Corrigendum

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On page 2686 an error appeared in the second sentence of the second paragraph of the Results section in both the on-line and print versions of this paper, which reads:

However in both winter and summer, the U_{crit}s of these groups were significantly higher than those for the groups acclimated to the reversed seasonal temperatures (5°C in winter, 15°C in summer).

The correct sentence should read:

However in both winter and summer, the U_{crit}s of these groups were significantly higher than those for the groups acclimated to the reversed seasonal temperatures (15°C in winter, 5°C in summer).

The authors apologise for any inconvenience caused.

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The effects of acclimation to reversed seasonal temperatures on the swimming performance of adult brown trout *Salmo trutta*

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Summary

Adult brown trout (*Salmo trutta*) were acclimatised to and maintained at seasonal temperatures (5°C in winter; 15°C in summer) and acclimated to reversed seasonal temperatures (15°C in winter; 5°C in summer) while exposed to the natural (i.e. seasonally variable) photoperiod. The mean critical swimming speeds (U_{crit}) of animals acclimatised to the seasonal temperatures were similar, but more than 30% greater than those for fish acclimated to the reversed seasonal temperatures. The lower values of U_{crit} that accompanied acclimation to reversed seasonal temperatures appeared largely to result from the inability of white muscle to function maximally, since the concentrations of lactate and ammonia in white muscle of fish swum to U_{crit} at reversed seasonal temperatures were significantly lower than those in fish swum at seasonal temperatures. These observations, together with biochemical and morphometric attributes of muscle tissue, suggest that swimming ability is influenced, at least in part, by seasonal factors other than temperature. These data have important implications for the design of experiments using fish that experience predictable, usually seasonal, changes in their natural environment (temperature, dissolved oxygen, changes in water levels, etc.).

Key words: brown trout, *Salmo trutta*, swimming, morphometry, temperature.

Introduction

Critical swimming speed (U_{crit}; Brett, 1964) is an indicator of swimming capacity and can therefore reflect the physiological status of active fish (see Plaut, 2001). In some species, U_{crit} is reduced when fish are suddenly exposed to temperatures that are significantly higher or lower than their acclimation temperature (Randall and Brauner, 1991), while other species are more tolerant (Jones and Sidell, 1982). However, with full acclimation, swimming performance can be improved at lower temperatures, as is the case for the goldfish, *Carassius auratus* (Rome et al., 1985), but often remains lower than that observed in individuals of the same species that are acclimated to a higher temperature.

At lower temperatures, the metabolic rate is reduced and this may reduce locomotory capacity, due to decreased contractile rates in both the red and white swimming muscles and the heart (Vornanen, 1994). As a compensatory response to exposure to low environmental temperature, biochemical and morphological changes occur in the slow oxidative (red) muscle fibres in a variety of species including salmonids. These changes include increases in the relative proportion and in the capillary density of these fibres, increases in both mitochondrial densities and mitochondrial cristae surface densities (and hence aerobic enzyme activity), decreases in the length of the diffusion path between the sarcoplasmic and mitochondrial compartments, and changes in the proportions of muscle enzymatic and myosin heavy chain isoforms (see Johnston, 1982; Jones and Sidell, 1982; Blier and Guderley, 1988; Egginton and Sidell, 1989; Londraville and Sidell, 1996; Cordiner and Egginton, 1997; Egginton and Cordiner, 1997; Guderley and St-Pierre, 2002; Watabe, 2002). In addition, acclimation to lower temperatures is often accompanied by an increase in the lipid content of aerobic muscle, which may enhance oxygen transport as well as acting as an intracellular oxygen store (Hoofd and Egginton, 1997).

In most studies where swimming performance has been investigated, the water temperatures and photoperiods under which experimental animals have been maintained have been quoted. However, few have mentioned at what time of the year such experiments have been performed or whether water quality (pH, dissolved ions, etc.) was maintained constant throughout the experimental period. Also, it is rarely indicated whether the experiments were carried out at seasonally appropriate temperatures and photoperiods.

Previous studies (Butler et al., 1992; Beaumont et al., 1995; Day and Butler, 1996) have revealed that adult brown trout, *Salmo trutta*, can maintain their swimming performance (as determined by U_{crit}) independently of the seasonal temperature to which they were acclimatised (5°C in winter; 15°C in summer). In these experiments, no attempt was made to control

photoperiod, so that all animals were also exposed to the natural (i.e. seasonal) light/dark cycle. Similar results were obtained for white crappie (*Pomonis annularis*) acclimated to three different temperatures and exposed to five different photoperiods (Smiley and Parsons, 1997). To date, this latter study, together with that carried out by Kolok (1991) on large-mouth bass, *Micropterus salmoides*, appear to be the only ones where the determination of U_{crit} has been performed under conditions where both photoperiod and environmental temperature have been manipulated. However, there have been other studies on the effects of season and thermal acclimation on the locomotory apparatus. For example, Guderley et al. (2001) demonstrated the effects of these factors on the speed of locomotion of the three-spine stickleback (*Gasterosteus aculeatus*) during experimentally elicited startle responses, and Kilarski et al. (1996) demonstrated the effects of season on short-term thermal acclimation and on changes in the inner mitochondrial membranes of oxidative ('red') skeletal muscle of crucian carp (*Carassius carassius*).

The primary purpose of the present study was to see whether the ability of brown trout to maintain swimming performance at 5°C or 15°C was independent of season while other environmental variables (water quality, stocking density and the availability of food) were maintained constant and the animals exposed to the natural (i.e. seasonally changing) photoperiod (Butler et al., 1992; Butler and Day, 1993; Day and Butler, 1996). Briefly, fish were acclimatised to both seasonal temperatures (5°C in winter, 15°C in summer) or acclimated to reversed seasonal temperatures (15°C in winter, 5°C in summer) in moving water and then swum in a variable-speed water channel up to their U_{crit}. Tissue samples were subsequently taken for biochemical and morphometric analysis to determine if any observable differences in data obtained from these analyses could be related to any differences in U_{crit}. A preliminary report of part of this study was given in Day and Butler (1999).

Materials and methods

Animal husbandry

Adult (non-breeding) brown trout *Salmo trutta* L. (mass 450–515 g; Table 1) were obtained from the Leadmill Trout Farm, Hathersage, Derbyshire, UK. As in our previous study (Day and Butler, 1996), the fish were initially placed in a large (2 m diameter) circular glass fibre 'training' tank (for details, see Butler et al., 1992) that was continually supplied (120 l h^{-1}) with dechlorinated Birmingham tap water ($[Ca^{2+}]$=130–200 μmol l^{-1}). In the middle of this tank was a smaller tank (diameter, 1 m; volume 400 l). Together, the two formed a 0.5 m wide × 0.6 m deep channel through which aerated water flowed at approximately 0.25 m s^{-1}. Several open-ended plastic tubes (internal diameter, 10 cm; length, 55 cm) were suspended mid-water in the channel to provide shelter for the fish. They invariably preferred to 'hide' in these but, to do so, they had to swim continuously against the direction of flow in order to maintain their station. The presence of both tubes and flowing water ensured that individuals were sufficiently 'trained' for the subsequent swimming experiments. The animals were left for a minimum of two weeks in this tank. After this initial period, the fish were transferred to a similar tank supplied with a continuous (3 l min^{-1}) supply of 'artificial lakewater' ($[Ca^{2+}]\approx$25 μmol l^{-1};), which was identical to that used in our previous studies (Butler et al., 1992; Day and Butler, 1996). The fish were left in this water for a minimum of a further 8 weeks. For animals that were maintained at reversed seasonal temperatures, water temperatures were initially either increased or decreased by approximately 1.0°C per day until the required acclimation temperature had been obtained. All fish were exposed to the natural (i.e. seasonally variable) photoperiod, within a laboratory with large windows, and fed twice daily to satiety on floating food pellets [Mainstream trout diet, B.P. Nutrition (UK) Ltd].

Experimental design

Data were obtained from four groups of 12 resting fish and four comparable groups of fish that were swum up to their U_{crit}. Each group was acclimatised to one of the 'seasonal' temperatures (5°C in winter, 15°C in summer) or acclimated to one of the reversed seasonal temperatures (15°C in winter and 5°C in summer) (Table 1). During the winter, all experiments were performed between mid-November and January, while during the summer, experiments were performed from mid-June to August. These periods correspond approximately to minimum and maximum daylengths of the palaearctic seasonal photoperiod and the minimum and maximum temperatures experienced by stockfish at the fish farm (Fig. 1).

Table 1. *Treatments and physical characteristics of brown trout used in the present study*

N	Season	Acclimation temperature (°C)	Total length (mm)	Mass (g)	Gonadosomatic index (% body mass)	Condition factor
12	Winter	5	344.40±4.31	481.7±11.01	0.42±0.09*	1.70±0.12
12		15	352.02±5.02	510.2±29.64	0.50±0.11	1.66±0.07
12	Summer	5	355.54±5.52	494.0±14.33	0.44±0.08	1.60±0.05
12		15	343.13±6.03	482.6±9.60	0.63±0.05*	1.55±0.06

Gonadosomatic index=100×[total gonad mass (g)]/[body mass (g)]%.
Condition factor=100 000×[body mass (g)/[fork length (mm)]^3.
*Significant difference between groups maintained at seasonal temperatures (P<0.05; N=12).

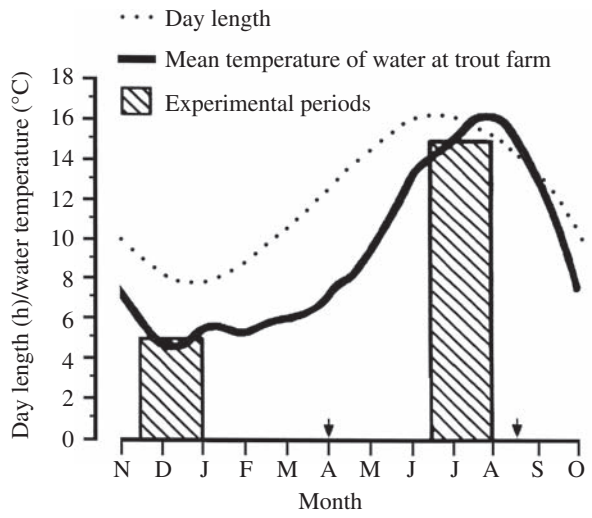

Fig. 1. The annual variation in day length and water temperature at Leadmill Trout Farm, from where stocks of adult brown trout were obtained, and the periods when all experiments were performed. Arrows indicate start of the acclimation periods, width of columns indicate experimental periods and height of columns indicate seasonal temperatures.

Experimental protocol and removal of tissue samples for biochemical and morphometric studies

After acclimatisation or acclimation, animals that were to be sampled were placed in a variable-speed, Blažka-type water channel for 6 days (Butler et al., 1992). Photoperiod and temperature, quality and rate of supply of the water were identical to those experienced by the animals during the acclimation period. After this experimental period, each fish was either sampled at rest or after being swum to its U_{crit}. The latter was determined by swimming for 15 min at 0.2 m s^{-1} and then for the same time at increasing 0.1 m s^{-1} increments until the animal ceased to swim (Butler et al., 1992). The fish was then immediately covered with a sheet of wet foam (which prevented it from struggling) before being removed from the water channel and killed (by a blow to the head followed by destruction of the brain). The fish was then quickly weighed and the heart rapidly excised.

Red muscle, white muscle and liver were immediately removed, weighed and freeze-clamped with aluminium tongs that had been pre-cooled in liquid N_2. Red muscle was removed as a thin 5–7 cm strip posteriorly from the left flank and finishing 3 cm from the base of the tail fin. White muscle was taken as a longitudinal block of tissue, approximately 6 cm in length and 1 cm in diameter, from deep epaxial (dorso-lateral) muscle in the same region. All tissue samples that were to be used for subsequent biochemical analyses were removed, processed and stored in liquid N_2 within approximately 90 s from the time of death of the animal. These were later ground to a fine powder under liquid N_2, prior to subsequent biochemical analysis.

Determination of gonadosomatic indices and condition factors

The condition factor for each fish was determined and the gonads removed and weighed for the determination of the gonadosomatic index (Anderson and Gutreuter, 1983) (see Table 1).

Muscle morphometry

The remainder of the red and white muscles from the left flank of each animal was dissected out separately and weighed. The accumulated muscle masses for both red muscle and white muscle were calculated and then doubled. Preliminary investigations had shown that contralateral differences between total masses for both red and white muscles did not vary by more than ±3.5%.

For detailed morphometric studies, whole blocks of tissue containing both red muscle and white muscle were removed from the right flank of the animal (within 5 min after death) by directly cutting deep through the skin in the region of the lateral line. These were then quickly coated in Tissue-Tek mountant medium (Gurr) and frozen in a small (50 ml) plastic beaker containing isopentane that had been previously cooled in liquid N_2. Sections of muscle (thickness, 10 μm) were cut at –20°C in a cryostat (Bright Instruments, UK) and stained for alkaline phosphatase activity at room temperature by the method of Ziada et al. (1984) so that blood capillaries became visible. These were then examined under a microscope fitted with a camera lucida attachment (Carl Zeiss) which projected images onto a digitising tablet (GTCO Corporation, Rockville, USA). All images were processed using Sigma Scan PC digitising software (Jandel Scientific California, USA). Mean muscle fibre cross-sectional areas, tissue capillary densities, and the mean number of capillaries per muscle fibre were determined with the aid of a randomly placed 'unbiased sampling' counting frame (Egginton, 1990).

Muscle biochemistry

Phosphofructokinase (PFK) and citrate synthase (CS) activities were assayed at 15°C in muscle samples taken from resting animals. PFK was assayed according to the method of Su and Storey (1994) at 340 nm using a Shimadzu UV-160A spectrophotometer fitted with a CPS240A temperature controller (Shimadzu Corp., Japan). CS activity was determined in the same samples at 412 nm by the method of Srere et al. (1963), as modified by Hansen and Sidell (1983). PFK was extracted by homogenising muscle samples on ice (3×15 s bursts at 20 500 r.p.m. with a Ultra-Turrax T25 homogeniser) in an extraction buffer containing 75 mmol l^{-1} Tris, 1 mmol l^{-1} EDTA, 2 mmol l^{-1} $MgCl_2$ and 2 mmol l^{-1} DTT (pH 7.4). This medium (minus the DTT) was also used to extract CS. Prior to analysis, all samples were clarified by centrifugation at 300 ***g*** for 5 min at 15°C. It should be noted that preliminary assays for both of these enzymes were carried out at 5°C but, for some reason, the data obtained were highly variable between aliquots of tissue homogenate. There was no sign of condensation at 5°C and the variability was eliminated when aliquots of homogenised samples were analysed at 15°C. All values of enzyme activities are given per unit wet mass.

Muscle glycogen and free glucose levels were determined

by the method of Keppler and Decker (1974). For determination of lactate concentrations, a 100–120 mg sample of frozen, powdered tissue was homogenised with ice-cold 1 mol l^{-1} perchloric acid (dilution factor 1:5, mass:volume). After centrifugation at 8000 ***g*** for 10 min, the supernatant was neutralised with 2 mol l^{-1} KOH and then assayed at 340 nm and 25°C by the method of Gutman and Wahlefeld (1974). Total muscle lipid content was determined by the method of Bligh and Dyer (1959). For total ammonia concentration [Tamm], weighed (approximately 100 mg) portions of frozen, powdered red muscle and white muscle were homogenised and deproteinised in ice-cold 1 mol l^{-1} perchloric acid (dilution factor 1:5 mass:volume) and then centrifuged at 10 000 ***g*** for 2 min to remove precipitated proteins. The supernatant was neutralised with 2 mol l^{-1} $KHCO_3$ (Kun and Kearney, 1984) and then analysed for total ammonia content ($[NH_3]+[NH^{4+}]$) using the Sigma 171-A diagnostic kit (Day and Butler, 1996).

Statistical analyses

All data were analysed by analysis of variance (ANOVA). Between-treatment comparisons were made using the *post-hoc* Tukey multi-comparison test (Zar, 1984), and significance was taken to be when $P<0.05$. When any variable is quoted as being 'different' from another, this means that the difference is statistically significant. All means are plotted with their standard errors (S.E.M.).

Results

The animals

The gonadosomatic indices (GS) for three of the four groups of fish were similar; the GS for fish maintained at 15°C in summer was greater than those for fish maintained at 5°C in summer, 5°C in winter and 15°C in winter. Condition factor did not differ between the four groups of animals (Table 1).

Swimming performance

There was no difference between the U_{crit}s of the two groups of fish swum at seasonal temperatures (5°C in winter, 15°C in summer; Table 2). However, in both winter and summer, the U_{crit}s of these groups were significantly higher than those for the groups acclimated to the reversed seasonal temperatures (5°C in winter, 15°C in summer). In winter, fish acclimated to 15°C exhibited a 32% lower U_{crit} compared with those acclimatised to 5°C, while in summer, fish acclimated to 5°C showed a 30% lower U_{crit} than those fish maintained at 15°C. At reversed seasonal temperatures, the mean U_{crit} of fish swum at 15°C in winter was 11% lower than that for fish swum at 5°C in summer (Table 2).

Morphometry

Masses of heart, red muscle and white muscles

While there was little effect of season or temperature on the mass of the white muscle, fish acclimated to 5°C had greater amounts of both red and heart muscles than those acclimated to 15°C, and the difference was greater in winter (Table 2).

Table 2. *Mean values (± S.E.M.) for* U_{crit}*, and morphometry and enzyme activity of red and white muscles in adult brown trout*

	Winter 5°C	Winter 15°C	Summer 5°C	Summer 15°C
U_{crit} (body lengths s^{-1})	1.95±0.07[a]	1.33±0.07[b]	1.47±0.06[b]	2.10±0.12[a]
Muscle mass (g)				
Red muscle	11.80±0.48	8.42±0.31[a]	9.98±0.30	8.29±0.48[a]
White muscle	211.99±9.68[a]	234.74±13.21[a]	249.22±17.88[a]	229.09±19.45[a]
Heart	0.63±0.02	0.47±0.03	0.55±0.01	0.39±0.01
Mean fibre cross-sectional area (μm^2)				
Red muscle	1414±78	1040±33[a]	1192±69	1056±41[a]
White muscle	4442±275[a,b]	4463±119[a]	4201±147[a]	4793±76[b]
Capillary density (capillaries mm^{-2})				
Red muscle	1512±59	1771±60[a]	2072±55	1795±30[a]
White muscle	230±8[a]	208±10[b]	222±17[a,b]	231±19[a]
Capillary-to-fibre ratio				
Red muscle	2.36±0.06[a]	2.09±0.07	2.32±0.03[a]	1.96±0.04
White muscle	2.05±0.05	1.88±0.10[a]	1.83±0.05[a]	1.72±0.12[a]
CS activity ($\mu mol\ min^{-1}\ g^{-1}$ wet mass)				
Red muscle	22.35±2.00	15.13±0.82	17.32±0.81	13.55±0.68
White muscle	4.12±0.11	2.71±0.32[a,b]	3.02±0.28[a]	2.26±0.15[b]
PFK activity ($\mu mol\ min^{-1}\ g^{-1}$ wet mass)				
Red muscle	4.01± 0.48[a]	3.71±0.28[a]	4.25±0.44[a]	7.23±0.61
White muscle	21.85±2.50[a]	20.74±2.43[a]	22.28±3.01[a]	35.52±3.45

Values with same letters are not significantly different from one another ($P<0.05$; $N=12$).

Thus, the amounts of red and heart muscles in fish acclimatised to 5°C in winter were 42% and 61% greater, respectively, than those in fish acclimatised to 15°C in summer, but in fish acclimated to 5°C in summer, the amounts of red and heart muscles were only 20% and 41% greater, respectively, than those in fish acclimatised to 15°C in summer.

Morphometry of red and white muscles

Again, there was little systematic difference in any of the measured parameters in white muscle between the different groups (Table 2), whereas mean fibre cross-sectional area of the red muscle showed a similar pattern to the amount of red muscle (see above). In fish acclimatised to 5°C in winter, mean fibre cross-sectional area of the red muscle was 34% greater than in fish acclimatised to 15°C in summer, but in fish acclimated to 5°C in summer, mean fibre cross-sectional area of the red muscle was only 13% greater than that in fish acclimatised to 15°C in summer (Table 2). Although capillary density was greatest in red muscle of fish acclimated to 5°C in summer and lowest in fish acclimatised to 5°C in winter, the mean number of capillaries per muscle fibre was approximately 15% greater in red muscle of fish acclimated to 5°C than in those acclimated to 15°C, irrespective of season (Table 2).

Enzyme activities

The activities of CS were approximately 5.5 times greater in red muscle than in white muscle (Table 2), and in both muscles showed similar patterns among the groups of fish to those seen with the amount of red muscle and mean fibre cross-sectional area for red muscle (see above). In fish acclimatised to 5°C in winter, CS activity in red and white muscles was 65% and 82%, respectively, higher than in those of fish acclimatised to 15°C in summer, but in fish acclimated to 5°C in summer, CS activity in red and white muscles was only 28% and 34% higher than in those of fish acclimatised to 15°C in summer (Table 2). The activities of PFK were between 5 and 5.5 times greater in white muscle than in red muscle in the various groups of fish (Table 2), but within each muscle, the activities were similar in all groups of fish, except in those acclimated to 15°C in summer. In this group, activity of PFK was approximately 80% higher than that in the other groups (Table 2).

Metabolic substrates and metabolites

In resting fish, the concentrations of glycogen in the liver were 7–10 times greater than those in white muscle and 8–18 times greater than those in red muscle (Fig. 2). In resting fish, there was no difference in the concentrations of glycogen in the red muscles between any of the groups of fish, whereas in white muscle and liver, the concentrations were greatest in fish acclimatised to 5°C in winter and lowest in fish acclimatised to 15°C in summer. In all tissues, the concentrations of glycogen were lower in fish that had swum to U_{crit} than in those at rest, but, after swimming to U_{crit}, the concentrations were not as low in fish acclimated to reversed seasonal temperatures as in those acclimatised to seasonal temperatures (Fig. 2).

Similarly, in resting fish, the concentration of free glucose

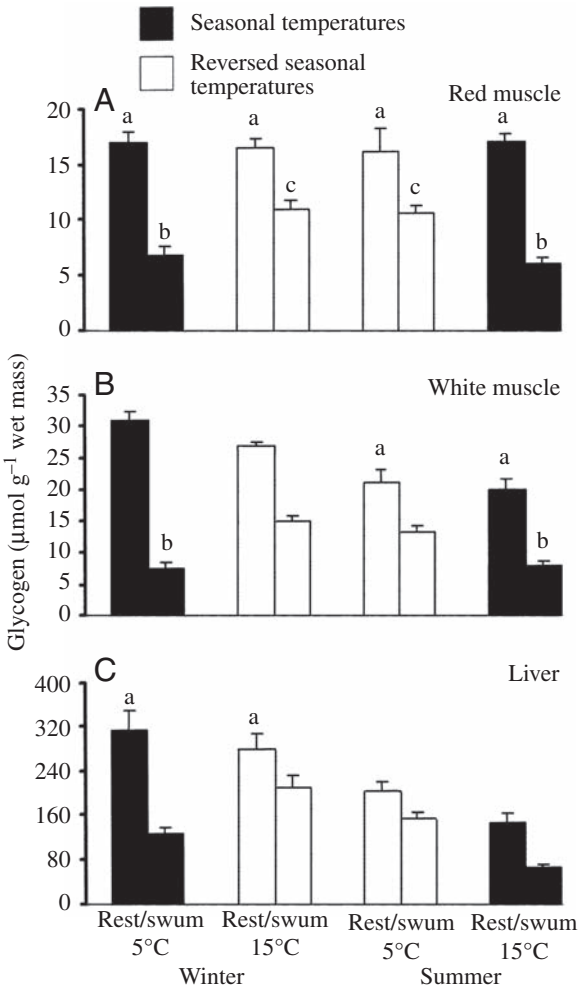

Fig. 2. Mean values (± S.E.M.) of glycogen concentrations in red muscle, white muscle and liver of adult brown trout sampled at rest or after swimming up to their critical swimming speed (U_{crit}). For the appropriate comparisons, values with the same letters are not significantly different from one another (P<0.05; N=12).

was greatest in liver and lowest in red muscle (Fig. 3), but, in this case, the concentrations in each tissue were similar in resting fish and were higher in fish swum to U_{crit}. However, the concentrations after swimming to U_{crit} were not as great in fish acclimated to reversed seasonal temperatures as in those acclimatised to seasonal temperatures (Fig. 3).

The concentrations of lipid in red muscle of resting fish were between 6 and 13 times higher than those in white muscle (Fig. 4). In white muscle of resting fish, lipid concentrations differed little between groups of fish, except for those acclimated to 5°C in summer, in which it was approximately 20% lower than in the other groups. However, in red muscle of resting fish, those acclimated to 5°C had greater concentrations of lipid than those acclimated to 15°C, and the difference was greater in summer. Lipid concentration in the red muscle of resting fish acclimatised to 5°C in winter was 110% greater than that in fish acclimatised to 15°C in summer, but in resting fish acclimated to 5°C in summer, lipid

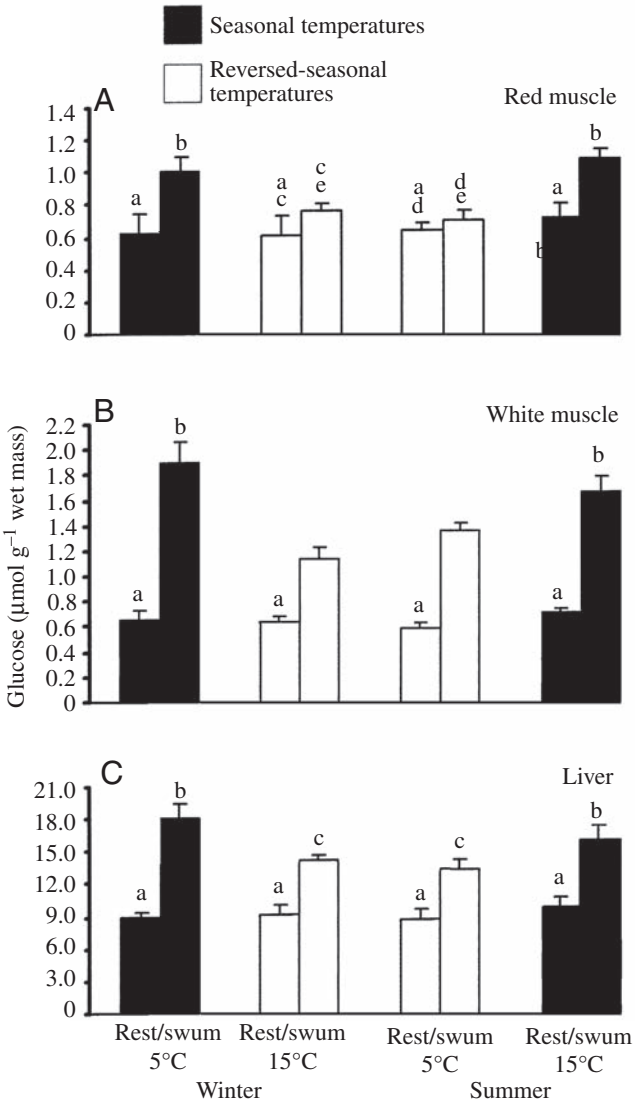

Fig. 3. Mean values (± S.E.M.) of free glucose concentrations in red muscle, white muscle and liver of adult brown trout sampled at rest or after swimming to critical swimming speed (U_{crit}). For the appropriate comparisons, values with the same letters are not significantly different from one another (P<0.05; N=12).

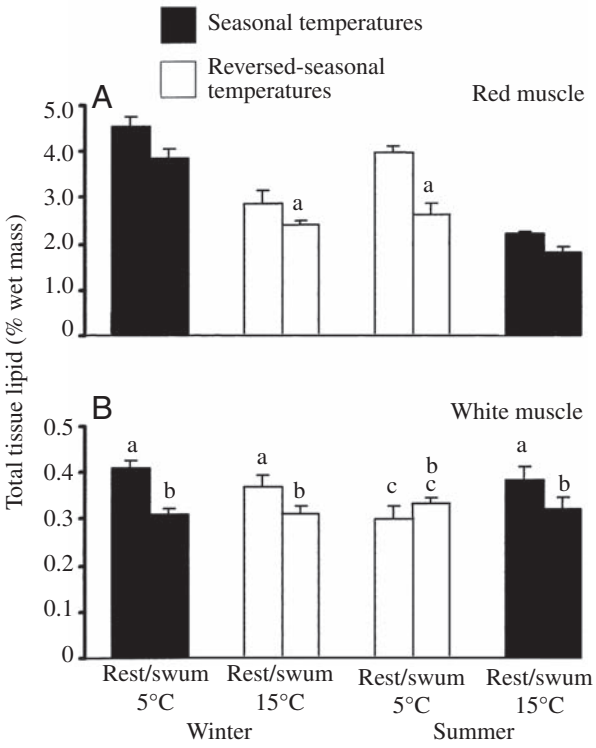

Fig. 4. Mean values (± S.E.M.) of total lipid levels in red muscle and white muscle of adult brown trout sampled at rest or after swimming to critical swimming speed (U_{crit}). For the appropriate comparisons, values with the same letters are not significantly different from one another (P<0.05; N=12).

concentration was only 83% greater than that in fish acclimatised to 15°C in summer. The concentrations of lipid were lower in both red and white muscles in all groups of fish swum to U_{crit} compared to those in the groups of resting fish, except in the white muscle of those acclimated to 5°C in summer, and the proportional reduction was similar in each case (Fig. 4).

In resting fish, the concentrations of lactate were between 150 and 200% greater in white muscle than in red muscle and, within each muscle, the concentrations were similar in all groups of fish (Fig. 5A,B). The concentrations of lactate in both muscles of fish that had swum to U_{crit} were higher than in those at rest and, in red muscle, the concentrations were similar in all groups. However, in white muscle, the concentrations of lactate in the fish swum to U_{crit} were 45–55% lower in those acclimated to reversed seasonal temperatures compared with those in fish acclimatised to seasonal temperatures (Fig. 5).

A similar pattern to that seen with lactate concentrations occurred with total ammonia [Tamm] in the muscles, with greater [Tamm] present in both muscles in fish swum to U_{crit} compared with those in resting fish (Fig. 5C,D). However, in white muscle, [Tamm] of fish swum to U_{crit} were lower in those acclimated to reversed seasonal temperatures than in those acclimatised to seasonal temperatures.

Discussion

In the present study, where both morphometric and swimming performance data were obtained, it is important that the experimental animals used were all of a similar size. It may also have been important that they were all in a similar state of sexual maturity, although published data correlating changes in states of sexual maturity to changes in morphometric variables are sparse. The condition factor for each of the four groups of fish that were used in this study did not differ. In addition, the GSI of three of these groups was similar, the exception being the GSI of the group maintained at 15°C in summer, which was 20–30% higher than the GSIs for the other groups. The change in GSI is small compared with those in

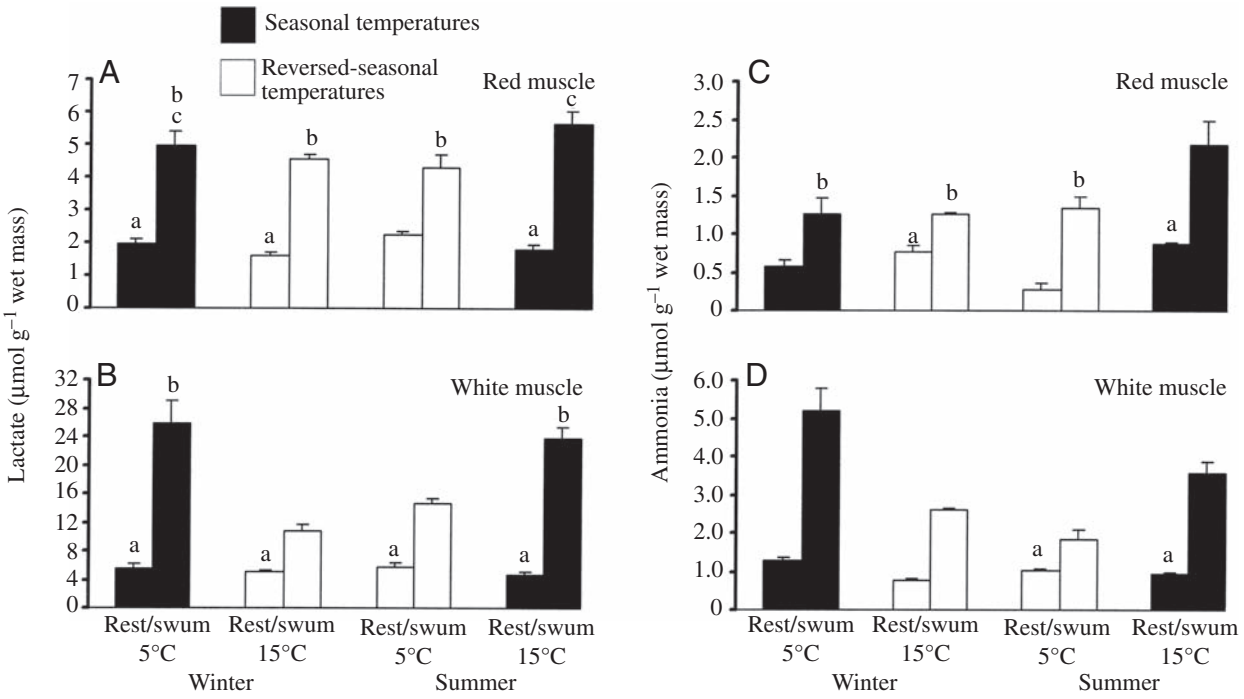

Fig. 5. Mean values (± S.E.M.) of lactate and ammonia concentrations in red muscle and white muscle of adult brown trout sampled at rest or after swimming to critical swimming speed (U_{crit}). For the appropriate comparisons, values with same letters are not significantly different from one another (P<0.05; N=12).

published data. For example, Taylor et al. (1996) reported that the GSI for the adult rainbow trout *Oncorhynchus mykiss* used in their experiments differed by more than 6-fold, from 0.48 for fish maintained at 4°C to 3.06 for fish acclimated to 18°C.

Swimming performance

The present study clearly demonstrates that acclimation to reversed seasonal temperatures lowers swimming performance (U_{crit}) in adult brown trout. This appears to be due, at least in part, to a reduction in the use of white muscle, as the increases in lactate and ammonia in white muscle in the fish swimming to U_{crit} at reversed seasonal temperatures were not as great as those observed in fish swum at seasonal temperatures. Thus, this reduction in swimming performance with acclimation to reversed seasonal temperatures cannot be explained by unusually high levels of plasma and muscle ammonia, as occurs in fish exposed to low pH (Beaumont et al., 2000). In addition, the animals held at reversed seasonal temperatures were sampled after having acclimated to these conditions over an extended period. Thus, other, comparatively long-term, changes must have been responsible for the differences in swimming performance between the groups of fish acclimatised to seasonal and acclimated to reversed seasonal temperatures.

Effects of acclimatisation to seasonal temperatures on muscle morphometry and biochemistry

There is increasing evidence that acclimation to low temperature causes many fish to undergo anatomical and biochemical changes to their locomotory apparatus. In the current study, the capillary-to-fibre ratio, mean cross-sectional fibre area and mass of red muscle were significantly greater in fish acclimatised to 5°C in winter than in those acclimatised to 15°C in summer.

The greater mass of red muscle in fish acclimatised to 5°C in winter than in those acclimated to 15°C in summer was the result of an increase in mean fibre cross-sectional area of this tissue. This phenomenon of 'bulking-up' of aerobic muscle at low temperature has been demonstrated in a number of species including *Carassius auratus* (Johnston and Lucking, 1978) and *Morone saxatilis* (Jones and Sidell, 1982). As observed in other species, including rainbow trout (Egginton and Cordiner, 1997), the capillary to red muscle fibre ratio increased with acclimation to the lower temperature in the present study, although the capillary density per unit area decreased due to the increase in fibre diameter. Such changes, together with the cardiac hypertrophy, could potentially increase blood flow to aerobic muscle, which would enhance aerobic metabolism of this tissue during winter.

White muscle changed little with acclimatisation to the lower winter temperature or to 5°C in summer. This is somewhat surprising, since it suggests that white muscle has low 'plasticity' in response to changes in abiotic conditions. Undoubtedly, white muscle must adapt to environmental temperature in some way, since there is no difference in U_{crit} between animals maintained at the two seasonal temperatures, despite a lack of change in the speed at which white muscle is recruited (approximately 1 body length s^{-1}; Day and Butler, 1996).

The phenomenon of increased aerobic enzyme activity at lower temperatures is well known and has been demonstrated in a number of species (see Introduction), where it appears to be related to an enhancement in the ability of the fish to utilise lipid as an energy source at low temperatures (Hazel and Prosser, 1974). The current study demonstrates that, when assayed at the same temperature (15°C), CS activities in both red and white muscles were greater in winter fish at 5°C than in summer fish at 15°C (see also Battersby and Moyes, 1998), and the reverse was true for PFK. Unfortunately, it did not provide an indication of *in situ* (physiological) activities of CS and PFK in the winter fish. For this, activities of both of these enzymes should have been determined at 5°C for the winter fish but, as we have previously indicated (see Results), we were unable to obtain consistent data at this temperature. Therefore, the data only demonstrate changes in absolute capacity and do not provide evidence for true thermal compensation.

Nathanailides (1996) used the term EQ_{-10} to describe the extent to which the activity of an enzyme increases with every 10°C decrease in acclimation temperature and compared this with the Q_{10} of the enzyme. If EQ_{-10} equals Q_{10}, there is perfect compensation. Assuming a Q_{10} of 1.46 for CS (Nathanailides, 1996), the ratio of EQ_{-10}/Q_{10} is 1.13 for seasonally acclimated brown trout and 0.88 for those at 5 and 15°C in summer. Thus, this analysis clearly demonstrates that compensation of CS activity is greater at a low seasonal temperature than at a low non-seasonal temperature.

Effects of acclimation to reversed seasonal temperatures on muscle morphometry and biochemistry

The differences between the variables measured in fish at seasonal and reversed temperatures indicate that environmental temperature is not the only factor responsible for these observations. 'Seasonal' effects also seem to be at work. For example, PFK levels in animals at 15°C were considerably lower in winter than in summer and indeed were no different from those observed in animals acclimated to 5°C in winter. In addition, at 5°C, the capillary density of red muscle was higher in summer compared with in winter, probably due to the observed decrease in fibre size. The activities of CS in red and white muscle, the masses of red and heart muscle and mean red muscle fibre cross-sectional area were all lower in fish acclimated to 5°C in summer than in those acclimatised to the same temperature in winter. This suggests that, at the lower temperature, full thermal compensation is only possible during winter.

It would appear, therefore, that the changes in muscle morphometry and biochemistry that accompany acclimation to reversed seasonal temperatures are 'incomplete', when compared with changes observed in fish acclimated to seasonal temperatures, or, at the very least, take longer than the duration of acclimation period used in the present study. Further evidence for this comes from a study by Kilarski et al. (1996) on the mitochondrial morphometrics of oxidative muscle of crucian carp that were acclimated for 6 weeks to 5°C and 25°C in both winter and summer. In this study, it was demonstrated that at 5°C the surface density of outer mitochondrial membrane per muscle was higher in summer than in winter. At 25°C the surface density of inner mitochondrial membrane per fibre and the surface density of the inner mitochondrial membrane were higher in summer than in winter. In addition, Bouchard and Guderley (2003) studied the time course of acclimation, as determined by red muscle mitochondrial enzyme activity and respirometry, of groups of rainbow trout that were either 'warm acclimated' (water temperature raised from 5°C to 15°C during winter) or 'cold acclimated' (water temperature lowered from 15°C to 5°C during summer). They found that warm acclimation appeared to be completed within 8 weeks whereas cold acclimation appeared to be incomplete after 10 weeks.

The only other comparable biochemical data for the effects of reversed seasonal temperatures on enzyme activity concern the three-spine stickleback, *Gasterosteus aculeatus* (Guderley et al., 2001). In this study, fish were acclimated to 8°C and 23°C in both spring and autumn, and enzyme activities were determined (at 10°C and 20°C) for pectoral and axial muscle. It was observed that, in spring, the highest level of PFK activity was observed in axial muscle from fish acclimated to the lower temperature in spring and assayed at 20°C (there was no difference at 10°C and no data were presented for the autumn experiment). This is the opposite of that observed in the current study for brown trout acclimated to 15°C in winter.

Guderley et al. (2001) also demonstrated that for pectoral (i.e. aerobic) muscle (in contrast to what was observed in axial muscle) there was no significant difference between the levels of CS activity between the two groups of fish that were acclimated to 8°C and 23°C in the spring. This again was the reverse of what we observed in the brown trout, where the highest levels in red muscle occurred at 5°C. In spite of these contradictory data (which may be related to differences in life cycle or taxonomy), both of these studies do suggest that a seasonally changing environmental temperature is not the only factor that influences changes in muscle morphology and physiology.

What is the primary influence on swimming performance?

The biochemical and morphological data in the present study provide evidence that full thermal compensation does not occur in the fish that are acclimated to reversed seasonal temperatures. Thus, there must have been a factor or factors in addition to temperature that were responsible for the differences in U_{crit} between the groups of fish acclimatised to 'seasonal' and acclimated to 'reversed seasonal' temperatures. In fact, with few possible exceptions (e.g. Staurnes et al., 1994), there appears to be little experimental evidence to support the idea that temperature alone can act as a *zeitgeber* in fish, although it can, in conjunction with photoperiod, influence physiological changes (McCormick et al., 2002). Seddon and Prosser (1997) concluded that acclimation to environmental temperature was 'not an all or none phenomenon' and may depend on a variety of seasonal factors, including time of collection, nutritional and reproductive state, and circannual cycles.

Photoperiod is probably the most important environmental cue influencing changes in the locomotory apparatus and U_{crit} of brown trout, since it has previously been demonstrated to be the dominant *zeitgeber* for several endogenous rhythms and physiological changes in other fish species including circadian rhythmicity in heart rate (Pennec and Le Bras, 1988) and the timing of smolting in anadromous salmonids (Hoar, 1988; Duston and Saunders, 1990). In addition, studies on the effects of light exposure on sexual maturation of the stickleback (*G. aculeatus*) have revealed endogenous daily and annual rhythms of changing photoreactivity (Baggerman, 1985). In this species, the onset of sexual maturation can be experimentally triggered by a short (2 h) exposure to light during the scotophase in winter. Because of its predictable annual variation, photoperiod undoubtedly functions as a synchroniser of such rhythms in the natural cycle. Assuming that such rhythms exist in brown trout, it would be intriguing to perform swimming experiments, such as those described in the present study, on fish that were subjected to both reversed seasonal temperatures and reversed seasonal photoperiods.

What other factors may influence swimming performance?

In addition to photoperiod, there are a number of naturally occurring, seasonally changing environmental factors that may have influenced adaptation of the locomotory apparatus of brown trout to unseasonal temperatures. One example is the annual and semi-annual variation in the earth's geomagnetic field (see Malin et al., 1999) to which animals, including salmonids, are known to be sensitive (see e.g. Yokoi et al., 2003; Diebel et al., 2000). So, it is conceivable that changes in geomagnetism could be an exogenous cue that, probably in combination with others, affects changes in the locomotory apparatus of brown trout. Indeed, there is increasing evidence that in migratory animals, including salmonids, photoreception and geomagnetic detection are inextricably linked (Deutschlander et al., 1999).

Endogenous biological clocks may also play an important role, since their powerful influence on organisms is well known. For example, Saether et al. (1996) demonstrated that arctic char (*Salvelinus alpinus*) showed seasonal changes in food consumption and growth that were endogenously driven, being unaffected by experimental manipulation of photoperiod. Perhaps it is the 'regularity' of one or more of these internal clocks that prevents the locomotory apparatus of brown trout from becoming fully adapted to unseasonal environmental temperatures.

The present study is the first to investigate the effects of acclimation to reversed seasonal temperatures on U_{crit} and the morphometrics and biochemistry of the locomotory apparatus of a salmonid fish. It illustrates the importance of ensuring that experiments performed on fish such as brown trout should be performed under conditions of temperature and photoperiod that approximate those occurring in the natural environment at the time of the year when they are performed. It is only under these conditions that the data produced from such studies will be of relevance to animals in their natural environment.

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