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SHORT COMMUNICATION

THE RELATIONSHIP BETWEEN INTRACELLULAR pH AND SEASONAL TEMPERATURE IN THE BROWN TROUT *SALMO TRUTTA*

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Early studies on reptiles demonstrated that plasma pH increases as body temperature falls (Robin, 1962). Rahn (1967) proposed that plasma pH in all poikilothermic vertebrates is regulated as body temperature changes so as to maintain a constant relative alkalinity, i.e. a constant $[OH^-]/[H^+]$ ratio, and Reeves (1972) suggested a way in which this could be achieved. Known as the 'imidazole alphastat hypothesis', it postulates that P_{CO_2} is regulated (by way of ventilation) so that the fractional dissociation (α) of the imidazole moiety of histidine is kept constant. As the pK' of imidazole changes with temperature in about the same manner as the neutral pH of water (Heisler, 1986), the alphastat hypothesis is consistent with that of constant relative alkalinity.

Although studies on a range of poikilothermic vertebrates have demonstrated a rise in plasma pH with decreasing temperature, there is much variability in $\Delta pH/\Delta t$ from $-0.001 units^{\circ}C^{-1}$ in varanid lizards to $-0.021 units^{\circ}C^{-1}$ in freshwater turtles (see Heisler, 1986, for a comprehensive list). As far as intracellular pH (pHi) is concerned, the variability is even greater, both within and among tissues and over different temperature ranges. In the turtle, *Pseudemys scripta*, $\Delta pH/\Delta t$ of muscle and liver are in accord with the alphastat hypothesis (Malan *et al.* 1976), although $\Delta pH/\Delta t$ of the heart was lower than predicted by the hypothesis. Moreover, Heisler (1986) reports a study on *Varanus exanthematicus* in which it was found that pHi of white muscle, cardiac muscle and oesophagus changed little (-0.003 to $-0.005 units^{\circ}C^{-1}$) with changes in temperature whereas $\Delta pH/\Delta t$ for plasma was $-0.010 units^{\circ}C^{-1}$. In a more recent study, Boutilier *et al.* (1987) not only found differences between tissues and between two species of amphibians, *Xenopus laevis* and *Bufo marinus*, but also that $\Delta pHi/\Delta t$ was non-linear in most tissues and that it tended to decrease over lower temperature ranges for all tissues in *Bufo* and for the heart in *Xenopus.* This non-linearity was not apparent for blood.

The non-linearity of the relationship between pHi (but not blood pH) and temperature was also noted in the study on the American eel by Walsh and Moon (1982), again with the slope decreasing over lower temperature ranges. They worked over the temperature range 5–20°C, and inspection of their data for red, white and cardiac muscles (their

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Figs 2 and 3) indicates that there are no significant differences between the values at 15 and 5°C. Indeed, it appears that for red and cardiac muscles the mean values at 5°C are lower than those at 10°C. These authors relate the relative acidity at low temperature in eel blood and tissues to that seen in hibernating mammals (Malan *et al.* 1973) and draw attention to the associated lower spontaneous activity in eels at low (winter) temperature. These authors thus allude to a seasonal aspect and relevance to their data.

Butler and Taylor (1975) found that pH of both arterial and mixed venous blood of dogfish, *Scyliorhinus canicula*, at their appropriate seasonal temperature had $\Delta pH/\Delta t$ values of $-0.014 unit^{\circ}C^{-1}$, and Heisler *et al.* (1980) found that, following long-term (22–28 days) acclimation of dogfish (*S. stellaris*) to different temperatures, $\Delta pH/\Delta t$ of white muscle was not significantly different from that of blood, while that of red muscle was greater and that of cardiac muscle was less. In fact, there was probably no significant change in pHi of cardiac muscle over the 10–20°C temperature range. As far as we are aware, there have been no data published on intracellular pH of teleost fish acclimated to their relevant seasonal temperatures. It was decided, therefore, to determine pH of plasma and of white, red and cardiac muscles of brown trout acclimated to 5°C in winter and 15°C in summer.

The methods employed were as described by Butler *et al.* (1992) and Butler and Day (1993). Briefly, brown trout, *Salmo trutta* (L.) (mass 320–520g), were obtained from Leadmill trout farm, Hathersage, Derbyshire, and kept for 2–4 weeks in large (1400l) circular glass fibre tanks through which dechlorinated Birmingham tapwater was flowing at a rate of $120 l h^{-1}$. The water was aerated vigorously and circulated around the tank using a spray bar which produced jets of water that were nearly horizontal when they hit the surface. Because the water was circulating around the tank, at approximately $0.25 m s^{-1}$, the fish had to swim to maintain position.

Following the initial acclimation period, the fish were transferred to a similar tank containing soft ($[Ca^{2+}]=25 \mu mol l^{-1}$) artificial lakewater (Dalziel *et al.* 1985) also circulating at $0.25 m s^{-1}$. Titanium cooling coils and small aquarium heaters maintained the temperature of the water in the two large tanks at 5°C in winter (November to March inclusive, 4–7°C at the trout farm) and 15°C in summer (June to mid-September, 12–16°C at the trout farm). The pH of the water was maintained at 7±0.1 (range). The fish were kept under these conditions for a further 2–4 weeks after the initial period in Birmingham tapwater. Throughout the acclimation period the fish were exposed to a natural photoperiod and were fed daily on floating pellets [Mainstream trout diet, B.P. Nutrition (UK) Ltd]. All uneaten pellets were removed from the tank an hour after feeding and no food was given the day before transfer of the fish to an experimental tank or during the experimental period.

After acclimation, fish were anaesthetized in buffered MS222 and the dorsal aorta was cannulated. The animals were placed into a tube 65cm long and 15cm in diameter through which aerated, artificial lakewater at the appropriate temperature flowed at a rate of $40 l h^{-1}$ and left for a total of 6 days. On the fifth day, the fish were infused with $0.22 MBq kg^{-1}$ [^{14}C]DMO and $0.74 MBq kg^{-1}$ ^{3}H-labelled mannitol. No less than 18 h later, 2–3ml of arterial blood was removed from the dorsal aorta for the determination of arterial blood pH and CO_2 content. $P_{a_{CO_2}}$ and bicarbonate concentration, $[HCO_3^-]$, were

calculated from these data using appropriate values of αCO_2 and pK' from the formulae presented by Boutilier *et al.* (1984). pHi values of the cardiac and of the red and white skeletal muscles were determined by the DMO method (Waddell and Butler, 1959; Heisler, 1975; Milligan and Wood, 1986*a*,*b*) with ^{3}H-labelled mannitol being used to determine extracellular fluid volume (ECFV). Details of the method are given by Butler and Day (1993).

The changes in arterial blood $[HCO_3^-]$, Pa_{CO_2} and pH with temperature (Figs 1, 2) are qualitatively similar to those found in other species of fish (Cameron, 1984). $\Delta pH/\Delta t$ for blood (-0.016unit$^\circ C^{-1}$) is similar to that found in rainbow trout (-0.017unit$^\circ C^{-1}$; Randall and Cameron, 1973), slightly greater than that reported for seatrout and catfish (-0.013units$^\circ C^{-1}$; Cameron, 1978; Cameron and Kormanik, 1982) and substantially greater than that found in the American eel (-0.008units$^\circ C^{-1}$; Walsh and Moon, 1982). It is clear from Fig. 2, however, that there are no changes in pHi of the three muscle tissues studied. This is similar to the situation in the reptile *Varanus* (see Heisler, 1986) and, as indicated earlier, in the American eel over the temperature range 5–15°C (Walsh and Moon, 1982). As the latter authors point out, eels are particularly inactive during the winter, burying themselves in bottom mud and not feeding below a temperature of 7–8°C. They speculate that the relative acidity in the muscles would cause changes in protein net charge state which could cause deactivation of enzymes and, therefore, reduced metabolic rate in these tissues. Such an argument will not hold for brown trout, which show only a slight reduction in resting metabolic rate and swimming ability in winter-acclimated fish, compared with those in fish acclimated to summer temperatures (Butler *et al.* 1992). These authors discuss the various biochemical and morphological adaptations that have been found to occur in fishes (mainly carp) acclimated to low

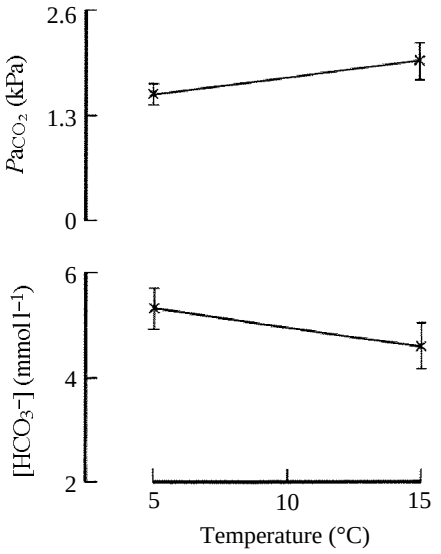

Fig. 1. Mean values ± S.E.M. of Pa_{CO_2} and $[HCO_3^-]$ in brown trout acclimated to 5°C (N=6) and 15°C (N=7).

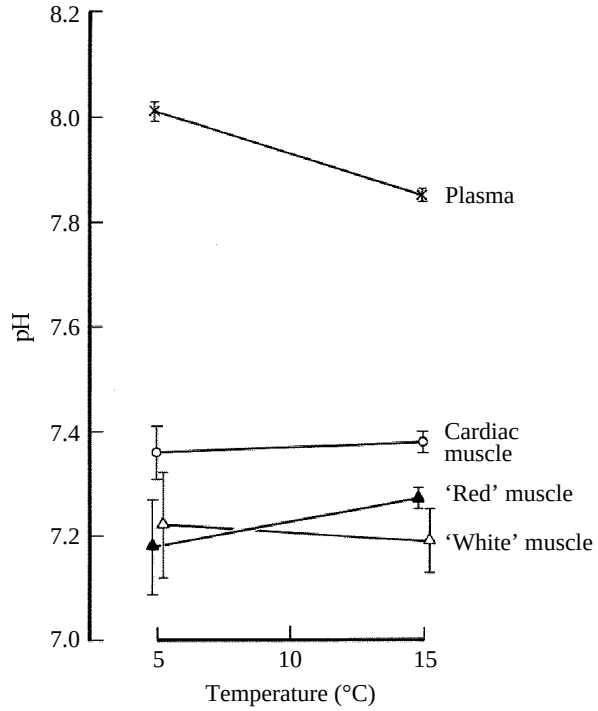

Fig. 2. Mean values ± S.E.M. of plasma pH and intracellular pH of white (△), red (▲) and cardiac (○) muscles of brown trout acclimated to 5°C (*N*=6) and 15°C (*N*=7).

temperatures and could explain the high swimming ability of winter-acclimated brown trout. In the context of this ability and the present data, Stevens and Godt (1990) were unable to demonstrate a significant advantage of a rise in pHi of muscles with a fall in temperature during locomotion in two species of amphibians.

This study not only supports the observations of a growing number of other workers, that pHi of some tissues can be regulated independently of extracellular pH, it also confirms the small body of evidence that, at least at the lower end of the temperature range, the pHi of some tissues, especially the muscles, may be completely independent of temperature. Whether there is a seasonal component to this phenomenon remains to be seen, as does its biological relevance, particularly in an animal that is capable of relatively high levels of swimming performance under winter conditions. It is interesting to note that the pHi of cardiac muscle in a number of species is unaffected by changes in temperature. It is also clear that regulation of intracellular pH in a number of tissues in many poikilothermic vertebrates cannot be explained on the basis of the imidazole alphastat hypothesis. In aquatic vertebrates, it appears that the relatively slow transfer of bicarbonate-equivalent ions is the important mechanism (Heisler, 1978; Heisler and Neuman, 1980).

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