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Metabolic recovery in herring larvae following strenuous activity

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Larvae of spring spawning Clyde herring *Clupea harengus* L. were reared at 5 and 12° C. Metabolism following burst swimming was studied in 7-day-old larvae at their respective rearing temperatures. Escape responses were repeatedly elicited using tactile stimulation for a period of 3 min. Larval herring were hard to fatigue and still responded to tactile stimuli after 3 min. Whole larvae were freeze-quenched in liquid nitrogen, either immediately after exercise, or after periods of recovery of up to 24 h. Samples were freeze-dried and analysed for whole body creatine (Cr), phosphocreatine (PCr), ATP, ADP, AMP, lactate, glucose, and glycogen using high performance liquid chromatography and enzymatic methods. The exercise regime resulted in a marked decrease in PCr, ATP and glycogen concentrations and an increase in creatine, glucose and lactate concentrations whereas there was no significant change in either AMP or ADP concentrations. The extent of phosphagen hydrolysis (approx. 110 to 15 μmol PCr g^{-1} dry body mass) and lactate accumulation (approx. 7 to 40 μmol lactate g^{-1} dry body mass) over the exercise period was similar at the two temperatures, consistent with a relatively constant degree of effort. The rates of recovery of PCr and ATP were essentially the same at 5 and 12° C; returning to resting levels after approximately 30 min. Lactate and glycogen concentrations were restored 60 min after exercise at both temperatures. Maximum lactate clearance rates (1·2 μmol min^{-1} g^{-1} wet muscle mass) were an order of magnitude faster than reported for adult fish in the literature.

Key words: fish; development; muscle; ATP; metabolism; lactate; exercise; temperature.

INTRODUCTION

The myotomes of newly hatched herring larvae *Clupea harengus* L., comprise a core of around 280 inner muscle fibres surrounded by a single superficial layer of smaller diameter muscle fibres (Batty, 1984; Vieira & Johnston, 1992). Superficial and inner muscle fibres contain high volume densities of mitochondria, e.g. 45 and 25% respectively for larvae reared at 8° C (Vieira & Johnston, 1992; Johnston & Horne, 1994). Larval muscle fibres contain developmental-stage specific isoforms of myosin heavy chains, troponin T, troponin I and myosin light chain 2, and represent different fibre types from those found in juvenile and adult stages (Crockford & Johnston, 1993).

In the early post-hatch stages, herring larvae undergo bouts of sinking and swimming and are almost continuously active providing light levels are sufficient for feeding (Blaxter & Staines, 1971). Muscle contraction during continuous swimming is powered by aerobic metabolism. Predation is a major source of mortality in fish larvae. Larvae respond to tactile stimulation with a C-start achieving escape speeds at hatching of 90–115 mm s^{-1} (7–9 body length s^{-1}) at 5° C and 175–190 mm s^{-1} (15–17 body length s^{-1}) at 17° C (Batty *et al.*, 1993).

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Since escape responses typically consist of less than 10 tail-beats it is likely that they are fuelled chiefly by the hydrolysis of phosphocreatine, which is subsequently replenished by aerobic metabolic pathways.

Temperature has a profound effect on the development of fish embryos and larvae. Recent morphological and ultrastructural studies have shown that developmental temperature influences muscle differentiation and the relative timing of organogenesis (Calvo & Johnston, 1992; Vieira & Johnston, 1992; Brooks & Johnston, 1993; Johnston, 1993). Calvo & Johnston (1992) reported that in turbot *Scophthalmus maximus* L., rearing temperature affected the distribution of muscle fibre types and glycogen storage levels at metamorphosis. There was a greater proportion of superficial red fibres and tonic muscle fibres in turbot reared at 17° C compared with those reared at 22° C. In herring larvae, development temperature influences the number and diameter of muscle fibres at hatching as well as the volume density and spatial organization of muscle fibre organelles (Vieira & Johnston, 1992; Johnston, 1993). For example, herring larvae reared at 15° C had a 64% greater volume density of mitochondria in the inner muscle fibres than larvae reared at 5° C, but had a similar volume density of myofibrils (Vieira & Johnston, 1992).

There have been relatively few studies on metabolism in fish larvae following burst swimming. Compared with juvenile and adult stages muscles in larvae have a relatively low glycolytic capacity and relatively high aerobic capacity, resulting in a more rapid recovery from strenuous exercise (Goolish, 1991; Krumschnabel & Lackner, 1992). The aim of the present study on larvae of the Atlantic herring was to investigate the influence of developmental temperature on metabolism following recovery from repeated bouts of escape swimming.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Adult mature herring were caught in the Firth of Clyde, Scotland in March 1993. Gonads were dissected from the fish, transported to the Dunstaffnage Marine Laboratory and the eggs from six females fertilized with the milt from five males on glass plates (Blaxter, 1968). The plates were transferred to 250-l seawater tanks in controlled temperature rooms maintained at 5 and 12° C with a 16 h L : 8 h D photoperiod. The main hatching occurred after approximately 27 days at 5° C and 12 days at 12° C. All experiments were conducted on 7–9-day-old larvae (yolk-sac partially absorbed).

EXERCISE, ACUTE TEMPERATURE TRANSFER AND SAMPLING PROTOCOLS

Groups of five to 10 1-week-old larvae were transferred to 11·5 cm diameter (1 cm deep) acrylic plastic dishes contained sea water at their respective rearing temperature. Escape swimming responses were repeatedly induced by tactile stimulation with a small glass pipette over a 3-min period. Separate groups of larvae were sampled prior to the exercise periods (resting controls), immediately after exercise, and after periods of recovery (5, 10, 15, 30, 60, 120 and 240 min and 12 and 24 h).

Each sample of larvae was blotted to remove excess salt water and then rapidly quenched in liquid nitrogen, freeze-dried over 24–36 h and stored with a desiccant at − 20° C until analysis. Samples were processed and analysed for whole body creatine, phosphocreatine, ATP, ADP, AMP, lactate, glucose and glycogen concentrations.

SAMPLE PROCESSING AND ANALYSIS

The freeze-dried larvae were weighed on a micro-balance (Mettler) and homogenized with a Duall glass homogenizer in 50–100 vol. 0·3 M perchloric acid and metabolites extracted for 10–15 min on ice. A 50-µl sample was removed for glycogen analyses and the remaining homogenate centrifuged at 13 000 ***g*** for 3 min. The pH of the supernatant was increased to 6·3 with a measured aliquot of K_2HPO_4 and precipitated perchlorate removed by centrifugation. The supernatant was stored on ice for the immediate determination of the high energy phosphates and later analysis of lactate and glucose.

High performance liquid chromatography (HPLC) was used to determine the concentrations of creatine (Cr), phosphocreatine (PCr) and the adenosine compounds, AMP, ADP and ATP in duplicate. The method used was a modification of the protocols described by Sellevold *et al.* (1986) and Moon *et al.* (1991) using a ODS-YMC (5 µm packing) 150 × 4·6 mm column. Metabolites were eluted isocratically with a mobile phase containing 215 mM KH_2PO_4, 2·3 mM tetrabutyl-ammonium hydrogen sulphate and 3·5% acetonitrile, pH = 6·25.

Lactate and glucose concentrations were measured by spectrophotometric methods using Sigma kits Nos 735 and 510, respectively. The assays were modified for use in a 300 µl × 96 well microplate. Samples were run in duplicate or triplicate and absorbances read on a Dynatech MR5000 plate spectrophotometer. Both assays were calibrated and validated with the appropriate standards and controls.

Glycogen was determined in neutralized homogenates by assaying glucose concentration after hydrolysis with amyloglucosidase (Bergmeyer, 1974).

DATA ANALYSIS

Results are presented as means ± S.E.M. Sample number refers to the number of pooled samples (each containing five to 10 individual larvae). The data were normally distributed. Significant differences ($P<0·05$) between resting-control larvae and the recovery times were determined using one-way analysis of variance and unpaired Student's *t*-tests (Minitab). Exponential or logarithmic equations were fitted to the recovery data (from 0 h; immediately after the exercise period) for lactate, Cr, PCr and ATP. Analysis of covariance was used to test for statistical differences between rates of recovery at 5 and 12° C using the regression lines obtained from plots of the natural log of metabolite concentration vs time.

RESULTS

Following tactile stimulation 7-day-old herring larvae exhibited C-starts followed by three to 12 tail-beats. After 3 min repeated stimulation larvae were still capable of escape responses although spontaneous swimming movements became less frequent.

There were no significant differences in the concentrations of each metabolite analysed in the resting larvae at the two rearing temperatures (Table I). At both 5 and 12° C, the 3 min intensive exercise period resulted in a marked decrease in whole body PCr concentrations and a corresponding increase in Cr concentrations (Fig. 1). The magnitude of the changes in these metabolites were similar for the two temperatures. The recovery of Cr approximated an exponential decay and PCr a logarithmic increase (Table II). There was no significant difference (ANCOVA) in the rates of recovery of PCr and Cr concentrations at 5 and 12° C (Fig. 1).

There was no change in whole body ADP or AMP concentrations with exercise at either temperature (Fig. 2). However, at both 5 and 12° C, ATP concentrations decreased by approximately 75% after exercise. ATP concen-

TABLE I. Resting metabolite levels in herring larvae reared at 5 and 12° C (mean ± S.E.M.; $n = 5–7$)

Metabolites (μmol g^{-1} dry body mass)	5° C	12° C
Creatine	88·3 ± 6·0	96 ± 6
Phosphocreatine	107·0 ± 11·1	112 ± 14
ATP	21·4 ± 1·5	20·5 ± 2·2
Lactate	5·83 ± 2·1	6·95 ± 1·3
Glucose	1·80 ± 1·50	2·82 ± 1·50
Glycogen	37·2 ± 3·4	39·6 ± 5·9

trations returned to resting levels after 60–120 min (Fig. 2). There was no significant difference in the rates of recovery of ATP at the two temperatures; the ATP recovery approximating a logarithmic relationship (Table II).

Whole body glycogen concentrations decreased and lactate concentrations significantly increased following 3 min burst swimming (Fig. 3). Lactate increased to maximum levels immediately after exercise, reaching 34·8 and 40·3 μmol g^{-1} dry body mass at 5 and 12° C, respectively. At both 5 and 12° C, glycogen concentrations returned to resting levels after 120 min and lactate levels returned to resting levels after 60 min (Fig. 3). There was no significant difference in the rates of recovery at the two temperatures (Table II). Glucose levels also increased with exercise and at 5° C remained elevated above control levels for 4 h post-exercise (Fig. 3).

DISCUSSION

METABOLIC CHANGES AFTER STRENUOUS EXERCISE

Following tactile stimulation escape responses are of short duration (three to 12 strides), and the fuel supply for muscle contraction is probably largely supplied by the breakdown of phosphocreatine. However, repeated stimulation for 3 min produces a significant accumulation of lactate of the order of 35 μmol g^{-1} dry mass or approximately six-fold over resting levels (Fig. 3). The majority of studies investigating lactate changes in fish with exercise have focused on muscle. One-week-old herring larvae have approximately 40% muscle mass (unpublished observations). If we assume that lactate production/catabolism in other tissues is negligible during short intensive exercise (especially immediately after a short exercise period) then a 15-fold increase in muscle lactate concentration occurred in the herring larvae; lactate increasing to approximately 15 μmol g^{-1} wet muscle mass (calculated using 40% muscle mass and 82·9% water content).

Ferguson *et al.* (1993) found that body size influenced the magnitude of the lactate elevation in the white muscle of rainbow trout *Oncorhynchus mykiss* (Walbaum). There was a positive correlation in the magnitude of the lactate elevation with body size; the larger the trout the greater the lactate increase after exhaustive exercise. Developmental stage also influences the size of the lactate

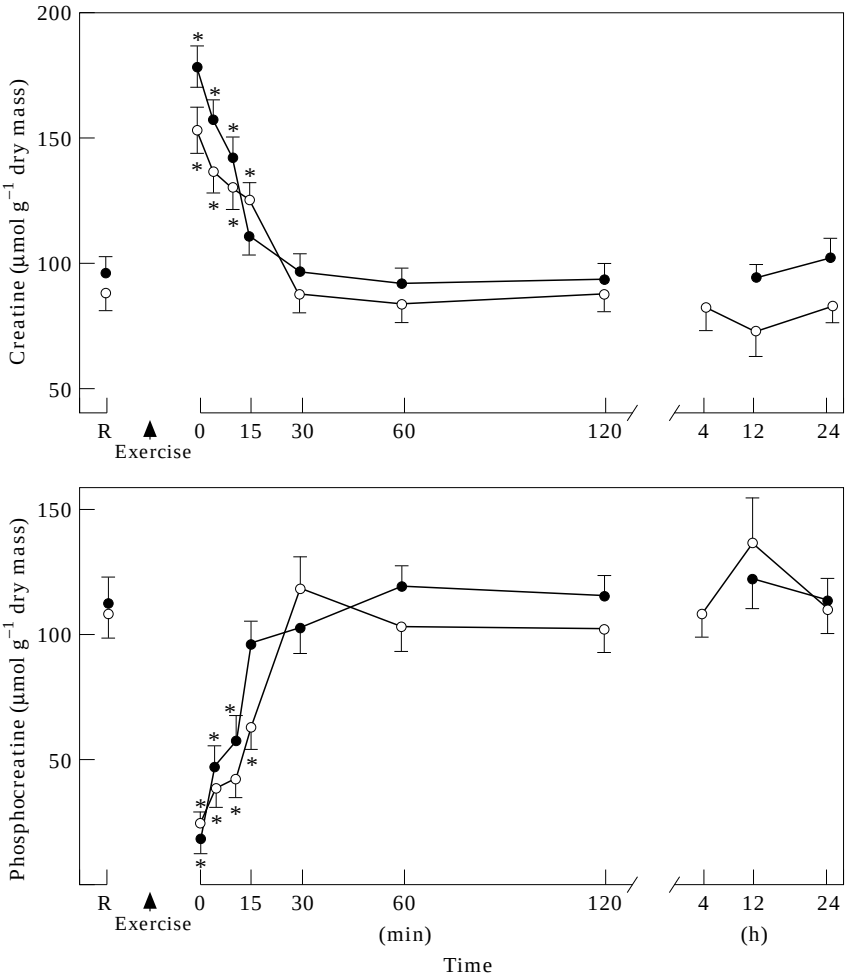

FIG. 1. Whole body phosphocreatine and creatine concentrations in herring larvae after strenuous exercise at 5° C (○) and 12° C (●). Arrowhead, the 3-min exercise period. R, resting controls; *, significant difference from resting controls; results are means ± S.E.M.

increase after strenuous exercise in other species. Krumschnabel & Lackner (1992) reported approximately a seven-fold increase in whole body lactate concentration in roach larvae *Rutilus rutilus* L., subjected to intensive forced activity, yet juvenile roach subjected to the same exercise regime showed a 33-fold increase in lactate concentration. This difference was attributed to a combination of low glycolytic and high oxidative capacity of the larvae. The H_4-isoenzyme of lactate dehydrogenase, which functions in the direction of lactate oxidation, is the dominant form in the muscle of roach larvae. It is slowly replaced with the M_4-isoenzyme of LDH, which reduces pyruvate, concomitant with the development of the gills and appearance of juvenile muscle fibre types (El-Fiky *et al.*, 1987).

In the present study, larvae were still able to elicit escape responses after 3 min stimulation, i.e. they were hard to fatigue which suggests that a high proportion

TABLE II. Metabolite recovery equations for herring larvae subjected to intensive exercise at 5 and 12° C

	Exponential decay	
Creatine		
5° C	$y = 172{\cdot}8\ e^{-0{\cdot}109x}$	$r = 0{\cdot}935$ ($P < 0{\cdot}01$)
12° C	$y = 195{\cdot}6\ e^{-0{\cdot}123x}$	$r = 0{\cdot}964$ ($P < 0{\cdot}01$)
Lactate		
5° C	$y = 50{\cdot}1\ e^{-0{\cdot}316x}$	$r = 0{\cdot}970$ ($P < 0{\cdot}01$)
12° C	$y = 48{\cdot}9\ e^{-0{\cdot}286x}$	$r = 0{\cdot}991$ ($P < 0{\cdot}01$)
	Logarithmic increase	
Phosphocreatine		
5° C	$y = 11{\cdot}70 + 47{\cdot}7 \ln x$	$r = 0{\cdot}936$ ($P < 0{\cdot}01$)
12° C	$y = 12{\cdot}77 + 54{\cdot}5 \ln x$	$r = 0{\cdot}975$ ($P > 0{\cdot}01$)
ATP		
5° C	$y = 2{\cdot}67 + 6{\cdot}27 \ln x$	$r = 0{\cdot}881$ ($P < 0{\cdot}01$)
12° C	$y = 2{\cdot}10 + 7{\cdot}47 \ln x$	$r = 0{\cdot}857$ ($P < 0{\cdot}01$)

y, Metabolite concentration (μmol g^{-1} dry body mass) and *x*, time (min). Correlation coefficient *r*. There were no significant differences in the rates of recovery of metabolites measured in herring larvae reared at 5 and 12° C (ANCOVA).

of their energy needs came from renewable (aerobic) sources. Since oxygen uptake during activity scales to bodymass$^{0{\cdot}75}$ it seems likely that aerobic metabolism pathways are able to fuel a higher proportion of the behavioural repertoire of larval than of adult stages (Goolish, 1991).

Strenuous exercise in adult fish results in ATP synthesis from the adenylate kinase reaction (2ADP ATP + AMP) which is displaced from equilibrium by the activation of AMP deaminase (AMP IMP + NH3) (Driedzic & Hochachka, 1978; Schulte *et al.*, 1992). Following exercise to exhaustion in *Notothenia coriiceps* Richardson, IMP increased from 1·2 to 4·1 μmol g^{-1} dry mass whereas AMP concentrations decreased four-fold (Dunn & Johnston, 1986). In contrast, AMP concentrations were not significantly changed by strenuous exercise in herring larvae which suggests that adenylate kinase and/or AMP deaminase enzymes are present in low concentrations (Fig. 2).

RECOVERY FROM STRENUOUS EXERCISE

Lactate produced in muscle can have several fates (as a gluconeogenic substrate, a glyconeogenic substrate, and/or an oxidative substrate). Evidence from several fish species points to the bulk of lactate produced in the white muscle during intensive exercise being metabolized *in situ* (Batty & Wardle, 1979; Tang & Boutilier, 1991; Moyes *et al.*, 1992; Girard & Milligan, 1992; Milligan & Girard, 1993). In the herring larvae, lactate recovers within 60 min whereas glycogen returns to resting concentrations more slowly (approximately 12 h), which suggests that a high proportion of the lactate produced is oxidized. A similar slower recovery of glycogen than lactate has also been reported for roach larvae (Krumschnabel & Lackner, 1992). Lactate may serve as a substrate for aerobic metabolism to re-establish metabolic homeostasis.

The lactate clearance rates in the herring larvae are particularly impressive and are some of the highest recorded for fish. Maximum clearance rates were in the

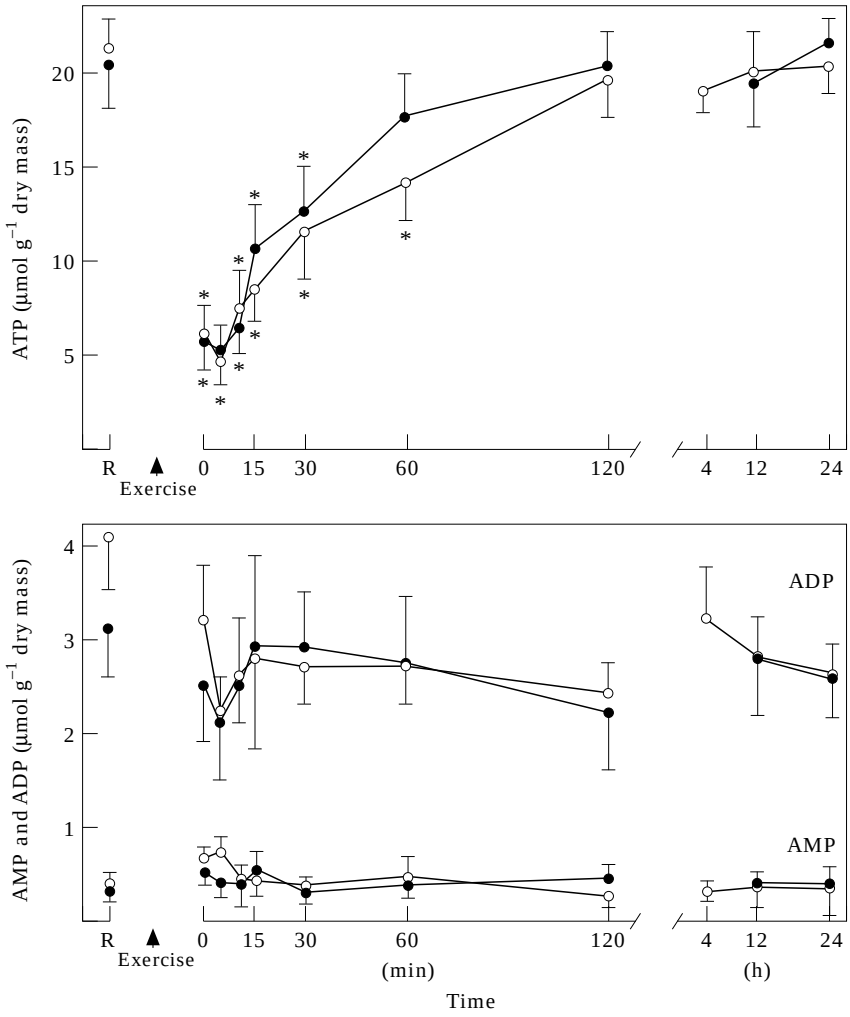

FIG. 2. Whole body ATP, ADP and AMP concentrations in herring larvae after strenuous exercise at 5° C (○) and 12° C (●). Arrowhead, 3-min exercise period. R, resting controls; *, significant difference from resting controls; results are means ± S.E.M.

order of 1·2 μmol min^{-1} g^{-1} wet muscle mass. Lactate clearance rates recorded for trout (body mass = 400–600 kg) white muscle are between 0·05–0·12 μmol min^{-1} g^{-1} wet muscle mass (Milligan & Wood, 1986; Moyes *et al.*, 1992). Only skipjack tuna (*Katsuwonus pelamis* L.) are comparable, metabolizing lactate at 1·3 μmol min^{-1} g^{-1} wet muscle mass, although at much higher temperatures (25° C) (Arthur *et al.*, 1992). The volume density of mitochondria in the inner muscle fibres of 1-day-old herring was 15·9% at 5° C and 26·1% at 15° C (Vieira & Johnston, 1992). In contrast, the volume density of mitochondria in the white muscle of adult teleosts is usually within the range 1–6% (Moon & Johnston, 1981). The higher densities of mitochondria in larval than adult muscle, therefore correlate with a more rapid metabolism of lactate following exercise.

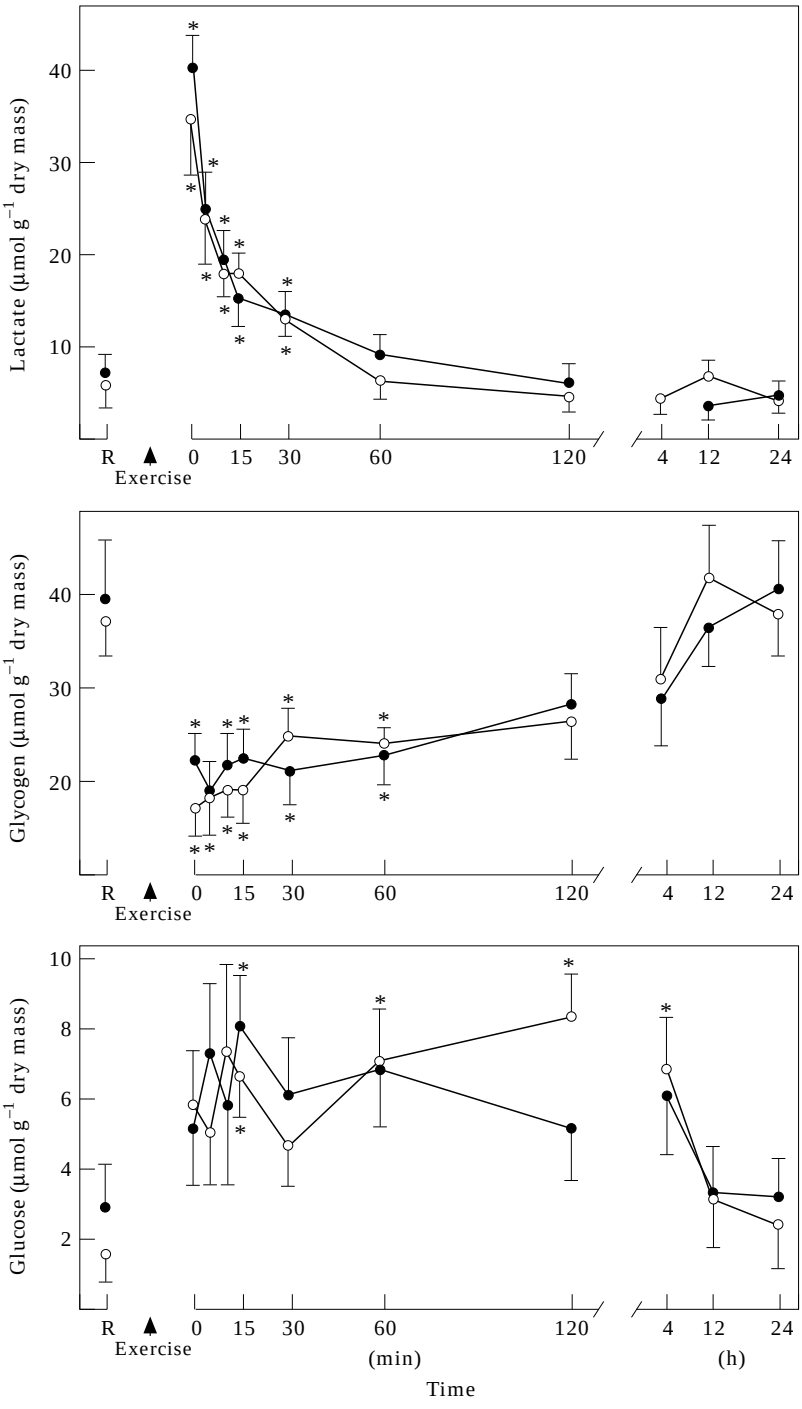

FIG. 3. Whole body lactate, glycogen and glucose concentrations in herring larvae after strenuous exercise at 5° C (○) and 12° C (●). Arrowhead, 3-min exercise period. R, resting controls; *, significant difference from resting controls; results are means ± S.E.M.

There are physiological, anatomical and behavioural differences between herring larvae reared at different temperatures (Batty & Blaxter, 1992; Vieira & Johnston, 1992; Johnston, 1993; Johnston *et al.*, 1995). Considering just the kinetic properties of enzymatic reactions with respect to temperature, one would expect recovery from intensive exercise to be faster in herring reared at 12 than at 5° C. However, there were no significant differences in the magnitude of the metabolite changes nor in the rates of recovery of metabolites in herring larvae reared at 5 and 12° C. Dalla Via *et al.* (1989) investigating the effect of temperature on the metabolic responses of juvenile *R. rutilus* to forced exercise, found that lactate and other glycolytic metabolites took approximately 2 h at 20° C and 8 h at 4° C to return to pre-exercise levels. Results from the larval herring are consistent with an increase in concentration of enzymes of energy metabolism in the 5° C relative to the 12° C reared fish.

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