

The effects of temperature on muscle pH, adenylate and phosphogen concentrations in *Oreochromis alcalicus grahami*, a fish adapted to an alkaline hot-spring

I. A. JOHNSTON*, F. B. EDDY† AND G. M. O. MALOIJ

Department of Animal Physiology, University of Nairobi, P. O. Box 30197, Chiromo, Nairobi, Kenya

(Received 8 March 1983, Accepted 30 March 1983)

The concentrations of phosphorylcreatine (PCr), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inorganic phosphate (Pi), pyruvate and lactate were determined in freeze-clamped fast muscle samples from *Oreochromis alcalicus grahami* a fish adapted to extreme alkalinity (~ pH 10.0) and high temperatures (Lake Magadi, Kenya). Specimens were analysed from both geothermally heated hot springs (35–37°C) and from isolated cool pools (28°C) and from stocks acclimated to 20°C in the laboratory. The ratios of (ATP)/(ADP) and (ATP)/(ADP) (Pi) decreased with increasing body temperature consistent with an increase in glycolysis and tissue respiration rates, respectively. The apparent equilibrium constant of creatine kinase (K_{CK}), (creatine)/(ATP)/(phosphorylcreatine) (ADP) was found to decrease with increasing temperature: 20.2 (20°C), 13.9 (28°C), 8.0 (37°C). A near constant muscle and blood pH (or slight increase in alkalinity with higher temperatures) was found regardless of body temperature (Blood pH 7.64, 7.74, muscle pH 7.27, 7.51 at 20°C and 35°C, respectively). These results are consistent with an unusual pattern of acid-base regulation in this species.

I. INTRODUCTION

The blood pH of fishes varies inversely with body temperature ($\Delta \text{pH}/dT^\circ \text{C} = 0.0189/^\circ \text{C}$) through the regulation of plasma bicarbonate concentrations (Randall & Cameron, 1973; Rahn & Garey, 1973; Reeves, 1977). A similar relationship between pH and temperature was found for the red and white muscles of the dogfish, *Scyliorhinus stellaris*, (Heisler *et al.*, 1976). The physiological significance of this pattern of acid-base regulation has been variously ascribed to attempts by the organism either to maintain a constant relative alkalinity (OH^-/H^+ ratio) or to maintain a constant fractional charge state of peptide-linked histidine residues (α_{im} or alaphostat hypothesis) (Howell *et al.*, 1970; Reeves, 1977). However, recent studies have shown that a number of species show significant departures from the alaphostat hypothesis (Heisler, 1982; Walsh & Moon, 1982).

It is of interest to extend these studies to species adapted to living under conditions of extreme alkalinity. The ecology and osmoregulation of one such species, *Tilapia grahami*, has been the subject of a number of investigations (Reite *et al.*, 1974; Johansen *et al.*, 1975; Maloij *et al.*, 1978; Eddy *et al.*, 1981). These fish are found in large numbers in the highly alkaline (pH 10.0) and

*Permanent address: Department of Physiology, University of St Andrews, St Andrews, Fife KY16 9TS, Scotland.

†Permanent address: Department of Biological Sciences, The University, Dundee DD1 4HN, Scotland.

warm waters (25–40° C) around the shores of Lake Magadi, Kenya (Reite *et al.*, 1974). Recently, the taxonomy of the cichlids has undergone revision and *T. grahami* has now been re-classified as *Oreochromis Alcolapia alcalicus grahamii* (Trewavas, 1982).

A variety of indirect techniques have been described to measure intracellular pH in fishes (see Reeves, 1977). Unfortunately, the small size (~12 cm total length [T.L.]) of *Oreochromis alcalicus grahami* excludes any method involving the use of chronically implanted cannulae.

In the present study, measurements of muscle metabolites and pH have been made on fish exposed to three different temperatures. The results on muscle pH are qualitatively comparable with measurements of blood pH obtained by cardiac puncture and suggest an unusual pattern of acid-base regulation.

II. MATERIALS AND METHODS

Fish were netted around the edge of Lake Magadi close to the pumping station of the Magadi Soda Company. Specimens were taken from pools fed directly from geothermally heated hot springs where the highest temperature exceeded 40° C, but the fish were commonly found in areas where the temperature averaged 35–37° C. Further specimens were taken from nearby pools, fed indirectly by the spring water, where the water had cooled and the mean temperature was about 28° C. The construction and arrangement of the pools suggested that movement of fish between them was highly unlikely except on the rare occasions when rainstorms caused temporary flooding (Coe, 1966). The fish were trapped so as to inflict a minimum of trauma, transferred to floating keep-nets in the same pools and left for several hours prior to sampling. Other fish were transported to the laboratories in Nairobi and maintained for 3–7 days in tanks of Magadi water at either 20° C or 35° C before use.

SAMPLING OF MUSCLES

Fish, mean length 9.3 ± 0.4 cm, and 12.4 ± 1.2 g body weight, were removed from the keep-net and freeze-clamped with tongs cooled in liquid nitrogen (–159° C). Fish that struggled during capture were returned to the pool and not taken for analysis. Using this freeze-clamping procedure the outer-layers of muscle tissues are frozen almost immediately and reliable measurements of tissue adenylates can be obtained (Walesby & Johnston, 1980).

Frozen samples of white fibres (200–300 mg) were pulverized in a mortar and pestle cooled in liquid nitrogen. The powder was extracted in 2.1 ml of 0.6 N perchloric acid (HClO₄) for 4 min at 0° C. Tissue debris was immediately removed by centrifugation (30 s at 2000 g) and an aliquot of the clear supernatant neutralized by approximately 400 µl of 2.2 M potassium carbonate (K₂CO₃). Complete neutralization was checked by the addition of a small amount of methyl orange indicator. Samples were divided into a small number of glass vials and stored frozen until analysis.

SAMPLING OF BLOOD

Blood sampling was carried out at the lakeside on large individuals, 5–15 g. The most successful method used was to draw blood directly from the heart. Fish were first rinsed in fresh water at the same temperature as the lake pools, then placed ventral side upwards in a plastic holder. A fine needle attached to a 100 µl Terumo syringe (heparinized) was used to penetrate and draw blood from the heart and pericardial cavity, usually 50–100 µl being obtained depending on the size of the fish, normally being completed in 1 min.

MEASUREMENT OF BLOOD AND MUSCLE PH

Immediately blood had been drawn its pH value was measured using a Radiometer micro pH electrode system (BMS mk 2) maintained at the appropriate temperature attached to

a Radiometer PHM7 mk 2 meter, powered by a Honda portable generator. Under these conditions the performance of the equipment closely matched that obtained in the laboratories. Each fish normally yielded sufficient blood for two determinations which were averaged. Muscle pH was determined directly in the same Radiometer digital acid-base analyser connected to a thermostatically controlled circulating water bath. Samples (~50 mg) were weighed, frozen and homogenized at 0°C with 5 ml g⁻¹ wet wt of a solution containing 145 mM potassium chloride (KCl) and 10 mM sodium chloride (NaCl) in a motor-driven glass-Teflon Potter-Elvehjem-type homogenizer. pH measurements of homogenates were performed at either 20°C, 28°C or 35°C and a reading taken over 1 min and extrapolated back to zero time. This method gives values of tissue pH which are dependent both on H⁺ activity and the buffering capacity of all the different cellular compartments, for example, cytosol, mitochondria, blood, interstitial fluid, etc. In the case of white skeletal muscle the cytosol makes the major contribution and pH values obtained largely represent an 'average' for this compartment.

ASSAY OF MUSCLE METABOLITES

The following assays were based on the oxidation or reduction of nicotinamide adenine dinucleotides (NAD) in coupled enzyme systems monitored at 340 nm using a Beckman Model DB spectrophotometer fitted with a strip chart recorder. Assays were carried out in duplicate and standards run for each assay batch. All metabolite concentrations were expressed as μmoles g wet wt⁻¹.

Adenosine triphosphate (ATP)

Adenosine triphosphate (ATP) was determined in a medium containing 60 mM triethanolamine-hydrochloric acid ([HOC₂H₄]₃N-HCl), pH 7.5, 5 mM magnesium chloride (MgCl₂), 8 mM glycerate-3-phosphate, 0.1% bovine serum albumin, 0.5 mM EDTA, 1 mM glutathione and 0.25 mM NADH. The reaction was started by simultaneous addition of 12 U phosphoglycerate kinase (from yeast) and 3 U glyceraldehyde phosphate dehydrogenase (from yeast), and was complete within 15 min.

Phosphorylcreatine (PCr)

Phosphorylcreatine (PCr) was determined in the same spectrophotometer cuvette by addition of 0.22 mM ADP (final concentration) and approximately 30 U creatine kinase (from rabbit muscle).

Adenosine diphosphate (ADP) and adenosine monophosphate (AMP)

Adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were assayed in a medium of 60 mM (HOC₂H₄)₃N HCl pH 7.6, 77 mM K₂CO₃, 1.0 mM phosphoenol pyruvate, 19 mM magnesium sulphate (Mg₂SO₄), 68 mM KCl, 0.33 mM NADH, 10 mU lactate dehydrogenase (from pig heart). The assay for ADP was started by addition of 12 mU of pyruvate kinase (from rabbit muscle). The AMP was subsequently assayed in the same cuvette by addition of 150 mU of adenylate kinase (from rabbit muscle).

Inorganic phosphate (Pi)

Inorganic phosphate (Pi) was determined in a coupled system based on the phosphorylation of glyceraldehyde-3-phosphate using glyceraldehyde-3-phosphate dehydrogenase (Guynn *et al.*; 1972). The reaction medium contained 30 mM tris-HCl pH 8.4, 50 mM KCl, 2 mM Mg₂SO₄, 0.1 mM EDTA, 5 mM glucose, 0.1 mM ADP, 0.1% bovine serum albumin, 1 mM NAD, 0.8 mM fructose diphosphate. To 25 ml of this cocktail were added 240 U glyceraldehyde phosphate dehydrogenase (from yeast), 200 U hexokinase (from yeast), 14 U aldolase (from rabbit muscle) and 675 U phosphoglycerate (from yeast), after which the cocktail was allowed to stand for 30 min at +4°C for endogenous Pi to be fully used. Assays were carried out on 20–40 μl of tissue extract in a final volume of 1 ml reaction medium.

Pyruvate

Pyruvate was assayed in a medium containing 100 mM $(\text{HOC}_2\text{H}_4)_3\text{N}$ pH 7.6, 0.25 mM NADH and excess lactate dehydrogenase (from rabbit muscle).

Lactate

Lactate was determined in a medium containing 150 mM hydrazine ($\text{H}_2\text{N}\cdot\text{NH}_2$), 400 mM glycine pH 9.2, 2.5 mM NAD, 20 U lactate dehydrogenase (from rabbit muscle).

Free creatine

Free creatine was determined colorimetrically as described by Eggleton *et al.*, 1953.

CALCULATIONS

Apparent equilibrium constants for the creatine kinase (K_{CK}) reaction, phosphorylation state ratio and adenylate energy charge were derived from the molar ratios of the relevant metabolites.

$$\text{Apparent } K_{\text{CK}} = \frac{(\text{creatinine}) (\text{ATP})}{(\text{creatinine phosphate}) (\text{ADP})}$$

$$\text{Phosphorylation state ratio} = \frac{(\text{ATP})}{(\text{ADP}) (\text{Pi})}$$

$$\text{Adenylate energy charge} = \frac{(\text{ATP}) + 0.5 (\text{ADP})}{(\text{ATP}) + (\text{ADP}) + (\text{AMP})}$$

CHEMICALS

All biochemical reagents were obtained from either the Sigma Chemical Co. (London) or Boehringer Mannheim (UK) Ltd. Other chemicals were B.D.H. (Poole) Ltd, analar grade.

STATISTICAL ANALYSIS

Results were compared using a student's *t*-test. Values of apparent K_{CK} , (ATP)/(ADP), phosphorylation state ratio and adenylate energy charge were subject to arcsine transformation.

III. RESULTS

The values obtained for blood pH and muscle pH and metabolite concentrations are shown in Table I. Lactate concentrations are somewhat lower in the 20° C than either the 28 or 35–37° C groups of fish. However, the similarities of both lactate/pyruvate ratios and free creatine concentrations would not indicate any major difference in the three groups with respect to handling stress or exercise. The values for lactate are within the range reported for 'rested' individuals in the literature (Black *et al.*, 1961).

Increasing temperature is associated with a decrease in ATP and an increase in ADP concentrations ($P < 0.01$) (Table I). The apparent equilibrium constant for K_{CK} decreased from 20.2 at 20° C to 8.0 at 35.36° C ($P < 0.001$) and had an intermediate value in fish collected from 'cool' pools (28° C). Ratios of (ATP)/(ADP) and phosphorylation state ratio decreased with increasing body temperature while energy charge remained high (0.84–0.95) (Table I).

At each temperature investigated, blood pH was found to be 0.2–0.3 units more alkaline than corresponding values of muscle pH (Table I). In both cases pH values were significantly higher in 35–36° C fish than those held at 20° C.

TABLE I. Concentrations of metabolites ($\mu\text{moles g wet wt}^{-1}$) apparent K_{CK} equilibrium constant and muscle and plasma pH in *O. alcalicus grahami* acclimated to three different temperatures

Parameter	Environmental temperature		
	Nairobi (20°C)	Magadi 'cool pool' (28°C)	Magadi 'hotspring' (35–37°C)
PCr	17.5 ± 2.2	15.3 ± 1.0	17.4 ± 1.9
Cr	25.6 ± 2.7	29.4 ± 1.9	27.8 ± 2.7
ATP	4.0 ± 0.4	3.4 ± 0.3	2.6 ± 0.4
ADP	0.29 ± 0.051	0.47 ± 0.024	0.52 ± 0.045
AMP	0.22 ± 0.027	0.26 ± 0.042	0.29 ± 0.022
Pi	6.1 ± 0.5	6.5 ± 0.7	5.0 ± 0.4
Pyruvate	0.084 ± 0.019	0.19 ± 0.030	0.18 ± 0.043
Lactate	4.88 ± 0.71	8.53 ± 0.47	7.81 ± 1.4
Lac/Pyr ratio	58.1	44.9	43.4
Blood pH	7.64 ± 0.055	ND	7.74 ± 0.06
Muscle pH	7.27 ± 0.035	ND	7.51 ± 0.057
Apparent K_{CK}	20.2	13.9	8.0
(ATP)/(ADP)	13.8	7.2	5.0
(ATP)/(ADP) (Pi)	2.26	1.11	1.0
Energy charge	0.95	0.88	0.84
(ATP) + 0.5(ADP)			
(ATP) + (ADP) + (AMP)			

ND = not determined.

IV. DISCUSSION

Values obtained for fast muscle ATP, AMP and ADP concentrations in *O. alcalicus grahami* are within the range reported for other fish (Beis & Newsholme, 1975; Thillart *et al.*, 1980; Walesby & Johnston, 1980) and show consistent changes in relation to body temperature. The relative concentrations of adenine nucleotides are thought to be important in the control of respiration rate in coupled mitochondria (Erecinska *et al.*, 1977) and in the carbon flux through glycolysis (Newsholme & Start, 1973) via their effects on key regulatory enzymes. The decreases in phosphorylation state and in (ATP)/(ADP) ratio with increasing temperature are consistent with an increase in cellular respiration and glycolysis, respectively. This parallels the expected direct effects of temperature on protein turnover, resting metabolism and routine activity and correlates with the observed locomotory behaviour of the fish which become considerably more active at higher temperatures.

Studies of rat brain metabolism during arterial hypoxemia and hypercapnia (Siesjo *et al.*, 1975) and of human muscle following isometric exercises have shown a positive linear correlation between the log apparent equilibrium constant of K_{CK} and tissue pH determined simultaneously by other methods (Sahlin *et al.*, 1975). Since temperature directly affects (ATP)/(ADP) ratios, the K_{CK} equilibria method cannot be used as a measure of intracellular pH in the present study.

TABLE II. Ionic composition and pH of Magadi water

Parameter	Units	Reference
pH	9.84 (35°C) 9.90 (20°C)	This study
Ionic strength	0.5	Eddy & Maloiy, unpubl. data
Na	210 mM	Eddy & Maloiy, unpubl. data
HCO ₃ ⁻	87 mM	Maloiy <i>et al.</i> , 1978

Measurements of intracellular pH are highly dependent on the analytical method used. The method used to obtain blood from *Oreochromis* for pH measurements was not ideal since the fish were stressed by removal from the water and the time taken for heart puncture. This is likely to result in lower pH values than might be obtained for unstressed fish. For example, Eddy *et al.* (1977) obtained a mean pH value of 7.67 at 20° C using unstressed rainbow trout, *Salmo gairdneri*, with a cannula to the ventricular aorta while at the same temperature Smit *et al.* (1981) obtained a mean value of pH 7.57 by heart puncture. The small size of the fish would seem to preclude any reasonable technique for obtaining blood samples from free-swimming unstressed individuals. However, in the present study the sampling procedure was identical at each temperature so that pH values will probably suffer from the same errors. It is noteworthy that the results for blood pH of *Oreochromis* shown in Table I are reasonably uniform and do not show the high variability seen in previous studies (Johansen *et al.*, 1975).

In the present study the pH data for blood are qualitatively similar to those for muscle in that they do not follow the expected relationship with temperature commonly seen in poikilotherms (Reeves, 1977). For example, blood pH is inversely related to temperature with $\Delta\text{pH}/\Delta T$ of around -0.0148 for dogfish, *Scyliorhinus stellaris*, (Heisler *et al.*, 1976); -0.018 for rainbow trout (Cameron & Randall, 1972) and about -0.017 for a variety of amphibia (Reeves, 1977). The blood pH of *Oreochromis* at 35° C is 7.74 and if this varied with temperature according to the relationship shown for trout, then blood pH would be 7.95 at 20° C which contrasts with the measured value of 7.64 (Table I). In view of the method of sampling these must be considered minimum figures and values 0.1 or 0.2 pH units higher may be predicted.

On acclimation of American eels, *Anguilla rostrata*, from 20 to 5° C, Walsh & Moon (1982) found that pH of venous blood decreased far less than predicted by the alaphstat hypothesis ($\Delta\text{pH}/\Delta T^{\circ}\text{C} = -0.0076$). In addition, evidence was obtained for different patterns of acid-base between intra- and extracellular compartments and between different tissues. In the case of red skeletal muscle, intracellular pH was almost constant over the range 5–20° C ($\Delta\text{pH}/\Delta T^{\circ}\text{C} = -0.0033$). Heisler (1982) found that transition from water-breathing to air-breathing in the tropical freshwater fish, *Synbranchus marmoratus*, resulted in an acidosis of the blood (~ 0.6 pH units) due to an increase in PaCO₂ from 6 to 26 mm Hg. In contrast, intracellular pH in white skeletal muscle and cardiac muscle was unaffected by the acidosis in the extracellular compartment due to a four-fold increase in intracellular bicarbonate concentration.

The data in the present study also point to an unusual acid-base regulation in alkaline hotspring fish which does not conform to that predicted by the alaphstat or relative alkalinity hypotheses. It is not unreasonable to look to the peculiar ionic composition and high pH to Magadi water for an explanation (Table II). The increased alkalinity of the blood at reduced body temperature in trout is thought to be the result of an elevation of plasma bicarbonate occurring via exchange processes at the gills (Randall & Cameron, 1975; Reeves, 1977). For example, a trout when warmed to 35° C would show arterial blood pH values of 7.6 or less and HCO_3^- contents of 2.3 mM (Reeves, 1977; Randall & Cameron, 1975). In fact, much higher values of 6–9 mM HCO_3^- are observed in *Oreochromis* at this temperature (Maloiy *et al.*, 1978). It seems possible that *O. alcalicus grahami* have evolved mechanisms to keep blood pH and HCO_3^- within the normal physiological range during excursions into cool pools. Under these conditions, maintenance of a blood pH below 8.0–8.7 at 20° C may take precedence over the regulation of a constant relative alkalinity. A variety of mechanisms could be envisaged including, differential rates for Na^+/H^+ , $\text{Na}^+(\text{NH}_4^+)$ and $\text{Cl}^-/\text{HCO}_3^-$ exchanges across the gill epithelium whereby acid is excreted (or base absorbed).

The failure to maintain a constant OH^-/H^+ ratio with changing temperature might be expected to result in an increase in the temperature dependence of sensitive kinetic parameters such as enzyme-ligand binding. For example, the K_m for pyruvate of goldfish, *Carassius auratus*, M_4 lactate dehydrogenase isoenzyme is less temperature sensitive when measured under conditions of constant relative alkalinity than under conditions of constant pH (Wilson, 1977). Alternatively, enzyme function in the tissues of alkaline hotspring fish may have evolved to be relatively independent of changes in OH^-/H^+ ratios as it presumably has in specialised cell compartments of other animals, etc. lysozymes.

This work was supported by travel grants from The Royal Society. I. A. J. is also grateful for an award from The Browne Fund of the Royal Society.

References

- Beis, I. & Newsholme, E. A. (1975). The contents of adenine nucleotides, phosphogen and some glycolytic intermediates in resting muscles from vertebrates and invertebrates. *Biochem. J.* **152**, 23–32.
- Black, E. C., Robertson, A. C. & Parker, R. R. (1961). Some aspects of carbohydrate metabolism in fish. In *Comparative Physiology of Carbohydrate Metabolism in heterothermic animals*. (A. W. Martin, ed.), pp. 89–124. Washington: Seattle University.
- Cameron, J. N. & Randall, D. J. (1972). The effects of increased ambient CO_2 on arterial CO_2 tension, CO_2 content and pH in rainbow trout. *J. exp. Biol.* **57**, 673–680.
- Coe, M. J. (1966). The biology of *Tilapia grahami* Boulenger in Lake Magadi, Kenya. *Acta Trop.* **23**, 146–177.
- Eddy, F. B., Lomholt, J. P., Weber, R. C. & Johansen, K. (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO_2 tension. *J. exp. Biol.* **67**, 32–47.
- Eddy, F. B., Bamford, O. S. & Maloiy, G. M. O. (1981). Na^+ and Cl^- effluxes and ionic regulation in *Tilapia grahami*, a fish living in conditions of extreme alkalinity. *J. exp. Biol.* **91**, 349–353.

- Egglton, P., Elsdon, S. R. & Gough, N. (1953). The estimation of creatine and of diacetyl. *Biochem. J.* **37**, 526.
- Erecinska, M., Stubbs, M., Miyata, Y., Ditre, C. M. & Wilson, D. F. (1977). Regulation of cellular metabolism by intracellular phosphate. *Biochim. biophys. Acta* **463**, 20-35.
- Guynn, R. W., Veloso, D. & Veech, R. L. (1972). Enzymatic determination of inorganic phosphate in the presence of creatine phosphate. *Anal. Biochem.* **45**, 277-285.
- Heisler, N., Weitz, H. & Weitz, A. M. (1976). Extracellular and intracellular pH with changes of temperature in the dogfish, *Scyliorhinus stellaris*. *Respir. Physiol.* **26**, 249-263.
- Heisler, N. (1982). Intracellular and extracellular acid-base regulation in the tropical freshwater teleost fish *Synbranchus marmoratus* in response to transition from water breathing to air breathing. *J. exp. Biol.* **99**, 9-28.
- Howell, B. J. (1970). Acid-base balance in transition from water breathing to air breathing. *Fedn. Proc. Fedn. Am. Socs exp. Biol.* **29**, 1130-1134.
- Johansen, K., Maloiy, G. M. O. & Lykkeboe, G. (1975). A fish living in extreme alkalinity. *Respir. Physiol.* **24**, 159-162.
- Maloiy, G. M. O., Lykkeboe, G., Johansen, K. & Bamford, O. S. (1978). Osmoregulation in *Tilapia grahami*, a fish in extreme alkalinity. In *Comparative Physiology of Water, Ions and Fluid Mechanics*. (K. Schmidt Nielsen, L. Bolis, S. P. H. Maddrell eds), pp. 229-238. Cambridge: Cambridge University Press.
- Newsholme, E. A. & Start, C. (1973). *Regulation in Metabolism*. New York: Wiley.
- Rahn, H. & Garey, W. F. (1973). Arterial CO₂, O₂, pH and HCO₃⁻ values of ectotherms living in the Amazon. *Am. J. Physiol.* **225**, 735-738.
- Randall, D. J. & Cameron, J. N. (1973). Respiratory control of arterial pH as temperature changes in rainbow trout *Salmo gairdneri*. *Am. J. Physiol.* **225**, 997-1002.
- Reeves, R. B. (1977). The interaction of body temperature and acid-base balance in ectothermic vertebrates. *A. Rev. Physiol.* **39**, 559-586.
- Reite, C. B., Maloiy, G. M. O. & Hasehaug, B. (1974). pH, salinity and temperature tolerance in Lake Magadi *Tilapia*. *Nature (Lond.)* **247**, 315.
- Sahlin, K., Harris, R. C. & Hultman, E. (1975). Creatine kinase equilibrium and lactate content compared with muscle pH in tissue samples obtained after isometric exercise. *Biochem. J.* **152**, 173-180.
- Siesjo, B. K., Folgergrova, J. & MacMillan, V. (1975). The effect of hypercapnia upon intracellular pH in the brain, evaluated by the bicarbonate-carbonic acid method and from the creatine phosphokinase equilibrium. *J. Neurochem.* **19**, 2483-2495.
- Smit, G. L., Hattingh, H. & Ferreira, J. T. (1981). The physiological responses of blood during thermal adaption in three freshwater fish species. *J. Fish Biol.* **19**, 147-160.
- Trewavas, E. (1982). In *Iclarm conference on the biology and culture of tilapias*. R. S. V. Pullin, R. H. Lowe-McConnell, eds). (In press).
- Thillart, G. van den, Kesbeke, F. & Waarde, A. van (1980). Anaerobic energy metabolism of goldfish (*Carassius auratus* L.). Influence of hypoxia and anoxia on phosphorylated compounds and glycogen. *J. Comp. Physiol.* **136**, 45-52.
- Walesby, N. J. & Johnston, I. A. (1980). Temperature acclimation in brook trout muscle: Adenine nucleotide concentrations phosphorylation state and adenylate energy charge. *J. comp. Physiol.* **139**, 127-133.
- Walsh, P. J. & Moon, T. W. (1982). The influence of temperature on extracellular and intracellular pH in the American eel, *Anguilla rostrata* (Le Sueur). *Resp. Physiol.* **50**, 129-140.
- Wilson, T. L. (1977). Interrelations between pH and temperature for the catalytic rate of the M₄ isoenzyme of lactate dehydrogenase (EC1.1.1.27) from goldfish (*Carassius auratus* L.) *Archs Biochem Biophys.* **179**, 378-390.