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EFFECTS OF ENVIRONMENTAL FACTORS ON EXERCISE IN FISH

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Summary

The critical swimming velocity of fish is affected by environmental conditions and the highest swimming speed is obtained only under specific circumstances. The mechanisms causing a reduction in exercise capacity depend on the type of environmental change. Acid waters exert an effect by reducing oxygen transport capacity, whereas reduced temperatures act largely on muscle contractility. Alkaline conditions and salinity change may affect both gas transport and muscle contractility.

A fish must operate over a wide range of internal and external conditions and must possess both short- and long-term mechanisms to maintain function under a wide variety of conditions. These mechanisms may be part of the immediate reflex arsenal available to the fish (for example, catecholamine release) or they may be mechanisms induced during acclimation. The nature of the acclimation process is an indication of what may be limiting exercise under a particular set of circumstances.

The concept of symmorphosis, especially when applied to ectotherms, needs to be viewed in a broader context. A symmetry of component parts may exist such that structures are designed to satisfy functional requirements for operation over a wide range of conditions, rather than optimally for a given set of conditions. This must involve compromises in design, especially for ectotherms such as fish. Thus, the more variable the conditions under which a system must operate, the less apparent it will be that symmorphosis exists between the component parts.

Introduction

Symmorphosis refers to the symmetry between component parts of a physiological system such that any one part will satisfy, but not exceed, the functional requirements of that system (Taylor and Weibel, 1981). Lindstedt and Jones (1987) rephrased this idea, stating that 'symmorphosis considers structures to be optimally designed to satisfy functional requirements'. In many situations the components of a system will serve several functions and therefore be subject to many, perhaps conflicting, forces. As Lindstedt and Jones point out, it is very difficult to identify the selective forces that have operated in the evolution of a particular system. The

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final outcome could be the end result of a series of compromises. Individuals must operate in a variety of conditions, both internal and external, that place constraints on systems. There are probably many compromises in design such that an animal can maintain function over a wide range of situations, although not performing any of the functions optimally. That is, many systems are not optimally designed for any one function and/or set of conditions, but represent a compromise between conflicting requirements. For example, acid conditions, which cause a reduction in blood oxygen capacity *via* the Root shift that results in the delivery of oxygen to the swimbladder and eye in teleost fish, could also reduce blood oxygen capacity and the delivery of oxygen to all tissues if the acidosis is general, as for example following exhaustive exercise.

Physiological systems have evolved to take into account probable operating conditions, based on the past experience of the species. Such information is stored in the genotype and expressed as the phenotype. Variability in the phenotype is perhaps an indication of weak and/or conflicting selective forces. Such variability is probably larger in ectotherms like fish, which must operate over a wide range of environmental temperature, pH and salinity, than in mammals. For this reason, it seems likely that symmorphosis is probably more applicable to mammals and birds than to fish and amphibians.

Fish acclimate to changes in the environment. Any adjustment made by the animal during this process is an indication of a possible rate-limiting step in a physiological system. Acclimation may involve direct regulation, that is a return to the original internal state, as seen in animals exposed to salinity change. The ionic composition of the body is disturbed by the environmental change and then returned to the original level by modulation of epithelial ion transport mechanisms. Alternatively, acclimation may involve indirect compensation, as seen in the response of fish to temperature change. In this case, the animal cannot regulate temperature but alters some other feature, such as muscle contractility, to maintain function. The variable that is changed provides an indication of a design limitation at the new temperature. Finally, animals may have only a limited capacity to compensate for environmental change, presumably because the animal has not evolved the required components. This may be the case when many fish species are exposed to acid conditions.

Exercise involves the interaction of many systems and it has been suggested that it provides an integrated measure of an animal's fitness for a particular environment (Nelson, 1989). There are many forms of exercise in fish, including sustained, prolonged and burst activities, often using either C-type or S-type fast starts. Burst activity and fast starts utilize local energy stores, are stereotypic, and are generally considered to be independent of environmental conditions, except where energy stores and/or muscle contractility have been affected. The effects of changes in the environment on these forms of activity, however, have not been investigated in detail.

Aerobic exercise (prolonged and sustained swimming) is initially affected by changes in the environment (Fig. 1). Trout can swim faster in water of neutral p

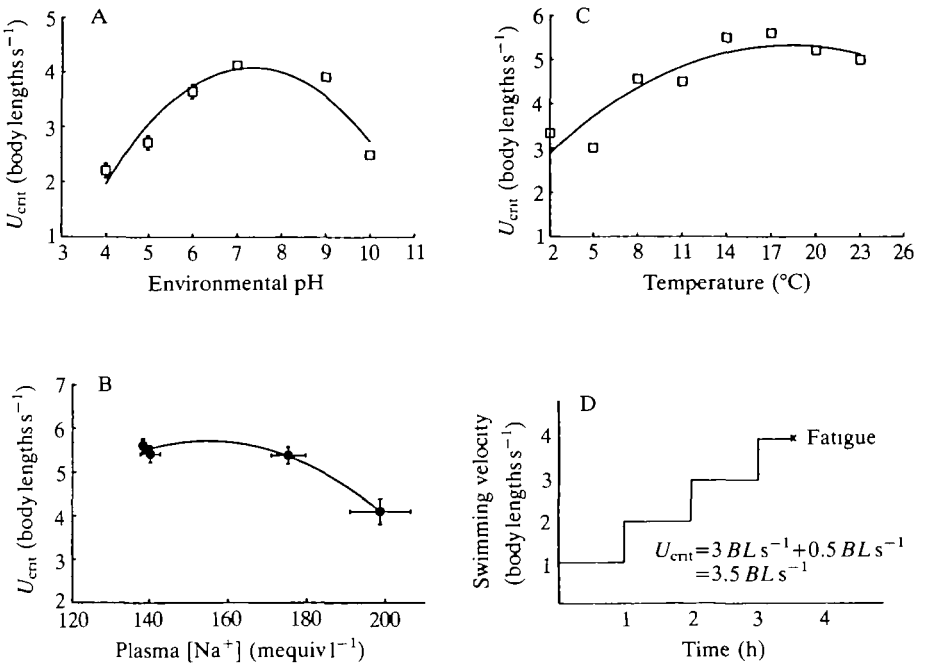

Fig. 1. The effect of (A) environmental pH (N=5) (modified from Ye and Randall 1991), (B) elevated plasma Na^+ concentration (N=6) (C. J. Brauner, J. M. Shrimpton and D. J. Randall, unpublished data) and (C) water temperature (N=2, no error bars) (modified from Griffiths and Alderdice, 1972) on the critical swimming velocity (U_{crit}) of various salmonid species. (D) Calculation used to determine U_{crit}. Bars show ±S.E.M. BL, body length.

than in acid or alkaline water. Similar relationships can be seen between swimming speed and temperature or plasma sodium concentration. Hypoxic water also reduces swimming speed. That is, changes in the environment can have a marked effect on aerobic swimming performance in fish.

The aerobic swimming performance in fish is affected by muscle contractility and/or the rate of gas transfer. Proponents of symmorphosis might argue that the component parts of this system would be balanced such that any link in the chain was no larger than any other. This seems to be true for blood oxygen-carrying capacity. Davis *et al.* (1963) showed that, in general, hyperoxia did not increase swimming performance in salmonid fish and Jones (1971) showed that critical swimming velocity (U_{crit}) was reduced in rainbow trout if blood oxygen content was reduced or if the fish was exposed to hypoxia. That is, blood oxygen capacity is matched to the other components of the system and is sufficient for, but does not exceed, the requirements for maximum aerobic swimming performance and, therefore, is consistent with the concept of symmorphosis. This may not be so under all conditions, however, and each component of the gas exchange/muscle contraction system may be affected differentially by environmental change. For

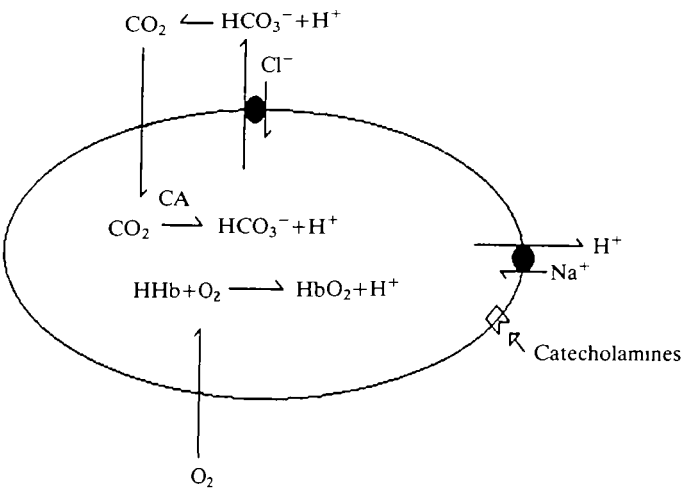

Fig. 2. The regulation of erythrocytic pH during a metabolic acidosis, following adrenergic stimulation. The increased Na^+/H^+ transfer transports protons out of the erythrocyte at a greater rate than the Jacobs–Stewart cycle replaces them, owing to the absence of carbonic anhydrase (CA) in the plasma. Hb, haemoglobin.

example, the reduction in swimming performance as a result of water acidification may be due to changes in blood oxygen-carrying capacity, whereas that associated with a change in temperature can be explained by effects on muscle contractility.

Fish increase gas transfer during exercise by increasing water and blood flow, as well as by increasing diffusing capacity and oxygen extraction in the tissues (Jones and Randall, 1978), as in other vertebrates. Gas transfer in fish, however, has many important characteristics that differ from those found in other vertebrates. Many teleosts have blood with a marked Root shift; that is, a reduction in blood pH results in a decrease in haemoglobin oxygen capacity. This is important for the transfer of oxygen into the swimbladder, where a local acidosis liberates oxygen from haemoglobin. Should the acidosis become general, however, as occurs following exhaustive activity, then blood oxygen-carrying capacity is reduced and a reduction in swimming performance might be anticipated. This does not occur because the liberation of catecholamines into the blood maintains erythrocytic pH in the face of a blood acidosis, and no Root shift is observed (Fig. 2). This increase in erythrocytic pH is caused by a β-adrenergic activation of Na^+/H^+ transfer across the red blood cell membrane that results in a disequilibrium of the proton gradient across the erythrocyte membrane, raising intracellular pH. The erythrocytic membrane is relatively impermeable to protons and so acid is transferred between the plasma and the red blood cell *via* the Jacobs–Stewart cycle. The increase in red blood cell pH is not immediately short-circuited by the Jacobs–Stewart cycle, because the plasma carbon dioxide hydration/dehydration reaction is uncatalysed and the rate of proton transfer is much faster than the plasma bicarbonate dehydration reaction velocity (Forster and Steen, 1969; Motais *et al.*

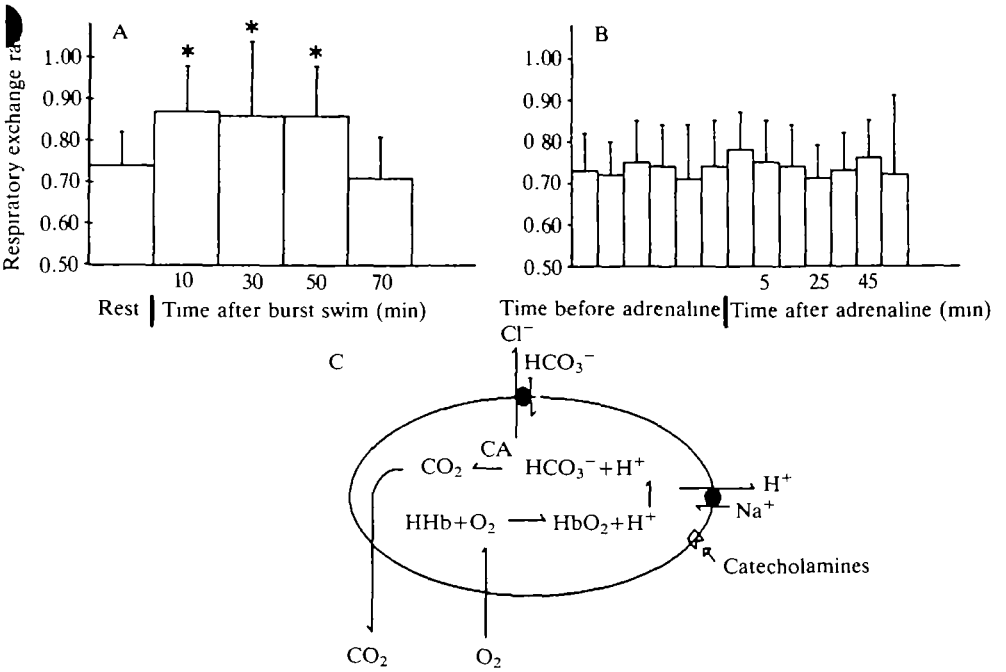

Fig. 3. Respiratory exchange ratio (RE) (A) prior to and following a burst swim and (B) prior to and following adrenaline infusion in fish at rest (modified from Steffenson *et al.* 1987). Bars show S.D. (N=6). Asterisks indicate values significantly different from resting value ($P \leq 0.025$). (C) Maintenance of CO_2 excretion during adrenergic stimulation of the erythrocyte. Protons essential for HCO_3^- dehydration are released from the haemoglobin upon oxygenation. CA, carbonic anhydrase; Hb, haemoglobin.

1989; Nikinmaa *et al.* 1990). This means that, unlike the situation in mammals, the capillary endothelium in fish cannot contain carbonic anhydrase activity because if it did, the catecholamine-stimulated rise in erythrocytic pH would be short-circuited and blood oxygen-carrying capacity would be reduced. Thus, all carbon dioxide transfer must occur through the red blood cell, as shown by Perry *et al.* (1982), and there must be a tight coupling of oxygen uptake and carbon dioxide excretion. Haemoglobin oxygenation supplies the protons for bicarbonate dehydration and, therefore, carbon dioxide excretion. Within the red blood cell this occurs at the catalysed rate and so during the rapid transit of blood through the gills, carbon dioxide excretion is maintained, even though the erythrocytes may be stimulated by catecholamines (Steffensen *et al.* 1987). Haemoglobin oxygenation will not only supply protons for bicarbonate dehydration but could also activate Na^+/H^+ exchange (Motais *et al.* 1989) and some reduction in carbon dioxide excretion may occur (Fig. 3). The extent of this reduction will depend on the relative rates of Na^+/H^+ exchange and the catalysed bicarbonate dehydration reaction during the early phases of oxygenation and subsequently on the relative rates of Na^+/H^+ transfer and Cl^-/HCO_3^- exchange. There would appear to be some adrenergically mediated expulsion of the protons liberated from the

haemoglobin into the plasma because there is a large, but slow, increase in blood Pa_{CO_2} after exhaustive exercise.

Aquatic vertebrates, unlike those on land, have muscle membranes that are permeable to ammonium ions as well as ammonia (Wright *et al.* 1988). In addition, during exhaustive exercise in fish the muscle adenylate pool is metabolised, resulting in ammonia production in the muscle (Mommsen and Hochachka, 1988). Fish muscle ammonia levels are relatively high and the ammonium ion permeability may be functionally connected to the large production of ammonia that occurs in fish during violent exercise, enhancing excretion from the muscle and reducing the possibility of an impairment of contractility due to increased ammonia levels.

Maximum aerobic swimming performance in fish (critical swimming velocity) can be seen as a series of compromises between conflicting functions. How then do changes in the environment affect exercise performance in fish?

Water pH

Swimming is reduced under acid conditions; exposure of the fish to water at pH 4 causes inhibition of proton excretion and a reduction in sodium uptake, resulting in a gradual reduction of plasma NaCl level associated with an acidosis if the fish is in soft (low-calcium) water (Packer and Dunson, 1970; Dively *et al.* 1977; Booth *et al.* 1982; McKeown *et al.* 1985; McDonald, 1983; Ye *et al.* 1991). The reduction in ionic content is a function of both a reduced influx and an elevated efflux of the ions (Wright and Wood, 1985; Booth *et al.* 1982). At rest, this osmoregulatory disturbance is correlated with a haemoconcentration due to an increase in the number of circulating red blood cells, an increase in red blood cell volume and a decrease in total blood volume through a reduction in plasma volume (Milligan and Wood, 1982). Milligan and Wood (1982) have suggested that the increase in haematocrit may cause an increase in blood viscosity and dorsal aortic pressure. In amphibians, a reduction in blood volume through dehydration or haemorrhage reduces cardiac output, primarily through a reduction in stroke volume, and reduces maximal oxygen consumption (Hillman, 1987; Hillman and Withers, 1988). Thus, it seems probable that the haematological alterations that are seen in concert with a reduction in plasma ion concentrations may reduce oxygen delivery to the tissues, especially during exercise.

The acidosis in response to acid-water exposure does not cause catecholamine release (Ye *et al.* 1991), presumably because changes are slow compared with those observed following exhaustive exercise, and are not sufficient to invoke catecholamine release. Thus, acid exposure results in a fall in red blood cell pH (Fig. 4), presumably reducing blood oxygen content *via* a Root shift. The reduced blood oxygen content presumably limits maximum aerobic capacity and contributes to the decrease in U_{crit} seen in fish exposed to acid waters. The acidosis that follows exhaustive exercise does not limit subsequent episodes of aerobic exercise

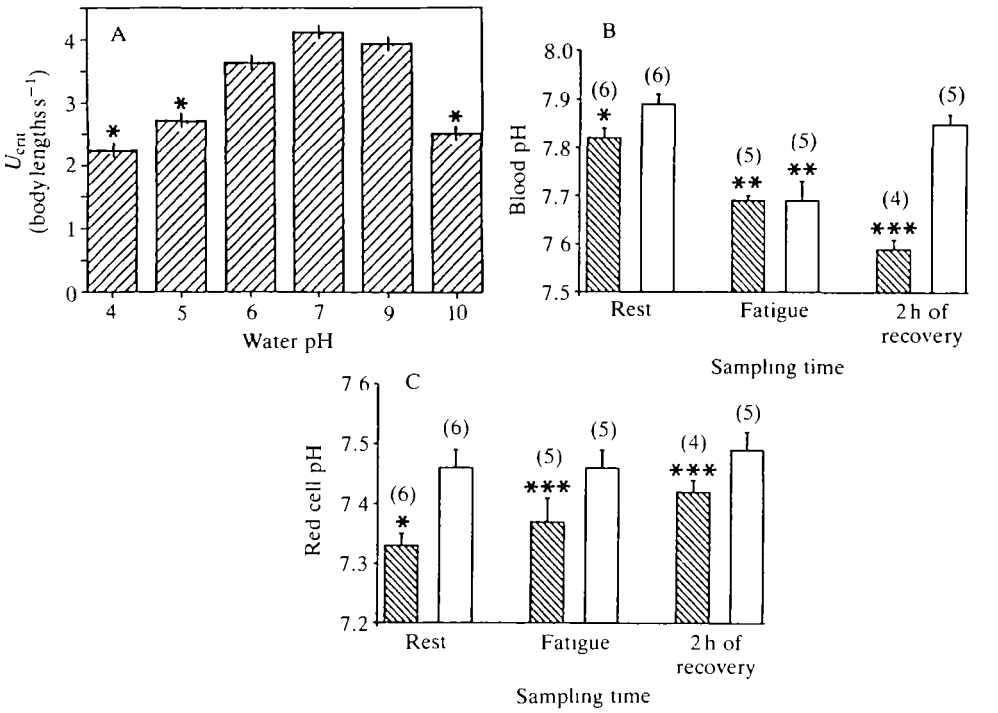

Fig. 4. (A) The effect of environmental pH on critical swimming velocity U_{crit} in adult rainbow trout. * Significantly different from the value at pH 7 ($P \leq 0.05$). (B,C) The effect of two environmental pH values (4, diagonally hatched columns, and 7, open columns) on (B) blood pH (pHe) and (C) red blood cell pH (pHi) during exercise and recovery in adult rainbow trout (modified from Ye and Randall, 1991). Bars show ±S.E.M. ** Significantly different from respective resting level; *** significantly different from both resting and pH 7 value. Numbers in parentheses indicate sample size.

(Randall *et al.* 1987; Fig. 5) because oxygen content is maintained by catecholamine release (Primmett *et al.* 1986), which elevates red blood cell pH.

Although the capillary endothelia of fish appear not to have carbonic anhydrase activity, fish muscle does have the typical vertebrate type III version (Sanyal *et al.* 1984). The cellular location of this carbonic anhydrase is not known in fish, but it may be on the sarcolemma, as well as in the intracellular compartment, as in mammals. This would ensure a rapid carbon dioxide hydration/dehydration reaction in the extracellular space surrounding the muscle. Thus, carbon dioxide excreted from the muscle could acidify the surrounding fluid and enhance NH_4^+ excretion by acid trapping (Fig. 6). This was tested by Y. Tang, H. Lin and D. J. Randall (in preparation), who showed that bicarbonate infusion reduced ammonium movement out of the muscle (Fig. 6). Fish exposed to alkaline conditions show a marked blood alkalosis, which may also lead to accumulation of muscle ammonia, exacerbated by a rise in blood ammonia level (Lin and Randall, 1990). Muscle membranes are permeable to ammonium ions in fish (Wright *et al.* 1988).

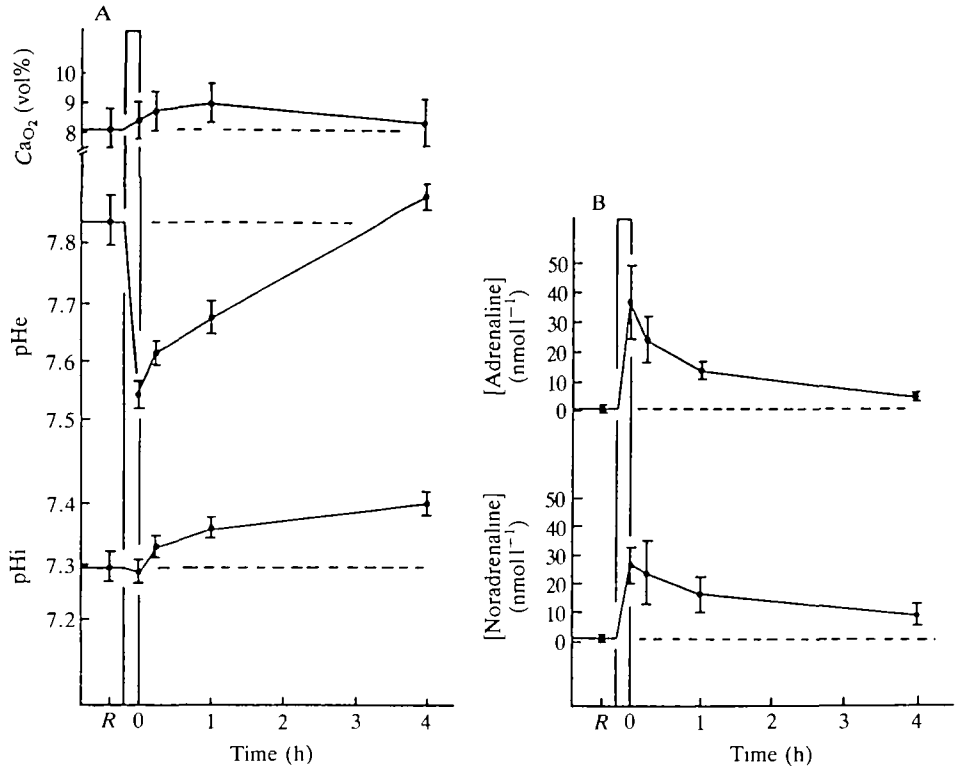

Fig. 5. The effect of a burst swim and subsequent recovery on (A) arterial oxygen content (Ca_{O_2}), blood pH (pHe) and red blood cell pH (pHi) and (B) plasma adrenaline and noradrenaline concentrations. R, rest; bar, burst activity to exhaustion; 0 h, start of recovery at 80% U_{crit} (from Primmett *et al.* 1986). Bars show ±S.E.M. (N=13).

This is associated with a reduction in the muscle adenylate pool following exhaustive exercise (Mommsen and Hochachka, 1988), resulting in large increases in muscle ammonia levels. Exposure of fish to alkaline conditions results in blood ammonia accumulation (Wright and Wood, 1985; Lin and Randall, 1990) and a blood alkalosis (Ye *et al.* 1991). Both will tend to increase muscle ammonia content and this may be the cause of impaired swimming in trout exposed to alkaline waters. There is, however, no direct evidence for an effect of elevated ammonia levels on muscle contractility. It is possible that ammonia may limit exercise because of a neural rather than a muscular impairment. At high concentrations, ammonia causes convulsions in fish (Hillaby and Randall, 1979), as in other vertebrates, indicating the possibility that at lower levels there may be some impairment of neural function which, in turn, may limit exercise.

Temperature

Acute exposure (approximately 24 h) to temperatures above and below acclim

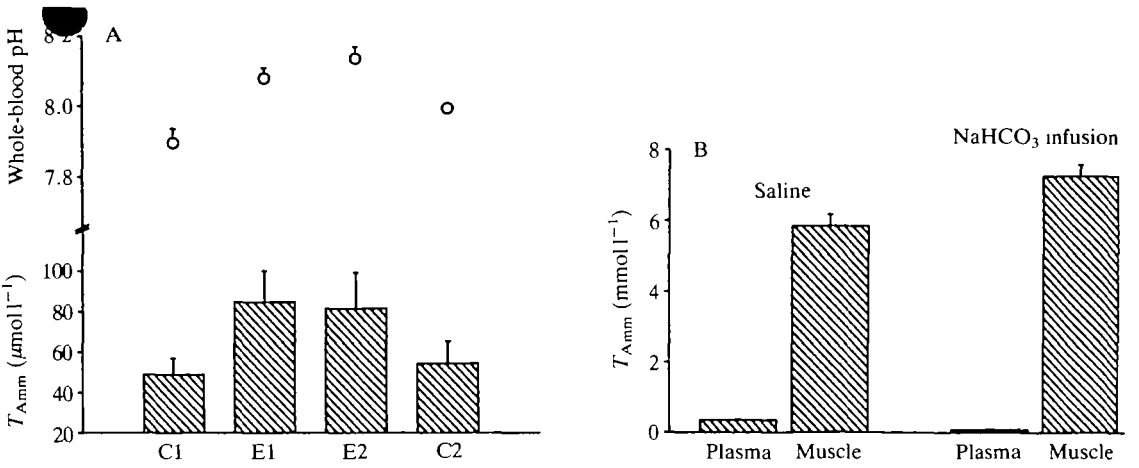

Fig. 6. (A) Whole-blood pH and plasma total ammonia concentrations (T_{Amm}) in rainbow trout in neutral water (C1); alkaline water (pH=9.9–9.6) for 45 min (E1) and 90 min (E2), and following 30 min in neutral water (C2) (modified from Lin and Randall, 1990). (B) Plasma and muscle T_{Amm} 15 min after exhaustive exercise in fish injected with saline or $NaHCO_3$ (from Y. Tang, H. Lin and D. J. Randall, in preparation). Bars show S.E.M. (N=6)

ation temperature results in a reduction in U_{crit}. The temperature-induced reduction in aerobic swimming performance could stem from changes in the external environment, such as viscosity of the medium or oxygen availability, but more probably it is caused by changes in the internal environment, such as oxygen delivery to, or power produced by, the muscle. The relationship between U_{crit} and temperature is described by a bell-shaped curve, whereas viscosity and water oxygen content decrease with an increase in temperature, and the changes are relatively minor over the range of temperatures tolerated by the fish. Limitations to aerobic swimming performance are, therefore, probably the result of internal changes associated with temperature, such as the oxygen-carrying capacity of the blood or reductions in muscle contractility.

In temperatures above that of acclimation, O_2-carrying capacity of the blood could be reduced through a rightward shift of the haemoglobin oxygen dissociation curve. In addition, the oxygen content of the external environment will be reduced as the solubility of oxygen in water varies inversely with water temperature. Thus, an increase in temperature could reduce the oxygen-carrying capacity of the blood and limit oxygen delivery to the tissues. The converse is true, however, for fish in cold water, where the level of dissolved oxygen in the external environment will increase, as will the haemoglobin oxygen-affinity of the blood. Thus, one might expect U_{crit} to increase with decreasing temperature, a characteristic that is not noted. Thus, although temperature-induced limitations in oxygen delivery to the tissues may be responsible for a reduction in U_{crit} of fish swimming in warmer water, this does not appear to be the case for those in cold water.

The reduction in U_{crit} at low temperatures may be due to a reduction in power generated by the muscle, resulting in a 'compression of recruitment order' (Rome *et al.* 1985). In acclimated fish there is a gradual recruitment of the red muscle fibres as swimming velocity increases, until all the red muscle fibres are being utilized. Upon complete utilization of the aerobic fibres, any increase in velocity is accomplished through the sequential recruitment of anaerobic fibres. The first use of anaerobic fibres in carp occurs at approximately 36 % of the maximal muscle contraction velocity (V_{max}) (Rome *et al.* 1990; Rome and Sosnicki, 1990), and the relative contribution of anaerobic muscle to the propulsion increases with velocity. Carp acutely exposed to cold water show a reduction in the force and power generated by the red muscle fibres, but the power required to swim at a given speed does not change. Thus, animals in cold water swimming at $2\,BL\,s^{-1}$ (where BL is body length) will require a greater proportion of red muscle fibres than will the fish at warmer temperatures. Cold-water fish will have recruited all their red muscle fibres at a lower swimming velocity than will a fish in warmer water and the recruitment of white muscle fibres will occur earlier. The recruitment order of the muscle fibre types in fish in cold water is similar to that seen in warmer water and the point at which white muscle fibres are recruited is still 36 % of V_{max}, but this all occurs over a smaller range of speeds. Thus, the compression in recruitment order results in the animals reaching fatigue at a lower swimming velocity.

Reductions in swimming speed at high temperatures are less than those at low temperatures. Calculated reductions due to decreases in oxygen delivery for fish in warm water are likely to be less than the reductions in cold water calculated from *in vitro* changes in muscle contractility. Thus, predictive reductions in U_{crit} based on these features mimic those determined in whole-animal studies.

Thermal acclimation to low temperatures results in adjustments of the muscle, including an increase in the relative proportion of red to white muscle in goldfish and striped bass and an increase in mitochondrial density, and thus the associated mitochondrial enzymes, within that red muscle (see review by Sidell and Moerland, 1989). The increased oxidative potential of the red muscle will act to offset the temperature-induced reduction in the biochemical reaction rates, which probably reduce the force and power of muscular contraction. Striped bass acclimated to 9°C swam faster following acute transfer to 15°C than those acclimated to 25°C and swum at 15°C (Sisson and Sidell, 1987). This indicates that modifications at the cellular level during acclimation allow the animal to compensate for the acute reductions in swimming velocity seen with a reduction in temperature. These changes in muscle contractility occurring during acclimation to low temperatures support the notion that muscle contractility limits exercise in animals acutely exposed to reductions in temperature.

Salinity

Rapid transfer of juvenile coho salmon to full-strength sea water results in a

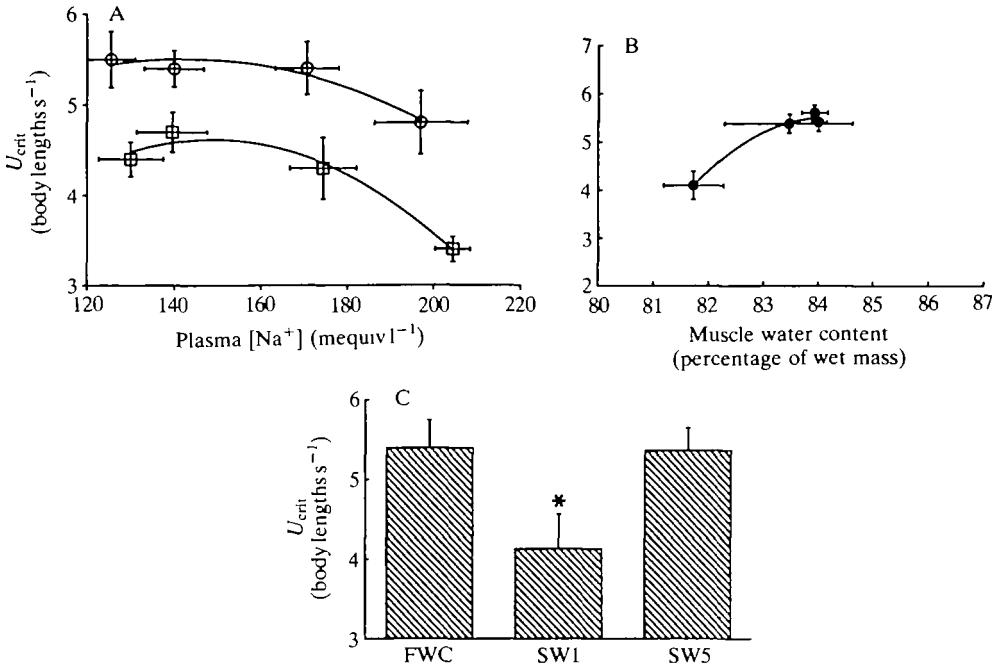

Fig. 7. (A) The correlation between U_{crit} and elevated plasma Na^+ concentrations in hatchery (□) and wild (○) coho salmon smolts. (B) U_{crit} is also correlated with muscle water content in juvenile parr. (C) 1 day of seawater exposure (SW1) reduced U_{crit} relative to that determined in fresh water (FWC). However, 5 days of exposure to full-strength sea water (SW5) was time enough to restore U_{crit} (C. J. Brauner, J. M. Shrimpton and D. J. Randall, unpublished data). Bars show ±S.E.M. (N=6). An asterisk indicates that a value is significantly different from the value in fresh water ($P \leq 0.05$).

reduction in U_{crit}. The degree to which the swimming capacity is reduced is dependent upon the degree to which plasma ion concentrations become elevated (Figs 1B, 7). As plasma ion levels become elevated there is an increase in plasma volume and a reduction in the muscle water content. In amphibians, hypernatraemic blood reduces cardiac contractility (Hillman, 1984) and thus cardiac output. Decreases in haematocrit due to an expanded blood volume and the direct effect of elevated ion concentrations on haemoglobin oxygen-affinity may also contribute to a reduction in oxygen delivery to the tissues.

The reduced muscle water content may also affect muscle contractility. Homsher *et al.* (1974) found a reduction in the force generated in stimulated frog skeletal muscle bathed in hypertonic solutions. Hyperosmotic infusions in dogs result in a reduction in muscle water content and an intracellular alkalosis (Makoff *et al.* 1970).

Exposure to sea water of distinct phenotypes of coho salmon from the same

brood stock results in a difference in the degree to which the animals are affected The reduced osmoregulatory ability of a group of hatchery-reared coho salmon, in which there was a larger reduction in muscle water content and haematocrit, resulted in greater impairment of swimming performance relative to their wild counterparts in water of the same composition (Fig. 7A).

Fish transferred to saline waters eventually acclimate and aerobic swimming capacity is restored. Acclimation involves an increased osmoregulatory capacity through the recruitment of chloride cells in the gills, which restore ion concentrations within the plasma to the level in animals maintained in fresh water. This substantiates the view that reductions in swimming performance are related to osmotic changes in the body.

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