THERMOACCLIMATORY VARIATIONS IN THE MICROENVIRONMENT OF HEMOGLOBIN IN THE RAINBOW TROUT, *Salmo gairdneri*, AND THE CARP, *Cyprinus carpio*

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ABSTRACT

Several inorganic substances (e.g., Cl^-, Mg^{2+}, Ca^{2+}, H^+) are potent negative modulators of hemoglobin-oxygen affinity. To evaluate the possibility that potentially adaptive changes in the red cell ionic environment of hemoglobin may take place during acclimation of fishes to increased environmental temperature, hematological status (hemoglobin, hematocrit, red cell numbers, mean erythrocytic volume and hemoglobin content), plasma and packed red cell electrolyte levels (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-) were evaluated in summer and winter populations of the stenothermal rainbow trout, Salmo gairdneri, following acclimation to 2°, 10°, 18°C, and in a spring population of eurythermal carp, Cyprinus carpio, held at 2°, 16° and 30°C. From these data cell ion concentrations and ion:hemoglobin ratios were estimated. In view of the role of red cell carbonic anhydrase in the reductions of blood CO_2 tensions and the recruitment of Na^+ and Cl^- lost by fishes, a preliminary investigation of thermoacclimatory changes in the activity of this system in rainbow trout erythrocytes was conducted.

Few changes in hematological status were encountered following acclimation. There was, however, some evidence of weight-specific differential hematological response in carp. This lead to markedly greater increases in hemoglobin, hematocrit and red cell numbers in smaller rather than in larger specimens at higher temperatures; variations which were well correlated with changes in plasma Ca^{2+}.

Plasma composition in summer trout was not altered by acclimation. In winter trout plasma Na^+ and K^+ increased at higher temperatures. Carp were characterized by increases in plasma calcium, and reductions in sodium and magnesium under these conditions. Several significant seasonal differences in plasma ion levels were observed in the trout.

In trout, only erythrocytic K^+ and K^+:Hb were altered by acclimation, rising at higher temperatures. In carp Na^+, Na^+:Hb, $C\ell^-$ and $C\ell^-$:Hb increased with temperature, while Mg^{2+} and Mg^{2+}:Hb declined. Changes in overall ionic composition in carp red cells were consistent with increases in H^+ content. In both species significant reciprocal variations in $C\ell^-$ and Mg^{2+} were found. In mammalian systems increases in $C\ell^-$ and H^+ reduce hemoglobin-oxygen affinity by interaction with hemoglobin. Reduction in Mg^{2+} maximizes organophosphate modulator availability by decreasing ATP·Mg^{2+} complex formation. Thus, the changes observed may be of adaptive value in reducing hemoglobin-oxygen affinity, and facilitating oxygen release to cells at higher temperatures. Trout appear to maintain a high chloride-low magnesium state over the entire thermal tolerance zone. Carp, however, achieved this state only at higher temperatures.

In both species mean erythrocytic volume was decreased at higher temperatures and this may facilitate branchial oxygen loading. Since mean erythrocytic volume was inversely related to red cell ion content, it is hypothesized that reductions in cell volume are achieved by export of some unidentified solute or solutes.

Variations in the carbonic anhydrase activity that could be attributed to the thermoacclimatory process were quite modest. On the other hand, assays performed at the temperature of acclimation showed a large temperature effect where under in vivo conditions of temperature fish acclimated to higher temperatures might be expected to have higher activities. Furthermore, since hematocrit increased with temperature in these fish, while carbonic anhydrase is present only in the erythrocyte, the whole blood levels of this enzyme are expected to increase and further augment the temperature effect. This, in turn, could aid in the reduction of CO_2

tension and increase the production of H^+ and HCO_3^- used in the active uptake of Na^+ and $C\ell^-$ at higher temperatures.

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INTRODUCTION

Exposure to increased environmental temperature confronts the teleost fish with the problem of satisfying much-heightened oxygen requirements under conditions of decreased oxygen availability (Houston, 1973). Resolution of this problem is complicated by a number of inherent limitations in the systems which are responsible for oxygen uptake from water and its eventual delivery to cellular usage sites. Under circumstances of enhanced oxygen demand the terrestrial vertebrate commonly responds by increasing ventilatory activity. As discussed by Randall (1970), such responses are less appropriate in the case of fishes. Operation of the massive ventilatory musculature imposes significant metabolic costs. Associated with increases in flow velocity, there is also a reduction in exposure time to lamellar exchange surfaces. Much of the additional flow is passed through 'dead space' regions in the opercular area rather than over lamellae, and the net result is a decrease in oxygen extraction efficiency as ventilatory flow rises.

Much the same is true of increases in branchial perfusion by the amplification of cardiac output. Furthermore, to be effective an increase in output must be closely linked to corresponding changes in ventilation, for efficiency falls rapidly as the branchial flow:branchial perfusion ratio increases or decreases past an optimum value (Heath and Hughes, 1973). Because of this, the degree to which cardiac output can be usefully increased is limited by the extent to which it is practical to increase ventilatory flow.

Although some evidence suggests that teleost fishes possess hormonally-

regulated alternative bloodflow pathways through the gills, and can alter the exchange area to amplify oxygen uptake (Randall, 1970), these changes would result in increased rates of endosmosis and electrolyte loss. Accordingly, the animal would be faced with the necessity of expending energy in regulating water-electrolyte balance.

However, the fish may also respond by adjustments at the hematological level which enhance oxygen-carrying capacity. Since the overall oxygen uptake is a function of both blood oxygen-carrying capacity and branchial ventilation-perfusion-area relations (Rahn, 1966), even modest increases in capacity, when coupled with increases in ventilation and perfusion can prompt substantial increases in oxygen uptake. Hematological response would seem to offer more advantages and fewer disadvantages than adjustments in exchanger system function. Extensive investigation has, however, failed to demonstrate that such responses play a major role in resolution of the temperature-oxygen demand problem. When variations are seen, they are normally modest in magnitude. Inconsistent findings have also been reported, even in studies involving the same species (Houston and Cyr, 1974; Houston et al., 1976). In some instances the changes observed have even been seemingly anti-adaptive in nature, e.g. decreased hemoglobin levels at increased temperature (Grigg, 1969).

It is, however, possible that more subtle responses operate at this level. Teleostean hemoglobins normally occur as families of electrophoretically-distinguishable components which, in some instances at least, differ markedly in their transport characteristics (Riggs, 1970). Thermal acclimation* is associated with changes in the abundance of specific polymorphs (Houston et al., 1976), and it is tempting to speculate that this

* a situation where a new steady state position is reached with respect to temperature

may represent a form of selective modification which has adaptive significance. Furthermore, oxygen affinities of fish hemoglobins can be modulated by pH, inorganic and organophosphate compounds. Accordingly, the coupling of appropriate changes in hemoglobin system elements with suitable variations in the modulatory microenvironment within the erythrocyte, could well lead to transport modifications of significant importance.

In general, research on the environmental modulation of fish hemoglobins has emphasized erythrocytic changes in organophosphate levels (Wood and Johansen, 1972; Powers, 1974; Wood et al., 1975; Weber et al., 1976a and b). It is likely that other means of modulating hemoglobin play an important role in response. Inorganic electrolytes such as chloride are strong effectors of hemoglobin-oxygen affinity (Benesch et al., 1969; Rollema et al., 1975), co-operativity and Bohr effect (Gillen and Riggs, 1972). Magnesium and calcium are known to complex ATP*, preventing it from reacting with hemoglobin and modifying hemoglobin-oxygen affinity (Bunn et al., 1971). Likewise, monovalent cations such as sodium and potassium effect chloride-ATP hemoglobin interactions (Rossi-Fanelli et al., 1961b; Bunn et al., 1971).

In spite of the influence which these electrolytes have upon hemoglobin functions, they have never been studied in relation to teleostean responses to environmental stress in which modulation might be critically important. Indeed the erythrocytic electrolyte environment of hemoglobin has never been adequately defined in any fish species. It is at least possible that erythrocytic ionic composition could be modified during thermal acclimation so as to confer adaptive advantages under specific temperature conditions. To assess this possibility hematological data (blood hemoglobin, hematocrit,

*ATP - adenosine triphosphate

red cell numbers, mean erythrocytic volume and hemoglobin content), plasma and packed erythrocytic electrolyte levels were obtained for summer and winter populations of rainbow trout, Salmo gairdneri, acclimated to 2°, 10° and 18°C, and carp, Cyprinus carpio, held at 2°, 16° and 30°C. Red cell electrolyte concentrations and ion:hemoglobin ratios were then calculated from this data. The electrolyte modifications observed have been interpreted in relationship to hemoglobin function and from the viewpoint of cell volume regulation, and the control of cellular metabolism. The analysis of estimated potential differences across the erythrocyte membrane in relation to thermal acclimation has provided a basis for a fresh view of ionic regulation by trout and carp erythrocytes.

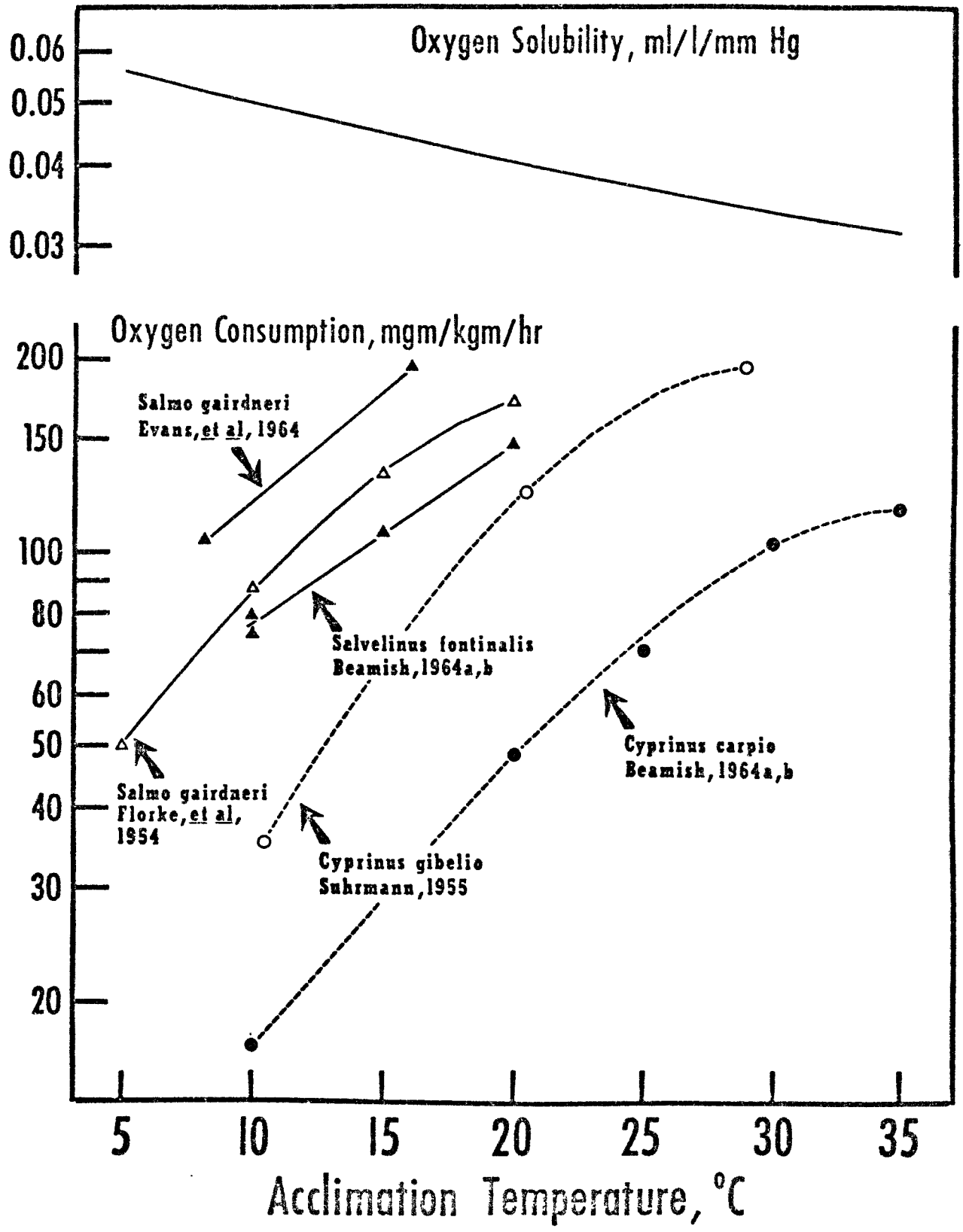
Increases in oxygen consumption with temperature produce increases in CO_2 evolution. In spite of this rainbow trout maintain low blood P_{CO_2} tensions over wide temperature ranges (Cameron, 1971). In view of the importance carbonic anhydrase plays in this process as well as in ion regulation by fish (Maetz, 1971), and appreciating the homeostatic difficulties thermal stress places on these processes, erythrocytic carbonic anhydrase was assayed in rainbow trout acclimated to 2°, 10° and 18°C to see if adaptive, thermoacclimatory modifications of this enzyme occurred in the erythrocyte.

Literature Review

Few if any environmental factors influence biological activity more than temperature, and the poikilotherms, of course, are potentially very sensitive to the effects of environmental temperature change on body functions. Such organisms, however, frequently exhibit various types of compensation which enable them to remain active over relatively broad

Fig. 1. Oxygen availability and oxygen consumption in representative cyprinid and salmonid species following thermal acclimation (from Houston 1973).

Fig-1


Oxygen Solubility, ml/l/mm Hg
0.06
0.05
0.04
0.03
Oxygen Consumption, mgm/kgm/hr
200
150
100
80
60
50
40
30
20
Salmo gairdneri
Evans, et al, 1964
Salmo gairdneri
Florke, et al,
1954
Selvelinus fontinalis
Beamish, 1964a,b
Cyprinus carpio
Beamish, 1964a,b
Cyprinus gibelio
Suhrmann, 1955
5
10
15
20
25
30
35
Acclimation Temperature, °C

temperature ranges.

In the case of teleost fishes, increases in water temperature place special stress upon the cardiovascular-respiratory system. This must satisfy the increased demand for oxygen imposed by a higher metabolic activity despite decreased oxygen solubility (Fig. 1). Such demands are not small. The carp, Cyprinus carpio, for example, increases metabolism (as measured by O_2 uptake) by over 700% as temperature is elevated from 10° to 30°C. Oxygen solubility, however, decreases by 31% over this range. Fish such as the rainbow trout, Salmo gairdneri, although more stenothermal than carp, increase O_2 consumption with temperature at a rate (mg $O_2 \cdot kgm^{-1} \cdot hr^{-1} \cdot °C^{-1}$) two to three times that of carp. Furthermore, goldfish, Carassius auratus, carp and brook trout, Salvelinus fontinalis, have been shown to maintain constant oxygen consumption rates at various temperatures over relatively broad ranges of oxygen tensions (Graham, 1949; Beamish, 1964).

The ability of fish to meet the increased oxygen demands imposed by higher temperatures is partly due to the fact that increases in temperature produce decreases in water viscosity and increases in O_2 diffusion rate. These facilitate water flow across the gills, and increase the rate at which O_2 enters the blood (Randall, 1970). However, much of the ability of fishes to meet temperature-induced increases in oxygen demand depends on the compensatory responses of the cardiovascular-respiratory complex. With this in mind, this literature review has been divided into sections dealing with the various adaptations taking place at different levels of this system in response to increases in water temperature.

A corollory feature of adaptations which permit the organism to deliver

more oxygen at higher temperatures is the need to handle increased carbon dioxide loads. One of the central factors enabling fish to do this is the carbonic anhydrase enzyme system. The functions of this enzyme within fish will not be discussed within the Literature Review, but are considered within the Results and Discussion sections.

Adaptations of the Cardiovascular-Respiratory System to the Increased Oxygen Demands of Higher Temperatures

Of all the systemic responses to temperature, perhaps the best studied are those involving the branchial exchanger system. This system is associated with the transfer of water over the gills and into the blood. Four potentially modifiable factors in this system can increase oxygen uptake with temperature. (1) The volume flow of water over the gills per unit time ($\dot{V}_g$) can be increased to present more oxygenated water to the gills per unit time. (2) The volume flow of blood per unit time ($\dot{Q}_g$) can be increased to present more blood at the exchange surface. This, too, would increase oxygen transfer across the gill by enhancing the water:blood O_2 tension gradient. (3) Exchange area (A) can also be increased to amplify oxygen transfer at any specific gradient. (4) Reduction in the mean diffusion pathway (ΔX) can also increase the transfer rate of oxygen.

These interactions are approximated by the following ventilation (1), perfusion (2) and diffusion (3) equations (Hughes and Shelton, 1962; Hughes, 1964; Randall *et al.*, 1967; Randall, 1970; Mearow, 1975):

$$(1)\quad \dot{V}_{O_2} = \dot{V}_g \cdot \alpha w_{O_2}(P_{iO_2} - P_{eO_2})$$

$$(2)\quad \dot{V}_{O_2} = \dot{Q}_g \cdot \alpha b_{O_2}(P_{aO_2} - P_{vO_2})$$

$$(3)\quad \dot{V}_{O_2} = \frac{D'A\Delta P_{O_2}}{\Delta X \cdot 760}$$

where $\dot{V}_g$ = water flow across the gills (ml/min)

$\dot{Q}_g$ = blood flow through the gills (ml/min)

P_{iO_2} = inspired water oxygen tension (mm Hg)

P_{eO_2} = expired water oxygen tension (mm Hg)

P_{aO_2} = arterial oxygen tension (mm Hg)

P_{vO_2} = venous oxygen tension (mm Hg)

ΔP_{O_2} = oxygen gradient between the blood and water across the gill epithelium (mm Hg)

αw_{O_2} = water oxygen solubility (ml/litre/mm Hg)

αb_{O_2} = blood oxygen capacity (ml/litre/mm Hg)

D' = Krogh permeation coefficient for oxygen diffusion within the gill epithelium (Krogh, 1949; as in Randall, 1970) (ml/min/cm^2/cm/760 mm Hg)

A = area of the secondary lamellae (cm^2)

ΔX = thickness of the gill epithelium (cm)

In equations (1) and (2) $\dot{V}_{O_2}$ is expressed in ml O_2/min, while in equation (3) the units of $\dot{V}_{O_2}$ are ml O_2/min/cm^2 (gill area).

Changes in ventilation and perfusion with temperature have been described by Hughes and Roberts (1970) for rainbow trout. When water temperature was increased at a rate of 1°C/3 min the trout responded by increasing ventilation and heart rate, and also the amplitude of the pressure changes within the respiratory cavities. The latter observation indicates the likelihood of increases in ventilatory stroke volume. Heath and Hughes (1973) subjected the same species to slower warming rates (1.5°C/hr), and found that ventilatory and cardiac rates increased nearly synchronously from 80 to 110 cycles/min between 15° and 23°C; these functions having

thermal coefficients (Q_{10}) of 1.4 and 1.6 respectively. Again, increased ventilatory frequency was coupled with increases in ventilatory stroke volume. These responses were thought to facilitate oxygen uptake, and to permit satisfaction of substantial increases in oxygen consumption (from 110 mg O_2/kg/hr at 15°C to 280 mg O_2/kg/hr at 23°C). Increases in ventilation and/or perfusion with temperature have also been reported in the sockeye salmon, Oncorhynchus nerka, (Brett, 1964), the lingcod, Ophiodon elongatus, (Randall, 1968), and the bluegill sunfish, Lepomis macrochirus (Spitzer et al., 1969).

Although changes in ventilation and perfusion take place and their importance cannot be doubted, they involve substantial metabolic costs. The amplification of $\dot{V}_g$ and $\dot{Q}_g$ becomes self-limiting when the O_2 cost of operating cardiac and ventilatory muscles exceeds the gain in O_2 acquired.

Heath and Hughes (1973) found that ventilation and heart rate in rainbow trout increased up to 23°C, but at temperatures above this (to approx. 26°C) ventilatory rate levelled off, as did the oxygen consumption, while the heart rate decreased until death occurred. The onset of cardiac failure at high temperature was seen when the heart missed every fourth cycle. The actions of the opercular and buccal ventilatory pumps became dissynchronized, and pressure reversals (coughs) were common. Hughes and Roberts (1970), studying the same species but using more rapid temperature changes, reported similar results except that the respiratory aberrations previously stated occurred at higher temperatures.

Brett (1964) found that maximum sustained swimming activities of sockeye salmon were maintained at equilibrium* at 15°C. If oxygen tensions were raised or lowered, the result was either an increased or decreased

* i.e., swimming activity could no longer be increased

activity. Randall (1970), in his discussion of Brett's work, concluded that either the cost of delivering oxygen to the gills or the transfer factor for oxygen* (T_{O_2}) was limiting oxygen uptake.

The potential benefits of increases in ventilation are limited in other ways. Increases in $\dot{V}_g$ are normally accompanied by reductions in efficiency of extraction. This is in part because increases in ventilation produce a situation in which larger percentages of the total water irrigating the gills is not brought into close contact for long enough duration to allow gas exchange to take place. Randall (1970) describes this as 'shunted water', and has divided overall ventilatory flow into three categories. The diffusional deadspace volume involves water not allowed to reach O_2 equilibrium with the blood because of wide spacing of secondary lamellae or rapid flow over the gills. The distributional dead space volume involves water delivered to parts of the respiratory surface where the blood has already been saturated with oxygen. Water passing between filaments and not in contact with the respiratory epithelium is termed the anatomical dead space volume. Randall (1970) has calculated that all of these 'dead volumes' increase as $\dot{V}_g$ increases in trout. The smallest increases were found in diffusional dead space volume. This rose from 1% to 7% of $\dot{V}_g$ as $\dot{V}_g$ increased from 1 to 10 ml·sec^{-1}. On the other hand, the sum of the distributional and anatomical dead space increased from a value of near 0 to 60% of $\dot{V}_g$ as $\dot{V}_g$ increased from 1 to 3 ml·sec^{-1}, indicating that most of the water flowing over the gills at high ventilatory rates is actually of little value to the fish.

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*The transfer factor for a gas is a measure of the ability of the gills to transfer that gas per unit gradient. The transfer factor for oxygen T_{O_2} is defined as $T_{O_2} = \dot{V}_{O_2}/\Delta P_{O_2}$ (Randall, 1970).

Increases in ventilation and cardiac rate can be detrimental in a number of other ways. Increased muscular activity in some species has been thought to correlate with reductions in blood pH so large that oxygen loading at the gills is hindered (Powers, 1972).

Similarly, the use of alternate circulatory pathways within the gill (Steen and Kruysse, 1964; Randall, 1970) to increase exchange area, when coupled with increased perfusion and ventilation, would inevitably lead to increases in electrolyte and water fluxes. These, in turn, require some form of compensatory response.

In view of the limitations, primary costs, and secondary stresses associated with modifications in branchial exchanger system function, it was thought by several investigators, including Anthony (1961) and Houston and DeWilde (1968), that other changes involving less stress might be utilized by fish to resolve the temperature-oxygen problem. Initially changes in hematological parameters were considered. It was felt that appropriate changes, in conjunction with branchial exchanger responses, could greatly facilitate oxygen uptake.

Modifications in Oxygen Carrying Capacity and Diffusion Characteristics of the Blood

Blood has several characteristics amenable to potentially adaptive modifications. Oxygen carrying capacity can, for example, be increased by increasing hemoglobin levels. This can be accomplished by increasing the number of red blood cells, without altering cell hemoglobin content, or by increasing cellular hemoglobin content without increasing erythrocyte numbers. Either modification, or a combination of both, would increase the amount of oxygen transported by the blood from gill to tissue

areas. Secondly, erythrocyte volume can be reduced. The significance of this form of response is clearly suggested by the work of Holland and Forester (1966) on the relationship between uptake velocity constant (K_C)* of O_2 and erythrocyte volume. K_C was found to decrease as cell volume increased. This effect is substantial. Goat erythrocytes with a MEV** of 20 μ^3 had a mean K_C value of 150 $mM^{-1}sec^{-1}$, K_C decreased to 80 $mM^{-1}sec^{-1}$ in 40 μ^3 rabbit erythrocytes, and in frog corpuscles with a MEV of 680 μ^3, K_C was only 19 $mM^{-1}sec^{-1}$. If the red blood cell is equated to a sphere, K_C values are significantly correlated with the reciprocal of the radius. No evidence of species differences in hemoglobin reaction rates or membrane diffusion properties for oxygen was apparent, indicating that it is the reduction in diffusion distance which facilitates O_2-Hb combination. Thus, in acclimation to higher temperature conditions, it would be of some benefit to the fish to package equivalent amounts of hemoglobin in smaller erythrocytes since this would increase the oxygen uptake velocity. Thus, most appropriate adaptive hematological responses to increased temperature entail increases in total hemoglobin, reduction in MEV and increases in erythrocytic numbers and mean erythrocytic hemoglobin content.

Although such changes would presumably be energetically less demanding than responses at the branchial exchanger level, and would also produce fewer deleterious side effects (e.g., endosmosis and salt depletion), evidence of their occurrence is both unclear and conflicting. For instance, Houston and Cyr (1974) found that goldfish acclimated to 2°, 20° and 35°C significantly increased both hemoglobin and hematocrit levels at higher temperatures. Catlett and Millich (1976) also found increased red blood

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*$K_C = \frac{d[HbO_2]}{dt} \cdot ([Hb][O_2])^{-1}$ **mean erythrocyte volume

cell counts and hematocrit in this species, but little change in mean erythrocytic volume. Similarly, Houston and Rupert (1976) found moderate but significant increases in both hematocrit and blood hemoglobin levels in 30°C as compared to 5°C-acclimated goldfish. Anthony (1961), however, reported that goldfish acclimated to 5°, 6°, 26° and 30°C showed slight decreases in erythrocyte numbers. Both hematocrit and mean erythrocytic volume increased between 5° and 26°C, and then fell off to lower values at 30°C than were seen in the cold-acclimated fish. Despite these changes the overall oxygen carrying capacity of the blood was not significantly altered. Likewise, Houston et al. (1976) observed little change in either hemoglobin or hematocrit values in goldfish acclimated to 5° and 30°C, and Faulkner and Houston (1966) found no significant variation in hematocrit, hemoglobin or erythrocyte numbers in goldfish held at 20° and 30°C.

In studies upon carp, Houston and DeWilde (1968) found that acclimation to temperatures between 2° and 33°C lead to increases in red cell number, packed cell volume and hemoglobin concentration, and some decrease in mean erythrocytic volume with temperature. Houston et al. (1976), however, found no significant changes in hematocrit or hemoglobin in carp acclimated to 5° and 30°C.

In rainbow trout, Houston and Cyr (1974) found quite large increases in both hematocrit and hemoglobin at higher temperatures in trout acclimated to 2°, 10° and 18°C. DeWilde and Houston (1967), however, reported few significant thermoacclimatory changes in this species. Powers (1974) observed large increases in the hematocrit of Catostomus clarkii with temperature, while Houston et al. (1976) found few hematological variations in a related species, Catostomus commersoni, following acclimation to 3°, 10° and 20°C.

The available literature on thermoacclimatory changes in erythrocyte numbers, volumes and/or blood hemoglobin contents is clearly inconsistent, and presents no general pattern. Where significant hematological changes take place (Houston and Cyr, 1974), they are almost certainly of some benefit to the fish in satisfaction of increased oxygen demands. However, even studies on the same species are inconsistent (Anthony, 1961; Faulkner and Houston, 1966; Houston and Cyr, 1974; Houston et al., 1976), and the small magnitude of the variation encountered in others (Houston and DeWilde, 1968) indicates that they are probably of little adaptive value. Thus, earlier studies suggest that hematological responses involving increases in blood oxygen-carrying capacity are not extensively utilized by fishes challenged by temperature-induced increases in oxygen demand. However, as pointed out in the succeeding section, alternative responses at the hematological level may well be of significant value.

Molecular Adaptations within the Hemoglobin System

Hochachka and Somero (1973), in describing various strategies of biochemical adaptation, note three general molecular mechanisms by means of which adaptation may be achieved. These involve, (1) variations in the type of macromolecules present, (2) adjustment in the amounts or concentration of macromolecules, (3) modulation of the functions of macromolecules. Although molecular aspects of hemoglobin adaptation to temperature in fishes have only recently been studied, present evidence suggests that the hemoglobin system may have the capacity to adapt in all three ways when the animal is faced with a thermal stress.

Most teleost fishes possess a number of electrophoretically distinct hemoglobin polymorphs (Table 1). A number of studies indicate that in-

Table 1: Electrophoretic Patterns Obtained from Various Species of Fish

SPECIES	NUMBER OF COMPONENTS	ELECTROPHORETIC TECHNIQUE
Carp, Cyprinus carpio	3	Starch gel
Carp, Cyprinus carpio	3	Sephadex
Goldfish, Carassius auratus	3	Paper
Goldfish, Carassius auratus	3	Starch gel
Goldfish, Carassius auratus	3	Acrylamide gel
Chinook salmon, Onchorhynchus tshawytscha	2	Moving boundary
Sockeye salmon, Onchorhynchus nerka	7	Starch gel
Chum salmon, Onchorhynchus keta	9	Starch gel
Atlantic salmon, Salmo salar	9	Starch gel
Rainbow trout, Salmo gairdneri	3	Moving boundary
Rainbow trout, Salmo gairdneri	11	Starch gel
Rainbow trout, Salmo gairdneri	7	Starch gel
Rainbow trout, Salmo gairdneri	9	Acrylamide gel
Rainbow trout, Salmo gairdneri	6	Cellulose acetate
Brook trout, Salvelinus fontinalis	3	Cellulose acetate
Brook trout, Salvelinus fontinalis	15	Starch gel
Smallmouth bass, Micropterus dolomieui	3	Cellulose acetate
Largemouth bass, Micropterus salmoides	4	Cellulose acetate
Catfish, Parasilurus asotus	4	Starch gel
Brown bullhead, Ictalurus nebulosus	7	Acrylamide gel
Cod, Gadus morrhua	3	Agar
Plaice, Pleuronectes platessa	5	Agar
Flounder, Platichthys flesus	5	Agar
Green sunfish, Lepomis cyanellus	6	Starch gel

(from Mearow, 1975)

dividual fractions may differ in their transport characteristics, and that the presence of certain polymorphs may offer distinct advantages under certain environmental conditions (Riggs, 1970; Powers, 1972; Iuchi, 1973b; Perez and Maclean, 1976). However, only recently have quantitative and qualitative changes in hemoglobin polymorphism been demonstrated in relation to thermal acclimation. Houston and Faulkner (1966) using cellulose strip chromatography found that goldfish acclimated to temperatures above 12°C tended to produce a new electrophoretically-distinct hemoglobin. This was subsequently confirmed by Houston and Cyr (1974), Houston and Rupert (1976) and Houston et al. (1976) using polyacrylamide gel electrophoresis. At acclimation temperatures between 20° and 35°C the goldfish exhibits three hemoglobin polymorphs, while at 2° and 5°C only two hemoglobins are found. It was also found that of the two hemoglobins present at all temperatures one increased, while the other decreased in concentration with temperature. Houston and Cyr (1974) observed significant increases in blood hemoglobin levels with temperature and speculated that these changes could be channelled to selective modifications that might lead to the preponderance of polymorphs suited to a particular temperature regime. Houston et al. (1976) found that even in instances where little significant change in overall hemoglobin concentration took place, acclimation produced significant modifications in the relative abundancies of specific polymorphs.

Changes in hemoglobin polymorphism with temperature are not limited to goldfish. They have been demonstrated in rainbow trout (Houston and Cyr, 1974), carp, pumpkinseed sunfish, Lepomis gibbosus, white sucker, Catostomus commersoni, and carp-goldfish hybrids (Houston et al., 1976), while modest changes in one of the seven hemoglobin components of the brown

bullhead, Ictalurus nebulosus, have also been reported (Grigg, 1969). In these species, however, variations occurred only in terms of abundance. The goldfish is, at present, unique in the exhibition of qualitative adjustment in polymorphs.

It is tempting to conclude that such thermal modifications in hemoglobin polymorphism may be of adaptive value. At present such a conclusion would be premature, since the transport functions of hemoglobin are strongly modulated by the internal microenvironment of the erythrocyte. Only if this environment is defined in relation to acclimation can the transport capacities of specific polymorphs be assessed in any physiologically-realistic fashion. Accordingly, in the next section of this Literature Review, consideration is given to the modulation of hemoglobin function by cellular agencies.

The Molecular Basis of Hemoglobin Function and Possible Sites for Hemoglobin Modulation

Prior to any consideration of microenvironmental effects upon hemoglobin-oxygen affinity, the molecular basis of hemoglobin function must be discussed. Hemoglobins exhibiting co-operativity in oxygen binding consist of four protoporphyrin IX-containing peptides. These usually take the form of 2α and 2β subunits held together by electrostatic, van der Waals and hydrogen bonding interactions. The hemoglobins of different species have chains varying in amino acid sequence and, likewise, in type and degree of secondary and tertiary binding. These variations manifest themselves in different oxygen affinity characteristics. Despite such differences, evidence now available indicates that vertebrate hemoglobins having sigmoidal oxygenation characteristics (i.e., co-operativity) and exhibiting pH

effects (Bohr effect) contain invariant residues (Gillen and Riggs, 1972; Powers and Edmunson, 1972a, b; Weissbluth, 1974; Coates, 1975). Of these, valine at the N-terminal, and the second last tyrosine residue in the α- and β-chains, as well as the C-terminal arginine and histidine of the α- and β-chains respectively are considered to be of major importance.

Changes in hemoglobin conformation upon oxygenation have been described by Perutz (1970a, b). When hemoglobin is in the deoxygenated state oxygen binding to α-heme pockets is favored over β-heme pockets. The oxygenation of an α-chain heme produces changes in tertiary structure which break polar bonds between it and the non-oxygenated α-chain. The result of this is that the vibrational freedom of the deoxy α-chain is increased, and consequently the second α-chain is oxygenated much more readily than the first. Oxygenation also sequentially breaks polar bonds within β-chains. When both α-chains have been oxygenated, the β-chains, which in totally deoxygenated hemoglobin have such restrictive heme pockets that O_2 cannot enter, have enough fluidity to permit oxygenation. Oxygenation of one β-chain influences its own tertiary structure, and favors release of 2,3DPG* or ATP bound between the two β chains. As a result, the last β-chain is 80 times easier to oxygenate than the primary α-chain. During the sequential breaking of tertiary bonds H^+ ions (or Bohr protons) are released.

The physiological importance of this process can be seen by reference to hemoglobin-oxygen equilibrium curves. Co-operativity leads to sigmoidal curve relationships between oxygenation and oxygen tension in which relatively small changes in oxygen tension, such as those which are seen between arterial and venous blood, lead to relatively large changes in hemoglobin

*2,3 diphosphoglycerate

oxygenation and deoxygenation. In the absence of co-operativity hemoglobin has the hyperbolic characteristic seen in myoglobin (Weissbluth, 1974) and the di-heme hemoglobins of the lamprey, Petromyzon marinus (Riggs, 1970).

Deoxygenation within capillaries is also facilitated by the decreases in pH which stem from production of lactate and the conversion of CO_2 to H_2CO_3 by carbonic anhydrase which forms H^+ and HCO_3^- within erythrocytes near metabolizing tissues. These lead to increases in hydrogen ion concentration which tend to reverse proton release and favor deoxygenation. Because of this, there is an additive deoxygenation effect; more deoxygenation takes place than would be the case with reductions in oxygen tensions alone.

Hemoglobin-oxygen affinity can be modulated at a number of other possible sites as well. Variations in erythrocytic organophosphate concentrations also effect hemoglobin-oxygen affinity. DPG is the major organophosphate within mammalian erythrocytes (Benesch and Benesch, 1969) while ATP, and in some cases GTP*, seems to be the more important organophosphate modulators in fish (Gillen and Riggs, 1971; Peterson and Poluhowich, 1975; Kaloustian and Poluhowich, 1976; Weber et al., 1976a). Increasing organophosphate concentrations within erythrocytes favors the deoxy form of hemoglobin, since cross binding between β chains is maximized, and oxygen affinity is decreased.

Both co-operativity and the Bohr effect result from the presence of positive and negative interactions of heme chains as well as freely dissociable proton groups. It is therefore not surprising that charged electrolytes also influence hemoglobin-oxygen affinity. For example, high concentrations of salts, such as NaCl, tend to dissociate hemoglobin into

*guanosine triphosphate

half molecules, evidently by neutralizing salt bridges between peptides. This tends to decrease co-operativity, and increase oxygen affinity (Rossi-Fanelli et al., 1961a). At physiological levels, however, electrolytes tend to act in a more specific fashion and often with quite different effects.

Chloride

Studies on human hemoglobin have shown that increasing chloride concentration from 5 mM to 100 mM at intracellular pH levels (i.e., 7.3) doubles the number of Bohr protons released per tetramer upon oxygen ligation. Presumably chloride ions bind near the amino acids responsible for proton release, and lower the pK values of proton groups (Bruin et al., 1974; Rollema et al., 1975). This leads to three consequences. First, the Bohr effect is enhanced. Since increased numbers of protons are given off upon oxygenation, lowering pH produces greater changes in hemoglobin-oxygen affinity. Second, the co-operativity of the hemoglobin peptides is enhanced following the sequential ligation of oxygen, since the breaking of salt bridges results in increased probability of deprotonizing appropriate amino acid residues and increasing the fluidity of chains involved in further oxygenations. Finally, since the number of protons released upon oxygenation (ΔZ_β) is directly related to the oxygen tension needed to saturate hemoglobin at a given pH*, changes in chloride concentration significantly modulate the range over which oxygenation takes place.

The effects of changing chloride levels of hemoglobin solutions are quantitatively quite dramatic. Benesch et al. (1969) found that at pH 7.30

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*i.e., for human hemoglobins, Rollema et al. (1975) describes the following relation

$$\frac{\log P_{50}}{pH} = \frac{1}{4} \Delta Z_\beta ,$$

where P_{50} is the O_2 tension needed to produce 50% saturation of hemoglobin.

increasing sodium chloride from 10 to 100 mM increased the log P_{50} of dialysed human hemoglobin from 0.1 to 0.5. The significance of this is obvious. At 10 mM NaCl and a log P_{O_2} of 0.2, hemoglobin is 80% saturated. At the same oxygen tension in 100 mM NaCl only 20% saturation can be attained. Similar effects of chloride on human hemoglobin P_{50} values have been demonstrated by Rossi-Fanelli et al.(1961b) and Antonini et al. (1962). These authors also measured changes in the Hill coefficient n, and showed that co-operativity increased with chloride concentration.

Chloride effects are not limited to human hemoglobin. Under pH conditions similar to those in fish erythrocytes, Gillen and Riggs (1972) found that log P_{50} of stripped carp hemoglobins increased from 0 to 0.4 with an increase in co-operativity from n = 1.5 to 2.0 in 100 as compared to 0 mM NaCl. Reductions in pH amplified the decrease in oxygen affinity produced by the salt, indicating the likelihood of an increased Bohr effect. Even the hemoglobins of the eel, Anguilla rostrata, regarded by Weber et al.(1976a) as being salt-insensitive, show a significant chloride effect. Kaloustian and Poluhowich (1976) found that if the eel hemoglobins were stripped of organophosphates and NaCl was increased from 20.4 to 60.1 mEq/ℓ (pH 7.0, 10°C) oxygen saturation was decreased by 20% at oxygen tensions of 5 mm Hg. Of greater importance, it was found that at various ATP and GTP concentrations NaCl decreased hemoglobin-oxygen affinities (as measured by P_{50} values) to a greater extent than in organophosphate-free solutions.

Monovalent Cations

Monovalent cations at physiological levels seem to produce little if any effect upon hemoglobin. Bruin et al. (1974) and Rollema et al. (1975)

studied the effects of chloride using KCl as a Cl^- source and NaOH and HCl combinations to arrive at appropriate buffer pH values. Both studies revealed that cationic concentrations in the media had little effect. This conclusion has been partially substantiated by Benesch et al. (1969) who obtained comparable chloride effects using NaCl rather than KCl as a chloride source. Rossi-Fanelli et al. (1961b) reported that KCl tended to produce slightly larger P_{50} shifts than were obtained with NaCl or TRIS-HCl at similar concentrations and pH. Bunn et al. (1971), however, found that hemoglobin solutions containing 100 mM chloride, and either 0.1 M K^+ or 0.1 M Na^+ showed little difference in oxygen affinities unless organophosphates DPG or ATP were present (at 1 mM/ℓ). The latter study also revealed that K^+ in combination with Cl^- and organophosphates produced lower hemoglobin-oxygen affinities than similar solutions having Na^+ rather than K^+. The greatest effect was seen when ATP was the organophosphate in solution. The differing effects of Na^+ and K^+ on hemoglobin-oxygen affinity may be a result of differential interactions with ATP and/or chloride rather than a consequence of any direct effect on the hemoglobin molecule.

Divalent Cations

Divalent cations such as magnesium and calcium tend to have more influence upon hemoglobin-oxygen affinity than do monovalent cations. Bunn et al. (1971), studying Mg^{+2} hemoglobin and ATP interactions, found that Mg^{+2} (0 to 30 mM) had little effect upon oxygen affinity in the absence of ATP or DPG. However, in the presence of 1 mM ATP oxygen affinity was increased from a P_{50} value of 8.0 to 3.6 when the magnesium concentration was increased from 0 to 7 mM. At Mg^{+2} levels of 7 mM in the presence of

ATP hemoglobin-oxygen affinities approximated those of stripped hemoglobin. Bunn et al. (1971) hypothesized that magnesium acts by binding ATP, and preventing its interaction with hemoglobin. Similar effects were also obtained when Mg^{+2}, DPG and hemoglobin oxygen interactions were studied. Of added importance is the fact that the interaction of these organophosphates with Mg^{+2} and hemoglobin depends on hemoglobin oxygenation state. In the oxy form there is virtually no binding of ATP (or DPG) to hemoglobin, and 80% of the ATP present is complexed with Mg^{+2}. Deoxyhemoglobin, on the other hand, has a high affinity for ATP. It competes with magnesium for the organophosphate, and this results in a decrease in Mg^{+2}-ATP, coupled with an increase in ATP-Hb. The result of this is that there is a strong shift from the low-affinity deoxy state hemoglobin to high oxygen affinity in the presence of oxygen. If such shifts take place between the areas of oxygen uptake and release, oxygen transfer would, of course, be greatly facilitated. Bunn et al. (1971) suggest that the magnitude of the affinity change may actually be greater than predicted on the basis of the foregoing considerations since venous-arterial pH differences will amplify affinity effects, as would the decrease in pH at venous levels. On the venous side of the system, decreases in pH would tend to protonate ATP, reducing its affinity for magnesium and providing extra phosphate hemoglobin interaction which would decrease Hb-O_2 affinity and facilitate O_2 release.

Calcium apparently acts in an analogous fashion. However, its actions are weaker, and consequently produce less effect upon Hb-O_2 affinity (Bunn et al., 1971). In addition, it is present in very small concentrations (< 1 mM within erythrocytes), and much of this is lipid-bound. Consequently,

it seems unlikely that calcium plays any direct role in the modulation of hemoglobin-O_2 affinities in the erythrocyte.

Environmentally-Induced Modulation of Hemoglobin-Oxygen Affinities

Both temperature and oxygen tension influence erythrocytic levels of molecules affecting loading and unloading of oxygen by hemoglobin. For example, Wood and Johansen (1972) found that when eel, Anguilla anguilla, were held under hypoxic conditions (P_{O_2} = 15-30 mm Hg) erythrocytic ATP levels decreased. Weber et al. (1976a) in their studies on this species found that GTP was the most abundant organic phosphate within the erythrocyte, and that it also decreased under conditions of hypoxia. Decreases in erythrocytic organophosphate levels under hypoxic conditions have also been reported for Pleuronectes platessa (Wood et al., 1975). Since such modifications increase hemoglobin-oxygen affinity, these authors concluded that the response was adaptive, and would facilitate oxygen loading at the gills.

In mammals the lowering of arterial oxygen tension (e.g. at high altitudes) triggers an opposite response than reported for fishes; 2,3-DPG, the principal organophosphate of the mammalian erythrocyte, increases and prompts decreases in hemoglobin-oxygen affinity (Lenfant et al., 1968). This was also thought to represent an adaptive strategy since oxygen unloading at the tissue level would be facilitated, while still allowing hemoglobin to be saturated at the lungs. This response may, however, actually prove to be anti-adaptive since increasing hemoglobin oxygen affinity by artificial means (e.g. cyanate treatment) increases survivorship of rats under hypoxic conditions (Eaton, 1974).

In assessing the effects of thermoacclimation upon hemoglobin-oxygen

affinity, Grigg (1969), Eaton (1974) and Powers (1974) concluded that increases in water temperature through their effects on oxygen solubility were comparable to hypoxia. Therefore, it was hypothesized that hemoglobins which could be modulated to increased oxygen affinity with temperature would be of adaptive advantage.

The few studies in which hemoglobin-modulation has been examined in relation to temperature are notable for a diversity of results and interpretations. Weber et al. (1976b), for example, reported that ATP, the main organic phosphate of the rainbow trout erythrocyte, did not change significantly following acclimation to 5°, 15° and 22°C. Hemoglobin-oxygen affinity, as measured on whole blood samples at 15°C over a wide pH range, was not significantly altered in relation to acclimation temperature. When whole blood hemoglobin oxygen affinities were measured at acclimation temperatures, those animals held at 22°C had dramatically reduced oxygen affinities by reference to 5°C specimens, confirming the earlier findings of Eddy (1971) with respect to the temperature sensitivity of trout hemoglobins.

It is difficult to visualize any advantage associated with increasing hemoglobin oxygen affinities at higher temperatures in many species. Grigg (1969) has argued such a case for the brown bullhead, Ictalurus nebulosus. This species has high hemoglobin-oxygen affinities. Under realistic conditions 50% saturation can be achieved at oxygen tensions of only 6 mm Hg. In such cases reduction of oxygen affinity to facilitate oxygen unloading at the tissue level would constitute the more appropriate adaptive response. Grigg (1969) did not consider this interpretation, although it is, in fact, consistent with his reported results. If pH conditions within the bullhead

erythrocyte are comparable to those reported by Steen and Turitzin (1968) and Wood and Johansen (1973), i.e. 6.8-7.0, Grigg's specimens, when acclimated to 9°C, probably had P_{50} values of approximately 6 mm Hg, whereas those fish at 24°C may have exceeded 15 mm Hg. In other words, under *in vivo* temperature conditions there was, in fact, a substantial reduction in affinity. Similarly, Eddy (1973) found that the tench, *Tinca tinca*, acclimated at 5°, 13° and 20°C, was characterised by hemoglobin oxygen affinity decreases with temperature. P_{50} values were 0.5, 2 and 3 mm Hg at 5°, 13° and 20°C respectively.

In view of these experimental results, there are grounds, therefore, for the suggestion that the acclimation of teleosts to increased temperature may involve reductions in hemoglobin-oxygen affinity under *in vivo* conditions which would favor unloading at the tissue level rather than increases in affinity facilitating oxygen loading at the gills.

MATERIALS AND METHODS

1. Origin and Maintenance of Experimental Animals

Rainbow trout having a mean weight of 227±12.1 * g were used in the seasonal, thermoacclimatory electrolyte study. Trout used for carbonic anhydrase assays had an average weight of 290±13.7 g. All fish were purchased from a local supplier (Goosen's Trout Farm, Otterville, Ontario) and were taken from the same pond stock. The first shipment of fish for the electrolyte study was received in March, 1975 and sampled during July and August of 1975. The second batch of fish was received in August, and sampled in November to December of 1975. Fish used for the carbonic anhydrase study were purchased in May, 1976 and sampled during August and September, 1976.

Carp used in the thermoacclimatory electrolyte study had a mean weight of 135±26.4 g. These were seined from the Virgil Dam Reservoir, Virgil, Ontario during the summer and fall of 1975, and sampled during May of 1976.

Each batch of fish was inspected upon arrival, and equally distributed between three constant-flow 500 ℓ tanks (Frigid Units Inc., Toledo, Ohio, model LS-700). Water temperatures were controlled by means of model BHL-1076 coolers (Frigid Units Inc.) used in conjunction with 1000 watt stainless steel heating coils regulated via a water temperature sensor, linked to locally-designed and constructed controller units. The coolers served two other functions in that they constantly cycled tank water thru filters and splash-aerated the water. Supplementary dechlorinated water inflows were provided, and tank water exchanged three times daily. Oxygen levels never dropped below 90% saturation for any particular water temperature.

* ± 1 standard error

Carp were kept in 1000 ℓ holding tanks (Frigid Units Inc., RT-430) supplied with constant flow of room temperature aerated water until they could be transferred to the LS-700 acclimation tanks.

Initially all trout were maintained at hatchery water temperature. Carp were also transferred in such a manner so as to avoid thermal shock. All fish were fed daily, ad libitum, on Purina Trout Chow (Purina Industries). Following resumption of normal feeding and other activities, water temperatures were altered by ≏0.5° per day until acclimation temperatures of 2°, 10° and 18°C for rainbow trout, and 2°, 16° and 30°C for carp were reached. Fish were then maintained for no less than a month at their respective acclimation temperatures prior to sampling. A 12 hour light/ 12 hour dark photoperiod cycle was used throughout the study.

2. Sampling Procedure

Chemical anesthetization prior to sampling was rejected due to the plasma electrolyte changes associated with this procedure (Houston et al., 1971). Fish were stunned by a blow to the head, and blood drawn from the caudal vessels into syringes rinsed with ammonium heparin (Sigma Chemical Co., St. Louis, Mo., 50,000 units). The freshly-drawn blood was then used in the analytical procedures outlined in the following pages.

3. Hematological Determinations

(a) Hematocrit (PCV)

Hematocrit determinations were performed in triplicate with Fisher microhematocrit tubes. Separations were carried out by centrifugation at 13,000 RCF (relative centrifugal force) for five minutes in an Adams

microhematocrit centrifuge. An Adams microhematocrit reader was used to determine packed cell volumes.

(b) Hemoglobin Determinations (Hb)

Hemoglobin was determined in duplicate by the alkaline hematin method as outlined by Anthony (1961). A 20 µℓ volume of fresh whole blood was added to 5.0 ml of 0.1 N NaOH. A standard curve was prepared by placing 20 µℓ, 10 µℓ and 5 µℓ volumes of Hemotrol (Clinton Laboratories, Santa Monica, Ca.) containing 16.4 g/100 ml stabilized human hemoglobin into separate cuvettes. Standards and samples were placed in a boiling water bath for 5.0 minutes and, after cooling, absorbances were read at 560 nm using a Bausch and Lomb Spectronic 700 spectrophotometer. Assuming a molecular weight of 64,500 gHb/mole (Lehninger,1970), molar concentrations of hemoglobin per litre of blood were calculated. On the basis of the corresponding hematocrit value corrected for 'trapped plasma' (described later) the molar concentration of hemoglobin per litre of packed erythrocytes was determined.

(c) Red Blood Cell Count (RBC)

A volume of well-mixed blood was drawn into a blood diluting pipette, and diluted 1:100 using isotonic Hesser's saline (Hesser, 1960). A small volume was blown from the pipette to clear unmixed fluid from the pipette stem, and aliquots applied to the duplicate chambers of a hemocytometer (American Optical, Buffalo, N.Y.). Four peripheral and one central squares of the duplicate chambers were counted at 400X magnification, and the mean count per square obtained. Since each secondary square has an area of 0.04 mm^2 and is 0.1 mm deep, multiplying the count by 25,000 gave the number of erythrocytes per mm^3 of blood.

(d) Mean Erythrocytic Volumes (MEV)

Mean erythrocytic volume was calculated using the formula:

$$MEV\ (\mu^3) = PCV(10)/\text{erythrocyte number}(10^6/mm^3)$$

from Houston (1975).

4. Cation Determinations

Freshly-drawn blood was separated into two containers. One was used for whole blood determinations, and the other centrifuged immediately (5000 g, 5 minutes). After centrifugation plasma was carefully removed with a capillary pipette, and stored in a sealed plastic vial at -80°C. It proved difficult to remove all of the plasma without disturbing the erythrocytes. Such disturbances must be avoided since they cause hemolysis, and lead to overestimations of plasma Mg^{+2} and K^+ levels. Therefore, plasma was withdrawn until only a small layer remained, and this was then absorbed with the tip of a cotton swab. Once separated, plasma and erythrocytes can be stored at -80°C for at least one year without significant changes in ionic composition.

The small volumes of sample obtained required development of procedures whereby all cation determinations could be made on one dilution of each fraction. Double-distilled water was used throughout in making up solutions and samples were prepared in duplicate for each specimen.

In the case of whole blood or packed erythrocytes, 100 µℓ of well-mixed blood, or thawed erythrocytes, was pipetted into 5.0 ml of water. This was vortexed for 4 minutes and allowed to stand for 1 hour at 2°C. After this period, 5.0 ml of 15.214 g $SrCl_2 \cdot 6\ H_2O/\ell\ H_2O$ was pipetted into the tube, and the tube vortexed for a further 4 minutes. Cell debris was removed by centrifugation in a clinical desk-top centrifuge (1,500 g, 15 minutes). The clear red supernatant was transferred to a separate test tube and refrigerated (2°C) prior to analysis. All subsequent determinations

were made on the same day to avoid refreezing and thawing.

In the case of the plasma, 100 µℓ of plasma was pipetted into 10.0 ml of 7.607 g $SrCl_2 \cdot 6\ H_2O$/ℓ H_2O, shaken and stored in the same manner.

This method of sample preparation was found to yield satisfactory results for ionic determinations of the fractions. For erythrocytes and whole blood the use of harsher tissue extractions employing a nitric acid digest (Whittam, 1955; Little, 1964; Houston and Mearow, 1978) produced neither more complete extractions nor more consistent results.

Atomic absorption spectroscopy was used for Mg^{+2} and Ca^{+2} analyses, but proved unsuitable for K^+. Both the latter, and Na^+ were analyzed by the emission technique. Part of the difficulty in applying the former method stems from the fact that if standards containing K^+ but no Na^+ are used to determine unknown K^+ levels of blood, there is a significant overestimation of the values. This is due to the fact that all blood fractions contain considerable amounts of both Na^+ and K^+, and the presence of Na^+ in the sample produces positive interference, and consequently larger optical density readings during K^+ determinations, than if only K^+ was present in the blood. If standards having only K^+ are used for blood determinations the result is an unequal comparison. The use of the emission mode for Na^+ and K^+ analyses leads to little cross interference, improved sensitivities and near-linear calibration curves over the range of concentrations used. While emission spectroscopy is better suited to Na^+ and K^+ analyses, atomic absorption produces better resolution of Mg^{+2} and Ca^{+2}. For this reason each mode was used for the cationic determinations it best suited.

Standards were prepared from BDH single-element Atomic Absorption

Standards (BDH Chemicals, Toronto, Ontario), and contained exactly the same $SrCl_2$ levels as sample preparations. Determinations were made with a Unicam SP-90 Spectrophotometer at the settings listed in the following table.

Table 2

Atomic Absorption Spectrophotometer Settings

Mode	Ion	Wave-length (mμ)	Slit-width (mm)	Burner height (cm)	Air flow ℓ/min	Fuel flow cc/min	Lamp current (mA)
Emission	Na^+	589.0	0.08	2.0	5	1000	0
	K^+	766.5	0.10	2.0	5	1000	0
Atomic	Mg^{+2}	422.7	0.10	1.0	5	1000	12
Absorption	Ca^{+2}	285.2	0.08	1.0	5	1200	12

Optical density values were recorded using a Fisher Recordal Series 5000 recorder.

During all determinations the instrument was blanked with a solution of 7.532 g $SrCl_2 \cdot 6\ H_2O$/ℓ H_2O between each measurement. A series of 6 standards for the appropriate fraction were measured between each group of 10 samples to check for drift. The concentrations of the samples were determined from a calibration curve of the standards aspirated before and after the samples.

Electrolyte concentrations in carp and rainbow trout erythrocytes have never previously been determined. Consequently, there was no data available for comparison. Because of this, the check method described by Bugyi et al. (1969) was undertaken. If ionic determinations are made on plasma, packed

erythrocytes and whole blood, and if the packed cell volume is known, then a determined whole blood ionic value can be compared with calculated blood values, where the calculated value for an ion x is:

$$WB_{cx} = (P_x) \cdot (1\text{-PCV}) + (E_x) \cdot (PCV)$$

and WB_{cx} = calculated concentration of ion in whole blood in mEq/ℓ blood,

(P_x) = plasma concentration of x, mEq/ℓ plasma,

(E_x) = erythrocyte concentration of x, mEq/ℓ cells, and

PCV = the fraction of blood occupied by erythrocytes.

If discrepancies between the calculated and determined values occur, this indicates that analytical errors must exist in one or more of the blood fractions or the PCV. For 57 to 63 fish (summer rainbow trout) on which 4 cationic determinations were performed, the average error for Na^+ was 2.28% with 95% of the normalized distribution of errors being between 3.57% to 0.99%. K^+ produced a mean error of 2.05% with the 95% confidence limits lying between 3.45% to 0.65%. Determinations of Mg^{+2} and Ca^{+2} produced somewhat larger mean errors (4.34% and 3.97% respectively), but in view of the small levels of Mg^{+2} and Ca^{+2} present in the blood these represent relatively small quantitative errors in determination.

5. Chloride Determinations

Chloride was determined using a Buchler-Cotlove chloridometer (Buchler Instruments Inc., Fort Lee, N.J.). Plasma, whole blood, packed erythrocytes and standards were prepared in triplicate and treated in the same manner. The standard used was Versatol (General Diagnostics, Morris Plains, N.J.), an artificial human serum containing 103 mEq/ℓ Cl^-. 20 μℓ volumes were pipetted into 2.0 ml of 0.1 N HNO_3 + 10% acetic acid solution. Plasma pre-

parations were analyzed immediately, while erythrocyte and whole blood preparations were sealed and allowed to digest for 12 hours. Standards were prepared for each set of unknowns, and although no digestion period is necessary prior to determination, the standards used in determinations of erythrocytic or whole blood chloride were allowed to stand for 12 hours as well. Just prior to analysis, 3 drops of gelatin reagent (6.2 g of a reagent containing 60:1:1 gelatin:thymol blue:thymol/ℓ H_2O, prepared by Buchler Instruments) were added to each sample cuvette.

The Buchler-Cotlove method involves the release of Ag^{+2} into the solution and simultaneous measurement of solution conductivity. Ag^{+2} reacts with Cl^- to form $AgCl_2$. The endpoint of the titration is reached when all chloride is in the form of $AgCl_2$ and free Ag^{+2} accumulates, increasing solution conductivity. Since Ag^{+2} is released at a constant rate into the solution, the time taken to reach the endpoint is linearly proportional to chloride concentration. In the present study the endpoint of titration was met when the solution conductivity reached 10 mA.

In all cases blanks containing 20 μℓ distilled H_2O + 2.0 ml 0.1 N HNO_3, 10% acetic acid + 3 drops gelatin reagent were first run to 'condition' the Ag^{+2} electrode and provide a stable 'blank' time reading. Subsequently, standards followed by ten samples were run in triplicate, and titration times recorded. The chloride concentration of the unknown sample was calculated as follows:

$$\text{Sample concentration (mEq/ℓ)} = \frac{T_s - T_b}{T_{std} - T_b} \times \text{Std. concentration}$$

where T_s = titration time of sample (sec)

T_b = titration time of blank (sec)

T_{std} = titration time of standard (sec), and

Std. concentration = 103 (mEq/ℓ, Cl^-)

6. Water Determinations

Plasma and erythrocytic water were determined in duplicate. A 50 µℓ volume of packed cells or plasma was weighed and dried for 24 hrs at 70°C, then 48 hrs at 103°C. Water content was then calculated, and expressed as a % of the total weight as well as the volume of H_2O occupying a volume of plasma or erythrocytes. In the case of packed erythrocytes the volume of H_2O contributed by the trapped plasma was compensated for.

7. 'Trapped Plasma' Factor

Cation and chloride determinations made on samples of packed erythrocytes involve an error due to plasma trapped within the interstices of the cells. To compensate for this, the percent volume of plasma occupying the erythrocyte column was determined. The detailed procedure is outlined in Appendix 1. This involved additions of radioactive, erythrocyte-impermeable polyethylene glycol-^{14}C (m. wt. 4000) to a volume of blood, separation of the blood into plasma and erythrocyte fractions as for ionic determinations, and determination of the radioactivity of each of these fractions. After compensation for quenching imparted by the coloration of the plasma or erythrocytes, the radioactivity present in a volume of erythrocytes was equated to the volume of plasma, and the % volume of plasma occupying the packed erythrocytes determined.

The possibility that erythrocytes of small mean volume might pack differently than larger cells was also considered. In this case the plasma trapping factor was determined for five samples of rainbow trout erythro-

cytes whose MEV varied from 230 μ^3 to 311 μ^3. No correlation between the plasma trapped and MEV was found. The mean value for the trapped plasma was 2.82%. Catlett and Millich (1976) using ^{14}C-inulin reported a trapping factor of 3% in goldfish with MEV of 190 μ^3. Human erythrocytes, having volumes of approximately 80 μ^3, have trapping factors of 2-5% (Bugyi et al., 1969). Therefore, it appears that MEV is not an important variable in this correction factor. In view of this, a trapping factor for carp erythrocytes (MEV of between 190 and 250 μ^3) was not calculated and the trout factor of 2.82% was used.

Electrolyte levels for a measured volume of erythrocytes were corrected for the trapped plasma using the following equation:

$$\text{Correct level of electrolyte (mEq/}\ell\text{ cells)} = \frac{(E) - (P \cdot 0.0282)}{0.9718}$$

where E = mEq of electrolyte/ℓ of non-corrected packed cells, and

P = mEq of electrolyte/ℓ of plasma.

8. Carbonic Anhydrase

(a) Sample Preparation

Triplicate hematocrit readings were obtained for freshly drawn, well-mixed blood. On the basis of these values, duplicate samples were prepared with sufficient whole blood to provide 0.125 ml of packed erythrocytes. These were centrifuged (5000 g, 5 minutes, 3°C) and the plasma removed and stored at 2°C. The packed cells were washed 4 times in 0.8% saline with intervening centrifugations (5000 g, 5 minutes, 3°C). After the fourth wash they were resuspended in 200 $\mu\ell$ of saline, and transferred to graduated centrifuge tubes. Double-distilled H_2O was added to a final volume of 5.0 ml, the suspension vortexed for 4 minutes and centrifuged at 30,000 g for

30 minutes at 2°C. Although the alevin stages of rainbow trout (approximately 25 days old) possess erythrocytes with extensive tubular networks and pronounced nuclei, adults of the size type used in the present study are characterized by red cells having few or no organelles and reduced nuclei (Iuchi, 1973a). It seemed likely, therefore, that the pellet brought down by centrifugation consisted mainly of membranous material. Centrifugation of the supernatant at 100,000 g for 45 minutes failed to bring down any further material, suggesting that this fraction consisted primarily of non-membranous components.

In some instances assays performed on non-mammalian erythrocytes have involved only the supernatant fraction; the membrane fraction has been discarded (e.g., Mashiter and Morgan, 1975). This is largely because such procedures have been based on those previously performed on mammalian erythrocytes where carbonic anhydrase is present only in the cytosol. Preliminary work within this laboratory, as well as that of Haswell (1977), suggested that there may be a membrane-situated form of this enzyme in rainbow trout erythrocytes. In view of this, the membrane fractions were also retained and prepared for carbonic anhydrase activity determinations.

After centrifugation, pellet and supernatant were separated, and the pellet washed 3 times with 2.5 ml aliquots of distilled water. Wash waters were monitored for carbonic anhydrase activity and protein. Modest activity was inconsistently observed only after the first wash. The washed pellet was then brought to 2.5 ml with distilled H_2O, and vigorously vortexed until the solution became clear. To remove bubbles which had formed during this procedure, the solution was centrifuged at 30,000 g for 15 minutes. Once prepared in this manner, further centrifugations did not

bring down material. Microscopic examinations indicated that this fraction contained very finely divided membrane with no sealed vesicles. Since interest at this stage was focussed only upon the contribution of 'membrane' and 'cytosol' components to total carbonic anhydrase activity, no further purification was undertaken.

(b) Assay Procedure

Assays were performed in duplicate on each of two preparations of cytosol and membrane fractions. Each was assayed at both the appropriate acclimation temperature (2°, 10° or 18°C) and a common assay temperature (25°C). Assessment could then be made of enzyme activity under *in vivo* temperature conditions, as well as enzyme activity modifications that result from thermal acclimation.

The reaction mixture used was based on the ability of carbonic anhydrase to break down p-nitrophenyl acetate to nitrophenol and nitrophenolate. Both products absorb light equally at 348 nm; therefore, product formation can be readily monitored and converted to μM substrate catalyzed. Since other esterases present in cells can also break down p-nitrophenyl acetate, carbonic anhydrase activity was determined by estimating the total esterase activity (including that of carbonic anhydrase), and carrying out a second assay with the same enzyme preparation prepared with a carbonic anhydrase inhibitor, acetazolamide, present. The difference in substrate conversion between the two assays was attributed to carbonic anhydrase.

Assays without inhibitor were carried out in the following manner. A 0.2 ml volume of cytosol or membrane preparation was pipetted into 0.8 ml of 125 mM Tris-H_2SO_4 buffer (pH 7.5) and pre-incubated at the appropriate temperature for 2 minutes. To this was added 1.0 ml of 3 mM p-nitrophenyl

acetate, in 3% aqueous acetone. The reaction mixture was allowed to pre-incubate for 20 seconds (which is adequate for thermal equilibration) and then drawn into the temperature-controlled cell of the Spectronic 700. Changes in absorbance were monitored for 4 minutes at 348 nm using a Fisher Recordall series 5000 recorder. Assays involving inhibitor were carried out in the same manner for each enzyme preparation using 0.8 ml of 125 mM Tris-H_2SO_4 buffer (pH 7.5) containing 125 mM acetazolamide.

The assay presents a number of problems worthy of mention. The substrate p-nitrophenyl acetate is not sufficiently soluble to give the concentrations required for V_{max} activities. Consequently, enzyme velocity is governed by the substrate concentration of the assay. If the reaction is allowed to proceed for long periods of time it reduces the substrate concentration and therefore measured activity. This can be largely overcome by allowing the enzyme to act for relatively brief periods of time, i.e., intervals of not more than a few minutes (P. Nicholls, personal communication). Therefore, activity calculations were made only on the linear portion of the absorbance vs. time curve found immediately after introduction of the reaction mixture into the flowthrough cell and attainment of the assay temperature (a matter of seconds). The maximal breakdown of substrate was, in all cases, less than 0.6%/minute, and substrate concentration was not seriously diminished. Thus, the velocity of the reaction did not seriously change with time. Since all preparations were compared in the same manner and activities calculated from the same sections of the absorbance vs. time curve, the results are regarded as being at least comparable to each other.

Protein determinations used in calculating specific activity values

were performed by a modified Lowry technique (Albro, 1975). Since the red blood cells contain a great deal of hemoglobin which is not associated with any enzyme activity, the hemoglobin content of each fraction was determined using the technique of Anthony (1961) previously described. Hemoglobin content was then subtracted from total protein and specific activities calculated on the basis of non-hemoglobin protein.

9. Statistical Analysis

All statistical analyses were carried out using a Wang 2200 desk top computer (Wang Laboratories Inc., Tewksbury, Mass.). Single classification analysis of variance was used to evaluate differences between groups. All data were subjected to logarithmic or arc-sin transformation prior to analysis and significance attributed to differences at the $P < 0.05$ or $P < 0.01$ level.

Both linear and geometric regression analysis was performed using the least squares method. In the case of linear regressions, data points (X,Y) were fitted to the line $Y = A + BX$. When geometric regressions were performed, data points were fitted to the curve $Y = AX^B$ by taking the log of both sides of the equation [Log(Y) = Log(A) + Blog(X)] and reducing the problem to a linear regression where data points (X',Y') are fitted to $Y' = A' + BX'$ and $Y' = LogY$, $X' = LogX$ and $A' = LogA$.

The coefficients of correlation for the regressions were then analyzed to determine the significance of the fit. Again, significance was attributed only to fits at the $P < 0.01$ or $P < 0.05$ levels.

RESULTS AND DISCUSSION

Hematological Variation Following Thermal Acclimation

Rainbow Trout

Thermoacclimatory and seasonal hematological variations in rainbow trout are summarized in Table 3 and Appendices 2 and 3 . The hematology of rainbow trout showed little change with either temperature or season. Mean blood hemoglobin levels (Table 3a) of summer fish acclimated to 2°, 10° and 18°C, and winter fish at 2° and 10°C were within 2% of each other. The most extreme variation seen occurred in winter fish held at 10° and 18°C. In this case mean hemoglobin decreased from 8.22±0.259 (10°C) to 7.59±0.432 g%, but the difference was not significant. Like hemoglobin, hematocrit showed no significant change with temperature or season (Table 3b). Although mean values for summer fish were slightly higher than those of groups sampled during the winter, the maximum differences were only 30.8±1.57 (winter, 18°C) and 33.7±0.716 (summer, 10°C).

Summer-sampled fish exhibited what might be regarded as 'appropriate' hematological response in that larger numbers of cells (Table 3c) having smaller volumes (Table 3d) were found at 10° as compared to 2°C ($P < 0.05$). Such trends should increase the rate of oxygen diffusion into the cells by decreasing the diffusion pathway (Holland and Forester, 1966), and would therefore facilitate oxygen loading at the gills. However, the overall oxygen-carrying capacity of the blood, which is governed by the hemoglobin content was little altered and the mean erythrocytic hemoglobin content actually decreased at 10° as compared to 2°C ($P < 0.01$, Table 3e). The summer series of fish showed no significant changes in red blood cell

Table 3

A summary of hemoglobin (Hb, g/100 ml), hematocrit (PCV, %), erythrocyte numbers (RBC, $x10^6/mm^3$), mean erythrocytic volume (MEV, μ^3) and hemoglobin content (MEHbC, mM/cell $x10^{-12}$) of thermally-acclimated rainbow trout (Salmo gairdneri). Reported as the mean ± 1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Significance		
		2°C	10°C	18°C	2°C	10°C	18°C
Hb 3a	Summer	8.12±0.228	8.20±0.207	8.07±0.274	N.S.	N.S.	N.S.
	Winter	8.06±0.253	8.22±0.259	7.59±0.432	N.S.	N.S.	N.S.
					*N.S.	N.S.	N.S.
PCV 3b	Summer	32.8±1.11	33.7±0.716	33.2±1.03	N.S.	N.S.	N.S.
	Winter	31.5±0.927	31.6±1.06	30.8±1.57	N.S.	N.S.	N.S.
					*N.S.	N.S.	N.S.
RBC 3c	Summer	1.19±4.76x 10^{-2}	1.36±4.37x 10^{-2}	1.33±5.64x 10^{-2}	p<0.05	N.S.	N.S.
	Winter	1.32±5.25x 10^{-2}	1.23±5.97x 10^{-2}	1.28±5.93x 10^{-2}	N.S.	N.S.	N.S.
					*N.S.	N.S.	N.S.
MEV 3d	Summer	277±9.83	252±6.31	255±12.1	p<0.05	N.S.	N.S.
	Winter	241±6.69	263±8.60	242±6.50	p<0.05	p<0.05	N.S.
					*p<0.01	N.S.	N.S.
MEHbC 3e	Summer	1.07±3.23x 10^{-2}	0.939±2.11 x10^{-2}	0.991±5.07x 10^{-2}	p<0.01	N.S.	N.S.
	Winter	0.956±2.75x 10^{-2}	1.07±3.89x 10^{-2}	0.923±2.66x 10^{-2}	N.S.	p<0.01	N.S.
					*p<0.01	p<0.01	N.S.

*significant differences between seasons

numbers, volume or hemoglobin content between 10° and 18°C, although the mean erythrocytic hemoglobin content decreased slightly.

Winter fish exhibited some significant changes in hematological status, but these did not, on the whole, appear to be consistent with adaptive adjustment. For example, red cell count (Table 3c) showed no significant change with acclimation temperature. Mean erythrocytic volume (Table 3d) increased significantly between 2° and 10°C ($P < 0.05$), but then decreased between 10° and 18°C ($P < 0.05$). Cell volumes at 18°C were not significantly different from those found in fish acclimated to 2°C. A similar trend was observed in mean erythrocytic hemoglobin content (Table 3e). This increased between 2° and 10°C but decreased significantly ($P < 0.01$) at 18°C to mean values below those observed at 2°C. Although some significant seasonal differences in mean erythrocytic volume and hemoglobin content were found (Tables 3d and 3e respectively), these were inconsistent in nature.

Carp

Hematological data on carp acclimated to 2°, 16° and 30°C are summarized in Table 4 and Appendix 4. Like trout, carp showed few significant hematological changes with acclimation. Unlike the trout, however, the trends exhibited were consistent with adaptive response, and in many cases, approached significance. The most obvious of these occurred between 16° and 30°C. Mean hemoglobin levels (Table 4a), packed cell volumes (Table 4b) and red cell counts (Table 4c) increased with temperature, and would be expected to slightly amplify oxygen carrying capacity of the blood. Coupled with these was a significant ($P < 0.05$) reduction in the mean erythrocytic volume (Table 4d) between 16° and 30°C which should facilitate

Table 4

A summary of hemoglobin (Hb, g/100 ml), hematocrit (PCV, %), erythrocyte numbers (RBC, $x10^6/mm^3$), mean erythrocytic volume (MEV, μ^3) and hemoglobin content (MCHbC, mM/cell $x10^{-12}$) of thermally-acclimated carp (Cyprinus carpio). Reported as the mean ± 1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Significance		
		2°C	16°C	30°C	2°C	16°C	30°C
Hb 4a	Spring	7.51±0.214	7.54±0.264	8.18±0.362	N.S.	N.S.	N.S.
PCV 4b	Spring	32.9±0.951	32.3±1.16	33.4±1.51	N.S.	N.S.	N.S.
RBC 4c	Spring	1.48±5.37x 10^{-2}	1.48±6.58x 10^{-2}	1.67±7.90x 10^{-2}	N.S.	N.S.	N.S.
MEV 4d	Spring	226±9.94	222±8.36	202±5.53	N.S.	N.S.	$p<0.05$
MCHbC 4e	Spring	0.795±2.47x 10^{-2}	0.802±4.12x 10^{-2}	0.767±2.27x 10^{-2}	N.S.	N.S.	N.S.

oxygen diffusion into the cell at higher temperatures. Interestingly, mean erythrocytic hemoglobin content (Table 4e) decreased slightly between these temperatures.

In general, the data obtained for rainbow trout compared well with earlier observations on this species (DeWilde and Houston, 1967; McCarthy et al., 1973; Denton and Yousef, 1975), while those for carp were well within ranges reported by Houston and DeWilde (1968) and Houston et al. (1976).

Seasonal and thermoacclimatory variations observed in trout were generally consistent with those noted by DeWilde and Houston (1967) in summer and fall-winter groups of this species following acclimation to temperatures ranging from 3° to 21°C. It should be noted, however, that DeWilde and Houston (1967) found pronounced trends. Red blood cell count and blood hemoglobin increased, and the mean erythrocytic volume decreased slightly at higher acclimation temperatures. The thermoacclimatory variations in carp noted in the present study were remarkably consistent with the results obtained by Houston and DeWilde (1968), who also reported modest, mostly non-significant increases in blood hemoglobin, hematocrit and erythrocyte numbers with some decrease in mean erythrocyte volume at increased temperatures.

The absence of any marked change in the oxygen carrying capacity of the blood with thermal acclimation has been reported in other species of fish as well. Thermal acclimation produced little change in the hematology of goldfish (Anthony, 1961; Faulkner and Houston, 1966; Houston et al., 1976) or in bluegill sunfish, Lepomis gibbosus, white sucker, Catostomus commersoni, and goldfish x carp hybrids (Houston et al., 1976). However, Houston and Cyr (1974) and Houston and Rupert (1976) observed significant

increases in the hemoglobin content and packed cell volume in goldfish with acclimation temperature, and in the case of Houston and Cyr (1974) such responses were also exhibited by rainbow trout.

The lack of consistency in response to ostensibly similar conditions raises a number of questions for which adequate answers are not yet available. One factor which may at least partially determine whether a fish will respond to a thermal stress is weight. When metabolism (M, kcal/time) is plotted against weight (W, kg), a geometric relationship often results. This can be described by the relationship $M = kW^b$, where k is a constant (kcal/kg/time) and b an exponent. Table 5 (from Prosser, 1973) summarizes exponents relating weight to metabolism in a number of species. A plot of oxygen consumption per weight versus weight for goldfish clearly reveals that smaller fish have higher metabolic rates per unit body weight than do larger fish (Fig. 2). Although the relationship of animal size and metabolic rate is complex, such observations are in part due to two factors. With growth there is a disproportionate increase of tissues of low metabolic activity (bone, fat, and connective tissue) as compared to those of higher metabolic activity such as liver, kidney, gill and muscle (Prosser, 1973; Denton and Yousef, 1976). Second, within the same species existing evidence suggests that the tissues of smaller animals possess larger oxidative capacities than do larger ones (Dehnel, 1958; Prosser, 1973).

Weight-specific differences in metabolic activity imply that the oxygen requirements of smaller specimens would be increased more than those of larger animals for any given rise in ambient temperature (see Fig. 2). Thus, hematological compensation in the form of an increase in the blood oxygen carrying capacity might well be more pronounced in smaller

Fig. 2. The effect of body size on weight-specific standard oxygen consumption of goldfish acclimated to 10° and 35°C. Plots are calculated from the data of Beamish and Mookerjii (1964).

Table 5. The coefficients b and K for various species of fish relating weight (W, kg) to metabolism (M, kcal·hr^{-1}), according to the equation $W = KM^b$ (from Prosser, 1973).

Fig. 2

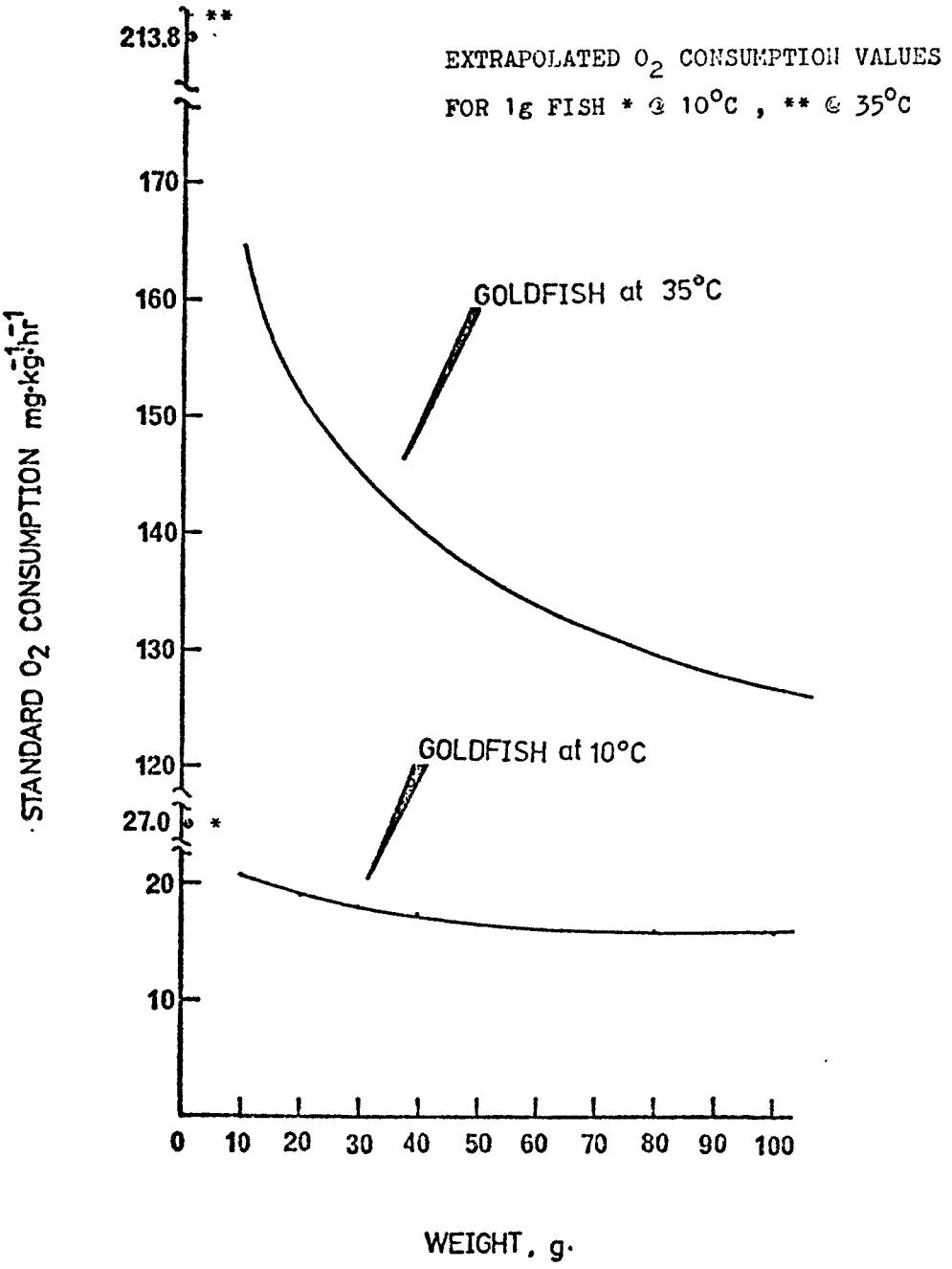


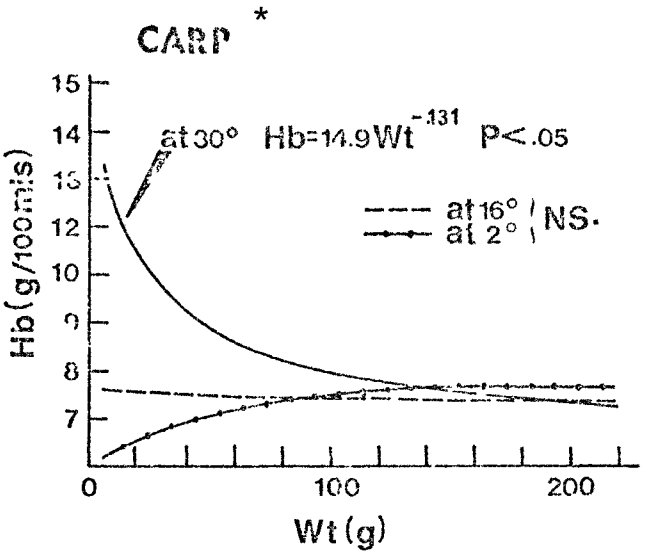
Table 5

Animals	*Size Range*	*b*	*K*
Fish			
salmon *Oncorhyncus*[56]	3–1400 g	0.97 (active) 0.78 (standard)	
goldfish[197]	10–400 g	0.85	
goldfish[16]		0.86	
Gadus[16]		0.70	
starry flounder[16]		0.86	
Squalus[15]		0.74	
lingcod[16]		0.78	
Antarctic *Notothenia*[170]	50–2000 g	0.785	0.235
ammocoetes of lamprey[50]	0.14–3.49 g	0.72	0.054

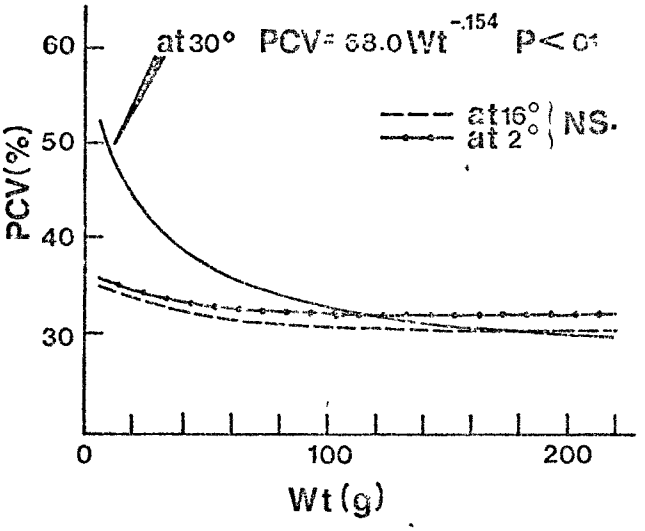
Fig. 3a to c. The weight-specific variations in blood hemoglobin (Hb), hematocrit (PCV) and red blood cell count (RBC) of carp acclimated to 2°, 16° and 30°C. Shown are the best fitting plots of geometric regressions performed on the data along with the significance levels of the correlation. Additional information describing the plots can be found in Appendix 37.

Fig 3

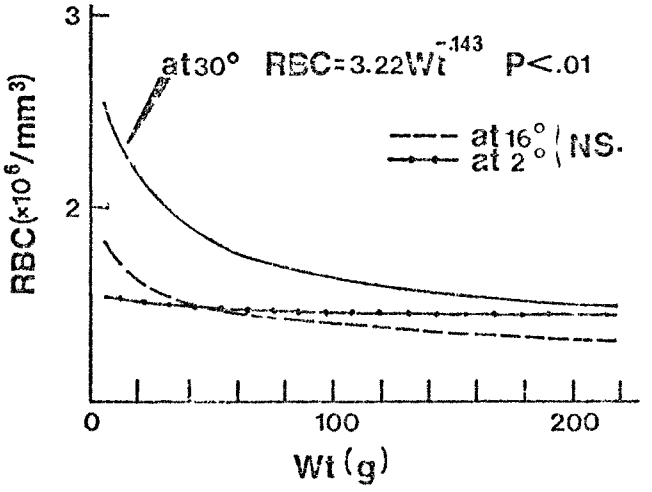
3a



3b



3c



* carp in the weight range of 17.3-444.8 g. were used to calculate the regressions

animals.

To examine this possibility, geometric and linear regressions were calculated for the relationships between the blood parameters measured and specimen weight at each acclimation temperature. The most interesting results were found for carp, and these are summarized in Figures 3(a) to (c). At temperatures of 2° and 16°C weight was not significantly correlated with hemoglobin content (Fig. 3a), hematocrit (Fig. 3b) or red cell count (Fig. 3c). At 30°C, however, the best fitting lines of all parameters decreased in a geometric fashion with increases in weight. Correlation coefficients were significant at the $P < 0.05$ or $P < 0.01$ level. This is, of course, consistent with weight-specific differences in the oxygen needs of smaller fish. The curves obtained have much the same characteristics as those depicted in Fig. 2 where weight-specific oxygen consumption is plotted as a function of weight.

If these results are of general application, they may account in large measure for earlier inconsistent findings. For example, 20 g specimens might show large changes in blood hemoglobin at acclimation temperatures 2°, 16° and 30°C, while fish at weights in excess of 100 g would exhibit little response (Fig. 3a). This would be the case for other hematological parameters as well. In this respect it is interesting to note that in this laboratory, using similar techniques, Houston and Cyr (1974) found marked and significant increases in hemoglobin content with acclimation to 35° as compared to 2°C in goldfish ranging in weight from 12.7 to 15.3* g. Houston and Rupert (1976) using goldfish in the 22 to 38 g range found moderate but still significant increases in hemoglobin with temperature,

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*ranges in these cases are expressed as the 95% confidence interval

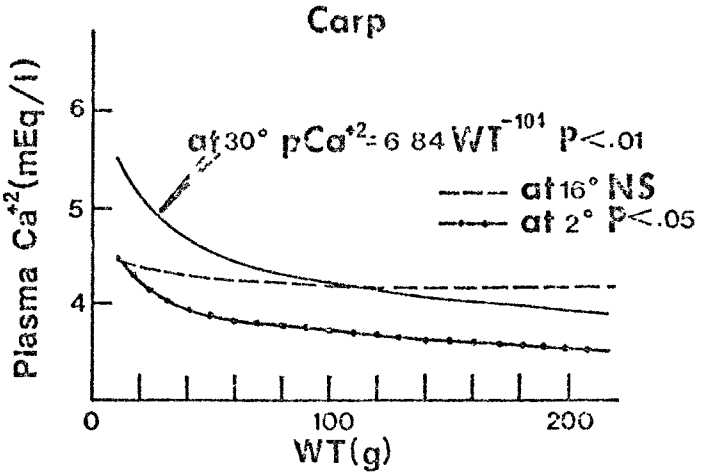
Fig. 4a. The weight-specific variation of plasma calcium in carp acclimated to 2°, 16° and 30°C. Shown are the best fitting plots of geometric regressions performed on the data along with the significance levels of the correlations.

Figs. 4b to d. Geometric regression of the hematological parameters (blood hemoglobin (Hb), hematocrit (PCV) and red blood cell count (RBC)) against plasma calcium levels of carp acclimated to 30°C. The best fitting plot of the geometric regression is shown, along with the significance level of the correlation. The units for the hematological parameters are the same as those in Fig. 3.

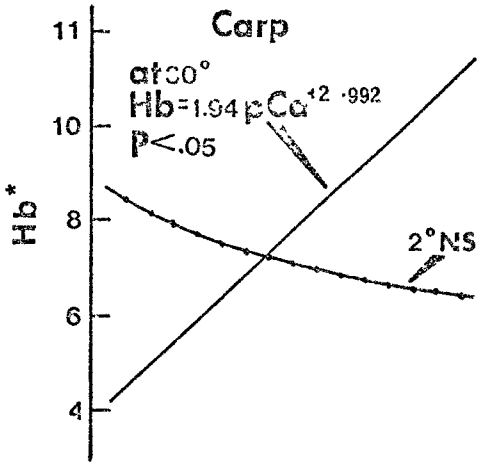
Additional information describing the plots can be found in Appendix 37.

Fig 4

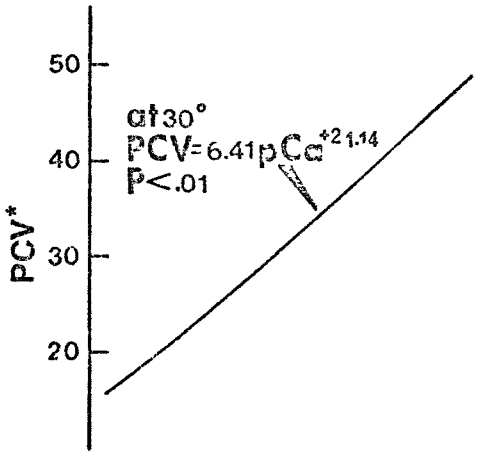
4a



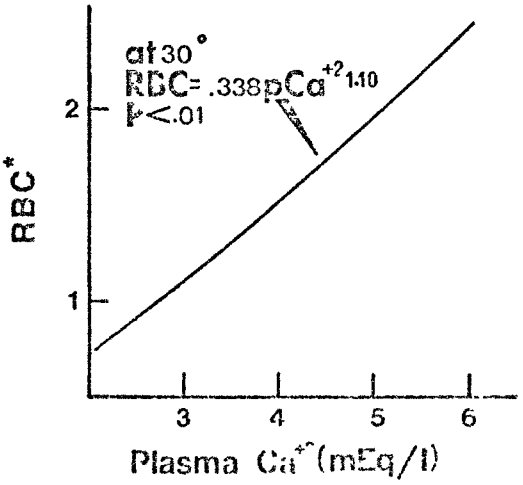
4b



4c



4d



while Houston et al. (1976) using larger goldfish (44.1 to 60.6* g) found no significant ($P < 0.05$) differences in animals held at 5° and 30°C. These observations are consistent with the type of variation suggested by Fig. 3a.

There is some evidence (Perris, 1971) that hemopoesis is related to plasma calcium levels, and that increases in red cell abundance can be related to corresponding changes in plasma calcium. Therefore, a similar analysis of weight-related variation in calcium was also carried out for carp. When plasma calcium was geometrically regressed against fish weight (Fig. 4a), values for specimens acclimated to 30°C produced inverse relationships similar to those encountered with hematological parameters. To see if hematological response could be related to plasma calcium, regression equations were then calculated for hemoglobin (Fig. 4b), hematocrit (Fig. 4c) and red cell count (Fig. 4d) against calcium. Direct and significant ($P<0.05$, $P < 0.01$) correlations were observed for each of the hematological parameters of 30°C specimens. Interestingly, in the one case where hemoglobin increased with weight (Fig. 3a, 2°C) the plot of plasma calcium vs hemoglobin was inverse in character (Fig. 4b, 2°C). This would also be consistent with a presumed relationship between plasma calcium and hematological response.

Although little literature is available on the relationship between plasma calcium and hemopoetic activity in the tissues of fish, relationships of this type have been found in mammalian systems. Perris (1971), in reviewing his own experiments, indicated that calcium strongly effects the events leading to mitosis. For example, in rats, periods of maximal

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*ranges in these cases are expressed as the 95% confidence interval

growth were shown to coincide with increases in thymus and bone marrow mitotic activity and these paralleled increases in ionized plasma calcium levels. Further experiments indicated that under *in vivo* and *in vitro* conditions mitotic activity in these tissues could be increased by artificially elevating calcium levels. Increased calcium levels were also seen after acute blood loss, and were linked to increases in red blood cell production. Intraperitoneal injection of $CaCl_2$ into rats resulted in a surge of reticulocyte formation in bone marrow, and increased ^{59}Fe incorporation into red blood cells. In most *in vivo* experiments parathyroid hormone administration (which is known to increase plasma Ca^{+2}) prompted the same effects as $CaCl_2$ injection. Experiments indicated that increases in plasma calcium produce increased cellular cyclic AMP levels which, in turn, stimulate phosphorylation of histones, permitting replication of DNA and mitosis.

The correlations observed between hematological status and plasma calcium tend to support the view that in fish, as in mammals, the hemopoetic system may be Ca^{+2}-triggered. The basis of the observed size differential remains uncertain at present. It is appropriate to note, however, that smaller animals are characterized by more rapid growth rates than larger specimens. Since growth is associated with a rapid deposition of bone which relies on plasma calcium, smaller animals are perhaps developmentally pre-adapted, in a sense, to more pronounced hematological response by virtue of their higher plasma calcium levels. The nature of calcium metabolism and regulation by fishes is, however, not well understood. Much the same is true of the basis for the temperature-related differences in calcium response encountered. What does seem clear, how-

Table 6a and b

Linear regression analysis of the hematological parameters and plasma Ca^{+2} (y) against weight (x) in thermally-acclimated rainbow trout (Salmo gairdneri). The data reported is n (sample size), cc (coefficient of correlation), as well as the level of significance (NS., $p<0.05$, $p<0.01$) and the best fitting line of the relationship.

(a)

Parameter Regressed Against Weight (g)	Season	Acclimation Temperature 2°C	10°C	18°C
Hb (g/100 ml blood)	Summer	n = 17 cc = 0.003 NS. y = -0.00005x + 8.13	n = 17 cc = 0.332 NS. y = -0.0043x + 8.84	n = 18 cc = 0.300 NS. y = -0.008x + 9.05
	Winter	n = 27 cc = 0.078 NS. y = 0.002x + 7.49	n = 21 cc = 0.1495 NS. y = 0.003x + 7.19	n = 20 cc = 0.5081 p<0.05 y = 0.018x + 2.37
PCV %	Summer	n = 17 cc = 0.563 p<0.05 y = -0.050x + 39.5	n = 17 cc = 0.380 NS. y = -0.017x + 36.3	n = 18 cc = 0.478 p<0.05 y = -0.047x + 38.8
	Winter	n = 27 cc = 0.097 NS. y = -0.009x + 34.1	n = 21 cc = 0.093 NS. y = 0.008x + 28.9	n = 20 cc = 0.403 NS. y = 0.052x + 15.67
RBC $x10^6/mm^3$ blood	Summer	n = 17 cc = 0.131 NS. y = -0.0005x + 1.26	n = 17 cc = 0.558 p<0.05 y = -0.0015x + 1.59	n = 18 cc = 0.429 NS. y = -0.002x + 1.63
	Winter	n = 27 cc = 0.002 NS. y = 0.00001x + 1.31	n = 21 cc = 0.155 NS. y = 0.0008x + 0.981	n = 20 cc = 0.347 NS. y = 0.002x + 0.787
Plasma Ca^{+2} mEq/l	Summer	n = 17 cc = 0.008 NS. y = 0.00009x + 4.64	n = 17 cc = 0.046 NS. y = -0.0006x + 4.74	n = 17 cc = 0.235 NS. y = 0.004x + 4.19
	Winter	n = 24 cc = 0.060 NS. y = 0.0007x + 4.82	n = 18 cc = 0.310 NS. y = -0.001x + 5.54	n = 15 cc = 0.342 NS. y = 0.004x + 3.66

Linear regression analysis of plasma Ca^{+2} (y) against the hematological parameters (x) in thermally-acclimated rainbow trout (Salmo gairdneri). Reported in the same manner as above.

(b)

Parameter Regressed Against Plasma Ca^{+2} (mEq/l)	Season	Acclimation Temperature 2°C	10°C	18°C
Hb (g/100 ml blood)	Summer	n = 15 cc = 0.269 NS. y = 0.191x + 3.13	n = 17 cc = 0.238 NS. y = 0.234x + 2.73	n = 17 cc = 0.244 NS. y = 0.146x + 3.50
	Winter	n = 24 cc = 0.253 NS. y = 0.127x + 3.98	n = 18 cc = 0.249 NS. y = 0.062x + 4.61	n = 18 cc = 0.673 p<0.01 y = 0.211x + 3.25
PCV %	Summer	n = 15 cc = 0.341 NS. y = 0.047x + 3.14	n = 17 cc = 0.124 NS. y = 0.035x + 3.46	n = 17 cc = 0.242 NS. y = 0.040x + 3.36
	Winter	n = 24 cc = 0.223 NS. y = 0.029x + 4.11	n = 18 cc = 0.438 NS. y = 0.026x + 4.30	n = 18 cc = 0.605 p<0.01 y = 0.053x + 3.23
RBC $x10^6/mm^3$	Summer	n = 15 cc = 0.570 p<0.01 y = 1.87x + 2.44	n = 17 cc = 0.338 NS. y = 1.58x + 2.51	n = 17 cc = 0.079 NS y = 0.236x + 4.36
	Winter	n = 24 cc = 0.010 NS. y = 0.022x + 4.99	n = 18 cc = 0.051 NS. y = 0.051x + 5.04	n = 18 cc = 0.434 NS. y = 1.01x + 3.58

* cc = r

ever, is that these characteristics, whatever their nature, apparently have some potential value in terms of hematological response to temperature in carp.

Comparable analysis of data for rainbow trout lead to inconclusive results. Geometric and linear regression analysis (of which the latter is shown in Table 6) showed poor correlations, and few differences between acclimation temperatures. Many factors may account for this. The trout used in the study were more homogeneous in weight than carp. Only 10 out of 120 fish were less than 100 g (40% of the carp were in this weight range); the weight range in carp showing the largest weight-dependent variation in hematology. Hence, weight-dependent variations in trout may have been masked by the use of an inappropriate weight range of specimens. In addition, this species may not be characterized by comparable responses. An important point that should also be made is that even if trout did show weight-hematological relationships, other factors must play an important part in determining whether this species will respond hematologically to temperature change since recent studies (Murphy and Houston, 1977; Smeda and Houston, 1978) have indicated some appropriate changes in hematocrit can occur in trout well over 200 g. Such responses may be linked to sampling season.

In conclusion, rainbow trout, acclimated to 2°, 10° and 18°C, and carp held at 2°, 16° and 30°C showed little hematological response following acclimation. The lack of any marked hematological change with acclimation has been observed in these species previously (DeWilde and Houston, 1967; Houston and DeWilde, 1968), as well as in other species (Houston et al., 1976). Although such changes are of potential value when

they occur, their absence or inconsistency suggests that hematological responses probably do not play a major role in the resolution of the temperature oxygen demand problem. By exclusion, at least, this points to the probable importance of other modifications at the branchial exchanger level or in terms of more subtle hemoglobin changes. The data did, however, indicate a possible influence of weight in hematological response to increased temperature by carp; a relationship that was absent in rainbow trout.

Thermoacclimatory Variations in Plasma Electrolyte Levels

Data pertaining to thermoacclimatory plasma electrolytes are summarized in Appendices 5 to 9 , Table 7, and Figs. 5 and 6. From Table 7, it can be seen that plasma water changed very little with thermal acclimation in both rainbow trout and carp. Although statistical significance was found in two cases (i.e., summer trout between 10°-18°C, $P < 0.05$; seasonal change between summer and winter trout at 10°C, $P < 0.05$), the changes involved were small. Differences between the means were actually less than 3% in both cases.

Rainbow Trout

Acclimation of trout produced little change in plasma electrolyte concentrations. For example, acclimation to 2°, 10° and 18°C leads to no significant changes in plasma Cl^-, Ca^{+2} or Mg^{+2} levels between temperatures (Figs. 5b, 5d and 5e respectively). Similarly, summer trout showed no significant thermal change in plasma Na^+ (Fig. 5a). However, in winter sampled specimens, sodium at 2°C was significantly lower than that at 10° and 18°C. In these cases, the 2° and 10°C groups were significantly

Table 7

Plasma water content ($\ell_{H_2O}/\ell_{plasma}$) in rainbow trout (Salmo gairdneri) acclimated to 2°, 10° and 18°C, and carp (Cyprinus carpio) acclimated to 2°, 16° and 30°C. Reported as the mean ± 1 standard error of the mean.

Species	Season	Acclimation Temperature			Signif. Diff.		
		2°/2°	16°/10°	30°/18°	2°/2°	16°/10°	30°/18°
Trout	Summer	0.945±0.005	0.957±0.004	0.938±0.007	N.S.	p<0.05	N.S.
	Winter	0.938±0.003	0.940±0.002	0.937±0.003	N.S.	N.S.	N.S.
					*N.S.	p<0.05	N.S.
Carp	Spring	0.947±0.004	0.943±0.002	0.945±0.005	N.S.	N.S.	N.S.

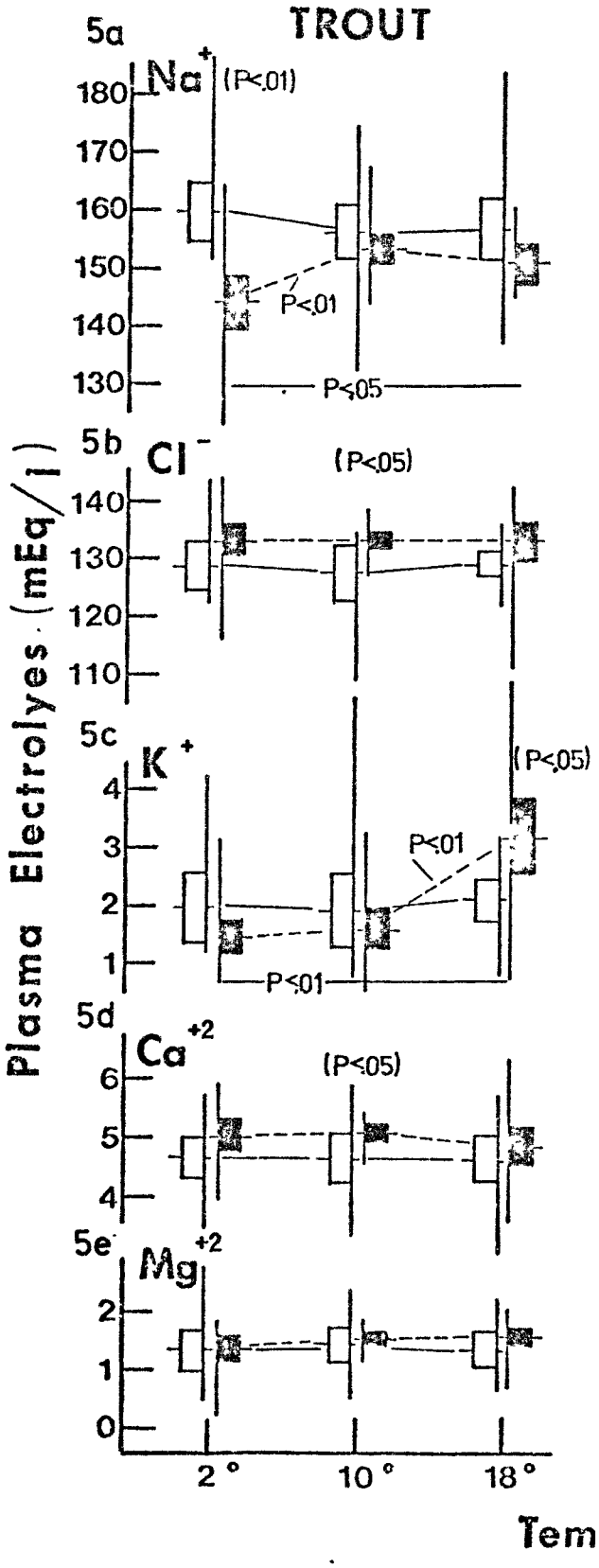
*significant differences between seasons

Figs. 5a to e. Plasma electrolyte levels ($mEq \cdot \ell^{-1}$, plasma) of rainbow trout acclimated to 2°, 10° and 18°C. The white bar indicates summer sampled trout while the black bar represents trout sampled in the winter.

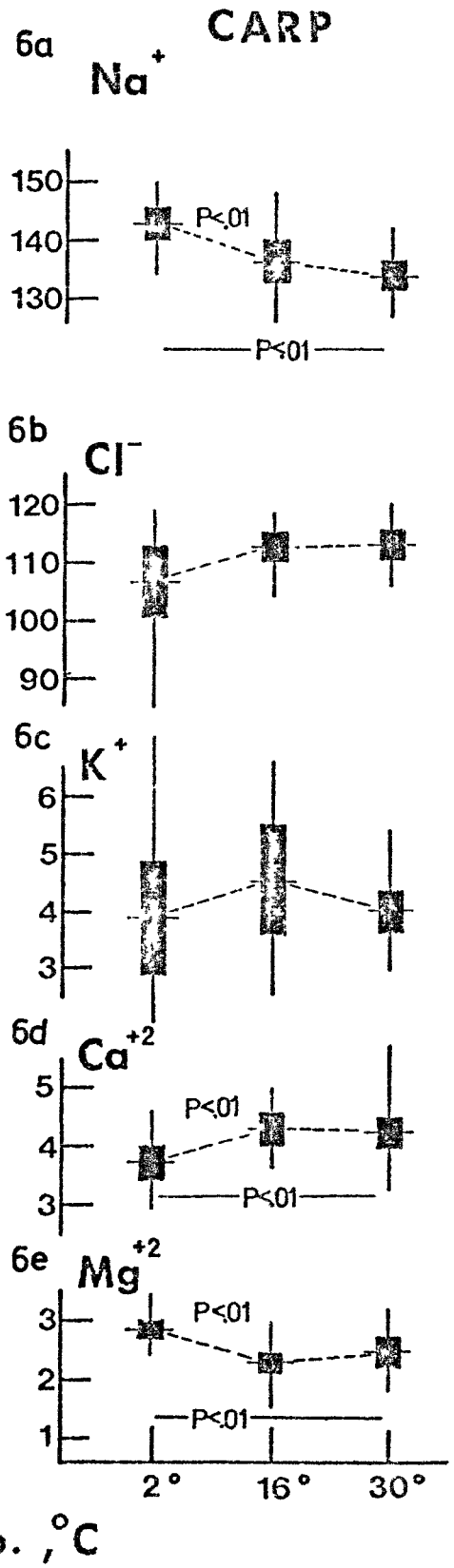
Figs. 6a to e. Plasma electrolyte levels ($mEq \cdot \ell^{-1}$, plasma) of carp acclimated to 2°, 16° and 30°C.

For both figures, the horizontal line represents the mean, vertical line the range and the vertical bar, the 95% confidence interval of the mean. In the case of trout, the level of significant difference between seasonal pair comparisons is shown in brackets while in both carp and trout the significant difference level between acclimation temperatures is placed between the groups being compared. The absence of a P value indicates that a comparison was not significant at the $P < 0.05$ level.

Fig·5


TROUT
5a
Na+
(P<.01)
180
170
160
150
140
130
P<.01
P<.05
5b
Cl⁻
(P<.05)
140
130
120
110
5c
K+
(P<.05)
4
3
2
1
P<.01
P<.01
5d
Ca+2
(P<.05)
6
5
4
5e
Mg+2
2
1
0
2°
10°
18°
Plasma Electrolyes (mEq/l)

Fig·6


CARP
6a
Na+
150
140
130
P<.01
P<.01
6b
Cl⁻
120
110
100
90
6c
K+
6
5
4
3
6d
Ca+2
5
4
3
P<.01
P<.01
6e
Mg+2
3
2
1
P<.01
P<.01
2°
16°
30°
Temp. ,°C

different at the $P < 0.01$ level, while comparisons between 2° and 18°C were significant at the $P < 0.05$ level. It should, however, be noted that in no case were increases greater than 9% encountered.

The most dramatic modifications occurred in plasma potassium. Although no significant changes were found in summer trout, winter specimens at 18°C had mean levels twice those present at 2° and 10°C (Fig. 5c). Significance in excess of the $P < 0.01$ level was seen in both comparisons.

As was the situation with thermal acclimation, only minor seasonal changes in plasma electrolyte levels were observed. Significant differences were found in certain isolated groups (i.e., Fig. 5a, Na^+, 2°; $P < 0.01$; Fig. 5b, Cl^-, 10°; Fig. 5c, K^+, 18°; Fig. 5d, Ca^{+2}, 10°; $P < 0.05$ in all cases), but differences in mean levels of Na^+, Cl^- and Ca^{+2} were minor. In the case of potassium, however, a large seasonal difference at 18°C was encountered. Potassium levels were approximately 50% higher in winter than in summer fish.

Carp

Carp had higher plasma levels of K^+ and Mg^{+2} than the trout. On the other hand, plasma concentrations of Na^+, Cl^- and Ca^{+2} were lower in this species (Fig. 6). Acclimation lead to variations in plasma electrolytes that were quite different from those observed in trout. For example, in trout, plasma Na^+ remained constant or increased at higher temperatures. In carp, Na^+ levels decreased with temperature to the extent where concentrations at 2°C were significantly different from those present at 16° and 30°C (Fig. 6a, $P < 0.01$ in both cases). As in trout there was no significant change in plasma chloride with acclimation (Fig. 6b). In contrast to winter trout, carp exhibited no significant change in plasma potassium with

temperature (Fig. 6c). Both divalent cations remained stable in trout while in carp plasma Ca^{+2} at 16° and 30°C was significantly higher than that found at 2°C (Fig. 6d, $P < 0.01$ in both cases) and Mg^{+2} at 16° and 30°C was significantly lower than at 2°C (Fig. 6e, $P < 0.01$ in both cases).

The plasma values presented in Figs. 5 and 6 agree well with seasonal and thermal changes reported in earlier studies. The lack of any large acclimatory or seasonal changes in rainbow trout Na^{+} and Cl^{-} (Figs. 5a and 5b) is consistent with the findings of Houston et al. (1968) who studied summer and winter populations of this species at six acclimation temperatures ranging between 3° and 21°C. In the study performed by Houston et al. (1968) all groups maintained mean sodium between 140 to 150 mEq/ℓ while chloride values were within 120 and 130 mEq/ℓ. In another study (Murphy and Houston, 1977) in which the influence of photoperiod-temperature interactions upon ion balance were examined in a winter population of rainbow trout, neither acclimation to temperatures between 2° and 18°C nor photoperiod produced dramatic changes in these electrolytes. Although some changes significant at the $P < 0.01$ level were noted, mean differences in plasma Na^{+} and Cl^{-} were always less than 10 mEq/ℓ (≃7% change in magnitude).

Existing evidence indicates that the cyprinid fishes are also able to maintain near constant levels of both plasma Na^{+} and Cl^{-} over broad temperature ranges. For example, in goldfish plasma concentrations of Na^{+} and Cl^{-} changed less than 10 mEq/ℓ at acclimation temperatures between 5° and 25°C (Prosser et al., 1970). Similarly, Houston et al. (1970) found that fall carp acclimated to four temperatures between 2° and 33°C maintained mean plasma Na^{+} levels within a 10 mEq/ℓ range. Acclimation of this species

to 7° and 27°C also failed to produce significant differences in sodium levels in winter sampled fish, and little change in plasma chloride in fall fish.

The changes in plasma Na^+ and Cl^- found in the present study in carp (Fig. 6) are consistent with the data presented by Houston et al. (1970). In the case of plasma chloride (Fig. 6b) no significant change with temperature occurred; although Na^+ was modified with temperature, the changes did not exceed 6%. The small decreases in plasma sodium observed with increases in temperature in the present study may be the result of increased sodium uptake by the erythrocytes at higher temperatures (see Fig. 8d).

Rainbow trout at a constant photoperiod, and sampled during summer and winter, exhibited similar plasma levels of sodium and chloride as trout sampled during the summer and winter with no photoperiod control (Houston et al., 1968). In view of this, as well as the lack of any large differences in the values obtained in the present study and that of Murphy and Houston (1977), it seems probable that season and photoperiod exert little influence upon either of these major plasma electrolytes.

The maintenance of near constant levels of Na^+, Cl^- and water in both rainbow trout and carp (Figs. 5 and 6, a and b; Table 7) was somewhat surprising. All pass more or less readily across the gills, and large concentration differences exist between the plasma and external medium. Increases in branchial ventilation and perfusion at higher temperatures enhance water electrolyte fluxes across the gills (Randall et al., 1972). Some appreciation of the homeostatic feat involved in maintaining constant levels of these electrolytes (a process that involves the renal excretion of water (McKay, 1974) and the active uptake of Na^+ and Cl^- at the gills)

can be gathered from water-electrolyte flux studies. For example, Motais and Isaia (1970) found that 60 g eels* acclimated to 5°C had a water turnover amounting to about 14% of the total body water per hour. Acclimation to temperatures of 25°C increased this nearly three times to a turnover of 41%·hr^{-1}. Likewise, Isaia (1972) found that goldfish replaced their body water at a rate of 25.2%·hr^{-1} at 5°C. When acclimated to 25°C water turnover was 1.35 times the total body water content per hour. Maetz (1972) found that the unidirectional sodium uptake of goldfish (presumed to counteract the loss of this ion) decreased by approximately 60% when fish acclimated to 16° were transferred to 6°C. Cameron (1976) found that transferring Arctic grayling, Thymallus arcticus, acclimated at 10° into 17°C water produced a 70% and 258% increase respectively in sodium and chloride uptake. Since it is likely that the same types of compensatory pressures were placed on both carp and trout within the present study, the ability of these fish to maintain mean plasma water levels within 3% (Table 7) and mean sodium and chloride changes within 10% (Figs. 5 and 6, a and b) constitutes a remarkable homeostatic feat.

Magnesium, calcium and potassium losses across the gills are probably small. In the case of these electrolytes there is little gradient between plasma and the external medium. In freshwater, Mg^{+2} concentrations particularly are often such that external levels of this ion are actually higher than those found in most fish plasmas (Hutchinson, 1957). Furthermore, potassium has been shown to be highly impermeable across the gill epithelium, a condition that likely exists with Mg^{+2} and Ca^{+2}.

The marked differences between summer and winter trout with respect to temperature-related changes in potassium (Fig. 5c) raises an interesting

* Anguilla anguilla

problem. In summer fish, no significant differences were found with temperature. However, in winter fish acclimation to 18°C was associated with a near doubling in potassium by comparison with the levels seen at 2° and 10°C. This anomaly was first observed by Houston et al. (1968). Summer sampled fish acclimated to temperatures between 3° and 21°C held potassium levels within 0.5 mEq/ℓ. Among winter fish, however, potassium rose from approximately 2.5 mEq/ℓ at 3°C to about 6 mEq/ℓ at 21°C. The significance of this is uncertain. It occurs only in winter trout at unseasonably high temperatures, and may indicate nothing more than a breakdown in regulation under anomalous temperature conditions. Furthermore, such a response may have deleterious effects. Heath and Hughes (1973) cited that high potassium levels possibly produce bradycardia in rainbow trout at high temperatures, forcing the heart into a diastole, or relaxed state.

In the rainbow trout plasma Ca^{+2} and Mg^{+2} did not vary with temperature or season (Figs. 5d and e). This is consistent with the limited data reported by Houston et al. (1968). Carp at 2°C, on the other hand, had higher levels of Mg^{+2} and lower Ca^{+2} concentrations than were present at 16° and 30°C (Figs. 6d and e, $P < 0.01$). It should, however, be pointed out that quantitatively these differences were small and their physiological significance, if any, is minimal, especially since reports on other thermal acclimatory studies of carp (Houston, 1973) indicate that there is little change in these electrolytes with temperature.

Thermoacclimatory Variations in Erythrocytic Electrolyte Levels

Electrolyte concentration within a cell may be altered by modifications in the partitioning of ions between the interior and exterior of the cell and by changes in cell water content. In both rainbow trout and carp,

Table 8

Erythrocytic water content (l_{H_2O}/l_{cells}) in rainbow trout (Salmo gairdneri) acclimated to 2°, 10° and 18°C, and carp (Cyprinus carpio) acclimated to 2°, 16° and 30°C. Reported as the mean ± 1 standard error of the mean.

Species	Season	Acclimation Temperature			Signif. Diff.		
		2°/2°	16°/10°	30°/18°	2°/2°	16°/10°	30°/18°
Trout	Summer	0.783±0.016	0.799±0.008	0.781±0.008	N.S.	N.S.	N.S.
	Winter	0.801±0.006	0.789±0.005	0.782±0.007	N.S.	N.S.	p<0.05
					*N.S.	N.S.	N.S.
Carp	Spring	0.763±0.009	0.748±0.010	0.756±0.006	N.S.	N.S.	N.S.

*Significant differences between seasons.

erythrocytic water content showed little change with thermoacclimation (Table 8). Consequently, observed electrolyte changes were attributed to changes in ion levels within the erythrocytes.

Representation of cellular electrolyte concentrations in terms of mEq/ℓ_{H_2O} is not accurate in all cases. It gives acceptable values for Na^+, K^+ and Cl^- concentrations, since these ions appear to occupy the total H_2O space of the cell (Gary-Bobo and Solomon, 1968). Concentrations of Ca^{+2} and Mg^{+2} present in cell water, however, are not depicted correctly since much of the Ca^{+2} and some of the Mg^{+2} is bound and therefore not dissolved in water (Bunn et al., 1971). Because of this the results reported in this section have been expressed in terms of mEq electrolyte per litre of cells. This is the more appropriate statement of cell ion levels since it makes no reference to the distribution within the cell. It should be noted, moreover, that neither the character of the variations observed nor differences in significance were found following the expression of values in mEq/litre of packed cells as compared to mEq/litre cell water. Thus, the interpretation of the results does not change with the units used. Appendices 15 to 19 provide values for red cell electrolyte levels expressed in mEq/ℓ_{H_2O}. These were required for the calculation of Nerst potentials, and in the discussion of some aspects of hemoglobin function. Thus, reference to ionic levels in mEq/ℓ_{H_2O} will be made when it is appropriate.

Rainbow Trout

Data for rainbow trout are summarized in Figs. 7a to e and in Appendices 10 to 12. These animals maintained erythrocytic levels of chloride (Fig. 7a), magnesium (Fig. 7b) and calcium (Fig. 7c) at relatively

Figs. 7a to e. Erythrocytic electrolyte levels ($mEq \cdot \ell^{-1}$, packed cells) of rainbow trout acclimated to 2°, 10° and 18°C. The white bar indicates summer sampled trout while the black bar represents trout sampled in the winter.

Figs. 8a to e. Erythrocytic electrolyte levels ($mEq \cdot \ell^{-1}$, packed cells) of carp acclimated to 2°, 16° and 30°C.

For both figures, the horizontal line represents the mean, vertical line the range and the vertical bar, the 95% confidence interval of the mean. In the case of trout the level of significant difference between seasonal pair comparisons is shown in brackets while in both carp and trout the significant difference level between acclimation temperatures is placed between the groups being compared. The absence of a P value indicates that a comparison was not significant at the $P < 0.05$ level.

Fig. 7

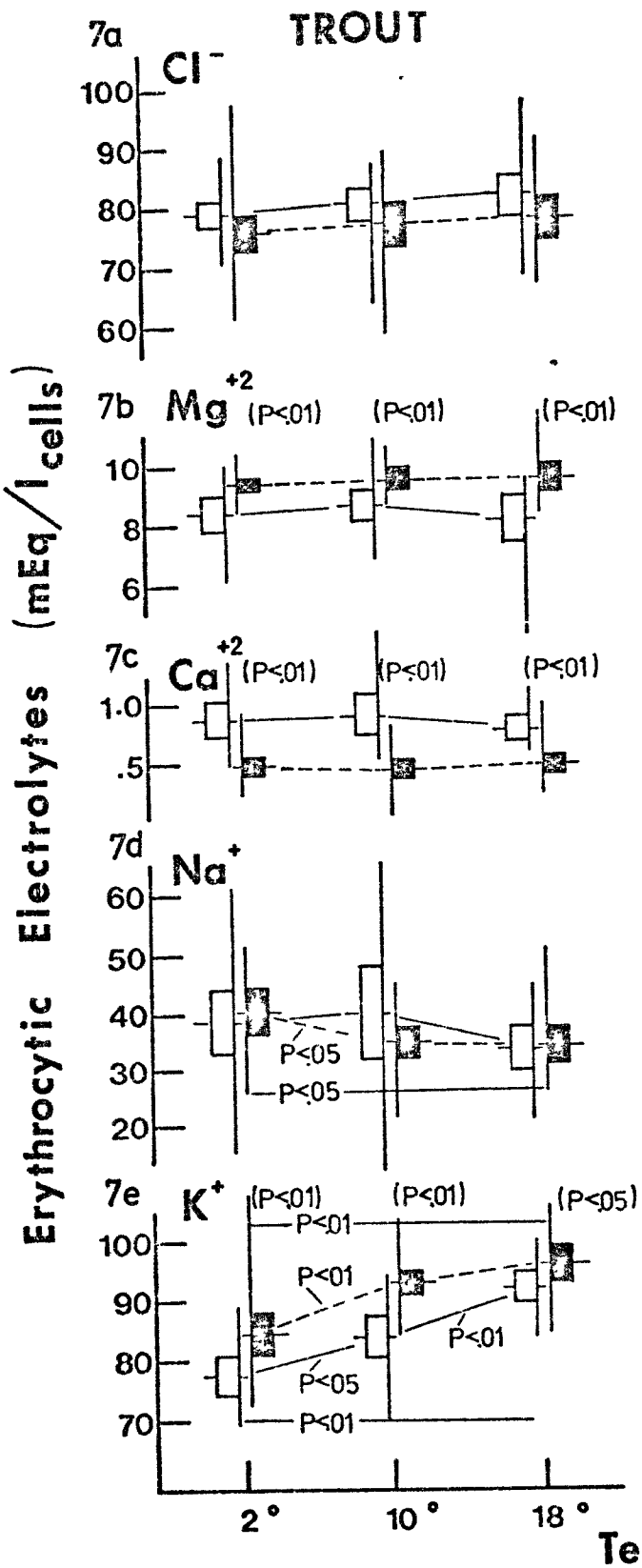
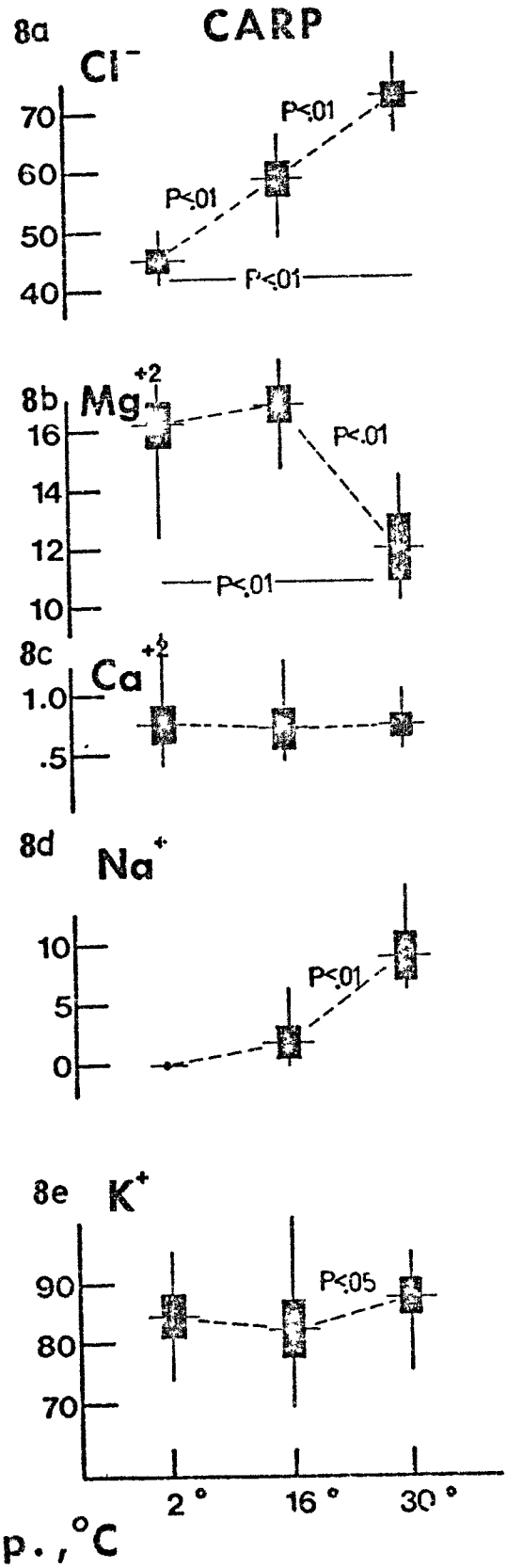

TROUT
7a
Cl⁻
100
90
80
70
60
7b
Mg⁺²
(P<.01)
(P<.01)
(P<.01)
10
8
6
7c
Ca⁺²
(P<.01)
(P<.01)
(P<.01)
1.0
.5
7d
Na⁺
60
50
40
30
20
P<.05
P<.05
7e
K⁺
(P<.01)
(P<.01)
(P<.05)
P<.01
P<.01
P<.01
P<.05
P<.01
100
90
80
70
2°
10°
18°
Temp., °C
Erythrocytic Electrolytes (mEq/l cells)

Fig. 8


CARP
8a
Cl⁻
70
60
50
40
P<.01
P<.01
P<.01
8b
Mg⁺²
16
14
12
10
P<.01
P<.01
8c
Ca⁺²
1.0
.5
8d
Na⁺
10
5
0
P<.01
8e
K⁺
90
80
70
P<.05
2°
16°
30°

constant values over the range of acclimation temperatures used. No significant changes with temperature were found. Erythrocytic sodium (Fig. 7d) on the other hand, was lower at 18° than at 2°C in both summer and winter test groups. In the case of winter fish, significant ($P < 0.05$) decreases in sodium were observed between 2° and 10°C, and 2° and 18°C. Summer fish maintained sodium levels constant between 2° and 10°C, but exhibited lower levels at 18°C. The latter decrease approached significance (i.e., it was significant at the $P < 0.10$, but not at the $P < 0.05$ level). Acclimation affected potassium levels more markedly (Fig. 7e). Summer fish were characterized by near linear increases in cell potassium with increasing temperature; the differences encountered being significant at each successive acclimation level. Winter fish exhibited a similar trend. Values for 10° and 18°C acclimated fish were significantly higher than the levels seen in 2°C fish.

Summer and winter groups exhibited some differences in electrolyte levels, although these did not alter the nature of the thermal response. Indeed, only in the case of chloride (Fig. 7a) and sodium (Fig. 7d) were significant seasonal variations absent. Levels of magnesium (Fig. 7b) and potassium (Fig. 7e) were significantly lower ($P < 0.01$ in nearly all cases) and calcium levels higher ($P < 0.01$ in all cases) in summer as compared to winter trout at all acclimation temperatures. It should be pointed out, however, that although significant seasonal differences existed, the actual magnitudes of these differences were again quite modest, and may have little real physiological relevance.

Carp

Data for carp are summarized in Figs. 8a to e and Appendices 13 and 14.

Carp erythrocytes were characterized by somewhat lower levels of chloride (Fig. 8a) and sodium (Fig. 8d), and higher levels of magnesium (Fig. 8b) than the erythrocytes of trout. Calcium (Fig. 8c) and potassium (Fig. 8e) levels were nearly identical in both species.

Major differences between the species were found with respect to the magnitude and nature of the electrolyte changes accompanying acclimation. Chloride and magnesium, which are important modulators of hemoglobin-oxygen affinity, were relatively stable in rainbow trout, but exhibited marked variation in carp. Chloride increased in near-linear fashion with temperature. Carp at 30°C had 1.6 times the chloride level found at 2°C (Fig. 8a). Differences significant at the $P < 0.01$ level existed between all pairs of acclimation temperatures. At 30°C magnesium was significantly reduced ($P < 0.01$), and was 25 to 30% below the values seen at 2° and 16°C. As was the case with rainbow trout, calcium levels were remarkably stable; mean values, remained within ±0.05 mEq/ℓ_{cells} between acclimation temperatures (Fig. 8c).

Erythrocytic sodium exhibited interesting thermoacclimatory variations (Fig. 8d). At 2°C only one out of the 13 carp sampled gave evidence of any sodium within the erythrocyte (Appendix 14, CP-27, 0.86 mEq/ℓ_{cells}). Among animals held at 16°C, nine showed appreciable amounts of sodium, while three fish apparently had none (Appendix 14). At 30°C, erythrocytic sodium was sharply elevated, and all fish sampled had at least some sodium (mean level 8.77±0.843 mEq/ℓ_{cells}).

Variations in potassium with temperature also differed in the two species. In trout potassium increased sharply at higher acclimation temperatures while in carp this ion remained reasonably constant with temperature (Fig. 8e). At 30°C cell potassium significantly increased

above levels found at 16°C; however, the actual mean concentration change was not great.

These electrolytes serve three primary functions in the erythrocyte. They are involved in volume regulation, as effectors of metabolism and modulators of hemoglobin-oxygen affinity. Taking into account the adaptive requirements of thermoacclimation, some speculations upon the consequences of the changes observed in carp and trout would seem to be warranted.

Erythrocytic Electrolytes and Volume Regulation

One of the more consistent hematological features of thermal acclimation is a greater or lesser reduction,but seldom an increase in mean erythrocytic volume at acclimation to higher temperatures. The adaptive value of this in terms of red cell oxygen loading was noted earlier. Among higher vertebrates the adjustment of erythrocyte volume normally involves the transfer of ions and active sodium transport plays a particularly important role in this process (Macknight and Leaf, 1977). To examine the involvement of electrolytes in the regulation of red cell volume in fishes, values for mean erythrocytic volume were regressed against all individual electrolytes and electrolyte summations on a per temperature basis and, in the case of trout, for the two seasonal groups considered. In no case was the anticipated positive correlation between cell volume and cell electrolyte levels found. Similarly, there was little correlation with plasma electrolyte levels. Plots of MEV against Σ cellular cations and chloride (Fig. 9) for summer trout and carp, indicated that cell volume actually decreased with increased electrolyte levels. This relationship is clearly incompatible with the hypothesis that either species alters MEV through manipulation of cellular ion levels, and points to the likelihood that cell volume control

Fig. 9. Linear regressions of mean erythrocytic volume against the sum of the erythrocytic cations (Na^+, K^+, Mg^+ and Ca^{+2}) plus chloride for carp acclimated to 2°, 16° and 30°C (Fig. 9a) and summer rainbow trout acclimated to 2°, 10° and 18°C (Fig. 9b). Shown is the best fitting line and its equation, the sample size (N), the coefficient of correlation (c.c.) and the significance level of the correlation ($P < 0.05$, $P < 0.01$). Additional information describing the plots can be found in Appendix 37.

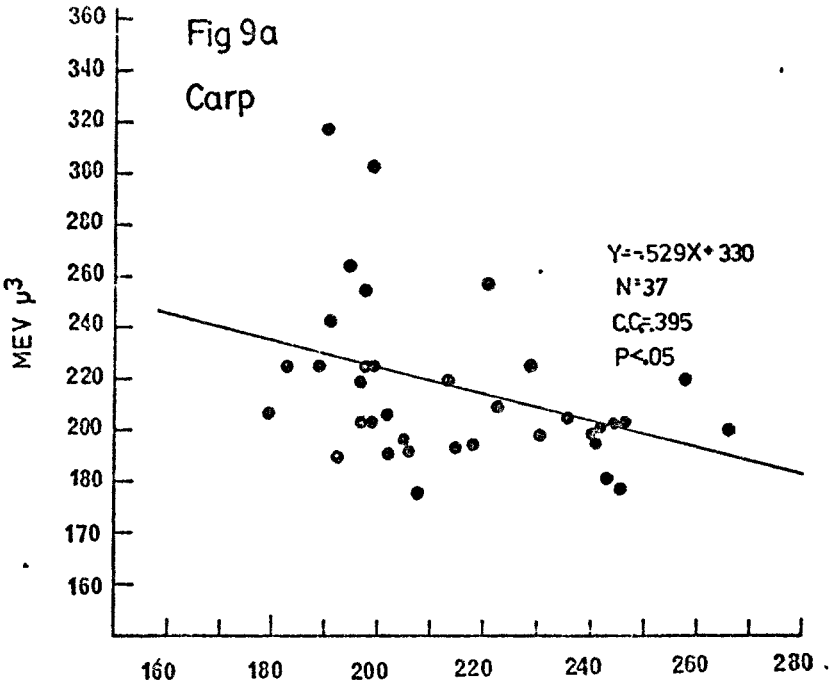


Fig 9a
Carp
Y=-.529X+330
N=37
C.C=.395
P<.05
MEV μ3
360
340
320
300
280
260
240
220
200
180
170
160
160
180
200
220
240
260
280
SUM OF CATIONS + CHLORIDE
mEq/l H2O

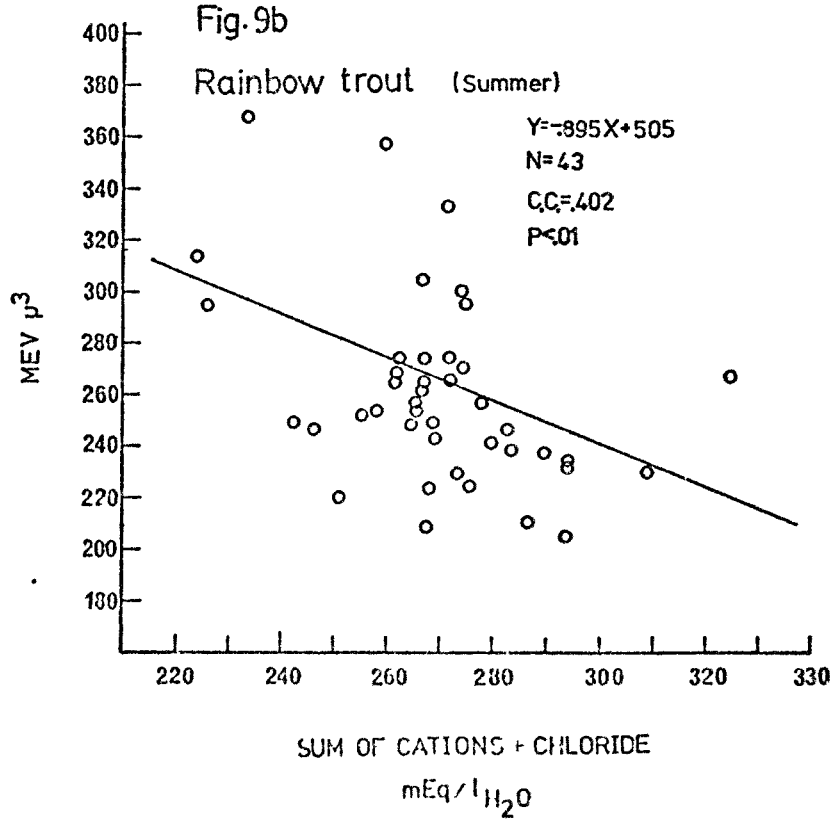


Fig. 9b
Rainbow trout (Summer)
Y=-.895X+505
N=43
C.C=.402
P<.01
MEV μ3
400
380
360
340
320
300
280
260
240
220
200
180
220
240
260
280
300
320
330
SUM OF CATIONS + CHLORIDE
mEq/l H2O

may involve other osmotically active solutes.

Such a system has, in fact, been identified in the flounder* (Fugelli, 1967). These animals undergo a 20% decrease in plasma osmolarity when migrating from salt to fresh water. If the erythrocytes of saltwater specimens are placed in plasma diluted by 20% they initially swell. However, volume is then regulated through the export of as yet unidentified ninhydrin-positive substances. Cell volume regulation by the adjustment of organic solute levels is not uncommon. Nerve cells from the freshwater crab, Eriochoir, increase free amino acids in concentrated saline (Gilles and Schonffeniels, 1969). In fact, many marine invertebrate animals osmoregulate when faced by varying salinity conditions by modifying cellular concentrations of nitrogenous organic molecules (reviewed by Prosser, 1973).

The control of erythrocyte volume through the adjustment of organic rather than inorganic ion levels would seem to offer distinct advantages. For example, electrolytes are known to affect metabolism (Bygrave, 1967) as well as the oxygen binding properties of hemoglobin. Intracellular electrolyte levels could be modified to suit these functions, rather than the functions relying on the particular ionic environment needed to regulate volume.

The independence of electrolyte concentrations and cell volumes may be of benefit in other ways. In carp particularly, and also in summer rainbow trout, the erythrocytic Σ cations as well as the mean Σ cations + chloride increased with acclimation temperature. These parameters either decreased slightly, or remained constant in plasma (Tables 9A and B). Such a situation might be expected to produce osmotic increases in cell volume. In fact, as Table 9C indicates, mean erythrocytic volume decreases

* Pleuronectes flesus

Table 9

The Σ cations and the Σ cations + chloride present in the erythrocytes and plasma as well as the mean erythrocytic volume in carp (Cyprinus carpio) acclimated to 2°, 16° and 30°C and summer rainbow trout (Salmo gairdneri) acclimated to 2°, 10° and 18°C. Reported as the mean ± 1 standard error of the mean.

A. Erythrocytic Electrolytes

Species	Measurement	Acclimation Temperature 2°/2°	16°/10°	30°/18°	Signif. Diff. 2°/2°	16°/10°	30°/18°
Carp	Erythrocytic Σ cations mEq/ℓ H_2O	134±2.43	136±4.15	145±3.20	N.S.	N.S.	p<0.05
R. Trout (Summer)		163±4.49	166±3.46	173.0±1.98	N.S.	N.S.	p<0.05
Carp	Erythrocytic Σ cations + chloride mEq/ℓ H_2O	194±2.23	214±4.44	241±4.12	p<0.01	p<0.01	p<0.01
R. Trout (Summer)		266±6.39	267±5.00	278±3.94	N.S.	N.S.	N.S.

B. Plasma Electrolytes

Carp	Plasma Σ cations mEq/ℓ plasma	154±1.67	147±1.40	145±1.24	p<0.01	N.S.	p<0.01
R. Trout		168±2.85	164±2.16	162±2.16	N.S.	N.S.	N.S.
Carp	Plasma Σ cations + chloride mEq/ℓ plasma	261±3.51	261±2.24	258±2.13	N.S.	N.S.	N.S.
R. Trout (Summer)		299±3.65	291±4.36	292±2.15	N.S.	N.S.	N.S.

C. Mean Erythrocytic Volume

Carp	Erythrocyte Volume μ^3	226±9.94	222±8.36	202±5.53	N.S.	N.S.	p<0.05
R. Trout (Summer)		277±9.83	252±6.31	255±12.1	p<0.05	N.S.	N.S.

with increased temperatures. Hence, a situation where fish acclimated to higher temperatures and oxygen need having larger erythrocytes, with decreased oxygen diffusion capabilities (Holland, 1970) is avoided and, in fact, somewhat improved by a decrease in cell volume.

Erythrocytic Electrolytes and Metabolism

The ionic modifications observed with thermal acclimation would almost certainly affect erythrocytic metabolism, although at present only the most general speculations as to what these effects might be can be made. Bygrave (1967), in discussing metabolic control by ions, divided the major cations into two major groups. Magnesium and potassium were considered to be stimulators of enzyme activity, while calcium and sodium had the opposite effect. Glycolytic phosphate transferring enzymes were found to be particularly sensitive to electrolyte modulation. In this case magnesium binds either to an adenine nucleotide or to the enzyme itself and forms a ternary enzyme-Mg^{+2}-substrate complex. Increasing magnesium in preparations containing pure enzymes tends to increase their activity. Among the glycolytic enzymes falling into this category are pyruvate kinase, fructokinase and phosphofructokinase. In the case of other enzymes, such as enolase, magnesium binds to the enzyme and activates catalysis by producing a conformational change. In the instance of *E. coli* phosphofructokinase, magnesium affects enzyme conformation and also acts as a substrate activator. In situations where Mg^{+2} forms ternary complexes, and especially in the case of pyruvate kinase, potassium, although it plays no role in phosphate transfers, controls the ternary enzyme structure, acting as an activator.

All of the enzymes mentioned are inhibited by calcium; largely through

competition of calcium for magnesium binding sites. Sodium exhibits a co-operative effect with calcium. Calcium also inhibits glycolysis in another way. Inhibition of pyruvate kinase results in a buildup of phosphoenolpyruvate which, by mass action, promotes the accumulation of 3-phosphoglycerate. Both of these inhibit fructokinase and hexokinase, and limit carbon flow through the glycolytic chain. Calcium can also uncouple glycolysis from the citrate cycle by inhibiting pyruvate carboxylase, a mitochondrial enzyme leading pyruvate into the cycle.

On the basis of data presented by Bygrave (1967), one might expect that increases in the cellular potassium and decreases in sodium (Figs. 7e and d) would favor some increase of the glycolytic metabolism in summer and winter trout acclimated to higher temperatures. Since winter trout were characterized by higher erythrocytic magnesium (Fig. 7b) and potassium (Fig. 7e) and lower calcium concentrations (Fig. 7c) than summer fish, metabolic activity should also be favored in the former group.

Carp differed from trout in that higher acclimation temperatures were associated with major decreases in magnesium (Fig. 8b), and substantial increases in sodium content (Fig. 8d). Under these circumstances lower acclimation temperatures should favor glycolytic activity. In this respect, data on carp and seasonal differences between summer and winter trout are consistent with the results of enzyme assays, amino acid incorporation studies and studies of metabolic pathways indicating that intermediary metabolism is reorganized so that the metabolic capabilities of cold-acclimated fish are increased over warm-acclimated animals (Caldwell, 1969; Dean, 1969; Hochachka, 1973). Such adaptations are presumed to represent adaptive strategies offsetting the direct effects of temperature

reductions on enzyme activity.

Although it is interesting to speculate upon the effects electrolytes may have on erythrocyte metabolism, it should be pointed out that few studies have been made on fish erythrocytes. Until the characteristics of the erythrocytic metabolism are defined it is impossible to definitely say that the patterns of ionic inhibition and stimulation outlined by Bygrave (1967) hold true for fish red cells.

Potential Electrolyte Modulation of Hemoglobin Function

As indicated in the literature review, chloride, magnesium and, to a lesser extent, calcium play important roles in the modulation of hemoglobin-oxygen affinity, rivalling the organophosphates in modulatory importance (Rossi-Fanelli et al., 1961b; Antonini et al., 1962; Benesch et al, 1969; Bunn et al, 1971; Gillen and Riggs, 1972; Kaloustian and Poluwich, 1975; Rollema et al, 1975).

Chloride, at appropriate concentrations and pH, affects hemoglobin by increasing the number of Bohr protons released between the oxy- and deoxy-states, and in this way raises P_{50} values (i.e., decreases Hb-O_2 affinity). In some cases it also increases co-operativity. Magnesium and calcium bind to ATP (the principal teleostean organophosphate modulator of Hb-O_2 affinity), and prevent its action upon hemoglobin. In this way they increase hemoglobin-oxygen affinity.

In Figures 10a to c and 11a to c, erythrocytic chloride, magnesium and calcium have been plotted as ratios with hemoglobin (mEq ion:mM Hb) for rainbow trout and carp. In trout no significant temperature changes in Cl:Hb (Fig. 10a), Mg:Hb (Fig. 10b) or Ca:Hb (Fig. 10c) were found. At all acclimation temperatures, however, summer fish had higher mean Cl^-:Hb,

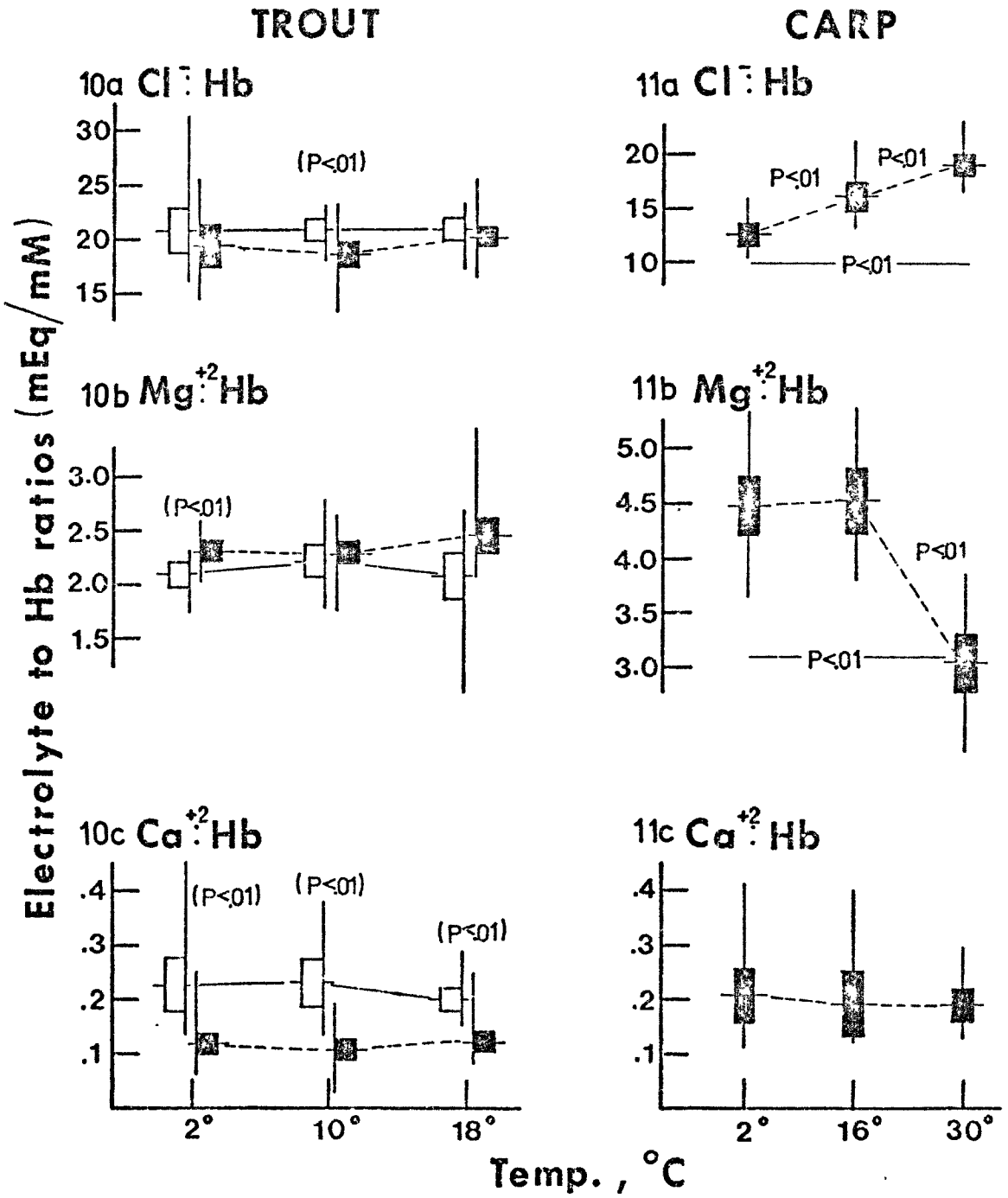
Fig. 10a to c. Erythrocytic electrolyte to hemoglobin ratios (mEq electrolyte:mM Hb) of rainbow trout acclimated to 2°, 10° and 18°C. The white bar indicates summer sampled trout while the black bar represents trout sampled in winter.

Fig. 11a to c. Erythrocytic electrolyte to hemoglobin ratios (mEq electrolyte:mM Hb) of carp acclimated to 2°, 16° and 30°C.

For both figures, the horizontal line represents the mean, vertical line the range and the vertical bar the 95% confidence interval of the mean. In the case of trout the level of significant difference between seasonal pair comparisons is shown in brackets while in both carp and trout the significant difference level between acclimation temperatures is placed between the groups being compared. The absence of a P value indicates that a comparison was not significant at the $P < 0.05$ level.

Fig·10

Fig·11


TROUT
CARP
10a Cl⁻ : Hb
11a Cl⁻ : Hb
10b Mg⁺² : Hb
11b Mg⁺² : Hb
10c Ca⁺² : Hb
11c Ca⁺² : Hb
Electrolyte to Hb ratios (mEq/mM)
(P<.01)
P<.01
2°
10°
18°
16°
30°
Temp., °C

Ca^{+2}:Hb and lower Mg^{+2}:Hb ratios than was the case in winter fish. In the instance of Cl^{-}:Hb and Mg^{+2}:Hb differences were small. Significant differences ($P < 0.01$ in both cases) were encountered only at 10°C (Cl^{-}:Hb) and 2°C (Mg^{+2}:Hb). Ca^{+2}:Hb ratios for summer trout were significantly higher ($P < 0.01$) at all acclimation temperatures than those of winter fish.

Unlike rainbow trout, carp exhibited large changes in electrolyte: hemoglobin ratios with acclimation. The Cl^{-}:Hb ratio increased with acclimation temperature (Fig. 11a); significant changes occurring between all acclimation temperatures. Mg:Hb ratios decreased significantly at 30°C by comparison with 2° and 16°C (Fig. 11b). The magnitudes of these changes were relatively large. For example, the Cl^{-}:Hb ratio at 30°C was 1.5 times that at 2°C. In the case of the Mg^{+2}:Hb ratios, the mean value found at 30°C was only 3/4 of those at 2° and 16°C. As in rainbow trout Ca^{+2}:Hb ratios were little altered by acclimation (Fig. 11c).

From these results it appears that the rainbow trout has only limited ability to adaptively modify the microenvironment of hemoglobin. Even in those instances where seasonal differences in electrolyte:hemoglobin ratios occurred, the higher Ca^{+2}:Hb (Fig. 10c) and Cl^{-}:Hb (Fig. 10a) and lower Mg^{+2}:Hb (Fig. 10b) levels in summer over winter samples were so small as to be of doubtful physiological significance. Furthermore, the increased Hb-O_2 affinity that might be produced by higher Ca^{+2}:Hb ratios in summer fish would be opposed by a reduction in affinity produced by the Mg:Hb and Cl:Hb changes in this group.

Carp, on the other hand, exhibited a marked increase in cell chloride and chloride to hemoglobin ratio at higher acclimation temperatures (Figs. 8a and 11a respectively), coupling this with a sharp reduction in magnesium

content and Mg^{+2}:Hb ratio at 30°C (Fig. 8b and 10b respectively). These variations would strongly favor reductions in hemoglobin-oxygen affinity at higher acclimation temperatures.

Although increases in chloride would directly reduce affinity, the magnitude of the modulatory effect is highly dependent upon co-existing conditions of pH. The pH of the carp red blood cell has not yet been measured. Plasma pH, however, varies inversely with temperature according to the relationship, pH = 8.08 - 0.0129 T/°C) (Rahn, 1971; personal communication to A. H. Houston). Accordingly, carp acclimated to 2°, 16° and 30°C would have plasma pH values of 8.06, 7.88 and 7.70 respectively. Wood and Johansen (1973) found that erythrocytic pH in eel was related to that of plasma, but was between 0.2 to 0.4 pH units below the plasma value. If the carp erythrocyte approximates this situation, red cell pH values of about 7.8, 7.6 and 7.4 would be expected at 2°, 16° and 30°C respectively. If such is the case, carp red cell pH and chloride as expressed in mEq/ℓ_{H_2O} fall well within the ranges (pH 7.0 to 8.5; chloride 0-100 mM/ℓ) reported by Rollema et al. (1975) to be particularly effective in increasing the release of Bohr protons, reducing hemoglobin-oxygen affinity (Table 10). Similarly, if a portion of erythrocytic magnesium is bound, cellular levels of this ion (Table 10) and predicted pH conditions are likely comparable to those at which magnesium interaction with ATP is maximal (0 to 14 mEq/ℓ, pH 7.3; Bunn et al., 1971). Thus ionically induced reductions in hemoglobin oxygen affinity at higher temperatures are highly probable.

Furthermore, reduction in pH and increases in temperature themselves lead to reduction in the Hb-O_2 affinity, and would add to ion-induced effects. Indeed, temperature, pH and electrolyte variations may well

Table 10

The levels of Mg^{+2} and Cl^{-} present per litre of cell water in carp (Cyprinus carpio) acclimated to 2°, 16° and 30°C, and rainbow trout (Salmo gairdneri) acclimated to 2°, 10° and 18°C. Reported as the mean ± 1 standard error of the mean.

Species	Measurement	Season	Acclimation Temperature 2°/2°	16°/10°	30°/18°	Signif. Diff. 2°/2°	16°/10°	30°/18°
Carp	Cl^{-} (mEq/l H_2O)	Spring	60.0±1.05	77.8±1.89	96.0±1.62	p<0.01	p<0.01	p<0.01
R. Trout		Summer	100±2.20	101±1.61	105±2.39	N.S.	N.S.	N.S.
R. Trout		Winter	94.5±1.89	97.2±2.12	99.0±2.19	N.S.	N.S.	N.S.
Carp	Mg^{+2} (mEq/l H_2O)	Spring	21.3±0.620	22.5±0.566	15.7±0.651	N.S.	p<0.01	p<0.01
R. Trout		Summer	10.7±0.487	10.8±3.15	10.5±0.482	N.S.	N.S.	N.S.
R. Trout		Winter	11.6±0.171	12.1±0.241	12.2±0.276	N.S.	N.S.	N.S.

interact synergistically rather than additively to promote reductions in Hb-O_2 affinity. In the case of pH, at least, a double effect is probable. Increases in hydrogen ion concentration with temperature would be expected to affect hemoglobin directly (Bohr effect). In addition, Mg^{+2}-ATP binding is reduced as pH decreases and this enhances organophosphate availability for modulatory purposes, and decreases Hb-O_2 affinities to an even greater extent (Bunn et al., 1971).

Table 10 indicates that the mean Cl^- levels in the rainbow trout red cell are above those of carp, while magnesium concentrations are lower. Only at 30°C did erythrocytic Cl^- and Mg^{+2} levels in carp approach those of rainbow trout. It will be recalled that the latter maintained relatively constant levels of these electrolytes over the 2° to 18°C range. Since Rollema et al. (1975) found that the maximal effect of chloride in reducing Hb-O_2 affinities occurs below 100 mM/ℓ, the trout erythrocyte, which contains approximately 100 mM/ℓ Cl^- and low Mg^{+2} levels, could be considered to have an ionic environment preadapted to low Hb-O_2 affinities over the entire temperature range tolerated by this species. This is consistent with the findings of Weber et al. (1976b) who indicated that whole blood oxygen affinities (when tested under constant temperature) changed little in thermally-acclimated trout. In addition, Prosser (1973) has summarized P_{50} values for a variety of teleost and concludes that the whole blood of rainbow trout has lower Hb-O_2 affinities than are seen in most other species of fish (Table 11).

Environmental Significance of the Electrolyte Changes in Carp and Rainbow Trout Erythrocytes

The findings of the present study, which suggest that conditions in

Fig. 12. The maximal oxygen solubility and corresponding oxygen tensions of water at 1 atmosphere pressure under various temperature conditions. The data used to plot O_2 solubility was taken from standard tables while O_2 tensions were calculated as outlined by Pierce *et al.* (1973).

Table 11. P_{50} values for the whole blood of various species of fish under representative *in vivo* temperature, CO_2 tension conditions (from Prosser, 1973).

Fig·12

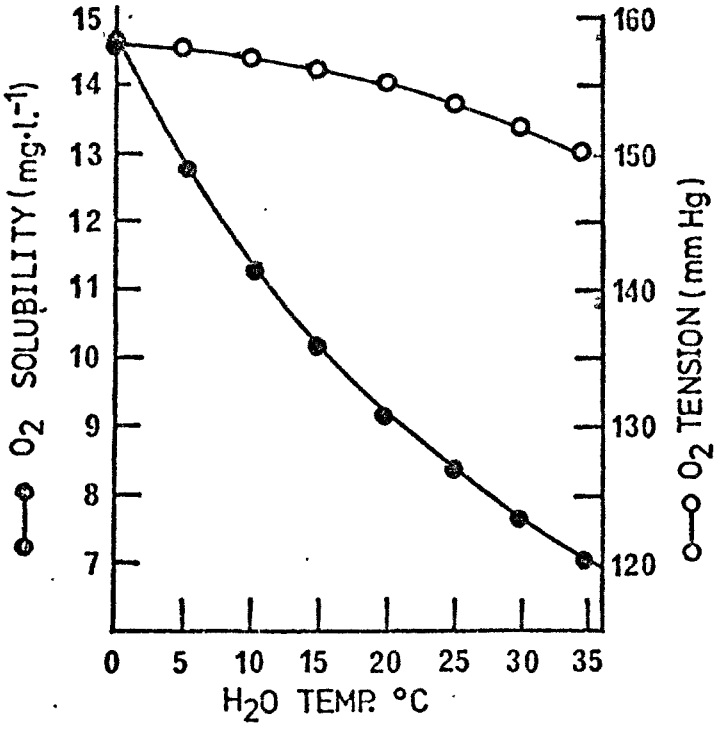


Table 11

Animal	P_{50} *Po_2 in mm Hg*	*O_2 capacity ml O_2/100 ml blood*
Fishes		
pike		9.0
goldfish[6]		10.7
rainbow trout[29]	30 (7–8 mm CO_2, 15°C)	
salmon	19 (1–2 mm CO_2, 15°C)	
brook trout[29]	7 (1 mm CO_2, 0°C)	
	21 (1 mm CO_2, 25°C)	
electric eel	12 (0 CO_2, 18°C)	19.7
	14 (7.4 mm CO_2)	
Japanese eel[212] Hb E_1	2.1 (7.0 mm CO_2, 20°C)	2.1
Hb E_2	14 (7.0 mm CO_2, 20°C)	1
Trematomus	21.5 (0.1 mm CO_2, −1.5°C)	5.3–7.7
mackerel	16 (1 mm CO_2, 20°C)	15.7
carp	5 (1–2 mm CO_2, 15°C)	12.5
catfish	1.4 (0–1 mm CO_2, 15°C)	13.3
Bagrus (deep FW)	1.5 (0 mm CO_2)	
Lates (high O_2 FW)	17 (0 mm CO_2)	
Neoceratodus[116]	11 (3.5 mm CO_2)	7.7
Protopterus[116]	10 (6 mm CO_2, 25°C)	6.8
Lepidosiren[95]	10.5 (6 mm CO_2, 23°C)	4.9–6.8
Symbranchus[95]	5–6	14.7
Scyliorhynus[150]	12 (7.0 mm CO_2, 17°C)	4.5
Squalus[115]	17 (0.5 mm CO_2, 11°C)	4.35
Squalus adult	16.4 (7.3 mm CO_2, 12°C)	
fetus	10.6	
Lampetra planeri[9] adult	0.77 (Hb 5 mg/ml)	
larva	0.37	
Eptatretus[123] Hb	2–4	1
Myxine[123] Hb	8 (7.5 mm CO_2, 25°C)	1
Petromyzon[123]	14–20	1.2
Ichthyomyzon[123] adult	17–19 (25°C)	1.0

the ionic microenvironment of hemoglobin are consistent with low hemoglobin oxygen affinity in trout at all temperatures, and reduced affinity in warm acclimated carp are at variance with the conclusions of some earlier work. As indicated in the Literature Review experiments performed on fish exposed to hypoxic conditions suggest that erythrocytic organophosphate levels are adaptively reduced to increase Hb-O_2 affinities, and therefore facilitate O_2 loading at the gills. Eaton (1974), Grigg (1969) and Powers (1974) have concluded that since oxygen solubility decreases with increased temperatures fish are faced with essentially hypoxic circumstances in which increases in Hb-O_2 affinity would be of adaptive value. Their experiments do not, however, confirm that under in vivo conditions fish respond in such a manner. Indeed, the data reported by Grigg (1969) in support of this contention can actually be interpreted as indicating that under in vivo cellular conditions oxygen affinity would be reduced rather than increased at higher temperatures.

While it is true that oxygen solubility decreases with increasing temperature, this is partially offset by increases in activity.* Because of this, oxygen tensions change little as temperature increases (Fig. 12). Consequently, the extent of hemoglobin saturation with oxygen at the gills depends more upon factors such as blood flow, ventilation and gill exchange area than it does upon O_2 solubility in the water.

Fish hemoglobins typically have much higher oxygen affinities than are seen in mammalian systems. Those of salmonids are among the lowest known in fishes (Table 11), and it might be expected that these species would have some difficulty in saturating their hemoglobins under hypoxic circumstances or at high temperatures. The results of studies by Heath and Hughes (1973) on rainbow trout are of particular interest in this context. Heath

* thermal movement of O_2

and Hughes (1973) rapidly increased the water temperature of rainbow trout from 14° to 27°C. This produced a situation in which (1) the effect of temperature reduces Hb-O_2 affinity; (2) due to the lack of time, acclimation cannot occur and ventilation, perfusion adjustments to increase O_2 uptake are least efficient; (3) cardiac output increases and blood has a reduced contact time with the respiratory surface; and (4) oxygen solubility decreases. All of these might be expected to produce decreases in oxygen content of blood leaving the gills. Surprisingly, however, only minor decreases in arterial oxygen content took place. On the other hand, venous oxygen content decreased 62% of arterial oxygen values at 15°C, to near zero at 23°C, i.e., venous blood unloaded almost all of its oxygen. It is at the venous system that a hemoglobin with reduced oxygen affinity is most beneficial.

Hemoglobin-oxygen affinity in carp is much higher than that in trout; 50% saturation of whole blood can be achieved at oxygen tensions as low as 5 mm Hg (Table 11), and 95% saturation at 25 mm Hg, P_{O_2} (Randall, 1970). In fact, the Hb-O_2 affinity of this species is so high that it would have twice the oxygen tensions needed for 95% saturation if it was present within the human venous system where oxygen unloading takes place. It would be of little advantage to the carp to increase affinity at higher temperatures, especially since its gill structure and ventilatory systems ensure more efficient hemoglobin oxygenation than is the case in trout. On the other hand, tight oxygen binding to hemoglobin could impede oxygen release. The type of modulation suggested by the changes in red cell chloride and magnesium, namely that Hb-O_2 affinity should decrease with temperature in carp, thus seems highly appropriate.

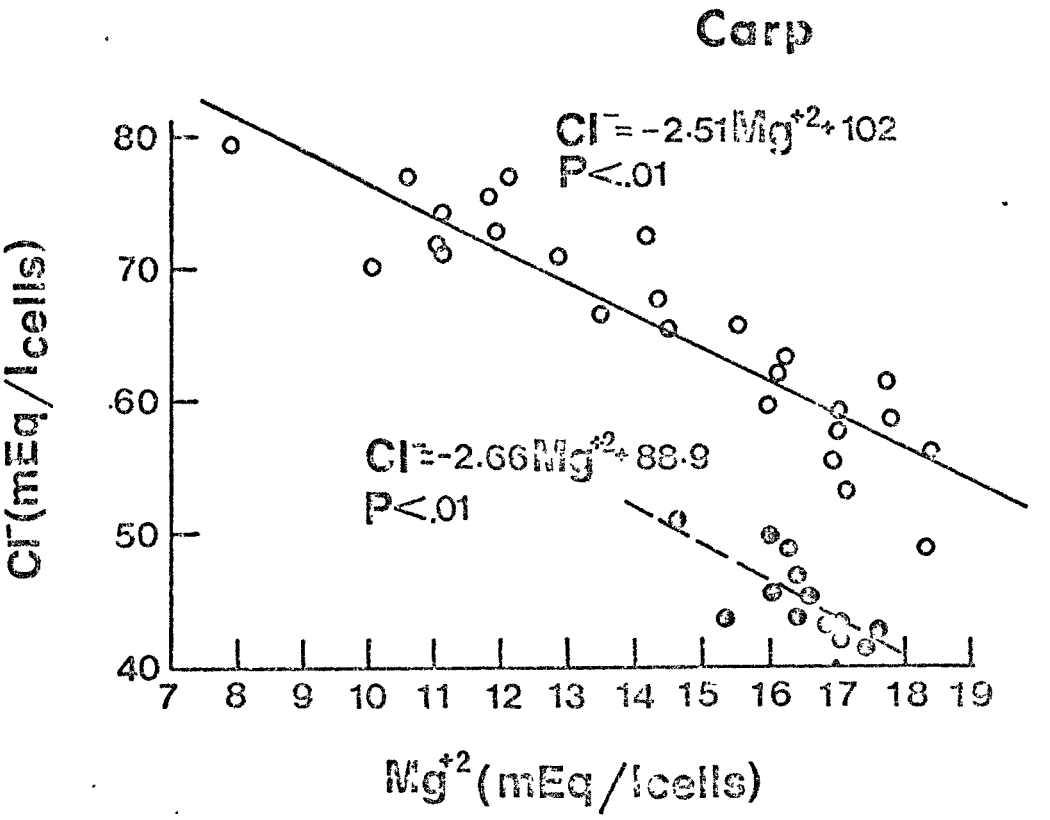
Table 12. Linear regression analysis of erythrocytic chloride (y, mEq/ℓ cells) against cell magnesium (x, mEq/ℓ cells) in thermally acclimated carp (Cyprinus carpio). The data reported are the sample size (n), coefficient of correlation (cc), significance of the correlation, and the equation of the best fitting line.

Acclimation Temperature		
2°C	16°C	30°C
n = 13 cc = 0.707 P<0.01 y = -2.66x + 88.9	n = 13 cc = 0.737 P<0.01 y = -3.19x + 113	n = 14 cc = 0.637 P<0.05 y = -1.39x + 89.1

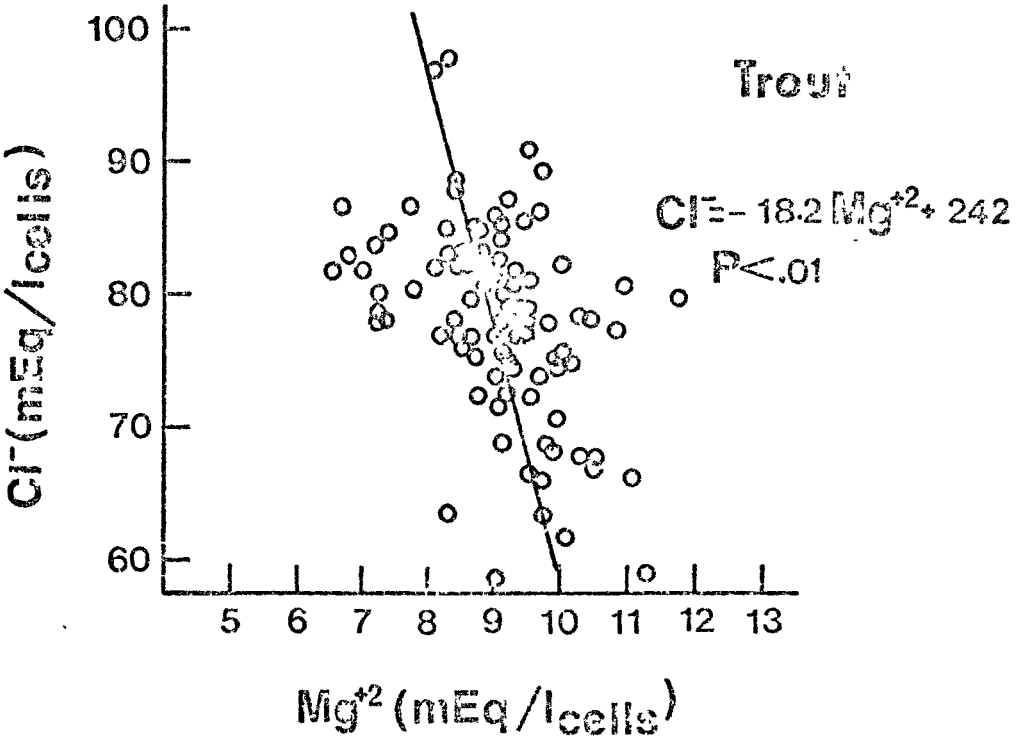
Fig. 13. Relationship between red cell chloride and magnesium ($mEq \cdot \ell^{-1}$, packed cells). Fig. 13a shows the plots obtained for carp acclimated to 2°, 16° and 30°C. The closed circles represent carp acclimated to 2°C while carp acclimated to 16° and 30°C are indicated by open circles. Fig. 13b shows the plot obtained for summer and winter rainbow trout acclimated to 2°, 10° and 18°C. In both diagrams the best fitting lines, their equations, and the significance level of the correlation ($P < 0.05$, $P < 0.01$) are shown. Additional information describing the plots can be found in Appendix 37.

Fig 13

13a



13b



Trout have an unusually high oxygen demand even at low temperatures (Fig. 1). These approximate the oxygen demands of carp at high temperatures. Furthermore, the Q_{10} of oxygen consumption is three times that of carp.* In view of this, and the narrow temperature ranges inhabited by trout, an erythrocytic ionic environment which facilitates unloading of oxygen under all circumstances offers clear advantages. Consistent with this, erythrocytic chloride and magnesium levels are such that, although they are not modified with temperature, they do favor low Hb-O_2 affinity.

Since increases in erythrocytic chloride and reductions in magnesium are conducive to reduction in Hb-O_2 affinity, it would be of some advantage to the animal if these were reciprocally related within the red cells. To ascertain if this was the case correlation analysis was carried out. As would be expected the most obvious relationships were obtained with data from carp (Table 12, Fig. 13a). Correlations significant at $P < 0.01$ level were found for each acclimation group, with values for 16° and 30°C animals comprising a single population distinct from the 2°C fish. Rainbow trout showed more variability, but nonetheless also showed a significant inverse relationship (Fig. 13b). In contrast to carp the various trout seasonal and acclimation groups were not distinct from the others. Not only are these relationships of physiological interest, but they raise the possibility that the forces modifying the concentration of one ion might prompt reciprocal changes in the other electrolyte.

Chloride and Magnesium Partitioning within the Fish Erythrocyte

Any attempt to account for the mechanisms underlying cellular electrolyte changes is rendered difficult because of the complex relationships governing ion distribution within the erythrocyte, and between the erythro-

* i.e., see Fig.1.

cyte and its plasma environment. Nevertheless, insights can be gained by considering the principal factors involved. For example, when ionic species in solution are separated by a differentially permeable membrane, which the ions can penetrate and a charged impermeable protein is added to one side of the system, a Gibbs-Donnan equilibrium situation is created. The electrolytes arrange themselves so that electroneutrality is established on both sides of the membrane, and the ratio of cations, inside to outside, equals that of anions, outside to inside.

To establish electroneutrality on both sides of the membrane, concentration disparities must exist between the two compartments. These are maintained by a potential difference across the membrane. The magnitude of the potential difference (V), i.e., the equilibrium potential, can be estimated using the Nerst equation,

$$E_p = \frac{2.303\ RT}{ZF} \log\left(\frac{[\text{cation a}]_o}{[\text{cation a}]_i} \text{ or } \frac{[\text{anion b}]_i}{[\text{anion b}]_o}\right)$$

where E_p = equilibrium potential (Volts)

R = gas constant (8.31 joules/mole-deg. absolute)

Z = valency

F = Faraday constant (96,500 C/mole)

T = temperature (deg.absolute)

The Nerst potential reflects the disparity of charge between the plasma and cellular compartments.

Human erythrocytes have extensively been studied in terms of ion permeabilities and equilibrium potentials. In human red cells the major impermeable protein is hemoglobin which carries a -10 mmole/mmole Hb·pH negative charge (Dalmark, 1976). Membrane permeability to cations other

than H^+ is not high, whereas anions, of which chloride is the major constituent, are relatively permeable. The permeability of chloride, for example, is 10^7 times that of sodium and potassium at 37°C (Dalmark, 1976). Monovalent cation distribution is largely determined by a (Na^+-K^+) active transport system (Hoffman, 1966). Chloride distribution is apparently passive, and has been used as a basis for the estimation of potential differences between the interior and exterior of mammalian, bird and fish erythrocytes (Armstrong, 1971). In the case of human red blood cells, values obtained in this way are well correlated with electrode probe measurements of the equilibrium potential (Armstrong, 1971).

Equilibrium potentials were calculated on the basis of the distribution ratios for the major red cell cations and chloride in carp and trout, and these values are summarized in Appendices 25 to 29. As is the case with human erythrocytes, sodium distributions lead to large calculated potentials which are inside-positive, whereas potassium distributions give inside-negative results. This is what would be expected if a (Na^+-K^+) pump was present in carp and trout erythrocytes that pumped sodium out, and potassium into the cell. Potentials estimated from calcium values suggest the likelihood of a Ca^{+2} pump which functions to transport Ca^{+2} out of the cell. Chloride distributions produce values similar to those of human erythrocytes, and probably reflect actual transmembrane potentials.

Carp

In the case of carp chloride equilibrium potentials suggest that the interior of the erythrocyte becomes less negative as temperature increases (Fig. 14). The differences observed between acclimation temperatures were significant at the $P < 0.01$ level. This decrease in negativity may reflect

Fig. 14. The transerythrocytic equilibrium potential (E_p) as calculated from the chloride distribution between the erythrocyte and plasma of thermally-acclimated carp. The horizontal line represents the mean; the vertical bar is the 95% confidence interval of the mean. The level of significant difference between acclimation temperatures is placed between the groups being compared.

Fig. 14

Carp

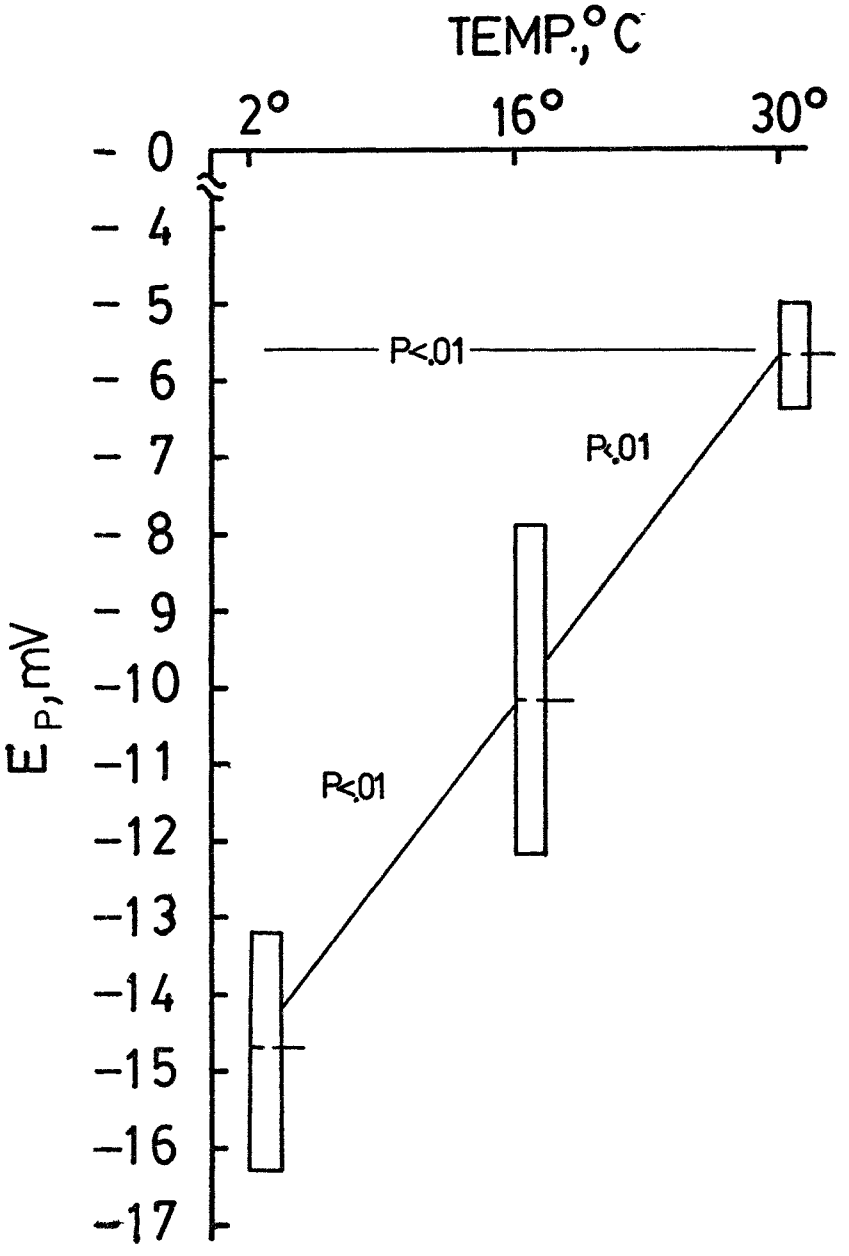


Fig. 15. The relationship of red cell magnesium ($mEq \cdot \ell^{-1}$, packed cells) and the transerythrocytic equilibrium potential (E_p) of thermally-acclimated carp. Shown are three regressions for various acclimation groups, their best fitting plots, and the significance level of the correlation.

Fig. 16. The relationship of red cell magnesium ($mEq \cdot \ell^{-1}$, packed cells) and the transerythrocytic equilibrium potential (E_p) of summer and winter rainbow trout acclimated to 2°, 10° and 18°C. Shown is the best fitting plot, its equation, and the significance level of the correlation.

In both diagrams the negative sign of E_p indicates the inside of the erythrocyte is negative with respect to the plasma. Regressions were calculated using the absolute value of E_p.

Additional information describing the plots can be found in Appendix 37.

Fig. 15

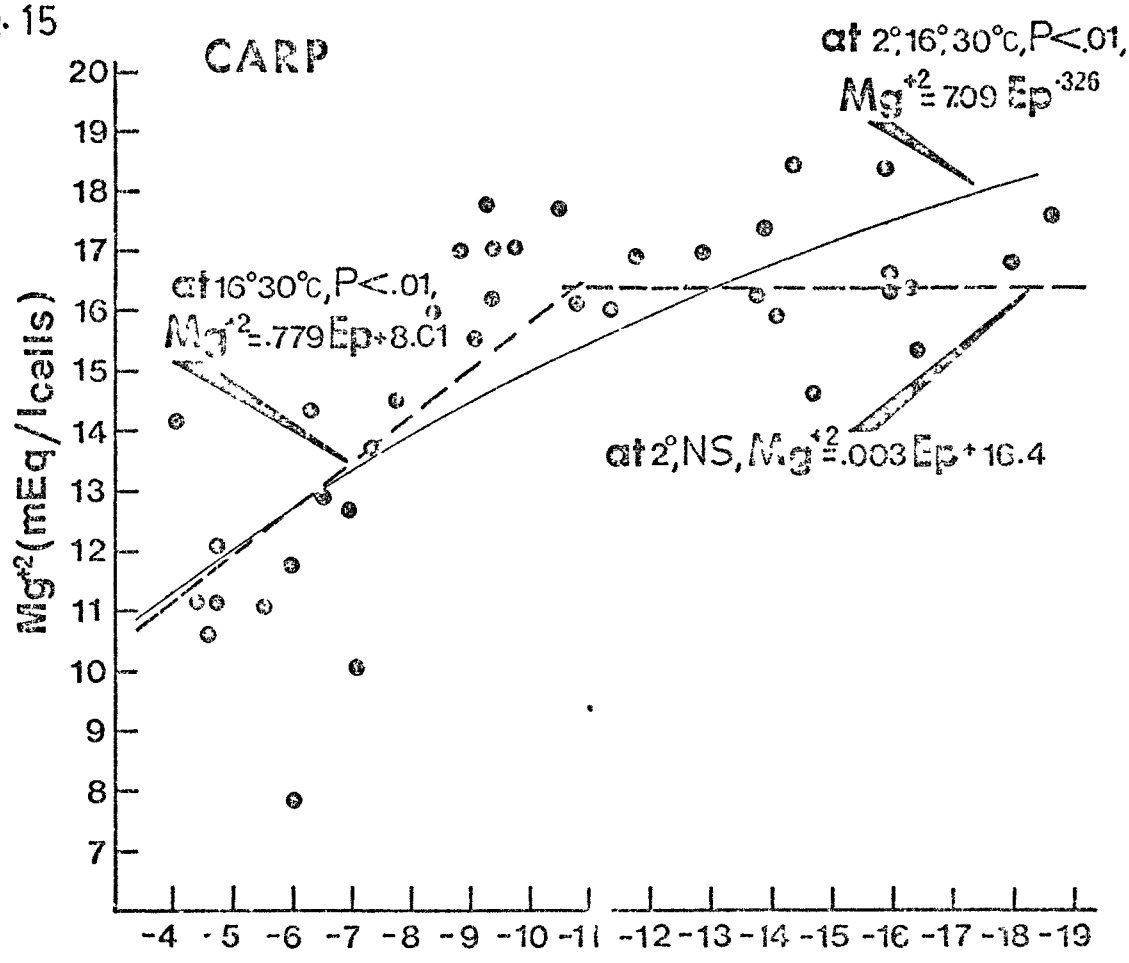
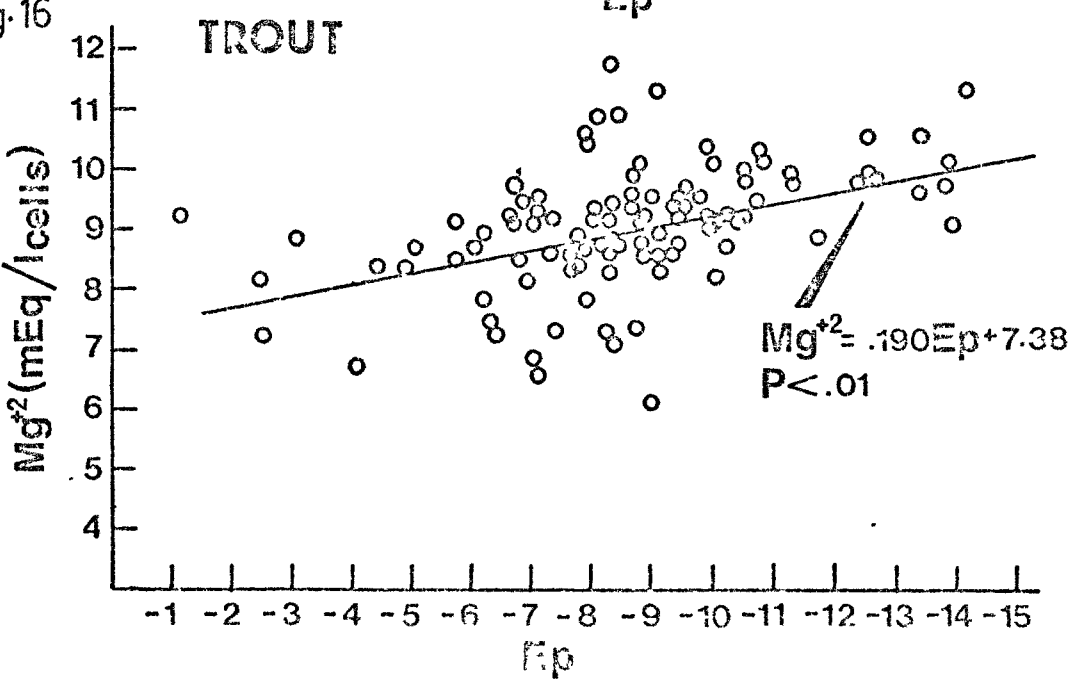

CARP
at 2°,16°,30°c,P<.01,
Mg+2 = 7.09 Ep·326
at 16°30°c,P<.01,
Mg+2=.779Ep+8.01
at 2°,NS,Mg+2=.003Ep+16.4
Mg+2(mEq/lcells)
Ep

Fig. 16


TROUT
Mg+2= .190Ep+7.38
P<.01
Mg+2(mEq/lcells)
Ep

changes in plasma pH since, as noted earlier, this varied inversely with temperature (Rahn, 1971, personal communication to A. H. Houston). Hemoglobin, by virtue of its negativity, would act as a buffer, binding H^+ ions and decreasing its own negative charge.

Regardless of how they are brought about, changes in transmembrane potential could have significant effects upon the partitioning of cellular electrolytes. In the case of magnesium, an active pump has not yet been identified in any species of erythrocyte. Thus, despite its low permeability (Wittham, 1964), diffusable Mg^{+2} may distribute itself in electrochemical equilibrium. Quite obviously under these circumstances its distribution will be the reverse of that of chloride. This may account, in part at least, for the inverse relationship between chloride and magnesium in both carp and rainbow trout erythrocytes (Fig. 13a and b).

If diffusable Mg^{+2}, in fact, is passively distributed variations in potentials should be accompanied by changes in cellular Mg^{+2} levels. To examine this hypothesis cell Mg^{+2} values were plotted against chloride equilibrium potentials (E_p). Carp (Fig. 15, 2°, 16°, 30°C) were characterized by a geometric relationship between cell Mg^{+2} and E_p; Mg^{+2} varied, as predicted, in relation to the transmembrane potential, increasing as the degree of negativity inside to outside increased. The correlation coefficient obtained was significant at the $P < 0.01$ level.

It should be noted that Fig. 15 includes data of carp acclimated to three different temperatures. The majority of the curve parallelling the x-axis (Fig. 15, 2°) represents fish acclimated to 2°C. The reason for the absence of variation in cell Mg^{+2} with E_p in this section of the curve is unknown. One possible explanation is that at temperatures between 16° and 2°C the

carp erythrocyte membrane may undergo a phase transition making it highly impermeable to Mg^{+2}, so that variations in membrane charge are unable to influence magnesium distribution. Curve sections for values from 16° and 30°C carp show near linear changes in cell Mg^{+2} with charge, and these could be fitted with a straight line equation (Fig. 15, 16° and 30°C). An alternative explanation could be that the proportion of total Mg^{+2} bound to protein, ATP etc., may vary with temperature so that more magnesium is bound within the cell at 2°C than at 16° or 30°C. Consequently, at 2°C less Mg^{+2} would be in the diffusable category. In this regard it is of interest to note that the concentration of H^{+}, which can compete with Mg^{+2} binding sites, increases with temperature in carp plasma.

If cellular Mg^{+2} content and transmembrane potential (E_p) are related, cellular changes in Mg^{+2} that occurred with acclimation can be explained in terms of the changes in E_p. For example, carp acclimated to 2°, 16° and 30°C had mean values of E_p that are -14.7±0.719, -10.2±0.999 and -5.71±0.309 respectively (see Fig. 14). If these values of E_p are traced out for respective Mg^{+2} values on Fig. 15, they approximate the mean levels of cellular Mg^{+2} found at 2°, 16° and 30°C (16.2±0.357, 16.8±0.312 and 11.9±0.446 mEq Mg^{+2}/ℓ_{cells} respectively, Fig. 8b). In other words, perhaps the driving force influencing Mg^{+2} out of the cell at higher temperatures is a change in the transmembrane potential. Since by virtue of its calculation, increases in E_p are consistent with higher $[Cl^{-}]_{RBC}/[Cl^{-}]_{plasma}$ ratios, as cellular Mg^{+2} decreases with thermoacclimation to high temperatures increases in erythrocytic Cl^{-} are encountered.

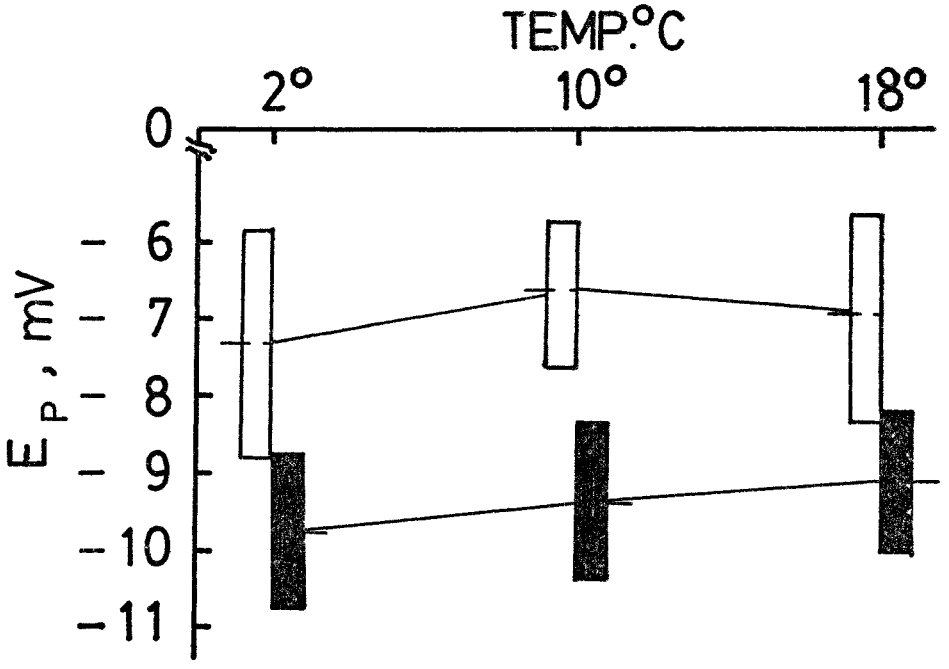
Rainbow Trout

Like carp, rainbow trout displayed an inverse relationship between cell

Fig. 17. The transerythrocytic equilibrium potential (E_p) as calculated from the chloride distribution between the erythrocyte and plasma of thermally-acclimated rainbow trout. The dark bars represent winter trout while the white bars indicate trout sampled in the summer. The horizontal line represents the mean; the vertical bar is the 95% confidence interval. No significant differences were found between the acclimation temperatures in either summer or winter trout.

Fig.17

Rainbow trout



Mg^{+2} and Cl^- (Fig. 13b). As well, the Mg^{+2} content also varies with the equilibrium potential across the erythrocyte (Fig. 16). In contrast with the situation in carp, however, acclimation did not lead to marked changes in erythrocytic chloride and magnesium levels (Fig. 6a to c). Consistent with this there was little evidence of a significant change in equilibrium potentials (Fig. 17).

The lack of any modifications in the equilibrium potential with acclimation in rainbow trout erythrocytes requires some discussion. In the case of carp it was suggested that changes in E_p might be produced by decreases in pH with increased acclimation temperature. The absence of change in rainbow trout erythrocyte transmembrane potential could be taken to mean that blood pH does not change or changes very little with acclimation. In this regard, although one study (Houston et al., 1968) has found little change in the plasma pH of rainbow trout acclimated to temperatures between 3° and 21°C, the majority of evidence indicates that trout blood pH decreases at higher acclimation temperatures (Randall and Cameron, 1973; Eddy, 1974; Reeves, 1977). If the latter is true, it becomes necessary to account for the absence of changes in E_p with acclimation to higher temperatures.

One possible explanation could be that trout hemoglobin has different characteristics than that of carp, i.e., that changes in pH may not alter the negativity of the protein. Some evidence does, in fact, suggest that rainbow trout hemoglobins are unique in some respects to those of other fish. For example, a number of their hemoglobins lack any Bohr effect because of the absence of certain protonizable amino acid groups. Powers (1972) believed that the presence of such hemoglobins was of adaptive

advantage in fish with a high metabolism. During periods of high activity blood pH could decrease to an extent that oxygen loading at the gills would be hindered. The presence of hemoglobins lacking a Bohr effect would, of course, insure oxygenation under these circumstances. It is at least possible that in rainbow trout this adaptation is taken a step further so that decreases in pH do not effect protein charge which might promote decreases in cellular magnesium and increases in chloride, since this would further reduce Hb-O_2 affinity.

In conclusion, rainbow trout show little modification in erythrocytic electrolyte levels other than potassium with thermal acclimation. Carp exhibited large modifications in the levels of Na^+ as well as Cl^- and Mg^{+2}. The latter two ions are important modulators of hemoglobin oxygen affinity. The electrolyte changes observed have been assessed in relation to their possible role in the maintenance of erythrocytic volume, and their effects upon red cell metabolism and hemoglobin function.

The data suggests that the manipulation of ion levels probably does not play a major role in the regulation of cell volume. Like the flounder, carp and rainbow trout may govern cell volume through other mechanisms involving organic solutes. Use of non-electrolytes to control volume offers several advantages, for the animal may adjust cell volume to promote oxygen loading while simultaneously altering ionic levels to suit various cell functions at different acclimation temperatures.

The variations observed may produce a cell environment favoring glycolysis in winter as compared to summer trout and in cold-acclimated carp. In this respect the results obtained are consistent with those of numerous metabolic studies which indicate that cold-acclimated fish modify metabolism

to achieve higher activities at lower temperatures. Such adaptations are thought to offset the direct effects of reduced temperature upon metabolic processes.

The most interesting aspect of thermoacclimatory response by the red cell is associated with the effects which changes in ionic composition may have on hemoglobin function. The trout, which shows little change in functionally important erythrocytic electrolytes with temperature or season, maintains cell electrolytes at levels conducive to a low hemoglobin-oxygen affinity state. This is consistent with the finding that trout hemoglobin P_{50} values are higher than those of other species of fish. This may be of advantage to the trout since they rely heavily upon venous deoxygenation to compensate for increased oxygen need.

Carp exhibited much larger changes in erythrocyte chloride and magnesium levels with temperature. The changes observed should produce reductions in hemoglobin-oxygen affinity with increasing temperature. In view of the very efficient gill structure of the carp, and the high oxygen affinity characterizing their hemoglobins, decreases in hemoglobin oxygen affinity should offer the advantage of prompting O_2 release at the tissue level, while still allowing for effective oxygenation at the gills.

In both species cell Cl^- and Mg^{+2} are inversely related, and appear to be altered in relation to the membrane potential changes accompanying acclimation. The mechanism leading to such variations in potential are not, however, clear.

Erythrocytic Carbonic Anhydrase in Thermally Acclimated Trout

Data pertaining to the thermoacclimatory changes in the carbonic anhydrase of the trout red cell are summarized in Appendices 30 to 36 and are

graphically displayed in Figs. 18 to 21.

Hematocrit and Erythrocytic Protein

In contrast to previous findings, rainbow trout used in the carbonic anhydrase assays exhibited significant ($P < 0.01$) increases in packed cell volume at 18°C (42.1±1.70%) over corresponding values at 2° and 10°C (33.2±0.965 and 33.8±1.11% respectively; Fig. 18a). Non-hemoglobin membrane protein of the erythrocytes expressed in mg protein/ml erythrocytes (Fig. 18b) was also constant at 2° and 10°C (34.4±1.44 and 34.2±2.31 respectively), while significantly lower values were found in 18°C acclimated trout (25.5±1.30). A similar trend was seen in the non-hemoglobin cytosol protein (Fig. 18c). Little variation in mean levels occurred between 2° and 10°C while a non-significant mean decrease was encountered at 18°C. Consequently the total non-hemoglobin protein of the erythrocytes (Fig. 18d) remained relatively constant between temperatures of 2° and 10°C but decreased significantly ($P < 0.05$) at 18°C.

Specific Activity

Specific activities of membrane and cytosol fractions are shown in Fig. 19. At the common assay temperature (25°C) the specific activity of the membrane fraction (Fig. 19a) increased with acclimation temperature. The breakdown rates of p-nitrophenyl acetate ($\mu M \cdot min^{-1} \cdot g^{-1}$) were 34.8±1.56, 39.1±2.38 and 52.7±2.75 at 2°, 10° and 18°C respectively. The activities of preparations from 18°C fish were significantly ($P < 0.01$) higher than those of the 10° and 2°C groups. In incubations conducted at actual acclimation temperatures (Fig. 19a) carbonic anhydrase showed even larger increases in specific activity; the mean activity at 18°C was about 180%

Fig. 18. The physical characteristics of blood from rainbow trout acclimated to 2°, 10° and 18°C. The packed cell volume of the blood (PCV, Fig. 18a) as well as the membrane, cytosol and total erythrocytic non-heme protein ('Membrane', Fig. 18b; 'Cytosol', Fig. 18c; 'Total', Fig. 18d) present in 1.0 ml of erythrocytes. The horizontal line represents the mean, the vertical bar is the 95% confidence interval of the mean. The level of significant difference between acclimation temperatures is placed between the groups being compared. The absence of a P value indicates that the comparison is not significant at the $P < 0.05$ level.

Fig. 16

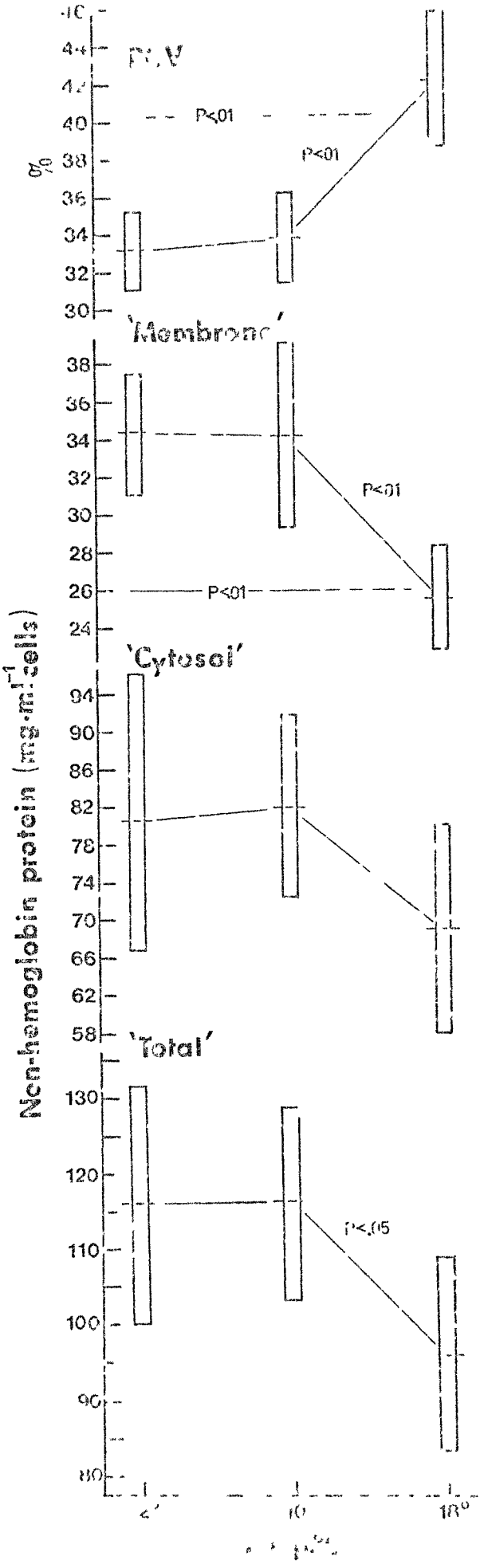
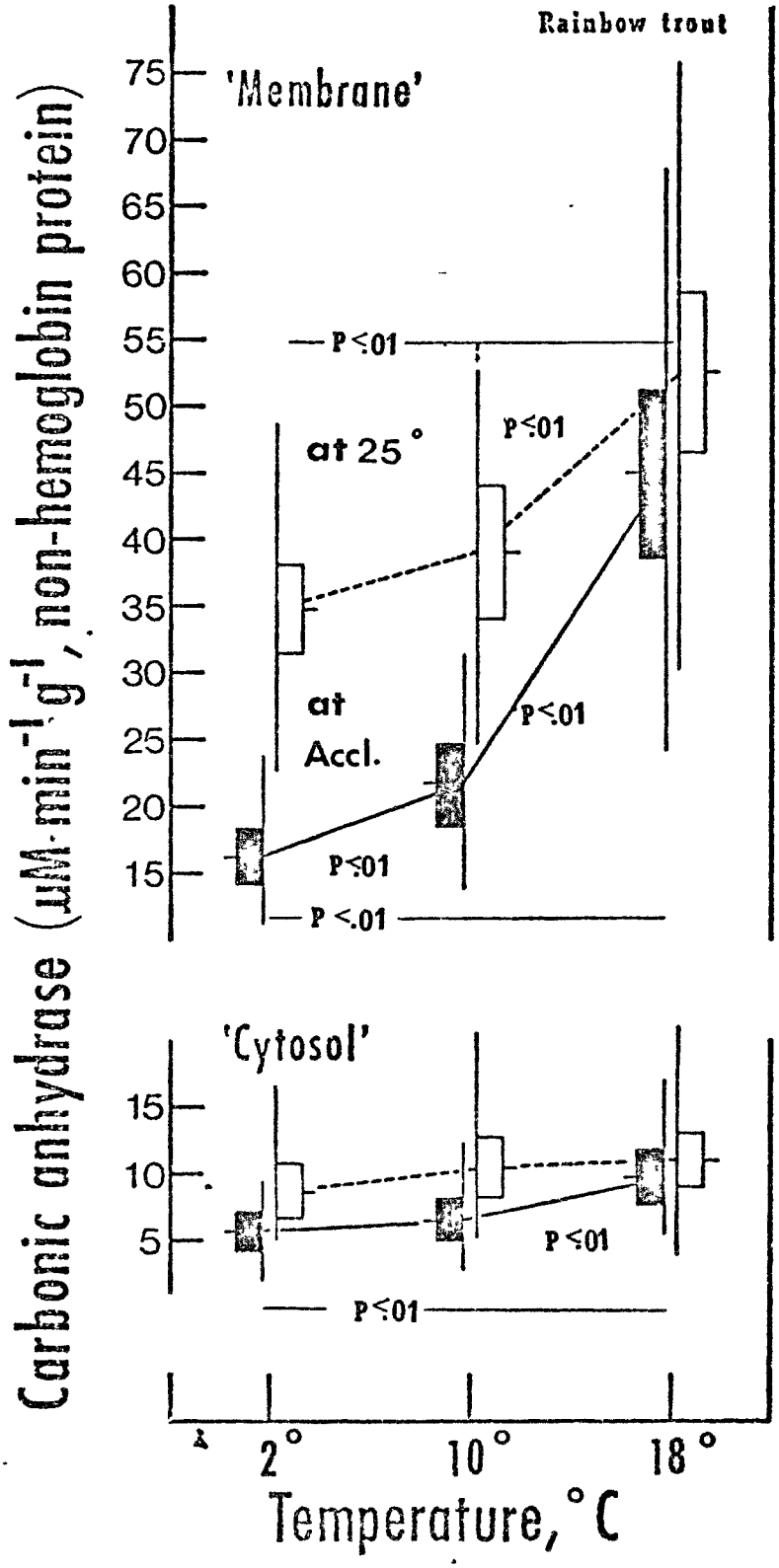

18a
18b
18c
18d
%
P<01
P<01
'Membrane'
P<01
P<01
'Cytosol'
'Total'
P<.05
Non-hemoglobin protein (mg·ml⁻¹cells)

Fig. 19a and b. Specific activity ($\mu M \cdot min^{-1} \cdot g^{-1}$, non-hemoglobin protein) of 'membrane' (Fig. 19a) and 'cytosol' (Fig. 19b) preparations from erythrocytes of rainbow trout acclimated to 2°, 10° and 18°C. The horizontal line represents the mean, vertical line the range, and the vertical bar the 95% confidence interval of the mean. The black bars represent assays performed at the temperature of acclimation while the white bars indicate assays performed at 25°C. The level of significant difference between acclimation temperatures is placed between the groups being compared.

Fig.19



19a

19b

higher than that seen at 2°C. Significant differences ($P < 0.01$) were observed between each pair of temperatures.

Unlike membrane activity, cytosol activity changed little when preparations were assayed at 25°C. Mean values tended to increase at higher acclimation temperature (2°C -8.7±0.84, 10°C -10.4±1.05, 18°C -11.1±1.02 $\mu M \cdot min^{-1} \cdot g^{-1}$), but differences were not significant. When these fractions were assayed at acclimation temperature (Fig. 19b), it was found that activities significantly increased with temperature. Mean specific activity in 18°C fish was 73% above that of 2°C fish, and significant differences ($P < 0.01$) were found in comparison of the 2° and 18°C, and 10° and 18°C acclimation groups.

In many studies on enzyme activities, only specific activity measurements are reported. When changes in specific activity are found after experimental treatment of test animals, these are usually taken as indications that enzyme levels or types have changed, or as is the case in certain membrane-bound enzymes, that differential lipid stimulation may have occurred. Very often little attempt is made to assess whether or not the actual protein content has changed. In fact, it is not an uncommon procedure when fractional protein contents change to dilute these to a common concentration before proceeding with the assay. This can lead to spurious changes in specific activity in cases where, in fact, enzyme activity *per se* has changed little. For example, at constant enzyme levels and substrate breakdown rates, an increase in total but not enzymatic protein will tend to produce reductions in calculated specific activity values. In such instances changes in specific activity offer little information useful in the assessment of physiological function. In the present study the protein content of membrane and cytosol

fractions changed with thermal acclimation (Figs. 18b and c). Since it was of some importance to know the carbonic anhydrase activities actually available to the fish at the different acclimation temperatures, activities were also calculated and expressed per ml of packed erythrocytes ($\mu M \cdot min^{-1} \cdot ml\ eryth^{-1}$; Fig. 20).

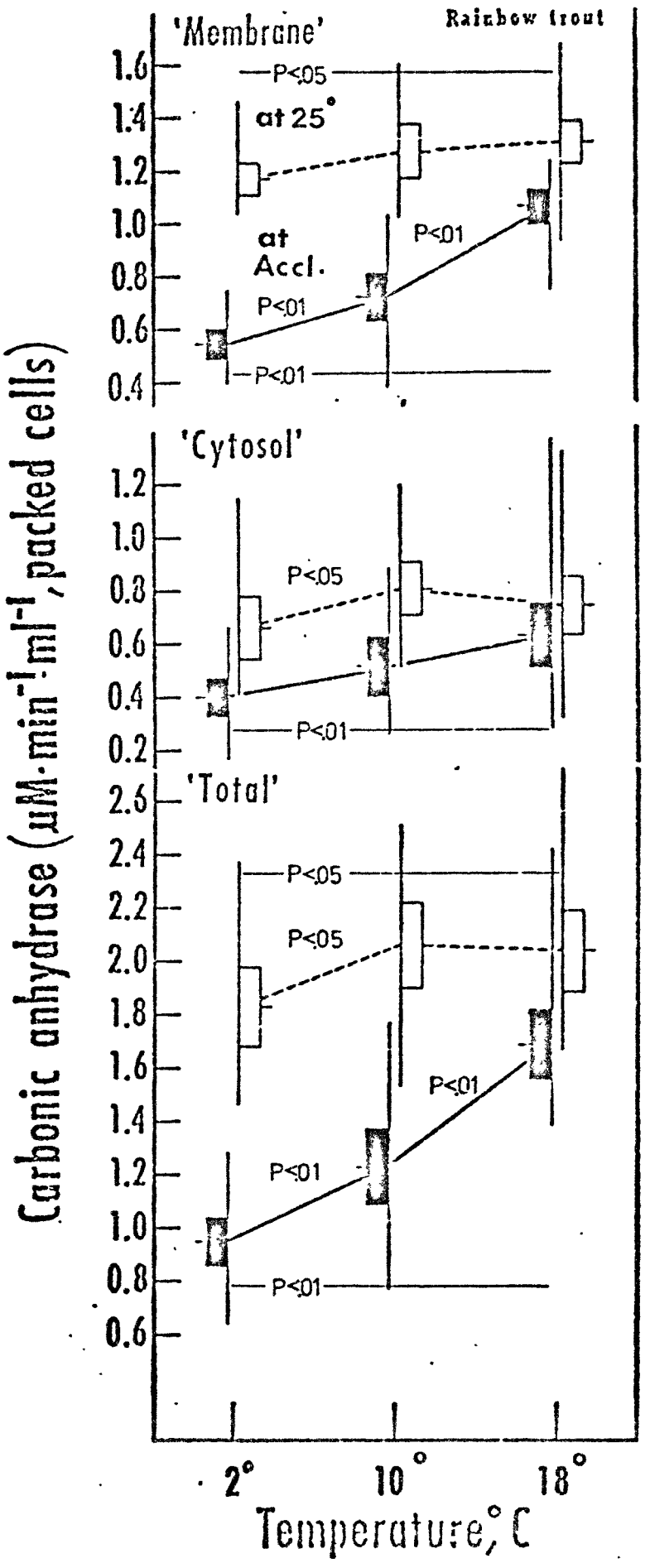
Cell Activities

Carbonic anhydrase activity present in the membranes of 1 ml of packed cells for assays conducted at 25°C is shown in Fig. 20a. When expressed in this manner, activity values did not differ greatly in relation to acclimation state as had been the case with specific activity. Small significant ($P < 0.05$) differences were seen only between 2° and 18°C groups. On the other hand, substantial increases in membrane activity were observed when assays were performed at acclimation temperatures (Fig. 20a). Under these conditions, significant differences ($P < 0.01$) were encountered between all acclimation groups. Cytosol activities, expressed per ml of erythrocytes were lower than those of membrane fractions (Fig. 20b), and approximately 40% of the total activity appeared to be associated with this fraction. In the assays conducted at 25°C (Fig. 20b), cytosol carbonic anhydrase showed no consistent change with acclimation. Activities in 10°C groups were significantly ($P < 0.05$) higher than those acclimated to 2°C, while fish at 18°C were comparable to those acclimated to 2°C. When assayed at the acclimation temperature, cytosol activity increased in a near linear fashion with temperature, with significant differences ($P < 0.01$) occurring between 2° and 18°C (Fig. 20b). It is clear from Fig. 20 that increases in temperature had a greater effect upon membrane fractions than was the case with cytosol.

Fig. 20c indicates the total (i.e., cytosol + membrane) carbonic anhy-

Figs. 20a to c. Activity per ml of erythrocytes ($\mu M \cdot min^{-1} \cdot ml^{-1}$, packed cells) of 'membrane' (Fig. 20a), 'cytosol' (Fig. 20b) and the whole erythrocyte, 'total' (Fig. 20c), of rainbow trout acclimated to 2°, 10° and 18°C. The horizontal line represents the mean, vertical line the range, and the vertical bar the 95% confidence interval of the mean. The black bars represent assays performed at the temperature of acclimation while the white bars indicate assays performed at 25°C. The level of significant difference between acclimation temperatures is placed between the groups being compared.

Fig·20



20a

20b

20c

drase activity present in trout erythrocytes. Assays conducted at 25°C suggest that rainbow trout do not greatly modify activity with acclimation. Values for specimens held at 10° and 18°C, for example, were only about 12% above those seen in 2°C-acclimated animals. This difference was, however, significant ($P < 0.05$). On the other hand, the activity values obtained under actual acclimation temperature conditions were sharply higher at 18°C than at 2°C, and significant differences ($P < 0.01$) were seen between all acclimation groups.

Discussion of Carbonic Anhydrase Data

Red cell carbonic anhydrase in fishes serves two important functions. First, it has been implicated in the branchial ion exchange process (H^+:Na^+, HCO_3^-:Cl^-) of rainbow trout (Kerstetter et al., 1970; Kerstetter and Kirshner, 1972) as well as in other fish (Maetz, 1956; Maetz and Garcia-Romeu, 1964; Dejours, 1969; Maetz, 1971). In such cases carbonic anhydrase is thought to fuel exchange through provision of H^+ and HCO_3^- ions. Consistent with this, acetazolamide inhibition of this enzyme in rainbow trout (Kerstetter et al., 1970) and goldfish (Maetz, 1956) leads to reductions in Na^+ and Cl^- uptake.

Not only might these transport systems be important in the recruitment of sodium and chloride but indirect evidence exists that the transfer of CO_2 from certain fish may rely heavily on these exchange processes. For example, if the external media of goldfish is changed from NaCl to Na_2SO_4, CO_2 evolution sharply decreases and stops, in spite of the fact that oxygen consumption remains steady. If after a time the fish are returned to NaCl media a surge of Cl^- uptake and HCO_3^- loss occurs (Dejours, 1969). Dejour's explanation of this phenomena was that an obligatory Cl^-:HCO_3^- exchange

mechanism exists at the gills and Cl^- ions are required for HCO_3^- output. This also points to the possibility that CO_2 may actually be lost from the gills only as H^+ and HCO_3^-.

The second principal function of carbonic anhydrase lies in the reduction of CO_2 tensions in the blood by a breakdown of molecular CO_2. Through this reaction and carbamino formation, CO_2 tensions in trout are maintained at levels between 3.5-6 mm Hg (Cameron, 1971). Simple calculations indicate the importance of this. On the basis of data from various sources summarized by Albers (1970), rainbow trout blood would be expected to carry a standing level of CO_2 of approximately 6 mmoles/litre in various forms (i.e., $H^+ + HCO_3^-$, carbamino reaction with hemoglobin, molecular CO_2). If such a quantity of CO_2 was carried only in molecular form in distilled water, at 20°C it would exert a partial pressure of 116 mm Hg. In trout blood, the P_{CO_2} levels would be even higher since the solubility coefficient of CO_2 decreases with increased ionic strength. Such high P_{CO_2} levels might be expected to have serious physiological consequences. For example, rainbow trout blood shows a very strong Root effect. At 5°C and P_{O_2} levels of 100 mm Hg, with no CO_2 present, trout blood is 95% saturated. Increasing P_{CO_2} to values of 7-10 mm Hg at the same oxygen tension decreases saturation to 60% (Beaumont, 1968). Normally these small levels of P_{CO_2} (7-10 mm Hg) can be handled by the fish quite easily since at the gills the removal of CO_2 is such that trout blood leaving the gills is seldom below 95% saturation (Randall, 1970). This suggests that O_2 loading problems do not occur. However, if P_{CO_2} levels were allowed to rise to levels above 116 mm Hg, serious loading problems due to the Root effect would be envisaged. At the tissue level high CO_2 tensions could

reversibly inhibit Krebs cycle intermediates evolving CO_2. In addition, increases in blood CO_2 levels are known to effect the control of breathing in fish (Hughes and Shelton, 1962).

In rainbow trout acclimated to 18°C oxygen consumption rates are 100% higher than in fish acclimated to 2°C. Presumably this increase in oxygen consumption is coupled with higher rates of CO_2 evolution by the tissues. Since passive electrolyte efflux is proportional to branchial irrigation, perfusion and effective exchange area (Randall et al., 1972), exposure to increased temperature would be expected to prompt reductions in the electrolyte levels and increases in CO_2 tension. Marked increases in blood CO_2 tension are not seen at higher temperatures (Cameron, 1971; Randall and Cameron, 1973) and the changes in plasma electrolyte levels that do take place are relatively modest. Quite possibly trout resolve both problems through the amplification of carbonic anhydrase activity to form H^+ HCO_3^-, employing the latter to drive the exchange uptake of Na^+ and Cl^-.

As discussed previously, very little evidence exists that quantitative or qualitative changes in carbonic anhydrase have taken place in the trout erythrocyte with thermal acclimation. Although measurements of carbonic anhydrase per ml of packed erythrocytes at assay temperatures of 25°C (Fig. 20) produced some significant changes in activity with thermal acclimation, the modifications were quite modest. For example, the activities present in the whole erythrocyte increased less than 12% at higher over lower acclimation temperatures. In view of this, increasing the level of carbonic anhydrase within the erythrocyte and/or synthesis of a more active form of this enzyme plays only a minor role in increasing carbonic anhydrase activity at higher acclimation temperatures.

Arrhenius plots* made on a limited number of membrane preparations provided no evidence that acclimation temperature leads to marked changes in inflection points. Fish acclimated to 2° and 18°C showed inflection points at 16° and 18°C respectively, indicating that the lipid environment surrounding membrane carbonic anhydrase changes little with thermoacclimation. Such findings are also consistent with those of Houston and Mearow (1978, unpublished results) who, after extensive study of goldfish erythrocytic membrane bound carbonic anhydrase, found little change in Arrhenius inflections with thermal acclimation.

Large potentially adaptive increases in enzyme activity did take place when the assays were performed under in vivo temperature conditions, namely at the temperature of acclimation. At 18°C the membrane activity of the whole erythrocyte was 94% higher than at 2°C (Fig. 20a, at temperature of acclimation), cytosol activity increased 54% (Fig. 20b, at temperature of acclimation), while the total erythrocytic activity was 74% higher (Fig. 20c, at temperature of acclimation) at 18° over 2°C. Significance at the $P < 0.01$ level was reached between all acclimation temperatures within the membrane fraction and at the level of the whole erythrocyte while cytosol showed significant differences ($P < 0.01$) only between 2° and 18°C groups. Since carbonic anhydrase is concentrated mainly within the blood rather than in the tissues of rainbow trout (Houston and McCarty, 1978), such large increases in the activity under in vivo temperature conditions may be of importance in reducing CO_2 tensions and providing increased amounts of H^+ and HCO_3^- for Na^+ and Cl^- exchange at higher temperatures.

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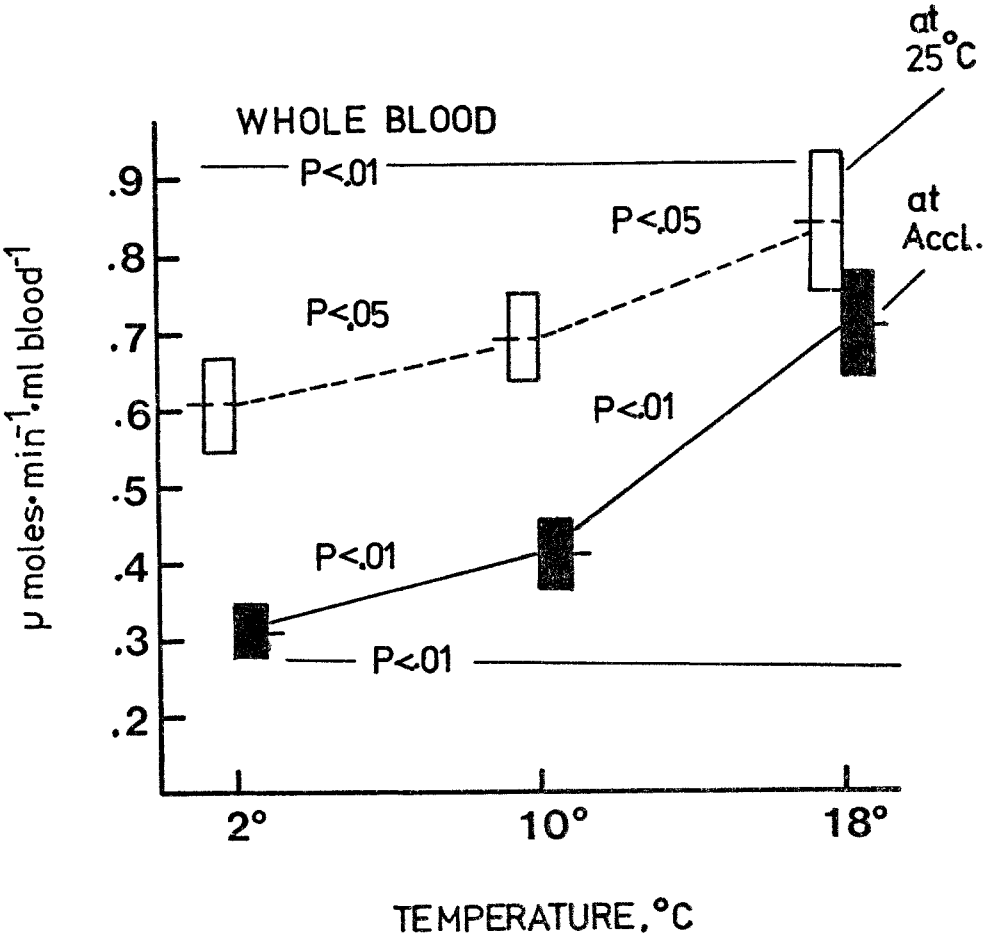
*a plot of -log of activity vs $\frac{1}{\text{absolute temp.}}$

Fig. 21. Calculated activity of whole blood (μmoles$\cdot$min$^{-1}\cdot$ml blood^{-1}) from rainbow trout acclimated to 2°, 10° and 18°C. The horizontal line represents the mean, the vertical bar is the 95% confidence interval of the mean. The black bars represent assays performed at the temperature of acclimation while the white bars indicate assays performed at 25°C. The level of significant difference between acclimation temperatures is placed between the groups being compared.

Fig·21


WHOLE BLOOD
P<.01
P<.05
P<.05
P<.01
P<.01
P<.01
at 25°C
at Accl.
.9
.8
.7
.6
.5
.4
.3
.2
2°
10°
18°
μ moles·min⁻¹·ml blood⁻¹
TEMPERATURE, °C

Under in vivo conditions in the present study, not only would temperature produce an effective increase in carbonic anhydrase activity, but since experiments have shown that trout carbonic anhydrase is present solely in the erythrocyte (Smeda, 1976, unpublished results; Haswell, 1977), the large increase in hematocrit (Fig. 18a) with acclimation temperature would be expected to increase the whole blood level of this enzyme. Fig. 21 shows the expected levels of carbonic anhydrase activity in the blood ($\mu M \cdot min^{-1} \cdot ml$ blood) at a common assay temperature of 25°C as well as at the temperature of acclimation, using in vivo levels of hematocrit to calculate the activity. Under these conditions the activity present at 18°C is 127% higher than that at 2°C. Significant differences ($P < 0.01$) exist between all acclimation temperatures.

Even though hematocrit alterations with temperature may not always occur (see Table 3b), other modes of increasing the amounts of carbonic anhydrase may exist. For example, Houston and DeWilde (1969) have shown that the blood volume of the brook trout, Salvelinus fontinalis, increases in warm acclimated animals. If such a response occurred in rainbow trout and if hematocrit remained constant, it would increase the total amount of carbonic anhydrase available to the fish at higher temperatures.

The lack of large qualitative or quantitative modifications of erythrocytic carbonic anhydrase with acclimation (Fig. 20, at 25°C) is consistent with certain features that may be suspected of the trout erythrocyte. These erythrocytes may have certain characteristics in common with their mammalian counterparts, one of these being the lack of synthetic capabilities to produce new enzymes and regenerate membrane material. If such is the case, modifications in erythrocytic enzyme composition could only be

accomplished by producing new erythrocytes. Since the rainbow trout erythrocyte has a long life span (220 days; Iuchi, 1973a), if fish were subjected to a rapid seasonal or diurnal temperature change, they may be incapable of modifying their blood composition at a rapid enough rate to compensate for the stress. In such a situation, perhaps the most efficient system is one that would respond nearly instantaneously to a temperature change. Therefore, in having a temperature-sensitive carbonic anhydrase enzyme (that increases in activity with temperature), a rise in temperature producing increased tissue CO_2 evolution would be compensated for immediately by increases in carbonic anhydrase activity.

SUMMARY

1. To evaluate the possibility that thermal acclimation is accompanied by adaptive alterations in the erythrocytic ionic environment of hemoglobin, hematological data (blood hemoglobin content, hematocrit, red blood cell numbers, mean erythrocytic volume and hemoglobin content), plasma and packed erythrocytic electrolyte levels (Na^{+}, K^{+}, Mg^{+2} and Ca^{+2}) were obtained for summer and winter sampled rainbow trout acclimated to 2°, 10° and 18°C and carp acclimated to 2°, 16° and 30°C. Red cell electrolyte concentrations and ion:hemoglobin ratios were then calculated.
2. Rainbow trout and carp showed very little hematological response following thermal acclimation. Aside from higher acclimation temperatures producing a modest increase in red cell numbers in summer trout and small decreases in red cell volume in both summer trout and carp, no significant differences were found with temperature. The data did, however, indicate a possible influence of weight and plasma Ca^{+2} upon hematological response to temperature in carp; a relationship that was absent in rainbow trout.
3. Plasma analysis indicated that rainbow trout were characterized by seasonal differences in response to temperature. The largest thermal modifications observed were in winter trout where plasma sodium and potassium were elevated at higher temperatures. Summer trout on the other hand exhibited no significant thermal-acclimatory change in any of the five electrolytes measured. In carp, increases in acclimation temperature produced a decline in plasma sodium and magnesium while calcium levels increased. In spite of a fair number of comparisons that showed statistically significant modifications with temperature and/or season, all the changes observed except those involving potassium were small in nature, and of doubtful physiological

significance. The increase in plasma potassium that was observed occurred under anomalous seasonal temperature conditions and could be the result of a breakdown in the regulation of this electrolyte, rather than an adaptive strategy.

4. The most obvious variation in the ionic composition of the rainbow trout erythrocyte was an increase in potassium content at higher temperatures. In carp, potassium levels were stable within the erythrocyte while sodium and chloride concentrations rose sharply and magnesium decreased following acclimation to higher temperatures. The electrolyte changes observed were assessed in relation to the possible roles they play in the maintenance of erythrocytic volume, and their effects upon red cell metabolism and hemoglobin function.
5. The inverse relationships found between red cell volume and total electrolyte levels in the cell indicates that manipulation of ionic levels probably does not play an important role in the regulation of cell volume. This is consistent with thefindings of other experiments that indicate organic solutes within the erythrocyte play an important role in this regard. The use of non-electrolytes to control volume offers some distinct advantages since electrolyte levels can be modified to suit cell and hemoglobin function without producing detrimental volume changes.
6. The variations in erythrocytic electrolytes observed may produce a cell environment favoring glycolysis in winter as compared to summer trout and in cold acclimated carp. This could be an adaptation to offset the direct effects of reduced temperature upon metabolic processes.
7. The most significant aspects of the thermoacclimatory changes in the red cell electrolytes are in the effects they may have on hemoglobin function.

Trout maintained all electrolytes at levels conducive to a low oxygen affinity state throughout all acclimation temperatures. This may be of advantage to the trout since they have high O_2 consumptions at all temperatures and rely heavily upon venous deoxygenation to compensate for oxygen need. Carp, on the other hand, modified erythrocytic chloride and magnesium levels with temperature in a way that would produce reductions in hemoglobin oxygen affinity with increased temperature. Such a response would facilitate the venous unloading of oxygen under conditions of increased oxygen need.

8. In both species cell chloride and magnesium are inversely related and appear to be altered in relation to the transmembrane potential changes accompanying acclimation.
9. Erythrocytic membrane and cytosol carbonic anhydrase present in rainbow trout acclimated to 2°, 10° and 18°C were assayed, at the temperature of acclimation, and at 25°C, to evaluate the possibility that thermal acclimation results in the adaptive modifications of this enzyme within the erythrocyte.
10. It was found that trout acclimated to 18°C had higher hematocrits and lower membrane and cytosol protein levels than fish present at 2° and 10°C.
11. When assayed at 25°C, the membrane fractions of fish acclimated to 18°C showed considerably higher specific activities over fish acclimated to 2° and 10°C. The significant differences were amplified even further when each membrane extract was assayed at the temperature of acclimation. Although the cytosol specific activities changed very little with acclimation temperature when assayed at 25°C, again when assayed at the temperature of acclimation, the effect of temperature was to amplify differences between

acclimation groups.

12. Since membrane and cytosol protein varied with acclimation, alterations in specific activity do not necessarily indicate functional changes in carbonic anhydrase. In view of this, the activities present per ml of packed erythrocytes were calculated. It was found that the differences in activity that could be attributed to the thermoacclimatory process was quite modest (<10% change in mean levels of activities). On the other hand, assays performed at the temperatures of acclimation showed a large temperature effect where under in vivo conditions of temperature, fish acclimated to higher temperatures might be expected to have higher activities per ml of erythrocytes. Furthermore, since hematocrit increases with temperature, the whole blood levels of this enzyme are expected to increase and further augment the temperature effect.
13. It was concluded that although very little qualitative or quantitative change in carbonic anhydrase took place within the trout erythrocyte, increases in temperature followed by raised hematocrits (when they occur) would produce higher enzyme activities. This, in turn, could aid in the reduction of CO_2 tension and increase the production of H^+ and HCO_3^- used in the active uptake of Na^+ and Cl^-.

BIBLIOGRAPHY

Albro, P. W. (1975) Determination of protein in preparations of microsomes. Anal. Biochem., 64: 485-493.

Anthony, E. H. (1961) The oxygen capacity of goldfish (*Carassius auratus* L.) blood in relation to thermal environment. J. Exp. Biol. 38: 93-107.

Antonini, E., J. Wyman, A. Rossi-Fanelli and A. Caputo (1962) Studies on the relationships between molecular and functional properties of hemoglobin. III. The influence of salts on the Bohr effect in human hemoglobin. J. Biol. Chem., 237: 2773-2777.

Armstrong, W. M. (1971) The cell membrane and biological transport. In *Physiology*. Edited by E. E. Selkurt. Little, Brown and Co., Boston. pp. 1-37.

Beamish, F. W. H. (1964) Respiration of fishes with special emphasis on standard oxygen consumption. III. Influence of oxygen. Can. J. Zool., 42: 355-366.

Beamish, F. W. H. and P. S. Mookherjii (1964) Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on the respiration of goldfish, *Carassius auratus* L. Can. J. Zool., 42: 161-175.

Beaumont, C. (1968) Unpublished data presented by D. J. Randall (1970). Gas exchange in fishes. In *Fish Physiology*. Vol. 4. Edited by W. S. Hoar and D. J. Randall. Academic Press, New York. pp. 272.

Benesch, R. and R. E. Benesch (1969) Intracellular phosphates as regulators of oxygen release by haemoglobin. Nature, 221: 618-622.

Benesch, R. E., R. Benesch and C. I. Yu (1969) The oxygenation of hemoglobin in the presence of 2,3-diphosphoglycerate. The effect of temperature, pH, ionic strength and hemoglobin concentration. Biochemistry, 8: 2567-2571.

Brett, J. R. (1964) The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can., 21: 1183-1226.

Bruin, S. H., H. S. Rollema, L. H. M. Janssen and G. A. J. van Os (1974) The interaction of chloride ions with human hemoglobin. Biochem. Biophys. Res. Comm., 58: 210-215.

Bugyi, H. I., E. Magnier, W. Joseph and G. Frank (1969) A method for measurement of sodium and potassium in erythrocytes and whole blood. Clin. Chem., 15: 712-719.

Bunn, H. F., B. J. Ransil and A. Chao (1971) The interaction between erythrocyte organic phosphates, magnesium ion and hemoglobin. J. Biol. Chem., 246: 5273-5279.

Bygrave, F. L. (1967) The ionic environment and metabolic control. Nature, 214: 667-670.

Caldwell, R. S. (1969) Thermal compensation of respiratory enzymes in tissues of goldfish (*Carassius auratus* L.). Comp. Biochem. Physiol., 31: 79-93.

Cameron, J. N. (1971) Oxygen dissociation characteristics of the blood of the rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., 38A: 699-704.

Cameron, J. N. (1976) Branchial ion uptake in Arctic grayling: resting values and effects of acid-base disturbance. J. Exp. Biol. 64: 711-725.

Catlett, R. H. and D. R. Millich (1976) Intracellular and extracellular osmoregulation of temperature acclimated goldfish, *Carassius auratus* L. Comp. Biochem. Physiol., 55A: 261-269.

Coates, M. L. (1975) Hemoglobin function in vertebrates, an evolutionary model. J. Mol. Eval., 6: 285-307.

Dalmark, M. (1976) Chloride in the human erythrocyte. Prog. Biophys. Molec. Biol., 31: 145-164.

Dean, J. M. (1969) The metabolism of tissues of thermally acclimated trout (*Salmo gairdneri*). Comp. Biochem. Physiol., 29: 185-196.

Dehnel, P. A. (1958) Photoperiod, temperature and salinity effects on the oxygen consumption of crabs. Nature, 181: 1415-1417.

Dejours, P. (1969) Variations of CO_2 output of a freshwater teleost upon change of the ionic composition of water. J. Gen. Physiol., 47: 1209-1227.

Denton, J. E. and M. K. Yousef (1975) Seasonal changes in hematology of rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., 51A: 151-153.

Denton, J. E. and M. K. Yousef (1976) Body composition and organ weights of rainbow trout, *Salmo gairdneri*. J. Fish Biol., 8: 489-499.

DeWilde, M. A. and A. H. Houston (1967) Haematological aspects of the thermoacclimatory process in the rainbow trout. J. Fish. Res. Bd. Can., 24: 2267-2281.

Eaton, J. W. (1974) Oxygen affinity and environmental adaptation. Ann. N. Y. Acad. Sci., 241: 491-497.

Eddy, F. B. (1971) Blood gas relationships in rainbow trout, *Salmo gairdneri*. J. Exp. Biol., 55: 695-711.

Eddy, F. B. (1973) Oxygen dissociation curves of the blood of the tench, *Tinca tinca*. J. Exp. Biol., 58: 281-293.

Eddy, F. B. (1974) *In vitro* blood carbon dioxide of the rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol., 47A: 129-140.

Falkner, N. W. and A. H. Houston (1966) Some haematological responses to sublethal thermal shock in the goldfish, *Carassius auratus*. J. Fish. Res. Bd. Can., 23: 1109-1120.

Fugelli, K. (1967) Regulation of cell volume in flounder (*Pleuronectes flesus*) erythrocytes accompanying a decrease in plasma osmolarity. Comp. Biochem. Physiol., 22: 253-260.

Gary-Bobo, C. M. and A. K. Solomon (1968) Properties of hemoglobin solutions in red cells. J. Gen. Physiol., 52: 825-853.

Gillen, R. C. and A. Riggs (1971) The hemoglobins of a freshwater teleost, *Cichlasoma cyanogluttatum*. I. The effects of phosphorylated organic compounds upon oxygen equilibria. Comp. Biochem. Physiol. 36B: 585-595.

Gillen, R. G. and A. Riggs (1972) Structure and function of the hemoglobins of the carp, *Cyprinus carpio*. J. Biol. Chem., 247: 6039-6046.

Gilles, R. and E. Schaffeniels (1969) Osmotic regulation in crab nerves. Comp. Biochem. Physiol., 31: 927-939.

Graham, J. M. (1949) Some effects of temperature and oxygen pressure on the metabolism and activity of a speckled trout, *Salvelinus fontinalis*. Can. J. Res. Sect. D, 27: 270-288.

Grigg, G. C. (1969) Temperature-induced changes in the oxygen equilibrium curve of the blood of the brown bullhead, *Ictalurus nebulosus*. Comp. Biochem. Physiol., 28: 1203-1223.

Haswell, M. (1977) Carbonic anhydrase in flounder erythrocytes. Comp. Biochem. Physiol., 56A: 281-282.

Heath, A. G. and G. M. Hughes (1973) Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). J. Exp. Biol., 59: 323-338.

Hesser, E. F. (1960) Methods for routine fish haematology. Progve. Fish Cult., 22: 164-171.

Hochachka, P. W. (1973) Comparative intermediary metabolism. In *Comparative Animal Physiology*. Edited by C. L. Prosser. W. B. Saunders Co., Philadelphia. pp. 212-278.

Hochachka, P. and G. Somero (1973) *Strategies of Biochemical Adaptation*. W. B. Saunders Co., Philadelphia.

Hoffman, J. F. (1966) The red cell membrane and the transport of sodium and potassium. Amer. J. Med., 41: 666-680.

Holland, R. A. B. (1970) Factors determining the velocity of gas uptake by intracellular hemoglobin. In *Blood Oxygenation*. Edited by D. Hershey. Plenum Press, New York. pp. 1-23.

Holland, R. A. B. and R. E. Forester (1966) The effects of size of red blood cells on the kinetics of their oxygen uptake. J. Gen. Physiol., 49: 727-742.

Houston, A. H. (1973) Environmental temperature and the body fluid system of the teleost. In *Responses of Fish to Environmental Changes*. Edited by W. Chavin. Charles C. Thomas, Springfield. pp. 87-162.

Houston, A. H. (1975) *Fundamental Concepts in Modern Biology*. Brock University Press, St. Catharines.

Houston, A. H. and D. Cyr (1974) Thermoacclimatory variation in the haemoglobin systems of goldfish (*Carassius auratus*) and rainbow trout (*Salmo gairdneri*). J. Exp. Biol., 61: 455-461.

Houston, A. H. and M. A. DeWilde (1968) Thermoacclimatory variations in the haematology of the common carp, *Cyprinus carpio*. J. Exp. Biol., 49: 71-81.

Houston, A. H. and M. A. DeWilde (1969) Environmental temperature and the body fluid system of the freshwater teleost. III. Hematology and blood volume of thermally-acclimated brook trout, *Salvelinus fontinalis*. Comp. Biochem. Physiol. 28: 877-895.

Houston, A. H. and L. S. McCarty (1978) Carbonic anhydrase (acetazolamide-sensitive esterase) activity in the blood, gill and kidney of the thermally-acclimated rainbow trout, *Salmo gairdneri*. J. Exp. Biol., 73: 15-27.

Houston, A. H. and K. M. Mearow (1978) [^{14}C] PEG-4000, chloride, chloride/potassium and sodium spaces as indicators of extracellular phase volume in the tissues of rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., in press.

Houston, A. H. and R. Rupert (1976) Immediate response of the hemoglobin system of the goldfish, *Carassius auratus*, to temperature change. Can. J. Zool., 10: 1737-1741.

Houston, A. H., J. A. Madden and M. A. DeWilde (1970) Environmental temperature and the body fluid system of the freshwater teleost. IV. Ionic regulation in the carp, *Cyprinus carpio*, following thermal acclimation. Comp. Biochem. Physiol., 34: 805-818.

Houston, A. H., K. M. Mearow and J. S. Smeda (1976) Further observations upon the hemoglobin systems of thermally-acclimated freshwater teleosts: pumpkinseed (*Lepomis gibbosus*), white sucker (*Catostomus commersoni*), carp (*Cyprinus carpio*), goldfish (*Carassius auratus*) and carp-goldfish hybrids. Comp. Biochem. Physiol., 54A: 267-273.

Houston, A. H., J. A. Madden, R. J. Woods and H. M. Miles (1971) Variations in the blood and tissue chemistry of brook trout, *Salvelinus fontinalis*, subsequent to handling, anesthesia and surgery. J. Fish. Res. Bd. Can., 28: 635-642.

Houston, A. H., R. S. Reaves, J. A. Madden and M. A. DeWilde (1968) Environmental temperature and the body fluid system of the freshwater teleost. I. Ionic regulation in thermally-acclimated rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., 25: 563-581.

Hughes, G. M. (1964) Fish respiratory homeostasis. Sym. Soc. Exp. Biol., 18: 81-107.

Hughes, G. M. and J. L. Roberts (1970) A study of the effects of temperature changes on the respiratory pumps of rainbow trout. J. Exp. Biol., 52: 177-192.

Hughes, G. M. and G. Shelton (1962) Respiratory mechanisms and their nervous control in fish. Adv. Comp. Physiol. Biochem., 1: 275-364.

Hutchinson, E. G. (1957) *A Treatise on Limnology*. John Wiley and Sons Inc., London. pp. 541-574.

Isaia, J. (1972) Comparative effects of temperature on the sodium and water permeabilities of the gills of a stenohaline freshwater fish (*Carassius auratus*) and stenohaline marine fish (*Serranus scriba, Serranus cabrilla*). J. Exp. Biol. 57: 359-366.

Iuchi, I. (1973a) The post hatching transition of erythrocytes from larval to adult type in the rainbow trout, *Salmo gairdneri*. J. Exp. Zool., 184: 383-395.

Iuchi, I. (1973b) Chemical and physiological properties of the larval and adult haemoglobins in the rainbow trout, *Salmo gairdneri irideus*. Comp. Biochem. Physiol., 44B: 1087-1101.

Kaloustian, K. V. and J. J. Poluhowich (1976) The role of organic phosphates in modulating the oxygenation behaviour of eel hemoglobin. Comp. Biochem. Physiol., 53A: 245-248.

Kerstetter, T. H. and L. B. Kirschner (1972) Active chloride transport by the gills of rainbow trout (*Salmo gairdneri*). J. Exp. Biol., 56: 263-272.

Kerstetter, T. H., L. B. Kirschner and D. Rafuse (1970) On the mechanisms of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). J. Gen. Physiol. 56: 342-359.

Lenfant, C. J., J. Torrance, E. English, C. A. Finch, C. Reynafarje, J. Ramos and J. Faura (1968) Effect of altitude on oxygen binding by hemoglobin and on organic phosphate levels. J. Clin. Invest., 47: 2652-2657.

Little, J. R. (1964) Determination of water and electrolytes in tissue slices. Anal. Biochem., 7: 87-95.

MacKay, W. C. (1974) Effect of temperature on osmotic and ionic regulation in goldfish, *Carassius auratus*. J. Comp. Physiol., 88: 1-9.

MacKnight, A. D. C. and A. Leaf (1977) Regulation of cellular volume. Physiol. Revs., 57: 510-573.

Maetz, J. (1956) Les échanges de sodium chez le poisson, *Carassius auratus* L. Action d'un inhibiteur de l'anhydrase carbonique. J. Physiol. Paris. [As cited by Maetz (1971).]

Maetz, J. (1971) Fish gills: mechanisms of salt transfer in freshwater and seawater. Phil. Trans. Soc. London, 262: 209-249.

Maetz, J. (1972) Branchial sodium exchange and ammonia excretion in the goldfish, *Carassius auratus*. Effects of ammonia-loading and temperature changes. J. Exp. Biol. 56: 601-620.

Maetz, J. and F. Garcia-Romeu (1964) Mechanism of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus*. II. Evidence for NH_4/Na^+ and $HCO_3^-/C\ell^-$ exchanges. J. Physiol., London, 47: 1209-1227.

Mashiter, K. E. and M. R. J. Morgan (1975) Carbonic anhydrase levels in the tissues of flounders adapted to sea water and fresh water. Comp. Biochem. Physiol. 52A: 713-717.

McCarthy, D. H., J. P. Stevenson and M. S. Roberts (1973) Some blood parameters of the rainbow trout (*Salmo gairdneri*, Richardson). I. Kamloops variety. J. Fish Biol. 5: 1-8.

McCarty, L. S. and A. H. Houston (1977) Na^+:K^+ and HCO_3^--stimulated ATPase activities in the gills and kidneys of thermally acclimated rainbow trout, *Salmo gairdneri*. Can. J. Zool., 55: 704-712.

Mearow, K. M. (1976) Thermoacclimatory variations in hemoglobin systems of wild goldfish, *Carassius auratus*, carp, *Cyprinus carpio*, and their natural hybrid. B.Sc. Thesis, Brock University, St. Catharines, Ontario.

Motais, R. and J. Isaia (1972) Temperature-dependence of permeability to water and to sodium of the gill epithelium of the eel, *Anguilla anguilla*. J. Exp. Biol. 56: 587-600.

Murphy, P. G. F. and A. H. Houston (1977) Temperature, photoperiod and water-electrolyte balance in the rainbow trout, *Salmo gairdneri*. Can. J. Zool., 55: 1377-1388.

Perez, J. and N. Maclean (1976) The hemoglobins of the fish, *Sarotherdon mossambicus*: functional significance and ontogenetic changes. J. Fish. Biol., 9: 447-455.

Perris, A. D. (1971) The calcium homeostatic system as a physiological regulator of cell proliferation in mammalian tissues. In *Cellular Mechanisms for Calcium Transfer and Homeostasis*. Edited by G. Nichols and R. H. Wasserman. Academic Press, New York. pp. 101-126.

Peterson, A. J. and J. J. Poluhowich (1976) The effects of organic phosphates on the oxygen behaviour of eel multiple hemoglobins. Comp. Biochem. Physiol., 55A: 351-354.

Perutz, M. F. (1970a) The Bohr effect and hemoglobin combination with organophosphates. Nature, 228: 734-739.

Perutz, M. F. (1970b) Stereochemistry of co-operative effects of hemoglobin. Nature, 228: 727-734.

Pierce, R. J., R. L. Wiley and T. E. Wissing (1973) Interconversion of units in studies of the respiration of aquatic organisms. Progve. Fish Cult., 22: 164-171.

Poluhowich, J. J. (1972) Adaptive significance of eel multiple hemoglobins. Physiol. Zool., 45: 215-222.

Powers, D. A. (1972) Hemoglobin adaptation for fast and slow water habitats in Sympatric catostomid fishes. Science, 177: 360-362.

Powers, D. A. (1974) Structure, function and molecular ecology of fish hemoglobins. Ann. N. Y. Acad. Sci., 241: 472-490.

Powers, D. A. and A. B. Edmundson (1972a) Multiple hemoglobins of catostomic fish. I. Isolation and characterization of isohemoglobins from *Catostomus clarkii*. J. Biol. Chem., 247: 6686-6693.

Powers, D. A. and A. B. Edmundson (1972b) Multiple hemoglobins of catostomid fish. II. The amino acid sequence of the major α chain from *Catostomus clarkii* hemoglobins. J. Biol. Chem., 247: 6694-6707.

Prosser, C. L. (1973) *Comparative Animal Physiology*. W. B. Saunders Co., Philadelphia.

Prosser, C. L., W. Mackay and K. Kato (1970) Osmotic and ionic concentrations in some Alaskan fish and goldfish from different temperatures. Physiol. Zool., 43: 81-89.

Rahn, J. (1966) Aquatic gas exchange: theory. Resp. Physiol., 1: 1-12.

Randall, D. J. (1968) The functional morphology of the heart in fishes. Am. Zool., 8: 179-189.

Randall, D. J. (1970) Gas exchange in fishes. In *Fish Physiology*. Vol. 4. Edited by W. S. Hoar and D. J. Randall. Academic Press, New York. pp. 253-292.

Randall, D. J., D. Baumgarten and M. Malyusz (1972) The relationship between gas and ion transfer across the gills of fishes. Comp. Biochem. Physiol., 41A: 629-637.

Randall, D. J. and J. N. Cameron (1973) Respiratory control of arterial pH as temperature changes in rainbow trout, *Salmo gairdneri*. Am. J. Physiol., 225: 997-1002.

Randall, D. J., G. F. Holeton and E. D. Stevens (1967) Exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. Exp. Biol., 46: 339-348.

Reeves, R. B. (1977) The interaction of body temperature and acid-base balance in ectothermic vertebrates. Ann. Revs. Physiol., 39: 559-586.

Riggs, A. (1970) Properties of fish hemoglobins. In *Fish Physiology*. Vol. 4. Edited by W. S. Hoar and D. J. Randall. Academic Press, New York. pp. 209-252.

Roberts, J. L. and G. M. Hughes (1967) Gill ventilation and stress in trout. Am. Zool., 7: 731.

Rollema, H. S., S. H. de Bruin, L. H. M. Janssen and G. A. J. van Os (1975) The effects of potassium chloride on the Bohr effect of human hemoglobin. J. Biol. Chem., 22: 1333-1339.

Rossi-Fanelli, A., E. Antonini and A. Caputo (1961a) Studies on the relationships between molecular and functional properties of hemoglobin. I. The effects of salts on the molecular weight of human hemoglobin. J. Biol. Chem., 236: 391-396.

Rossi-Fanelli, A., E. Antonini and A. Caputo (1961b) Studies on the relations between molecular and functional properties of hemoglobin. II. The effect of salts on the oxygen equilibrium of human hemoglobin. J. Biol. Chem., 236: 397-401.

Smeda, J. S. and A. H. Houston (1978) Carbonic anhydrase (acetazolamide-sensitive esterase) activity in the red blood cells of thermally-acclimated rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., in press.

Spitzer, K. W., D. E. Marvin and A. G. Heath (1969) The effect of temperature on the respiratory and cardiac response of the bluegill sunfish to hypoxia. Comp. Biochem. Physiol., 30: 83-90.

Steen, J. B. and S. N. Turitzin (1968) The nature and biological significance of the pH difference across red cell membranes. Resp. Physiol., 5: 234-242.

Weber, R. E., G. Lykkeboe and K. Johansen (1976a) Physiological properties of eel haemoglobin: hypoxic acclimation, phosphate effects and multiplicity. J. Exp. Biol. 64: 75-88.

Weber, R. E., S. C. Wood and J. P. Lomholt (1976b) Temperature acclimation and oxygen-binding properties of blood and multiple haemoglobins of rainbow trout. J. Exp. Biol., 65: 33-345.

Weissbluth, M. (1974) Hemoglobin: co-operativity and electronic properties. In *Molecular Biology, Biochemistry and Biophysics*. Vol. 15. Edited by A. Kleinzeller, G. F. Springer and H. G. Wittmann. Springer-Verlag, New York.

Whittam, R. (1955) A convenient micromethod for the estimation of tissue chloride. J. Physiol., 128: 65P.

Whittam, R. (1964) *Transport and Diffusion in Red Blood Cells*. Edward Arnold Ltd., London.

Wood, S. C. and K. Johansen (1972) Adaptation of hypoxia by increasing HbO_2 affinity and decreasing red cell ATP concentration. Nature, New Biology, 237: 278-279.

Wood, S. C. and K. Johansen (1973) Organic phosphate metabolism in nucleated red cells: influence of hypoxia on eel HbO_2 affinity. Neth. J. Sea Res., 7: 328-338.

Wood, S. C., K. Johansen, and R. E. Weber (1975) Effects of ambient P_{O_2} on hemoglobin-oxygen affinity and red cell ATP concentrations in a benthic fish, *Pleuronectes platessa*. Resp. Physiol., 25: 259-267.

APPENDICES

Appendix 1. Determination of "Trapped Plasma" Correction

Two 1.0 ml aliquots of blood, freshly-drawn as previously described, were pipetted into separate cuvettes. Remaining blood was used for determinations of hematocrit and red blood cell count and calculation of mean erythrocytic volume.

Five µl of ^{14}C-labelled PEG-4000 (polyethylene glycol, 4000 M.W., Amersham/Searle, Arlington Heights, Ill.) at a radioactive concentration of 250 µ Ci/ml of 0.7% saline was introduced into one of the cuvettes. The other cuvette received 5 µl of non-radioactive 0.7% saline. Following mixing by inversion, these samples were centrifuged (5000 g, 5 min). Plasma was drawn off as close as possible to the erythrocyte layer, and any remaining plasma extracted with cotton swabs. Radioactive and non-radioactive plasma and erythrocytes were then used to prepare eight types of scintillation samples in triplicate.

Vial *E contained 50 µl radioactive erythrocytes + 5 µl, 0.7% saline.

Vial *P contained 50 µl radioactive plasma + 5 µl, 0.7% saline.

Vial E contained 50 µl nonradioactive erythrocytes + 5 µl, 0.7% saline.

Vial P contained 50 µl nonradioactive plasma + 5 µl, 0.7% saline.

Vial *B contained 50 µl nonradioactive saline (0.7%) + 5 µl of PEG-4000* @ 250 µCi/ml of 0.7% saline.

Vial B contained 55 µl nonradioactive, 0.7% saline.

Vial *BE contained 50 µl nonradioactive erythrocytes + 5 µl, PEG-4000* 250 µCi/ml of saline.

Vial *BP contained 50 µl nonradioactive plasma + 5 µl PEG-4000*250 µCi/ml of saline.

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*indicates the presence of radioactivity

In each case these were dissolved in 1.0 ml of NCS tissue solubilizer (Amersham/Searle) at 60°C and allowed to stand for 12 hrs. After this 50 µl of tissue decolorant was added (1 g benzoyl peroxide in 5.0 ml toluene), and the solution was allowed to stand for 1 hr at 40°C. Finally, 15 ml of 2:1 ACS/Xylene (Amersham/Searle) was placed in each scintillation vial, and the containers stored in the dark for 24 hrs prior to scintillation counting for 15 minutes using a Searle Delta 300, liquid scintillation counting system.

The scintillation vials were grouped into three categories: (1) *E and *P were used to calculate the trapping factor, (2) E, P and B, which had no introduced radioactivity, provided background counts for the various fractions, and (3) *B, *BE and *BP, which had exactly the same amounts of introduced radioactivity, were used to calculate the quenching of each fraction due to coloration.

Since the external standard ratio (ESR) (the degree of quench imparted to a barium standard built into the scintillation instrument) is the same for blood fractions *E and *P used to calculate the plasma trapping factor, and *BE and *BP respectively used to calculate the quench, the degree of quench occurring in *BE, *BP can be said to represent the quench found in *E and *P.

The amount of quench was calculated by comparing the dpm between *BE and *BP, and a blank vial *B containing 50 µl of saline instead of plasma or erythrocytes but the same amount of radioactivity. Therefore,

$$\text{Erythrocyte Quench (EQ)} = \frac{*BE - E}{*B - B}$$

where all variables are in dpm except EQ, which has no units;

$$\text{Plasma Quench (PQ)} = \frac{*BP - P}{*B - B}$$

where all variables are in dpm except PQ which has no units.

The radioactive count of the plasma and erythrocyte vials used to calculate the trapping factor could then be corrected for background count and quench. Therefore:

Plasma count (Pc) = *P - P where all variables are in dpm

Erythrocyte (Ec) = *E - E
count

Pc and Ec can be compensated for quenching by

non-quenched Pc (NPc) = $\frac{1}{PQ}$ x Pc where all units for variables are dpm except EQ and PQ.

non-quenched Ec (NEc) = $\frac{1}{EQ}$ x Ec

If it is assumed that the PEG-4000 is non-permeable into erythrocytes and indeed this is likely since it is commonly used in determining extracellular volumes, the radioactivity present in the 50 µl volume of erythrocytes (NEc) is due totally to the presence of plasma. Since a 50 µl volume of plasma produced NPc dpm then $\frac{NEc}{NPc}$ x 50 is the volume of plasma trapped in the 50 µl volume of erythrocytes and

$$\frac{\text{µl's of plasma trapped in erythrocytes}}{\text{50 µl erythrocytes}} \times 100$$

gives % plasma trapped in an erythrocyte column obtained by centrifugation at 5000 g.

Appendix 2 Weight (g), hemoglobin (Hb, g/100 ml), hematocrit (HCV, %), erythrocyte numbers (RBC, $\times 10^6/mm^3$), mean erythrocyte volume (MCV, μ^3) and mean hemoglobin content (MCHC, $pg/cell \times 10^{-10}$) of thermally acclimated summer rainbow trout (Salmo gairdneri).

Data Item	Fish Number	Weight	Hb	HCV	RBC	MCV	MCHC
Acclimated to 2°							
1	RT-6	153.5	7.7	30.1	1.21	248	0.993
2	RT-7	149.6	7.7	29.2	1.01	249	1.178
3	RT-12	132.6	7.8	30.2	1.14	264	1.061
4	RT-13	140.5	7.3	26.5	1.09	243	1.017
5	RT-14	196.9	6.8	25.3	0.950	266	1.165
6	RT-15	165.1	6.0	26.6	0.720	369	1.292
7	RT-22	108.1	9.0	33.4	1.13	295	1.239
8	RT-23	171.9	9.3	37.9	1.48	256	0.973
9	RT-24	124.9	9.1	36.9	1.03	358	1.369
10	RT-25	153.5	9.4	36.9	1.45	254	1.007
11	RT-26	200.0	8.2	31.5	1.43	220	0.888
12	RT-27	117.5	8.9	34.0	1.37	248	1.007
13	RT-28	40.1	8.2	34.5	1.27	270	1.000
14	RT-29	38.1	7.6	40.0	1.28	313	0.922
15	RT-30	35.6	8.2	40.3	1.28	314	0.992
16	RT-31	163.5	7.7	31.8	1.19	267	1.000
17	RT-32	133.0	9.1	32.8	1.18	278	1.195
Mean		134.5	8.1	32.8	1.19	277	1.07
Variance		2686.1	0.885	21.03	3.859×10^{-2}	1643	1.829×10^{-3}
S. Dev.		51.838	0.941	4.586	0.1964	40.53	0.1352
S. Err.		12.570	0.228	1.112	4.764×10^{-2}	9.832	3.280×10^{-2}
95% Confidence Interval	U	161.2	8.6	35.2	1.290	298	1.14
	L	107.9	7.6	30.5	1.037	256	1.00
Acclimated to 10°							
1	RT-4	183.0	6.5	27.2	1.14	258	0.886
2	RT-5	181.0	7.8	32.2	1.22	264	0.992
3	RT-10	203.0	7.0	29.2	1.08	270	1.009
4	RT-11	103.1	8.7	34.5	1.15	300	1.174
5	RT-16	218.0	9.4	33.4	1.56	214	0.836
6	RT-17	172.0	9.2	37.6	1.37	274	1.044
7	RT-18	142.1	8.2	33.5	1.38	243	0.920
8	RT-19	220.1	8.5	33.9	1.38	261	0.956
9	RT-20	186.5	7.7	34.3	1.32	294	0.902
10	RT-21	163.9	8.3	35.5	1.41	251	0.915
11	RT-35	129.6	8.6	34.1	1.37	249	0.971
12	RT-37	205.7	7.5	33.0	1.20	274	0.967
13	PT-38	184.5	8.7	38.0	1.50	253	0.900
14	RT-41	179.2	7.1	29.5	1.24	237	0.887
15	RT-42	29.2	9.5	35.0	1.71	205	0.960
16	RT-43	34.5	8.7	37.6	1.63	231	0.828
17	RT-44	17.9	8.0	34.8	1.53	227	0.810
Mean		150.2	8.2	33.7	1.36	252	0.959
Variance		4350.8	0.726	8.703	3.245×10^{-2}	676.1	7.538×10^{-3}
S. Dev.		65.961	0.852	2.950	0.1801	26.00	8.682×10^{-2}
S. Err.		15.998	0.207	0.7155	4.369×10^{-2}	6.306	2.106×10^{-2}
95 Confidence Interval	U	184.1	8.6	35.2	1.46	265	0.983
	L	116.3	7.8	32.2	1.27	239	0.894
Acclimated to 18°							
1	RT-1		8.8	38.0	1.27	297	1.071
2	RT-2	157.5	6.0	24.4	1.09	223	0.855
3	RT-3	167.5	9.3	37.0	0.87	425	1.655
4	RT-8	146.8	7.6	29.7	1.29	230	1.403
5	RT 9	83.5	11.2	43.5	1.65	264	1.055
6	RT-33	116.5	7.6	32.8	1.22	269	0.967
7	RT-34	182.0	7.8	34.0	1.02	333	1.186
8	RT-36	158.1	8.7	35.3	1.70	207	0.794
9	RT-39	145.5	7.7	32.1	1.43	224	0.832
10	RT-40	166.0	8.0	31.5	1.61	196	0.770
11	RT-45	33.6	8.0	36.7	1.47	250	0.844
12	RT 46	38.5	8.4	36.3	1.54	236	0.844
13	RT-47	39.7	9.2	37.7	1.56	241	0.917
14	RT 48	12.5	7.5	29.6	1.52	224	0.878
15	RT 49	153.5	6.6	28.0	1.05	225	0.971
16	RT 50	14.4	9.1	27.5	1.03	267	0.918
17	RT-51	121.9	7.9	31.0	1.26	246	0.968
18	RT-52	142.5	8.0	32.1	1.20	267	1.033
19	RT-53	155.5	8.5	33.7	1.60	211	0.863
Mean		126.6	8.1	33.2	1.33	255	0.991
Variance		2074.8	1.48	20.19	6.034×10^{-2}	2284	4.575×10^{-3}
S. Dev.		45.601	1.10	4.494	0.2456	47.80	0.2209
S. Err.		10.749	0.274	1.031	5.848×10^{-2}	12.10	5.066×10^{-2}
95 Confidence Interval	U	149.6	8.6	35.1	1.44	299	1.097
	L	103.5	7.5	31.0	1.21	229	0.884

[illegible] (Hb, [illegible]), [illegible] (PCV, [illegible]), [illegible] (MCV, [illegible]) [illegible] content (MCHC, [illegible]) of the [illegible] rainbow trout (Salmo gairdneri)

Data Item	Code Number		Weight	Hb	PCV	[illegible]	MCV	MCHC
Acclimated to [illegible]								
1	RT-56		270.0	8.53	31.7	1.33	233	0.992
2	RT-57		352.5	10.50	37.0	1.66	223	0.883
3	RT-58		310.5	6.87	27.0	1.16	232	0.922
4	RT-59		275.5	8.25	30.2	1.30	241	0.877
5	RT-60		316.0	9.72	37.6	1.41	267	1.071
6	RT-61		316.0	9.50	37.0	1.45	255	1.014
7	RT-64		275.5	6.32	25.8	1.11	232	0.893
8	RT-65		318.5	7.43	31.0	1.15	270	1.000
9	RT-68		316.0	5.50	21.7	0.882	246	0.967
10	RT-69		320.0	9.46	41.3	1.80	229	0.817
11	RT-74		234.5	8.76	32.6	1.61	202	0.845
12	RT-75		321.0	8.58	30.0	1.49	201	0.906
13	RT-76		404.5	9.00	32.5	1.24	262	0.806
14	RT-77		300.5	8.04	29.2	1.05	278	1.190
15	RT-90		288.5	9.46	37.3	1.62	230	0.907
16	RT-91		313.0	8.23	29.9	1.24	241	1.032
17	RT-92		226.5	7.50	31.1	1.47	212	0.789
18	RT-93		300.0	6.12	23.5	1.11	212	0.855
19	RT-94		327.5	8.22	31.9	1.49	214	0.852
20	RT-95		257.0	6.92	26.5	0.933	270	1.089
21	RT-96		328.5	8.70	32.3	1.29	250	1.047
22	RT-97		301.5	7.55	30.2	1.37	220	0.854
23	RT-116		250.5	6.11	33.7	1.02	232	0.928
24	RT-117		311.0	7.89	29.2	1.32	221	0.924
25	RT-119		254.5	7.33	27.9	1.23	227	0.927
26	RT-120		249.0	6.81	27.0	0.715	378	1.483
27	RT-121		196.0	10.11	39.7	1.86	213	0.844
Mean			287.6	8.06	31.5	1.32	241	0.956
Variance			2627.5	1.729	23.21	7.452×10^{-2}	1209	2.044×10^{-2}
S. Dev.			51.259	1.315	4.817	0.2730	34.77	0.1430
S. Err.			9.8648	0.2530	0.9271	5.253×10^{-2}	6.691	2.752×10^{-2}
95% Confidence		U	307.9	8.58	33.4	1.42	254	1.012
Interval		L	267.3	7.54	29.6	1.21	227	0.899
Acclimated to 10°								
1	RT-66		318.0	8.69	35.1	1.29	272	1.047
2	RT-67		420.0	10.69	41.0	1.61	255	1.031
3	RT-70		262.5	7.51	26.5	0.683	388	1.698
4	RT-71		385.3	8.98	34.5	1.34	257	1.037
5	RT-72		321.5	8.82	34.0	1.35	252	1.015
6	RT-80		356.0	6.41	25.5	0.930	274	1.069
7	RT-81		335.0	7.51	29.0	1.29	225	0.899
8	RT-84		370.0	7.89	30.0	1.33	226	0.917
9	RT-85		303.5	8.07	33.5	1.30	258	0.962
10	RT-86		392.0	6.51	25.0	0.988	253	1.022
11	RT-89		362.0	10.24	37.9	1.64	231	0.970
12	RT-102		332.0	7.95	28.1	1.04	270	1.183
13	RT-103		316.0	7.19	27.7	1.13	245	0.982
14	RT-104		267.0	8.25	30.7	1.28	240	1.000
15	RT-105		353.0	10.13	40.2	1.58	254	0.994
16	RT-110		238.0	8.43	31.2	1.35	231	0.970
17	RT-111		355.0	8.06	30.8	1.10	280	1.136
18	RT-112		253.0	7.76	30.8	0.94	326	1.273
19	RT-113		232.0	7.46	31.9	1.03	309	1.126
20	RT-114		363.5	6.63	23.2	0.85	273	1.212
21	RT-115		237.5	9.35	36.1	1.70	212	0.853
Mean			323.0	8.22	31.6	1.23	263	1.066
Variance			3116.8	1.406	23.44	7.493×10^{-2}	1554	3.170×10^{-2}
S. Dev.			55.828	1.186	4.841	0.2737	39.43	0.1781
S. Err.			12.183	0.2588	1.056	5.973×10^{-2}	8.603	3.885×10^{-2}
95% Confidence		U	348.5	8.76	33.8	1.35	281	1.148
Interval		L	298.6	7.63	29.4	1.10	245	0.985
Acclimated to 18°								
1	RT-54		240.0	7.99	28.8	1.00	288	1.100
2	RT-55		326.5	6.62	30.9	1.05	294	0.931
3	RT-62		355.0	8.32	32.0	1.37	234	0.942
4	RT-63		237.0	7.05	29.0	1.19	244	0.916
5	RT-73		359.0	13.04	49.7	1.88	264	1.074
6	RT-78		235.5	4.16	17.1	1.01	169	0.639
7	RT-79		360.0	7.14	27.2	1.21	225	0.917
8	RT-82		312.0	7.89	31.2	1.33	235	0.917
9	RT-83		342.5	7.13	25.7	1.43	201	0.776
10	RT-86		208.5	6.32	24.5	1.04	236	0.954
11	RT-87		295.5	7.61	30.8	1.24	248	0.952
12	RT-93		270.5	10.42	40.7	1.75	233	0.926
13	RT-99		[illegible]	5.29	20.0	0.875	228	0.937
14	RT-100		[illegible]	[illegible]	37.[illegible]	[illegible]	[illegible]	0.952
15	RT-101		[illegible]	7.25	33.0	1.41	237	0.794
16	RT-10[illegible]		[illegible]	[illegible]	32.0	[illegible]	246	0.9[illegible]
17	RT-107		1[illegible]0.0	[illegible]	[illegible]	[illegible]	218	0.[illegible]83
18	RT-1[illegible]		[illegible]	[illegible]	36.5	1.45	252	0.966
19	RT-10[illegible]		30[illegible].0	[illegible]	[illegible]	1.10	259	1.0[illegible]
20	RT-1[illegible]		[illegible]	[illegible]	[illegible]	0.9[illegible]3	263	1.054
Mean			[illegible]	[illegible]	[illegible]	[illegible]	24[illegible]	[illegible]
Variance			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
S. Dev.			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
S. Err.			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
95% Confidence		U	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Interval		L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Appendix 4 . Weight (g), hemoglobin (Hb, g/100 ml), hematocrit (PCV, %), erythrocyte numbers (RBC, $\times 10^6/mm^3$), mean erythrocytic volume (MEV, μ^3) and hemoglobin content (MEHbC, $nM/cell \times 10^{-12}$) of thermally-acclimated carp (*Cyprinus carpio*).

Data Item	Code Number		Weight	Hb	PCV	RBC	MEV	MEHbC
Acclimated to 7°								
1	CP-3		225.3	8.53	34.0	1.51	225	0.874
2	CP-6		444.8	8.41	38.0	1.57	242	0.824
3	CP-9		274.1	7.89	29.3	1.52	196	0.803
4	CP-12		114.3	8.40	32.9	1.89	175	0.691
5	CP-15		218.6	7.30	30.1	1.48	203	0.764
6	CP-18		132.8	6.88	29.5	1.16	254	0.922
7	CP-21		137.7	7.02	29.0	1.43	203	0.762
8	CP-24		273.8	6.52	28.0	1.36	206	0.743
9	CP-27		170.6	7.74	38.7	1.22	317	0.984
10	CP-30		58.2	8.20	34.4	1.82	189	0.698
11	CP-33		98.1	7.53	31.6	1.35	234	0.867
12	CP-36		40.9	8.07	36.1	1.61	224	0.776
13	CP-39		29.6	6.69	37.1	1.36	273	0.765
14	CP-42		24.1	5.98	31.0	1.42	218	0.653
Mean			160.2	7.51	32.9	1.48	226	0.795
Variance			14005	0.6388	12.65	4.037×10^{-2}	1384	8.506×10^{-3}
S. Dev.			118.34	0.7992	3.557	0.2009	37.20	9.223×10^{-2}
S. Err.			31.629	0.2136	0.9507	5.370×10^{-2}	9.942	2.465×10^{-2}
95% Confidence Interval		U	228.5	7.97	34.9	1.59	247	0.848
		L	91.9	7.05	30.8	1.36	204	0.741
Acclimated to 16°								
1	CP-2		163.2	5.84	26.1	1.05	249	0.861
2	CP-5		153.1	7.00	30.0	1.59	187	0.686
3	CP-8		208.0	7.89	40.3	1.54	262	0.792
4	CP-11		543	6.34	25.7	1.23	209	0.799
5	CP-14		222.9	8.38	30.7	1.36	226	0.958
6	CP-17		278.1	7.30	31.4	1.04	302	1.086
7	CP-20		219.5	8.31	31.3	1.59	197	0.811
8	CP-23		37.6	7.27	29.2	1.48	197	0.764
9	CP-26		31.9	7.85	34.2	1.53	224	0.797
10	CP-29		38.6	9.47	38.0	1.83	208	0.803
11	CP-32		24.8	7.42	31.5	1.64	192	0.701
12	CP-35		18.0	6.74	32.1	1.44	223	0.722
13	CP-38		21.8	6.90	32.4	1.48	219	0.723
14	CP-41		17.3	8.82	38.8	1.87	207	0.733
Mean			106.4	7.54	32.3	1.48	222	0.802
Variance			9133.7	0.9766	13.792	6.056×10^{-2}	978.6	1.158×10^{-2}
S. Dev.			95.571	0.9883	4.335	0.2461	31.28	0.1076
S. Err.			25.542	0.2641	1.159	6.577×10^{-2}	8.360	4.116×10^{-2}
95% Confidence Interval		U	161.5	8.11	34.8	1.62	240	0.865
		L	51.2	6.97	29.8	1.33	204	0.740
Acclimated to 30°								
1	CP-1		169.0	7.59	29.4	1.52	193	0.776
2	CP-4		318.4	6.55	29.8	1.30	222	0.785
3	CP-7		181.0	5.58	25.0	1.39	180	0.622
4	CP-10		177.5	6.42	26.1	1.19	219	0.856
5	CP-13		132.4	8.38	33.3	1.66	201	0.783
6	CP-16		156.3	8.49	33.5	1.66	202	0.795
7	CP-19		179.9	8.35	33.0	1.71	193	0.754
8	CP-22		151.3	8.41	35.9	1.82	197	0.714
9	CP-25		191.7	9.05	34.8	1.74	200	0.805
10	CP-28		179.6	7.67	27.7	1.38	200	0.862
11	CP-31		39.0	9.19	39.0	2.21	176	0.643
12	CP-34		45.1	9.78	38.5	2.14	180	0.710
13	CP-37		26.2	10.65	45.0	1.75	257	0.942
14	CP-40		21.6	8.47	37.0	1.86	204	0.704
Mean			140.7	8.18	33.4	1.67	202	0.767
Variance			6786.5	1.847	31.92	8.741×10^{-2}	427.5	7.223×10^{-3}
S. Dev.			82.381	1.359	5.650	0.2956	20.67	8.499×10^{-2}
S. Err.			22.017	0.3623	1.510	7.902×10^{-2}	5.526	2.271×10^{-2}
95% Confidence Interval		U	198.7	8.97	36.7	1.84	214	0.816
		L	93.1	7.40	30.2	1.50	190	0.717

Appendix 5. Summary of plasma electrolytes and the sum of all cations (mEq/litre plasma) of thermally-acclimated rainbow trout (Salmo gairdneri). Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Signif. Diff.		
		2°C	10°C	18°C	2°C	10°C	18°C
Na^+	Summer	160±2.31	156±2.27	157±2.50	N.S.	N.S.	N.S.
	Winter	144±2.09	154±1.26	151±1.79	p<0.01	N.S.	p<0.05
					*p<0.01	N.S.	N.S.
K^+	Summer	1.97±0.287	1.90±0.291	2.13±0.172	N.S.	N.S.	N.S.
	Winter	1.43±0.140	1.60±0.183	3.20±0.308	N.S.	p<0.01	p<0.01
					*N.S.	N.S.	p<0.05
Mg^{+2}	Summer	1.33±0.171	1.43±0.132	1.36±0.136	N.S.	N.S.	N.S.
	Winter	1.36±0.100	1.56±3.65x 10^{-2}	1.60±7.02x 10^{-2}	N.S.	N.S.	N.S
					*N.S.	N.S.	N.S.
Ca^{+2}	Summer	4.65±0.164	4.65±0.204	4.67±0.182	N.S.	N.S.	N.S.
	Winter	5.02±0.126	5.10±6.15x 10^{-2}	4.87±0.149	N.S.	N.S.	N.S.
					*N.S.	p<0.05	N.S.
Cl^-	Summer	129±1.95	127±2.26	129±0.993	N.S.	N.S.	N.S.
	Winter	133±1.18	133±0.735	133±1.54	N.S.	N.S.	N.S.
					*N.S.	p<0.05	N.S.
Σ cations	Summer	168±2.85	164±2.16	162±2.16	N.S.	N.S.	N.S.
	Winter	152±2.56	161±1.34	160±2.17	p<0.01	N.S.	p<0.05
					*p<0.01	N.S.	N.S.

*significant differences between seasons

Appendix 6. Plasma electrolytes and the sum of all cations (eq/litre plasma) of thermally-acclimated summer rainbow trout (Salmo gairdneri).

Data Item	Code Number		Na^{+}	K^{+}	Mg^{+2}	Ca^{+2}	Σ cations	Cl^{-}
Acclimated to 2°								
1	RT-6		187.0	3.06	1.53	4.56	196.2	132.2
2	RT 7		165.2	1.19	1.11	3.43	171.0	132.8
3	RT-12		155.9	3.23	0.56	4.20	163.9	130.5
4	RT-13		169.2	1.19	1.12	5.08	175.6	135.6
5	RT-14		160.9	0.90	0 74	4.00	166.5	143.4
6	RT-15		152.8	3.92	0.58	4.56	161.8	122.9
7	RT-22		159.0	1.22	0.94	4.72	165.9	133.3
8	RT-23		-	1.19	2.58	5.72	-	129.6
9	RT-24		154.7	1.36	1.56	-	-	127.8
10	RT 25		154.7	0.98	2.10	-	-	130.1
11	RT-26		156.4	1.41	1.76	5.60	165.2	132.3
12	RT-27		156.5	1.53	1.60	4.76	164.4	132.0
13	RT-28		-	-	1.77	4.96	-	125.0
14	RT-29		165.2	-	2.77	5.44	-	103.8
15	RT-30		154.1	2.04	0.65	3.96	160.7	127.0
16	PT-31		151.9	4.26	0.63	4.44	161.2	126.4
17	RT-32		155.9	2.04	0.63	4.28	162.9	125.7
Mean			159.9	1.97	1.33	4.65	167.9	128.8
Variance			79.945	1.231	0.4980	0.4026	97.292	64.296
S. Dev.			8.9412	1.109	0.7057	0.6345	9.8637	8.0185
S. Err.			2.3086	0.2865	0.1712	0.1638	2.8474	1.9448
95% Confidence Interval		U	164.8	2.58	1.69	5.00	174.2	133.0
		L	154.9	1.35	0.97	4.30	161.7	124.7
Acclimated to 10°								
1	RT-4		162.1	1.69	1.02	3.36	168.2	133.0
2	RT-5		156.4	1.32	1.66	4.28	163.7	128.8
3	RT-10		174.1	1.46	1.80	5.00	182.4	134.6
4	RT-11		161.1	1.44	1.31	4.22	168.1	130.0
5	RT-16		152.8	1.05	1.58	5.00	160.4	127.8
6	RT-17		156.4	1.79	0.82	4.03	163.1	131.5
7	RT-18		162.3	2.26	0.61	4.16	169.3	130.1
8	RT-19		160.3	1.19	1.50	6.00	169.0	132.8
9	RT-20		132.9	5.66	0.68	3.60	142.8	98.5
10	RT-21		154.7	2.29	2.03	4.08	163.1	109.5
11	RT-36		157.2	1.61	0 51	3.92	163.2	124.2
12	RT-37		146.6	2.04	1.20	4.84	154.7	129.8
13	RT-38		154.7	2.13	2.34	5.12	164.3	131 0
14	RT-41		156.4	0.796	1.90	5.96	165.1	133.5
15	RT-42		-	-	1.77	5.52	-	127.4
16	RT-43		155.3	1.82	1.80	5.92	164.8	130.3
17	RT-44		-	-	1.85	4.00	-	131.3
Mean			156.2	1.90	1.43	4.65	164.1	127.3
Variance			77.592	1.273	0.2957	0.7060	69.926	80.911
S. Dev.			8.809	1.128	0.5438	0.8402	8.3621	9.3276
S. Err.			2.274	0.2913	0.1319	0.2038	2.1591	2.2611
95% Confidence Interval		U	161.1	2.53	1.71	5.09	168.8	132.1
		L	151.4	1.28	1.15	4.22	159.5	122.5
Acclimated to 18°								
1	RT-1		-	-	-	-	-	-
2	RT-2		168.4	2.45	2.25	5.48	178.6	131.4
3	RT-3		183.9	-	1.77	5.28	-	121.9
4	PT-8		137.2	0.845	1.24	3.44	142.7	127.3
5	RT-9		161.5	3.23	1.46	5.04	171.7	123.5
6	RT-33		157.8	1.39	0.79	4.68	164.7	131.7
7	RT-34		154.7	2.35	1.26	5.52	163.8	130.7
8	RT-35		152.2	2.04	0.83	5.76	160.8	128.2
9	RT 39		150.9	2.89	1.31	4.56	159.7	132.2
10	RT-40		-	-	-	-	-	-
11	RT 45		159.0	1.44	0.68	4.68	165.8	126.5
12	RT 46		165.2	1.95	-	5.28	-	132.1
13	RT-47		165.2	2.12	1.02	3.48	171.8	124.5
14	RT-48		155.8	2.71	1.02	4.76	164.3	122.4
15	PT-49		149.7	1.34	1.96	4.40	157.3	133.2
16	PT 50		150.0	2.12	0.79	3.08	156.0	129.6
17	RT-51		155.9	2.47	1.82	4.56	164.0	132.2
18	RT-52		147.6	1.53	2.20	4.68	156.0	136.0
19	PT-53		150.6	3.13	0.86	4.68	159.3	130.1
Mean			156.8	2.13	1.36	4.67	162.4	129 0
Variance			106.51	0.4727	0.2445	0.5652	63.838	16 780
S. Dev.			10.320	0.6875	0.5428	0.7504	8.3269	4.0963
S. Err.			2.5030	0.1719	0.1357	0.1870	2.1677	0.99350
95% Confidence Interval		U	162.1	2 49	1.65	5.05	167.1	131.1
		L	151.5	1.76	1.07	4.28	157.8	126.9

Appendix 7 Plasma potassium, sodium and the sum of all cations (meq/litre plasma) of the gill-acclimated rainbow trout (almagamated).

Data Item	Code Number	Na^+	K^+	Mg^{2+}	Ca^{2+}	Σ cations	Cl^-
Acclimated to							
1	RT-56	-	-	-	4.77	-	137.5
2	RT-57	135.3	0.65	0.80	4.84	141.6	126.7
3	RT-58	141.	1.05	1.67	5.00	147.5	133.7
4	RT-59	140.7	1.79	1.55	5.03	158.1	139.9
5	RT-60	126.2	0.68	0.22	5.96	133.1	119.2
6	RT-61	129.8	1.61	0.18	5.96	137.6	128.1
7	RT-64	140.6	0.92	1.10	-	-	138.9
8	RT 65	147.9	-	-	4.90	-	129.0
9	RT 68	144.3	1.90	1.42	4.36	152.0	139.1
10	RT-69	152.5	3.14	1.87	5.43	162.9	134.7
11	RT-74	150.6	1.56	1.61	4.79	158.6	133.2
12	RT-75	151.6	-	-	5.03	-	125.9
13	RT-76	126.2	0.77	1.59	5.11	133.7	143.4
14	RT-77	142.0	1.19	1.24	4.74	159.2	132.4
15	RT-90	146.8	0.61	0.46	5.03	152.9	134.1
16	RT-91	147.9	0.80	1.70	7.06	157.5	132.4
17	RT-92	144.7	1.11	1.73	4.50	152.1	126.8
18	RT-93	149.5	1.17	1.16	4.24	156.1	141.0
19	RT-94	-	2.23	1.69	-	-	135.1
20	RT-95	164.0	1.87	1.66	5.37	172.9	139.7
21	RT-96	-	2.78	1.66	5.32	-	130.8
22	RT-97	156.5	1.70	1.85	4.99	165.0	136.7
23	RT-116	133.9	1.99	1.69	-	-	125.4
24	RT 117	145.9	1.83	1.37	4.46	153.6	135.3
25	RT-119	141.5	1.49	1.62	4.94	149.6	133.5
26	RT-120	150.9	0.90	1.69	4.74	158.2	138.0
27	RT-121	123.7	0.64	1.21	3.90	129.4	120.7
Mean		144.2	1.43	1.36	5.02	151.6	133.0
Variance		105.13	0.4671	0.2403	0.3937	130.89	37.838
S. Dev.		10.253	0.6834	0.4902	0.6274	11.441	6.1513
S. Err.		2.0929	0.1395	0.1001	0.1255	2.553	1.1839
95% Confidence Interval	U	149.5	1.72	1.57	5.29	156.9	135.4
	L	139.8	1.14	1.16	4.75	146.2	130.6
Acclimated to 10°							
1	RT-66	165.1	1.17	1.68	5.43	173.4	129.8
2	RT 67	151.6	0.67	1.54	5.27	159.1	128.5
3	RT-70	144.3	2.94	1.62	5.38	154.2	138.4
4	RT-71	148.3	0.57	1.42	5.16	156.0	137.2
5	RT 72	147.9	1.09	1.68	4.95	155.6	129.8
6	RT-80	156.2	1.01	1.42	4.94	163.6	134.1
7	RT-81	153.6	1.20	1.51	5.22	161.5	131.6
8	RT-84	155.7	2.49	1.29	4.82	164.3	129.3
9	RT-85	160.0	0.76	1.90	5.39	168.0	132.7
10	RT-88	147.9	1.23	-	4.84	-	131.7
11	RT-89	153.2	0.88	1.58	-	-	130.5
12	RT-102	149.5	3.20	1.20	4.53	158.4	131.0
13	RT-103	153.2	1.95	1.60	-	-	136.8
14	RT-104	150.5	1.37	1.46	5.07	158.4	134.0
15	RT-105	156.4	1.40	1.57	-	-	131.3
16	RT-110	166.8	-	1.68	4.83	-	136.4
17	RT-111	154.1	2.03	1.60	5.16	162.9	135.2
18	RT-112	156.0	1.86	1.79	5.41	165.1	138.1
19	RT-113	155.4	1.21	1.77	5.41	163.8	137.3
20	RT-114	152.2	3.23	1.54	4.88	161.9	134.5
21	RT-115	144.6	1.83	1.35	5.16	152.9	127.6
Mean		153.5	1.60	1.56	5.10	161.2	133.1
Variance		33.180	0.6698	2.959	6.816×10^{-2}	28.818	11.334
S. Dev.		5.7602	0.8184	0.1720	0.2611×10^{-2}	5.3682	3.3667
S. Err.		1.2570	0.1830	3.846×10^{-2}	6.154×10^{-2}	1.3420	0.73466
95% Confidence Interval	U	156.1	1.99	1.64	5.23	164.1	134.7
	L	150.9	1.22	1.48	4.97	158.3	131.6
Acclimated to 18°							
1	RT-54	-	4.86	2.03	5.64	-	135.2
2	RT-55	160.1	5.56	1.86	4.63	172.2	133.2
3	RT-62	151.3	3.11	1.23	5.16	163.8	138.5
4	RT-63	156.1	2.35	1.75	5.19	165.4	136.8
5	RT-73	156.1	0.782	0.936	6.34	164.2	139.2
6	RT-78	146.1	-	1.73	4.55	-	134.1
7	RT-79	154.6	2.75	1.65	4.80	163.9	133.8
8	RT-82	153.6	2.99	1.76	4.90	163.1	133.5
9	RT-83	153.6	2.20	1.65	4.84	162.3	136.2
10	RT-86	155.2	3.63	1.54	-	-	129.0
11	RT 87	152.2	1.56	1.64	4.59	161.0	142.0
12	RT 9	152.6	2.20	1.94	5.57	162.6	128.8
13	RT 99	144.2	3.53	1.61	4.38	153.7	132.8
14	RT 100	147.9	2.96	1.87	4.22	157.0	126.2
15	RT-101	150.4	1.67	1.75	-	-	145.6
16	RT-106	160.0	2.83	1.65	4.40	168.9	135.7
17	RT-107	126.5	4.42	0.788	3.56	135.2	111.2
18	RT-16	141.5	1.65	1.74	5.44	150.3	137.3
19	RT-109	156.4	.42	1.71	4.64	165.2	134.5
20	RT-118	136.1	4.19	1.69	4.72	157.1	137.3
Mean		151.0	3.20	1.69	4.97	160.4	132.8
Variance		91.6	1.709	2.05	0.512	77.281	47.575
S. Dev.		7.75	1.311	1.341	0.7153	8.7914	6.899
S. Err.		1.73	0.5077	7.6×10^{-2}	0.1349	2.1061	1.551
95% Confidence Interval	U	154.3	4.26	1.92	5.19	165.0	136.0
	L	147.7	2.14	1.46	4.75	156.0	129.6

Appendix 8. Summary of plasma electrolytes and the sum of all cations (mEq/litre plasma) of thermally-acclimated carp (Cyprinus carpio). Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Signif. Diff.		
		2°C	16°C	30°C	2°C	16°C	30°C
Na^+	Spring	143±1.30	136±1.50	134±1.18	p<0.01	N.S.	p<0.01
K^+	Spring	3.90±0.441	4.55±0.432	4.02±0.153	N.S.	N.S.	N.S.
Mg^{+2}	Spring	2.80±6.79x 10^{-2}	2.29±8.35x 10^{-2}	2.47±0.120	p<0.01	N.S.	p<0.01
Ca^{+2}	Spring	3.70±0.116	4.30±0.125	4.22±0.130	p<0.01	N.S.	p<0.01
Cl^-	Spring	106±2.91	112±1.12	113±1.28	N.S.	N.S.	N.S.
Σ cations	Spring	154±1.67	147±1.40	145±1.24	p<0.01	N.S.	p<0.01

Appendix 9. Plasma electrolytes and the sum of all cations (meq/litre plasma) of thermally-acclimated carp (Cyprinus carpio).

Data Item	Code Number		Na^+	K^+	Mg^{+2}	Ca^{+2}	Σ cations	Cl^-
Acclimated to 2°								
1	CP-3		141.1	3.65	3.10	3.54	151.4	115.4
2	CP-6		140.5	2.77	3.04	3.98	152.3	105.8
3	CP-9		140.7	2.45	2.60	3.46	149.2	118.2
4	CP-12		-	2.10	2.37	3.24	-	85.7
5	CP-15		139.0	2.99	2.79	3.41	148.2	93.7
6	CP-18		141.2	-	2.85	2.97	-	113.5
7	CP-21		134.7	3.12	2.65	3.52	144.9	112.4
8	CP-24		149.1	2.86	2.75	3.71	158.7	89.6
9	CP-27		144.8	5.48	2.86	3.76	156.9	110.5
10	CP-30		148.0	3.69	3.15	3.90	158.7	110.1
11	CP-33		136.9	4.78	2.75	3.46	147.9	94.9
12	CP-36		143.5	3.81	2.86	3.84	154.0	109.5
13	CP 39		146.3	7.96	3.39	4.51	161.2	110.4
14	CP-42		150.3	5.08	2.81	4.51	182.7	119.6
Mean			142.9	3.90	2.86	3.70	153.8	106.4
Variance			21.860	2.533	6.450×10^{-2}	0.1891	33.288	118.59
S. Dev.			4.676	1.592	0.2539	0.4349	5.7695	10.890
S. Err.			1.297	0.4414	6.787×10^{-2}	0.1162	1.6655	2.9104
95% Confidence Interval		U	145.7	4.86	3.00	3.95	157.4	112.7
		L	140.1	2.94	2.71	3.45	150.2	100.1
Acclimated to 16°								
1	CP-2		134.2	5.31	2.44	3.77	145.7	111.5
2	CP-5		139.0	2.62	2.13	3.66	147.4	114.1
3	CP-8		136.4	4.59	2.97	4.20	148.2	110.0
4	CP-11		135.9	2.97	2.13	3.84	144.8	104.5
5	CP-14		127.8	2.99	2.22	4.91	137.9	-
6	CP-17		132.1	7.53	2.89	3.94	146.5	113.1
7	CP-20		134.1	4.17	2.22	5.00	145.5	116.1
8	CP-23		142.5	4.43	2.26	4.09	153.3	115.2
9	CP-26		128.5	5.86	2.49	4.66	141.5	104.8
10	CP-29		146.0	3.40	2.06	4.13	157.6	115.1
11	CP-32		135.5	4.78	2.01	3.84	146.1	114.6
12	CP-35		137.9	3.38	2.13	4.41	147.8	112.2
13	CP-38		138.3	7.08	2.24	4.96	152.6	118.1
14	CP-41		-	-	1.89	4.84	-	112.0
Mean			136.2	4.55	2.29	4.30	147.3	112.4
Variance			29.146	2.428	9.758×10^{-2}	0.2352	25.62	16.372
S. Dev.			5.3987	1.558	0.3124	0.4849	5.0611	4.0463
S. Err.			1.4973	0.4322	8.348×10^{-2}	0.1246	1.4037	1.1222
95% Confidence Interval		U	139.4	5.49	2.47	4.58	150.4	114.9
		L	132.9	3.61	2.11	4.02	144.2	110.0
Acclimated to 30°								
1	CP-1		132.6	3.80	1.89	3.82	142.1	110.2
2	CP-4		130.7	3.64	2.65	4.03	141.0	106.4
3	CP-7		138.8	3.94	2.61	4.15	149.1	118.8
4	CP-10		-	3.84	2.02	3.30	-	115.7
5	CP-13		133.2	3.94	2.47	3.94	143.6	114.1
6	CP-16		129.1	4.14	2.41	4.11	139.8	110.5
7	CP-19		127.2	-	3.10	4.09	-	112.2
8	CP-22		128.7	3.68	2.49	3.90	138.8	113.7
9	CP-25		134.1	3.86	2.44	4.30	144.7	119.8
10	CP-28		137.8	4.00	1.81	4.10	147.7	-
11	CP-31		141.5	3.09	2.63	4.05	152.2	108.2
12	CP-34		135.0	4.24	1.91	4.51	145.7	109.9
13	CP-37		136.9	4.67	2.83	5.19	149.6	107.7
14	CP-40		135.2	5.41	3.32	4.70	148.6	120.0
Mean			133.9	4.02	2.47	4.22	145.2	112.9
Variance			18.029	0.3651	0.2015	0.2359	18.344	21.382
S. Dev.			4.2457	0.5424	0.4489	0.4857	4.2830	4.6247
S. Err.			1.1775	0.1532	0.1200	0.1298	1.2364	1.2827
95% Confidence Interval		U	136.5	4.35	2.73	4.50	148.0	115.7
		L	131.3	3.69	2.21	3.94	142.5	110.1

Appendix 10. Summary of erythrocytic electrolytes and the sum of all cations (mEq/liter cells) of thermally-acclimated rainbow trout (Salmo gairdneri). Reported as the mean ± standard error of the mean.

Measurement	Season	Acclimation Temperature 2°C	10°C	18°C	Signif. Diff. 2°C	10°C	18°C
Na^{+}	Summer	38.5±2.53	40.0±3.65	33.9±1.83	N.S.	N.S.	N.S.
	Winter	40.4±1.78	35.0±1.33	34.5±1.49	p<0.05	N.S.	p<0.05
					*N.S.	N.S.	N.S.
K^{+}	Summer	77.4±1.58	83.5±1.58	91.6±1.18	p<0.05	p<0.01	p<0.01
	Winter	84.4±1.60	92.7±0.964	95.5±1.33	p<0.01	N.S.	p<0.01
					*p<0.01	p<0.01	p<0.05
Mg^{+2}	Summer	8.40±0.289	8.60±0.259	8.14±0.362	N.S.	N.S.	N.S.
	Winter	9.33±0.120	9.49±0.188	9.54±0.200	N.S.	N.S.	N.S.
					*p<0.01	p<0.01	p<0.01
Ca^{+2}	Summer	0.867±6.60x 10^{-2}	0.894±8.28x 10^{-2}	0.776±3.74x 10^{-2}	N.S.	N.S.	N.S.
	Winter	0.481±3.69x 10^{-2}	0.430±3.89x 10^{-2}	0.468±3.78x 10^{-2}	N.S.	N.S.	N.S.
					*p<0.01	p<0.01	p<0.01
Cl^{-}	Summer	78.6±1.02	80.2±1.27	81.2±1.65	N.S.	N.S.	N.S.
	Winter	75.7±1.51	76.5±1.78	77.2±1.72	N.S.	N.S.	N.S.
					*N.S.	N.S.	N.S.
Σ cations	Summer	128±2.92	132±2.73	135±1.27	N.S.	N.S	p<0.05
	Winter	135±1.49	138±1.65	140±1.80	N.S.	N.S.	p<0.05
					*p<0.05	N.S.	p<0.05

*Significant differences between seasons

Appendix II. Erythrocytic electrolytes and the sum of all cations (m Eq/litre cells) of thermally acclimated [illegible] rainbow trout (salmo gairdneri)

Data Item	Code Number		Na^+	K^+	Mg^{+2}	Ca^{+2}	Σ cations	Cl^-
Acclimated to 2°								
1	RT-6		40.6	80.6	8.75	0.559	130.4	75.0
2	RT-7		46.9	72.3	7.29	0.950	127.3	78.6
3	RT-12		16.5	72.9	-	1.162	-	-
4	RT-13		43.4	81.1	8.75	0.495	133.7	80.4
5	RT-14		40.6	84.1	9.10	0.306	134.6	82.5
6	RT-15		31.3	70.0	7.28	0.550	109.2	77.3
7	RT-22		28.5	88.5	9.93	0.752	127.7	70.8
8	RT-23		61.0	72.6	8.12	0.833	142.6	82.0
9	RT-24		44.0	78.9	6.48	1.067	132.4	88.3
10	RT-25		42.8	82.6	7.83	0.759	133.9	80.2
11	RT-26		42.3	79.2	9.23	0.611	131.3	78.9
12	RT-27		43.7	83.1	9.08	1.035	136.9	75.4
13	RT 28		29.6	68.7	-	-		79.6
14	RT-29		42.0	-		1.365	-	81.3
15	RT 30		29.8	69.1	6.11	1.169	106.2	74.4
16	RT-31		33.2	76.9	9.23	0.962	120.3	78.3
17	RT-32		-	-	-	-	-	75.0
Mean			38.5	77.4	8.40	0.867	128.2	78.7
Variance			102.1	37.52	1.088	6.531×10^{-2}	110.61	16.69
S. Dev.			10.10	6.125	1.043	0.2556	10.517	4.085
S. Err.			2.526	1.582	0.2893	6.598×10^{-2}	2.9170	1.021
95% Confidence Interval		U	43.9	80.8	9.03	1.01	134.5	80.8
		L	33.1	74.0	7.77	0.726	121.9	76.5
Acclimated to 10°								
1	RT-4		54.1	77.6	7.23	1.471	140.4	83.6
2	RT-5		50.5	78.2	7.81	0.670	137.2	86.6
3	RT-10		44.1	84.1	6.85	0.600	135.7	82.5
4	RT 11		44.8	83.7	8.68	1.115	129.6	80.1
5	RT-16		40.6	88.4	8.68	0.694	138.4	83.2
6	RT-17		29.7	86.1	8.81	0.626	125.2	84.7
7	RT-18		17.6	92.5	10.87	0.521	120.9	77.4
8	RT-19		31.2	93.6	9.33	0.818	134.9	77.2
9	RT-20		13.7	86.1	8.34	0.739	108.9	63.9
10	RT-?		30.6	76.5	7.23	0.746	121.1	80.0
11	RT-36		26..	86.2	10.05	1.096	123.4	75.3
12	RT 37		46.9	81.2	8.58	0.703	137.4	83.0
13	RT-38		43.2	82.6	9.34	0.695	140.8	80.7
14	RT-41		64.3	70.3	8.47	1.309	144.4	84.1
15	RT-42		-	-	8.36	1.626	-	77.2
16	RT-43		51.8	86.1	8.96	0.870	147.7	84.1
17	RT-44		-	-	-	-	-	80.4
Mean			40.0	83.5	8.60	0.894	132.4	80.2
Variance			200.2	37.66	1.074	0.1097	111.91	27.57
S. Dev.			14.15	6.137	1.036	0.3313	10.579	5.251
S. Err.			3.653	1.584	0.2590	8.283×10^{-2}	2.7314	1.274
95% Confidence Interval		U	47.8	86.9	9.15	1.07	138.3	82.9
		L	32.1	80.1	8.05	0.717	126.5	77.5
Acclimated to 18°								
1	RT-1		-	-	-	-	-	-
2	RT-2		23.9	99.2	6.60	0.586	130.3	81.6
3	RT-3		23.6	97.5	9.79	0.592	131.5	68.0
4	RT-8		30.7	98.8	8.34	0.793	138.6	97.5
5	RT-9		33.9	92.6	4.15	0.994	131.6	80.1
6	RT-33		29.1	91.3	8.59	0.757	130.1	80.5
7	RT-34		31.2	96.6	9.52	0.733	138.1	78.1
8	RT-35		32.9	88.8	9.40	0.800	131.9	78.9
9	RT-39		42.7	83.2	8.38	1.119	135.9	83.1
10	RT-40		40.8	90.3	9.16	1.004	141.3	-
11	RT 45		-	-	-	-	-	-
12	RT-46		-	-	-	-	-	-
13	RT-47		40.1	85.7	6.73	0.644	133.2	86.6
14	RT-48		22.5	91.4	8.36	0.755	123.0	77.8
15	RT-49		30.4	90.9	9.12	0.716	131.1	85.1
16	RT 50		44.2	85.7	7.04	0.713	137.7	81.2
17	RT-51		33.4	94.5	9.26	0.641	136.8	76.8
18	RT-52		41.6	90.3	7.49	0.766	143.2	84.8
19	RT-53		39.1	89.7	9.50	0.607	138.1	76.8
Mean			33.9	91.6	8.14	0.776	134.5	81.2
Variance			53.41	22.243	2.099	2.233×10^{-2}	25.921	40.59
S. Dev.			7.309	4.7216	1.449	0.1494	5.0912	6.371
S. Err.			1.677	1.1804	0.3672	3.760×10^{-2}	1.2728	1.645
95% Confidence Interval		U	37.8	94.1	8.91	0.856	137.2	84.7
		L	30.1	89.1	7.37	0.697	131.7	77.6

Appendix I. Body electrolyte values of the [illegible] (milliequivalents per liter cell) of [illegible] acclimated to various temperatures (time constant)

Data No.	Code Number	Na^+	K^+	Mg^{++}	Ca^{++}	Cations	Cl^-
Acclimated to [illegible]							
1	RT-51	-	-	-	0.570		72.2
2	RT-52	35.7	68.1	9.41	0.345	153.4	80.7
3	RT-53	43.9	86.7	10.50	0.197	141.6	66.7
4	RT-54	49.5	80.0	9.83	0.905	138.3	81.6
5	RT-60	31.2	92.1	9.01	0.111	133.8	76.9
6	RT-61	30.7	99.2	8.19	0.159	133.3	96.6
7	RT-62	-	80.1	9.16	-	-	75.4
8	RT-63	-	-	-	0.678	-	-
9	RT-65	26.8	107.2	9.35	0.293	143.7	74.4
10	RT-69	40.7	97.0	8.73	0.415	146.8	82.5
11	RT-74	37.1	87.9	10.10	0.205	135.9	61.6
12	RT-75	-	-	-	0.307	-	73.9
13	RT-76	51.4	77.3	9.72	0.424	138.8	69.6
14	RT 77	39.6	85.2	9.72	0.290	134.3	65.7
15	RT-90	34.8	86.2	9.08	0.657	130.7	75.4
1	RT-91	41.4	84.9	9.59	0.660	136.6	81.1
17	RT-92	30.7	84.6	9.37	0.464	125.1	78.4
18	RT 93	51.2	78.3	9.09	0.655	139.2	81.2
19	RT-94	-	78.9	10.51	-	-	67.8
20	RT-95	43.9	81.4	8.45	0.247	134.0	88.0
21	RT-96	-	76.0	9.16	0.636	-	76.8
22	RT-97	-	85.8	8.64	-	-	82.1
23	RT-116	30.1	78.8	9.07	0.315	118.3	73.8
24	RT-117	48.8	76.5	8.71	0.303	134.3	76.6
25	RT-119	47.4	79.9	9.16	0.457	136.9	72.6
26	RT-120	50.7	79.8	9.91	0.434	140.8	75.3
27	RT-121	43.4	72.2	9.78	0.516	125.9	63.2
Mean		40.4	84.4	9.33	0.481	135.3	76.7
Variance		63.01	65.91	0.3152	3.263×10^{-2}	44.285	59.39
S. Dev.		7.938	8.118	0.5875	0.1807	6.6546	7.707
S. Err.		1.775	1.657	0.1199	3.688×10^{-2}	1.4880	1.511
95% Confidence Interval	U	44.1	87.8	9.57	0.557	138.4	78.8
	L	36.7	80.9	9.08	0.404	132.2	72.6
Acclimated to 10°							
1	RT-66	39.6	91.2	9.16	0.386	140.3	72.4
2	RT-67	37.8	95.2	10.95	0.790	144.7	80.4
3	RT-70	37.6	91.5	8.64	0.490	138.2	79.6
4	RT-71	36.7	90.9	9.59	0.369	141.6	77.4
5	RT-72	34.8	103.2	7.32	0.466	145.8	73.5
6	RT-80	45.5	93.5	10.01	0.386	149.4	82.1
7	RT 81	38.3	94.5	9.34	0.318	142.5	78.0
8	RT 84	34.5	97.3	9.04	0.467	141.3	83.6
9	RT-85	34.0	96.7	9.70	0.477	140.9	86.0
10	RT-88	-	96.7	-	0.390	-	66.7
11	RT-89	-	90.8	9.90	-	-	77.7
12	RT-102	22.7	95.4	11.30	0.116	129.5	59.1
13	RT-103	-	89.7	9.75	-	-	89.0
14	RT-104	35.6	91.6	10.09	0.396	137.7	74.9
15	RT-105	-	84.9	9.49	-	-	72.2
16	RT-110	-	-	8.73	0.260	-	84.8
17	RT-111	31.1	95.3	10.03	0.809	135.2	75.1
18	RT-112	31.1	89.7	9.13	0.275	130.2	84.1
19	RT-113	40.6	88.0	9.03	0.411	138.0	85.8
20	RT 114	31.4	86.2	9.10	0.504	127.2	71.8
21	RT-115	29.0	88.8	9.53	0.431	127.8	72.2
Mean		35.0	92.7	9.49	0.430	138.1	76.5
Variance		28.23	18.57	0.7083	2.713×10^{-2}	43.660	66.20
S. Dev.		5.313	4.310	0.8416	0.1647	6.6070	8.136
S. Err.		1.328	0.9637	0.1882	3.882×10^{-2}	1.6519	1.775
95% Confidence Interval	U	37.8	94.7	9.89	0.512	141.7	80.2
	L	32.2	90.6	9.10	0.348	134.6	72.8
Acclimated to 18°							
1	RT-54	-	96.2	9.65	0.593	-	68.2
2	RT-55	29.5	90.0	9.19	0.438	129.1	86.9
3	RT-64	27.2	105.2	9.58	0.393	142.4	66.5
4	RT-68	38.0	98.1	9.17	0.434	145.7	80.4
5	RT 73	50.2	91.7	9.55	0.372	151.8	90.8
6	RT-78	26.8	-	8.34	0.446	-	82.8
7	RT-79	27.2	101.4	9.25	0.416	138.3	73.3
8	RT-82	30.0	102.6	9.82	0.582	148.0	72.2
9	RT-83	35.6	99.5	8.57	0.971	144.6	76.5
10	RT-86	28.7	104.4	9.15	-	-	69.7
11	RT-87	42.3	97.4	9.51	0.509	143.7	85.3
12	RT-98	36.5	91.3	8.54	0.382	136.7	74.0
13	RT-99	37.5	96.6	10.44	0.445	141.0	76.2
14	RT-100	42.2	89.5	9.64	-		73.8
15	RT-101	27.7	89.3	10.30	0.332	127.6	67.6
16	RT-106	27.7	95.9	10.23	0.315	134.2	76.1
17	RT 107	41.4	94.6	11.98	0.245	137.0	66.2
18	RT-108	35.1	99.1	9.45	0.652	144.6	82.9
19	RT-109	[illegible]	90.6	8.[illegible]	0.498	132.4	84.9
20	RT[illegible]	32.2	92.7	11.77	0.603	145.2	74.6
Mean		34.5	96.[illegible]	9.84	0.469	140.	77.2
Variance		47.08	27.4	0.03	2.96×10^{-2}	57.115	52.19
S. Dev.		6.[illegible]	5.[illegible]	0	0.[illegible]	7.[illegible]	7.[illegible]
S. Err.		1.46	1.[illegible]	0	$3.[illegible] \times 10^{-2}$	1.[illegible]	1.726
95% Confidence Interval	U	37.[illegible]	98	9.[illegible]	0.[illegible]	143.[illegible]	80.[illegible]
	L	31.[illegible]	93.[illegible]	9.[illegible]	0.[illegible]	136.[illegible]	73.[illegible]

Appendix 13. Summary of erythrocytic electrolytes and the sum of all cations (mEq/litre cells) of thermally-acclimated carp (Cyprinus carpio). Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Signif. Diff.		
		2°C	16°C	30°C	2°C	16°C	30°C
Na^+	Spring	*0.07	1.67±0.561	8.77±0.843	**	p<0.01	**
K^+	Spring	84.5±1.68	82.1±2.27	87.8±1.52	N.S.	p<0.05	N.S.
Mg^{+2}	Spring	16.2±0.357	16.8±0.312	11.9±0.466	N.S.	p<0.01	p<0.01
Ca^{+2}	Spring	0.740±7.56x 10^{-2}	0.699±8.33x 10^{-2}	0.719±4.06x 10^{-2}	N.S.	N.S.	N.S.
Cl^-	Spring	45.1±0.846	58.4±1.38	72.5±0.990	p<0.01	p<0.01	p<0.01
Σ cations	Spring	102±1.79	102±2.58	110±1.86	N.S.	p<0.05	p<0.01

*mean

**proper analysis not possible

Appendix 10 Erythrocytic electrolyte and the sum of all cations (meq/litre cells) of thermally acclimated carp (cyprinus carpio).

Data Item	Code Number		Na^+	K^+	Mg^{+2}	Ca^{+2}	Σ cations	Cl^-
Acclimated to 2°								
1	CP-3		0	77.1	14.68	0.870	92.7	50.7
2	CP-6		0	86.8	15.38	0.765	103.0	43.5
3	CP-9		0	92.2	16.81	0.615	109.6	43.2
4	CP-12		0	89.35	17.11	0.721	107.2	41.9
5	CP-15		0	83.4	17.42	1.455	102.3	41.4
6	CP-18		0	95.1	17.63	0.538	113.3	42.9
7	CP-21		0	88.1	16.61	0.680	105.4	45.1
8	CP-24		0	79.4	16.08	0.641	96.2	45.4
9	CP-27		0.861	84.6	15.99	0.620	102.1	49.8
10	CP-30		0	81.5	16.31	0.583	98.4	49.0
11	CP-33		0	86.9	17.01	0.452	104.4	43.4
12	CP-36		0	80.4	16.39	0.441	97.3	46.8
13	CP-39		-	-	12.38	0.684	-	-
14	CP-42		0	73.8	16.40	1.236	91.5	43.6
Mean			0.07	84.5	16.16	0.740	101.8	45.1
Variance				36.61	1.7888	7.991×10^{-2}	41.485	9.309
S. Dev.				6.050	1.3374	0.2827	6.4409	3.051
S. Err.				1.678	0.35745	7.555×10^{-2}	1.7864	0.3462
95% Confidence Interval		U		88.2	16.93	0.903	105.7	47.0
		L		80.9	15.39	0.577	97.90	43.3
Acclimated to 16°								
1	CP-2		-	-	14.56	-	-	65.4
2	CP-5		2.28	85.1	16.95	0.676	105.0	55.3
3	CP-8		0	76.1	15.56	0.477	92.2	65.6
4	CP-11		0.193	88.1	17.11	0.605	106.0	53.0
5	CP-14		2.61	92.9	17.07	0.528	113.1	57.6
6	CP-17		3.73	76.7	16.15	0.504	97.1	62.0
7	CP-20		6.26	91.2	16.21	0.552	114.2	63.4
8	CP-23		2.59	85.7	17.03	0.611	106.0	59.3
9	CP-26		0.079	75.2	16.00	0.575	89.9	59.9
10	CP-29		0	86.9	18.46	0.590	106.0	56.3
11	CP-32		1.46	86.9	18.34	0.664	107.4	48.8
12	CP-35		0	73.0	17.80	1.319	92.1	59.0
13	CP-38		0.761	69.8	17.72	1.288	89.5	61.6
14	CP-41		-	-	-	-	-	50.8
Mean			1.67	82.1	16.84	0.699	101.5	58.4
Variance			3.777	62.05	1.2633	8.319×10^{-2}	79.764	26.6299
S. Dev.			1.943	7.877	1.1240	0.2884	8.9311	5.1604
S. Err.			0.5610	2.274	0.31173	8.326×10^{-2}	2.5782	1.3792
95% Confidence Interval		U	2.90	87.1	17.52	0.882	107.2	61.4
		L	0.430	77.1	16.16	0.516	95.9	55.4
Acclimated to 30°								
1	CP-1		-	75.4	14.19	-	-	72.5
2	CP-4		8.55	86.2	11.10	0.711	106.5	71.6
3	CP-7		13.10	85.8	11.84	0.747	111.6	75.2
4	CP-10		6.57	94.8	11.12	0.752	113.2	71.1
5	CP-13		5.88	90.9	10.66	0.635	108.1	77.0
6	CP-16		6.42	94.0	11.19	0.892	112.5	74.3
7	CP-19		6.48	80.9	10.07	0.630	98.1	70.0
8	CP-22		15.29	88.1	12.73	0.898	117.0	69.9
9	CP-25		10.45	92.8	12.12	0.695	116.1	76.9
10	CP-28		9.42	93.3	11.95	0.565	115.2	72.7
11	CP-31		8.48	89.9	14.36	0.671	113.4	67.5
12	CP-34		-	90.8	13.77	0.552	-	66.5
13	CP-37		7.52	82.6	12.92	0.566	103.6	70.9
14	CP-40		7.03	83.1	7.87	1.035	99.1	79.5
Mean			8.77	87.8	11.85	0.719	109.5	74.5
Variance			9.519	32.49	3.0323	2.156×10^{-2}	41.660	13.73
S. Dev.			2.911	5.700	1.7414	0.1462	6.4544	3.705
S. Err.			0.8426	1.523	0.4659	4.056×10^{-2}	1.8632	0.9903
95% Confidence Interval		U	10.63	91.1	12.85	0.868	113.6	76.7
		L	6.92	84.5	10.84	0.641	105.4	70.4

Appendix 15. Summary of erythrocytic electrolytes and the sum of all cations (mEq/litre cell H_2O) of thermally-acclimated rainbow trout (*Salmo gairdneri*). Reported as the mean ± standard error of the mean.

Measurement	Season	Acclimation Temperature 2°C	10°C	18°C	Signif. Diff. 2°C	10°C	18°C
Na^+	Summer	48.9±3.14	50.1±4.57	43.6±2.29	N.S.	N.S.	N.S.
	Winter	50.5±2.14	44.8±1.68	44.2±1.80	N.S.	N.S.	p<0.05
					*N.S.	N.S.	N.S.
K^+	Summer	98.6±2.66	104.9±2.30	118.5±1.875	N.S.	p<0.01	p<0.01
	Winter	105±2.48	118±1.46	123±2.28	p<0.01	N.S.	p<0.01
					*N.S.	p<0.01	N.S.
Mg^{+2}	Summer	10.7±0.487	10.8±3.15	10.5±0.482	N.S.	N.S.	N.S.
	Winter	11.6±1.71	12.1±0.241	12.2±0.276	N.S.	N.S.	N.S.
					*p<0.05	p<0.01	N.S.
Ca^{+2}	Summer	1.11±0.090	1.12±0.107	0.997±0.045	N.S.	N.S.	N.S.
	Winter	0.603±0.047	0.548±0.049	0.599±0.049	N.S.	N.S.	N.S.
					*p<0.01	p<0.01	p<0.01
Cl	Summer	100±2.20	101±1.61	105±2.39	N.S.	N.S.	N.S.
	Winter	94.3±1.89	97.2±2.12	99.0±2.19	N.S.	N.S.	N.S.
					*p<0.05	N.S.	N.S.
Σ cations	Summer	163±4.49	166±3.46	173±1.98	N.S.	N.S.	p<0.05
	Winter	170±2.33	177±2.28	177±3.18	p<0.01	N.S.	N.S.
					*N.S.	p<0.05	N.S.

*significant differences between seasons

Appendix 1n. Erythrocyte water (litre H_2O/litre cells), erythrocytic electrolyte, and the sum of all cations (mEq/litre cell H_2O) of thermally-acclimated summer rainbow trout (Salmo gairdneri).

Data Item	Code Number	Erythrocyte Water	Na^+	K^+	Mg^{+2}	Ca^{+2}	Σ cations	Cl^-
Acclimated to 2°								
1	RT-6	0.775	52.4	103.9	11.29	0.721	168.3	96.8
2	RT-7	0.765	61.3	94.5	9.53	1.110	166.4	102.7
3	RT-12	0.788	70.9	92.5	-	1.470	-	-
4	RT-13	0.796	64.5	101.9	10.99	0.621	168.0	101.0
5	RT-14	0.764	51.0	105.7	11.43	1.013	169.1	103.6
6	RT-15	0.796	39.3	87.9	9.15	0.729	137.2	97.7
7	RT-22	0.826	34.5	107.1	12.02	0.910	154.6	85.7
8	RT-23	0.806	75.7	90.1	10.07	1.039	176.9	101.7
9	RT-24	0.847	61.9	93.2	10.01	1.259	156.3	104.3
10	RT-25	0.806	53.1	102.5	9.71	0.942	166.1	99.5
11	RT-26	0.838	50.5	94.5	11.01	0.729	156.7	94.2
12	RT-27	0.797	54.8	101.3	11.39	1.299	171.8	94.6
13	RT-28	0.788	37.6	87.2	-	-	-	101.0
14	RT-29	0.788	53.3	-	-	1.732	-	103.2
15	RT-30	0.783	37.8	87.7	7.75	1.484	134.8	94.4
16	RT-31	0.611	54.3	125.8	15.10	1.574	196.9	128.1
17	RT-32	0.765	-	-	-	-	-	98.0
Mean		0.783	48.9	98.6	10.73	1.109	163.3	100.4
Variance		3.432×10^{-3}	157.8	106.0	3.0808	0.12093	262.17	77.285
S. Dev.		5.858×10^{-2}	12.56	10.30	1.7549	0.34775	16.192	8.7912
S. Err.		1.625×10^{-2}	3.140	2.659	0.48673	8.9768×10^{-2}	4.4908	2.1978
95% Conf. Interval	U	0.819	55.6	104.3	11.79	1.301	173.1	105.1
	L	0.749	42.2	92.9	9.66	0.916	153.5	95.7
Acclimated to 10°								
1	RT-4	0.788	68.7	98.5	9.18	1.867	178.2	106.1
2	RT-5	0.836	60.4	93.5	9.34	0.801	164.1	103.6
3	RT-10	0.795	55.5	105.8	8.62	0.755	170.7	103.8
4	RT-11	0.764	58.6	109.5	11.36	1.459	169.6	104.8
5	RT-16	0.755	53.8	117.1	11.50	0.919	183.3	110.2
6	RT-17	0.800	37.1	107.6	11.01	0.783	156.5	105.9
7	RT-18	0.805	21.1	114.9	13.50	0.633	150.2	96.1
8	RT-19	0.795	39.2	117.7	11.73	1.029	169.7	97.1
9	RT-20	0.764	17.9	112.7	10.91	0.967	142.5	83.6
10	RT-21	0.785	46.6	97.5	9.21	0.950	154.3	101.9
11	RT-36	0.817	31.9	105.5	12.30	1.341	151.8	92.2
12	RT-37	0.826	56.8	98.3	10.39	0.851	166.3	100.5
13	RT-33	0.857	56.2	96.4	10.90	0.811	164.3	94.2
14	RT-41	0.788	81.6	89.2	10.75	1.661	183.2	106.7
15	RT-42	0.788	-	-	10.61	2.063	-	98.0
16	RT-43	0.788	65.7	109.3	11.37	1.104	187.4	106.7
17	RT-44	0.788	-	-	-	-	-	102.0
Mean		0.799	50.1	104.9	10.79	1.124	166.1	100.8
Variance		8.798×10^{-4}	313.9	79.320	1.5895	0.18388	170.37	44.040
S. Dev.		2.966×10^{-2}	17.716	8.9062	1.2607	0.42881	13.393	6.636
S. Err.		8.227×10^{-3}	4.5742	2.2996	0.31518	0.10720	3.4580	1.6095
95% Conf. Interval	U	0.817	59.9	109.8	11.46	1.353	173.5	104.2
	L	0.781	40.3	100.0	10.12	0.896	158.7	97.4
Acclimated to 18°								
1	RT-1	-	-	-	-	-	-	-
2	RT-2	0.788	30.3	125.9	8.33	0.744	165.3	103.6
3	RT-3	0.786	30.0	124.0	12.46	0.753	167.3	86.5
4	RT-8	0.764	40.2	129.3	10.92	1.038	181.4	127.6
5	RT-9	0.807	42.0	114.7	5.14	1.231	163.1	99.3
6	RT-33	0.797	36.5	114.6	11.17	0.950	163.2	101.0
7	RT-34	0.756	39.2	121.4	11.96	0.920	173.5	98.1
8	RT-35	0.769	41.3	121.7	11.03	1.015	167.4	100.1
9	RT-39	0.794	53.8	104.8	11.18	1.409	171.1	104.7
10	RT-40	0.809	50.4	111.6	11.32	1.241	174.6	-
11	RT-45	-	-	-	-	-	-	-
12	RT-46	-	-	-	-	-	-	-
13	RT-47	0.798	50.9	108.8	8.54	0.817	169.0	109.9
14	RT-48	0.773	30.7	124.7	11.41	1.030	167.8	106.1
15	RT-49	0.735	41.4	123.7	12.41	0.974	178.4	115.8
16	RT-50	0.807	54.8	106.2	8.72	0.934	170.6	101.4
17	RT-51	0.755	44.2	125.2	10.94	0.849	181.2	101.7
18	RT-52	0.755	59.1	119.6	9.92	1.014	189.7	112.3
19	RT-53	0.750	62.1	115.6	11.35	1.076	184.1	102.4
Mean		0.781	43.6	118.5	10.4	0.997	173.0	104.7
Variance		9.219×10^{-4}	84.14	56.238	.7135	3.236×10^{-2}	62.764	65.68
S. Dev.		3.036×10^{-2}	9.172	7.4991	1.9273	0.1813	7.9224	8.107
S. Err.		7.940×10^{-3}	2.293	1.9746	0.43176	4.232×10^{-2}	1.9506	2.390
95% Conf. Interval	U	0.793	46.47	122.5	11.31	1.03	177.2	109.8
	L	0.754	41.70	114.5	9.46	0.900	168.8	99.6

Appendix I. Erythrocyte osmotic fragility (in NaCl solution) and plasma electrolyte concentrations of the blood of individuals (acclimated to 0°, 10° and 18°C) of the rainbow trout (Salmo gairdneri).

Data Item	Fish Number	Erythrocyte Index	Na^+	K^+	Mg^{++}	Ca^{++}	Σ cation	Cl^-
Acclimated to 0°								
1	RT-56	0.806	-	-		0.707	-	89.6
2	RT-57	0.817	43.1	103.2	11.55	0.472	163.3	91.8
3	RT-58	0.791	55.5	103.6	13.27	0.628	179.0	84.3
4	RT-59	0.779	60.9	101.4	11.77	1.159	174.2	102.4
5	RT-60	0.791	30.4	116.4	11.73	0.530	167.9	97.2
6	RT-61	0.776	39.6	127.8	10.55	0.244	178.2	124.5
7	RT-64	0.786	-	101.9	11.65	-	-	95.9
8	RT-65	0.745	-	-	-	0.843	-	-
9	RT-53	0.741	36.0	141.0	12.57	0.422	193.1	100.0
10	RT-70	0.827	49.2	117.3	10.56	0.503	177.5	99.8
11	RT-74	0.791	46.9	111.1	12.77	1.015	171.8	77.9
12	RT-75	0.796	-	-	-	0.886	-	92.8
13	RT-76	0.806	63.6	95.9	12.06	0.526	172.2	85.1
14	RT-77	0.786	50.4	108.4	12.37	0.369	171.5	83.6
15	RT-80	0.756	46.0	114.0	12.01	0.869	172.9	99.7
16	RT 81	0.848	48.8	100.1	11.31	0.602	161.1	95.6
17	RT-82	0.827	37.1	102.3	11.33	0.561	151.3	94.8
18	RT-33	0.786	65.1	99.6	11.55	0.833	177.1	102.3
19	RT-91	0.807	-	97.6	13.02	-	-	84.0
20	RT-95	0.827	53.1	98.4	10.22	0.299	162.0	106.4
21	RT-96	0.827	-	91.9	11.08	0.769	-	92.9
22	RT 97	0.847	-	101.3	10.20	-	-	96.9
23	RT-116	0.786	38.3	100.3	11.54	0.401	150.5	93.9
24	RT-117	0.817	59.7	93.6	10.66	0.371	164.4	93.8
25	RT-119	0.765	60.4	101.8	11.67	0.582	174.4	92.5
26	RT-120	0.806	62.9	99.0	12.30	0.538	174.7	93.4
27	RT-121	0.816	53.2	88.5	11.99	0.632	154.3	77.5
Mean		0.801	50.5	105.4	11.64	0.603	169.6	94.5
Variance		7.873x10-4	91.94	148.03	0.70082	5.236x10-2	108.58	92.93
S. Dev.		2.806x10-2	9.588	12.167	0.83715	0.2288	10.420	9.640
S. Err.		5.728x10-3	2.144	2.4835	0.17083	4.671x10-2	2.3300	1.891
95% Conf. Interval	U	0.813	55.0	110.5	12.00	0.700	174.4	98.4
	L	0.789	46.0	100.3	11.29	0.507	164.7	90.6
Acclimated to 10°								
1	RT-66	0.791	50.1	115.3	11.58	0.488	177.4	91.5
2	RT-67	0.817	46.3	116.5	13.40	0.967	177.1	98.4
3	RT 70	0.755	49.8	121.2	11.44	0.660	183.0	105.4
4	RT-71	0.796	48.6	125.3	12.70	0.489	187.5	102.5
5	RT-72	0.797	43.7	128.5	9.18	0.595	182.9	98.5
6	RT-60	0.791	57.5	118.2	12.65	0.491	188.9	103.8
7	RT-81	0.764	50.1	123.7	12.22	0.416	186.5	102.1
8	RT-84	0.755	45.7	128.9	11.97	0.605	187.2	77.6
9	RT 85	0.785	43.3	123.2	12.35	0.608	179.5	109.6
10	RT 88	0.806	-	120.0	-	0.484	-	82.8
11	RT-89	0.786	-	115.5	12.60	-	-	98.9
12	RT 102	0.765	29.7	124.7	14.77	0.152	169.3	77.3
13	RT-103	0.816	-	109.9	11.95	-	-	109.1
14	RT-104	0.817	43.6	112.1	12.35	0.485	168.5	91.7
15	RT-105	0.796	-	106.7	11.92	-	-	90.7
16	RT-110	0.806	-	-	10.83	0.326	-	105.2
17	RT-111	0.786	39.6	118.7	12.76	1.029	172.0	95.5
18	RT-112	0.796	39.1	112.7	11.47	0.345	163.6	105.6
19	RT-113	0.790	51.4	111.4	11.43	0.520	174.7	108.6
20	RT-114	0.745	42.1	115.7	12.21	0.677	170.7	96.4
21	RT-115	0.806	36.0	110.2	11.82	0.535	158.6	89.7
Mean		0.789	46.8	118.0	12.08	0.548	176.7	97.2
Variance		4.920x10-4	45.40	42.330	1.1662	4.285x10-2	82.760	94.06
S. Dev.		2.218x10-2	6.736	6.5100	1.0799	0.2070	9.0933	9.698
S. Err.		5.089x10-3	1.684	1.4557	0.24147	4.879x10-2	2.2746	2.116
95% Conf. Interval	U	0.799	48.4	121.0	12.59	0.651	181.6	101.6
	L	0.778	41.2	114.9	11.58	0.445	171.9	92.8
Acclimated to 18°								
1	RT-54	0.796	-	120.9	12.37	0.751	-	85.7
2	RT-55	0.786	37.5	114.5	11.69	0.567	164.2	110.6
3	RT-62	0.765	35.6	127.5	12.52	0.514	186.1	86.9
4	RT-63	0.744	51.1	131.9	12.33	0.593	145.7	103.1
5	RT-73	0.800	62.8	114.6	11.94	0.465	189.8	113.5
6	RT-78	0.791	33.9	-	10.54	0.504	-	104.7
7	RT-79	0.734	37.1	130.1	12.60	0.567	188.4	99.9
8	RT-87	0.806	44.7	127.3	10.94	0.722	183.6	89.6
9	RT-83	0.756	46.6	136.2	11.22	1.271	189.3	100.1
10	RT 86	0.735	39.0	142.0	12.45	-	-	93.5
11	RT-87	0.826	51.2	117.3	11.51	0.616	181.2	103.3
12	RT-98	0.817	43.5	111.3	10.45	0.463	166.7	93.0
13	RT 99	0.765	43.8	126.3	13.65	0.562	184.3	102.2
14	RT 100	0.776	53.0	111.2	12.11	-	-	92.7
15	RT 101	0.796	34.5	112	12.56	0.416	160.5	95.0
16	RT 108	0.791	35.0	121.2	13.06	0.293	169.7	93.7
17	RT-107	0.791	52.0	107.6	13.04	0.319	172.9	93.7
18	RT 109	0.757	44.4	12	11.65	0.732	181.4	111.5
19	RT 100	0.755	43.8	121.0	11.02	0.190	176.4	102.5
20	RT 118	0.776	40.6	122.6	15.59	0.611	199.7	104.4
Mean		0.772	44.2	122.1	12.24	0.515	176.8	99.0
Variance		3.166x10-4	61.1	51.1	1.3[illegible]	7.351x10-	111.1	95.05
S. Dev.		2.[illegible]x10-	7.[illegible]	7.[illegible]	1.[illegible]	0.[illegible]	10.721	9.74
S. Err.		5.[illegible]	1.77	2.[illegible]	0.[illegible]	9.17[illegible]x10-2	2.91	2.2
95% Conf. Interval	U	[illegible]	[illegible]	127.[illegible]	12.[illegible]	0.[illegible]	182.6	103.[illegible]
	L	0.[illegible]	40.[illegible]	117.[illegible]	11.[illegible]	[illegible]	170.[illegible]	95.[illegible]

Appendix 18. Summary of erythrocytic electrolytes and the sum of all cations (mEq/litre cell H_2O) of thermally-acclimated carp (Cyprinus carpio). Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Signif. Diff.		
		2°C	16°C	30°C	2°C	16°C	30°C
Na^+	Spring	*0.08	2.23±0.704	11.6±1.09	**	p<0.01	**
K^+	Spring	112±2.19	110±3.58	116±2.45	N.S.	N.S.	N.S.
Mg^{+2}	Spring	21.3±0.620	22.5±0.566	15.7±0.651	N.S.	p<0.01	p<0.01
Ca^{+2}	Spring	0.976±0.109	0.940±0.118	0.950±0.056	N.S.	N.S.	N.S.
Cl^-	Spring	60.0±1.05	77.8±1.89	96.0±1.62	p<0.01	p<0.01	p<0.01
Σ cations	Spring	134±2.43	136±4.15	145±3.20	N.S.	N.S.	p<0.05

*mean
**proper analysis not possible

Appendix 19 Erythrocytic water (litre H_2O/litre cells), erythrocytic electrolytes and the sum of all cations (μeq/litre cell H_2O) of thermally acclimated carp (*Cyprinus carpio*).

Data Item	Code Number	Erythrocyte Water	Na^+	K^+	Mg^{+2}	Ca^{+2}	Σ cations	Cl^-
Acclimated to ?								
1	CP-3	0.756	0	102.1	19.44	1.150	122.7	67.2
2	CP-6	0.765	0	113.5	20.10	1.000	134.6	56 9
3	CP-9	0.744	0	123.9	22.60	0.827	147.3	58.1
4	CP-12	0.735	0	121.6	23.27	0.981	145 9	62.4
5	CP 15	0.754	0	110.7	23.10	1.930	135 7	54.9
6	CP-18	0.785	0	121.1	22.46	0.762	144.3	54.6
7	CP-21	0.754	0	116.9	22.03	0.901	139.8	59.8
8	CP-24	0.785	0	101.2	20.48	0.817	122.5	57.8
9	CP-27	0.795	1.08	106 4	20.11	0.780	128.4	62.6
10	CP-30	0.764	0	106.6	21.34	0.763	128.7	64.1
11	CP-33	0.744	0	116.8	22.86	0.608	140.3	58.3
12	CP-36	0.786	0	102.3	20.85	0.561	123.7	59.4
13	CP-39	0.837	-	-	14.79	0.817	-	-
14	CP-42	0.683	0	108.1	24.01	1.81	133.9	63.8
Mean		0.763	0.08	111.6	21 25	0.979	134.4	60.0
Variance		1.241×10^{-3}		62.522	5.3845	0.1655	77.006	14.34
S. Dev.		3.523×10^{-2}		7.9071	2.3204	0.4069	8.7753	3.787
S. Err.		9.415×10^{-3}		2.1930	0.62017	0.1087	2.4338	1.050
95? Conf. Interval	U	0.784		116.4	22.59	1.214	139.7	62.3
	L	0.743		106.8	19.91	0.744	129.1	57.7
Acclimated to 16?								
1	CP-2	0.756	-	-	19.26	-	-	86.5
2	CP-5	0.734	3.11	116.0	23.09	0.921	143.1	75.3
3	CP-8	0.806	0	94.45	19.30	0.592	114.4	81.4
4	CP-11	0.713	0.271	123.6	24.00	0.849	148.7	74.3
5	CP-14	0.744	3.51	124.8	22.94	0.710	152.0	77.4
6	CP-17	0.796	4.69	96.4	20.29	0.633	122.0	77.9
7	CP-20	0.764	8.22	119.3	21.21	0.722	149.2	83.0
8	CP-23	0.685	3.79	125.5	24.93	0.694	155.2	86.8
9	CP-26	0 755	0.11	96.97	21.19	0.761	119.0	79.3
10	CP-29	0.785	0	110.8	23.51	0.752	135.0	68.3
11	CP-32	0.756	1.93	114.9	24.26	0.878	142.0	64.5
12	CP-35	0.756	0	96.5	23.54	1.740	121.8	78.0
13	CP-38	0.704	1.08	99.11	25 17	1.830	127.2	87.5
14	CP-41	0.734	-	-		-	-	69.2
Mean		0.748	2.23	109.9	22.51	0.940	135.8	77.8
Variance		1.382×10^{-3}	5.953	153.50	4.1667	0.1664	207.07	50.22
S. Dev.		3.717×10^{-2}	2.440	12.390	2.0117	0.4079	14.390	7 086
S. Err.		1.073×10^{-2}	0.7044	3.5766	0.56627	0.1177	4.1540	1.894
95% Conf. Interval	U	0.771	3.78	117.7	23.75	1.199	144.9	81.9
	L	0.724	0.68	102.0	21.28	0.681	126.7	73.7
Acclimated to 30?								
1	CP-1	0.734	-	102.7	19.33	-	-	98.8
2	CP-4	0.781	10.94	110.3	14.21	0.910	136.4	91.7
3	CP-7	0.764	17.22	112.3	15.50	0.978	146.0	98.4
4	CP-10	0.713	9.21	132.9	15.60	1.050	158 8	99.7
5	CP-13	0.755	7.79	120 4	14.11	0.841	143.2	102.0
6	CP-16	0.755	8.50	124.5	14.82	1.180	149.0	98.4
7	CP-19	0.780	8.30	103.8	12.91	0.909	125.8	89.7
8	CP-22	0.774	19.75	113.9	16.44	1.160	151.2	90.3
9	CP-25	0.727	17.43	128.2	16.74	0.900	160.4	106.2
10	CP-28	0.775	12 13	120.4	15 42	0.723	146.6	93.8
11	CP-31	0.735	11.54	122.3	10.54	0.913	154.3	91.8
12	CP-34	0.765	-	118.7	16.60	0 722	-	86.9
13	CP-37	0.776	9.69	106.5	16.64	0 729	123 5	91.4
14	CP-40	0.756	9.80	110.0	10.41	1 870	131.1	105.2
Mean		0.756	11.57	116 2	15 69	0 950	144.9	96.0
Variance		4.732×10^{-4}	14 219	84.182	5.9 41	3.006×10^{-2}	125.05	36.52
S. Dev.		2.189×10^{-2}	3 771	9.1751	2.4369	0 1977	11 033	6.043
S. Err.		5.841×10^{-3}	1 009	2.4521	0 65105	5.428×10^{-2}	3.202	1.615
95? Conf. Interval	U	0.768	13 75	121.5	17.10	1 060	151.9	99.5
	L	0.744	9.17	110.9	14 29	0 831	137.8	92.5

Appendix 20. Summary of erythrocytic concentrations of hemoglobin (mMHb/litre cells) and the relationship of erythrocytic electrolytes to hemoglobin (mEq/mMHb) of thermally-acclimated rainbow trout (Salmo gairdneri). Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature 2°C	10°C	18°C	Signif. Diff. 2°C	10°C	18°C
mMHb/litre Erythrocyte	Summer	$3.97\pm9.27\times10^{-2}$	$3.88\pm5.92\times10^{-2}$	$3.87+4.75\times10^{-2}$	N.S.	N.S.	N.S.
	Winter	$4.09\pm6.49\times10^{-2}$	$4.16+4.51\times10^{-2}$	$3.93\pm6.03\times10^{-2}$	N.S.	p<0.01	N.S.
					*N.S.	N.S.	N.S.
Na^{+}/mMHb	Summer	9.83±0.663	10.37±0.9811	8.69±0.496	N.S.	N.S.	N.S.
	Winter	9.93±0.420	8.48±0.386	8.83+0.401	p<0.01	N.S.	N.S.
					*N.S.	N.S.	N.S.
K^{+}/mMHb	Summer	19.4±0.288	21.6±0.429	23.4±0.353	p<0.01	p<0.01	p<0.01
	Winter	20.9±0.576	22.4±0.332	24.4±0.335	p<0.05	p<0.01	p<0.01
					*N.S.	N.S.	N.S.
Mg^{+2}/mMHb	Summer	$2.09\pm4.96\times10^{-2}$	$2.21\pm7.09\times10^{-2}$	$2.08+9.79\times10^{-2}$	N.S.	N.S.	N.S.
	Winter	$2.30\pm4.97\times10^{-2}$	$2.28\pm4.86\times10^{-2}$	$2.45\pm7.55\times10^{-2}$	N.S.	N.S.	N.S.
					*p<0.01	N.S.	N.S.
Ca^{+2}/mMHb	Summer	$0.226\pm2.31\times10^{-2}$	$0.229\pm1.99\times10^{-2}$	$0.198\pm9.42\times10^{-3}$	N.S.	N.S.	N.S.
	Winter	$0.117+9.40\times10^{-3}$	$0.104\pm9.39\times10^{-3}$	$0.119\pm9.26\times10^{-3}$	N.S.	N.S.	N.S.
					*p<0.01	p<0.01	p<0.01
Cl^{-}/mMHb	Summer	20.7±0.948	20.7±0.439	20.8±0.496	N.S.	N.S.	N.S.
	Winter	19.3+0.978	18.5±0.542	19.8±0.517	N.S.	N.S.	N.S.
					*N.S.	p<0.01	N.S.

*significant difference between seasons

Appendix VI Erythrocytic concentrations of hemoglobin (mM Hb/litre cells) and the relationship of erythrocytic electrolytes to hemoglobin (m'eq/mM Hb) of thermally acclimated summer rainbow trout (Salmo gairdneri).

Data Item	Code Number	Hb	Na^+	K^+	Mg^{+2}	Ca^{+2}	Cl^-
Acclimated to 3°							
1	RT-6	4.07	9.98	19.8	2.15	0.137	16.4
2	RT-7	4.19	11.19	17.3	1.74	0.202	18.8
3	RT-12	4.12	4.00	17.7	-	0.282	-
4	RT-13	4.39	9.89	18.5	1.99	0.113	18.3
5	RT-14	4.27	9.51	19.7	2.13	0.159	19.3
6	RT-15	3.60	8.69	19.4	2.02	0.161	21.6
7	RT-22	4.31	6.61	20.5	2.30	0.174	16.4
8	RT-23	3.91	15.60	18.6	2.08	0.214	21.0
9	RT-24	3.93	11.20	20.1	2.16	0.272	22.5
10	RT-25	4.07	10.52	20.3	1.92	0.186	19.7
11	RT-26	4.15	10.19	19.1	2.22	0.147	19.0
12	RT-27	4.18	10.45	19.9	2.17	0.248	18.0
13	RT-28	3.79	7.81	18.1	-	-	21.0
14	RT-29	3.04	13.82	-	-	0.449	26.7
15	RT 30	3.24	9.20	21.3	1.88	0.361	23.0
16	RT-31	3.85	8.62	20.0	2.40	0.250	31.2
17	RT-32	4.42	-	-	-	-	17.0
Mean		3.97	9.83	19.4	2.09	0.226	20.7
Variance		0.1462	7.044	1.248	3.201×10^{-2}	7.987×10^{-3}	14.38
S. Dev.		0.3823	2.654	1.117	0.1789	8.937×10^{-2}	3.792
S. Err.		9.272×10^{-2}	0.6635	0.2884	4.962×10^{-2}	2.307×10^{-2}	0.9481
95% Confidence Interval	U	4.17	11.24	20.0	2.20	0.275	22.8
	L	3.78	8.41	18.7	1.98	0.176	18.7
Acclimated to 10°							
1	RT-4	3.82	14.16	20.3	1.89	0.385	21.9
2	RT 5	3.87	13.05	20.2	2.02	0.173	22.4
3	RT-10	3.84	11.48	21.9	1.78	0.156	21.5
4	RT-11	4.03	11.11	20.8	2.15	0.277	19.9
5	RT-16	4.50	9.02	19.6	1.93	0.754	18.5
6	RT-17	3.91	7.60	22.0	2.25	0.160	21.7
7	RT-18	3.90	4.36	23.7	2.79	0.134	19.8
8	RT-19	4.01	7.78	23.3	2.33	0.204	19.3
9	RT-20	3.57	3.84	24.1	2.34	0.207	17.9
10	RT 21	3.74	9.79	20.5	1.93	0.199	21.4
11	RT-30	4.01	6.51	21.5	2.51	0.273	18.8
12	RT 37	3.62	12.96	22.4	2.37	0.194	22.9
13	RT 38	3.66	13.17	22.6	2.55	0.190	22.0
14	RT-41	3.84	16.74	18.3	2.21	0.341	21.9
15	RT 42	4.32	-	-	1.94	0.376	17.9
16	RT-43	3.69	14.04	23.3	2.43	0.236	22.8
17	RT-44	3.67	-	-	-	-	-
Mean		3.88	10.4	21.6	2.21	0.229	20.7
Variance		5.964	14.44	2.765	8.036×10^{-2}	6.360×10^{-3}	3.091
S. Dev.		0.2442	3.800	1.663	0.2835	7.975×10^{-2}	1.758
S. Err.		5.923×10^{-2}	0.9811	0.4294	7.087×10^{-2}	1.994×10^{-2}	0.4395
95% Confidence Interval	U	4.00	12.5	22.6	2.36	0.271	21.6
	L	3.76	8.3	20.7	2.06	0.186	19.7
Acclimated to 18°							
1	RT-1	3.68	-	-	-	-	-
2	RT-2	3.92	6.10	25.3	1.68	0.149	20.8
3	RT-3	4.00	5.90	24.4	2.44	0.148	17.0
4	RT-8	4.09	7.51	24.2	2.04	0.194	23.8
5	RT-9	4.12	8.23	22.5	1.01	0.241	19.4
6	RT-33	3.70	7.86	24.7	2.41	0.205	21.8
7	RT-34	3.66	8.52	26.4	2.60	0.209	21.3
8	RT-35	3.94	8.35	22.5	2.38	0.203	20.0
9	RT-39	3.81	11.21	21.8	2.33	0.293	21.8
10	RT-40	4.05	10.07	22.3	2.26	0.248	-
11	RT-45	3.48	-	-	-	-	-
12	RT-46	3.69	-	-	-	-	-
13	RT-47	3.90	10.28	22.0	1.73	0.165	22.2
14	RT-48	4.03	5.58	22.7	2.07	0.187	19.3
15	RT-49	3.75	8.11	24.2	2.43	0.191	22.7
16	RT 50	3.54	12.43	24.2	1.99	0.201	23.1
17	RT-51	4.05	8.95	23.3	2.04	0.159	19.0
18	RT-52	3.88	11.21	22.7	1.86	0.197	21.3
19	RT-55	4.21	9.29	21.3	2.02	0.192	18.2
Mean		3.87	8.69	23.4	2.08	0.193	20.8
Variance		4.291	3.939	1.993	0.1552	1.421×10^{-3}	3.689
S. Dev.		0.2071	1.984	1.412	0.3915	3.769×10^{-2}	1.921
S. Err.		4.752×10^{-2}	0.4361	0.3234	9.147×10^{-2}	8.648×10^{-3}	0.4409
95% Confidence Interval	U	3.97	9.74	24.2	2.29	0.218	21.8
	L	3.77	7.63	22.7	1.87	0.176	19.7

Appendix [illegible] Erythrocytic concentration of [illegible] ion (mEq/liter cell) and its relationship of erythrocytic analyte to [illegible] group (Eq/Eq Hb) on [illegible] acclimated to different temperatures (Alewife Reservoir)

Data Item	Code Number	Hb	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-
Acclimated to							
1	RT-56	4.28	-	-	-	0.155	16.9
2	RT-57	4.56	7.72	19.7	2.07	0.070	17.7
3	RT-5	4.03	10.76	21.	2.57	0.122	16.3
4	RT-58	5.74	12.97	21.4	2.51	0.243	21.8
5	RT-60	4.13	7.55	22.3	2.19	0.100	13.6
6	RT-61	4.09	7.51	24.3	2.00	0.046	23.6
7	RT-64	3.91	-	20.6	2.11	-	19.3
8	RT-65	3.72	-	-	-	0.166	-
9	RT-68	4.04	6.65	26.5	2.31	0.096	18.4
10	RT-69	5.66	11.12	26.5	2.39	0.113	40.1
11	RT-74	4.29	6.65	20.5	2.35	0.197	14.4
12	RT-75	4.63	-	-	-	0.066	16.0
13	RT-76	4.43	11.60	17.4	2.19	0.096	15.5
14	RT-77	4.41	8.98	19.3	2.20	0.066	14.9
15	RT-90	4.06	8.57	21.9	2.24	0.162	16.6
16	RT-91	4.41	9.39	19.3	2.17	0.154	18.4
17	RT-92	3.84	7.99	22.0	2.44	0.120	20.4
18	RT-93	4.16	12.31	18.8	2.18	0.157	19.5
19	RT-94	4.10	-	19.2	2.56	-	16.5
20	RT-95	4.15	10.58	19.6	2.04	0.060	21.2
21	RT-96	4.30	-	17.7	2.13	0.148	17.9
22	RT-97	3.97	-	21.6	2.18	-	20.7
23	RT-116	2.89	10.4	27.3	3.14	0.109	25.5
24	RT-117	4.30	11.3	17.8	2.03	0.070	17.8
25	RT-119	4.20	11.3	19.0	2.18	0.109	17.3
26	RT-120	4.04	12.5	19.8	2.45	0.107	18.5
27	RT-121	4.07	10.7	17.7	2.40	0.126	15.5
Mean		4.09	9.93	20.9	2.30	0.117	19.3
Variance		0.1136	3.533	7.964	5.933×10^{-2}	2.121×10^{-3}	24.89
S. Dev		0.3371	1.880	2.822	0.2436	4.605×10^{-2}	4.989
S. Err.		6.488×10^{-2}	0.4203	0.5761	4.972×10^{-2}	9.400×10^{-3}	0.9784
95% Confidence Interval	U	4.23	10.81	22.0	2.41	0.137	21.3
	L	3.96	9.05	19.7	2.20	0.098	17.3
Acclimated to 10°							
1	RT-66	3.96	10.00	23.0	2.31	0.097	18.3
2	RT-67	4.17	9.06	22.8	2.62	0.189	19.3
3	RT-70	4.50	8.36	20.3	1.92	0.111	17.7
4	RT-71	4.15	8.84	22.9	2.31	0.089	18.7
5	RT-72	4.15	8.36	24.9	1.76	0.112	18.9
6	RT-80	4.01	11.35	23.3	2.50	0.097	20.5
7	RT-81	4.12	9.30	22.9	2.27	0.077	18.9
8	RT-84	4.18	8.25	23.3	2.16	0.109	17.0
9	RT-85	3.84	8.85	25.2	2.53	0.124	22.4
10	RT-88	4.16	-	23.2	-	0.094	16.0
11	RT-89	4.32	-	21.0	2.29	-	16.0
12	RT-102	4.50	5.04	21.5	2.51	0.020	13.1
13	RT-103	4.12	-	21.8	2.37	-	21.6
14	RT-104	4.29	8.30	21.4	2.35	0.092	17.5
15	RT-105	4.02	-	21.1	2.36	-	18.0
16	RT-110	4.32	-	-	2.02	0.061	19.6
17	RT-111	4.18	7.44	22.3	2.40	0.194	18.0
18	RT-112	4.01	7.76	22.3	2.28	0.069	21.0
19	RT-113	3.74	10.86	23.5	2.41	0.110	22.9
20	RT-114	4.57	6.87	18.9	1.99	0.110	15.7
21	RT-115	4.13	7.02	21.5	2.37	0.104	17.5
Mean		4.16	8.48	22.4	2.28	0.104	18.5
Variance		4.278×10^{-2}	2.38	2.203	4.731×10^{-2}	1.552×10^{-3}	6.177
S. Dev.		0.2068	1.544	1.484	0.2175	3.940×10^{-2}	2.485
S. Err.		4.514×10^{-2}	0.3860	0.3319	4.864×10^{-2}	9.286×10^{-3}	0.5423
95% Confidence Interval	U	4.26	9.30	23.0	2.39	0.123	19.6
	L	4.07	7.66	21.7	2.18	0.084	17.3
Acclimated to 18°							
1	RT-54	3.93	-	24.5	2.51	0.152	17.4
2	RT-55	3.43	8.60	26.2	2.68	0.128	25.3
3	RT-62	4.14	6.57	25.4	2.31	0.034	16.1
4	RT-63	3.87	9.82	25.3	2.37	0.112	20.8
5	RT-73	4.18	12.01	21.9	2.28	0.039	21.7
6	RT-78	3.88	6.90	-	2.15	0.115	21.3
7	RT-79	4.26	6.48	24.1	2.20	0.099	17.5
8	RT-82	4.07	8.66	25.5	2.19	0.145	18.0
9	RT-83	3.96	8.94	25.0	2.15	0.244	19.2
10	RT-86	4.12	6.97	25.3	2.22	-	16.7
11	RT-87	3.94	10.74	24.7	2.47	0.129	21.6
12	RT-98	4.10	6.66	22.3	2.09	0.093	18.5
13	RT-99	4.22	7.94	22.9	2.47	0.105	18.5
14	RT-100	4.17	10.32	21.2	2.31	-	17.7
15	RT-101	3.39	7.96	25.6	2.95	0.095	19.4
16	RT-102	3.92	7.07	24.5	2.61	0.080	19.9
17	RT-107	3.92	12.76	20.3	3.23	0.077	20.6
18	RT-108	3.95	8.98	25.7	2.53	0.166	22.5
19	RT-109	3.72	8.75	24.0	2.20	0.103	22.5
20	RT-110	4.07	9.78	23.0	2.32	0.145	18.2
Mean		3.93	9.13	24.4	2.48	0.113	19.3
Variance		2.222×10	3.609	4.127	0.1113	1.561	1.220×10
S. Dev.		0.4801	1.900	2.031	0.3337	0.1249	3.493×10
S. Err.		6.913×10^{-2}	0.4243	0.4539	0.0745	0.0279	7.804
95% Confidence Interval	U	4.06	10.02	25.3	2.64	0.171	23.1
	L	3.79	8.24	23.4	2.32	0.055	15.4

Appendix 23. Summary of erythrocytic concentrations of hemoglobin (mMHb/litre cells) and the relationship of erythrocytic electrolytes to hemoglobin (mEq/mMHb), of thermally-acclimated carp (Cyprinus carpio). Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Signif. Diff.		
		2°C	16°C	30°C	2°C	16°C	30°C
mMHb/litre Erythrocyte	Spring	3.62±0.110	3.74±9.11x 10^{-2}	3.91±6.76x 10^{-2}	N.S.	N.S.	p<0.01
Na^{+}/mMHb	Spring	2.08x10^{-2}	0.419±0.137	2.28±0.239	**	p<0.05	**
K^{+}/mMHb	Spring	23.1±0.562	21.8±0.356	22.5±0 453	N.S.	N.S.	N.S.
Mg^{+2}/mMHb	Spring	4.49±0.120	4.52±0.132	3.03±0.121	N.S.	p<0.01	p<0.01
Ca^{+2}/mMHb	Spring	0.206±2.26x 10^{-2}	0.189±2.72x 10^{-2}	0.187±1.27x 10^{-2}	N.S.	N.S.	N.S.
Cl^{-}/mMHb	Spring	12.4±0.461	15.8±0.631	18.6±0.439	p<0.01	p<0.01	p<0.01

*mean

**proper analysis not possible

Appendix 24. Erythrocytic concentration of hemoglobin (mMHb/liter cells) and the relationship of erythrocytic electrolytes to hemoglobin (mEq/mMHb) of thermally acclimated carp (Cyprinus carpio).

Data Item	Code Number	Hb	Na^+	K^+	Mg^{+2}	Ca^{+2}	Cl^-
Acclimated to 7°							
1	CP-3	4.00	0	19.3	3.67	0.218	12.7
2	CP-6	3.52	0	24.7	4.37	0.217	12.4
3	CP-9	4.21	0	21.9	3.99	0.146	10.3
4	CP-12	4.07	0	21.9	4.20	0.177	10.3
5	CP-15	3.86	0	21.6	4.51	0.377	10.7
6	CP-18	3.73	0	25.5	4.73	0.160	11.5
7	CP-21	3.87	0	22.8	4.29	0.176	11.7
8	CP-24	3.11	0	26.5	5.17	0.206	14.6
9	CP-27	3.19	0.270	26.5	5.01	0.194	15.6
10	CP-30	3.80	0	21.4	4.29	0.153	12.9
11	CP-33	3.81	0	22.8	4.46	0.119	11.4
12	CP-36	3.56	0	22.6	4.60	0.124	13.1
13	CP-39	2.88	-	-	4.29	0.238	-
14	CP-42	3.08	0	24.0	5.32	0.401	14.2
Mean		3.62	2.08×10^{-2}	23.1	4.49	0.208	12.4
Variance		0.1687		4.111	0.2026	7.171×10^{-3}	2.763
S. Dev.		0.4108		2.028	0.4501	8.468×10^{-2}	1.662
S. Err.		0.1098		0.5624	0.1203	2.263×10^{-2}	0.4610
95% Confidence Interval	U	3.86		24.3	4.75	0.256	13.4
	L	3.38		21.9	4.23	0.159	11.4
Acclimated to 16°							
1	CP-2	3.56	-	-	4.09	-	18.4
2	CP-5	3.74	0.610	22.8	4.53	0.181	14.8
3	CP-8	3.12	0	24.4	4.99	0.153	21.0
4	CP-11	3.94	0.049	22.4	4.34	0.154	13.5
5	CP-14	4.30	0.599	21.3	3.92	0.121	13.2
6	CP-17	3.70	1.010	20.7	4.36	0.136	16.8
7	CP-20	4.24	1.480	21.5	3.82	0.123	15.0
8	CP-23	3.96	0.651	21.5	4.28	0.154	14.9
9	CP-26	3.67	0.022	19.9	4.36	0.157	16.3
10	CP-29	3.92	0	21.8	4.64	0.148	14.1
11	CP-32	3.76	0.388	23.1	4.88	0.177	13.0
12	CP-35	3.33	0	21.9	5.35	0.396	17.7
13	CP-38	3.40	0.224	20.5	5.21	0.379	18.1
14	CP-41	3.63	-	-	-	-	14.0
Mean		3.74	0.419	21.8	4.52	0.189	15.8
Variance		0.1161	0.2234	1.523	0.2255	8.853×10^{-3}	5.570
S. Dev.		0.3407	0.4727	1.234	0.4749	9.409×10^{-2}	1.329
S. Err.		9.105×10^{-2}	0.1365	0.3563	0.1317	2.716×10^{-2}	0.6303
95% Confidence Interval	U	3.94	0.720	22.6	4.81	0.250	17.1
	L	3.55	0.119	21.0	4.23	0.130	14.4
Acclimated to 30°							
1	CP-1	4.13	-	18.3	3.44	-	17.6
2	CP-4	3.64	2.35	23.7	3.05	0.195	19.7
3	CP-7	3.56	3.70	24.1	3.33	0.210	21.1
4	CP-10	3.32	1.68	24.2	2.84	0.192	18.1
5	CP-13	4.02	1.46	22.6	2.65	0.158	19.2
6	CP-16	4.05	1.59	23.2	2.76	0.220	18.3
7	CP-19	4.02	1.61	20.1	2.50	0.157	17.4
8	CP-22	3.73	4.10	23.6	3.41	0.240	16.7
9	CP-25	4.14	2.52	22.4	2.93	0.168	18.6
10	CP-28	4.42	2.13	21.1	2.70	0.128	16.4
11	CP-31	3.75	2.26	24.0	3.83	0.179	18.0
12	CP-34	4.06	-	22.4	3.39	0.136	16.4
13	CP-37	3.77	1.99	21.9	3.43	0.150	18.8
14	CP-40	3.55	1.98	23.4	2.22	0.252	22.4
Mean		3.91	2.28	22.5	3.03	0.197	18.6
Variance		6.387×10^{-2}	0.6960	2.877	0.2033	2.096×10^{-3}	2.732
S. Dev.		0.2527	0.8342	1.696	0.4508	4.577×10^{-2}	1.644
S. Err.		6.755×10^{-2}	0.2391	0.4703	0.1205	1.270×10^{-2}	0.4397
95% Confidence Interval	U	4.06	2.81	23.5	3.29	0.224	19.6
	L	3.77	1.75	21.5	2.77	0.169	17.7

Appendix 25 Summary of Nerst potentials (mV) across the erythrocytes of thermally-acclimated rainbow trout (Salmo gairdneri), as calculated using different electrolytes. Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature 2°C	10°C	18°C	Signif. Diff. 2°C	10°C	18°C
E_{Na^+}	Summer	+30.7±1.69	+30.7±2.57	+34.4±1.69	N.S.	N.S.	N.S.
	Winter	+26.5±1.03	+31.8±0.910	+33.1±1.11	p<0.01	N.S.	p<0.01
					*p<0.05	N.S.	N.S.
E_{K^+}	Summer	-94.8±3.37	-99.2±2.72	-99.9±2.75	N.S.	N.S.	N.S.
	Winter	-103±2.35	-106±2.78	-92.1±2.65	N.S.	p<0.01	p<0.01
					*N.S.	N.S.	N.S.
$E_{Mg^{+2}}$	Summer	-25.5±1.74	-25.3±1.62	-24.9±1.61	N.S.	N.S.	N.S.
	Winter	-27.2±1.66	-24.2±0.489	-24.9 0.783	N.S.	N.S.	N.S.
					*N.S.	N.S.	N.S.
$E_{Ca^{+2}}$	Summer	+18.1±1.33	+18.5±1.17	20.3±0.806	N.S.	N.S.	N.S.
	Winter	+26.4±1.05	+28.9±1.14	+27.5±0.915	N.S.	N.S.	N.S.
					*p<0.01	p<0.01	p<0.01
E_{Cl^-}	Summer	-7.78±0.698	-6.61±0.467	-6.96±0.631	N.S.	N.S.	N.S.
	Winter	-9.76±0.476	-9.35±0.484	-9.08-0.440	N.S.	N.S	N.S.
					*p<0.01	p<0.01	p<0.05

*significant differences between seasons

Appendix 26. Mean potentials (mV) across the erythrocyte of thermally-acclimated summer rainbow trout (*Salmo gairdneri*), as calculated using different electrolytes.

Data Item	Code Number	E_{Na^+}	$-E_{K^+}$	$-E_{Mg^{+2}}$	$E_{Ca^{+2}}$	$-E_{Cl^-}$
Acclimated to 2°						
1	RT-6	31.6	82.0	22.9	22.6	8.87
2	RT-7	24.6	102.3	24.5	14.2	7.43
3	RT-12	46.7	78.3	-	13.0	-
4	RT-13	27.9	104.2	26.4	25.5	8.19
5	RT-11	27.7	112.5	32.2	16.5	8.23
6	RT-15	33.0	72.9	32.4	22.1	6.26
7	RT-22	37.0	105.2	29.8	19.9	11.28
8	RT-23	-	101.3	15.5	20.8	6.98
9	RT-24	26.6	99.2	21.5	-	5.76
10	RT-25	27.0	108.5	17.3	-	8.00
11	RT-26	29.0	97.4	20.6	25.3	10.28
12	RT-27	27.0	97.9	22.2	16.4	10.00
13	RT-28	-	-	-	-	6.37
14	RT-29	23.1	-	-	14.2	1.45
15	RT-30	35.3	87.1	28.4	12.2	9.00
16	RT-31	25.8	78.8	36.9	13.0	1.07
17	RT-32	-	-	-	-	7.34
Mean		+30.7	-94.8	-25.5	+18.1	-7.28
Variance		40.11	158.7	39.54	22.96	7.799
S. Dev.		6.333	12.60	6.288	4.791	2.793
S. Err.		1.693	3.367	1.744	1.329	0.6982
95% Confidence Interval	U	+34.4	-87.6	-21.7	+21.0	-5.79
	L	+27.0	-102.1	-29.3	+15.2	-8.77
Acclimated to 10°						
1	RT-4	21.8	98.2	26.3	7.6	6.43
2	RT-5	24.1	102.9	20.6	20.9	6.23
3	RT-10	28.6	103.7	18.7	23.4	7.05
4	RT-11	25.4	104.8	25.9	13.5	6.07
5	RT-16	26.9	113.5	23.5	21.4	5.04
6	RT-17	36.5	98.5	30.9	20.8	3.09
7	RT-18	50.4	95.0	37.4	23.3	8.13
8	RT-19	35.4	111.0	24.6	22.0	8.68
9	RT-20	49.8	72.0	33.4	16.5	4.92
10	RT-21	30.0	90.7	18.1	18.2	2.52
11	RT-36	40.5	100.4	37.7	13.9	8.86
12	RT-37	24.2	93.4	25.8	21.7	7.31
13	RT-38	26.4	91.3	17.9	23.3	9.73
14	RT-41	17.2	113.7	20.5	16.3	6.82
15	RT-42	-	-	21.2	12.7	7.75
16	RT-43	22.3	96.5	21.8	21.2	6.22
17	RT-44	-	-	-	-	7.51
Mean		+30.7	-99.2	-25.3	+18.5	-6.61
Variance		99.35	110.6	41.76	21.82	3.703
S. Dev.		9.967	10.52	6.462	4.671	1.924
S. Err.		2.574	2.716	1.616	1.168	0.4667
95% Confidence Interval	U	+36.2	-93.3	-21.8	+21.0	-5.62
	L	+25.1	-105.0	-28.7	+16.1	-7.60
Acclimated to 18°						
1	RT-1	-	-	-	-	-
2	RT-2	44.2	97.6	15.9	25.6	7.17
3	RT-3	47.4	-	23.5	25.4	10.53
4	RT-8	31.6	125.3	26.9	15.4	0.78
5	RT-9	35.8	87.5	11.1	18.7	7.50
6	RT-33	39.2	108.1	31.9	21.3	9.18
7	RT-34	36.7	96.6	27.1	23.6	5.47
8	RT-35	33.2	101.7	33.0	22.2	6.99
9	RT-39	27.8	88.1	25.9	15.7	7.80
10	RT-40	-	-	-	-	-
11	RT-45	-	-	-	-	-
12	RT-46	-	-	-	-	-
13	RT-47	50.5	97.8	26.2	18.7	4.10
14	RT-48	41.6	95.2	29.9	19.6	4.42
15	RT-49	34.5	111.2	22.4	20.0	5.79
16	RT-50	27.5	95.9	29.0	16.8	8.38
17	RT-51	33.2	96.8	21.7	21.9	8.21
18	RT-52	24.5	107.7	18.1	20.0	6.33
19	RT-53	26.3	99.6	31.4	19.3	7.69
Mean		+34.4	-99.6	-24.9	+20.3	-6.96
Variance		42.82	105.7	39.07	9.749	5.975
S. Dev.		6.544	10.28	6.250	3.122	2.445
S. Err.		1.690	2.777	1.611	0.8062	0.6312
95% Confidence Interval	U	+38.0	-91.0	-21.5	+22.0	-5.61
	L	+30.8	-108.9	-28.4	+18.5	-8.31

Appendix 7 Activity terms (ΔF) from the cycle set of Table II, Intercepts (Intercept) and correlation coefficients for different elements

Data Item	Code Number	ΔF_{Na+}	ΔF_{K+}	ΔF_{Cl^-}	ΔF_{CaCl_2}	ΔF_{CO_2}
Acclimated to 5°						
1	RT-53	-	-		23.3	11.46
2	RT-57	29.2	119.2	30.6	30.0	8.00
3	RT-[illegible]	23.5	108.5	23.7	25.4	12.54
4	RT-58	23.5	93.2	22.3	18.7	9.57
5	RT-60	29.5	119.8	45.0	29.8	6.71
6	RT-61	29.9	101.8	45.1	38.6	2.47
7	RT-64	-	109.7	27.1	-	10.58
8	RT-65	-	-	-	21.7	-
9	RT-68	33.9	101.5	25.3	25.7	8.83
10	RT-69	28.2	84.4	19.9	28.9	8.47
11	RT-74	28.9	99.8	23.9	19.0	13.92
12	RT-75	-	-	-	31.4	9.10
13	RT-76	17.7	112.7	23.3	27.7	13.85
14	RT-77	27.6	105.5	26.5	31 0	12.31
15	RT-90	29.2	122.4	37.7	21.6	8.69
16	RT-91	28.0	112.7	21.6	26.6	9.43
17	RT-92	33.7	105.7	21.5	25.6	8.36
18	RT-93	21.1	103.9	26.5	19.6	8.74
19	RT-94	-	87.4	23.1	-	13.47
20	RT-95	28.3	92.4	20.8	35.0	7.97
21	RT-96	-	81.1	21.6	23.8	9.93
22	RT-97	-	95.6	46.8	-	9.37
23	RT-116	31.1	91.4	22.0	-	8.32
24	RT-117	22.7	91.7	23.5	30.2	10.25
25	RT-119	20.9	99.3	23.0	25.7	9.46
26	RT-120	22.0	110.0	22.9	26.4	10.56
27	RT-121	20.8	116.0	26.8	22.0	11.31
Mean		+26.5	-102.7	-27.2	+26.4	- 9.76
Variance		21.13	132.23	66.09	25.59	5.893
S. Dev.		4.596	11.499	8.129	5.058	2.428
S. Err		1.028	2 3472	1 659	1.055	0.4761
95% Confidence Interval	U	+28.6	- 97.6	-23.7	+28.6	- 8.78
	L	+24.3	-107.6	-30.6	+24.2	-10.74
Acclimated to 10°						
1	RT-66	31.0	110.0	22.6	30.3	10.45
2	RT-67	30.8	123.8	25.4	21.6	8.43
3	RT-70	27.5	89.1	23.0	26.4	8.25
4	RT-71	28.8	129.9	25.9	29.5	8.67
5	RT-72	31.8	114.8	19.7	27.1	8.76
6	RT-80	26.3	114.2	25.7	29.1	8.17
7	RT-81	28.3	112.1	25.0	31 3	7.13
8	RT-84	31.4	94.7	26.4	26.1	13.96
9	RT-85	33.2	122.9	22.2	27.3	5.99
10	RT-88	-	110.3	-	28.8	12.67
11	RT-89	-	117.1	24.4	-	5.69
12	RT-102	40.7	88.0	29.9	42.1	14.19
13	RT-103	-	97.1	23 9	-	6.72
14	RT-104	31.8	105.8	25.2	29.4	10.86
15	RT-105	-	104.0	23.9	-	10.63
16	RT-110	-	-	21.9	33.7	7.94
17	RT-111	34.7	97.6	24.5	20.4	10.04
18	RT-112	35.3	98.5	21.9	34.3	8.08
19	RT-113	23.3	103.9	22.1	29.2	7.07
20	RT-114	33.2	85.4	24.3	25.0	9.94
21	RT-115	35.1	98.7	25 9	28.2	9.79
Mean		+31.8	-106.1	-24.2	+28.9	- 9.35
Variance		13.25	154.74	4.772	23.20	4.924
S. Dev.		3.640	12.440	2.185	4.816	2.219
S. Err.		0.9099	2.7816	0.4885	1.135	0.4842
95% Confidence Interval	U	+33.7	-100.3	-23.2	+31.3	- 8.34
	L	+29.8	-111.9	-25.2	+26.5	-10.36
Acclimated to 18°						
1	RT-54	-	79.3	22.0	25.9	12.69
2	RT-55	38.3	73.9	22.1	27.5	6.62
3	RT-62	38.8	93.3	28.2	29.8	13.40
4	RT-63	35 4	99.5	23.7	28.2	7.40
5	RT-73	24.2	123.0	30.9	33.8	7.13
6	RT-78	38.2	-	21 9	26.4	7.76
7	RT-79	37 4	96.6	24.7	27.6	8.93
8	RT-82	32.7	92 3	23.5	24.9	11.76
9	RT-83	31.0	101 2	23.5	17.3	8.85
10	RT-86	36.7	89.9	25.2	-	10.10
11	RT-87	25.5	107.4	23.9	25.7	9.00
12	RT-98	33.5	90.5	20 1	32.0	9.15
13	RT-99	31.2	94.4	26 1	26.0	7.90
14	RT-100	27.5	89 1	22.5	-	9.55
15	RT-101	37.7	73 0	24 6	-	10 81
16	RT 1[illegible]	40.1	92.2	24 9	31.5	10 00
17	RT-107	24 3	77 9	35 7	31 5	9 10
18	RT-108	30.7	106.7	23 2	25.0	6.90
19	RT-109	33.6	87 5	24 7	26.6	6.19
20	RT-118	28.4	83.0	27.0	25.4	8 31
Mean		+33 1	- 1	-24 7	+27.5	9 63
Variance		23.53	154 [illegible]	12	11.23	8 871
S. Dev.		4.850	1 42	5.0	3.27	1 [illegible]67
S. Err		1 [illegible]4	[illegible]	[illegible]	[illegible]	0 [illegible]9
95% Confidence Interval	U	[illegible]	[illegible]	- 3	[illegible]	[illegible]
	L	[illegible]	[illegible]	- [illegible]	[illegible]	10 0[illegible]

Appendix 28. Summary of Nerst potentials (mV) across the erythrocytes of thermally-acclimated carp (Cyprinus carpio), as calculated using different electrolytes. Reported as the mean±1 standard error of the mean.

Measurement	Season	Acclimation Temperature			Signif. Diff.		
		2°C	16°C	30°C	2°C	16°C	30°C
E Na^+	Spring	+∞	+∞	+66.20±2.45	*	*	*
E K^+	Spring	-81.0±2.25	-79.5±3.06	-86.7±1.30	N.S.	p<0.05	p<0.05
E Mg^{+2}	Spring	-23.1±0.620	-27.6±0.699	-23.4±1.13	p<0.01	p<0.01	N.S.
E Ca^{+2}	Spring	+16.6±1.10	+20.4±1.24	+19.9±1.04	p<0.05	N.S.	p<0.05
E Cl^-	Spring	-14.7±0.719	-10.2±0.999	-5.71±0.309	p<0.05	p<0.05	p<0.01

*proper analysis not possible

Appendix 9. Nernst potentials (mV) across the erythrocytes of thermally-acclimated carp (Cyprinus carpio), as calculated using different electrolytes

Data Item	Code Number	$+E_{Na^+}$	$-E_{K^+}$	$E_{Mg^{+2}}$	$+E_{Ca^{+2}}$	$-E_{Cl^-}$
Acclimated to 2°						
1	CP-3	+∞	77.6	20.6	14.3	14.68
2	CP-6	+∞	86.2	21.5	17.3	16.46
3	CP-9	+∞	91.8	25.0	17.5	17.99
4	CP-12	+∞	94.3	26.0	15.1	9.34
5	CP-15	+∞	84.3	24.4	7.4	13.88
6	CP-18	+∞	-	23.8	16.8	18.60
7	CP-21	+∞	84.8	24.6	16.7	15.97
8	CP-24	+∞	83.5	23.3	18.4	11.30
9	CP-27	116.7	69.6	22.8	19.0	14.13
10	CP-30	+∞	78.6	22.2	19.8	13.73
11	CP-33	+∞	74.4	24.4	21.3	12.86
12	CP-36	+∞	76.3	22.7	23.6	16.11
13	CP-39	+∞	-	16.9	13.5	-
14	CP-42	+∞	71.1	24.7	11.5	16.25
Mean		+∞	-81.0	-23.1	+16.6	-14.72
Variance			60.60	5.380	16.940	6.7288
S. Dev			7.78	2.320	4.116	2.5940
S. Err.			2.25	0.6199	1.100	0.71945
95% Confidence Interval	U		-76.1	-21.8	+19.0	-13.15
	L		-86.0	-24.4	+14.2	-16.28
Acclimated to 16°						
1	CP-2	-	-	25.0	-	7.73
2	CP-5	96.0	93.0	29.0	17.9	11.73
3	CP-8	+∞	73.8	22.5	25.2	9.04
4	CP-11	156.0	91.6	29.5	19.4	9.72
5	CP-14	90.7	91.7	28.5	24.7	-
6	CP-17	84.6	62.0	23.5	23.5	10.77
7	CP-20	70.5	82.5	27.6	24.6	9.37
8	CP-23	92.1	81.5	29.0	19.8	8.80
9	CP-26	178.4	68.5	26.0	23.3	8.32
10	CP-29	+∞	85.4	29.6	21.5	14.45
11	CP-32	107.4	77.6	30.2	19.1	15.85
12	CP-35	+∞	82.1	29.2	12.3	10.46
13	CP-38	122.6	64.0	29.2	13.3	9.22
14	CP-41	-	-	-	-	13.87
Mean		+∞	-79.5	-27.6	+20.4	-10.01
Variance			112.4	6.364	18.60	12.963
S. D v.			10.60	2.523	4.312	3.6004
S. Err.			3.061	0.6997	1.245	0.99958
95% Confidence Interval	U		-72.7	-26.1	+23.2	7.84
	L		-86.2	-29.1	+17.7	-12.19
Acclimated to 30°						
1	CP-1	-	84.9	29.8	-	4.05
2	CP-4	66.4	87.4	21.1	20.3	5.55
3	CP-7	55.5	86.4	22.8	19.4	5.93
4	CP-10	-	91.7	26.3	15.4	4.73
5	CP-13	75.8	87.6	21.9	21.0	4.60
6	CP-16	72.4	87.5	23.0	17.0	4.42
7	CP-19	72.5	-	18.0	14.4	7.07
8	CP-22	49.9	83.6	24.2	16.3	6.97
9	CP-25	59.8	80.9	24.4	20.4	4.71
10	CP-28	64.7	87.5	27.3	23.2	-
11	CP-31	67.4	94.0	25.2	23.1	6.30
12	CP-34	-	85.8	28.7	24.5	7.36
13	CP-37	71.3	79.4	22.0	26.7	6.49
14	CP-40	72.5	76.1	18.6	17.4	6.01
Mean		+56.2	-86.7	-23.5	+19.9	- 5.71
Variance		66.11	22.11	17.90	14.11	1.241
S. Dev		8.131	4.702	4.231	3.757	1.114
S. Err.		2.452	1.304	1.131	1.042	0.7090
95% Confidence Interval	U	+71.7	-83.8	-21.0	+22.2	- 5.03
	L	+60.7	-89.5	-25.9	+17.7	- 6.38

Appendix 30. Summary of weight (g), hematocrit (PCV, %) and the nonheme protein (mg/ml erythrocytes) of erythrocyte membrane, cytosol and total erythrocyte fractions of thermally-acclimated rainbow trout (Salmo gairdneri). Reported as the mean±1 standard error of the mean.

Fraction	Acclimation Temperature			Significant Differences		
	2°C	10°C	18°C	2°C	10°C	18°C
Weight	296±15.0	290±15.2	286±10.66	N.S.	N.S.	N.S.
PCV	33.2±0.966	33.8±1.11	42.1±1.70	N.S.	p<0.01	p<0.01
Membrane Protein	34.4±1.44	34.2±2.31	25.5±1.30	N.S.	p<0.01	p<0.01
Cytosol Protein	81.4±6.59	82.1±4.50	69.1±5.21	N.S.	N.S.	N.S
Total Erythrocyte Protein	116±7.42	116±6.08	95.9±5.90	N.S.	p<0.05	N.S.

Appendix 31. Weight (g), hematocrit (PCV, %) and the membrane protein (mg/ml erythrocytes) of erythrocyte membrane, cytosol and total erythrocyte fractions of thermally-acclimated rainbow trout (Salmo gairdneri).

Data Item	Code Number	Weight	PCV	Membrane Protein	Cytosol Protein	Total Erythrocyte Protein
Acclimated to 7°C						
1	CAT-15	376	33.5	32.4	59.2	91.6
2	CAT-16	235	30.9	33.5	44.7	78.2
3	CAT-19	259	36.8	36.7	113.7	150.4
4	CAT-20	380	30.0	32.2	102.4	134.6
5	CAT-33	263	31.1	31.7	39.4	71.1
6	CAT-31	258	35.4	32.7	62.2	94.9
7	CAT-39	411	27.4	31.7	70.2	101.9
8	CAT-40	341	37.8	37.5	42.6	80.1
9	CAT-43	233	32.2	37.2	69.9	107.1
10	CAT-44	290	26.5	21.7	102.9	124.7
11	CAT-45	275	31.6	37.5	98.8	136.3
12	CAT-46	240	37.3	28.5	73.9	102.4
13	CAT-47	400	36.9	40.0	84.8	124.8
14	CAT-48	225	30.5	29.8	98.2	128.0
15	CAT-57	265	31.1	46.9	126.3	173.2
16	CAT-58(a)	223	32.4	40.4	113.8	154.2
Mean		296	33.2	34.4	81.4	115.8
Variance		4101	0.1490	33.19	760.0	880.43
S. Dev.		64.05	3.860	5.761	27.57	29.672
S. Err.		16.01	0.9650	1.440	6.892	7.4180
95% Confidence Interval	U	330	35.2	37.5	96.1	131.7
	L	262	31.1	31.3	66.6	100.0
Acclimated to 10°C						
1	CAT-17	392	36.1	34.5	90.5	125.0
2	CAT-18	284	32.2	28.9	61.1	90.0
3	CAT-21	333	42.2	29.6	76.3	105.9
4	CAT-22	340	30.7	26.8	51.7	78.5
5	CAT-35	342	26.3	42.9	53.8	96.7
6	CAT-36	270	38.7	29.1	66.3	95.4
7	CAT-37	298	32.5	41.0	96.3	137.3
8	CAT-38	312	34.6	27.4	83.4	110.8
9	CAT-58(b)	-	31.0	22.2	84.6	106.8
10	CAT-59	248	36.3	22.5	75.3	97.8
11	CAT-60	322	40.3	23.2	77.3	100.5
12	CAT-61	314	29.6	38.5	96.9	135.4
13	CAT-62	304	33.3	38.5	95.1	133.6
14	CAT-63	210	35.9	49.0	102.8	151.8
15	CAT-65	177	27.2	44.2	85.1	129.3
16	CAT-66	204	33.9	49.2	117.2	166.4
Mean		290	33.8	34.2	82.1	116.3
Variance		3469	0.1952	85.41	323.7	592.14
S. Dev.		58.90	4.418	9.242	17.99	24.334
S. Err.		15.21	1.105	2.310	4.498	6.0835
95% Confidence Interval	U	323	36.2	39.1	91.7	129.3
	L	257	31.4	29.3	72.5	103.4
Acclimated to 18°C						
1	CAT-2	229	39.8	14.7	-	-
2	CAT-6	224	28.8	28.9	73.9	102.8
3	CAT-3	272	30.7	-	-	-
4	CAT-7	362	49.8	25.3	43.8	69.1
5	CAT-8	269	35.2	22.8	33.2	56.0
6	CAT-9	267	46.9	-	24.3	-
7	CAT-10	-	54.3	17.9	70.4	88.3
8	CAT-10(b)	314	44.4	22.9	59.5	82.4
9	CAT-13	287	43.7	27.5	76.3	103.8
10	CAT-14	264	49.5	31.5	53.9	85.4
11	CAT-23	390	44.4	22.5	55.7	78.2
12	CAT-24	267	48.6	-	86.4	-
13	CAT-25	301	51.5	-	87.1	-
14	CAT-26	275	52.3	23.0	50.7	73.7
15	CAT-41	350	36.9	24.1	67.7	91.8
16	CAT-42	338	37.7	27.7	83.8	111.5
17	CAT-51	294	42.2	35.7	103.3	139.0
18	CAT-52	237	35.1	30.9	85.2	116.1
19	CAT-53	261	38.7	27.8	92.9	120.7
20	CAT-54	236	32.4	26.1	94.1	120.2
Mean		266	42.1	25.5	69.1	95.9
Variance		2161	0.5760	26.43	489.0	542.0
S. Dev.		46.49	7.590	5.195	22.11	22.85
S. Err.		10.66	1.697	1.299	5.512	5.900
95% Confidence Interval	U	309	45.7	28.3	80.1	108.6
	L	264	38.6	22.8	58.1	83.3

Appendix 32. Summary of specific activities (μmoles/min/g protein) of membrane and cytosol situated carbonic anhydrase of thermally-acclimated rainbow trout (Salmo gairdneri). Reported as the mean±1 standard error of the mean.

Fraction	Assay Temperature	Acclimation Temperature			Signif. Diff.		
		2°C	10°C	18°C	2°C	10°C	18°C
Membrane	@ 25°C	34.8±1.56	39.1±2.38	52.7±2.75	N.S.	p<0.01	p<0.01
	@ Acclimation Temperature	16.2±0.949	21.7±1.44	45.1±3.01	p<0.01	p<0.01	p<0.01
Cytosol	@ 25°C	8.7±0.839	10.4±1.05	11.1±1.02	N.S.	N.S.	N.S.
	@ Acclimation Temperature	5.6±0.53	6.5±0.71	9.7±0.90	N.S.	p<0.01	p<0.01

Appendix 33. Specific activities (μ of CO_2/min/mg protein) of membrane and cytosol situated carbonic anhydrase of thermally-acclimated rainbow trout (Salmo gairdneri).

Data Item	Code Number	Assay Temperature 25°C Membrane Bound	Assay Temperature 25°C Cytosol	Assay Temperature 2°C Membrane Bound	Assay Temperature 2°C Cytosol
Acclimated to 2°C					
1	CAT-15	39.3	7.2	15.2	6.0
2	CAT-16	37.9	10.3	16.8	7.4
3	CAT-19	33.8	5.9	15.9	3.4
4	CAT-20	35.1	6.1	14.4	4.0
5	CAT-33	35.0	16.3	14.8	8.6
6	CAT-35	32.1	10.4	14.8	5.9
7	CAT-39	39.1	5.8	23.7	7.6
8	CAT-40	30.1	13.3	21.1	7.1
9	CAT-43	39.2	12.2	14.1	9.4
10	CAT-44	46.7	5.5	22.6	2.2
11	CAT-45	29.0	6.8	10.6	5.6
12	CAT-46	36.8	5.7	19.4	2.6
13	CAT 47	31.0	7.3	11.1	5.9
14	CAT 48	40.6	11.7	15.8	5.9
15	CAT-57	22.8	5.1	12.5	3.5
16	CAT-58(a)	27.7	6.1	16.7	4.1
Mean		34.8	8.73	16.2	5.6
Variance		39.07	11.27	14.40	4.53
S. Dev.		6.250	3.356	3.794	2.13
S. Err.		1.563	0.8391	0.9436	0.532
95% Confidence Interval	U	38.2	10.5	18.2	6.7
	L	31.5	6.9	14.2	4.4
Acclimated to 10°C					
1	CAT-17	34.5	7.0	13.3	4.4
2	CAT-18	40.5	15.9	13.5	12.1
3	CAT-21	46.7	8.8	24.9	4.7
4	CAT-22	54.2	20.5	31.3	11.5
5	CAT-35	35.4	15.7	24.1	7.1
6	CAT-36	42.2	10.4	28.1	5.2
7	CAT-37	39.0	8.8	21.2	6.4
8	CAT 38	52.9	11.9	28.0	7.4
9	CAT 59(b)	47.3	8.1	24.6	7.1
10	CAT-59	45.4	6.8	23.6	3.1
11	CAT-60	47.5	9.1	27.8	5.3
12	CAT-61	29.1	6.1	19.2	5.6
13	CAT-62	32.7	7.9	18.5	2.8
14	CAT-63	26.3	9.3	18.2	8.5
15	CAT-65	27.1	13.9	16.1	10.0
16	CAT-66	24.8	5.4	14.1	3.5
Mean		39.1	10.4	21.7	6.5
Variance		90.23	17.49	32.93	8.00
S. Dev.		9.499	4.182	5.738	2.83
S. Err.		2.375	1.046	1.435	0.707
95% Confidence Interval	U	44.2	12.6	24.7	8.05
	L	34.0	8.1	18.6	5.04
Acclimated to 18°C					
1	CAT-2	75.6	-	-	-
2	CAT-6	56.8	13.1	43.5	13.6
3	CAT-3	-	-	67.7	-
4	CAT-7	58.1	11.0	37.7	11.6
5	CAT-8	62.2	9.6	46.2	16.9
6	CAT-9	-	21.0	76.4	16.1
7	CAT-10	59.7	8.9	53.6	8.6
8	CAT-10(b)	50.6	10.1	45.6	8.0
9	CAT-13	50.9	4.0	42.3	-
10	CAT 14	47.0	15.0	36.0	11.3
11	CAT-23	54.2	18.9	43.3	-
12	CAT-24	-	8.1	-	8.1
13	CAT 25	-	10.7	-	-
14	CAT 26	59.1	16.0	49.0	9.5
15	CAT-41	53.6	10.7	42.8	7.3
16	CAT 42	60.3	10.4	44.5	7.7
17	CAT 51	34.5	8.8	32.2	6.1
18	CAT 52	30.3	8.0	24.4	8.5
19	CAT 53	43.6	6.5	35.8	5.6
20	CAT 54	46.0	8.6	39.4	7.3
Mean		52.7	11.1	45.1	9.7
Variance		120.8	18.30	154.1	14.1
S. Dev.		10.99	4.303	12.41	3.69
S. Err.		2.743	1.075	3.010	0.929
95% Confidence Interval	U	58.3	13.3	51.5	11.7
	L	46.8	9.0	38.7	7.8

Appendix 34. Summary of cellular activity (μmoles/min/ml erythrocytes except in the case of whole blood which is μmoles/min/ml blood) of erythrocytic carbonic anhydrase in rainbow trout (Salmo gairdneri). Reported as the mean±1 standard error of the mean.

Fraction	Assay Temperature	Acclimation Temperature			Signif. Diff.		
		2°C	10°C	18°C	2°C	10°C	18°C
Membrane	@ 25°C	1.17±0.029	1.27±0.043	1.30±0.042	N.S.	N.S.	p<0.05
	@ Acclimation Temperature	0.545±0.028	0.715±0.042	1.06±0.029	p<0.01	p<0.01	p<0.01
Cytosol	@ 25°C	0.657±0.055	0.795±0.048	0.734±0.055	p<0.05	N.S.	N.S.
	@ Acclimation Temperature	0.404±0.035	0.515±0.060	0.625±0.057	N.S.	N.S.	p<0.01
Total Erythrocyte	@ 25°C	1.83±0.068	2.06±0.077	2.04±0.073	p<0.05	N.S.	p<0.05
	@ Acclimation Temperature	0.949±0.044	1.23±0.067	1.69±0.064	p<0.01	p<0.01	p<0.01
% of Total Activity of Erythrocyte Contributed by Membrane	@ 25°C	64.7±1.75	61.5±1.20	64.6±1.83	N.S.	N.S.	N.S.
	@ Acclimation Temperature	58.2±2.59	58.8±2.75	64.9±2.00	N.S.	N.S.	N.S.
% of Total Activity of Erythrocyte Contributed By Cytosol	@ 25°C	35.3±1.73	38.2±1.21	35.4±1.84	N.S.	N.S.	N.S.
	@ Acclimation Temperature	41.8±0.030	41.2±2.75	36.2±2.00	N.S.	N.S.	N.S.
Activity that would be present in Whole Blood	@ 25°C	0.608±0.028	0.691±0.027	0.840±0.043	p<0.05	p<0.05	p<0.01
	@ Acclimation Temperature	0.312±0.015	0.411±0.021	0.707±0.032	p<0.01	p<0.01	p<0.01

Appendix 35 Cellular activity (ml O2 / ml erythrocyte / hour) in the cells of whole blood (which contains 0.41 ml erythrocytes/ml blood) of erythrocytic carbonic anhydrase of individual Notothenia coriiceps (Cabo Evans) assayed at 25 C

Data Item	Code Number	Membrane Bound	Cytosol	Total Erythrocyte	Activity Contributed by Membrane Bound	Activity Contributed By Cytosol	Whole Blood
Acclimated to 2 C, Assayed at 25 C							
1	CAT-15	1.28	0.426	1.71	71.6	25.1	0.571
2	CAT-16	1.27	0.460	1.73	73.4	26.6	0.691
3	CAT-19	1.24	1.126	2.37	52.3	47.7	0.871
4	CAT-20	1.13	0.627	1.76	64.2	35.8	0.525
5	CAT-33	1.11	0.642	1.75	63.4	36.6	0.561
6	CAT-31	1.07	0.647	1.70	61.8	38.2	0.600
7	CAT-39	1.24	0.407	1.65	75.2	24.8	0.450
8	CAT-40	1.13	0.567	1.70	66.5	33.5	0.643
9	CAT-43	1.46	0.653	2.31	63.2	36.8	0.713
10	CAT-44	1.06	0.566	1.63	65.0	35.0	0.430
11	CAT-45	1.05	0.672	1.72	61.0	39.0	0.543
12	CAT-46	1.03	0.421	1.47	71.4	28.6	0.550
13	CAT-47	1.24	0.619	1.86	66.7	33.3	0.685
14	CAT-48	1.21	1.149	2.36	51.3	48.7	0.719
15	CAT-57	1.07	0.644	1.71	62.6	37.4	0.531
16	CAT-58(a)	1.12	0.694	1.81	61.9	38.1	0.587
Mean		1.17	0.657	1.83	64.7	35.3	0.608
Variance		1.317x10-2	4.878x10-2	7.347x10-2	0.4760	0.4760	1.289x10-2
S. Dev		0.1146	0.2203	0.2711	6.900	6.900	0.1136
S. Err.		2.866 10-2	5.502x10-2	6.776x10-2	1.725	1.725	2.839x10-2
95% Conf. Interval	U	1.23	0.775	1.97	68.4	39.0	0.668
	L	1.11	0.540	1.68	61.0	31.6	0.547
Acclimated to 10 C, Assayed at 25°C							
1	CAT-17	1.19	0.634	1.82	65.4	34.6	0.658
2	CAT-18	1.17	0.971	2.14	54.7	45.3	0.686
3	CAT-21	1.36	0.671	2.05	67.3	32.7	0.806
4	CAT-22	1.45	1.060	2.51	57.8	42.2	0.769
5	CAT-35	1.52	0.844	2.36	64.4	35.6	0.622
6	CAT-36	1.25	0.690	1.92	64.1	35.1	0.742
7	CAT-37	1.60	0.847	2.45	65.3	34.7	0.794
8	CAT-38	1.45	0.992	2.44	59.4	40.6	0.643
9	CAT-52(b)	1.05	0.685	1.74	60.3	39.7	0.538
10	CAT-	1.02	0.512	1.53	66.7	33.3	0.557
11	CAT-60	1.10	0.703	1.80	61.1	38.9	0.724
12	CAT-61	1.12	0.591	1.71	65.5	34.5	0.507
13	CAT-62	1.26	0.751	2.01	62.7	37.3	0.669
14	CAT-63	1.29	0.956	2.25	57.3	32.7	0.808
15	CAT-65	1.20	1.183	2.38	50.4	49.6	0.649
16	CAT-66	1.22	0.633	1.85	65.9	34.1	0.629
Mean		1.27	0.795	2.06	61.8	38.2	0.691
Variance		2.900x10-2	3.629x10-2	9.541x10-2	0.2320	0.2348	1.166x10-2
S. Dev		0.1705	0.1905	0.3089	4.816	4.846	0.1080
S. Err.		4.262 10-2	4.762x10-2	7.722x10-2	1.204	1.212	2.700x10-2
95% Conf. Interval	U	1.36	0.897	2.22	64.3	40.8	0.749
	L	1.1	0.694	1.90	59.2	35.6	0.634
Acclimated to 18°C, Assayed at 25 C							
1	CAT-2	1.11	0.560	1.67	66.5	33.5	0.663
2	CAT-6	1.64	0.969	2.61	62.8	37.2	0.750
3	CAT-3	1.43	1.310	2.74	52.2	47.8	0.840
4	CAT-7	1.47	0.482	1.95	75.4	24.6	0.970
5	CAT-8	1.42	0.319	1.74	81.6	18.4	0.612
6	CAT-9	1.41	0.511	1.92	73.4	26.6	0.909
7	CAT-10	1.07	0.627	1.70	62.9	37.1	0.900
8	CAT-10(b)	1.16	0.601	1.76	65.9	34.1	0.728
9	CAT-13	1.43	0.305	1.71	81.9	18.1	0.706
10	CAT-14	1.40	0.829	2.29	64.6	35.4	1.130
11	CAT-23	1.22	1.052	2.27	53.7	46.3	1.000
12	CAT-24	1.15	0.700	1.85	62.2	37.8	0.900
13	CAT-25	1.29	0.932	2.21	57.9	42.1	1.140
14	CAT-26	1.36	0.841	2.20	61.8	38.2	1.150
15	CAT-41	1.27	0.725	1.98	62.6	37.4	0.729
16	CAT-42	1.67	0.873	2.54	65.7	34.3	0.957
17	CAT-51	1.23	0.909	2.14	57.5	42.5	0.913
18	CAT-52	0.95	0.723	1.67	57.0	44.0	0.583
19	CAT-53	1.21	0.604	1.81	66.9	33.1	0.467
20	CAT-4	1.29	0.810	2.01	59.7	40.3	0.630
Mean		1.30	0.774	2.04	64.6	35.4	0.849
Variance		2.513 10-2	6.913x10-2	0.1067	0.0124	0.0256	6.632 10-2
S. Dev		0.1574	0.2487	0.3267	3.523	0.2029	0.1136
S. Err.		4.191x10-2	6.937x10-2	9.104x10-2	8.268	1.853	4.209 10-2
95% Conf. Interval	U	1.39	0.949	2.19	69.4	39.3	0.930
	L	1.2	0.601	1.89	60	31.6	0.750

Appendix 3o. Cellular activity (units/g wet/ml erythrocyte except in the case of whole blood which is in units of g wet/min/ml blood) of erythrocytic carbonic anhydrase of thermally acclimated rainbow trout (salmo gairdneri) assayed at the temperature of acclimation.

Data Item	Code Number	Membrane Bound	Cytosol	Total Erythrocyte	Activity Contributed By Membrane	Activity Contributed By Cytosol	Whole Blood
Acclimated to 2°C, Assayed at 2°C							
1	CAT-15	0.169	0.171	0.640	73.3	26.7	0.214
2	CAT-16	0.464	0.334	0.893	62.8	37.2	0.358
3	CAT-19	0.582	0.388	0.970	60.0	40.0	0.357
4	CAT-20	0.469	0.417	0.876	52.4	47.6	0.263
5	CAT-33	0.472	0.340	0.812	58.1	41.9	0.269
6	CAT-34	0.485	0.364	0.849	57.1	42.9	0.300
7	CAT-39	0.752	0.529	1.281	58.7	41.3	0.305
8	CAT-40	0.793	0.303	1.096	72.4	27.6	0.414
9	CAT-43	0.524	0.659	1.183	44.3	55.7	0.331
10	CAT-44	0.490	0.229	0.719	68.1	31.9	0.190
11	CAT-45	0.396	0.554	0.950	41.7	58.3	0.300
12	CAT-46	0.555	0.192	0.747	74.3	25.7	0.279
13	CAT-47	0.445	0.503	0.948	46.9	53.1	0.350
14	CAT-48	0.472	0.575	1.047	45.1	54.9	0.319
15	CAT-57	0.583	0.443	1.026	56.8	43.2	0.319
16	CAT-58(a)	0.675	0.463	1.138	59.3	40.7	0.369
Mean		0.545	0.404	0.949	58.2	41.8	0.312
Variance		1.251×10^{-2}	1.988×10^{-2}	3.094×10^{-2}	1.076	1.076	3.617×10^{-3}
S. Dev.		0.1118	0.1410	0.1759	10.37	10.37	6.014×10^{-2}
S. Err.		2.796×10^{-2}	3.525×10^{-2}	4.398×10^{-2}	2.594	2.594	1.504×10^{-2}
95% Conf. Interval	U	0.604	0.479	1.042	63.7	47.3	0.344
	L	0.485	0.329	0.855	52.7	36.3	0.280
Acclimated to 10°C, Assayed at 10°C							
1	CAT-17	0.456	0.396	0.852	53.5	46.5	0.308
2	CAT-18	0.389	0.738	1.13	34.3	65.7	0.362
3	CAT-21	0.731	0.356	1.09	67.1	32.9	0.458
4	CAT-22	0.836	0.612	1.45	57.7	42.3	0.414
5	CAT-35	1.034	0.380	1.41	73.3	26.7	0.372
6	CAT-36	0.819	0.345	1.16	70.6	29.4	0.450
7	CAT-37	0.867	0.616	1.48	58.6	41.4	0.482
8	CAT-38	0.765	0.620	1.39	55.0	45.0	0.479
9	CAT-58(b)	0.544	0.604	1.15	47.3	52.7	0.356
10	CAT-59	0.531	0.236	0.767	69.2	30.8	0.278
11	CAT-60	0.641	0.414	1.06	60.5	39.5	0.425
12	CAT-61	0.740	0.550	1.29	57.4	42.6	0.382
13	CAT-62	0.713	0.263	0.976	73.1	26.9	0.325
14	CAT-63	0.995	0.876	1.77	50.6	49.4	0.636
15	CAT-65	0.715	0.857	1.57	45.5	54.5	0.428
16	CAT-66	0.760	0.380	1.14	66.7	33.3	0.386
Mean		0.715	0.515	1.23	58.8	41.2	0.411
Variance		2.874×10^{-2}	3.954×10^{-2}	7.196×10^{-2}	1.212	1.212	7.313×10^{-3}
S. Dev.		0.1695	0.1989	0.2682	11.01	11.01	8.552×10^{-2}
S. Err.		4.239×10^{-2}	4.971×10^{-2}	6.706×10^{-2}	2.753	2.753	2.138×10^{-2}
95% Cont. Interval	U	0.805	0.621	1.37	64.6	47.1	0.456
	L	0.624	0.409	1.09	52.9	35.4	0.365
Acclimated to 18°C, Assayed at 18°C							
1	CAT-2	-	-	-	-	-	
2	CAT-6	1.25	1.01	2.26	55.3	44.7	0.651
3	CAT-3	1.17	0.874	2.04	57.4	42.6	0.628
4	CAT-7	0.912	0.478	1.39	65.6	34.4	0.715
5	CAT-8	1.06	0.568	1.63	65.0	35.0	0.371
6	CAT-9	1.19	0.341	1.53	77.8	22.2	0.720
7	CAT-10	0.958	0.590	1.55	61.8	38.2	0.841
8	CAT-10(b)	1.05	0.486	1.54	68.2	31.8	0.683
9	CAT-13	1.16	0.267	1.43	81.1	18.9	0.625
10	CAT-14	1.12	0.600	1.73	64.7	35.3	0.854
11	CAT-23	1.07	1.35	2.42	44.2	55.8	1.073
12	CAT-24	0.926	0.700	1.63	56.8	43.2	0.790
13	CAT-25	1.07	0.47	1.50	71.3	28.7	0.770
14	CAT-26	1.15	0.497	1.65	69.7	30.3	0.360
15	CAT-41	0.967	0.503	1.49	65.2	33.8	0.550
16	CAT-42	1.23	0.606	1.84	66.8	33.2	0.693
17	CAT-51	1.15	0.632	1.78	64.6	35.4	0.750
18	CAT-52	0.753	0.733	1.49	50.5	49.5	0.522
19	CAT-53	0.981	0.517	1.72	65.6	34.4	0.523
20	CAT-54	1.02	0.695	1.72	59.3	40.7	0.546
Mean		1.06	0.625	1.69	63.9	36.2	0.707
Variance		1.566×10^{-2}	6.981×10^{-2}	7.755×10^{-2}	0.7617	0.7652	1.902×10^{-2}
S. Dev.		0.1247	0.2660	0.2785	8.748	8.749	0.1379
S. Err.		2.861×10^{-2}	6.097×10^{-2}	6.38×10^{-2}	2.007	2.007	3.161×10^{-2}
95% Conf. Interval	U	1.12	0.763	1.83	68.0	40.4	0.774
	L	1.00	0.507	1.56	59.6	31.0	0.641

Appendix 57 Descriptive statistics of various regressions present in the Results and Discussion section. Shown is the figure being defined, type of regression performed (geometric, G, or linear, L), the number of pairs (x,y) regressed (N), coefficients A and B (for curve $y = A\,B^{x}$), the 0 and 1 degree coefficient (for $y = mx + b$), the coefficient of correlation and the standard error of the estimate.

Figure	Regression	N	A	B	0 Degree Coefficient	1 Degree Coefficient	Coefficient of Correlation	Standard Error
3a @ 30°C	G	14	14.9	-0.131	-	-	0.6408	0.1387
@ 16°C	G	14	7.67	-6.15×10^{-3}	-		4.981×10^{-2}	0.1364
@ 2°C	G	14	5.90	4.95×10^{-2}	-	-	0.4016	0.1043
3b @ 30°C	G	14	68.0	-0.154	-	-	0.7753	0.1113
@ 16°C	G	14	35.7	-2.62×10^{-2}	-	-	0.2098	0.1352
@ 2°C	G	14	30.4	-2.23×10^{-2}	-	-	0.1845	0.1097
3c @ 30°C	G	14	3.22	-0.143	-	-	0.6826	0.1361
@ 16°C	G	14	2.06	-8.24×10^{-2}	-	-	0.4965	0.1592
@ 2°C	G	14	1.54	-1.04×10^{-2}	-	-	6.892×10^{-2}	0.1359
4a @ 30°C	G	14	6.84	-0.104	-	-	0.7749	7.531×10^{-2}
@ 16°C	G	14	4.65	-1.38×10^{-2}	-	-	0.1876	0.1144
@ 2°C	G	14	5.12	-6.94×10^{-2}	-	-	0.5323	0.1019
4b @ 30°C	G	14	1.94	0.952	-	-	0.6570	0.1361
@ 16°C	G	14	3.18	0.589	-	-	0.5020	0.1181
@ 2°C	G	14	1.0	0.237	-	-	0.3138	0.1082
4c @ 30°C	G	14	6.41	1.14	-	-	0.7726	0.1118
@ 16°C	G	14	15.8	0.485	-	-	0.4031	0.1267
@ 2°C	G	14	16.5	0.4386	-	-	0.4737	9.80×10^{-2}
4d @ 30°C	G	14	0.338	1.10	-	-	0.7051	0.1320
@ 16°C	G	14	0.638	0.568	-		0.3605	0.1717
@ 2°C	G	14	1.31	8.76×10^{-2}	-	-	7.571×10^{-2}	0.1388
9a	L	37	-	-	330	-0.529	0.3951	29.89
9b	L	43	-	-	505	-0.897	0.4018	40.46
10a @ 16°,30°C	L	27	-	-	102	-2.51	0.9095	3.424
@ 2°C	L	13	-	-	88.9	-2.66	0.7066	2.255
10b	L	103	-	-	242	-18.2	0.3563	6.582
15 @ 16°,30°C	G	38	7.03	0.326	-	-	0.7375	0.1350
@ 16° 30°C	L	25	-	-	8.07	0.779	0.8011	1.806
@ 2°C	L	13	-	-	16.4	3.38×10^{-3}	1.061×10^{-2}	0.8469
16	L	108	-	-	7.38	0.190	0.4227	1.033