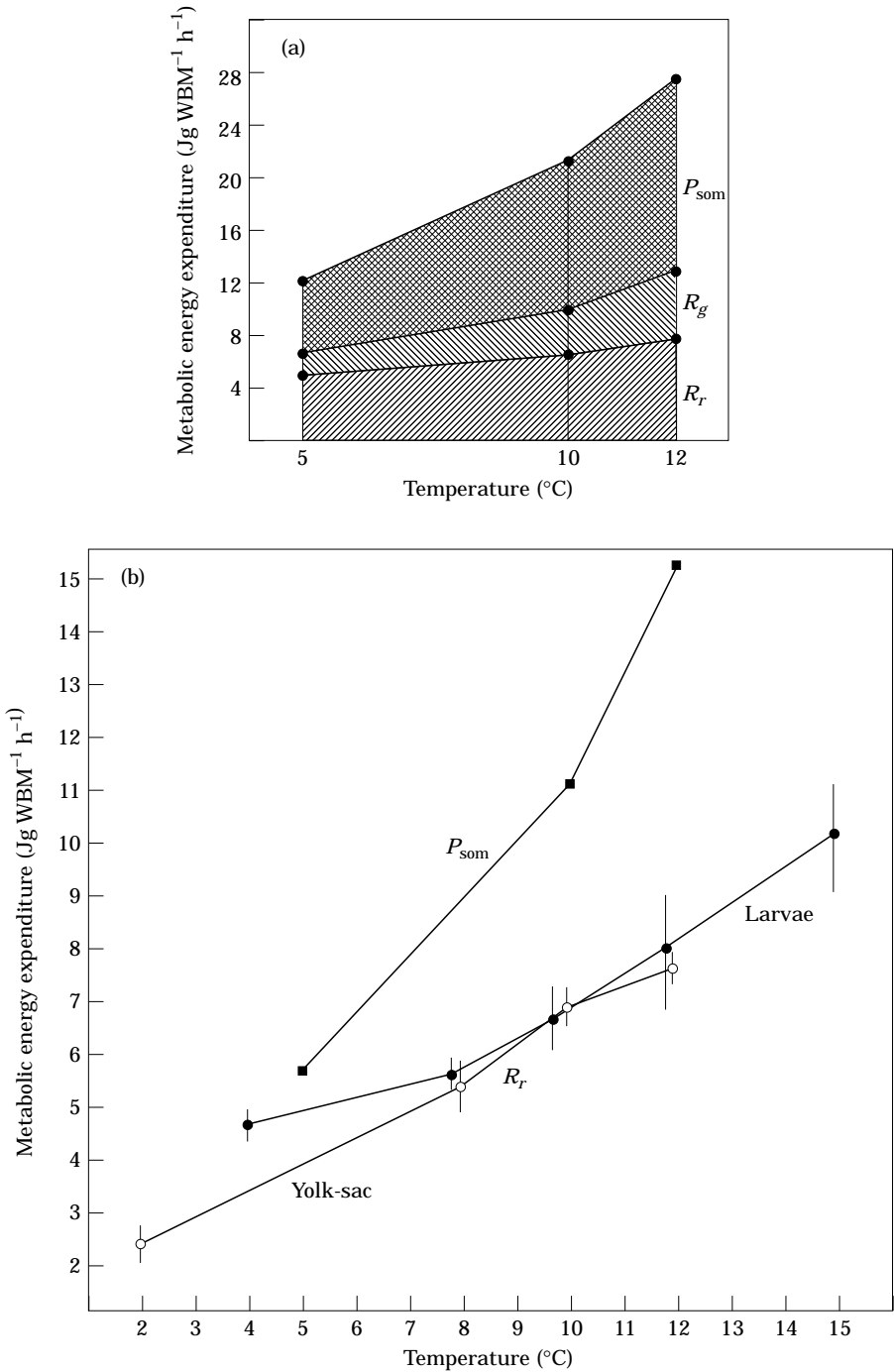


FIG. 3. Metabolic energy expenditures and somatic production ( $P_{som}$ ) in larvae of *C. lavaretus* at a range of temperatures. Oxygen consumption and growth expressed in energy equivalents, based on the following conversion factors: 1 mg DBM=22.7 J; 1  $\mu$ mol  $O_2$ =0.45 J. The energy budget (a) is based on the two sampling dates termed  $T_1$  and  $T_2$  in Table II.  $R_r$ , Routine rate of metabolic activity;  $R_g$ , metabolic cost of growth, calculated on the basis of growth rates by means of the calibration curve in Wieser (1994).

TABLE IV.  $Q_{10}$ -values for two temperature intervals of rates of change of physiological and morphological variables in larvae of *C. lavaretus* between approximately 360 (hatching) and 650–690 DD after fertilization (see Table II for details)

Variable (dimension)	Range of temperature (° C)	
	5→10	10→12
$R_r$ (J g WBM <sup>-1</sup> h <sup>-1</sup> )	1.8	2.45
$R_g$ (J g WBM <sup>-1</sup> h <sup>-1</sup> )	4.02	4.8
$R_{tot}$ (J g WBM <sup>-1</sup> h <sup>-1</sup> )	2.3	3.1
$dA$ (mm <sup>2</sup> day <sup>-1</sup> )	4.47	
$dl$ (mm day <sup>-1</sup> )	3.8	
$P_{som}$ (J g WBM <sup>-1</sup> h <sup>-1</sup> )	4.0	4.8
$C_m$ (J g WBM <sup>-1</sup> h <sup>-1</sup> )	3.0	3.9

Abbreviations as in Table III; in addition:  $A$ , total cross-sectional area of inner muscle fibres;  $l$ , total length.

1979; Wootton, 1990; Jobling, 1994). This difference is almost certainly due to the delayed development of the digestive apparatus in fish. In larvae the gut is still short and straight, a feature accompanied by poor feeding performance, fast evacuation rates (Kamler, 1992) and a general tendency of losing digestive enzymes with the faeces (Hofer & Nasir Uddin, 1985; Köck & Hofer, 1989). In consequence, fish larvae may always have to operate close to maximum digestive capacity and be less flexible in their responses to variations in food availability than older fish. This is also reflected in the indirect proportionality between assimilation efficiency and water temperature in marine larvae (Houde, 1989).

However, once food energy has been absorbed, fish larvae appear to be quite flexible in their ability to allocate metabolizable energy to different body functions according to priority of demand. This explains the pattern of energy allocation during periods of fast growth (Wieser, 1994) and is evident from the results of the present investigation which showed  $R_r$  to increase with a temperature coefficient of from 1.8 to 2.45 whereas  $P_{som}$  increased with a temperature coefficient of from 4.0 to 4.8 resulting in an increase of  $K_2$  from 46.3% at 5° C to 54.7% at 12° C (Table III). A similar ratio of increase has been found by Houde (1989) in marine fish, i.e. from 37% at 10° C to 48% at 30° C. A higher net conversion efficiency but with a similar temperature coefficient has been reported for *Rutilus rutilus* (L.) by Wieser *et al.* (1988). In embryos of *Oncorhynchus mykiss* (Walbaum) reared at 9 and 14° C, Kamler (1992) found developmental processes to proceed with a  $Q_{10}$ -value of approximately 3.0 whereas metabolic expenditures were almost temperature independent ( $Q_{10}=1.03-1.07$ ). As far as first-feeding larvae are concerned it should be pointed out, however, that the correct determination of  $K_2$  requires inclusion in the energy budget of the 'cost of growth' ( $R_g$ ). The present paper is one of the few in which this has been done for fish larvae (see also Wieser & Medgyesy, 1990a,b).

The hypothesis presented here is still quite speculative, resting on circumstantial evidence. If corroborated by more studies directed towards the analysis of defined populations it would present yet another example for the ontogenetic

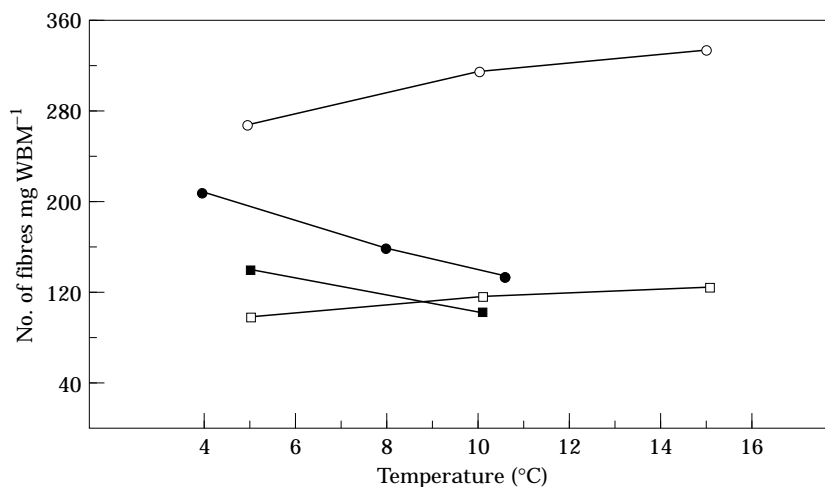


FIG. 4. Relationship between mass-specific fibre number and rearing temperature in larvae of four species of teleosts, 1 day after hatching. Data based on the following references: plaice (*P. platessa*, ○), Brooks & Johnston, 1993, estimated body weight 1.5 mg; herring (*C. harengus*, □), values represent averages of two investigations, Vieira & Johnston, 1992 and Johnston, 1993, estimated body weight 2.3 mg; salmon (*S. salar*, ●), Stickland *et al.* (1988) and Usher *et al.* (1994), estimated body weight 22 mg; white fish (*C. lavaretus*, ■), this investigation, body weight 6 mg. Body weights estimated on the basis of the total length measurements given in the references.

discontinuity marking the transition from the larval to the juvenile life stage in fish (Wieser, 1995).

Recent studies have shown that water temperature influences the process of differentiation of the swimming muscles (Stickland *et al.*, 1988; Vieira & Johnston, 1992; Johnston, 1993; Brooks & Johnston, 1993; Usher *et al.*, 1994). The major point emerging from these studies is that the two pathways leading to an increase of muscle mass, i.e. *hypertrophy* (increase of mass of individual fibres) and *hyperplasia* (increase of number of fibres) are differentially affected by temperature during embryonic development. In embryos reared at different temperatures total muscle mass at hatching is more or less independent of temperature. However, at one temperature the final mass may owe more to hypertrophy, at another temperature it may owe more to hyperplasia. Curiously, however, in the four species investigated so far temperature exerted opposite effects of muscle growth in the embryos (Blaxter, 1992): in plaice *Pleuronectes platessa* L., and herring *Clupea harengus* L. the lower temperature stimulated hypertrophy, the higher temperature hyperplasia, whereas in salmon *Salmon salar* L. and whitefish, the object of the present study, the opposite was the case (Fig. 4).

Usher *et al.* (1994), referring to a paper by Cheek & Hill (1970), put forward the following explanation based on energetic considerations of the finding that hyperplasia prevailed at the lower temperature: (1) in eggs provided with a fixed amount of energy supply in the form of yolk, the energy above maintenance expenditures is potentially available for biochemical syntheses; (2) since metabolic expenditures are positively related to temperature the scope for growth and differentiation may be greater at low than at high temperatures; (3) thus at low

temperatures the more expensive process of hyperplasia, requiring both protein synthesis and nuclear divisions, may be favoured, whereas at high temperatures hypertrophy, requiring only protein synthesis, would be the ontogenetic strategy of choice. This is a speculative suggestion which, however, is in line with the old observation that other meristic characters, particularly the number of vertebrae, show the same trend—numbers decrease with increasing temperature (Garside, 1970).

No explanation is readily available for the opposite case, the increase of fibre number with temperature, as observed in plaice and herring. However, the fact that we found the freshwater species *C. lavaretus* to display the same morphometric response to temperature as the other freshwater-derived species, *S. salar*, allows us to speculate that the response of the two marine species, *P. platessa* and *C. harengus*, may be related to the presence of large amounts of free amino acids in their eggs serving both as osmotic effectors and energy source (Fyhn & Serigstad, 1987; Thorsen *et al.*, 1993; Finn, 1994). If free amino acids provide a quantitatively significant source of easily available nutrients this might counteract the constraints on anabolic processes at higher temperatures characteristic of eggs without such an additional source of energy. This speculation is supported by two observations: firstly, that in free-feeding larvae and juveniles the constraint of high temperature on anabolic processes disappears, faster growth being associated with fibre hyperplasia (Weatherley & Gill, 1987; Higgins & Thorpe, 1990; Fig. 2 and Table II of the present paper); secondly, that in certain trout hybrids characterized by eggs with a much reduced yolk volume, the number of myomeres in the embryos at hatching is also reduced (Garside & Fry, 1959).

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