Comparative Studies of Hemoglobin Polymorphism, Oxygen Consumption, and Oxygen Equilibria of Hemoglobin Solution in Three Species of *Salvelinus*

by

Shiping Feng

A Thesis Presented to
The University of Manitoba
in Partial Fulfillment of the Requirements for the Degree of

Master of Science

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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ABSTRACT

Studies were conducted to identify the polymorphic hemoglobins and to compare the responses to temperature variation of hemoglobin P_{50} and standard metabolic rate in Arctic charr (Salvelinus alpinus), brook trout (S. fontinalis), and lake trout (S. namaycush).

At least 16 electrophoretically distinct hemoglobins were identified with 7 components common to all three species. Both Arctic charr and lake trout exhibited 5 anodic and 5 cathodic hemoglobins and the electrophoretic pattern of both species was invariant. Brook trout, however, exhibited two hemoglobin patterns, with the most common (84% of fish) consisting of 4 anodic and 7 cathodic components. The second hemoglobin pattern in brook trout was identical to the first except for the appearance of an additional minor anodic component. The total cathodic hemoglobin concentration in lake trout was significantly lower than that in Arctic charr and brook trout.

The oxygen affinities (P_{50}) of the hemoglobins of the three species were measured at three temperatures (5, 10, 16°C), three pH values (7.1, 7.5, 7.9), and four ATP levels (ATP:Hb = 0.00, 1.25, 2.50, 5.00 mol/mol). The hemoglobins of brook trout exhibited the lowest sensitivities to variations in temperature and pH. The oxygen binding characteristics of Arctic charr hemoglobins were similar to those of brook trout. At higher temperatures (10 and

16°C), the hemoglobins of lake trout had the lowest oxygen affinity and the largest Bohr effect. The Bohr effect ($\Delta \log p_{50}$ / ΔpH) at 16°C was 0.29, 0.33, and 0.39 for brook trout, Arctic charr, and lake trout, respectively. The apparent heat of oxygenation (ΔH $Kcalmol^{-1}$) at pH = 7.5 was -5.8, -10.2, and -15.3 for brook trout, lake trout, and Arctic charr, respectively. ATP reduced hemoglobin oxygen affinity in all species at 10°C and three pH levels. The response to ATP was similar in brook trout and Arctic charr but higher in lake trout.

Standard metabolic rate (SMR) was determined by closed-vessel respirometry for the three species at nominal temperatures of 5, 10, and 16°C over a body mass range of 7-390 g. The metabolic rates of the three species were found to increase with increasing water temperature and body mass. However, the correlation coefficients for mass and temperature were 0.664 and 0.024, 0.673 and 0.033, 0.895 and 0.039, for brook trout, Arctic charr, and lake trout, respectively. Estimated Q_{10} values were 1.8 for brook trout, 2.1 for Arctic charr, and 2.5 for lake trout over the 5 - 16°C temperature range.

The differences identified among the three species of Salvelinus with respect to hemoglobin heterogeneity, standard metabolic rate, and hemoglobin oxygen equilibrium characteristics were consistent with published values for the range of environmental conditions preferred or tolerated by each species.

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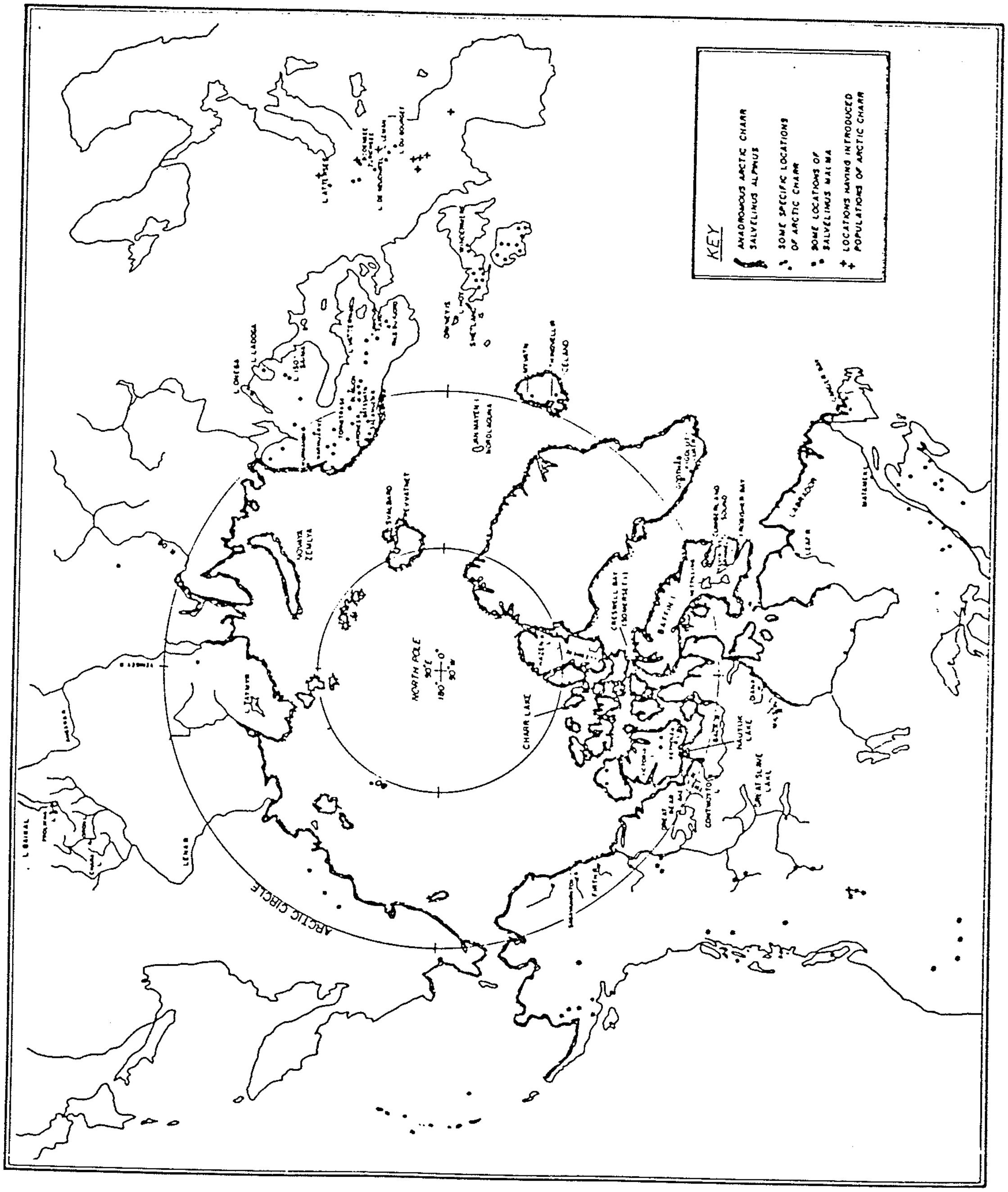
INTRODUCTION

Of the living representatives of the genus Salvelinus, Arctic charr (S. alpinus), lake trout (S. namaycush) and brook trout (S. fontinalis) are considered to be the most closely related since they diverged from a common ancestor probably before the Pleistocene (Behnke 1980; Savvaitova, 1980). Although considerable overlap exists, these three species generally occupy different aquatic habitats in terms of their temperature and dissolved oxygen ranges.

The geographic distribution and life history of Arctic charr are extremely complex. Johnson (1980) stated that the present distribution of Arctic charr undoubtedly reflects the geological events of the Pleistocene period, such as the advance and retreat of glaciers, the submergence and emergence of land forms under the weight of ice and the provision of pathways for dispersal by the formation of large proglacial lakes with ice damming of northerly flowing rivers. Generally, Arctic charr have been found in lakes, rivers and streams around the northern hemisphere in association with a restricted number of other fish species (Scott and Crossman, 1973) (Fig. 1), although relic populations have been identified in the north-east United states and Quebec (Johnson, 1980).

Arctic charr occur as both landlocked or nonmigratory and anadromous forms. Kornfield et al. (1981) suggested that impassable conditions resulting from the effects of climate caused the isolation

FIGURE 1. Sketch-map of the Polar regions showing the distribution of Arctic charr, _Salvelinus_ _alpinus_. The absence of marking in the Pacific Basin does not necessarily indicate that Arctic charr do not occur here, but its exact distribution must be determined by the appropriate investigations (_Adopted from Johnson_, 1980).


KEY
ANADROMOUS ARCTIC CHARR SALVELINUS ALPINUS
SOME SPECIFIC LOCATIONS OF ARCTIC CHARR
SOME LOCATIONS OF SALVELINUS MALMA
LOCATIONS HAVING INTRODUCED POPULATIONS OF ARCTIC CHARR
NORTH POLE
ARCTIC CIRCLE
CHARR LAKE
NAUTIK LAKE
GREAT SLAVE LAKE
CRESWELL BAY (SOMERSET I)
BAFFIN I
CUMBERLAND SOUND
LABRADOR
SVALBARD
NOVAYA ZEMLYA
JAN MAYEN I
ICELAND
L. LADOGA
L. ONEGA
L. BAIKAL
LENA

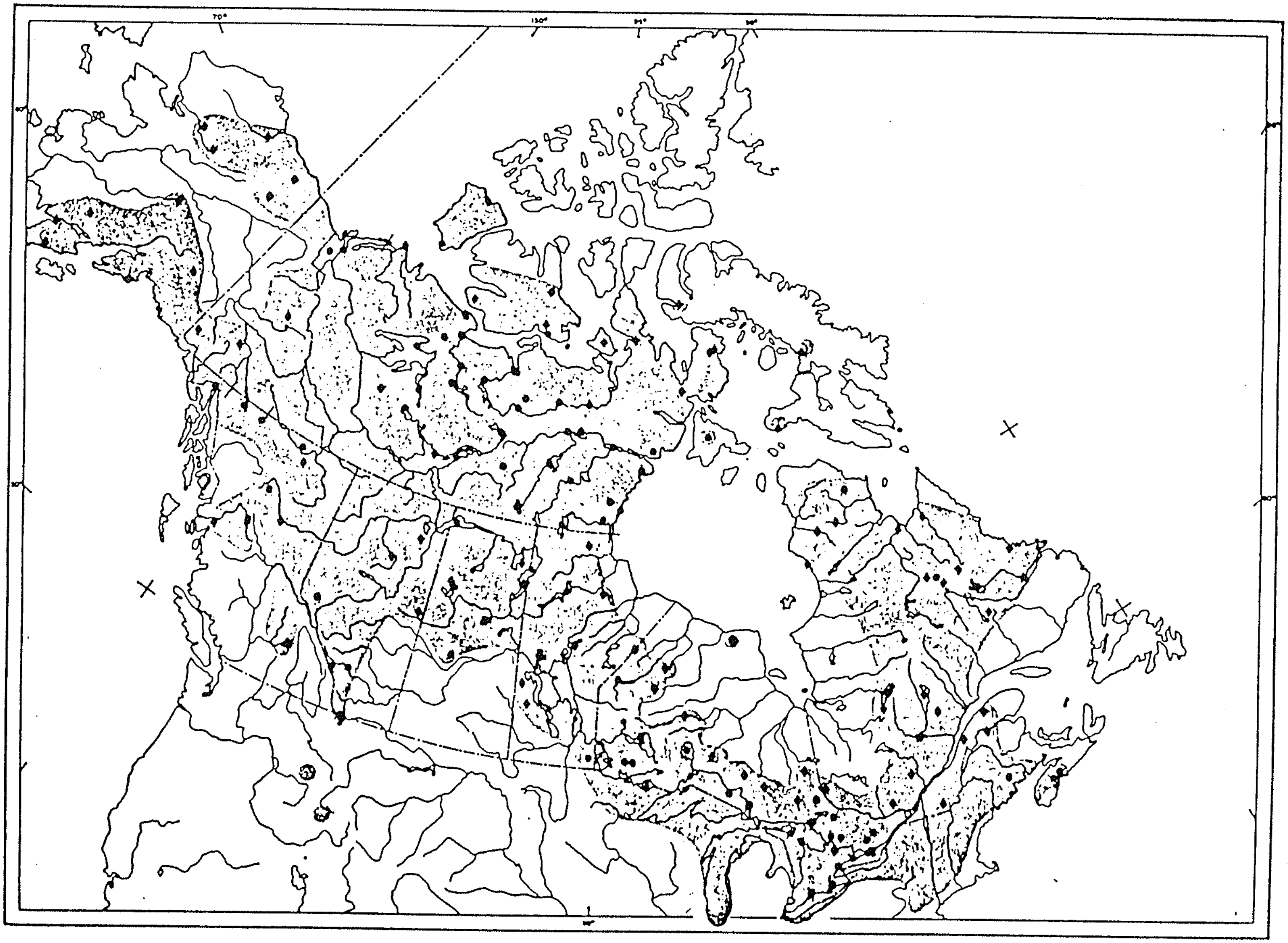
of nonanadromous stocks of Arctic charr. In anadromous stocks, the salinity tolerance of Arctic charr develops after 4 to 5 years of freshwater residence (Nielsen, 1961). Seaward migration of adults and subadults may occur annually or at longer intervals. The seaward migration of Arctic charr may start as early as mid-May or early June when rivers or lakes are still frozen. After their intensive feeding in the sea for a relatively short period Arctic charr return to freshwater in mid-August or early September (Moore and Moore, 1974; Johnson and Campbell, 1976; Johnson, 1980).

The most northerly distribution of Arctic charr may reflect its special capability to cope with cold environmental conditions although Arctic charr cannot tolerate seawater in combination with low temperature and must overwinter in freshwater (de Vries, 1971). In some high Arctic lakes such as Stanwell-Fletcher Lake, water temperature remains below 1.6°C throughout the year (Rust and Coakley, 1970). In small lakes in the Saqvaqjuac area, Arctic charr experience annual temperature extremes of 1.5 to 16°C (Dalton, 1981; Welch et al., 1987), which may reflect a normal environmental temperature range for this species. Although the incipient lethal temperatures for Arctic charr have not been precisely defined, it was reported that Arctic charr exhibit greatly reduced growth at temperature >17°C (Baker, 1983). Arctic charr exposed to 22°C for two days in net cages experienced approximately 50% mortality within two days and the remaining fish all died within three weeks despite a reduction of water temperature to 18°C (Giles, unpublished observation). Preferred water temperatures have not been established for Arctic charr, but the optimum growth temperature appears to be 13°C (Baker, 1983).

Lake trout are generally classified as a cold stenothermal species and occupy deeper lakes in northern North America (Behnke, 1980). In Canada this species is found in all provinces and territories except Prince Edward Island and insular Newfoundland (Scott and Crossman, 1973) (Fig. 2). Recently, lake trout have been introduced to Europe, South America and New Zealand (Behnke, 1980). Lake trout have a highly specialized mode of life with narrow environmental tolerances (Behnke, 1972). By making extensive vertical and horizontal movements, lake trout avoid changes in environmental temperature. High summer temperatures limit the distribution of lake trout and as surface waters warm these fish migrate to deeper water (Martin and Olver, 1980). The ultimate upper lethal temperature for lake trout was reported as 23.5°C (Gibson and Fry, 1954). A temperature range of 6 to 13°C is preferred by the lake trout (Martin and Olver, 1980; Ferguson et al., 1983). Lake trout are among the least tolerant to seawater of all North American salmonids (Rounsefell, 1958; Boulva and Simard, 1968).

Brook trout are endemic to northeastern North America (Behnke, 1980). This species has a worldwide distribution and has been introduced to Western North America, South America, Eurasia, and New Zealand (MacCrimmon and Campbell, 1969; MacCrimmon et al., 1971; Behnke, 1980). In Canada, brook trout populations are resident in all

FIGURE 2. The distribution of lake trout, Salvelinus namaycush. The shaded portion represent the total range of the species. Round spots indicate the existence of a specimen. Diamond-shaped spots represent literature references (Adopted from Scott & Crossman, 1973).

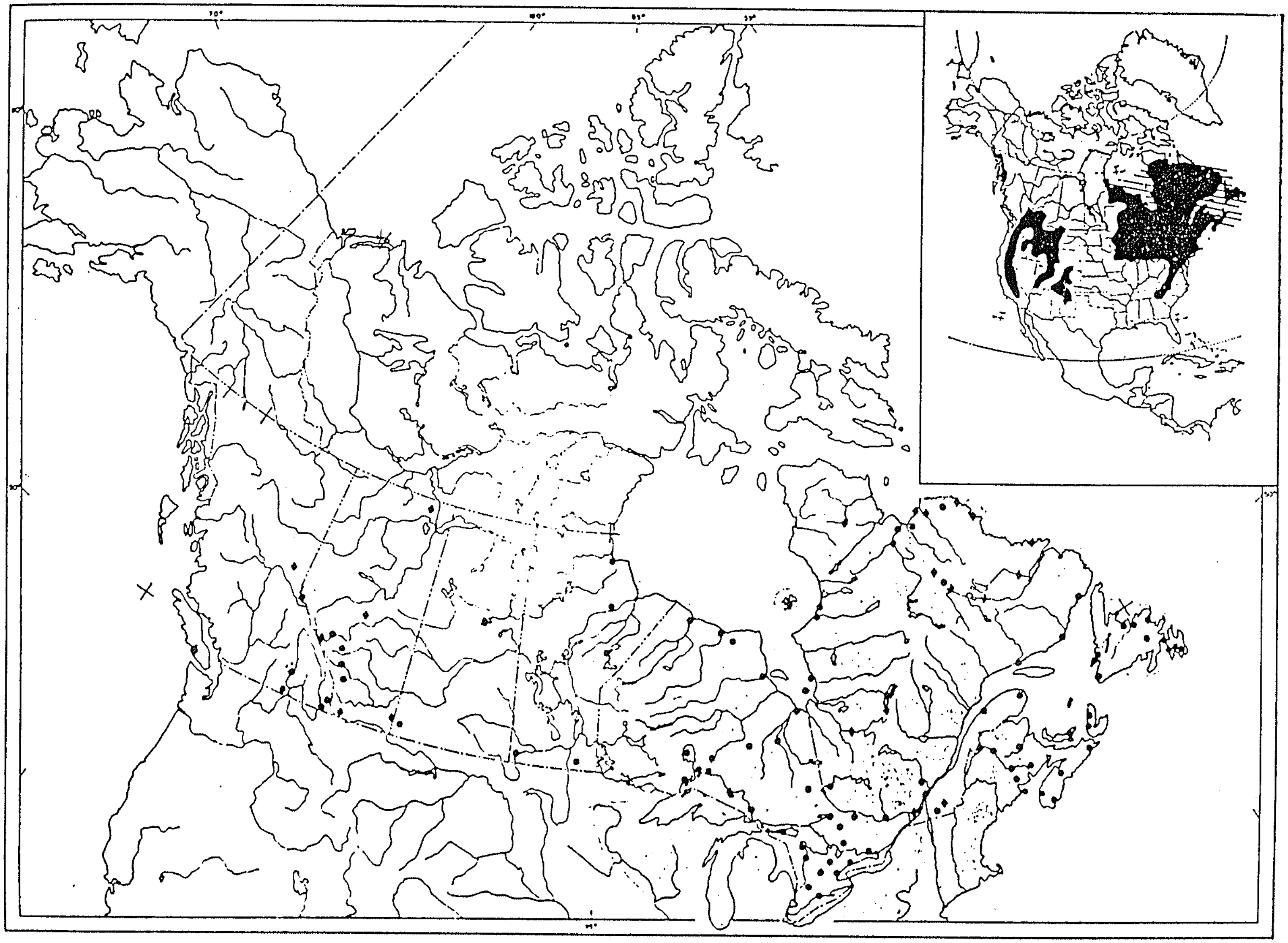

70°
130°

provinces (Scott and Crossman, 1973) (Fig. 3). Brook trout occur in cool, clear, and well-oxygenated rivers and streams, and less commonly in lakes. This species can either be sedentary or can migrate regularly between feeding, overwintering and spawning areas. The normal range of water temperature found in brook trout habitats is from 0 to 24°C (Ricker, 1932; Power, 1980). Under laboratory conditions the upper incipient lethal temperature is 25.3°C (Fry et al., 1946). A temperature range of 13-16.1°C provided optimum thermal conditions for the growth of brook trout (Baldwin, 1956; McCormick et al., 1972; Hokanson et al., 1973). The preferred temperature for brook trout is 14-19°C (Fisher and Elson, 1950; Cherry et al., 1975, 1977).

Numerous investigators have studied the relationship between the physiological characteristics of fish and the diversity of environmental habitats in which these animals live. Fish which occupy environmentally variable habitats are generally more tolerant of fluctuating conditions than species from more stable environments (Riggs, 1970; Powers, 1980). The identified physiological mechanisms that allow fish to adapt to environmental fluctuations in water temperature and dissolved oxygen include an increased capacity for altering ventilation rate (Wares and Ingram, 1979) and cardiac output (Smith and Jones, 1982; Randall and Daxboeck, 1982; Taylor and Barrett, 1985), as well as adjustments in the oxygen transport functions of blood (Holeton and Randall, 1967; Holeton, 1973; Scott and Rogers, 1981; Randall et al., 1982; Saint-Paul, 1984).

The physiological adaptations of fish blood to changes in

FIGURE 3. The distribution of brook trout, *Salvelinus fontinalis*. The shaded portion represent the total range of the species. Round spots indicate the existence of a specimen. Diamond-shaped spots represent literature references. The insert map represent the range of the species in North America (*Adopted from Scott & Crossman*, 1973).


70°
100°
85°
55°

temperature include: (1) increases in oxygen carrying capacity of blood resulting from increases in hemoglobin concentration, (2) changes in hemoglobin oxygen affinity, and (3) alterations in the intraerythrocytic levels of allosteric modifiers, such as ATP (adenosine triphosphate) and GTP (guanosine triphosphate) (Irving et al., 1941; Farghally et al., 1973; Houston, 1980; Powers, 1980; Wood, 1980; Smit et al., 1981; Smit and Hattingh, 1981; Yamamoto et al., 1983; Tun and Houston, 1986).

Increases in hematocrit and/or hemoglobin concentration of fish blood may be caused by hemoconcentration (Yamamoto et al., 1983) due to water shift from blood plasma to the tissues, elevated urinary water loss, swelling of erythrocytes, or release of erythrocytes into the circulating blood from the storage organs e.g., spleen. Generally, the hematological parameters in a fish reflect the ecological conditions of its habitat (Smit et al., 1981). For example, air-breathing fish and fish living in water of low environmental oxygen content have higher hemoglobin concentration (Mishra et al., 1977; Albers et al., 1981; Jensen and Weber, 1982; Wells et al., 1986). Moreover, hematocrit and hemoglobin concentration vary among teleosts, but in general are low in slow moving, sedentary, and benthic species, and high in active, predacious, and pelagic species (Larsson et al., 1976; Putnam and Freel, 1978; Wells et al., 1980).

The oxygen affinity of hemoglobin, as measured by the half-saturating concentration (P_{50}) of oxygen, is both directly and indirectly influenced by environmental temperature. The direct effect is due to the exothermic nature of the oxygen binding to hemoglobin. The indirect effect is governed via the Bohr effect by the inverse relationship between blood pH and temperature (Antonini & Brunori, 1981). The Bohr effect ($\Delta \log P_{50}/\Delta pH$) is a measure of the effect of hydrogen ion concentration or Pco_2 on hemoglobin oxygen affinity (Brittain, 1987 for review). Barcroft and King (1909) were the first to show that an increase in temperature will decrease the oxygen-affinity of hemoglobin. However, the effect of temperature on blood P_{50} varies in different species and may depend on the temperature ranges to which fish are exposed (Johansen & Lenfant, 1972; Nikinmaa et al., 1980).

The hemoglobin oxygen affinity of fish is also related to the availability of environmental oxygen. Fish living in hypoxic waters have higher blood oxygen affinities than fish living in more oxygenated waters (Johansen et al., 1978; Powers et al., 1979; Powers, 1980). Fonseca De Almeida-Val et al. (1985) reported that the same species collected from different environments had significantly different P_{50} values. These intraspecific differences indicated that the adaptive capabilities shown by fish are dependent on the complexity of environments. Riggs (1970) pointed out that hemoglobin P_{50} is also sensitive to the metabolic needs of fish. Fish such as trout are very active and have the highest oxygen requirements. The hemoglobins of these fish have a comparatively low oxygen affinity which facilitates the supply of oxygen to the tissues.

Intraerythrocytic organophosphates (ATP or GTP) can modify oxygen affinity of fish hemoglobin through two mechanisms. The direct or allosteric effect of this change is produced by selective binding to deoxygenated hemoglobin molecules. The indirect effect is a result of decreased intracellular pH at elevated ATP levels. This drop in pH is caused by an altered Donnan distribution of protons resulting in a Bohr shift of the oxygen dissociation curve (Wood, 1980). Nikinmaa and Soivio (1982) studying blood oxygen binding properties of hypoxic rainbow trout in vivo, observed a dramatic decrease in P_{50} in hypoxic fish and suggested this decrease of P_{50} resulted from the increase in red cell volume which causes a series of interrelated responses. The swelling of the erythrocytes decreased the concentrations of ATP and hemoglobin in the cell. This decrease changed the Donnan distribution of protons across the red cell membrane, thus increasing the intraerythrocytic pH and producing an increase in hemoglobin oxygen affinity. Modulation of hemoglobin P_{50} by nucleotide triphosphates has been reported in a wide variety of fish including eels (Wood and Johansen, 1972; Laursen et al., 1985; Andersen et al., 1985), mudsucker (van Aardt and Frey, 1986), Australian blackfish (Dobson and Baldwin, 1982), Antarctic fish (Tetens and Lykkeboe, 1984; Tetens et al., 1984), and salmonids (Tetens and Lykkeboe, 1981; Nikinmaa and Soivio, 1982). The relative importance of ATP versus GTP varies substantially among different species.

The hemoglobins of teleosts normally include a number of electrophoretically distinguishable components. These components have been attributed to the tetramer combinations of hemoglobin subunits (Gillen and Riggs, 1973; Powers, 1986). The hemoglobin multiplicity probably represents qualitative adaptations to the physical and chemical variations of the environment (Powers, 1974; van Vuren and Hattingh, 1978; Fyhn et al., 1979). Hattingh (1976) suggested that environmental conditions can influence the number and concentration of hemoglobin components in fish. Increases in total hemoglobin concentrations and individual hemoglobin isomorph abundance associated with increased temperature, hypoxia, and shortened daylight have been observed in salmonids, e.g. brook trout (Houston and de Wilde, 1969) and rainbow trout (Houston and Cyr, 1974; Tun and Houston, 1986). However,many studies have shown that qualitative and/or quantitative changes in the hemoglobin composition of adult fish are not related to environmental or physiological factors but may be related to ontogenetic factors (Tsuyuki et al., 1966; Yamanaka et al., 1967; Giles and Vanstone, 1976). Comparative studies have indicated that the electrophoretically distinct hemoglobin components of individual fish species exhibit different oxygen binding properties (Binotti et al., 1971; Weber et al., 1976a,b; Giles and Randall, 1980; Harrington, 1986). According to the functional properties of hemoglobins, fish can be divided into three major categories, as suggested by Weber et al. (1976a or b). Class I contains species with one or more hemoglobins, all of which are sensitive to both temperature and pH. Class II includes species with multiple hemoglobin components, some of which are similar to the class I hemoglobins, while other components are not affected by temperature and pH. In class III, fishes have hemoglobins that are pH sensitive but temperature insensitive.

Within certain limits, the oxygen uptake of fish increases with increasing temperature. At higher temperatures fish need more oxygen although the solubility of oxygen decreases with temperature. Therefore, the decrease in oxygen availability in the environment is a direct challenge to the oxygen uptake, transport, and delivery system of the fish. The decrease of dissolved oxygen in water will decrease the oxygen concentration gradient across fish gills. This limits oxygen saturation of the fish blood and may reduce oxygen delivery to the tissues (Hughes, 1981). As a result, the oxygen availability may affect energy available for fish activities. When oxygen supply is insufficient to meet the minimal energy demands for maintenance, fish will be unable to survive.

The adaptation at the organismic level to cope with the seasonal variations in dissolved oxygen and temperature includes two strategies. The first may be categorized as a behavioural strategy wherein the fish moves to a new location in order to maintain itself in its acceptable range of key environmental conditions. The second strategy is to defend the metabolic rate and extract more oxygen from the environment (Hochachka and Somero, 1974; Boutilier et al., 1988). Beamish (1990) pointed out that the metabolic rate of fish reflects not only environmental quality but also the physiological status of fish. Hence, the range of the adjustments in metabolic rate (MR) employed by fish can be assumed to vary among species which live in different environments.

The MR of fish can be determined indirectly from measurements of oxygen consumption. There are three levels of MR which are termed "standard", "routine", and "active" MR, respectively. The standard metabolic rate (SMR) is a measure of the heat production of a healthy, quiescent, and postabsorptive fish. Routine metabolic rate is the rate at which oxygen is consumed by a fish performing normal spontaneous movements. Active metabolic rate is the maximum sustained rate of heat production for a fish swimming steadily (Fry, 1971; du Preez, 1987; Brill, 1987).

The relationship between the MR (Vo_2) and fish body mass (M) is described by the power function $Vo_2 = aM^b$, where constants a and b are empirically determined (Winberg, 1956; Fry, 1971). Since Vo_2 is curvilinearly related to the mass of fish, a logarithmic transformation of both body mass and MR is used to linearize allometric data (Caulton, 1978; Tarby, 1981). By combining the mean rate of oxygen consumption for fish at specified water temperatures (T) into a multiple regression analysis, Tarby (1981) developed a model where $\log Vo_2 = a + b\log M + cT$. This equation assumes that the rate of metabolism is a function of the fish body mass and water temperature.

The hypothesis of this study is that variations in physiological characteristics expressed in Arctic charr, brook trout, and lake trout reflect differences in the environmental conditions preferred and/or tolerated by each species. The qualitative differences in the polymorphic hemoglobins may be related to the functional properties of hemoglobin in fish. In terms of metabolic oxygen requirements and blood oxygen equilibrium characteristics, the brook trout, a cold water eurytherm, should exhibit the lowest sensitivity to temperature variations while the lake trout, a coldwater stenotherm, should exhibit the greatest sensitivity. The objectives of this research were: (1) to compare the electrophoretic identity of the multiple hemoglobins of the three species, (2) to determine oxygen binding characteristics of each species' hemoglobins at different temperatures, pH values, and ATP levels, and (3) to define the SMR responses of the three species to a specified range of temperatures.

MATERIALS AND METHODS

Experimental Animals

Arctic charr (Salvelinus alpinus) were obtained from the Rockwood Aquaculture Research Centre at Gunton, Manitoba (tank No. N2C). These hatchery-reared Arctic charr originated from the artificial spawning of anadromous male and female charr from the Fraser River, Labrador (63°30′ W, 56°45′ N) in 1981. Lake trout (Salvelinus namaycush) were reared from eggs of wild fish collected at Clearwater Lake, Manitoba (101°00′ W, 54°00′ N) in 1987 and maintained at the Freshwater Institute, Winnipeg, Manitoba. Brook trout (Salvelinus fontinalis) were collected by electroshocking from the South Duck River in western Manitoba (100°40′ W, 52°48′ N) on June 10 and August 25, 1988. Mortality during collection and holding of the brook trout (a period of two years) was less than 2%. All three species were maintained at 10°C before they transferred to experimental tanks.

All fish were kept in the holding tanks (size = 160 L) supplied with dechlorinated freshwater at a controlled temperature of 10 ± 0.2°C at the Freshwater Institute. The 95% replacement time of water was 3.7 hours. Water pH was 7.8 and the measured chemical constituents of water in $\mathrm{mmol \cdot l^{-1}}$ were $Ca^{+2} = 0.5$, $Mg^{+2} = 0.2$, $Na^{+1} = 0.08$, $K^{+1} = 0.03$, $Cl^{+1} = 0.1$, and $HCO_3^{-1} = 1.6$. The fish were fed a daily ration of commercial trout pellets equivalent to 1% of their body mass, which slightly exceeded the maintenance requirements of these fish. The fish were also allowed several months to adjust to the new environmental conditions before they were used for the experimentation.

Identity of Hemoglobin Polymorphism

Electrophoresis

At least forty specimens of each species were used to determine hemoglobin patterns. All preparations were performed at 0-4°C. Individual fish were bled by syringe from the caudal vein. The blood was heparinized (40 U.S.P. units$\cdot$ml^{-1}) with ammonium-heparin (Sigma Chemical, St. Louis). The heparinized blood was centrifuged for 5 min at 4 °C. The plasma and leucocytes were removed by aspiration. Packed red blood cells were washed three times with cold 0.9% NaCl which was saturated with carbon monoxide. During the final wash the erythrocytes suspension was equilibrated with carbon monoxide to convert the hemoglobin to the carboxy-form. Hemolyzates were prepared by mixing the red blood cells with an equal volume of cold distilled water which was saturated with carbon monoxide. Cell debris was removed by centrifugation and the hemolyzate retained. Electrophoresis was conducted on all samples on the same day as the blood collection. Electrophoretic separation of the hemoglobins was carried out by using micro-starch gel method of Tsuyuki et al. (1966)

at pH 8.6. The time of electrophoresis was 90 min at a constant voltage of 200V at 0-1°C. The current did not exceed 3 mA per strip. Gels were stained in 0.1% Naphthol Blue Black in washing solution (acetic acid : methanol : distilled water; 1:5:5, by volume) for 30 min and were destained with washing solution.

Quantitative Analysis of Hemoglobin Components

The relative concentrations of individual hemoglobin isomorphs were estimated from photographic negatives of the gels by the procedure described by Giles and Rystephanuk (1989). The destained gels for each species were photographed using transmitted light with a Hasselblad camera (model 500c/n with 80 mm lens and extension tubes) and ILford FP4 ISO 125/22 black and white film. The negatives for all samples were scanned at 660 ηm in a Gilford Spectrophotometer (model 2400) equipped with a linear transport carriage, a 0.05 x 2.36 mm slit plate, and a Gilford variable span recorder. The area under each peak was determined with a Calcomp 9100 digitizer. Hemoglobin components were labelled according to their order from the point of origin. The first anodic band from origin was called A1 and the first cathodic band from origin was called C1, and so on. The relative mobility for each hemoglobin isomorph was expressed as a ratio of the travelled distance from the origin to the distance travelled by reference isomorphs common to all hemolyzates examined. The identity of these reference isomorphs was confirmed by electrophoresis of hemolyzates from all species side by side on single gels.

Hemoglobin-Oxygen Equilibrium

The blood for hemoglobin-oxygen equilibrium determinations was collected by syringe from 2 to 4-year-old fish. The heparinized blood collected from at least 10 fish for each species was then centrifuged at 4°C for 5 min. Packed red blood cells were washed three times with 0.9% NaCl and lysed in equal volumes of 0.01M Tris-HCl buffer. The phosphates of the hemolyzates for all species were removed by the method of gel filtration (Berman et al., 1971). The hemoglobin solution was chromatographed on a 2 X 22 cm column of the Sephadex G-25 Fine equilibrated with 0.01M Tris-HCl buffer containing 0.1M NaCl at pH 7.5. A 3-ml volume of hemoglobin solution was applied to the column and eluted at 0-1°C at a rate of 15 ml/h. The phosphate-stripped hemoglobin solutions were then adjusted to the desired pH (7.1, 7.5, or 7.9) by adding 0.01M Tris-HCl buffers at pH 8.6 or pH 2.5. After adjustment to the desired pH, the purified hemoglobin solutions were concentrated by molecular filtration using Centriprep Concentrators (Centriprep-10, GRACE Co.). The purified hemolyzates were adjusted to a hemoglobin concentration of 4.0-4.2 $g \cdot dl^{-1}$ prior to measurement of oxygen equilibrium. Methemoglobin concentration was estimated by comparison of the measured oxygen content of fully oxygen-saturated hemoglobin with the theoretical oxygen content (1.34 ml O_2/g Hb) for oxyhemoglobin (Edwards and Martin, 1967). Hemoglobin concentration was measured with Spectronic 710 spectrophotometer (BAUSCH & LOMB) at 540 ηm using the cyanmethemoglobin method (Evelyn and Malloy, 1938). The total phosphate concentration was determined by the method of Ames and Dubin (1960).

Oxygen equilibrium curves of the hemoglobin solutions were determined by the mixing method of Edwards and Martin (1967). The Po_2 of mixtures of oxygenated and deoxygenated hemoglobin was measured in a thermostated Radiometer PO_2 electrode (type E5046) at the equilibrium temperature with a PHM 71 acid-base analyzer. Aliquots (2-3 ml) of hemoglobin solution at a specified pH were equilibrated at each test temperature with water-saturated gas mixtures (0.339% carbon dioxide : balance nitrogen or 20.9% oxygen : 0.339% carbon dioxide : balance nitrogen; Linde analyzed gases). The oxygen content of each mixture was determined by the method of Tucker (1967) in a temperature-controlled tonometer at 32°C. The tonometer and the electrode were maintained at the equilibrium temperature by a Haake circulating water bath. The effects of three levels of organic phosphate on the oxygen equilibrium curve were determined by adding 0.76M ATP (ATP in 0.01M Tris-HCl buffer containing 0.1M NaCl) to hemoglobin solutions to give ATP:Hb molar ratios of 1.25:1, 2.5:1, and 5:1, respectively.

Estimates of the P_{50} and the extent of heme-heme interaction (n value) were determined from a least squares fit of the logarithm of the Hill approximation (Manwell, 1960)

$$Y = 100[(P/P_{50})^n \;/\; (1+(P/P_{50})^n)]$$

where Y is the percent of saturation, P is the oxygen partial pressure and n is the measurement of heme-heme interaction. The equilibrium curves for all hemoglobin solutions were corrected for oxygen dissolved in the buffer. The Bohr effect (Grigg, 1969) was calculated as

$$\phi = \Delta \log P_{50} \ / \ \Delta pH.$$

The apparent heat of oxygenation (Wyman, 1964), ΔH ($Kcalmol^{-1}$), was determined using the formula

$$\Delta H = 4.574 \ \Delta \log P_{50} \ / \ \Delta(1/T),$$

where T is in degrees Kelvin.

Standard Metabolic Rate

Standard metabolic rate (SMR) was measured at nominal water temperatures of 5, 10, and 16°C, respectively. A sample of 16 - 20 fish, ranging in weight from 7 to 390 g, were used for each test. The same group of fish for each species were tested at all three temperatures. Temperature was changed at the rate of 0.5°C per day and the fish were acclimated to the test temperature for at least four weeks prior to the measurement of SMR. Test fish were not fed for 24 hours before measurements were taken. Nominal chamber volumes were 0.5-1 l for fish < 50 g and 2-4 l for fish > 100 g.

The Vo_2 of resting fish was determined by closed-vessel respirometry. Glass vessels were fitted with inlet and outlet ports and immersed in a water bath at the specified test temperature. Temperature-controlled water was circulated within the bath to maintain a uniform temperature. During the test, the vessels were covered with black polyethylene to shield fish from visual disturbance. Fish were acclimated to the darkened vessels for 24 hours and air-saturated water (50 $ml.min^{-1}$) was directed through the vessels prior to the measurement of Vo_2. The ports were then closed and the partial pressure of oxygen in the water was measured at 0.5- to 1-h intervals with a Radiometer PHM-72 acid-base analyzer and a thermostated oxygen electrode (model E5046) regulated to the test temperature. Two to six measurements were made for each individual fish. For these measurements, 1-1.5 ml water samples were removed anaerobically from the vessels and the sample volumes were replaced by aerated water. The maximum error in calculated Vo_2 introduced by the sampling procedure was <2% (Giles, 1991). No correction was made for this error. The oxygen consumption by bacteria over the course of the test was below the limits of detection and no attempt was made to correct for this factor. The measurement of oxygen consumption was stopped when oxygen partial pressure dropped to 70-80 mmHg (48-55% of air saturation), in order to avoid possible hypoxic stress.

The estimated SMR for individual fish was calculated by the equation (Colt, 1980):

$$Vo_2 = \Delta Po_2 \cdot \alpha \cdot V$$

where Vo_2 = rate of oxygen consumption ($mg\ O_2.h^{-1}$); ΔPo_2 = rate of change in Po_2 ($mmHg.h^{-1}$): [initial Po_2 - final Po_2] / Po_{2s}, where Po_{2s} = Po_2 at saturation; α = solubility coefficient for oxygen in water at test temperature ($mg \cdot l^{-1}$); V = respirometer volume (l).

Statistical Analyses

All proportional data were subjected to an angular transformation prior to statistical analyses. Relative concentrations and mobilities were compared with the ANOVA procedure and the homogeneity of variance tested with Bartlett's X^2 test (Schlotzhauer and Littell, 1987).

Oxygen equilibria at each specified temperature, pH, and ATP level were analyzed using simple linear regression (Appendix G). The relationship among P_{50}, pH, ATP, and temperature were determined by multiple linear regression (Snedecor and Cochran, 1980).

Multiple linear regression coefficients, variance and covariance of the oxygen consumption (Appendix D) of each species at each test temperature were compared using the GLM procedure (SAS Institute Inc., 1985).

RESULTS

Hemoglobin Heterogeneity

The adult hemoglobin electrophoretic patterns of the three species studied were species-specific. At least one anodic component (A3 for all three species) and one cathodic component (C3, C4, and C5 in Arctic charr, lake trout, and brook trout, respectively) were common to all fish examined and were employed as reference components with mobilities of 100 for either the anodic or cathodic isomorphs. Arctic charr and lake trout had five anodic and five cathodic hemoglobin components, whereas brook trout had seven cathodic hemoglobin components and either four (phenotype A4), or five (phenotype A5) anodic components (Fig. 4). Of the 56 brook trout examined, 16 percent possessed phenotype A5 and the rest phenotype A4. For comparison purposes, brook trout with phenotype A4 and A5 were designated as brook trout A4 and A5, respectively.

The mean relative concentrations and mobilities of all hemoglobin components for the three species are summarized in Appendix A and graphically illustrated in Figure 5. The electropherograms of brook trout phenotypes A4 and A5 were similar, except for the presence of Hb A5 (relative concentration = 3.2%) in

FIGURE 4. Electrophoretic hemoglobin patterns for Arctic charr, lake trout, and brook trout. The width of bands represents the relative concentration (mean values were used).

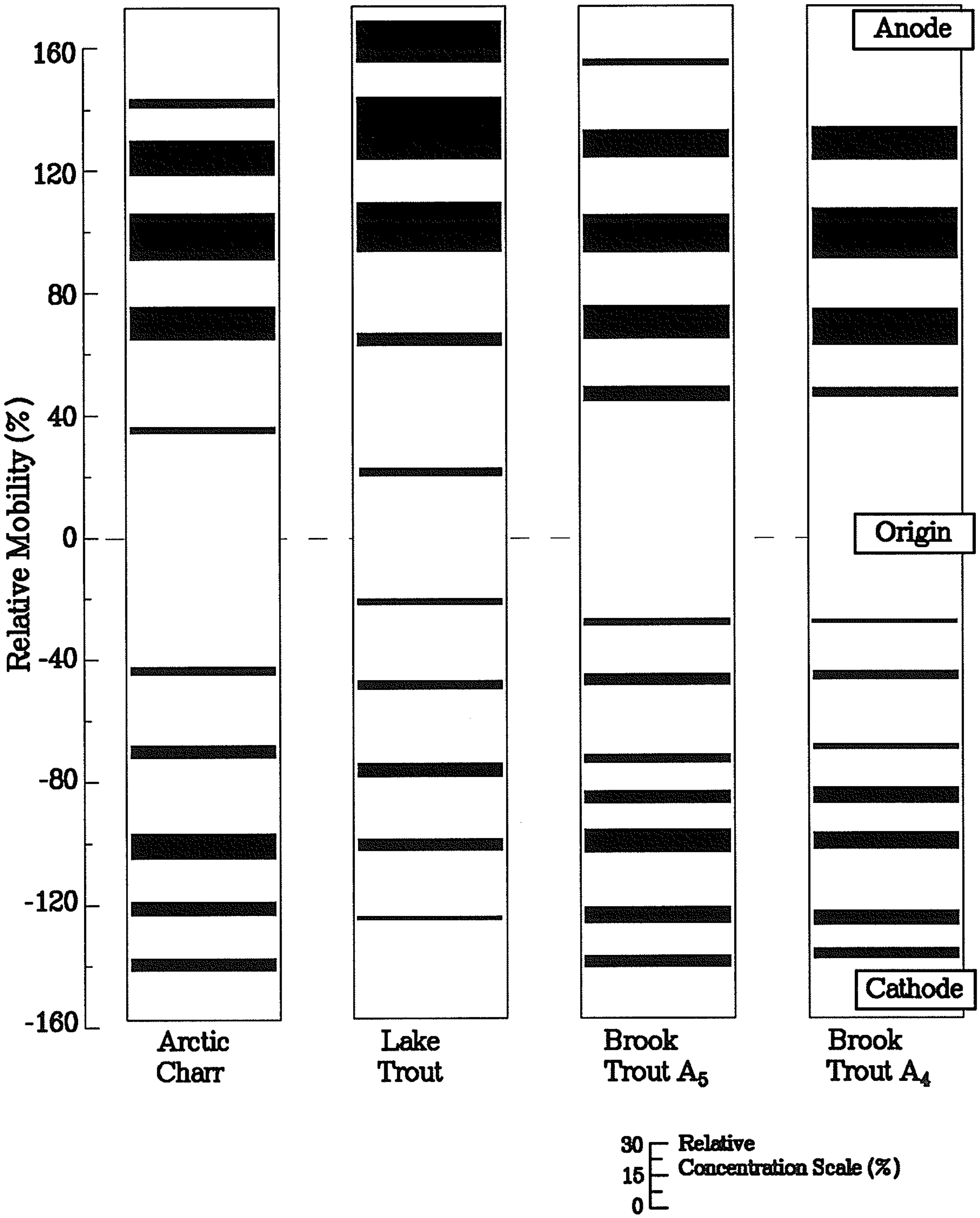
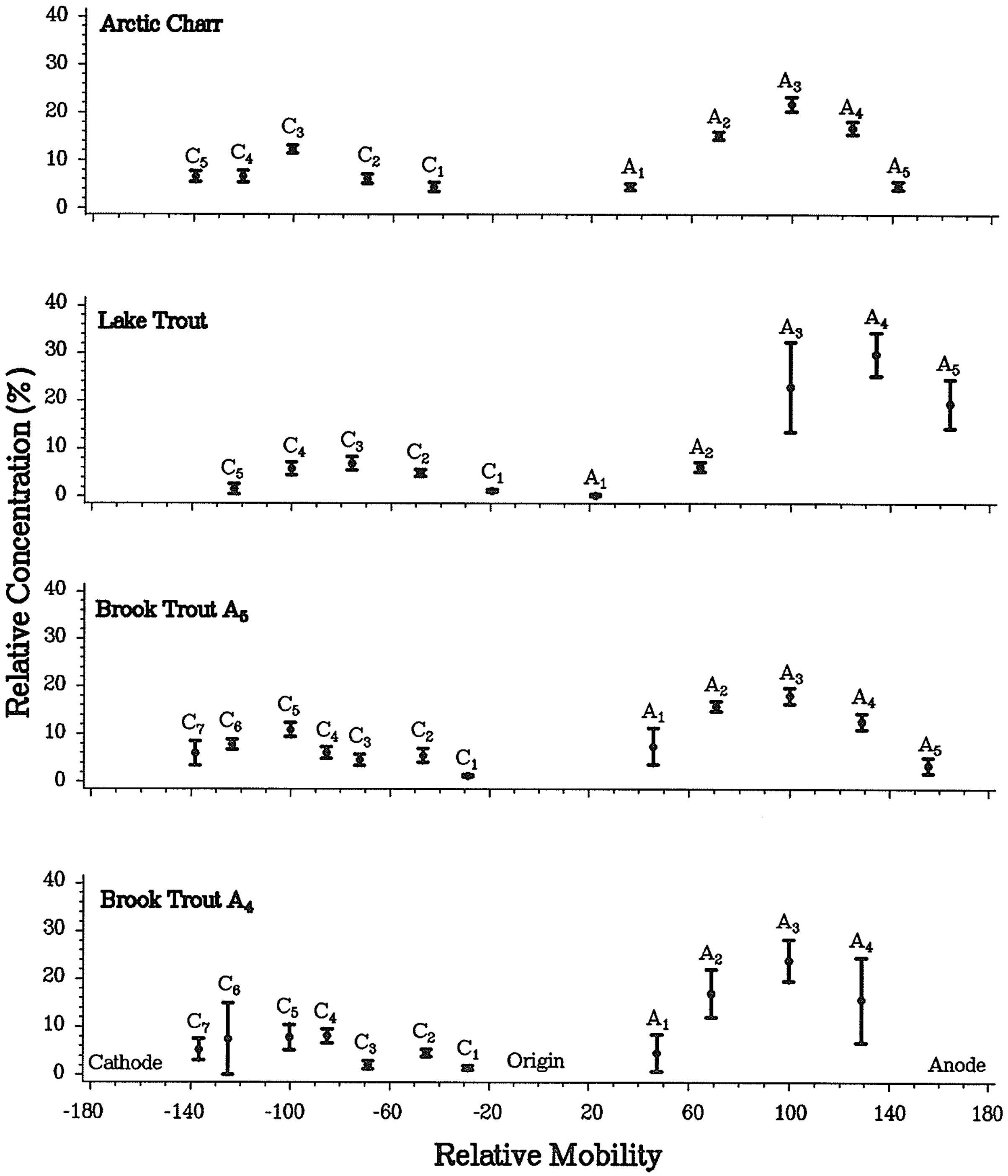

Anode
Origin
Cathode
Relative Mobility (%)
160
120
80
40
0
-40
-80
-120
-160
Arctic Charr
Lake Trout
Brook Trout A_5
Brook Trout A_4
30
15
0
Relative Concentration Scale (%)

FIGURE 5. Comparison of the relative mobility and relative concentration of hemoglobin components in Arctic charr, lake trout, and brook trout (means with 95% confidence interval).


Arctic Charr
Lake Trout
Brook Trout A5
Brook Trout A4
C7
C6
C5
C4
C3
C2
C1
A1
A2
A3
A4
A5
Cathode
Origin
Anode
Relative Concentration (%)
Relative Mobility
0
10
20
30
40
-180
-140
-100
-60
-20
20
60
100
140
180

brook trout A5. Although some hemoglobins displayed the same electrophoretic mobilities (e.g. A3 in all electropherograms; C3 in Arctic charr, C4 in Lake trout, and C5 in brook trout), the relative concentrations of individual hemoglobin components were different among the three species (Figs. 4 and 5).

In all fish studied, the total concentration of anodic hemoglobin components was higher than that of cathodic components (Fig. 6, Appendix A). There was no significant difference in the total concentrations of the cathodic hemoglobins between Arctic charr and brook trout ($P > 0.05$). The total concentration of the cathodic hemoglobins of lake trout, however, was significantly lower than that of Arctic charr and brook trout ($P < 0.05$).

Hemoglobin-Oxygen Equilibrium

Preparative Techniques

Gel filtration removed 96.6% of the total phosphates (Fig. 7). Methemoglobin, estimated from the rates of measured oxygen content of hemolyzates at 100% saturation to the theoretical value of 1.34 $ml.g^{-1}$, comprised an average of 4.1% of the total hemoglobins (Appendix B).

Since the hemolyzate from the mixture of blood of brook trout A4 and brook trout A5 was used for the test of oxygen-hemoglobin equilibrium, the comparison of oxygen binding characteristics between

FIGURE 6. Comparison of the total concentration of cathodic hemoglobin components in three _Salvelinus_ species.

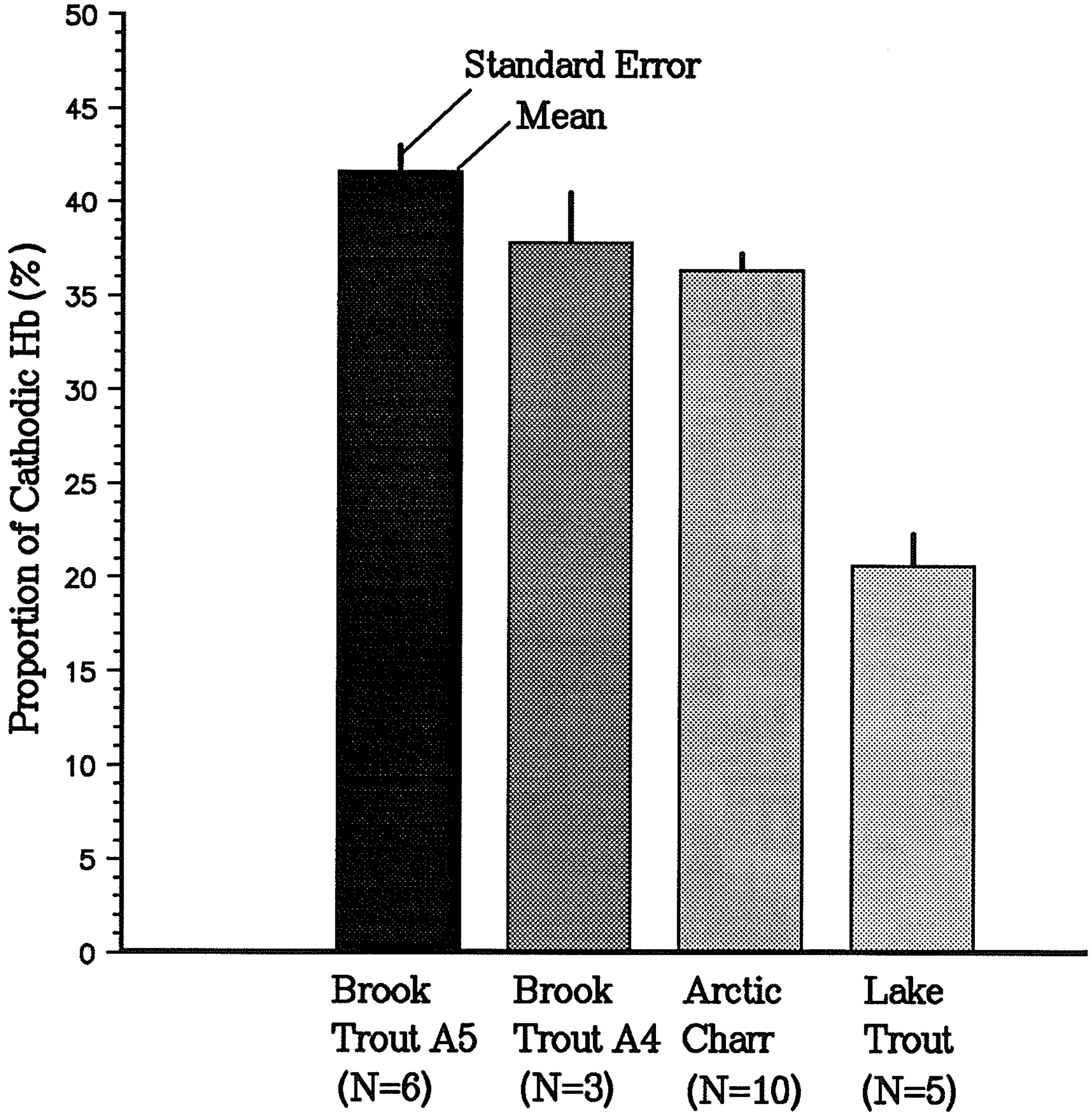
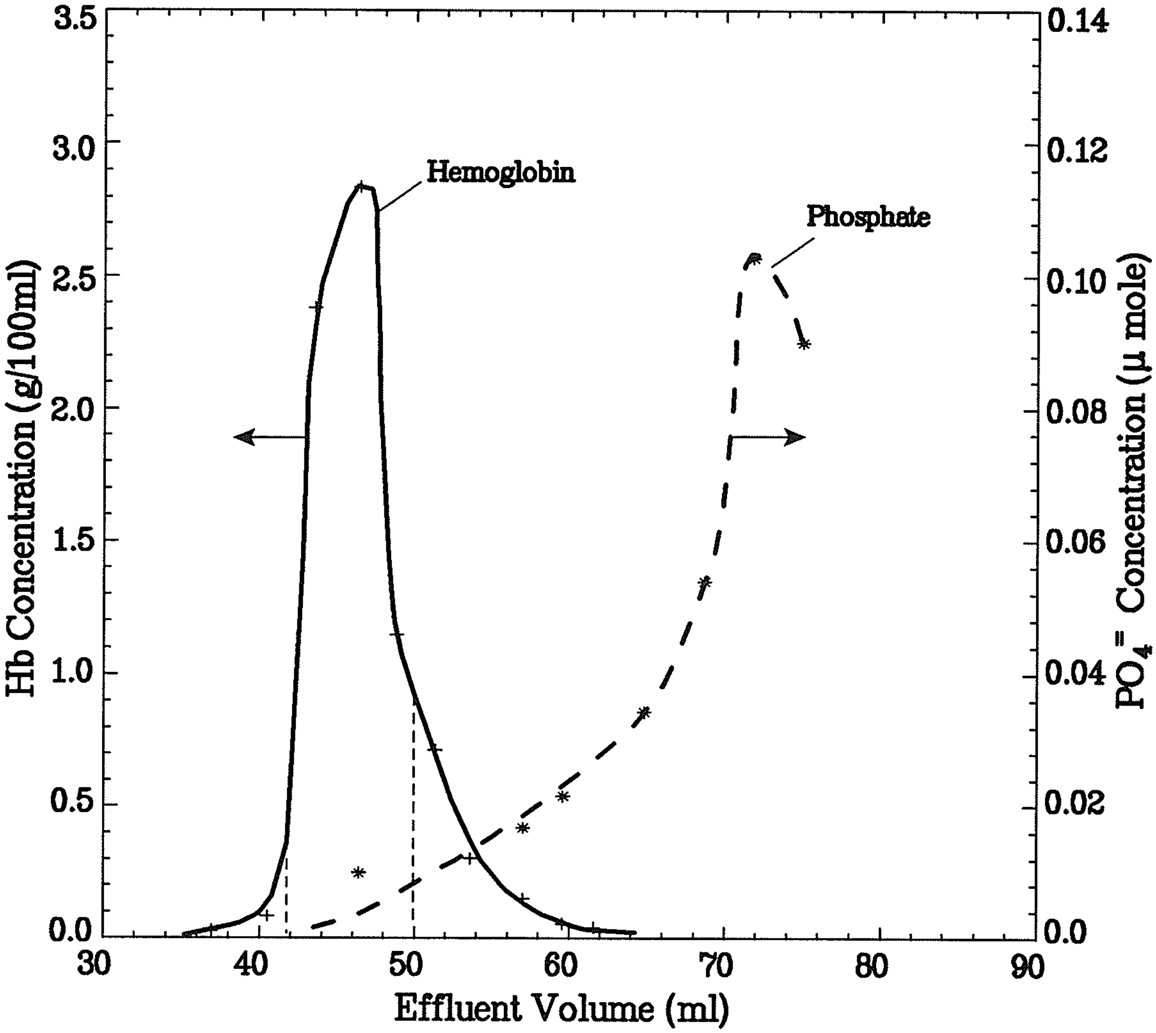

Proportion of Cathodic Hb (%)
0
5
10
15
20
25
30
35
40
45
50
Standard Error
Mean
Brook Trout A5 (N=6)
Brook Trout A4 (N=3)
Arctic Charr (N=10)
Lake Trout (N=5)

FIGURE 7. Gel filtration of Arctic charr hemoglobin solution: hemoglobin and phosphate concentrations versus effluent volume. Dashed vertical lines indicate the portion of the effluent employed in the oxygen-equilibrium studies.


3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.0
30
40
50
60
70
80
90
Hemoglobin
Phosphate
Hb Concentration (g/100ml)
PO$_4^=$ Concentration (μ mole)
Effluent Volume (ml)

two phenotypes of brook trout was carried out with whole blood. No significant difference was observed (Fig. 8).

Effects of pH and Temperature on Hemoglobin-Oxygen Affinity

Oxygen affinity of purified hemoglobin solutions decreased with increasing temperature and increased with increasing pH in all species (Fig. 9). Means and 95% confidence intervals for these results are presented in Appendix C.

The pH dependence of oxygen affinity as estimated by the Bohr shift was least at 10°C and highest at 5°C in all species (Table 1). However, brook trout hemoglobins exhibited the lowest Bohr shift at all temperatures while Arctic charr hemoglobins had the largest Bohr shift at 5°C. At 10 and 16°C the Bohr shift of lake trout hemoglobin exceeded that of both Arctic charr and brook trout.

The effect of temperature on hemoglobin-oxygen affinity determined by the apparent heat of oxygenation was lowest in brook trout, intermediate in lake trout and highest in Arctic charr at all test pH levels (Table 2). The pH sensitivity of ΔH was lowest in lake trout and highest in Arctic charr.

The effects of pH and temperature (T) on hemoglobin-oxygen affinity (P_{50}) were also determined by the relationship:

$$\log P_{50} = a + b\text{pH} + cT$$

where P_{50} is expressed in mmHg and T is in degrees Celsius. The multiple linear regression coefficients and variance analysis are

FIGURE 8. Comparison of oxygen equilibrium curves for two brook trout phenotypes.

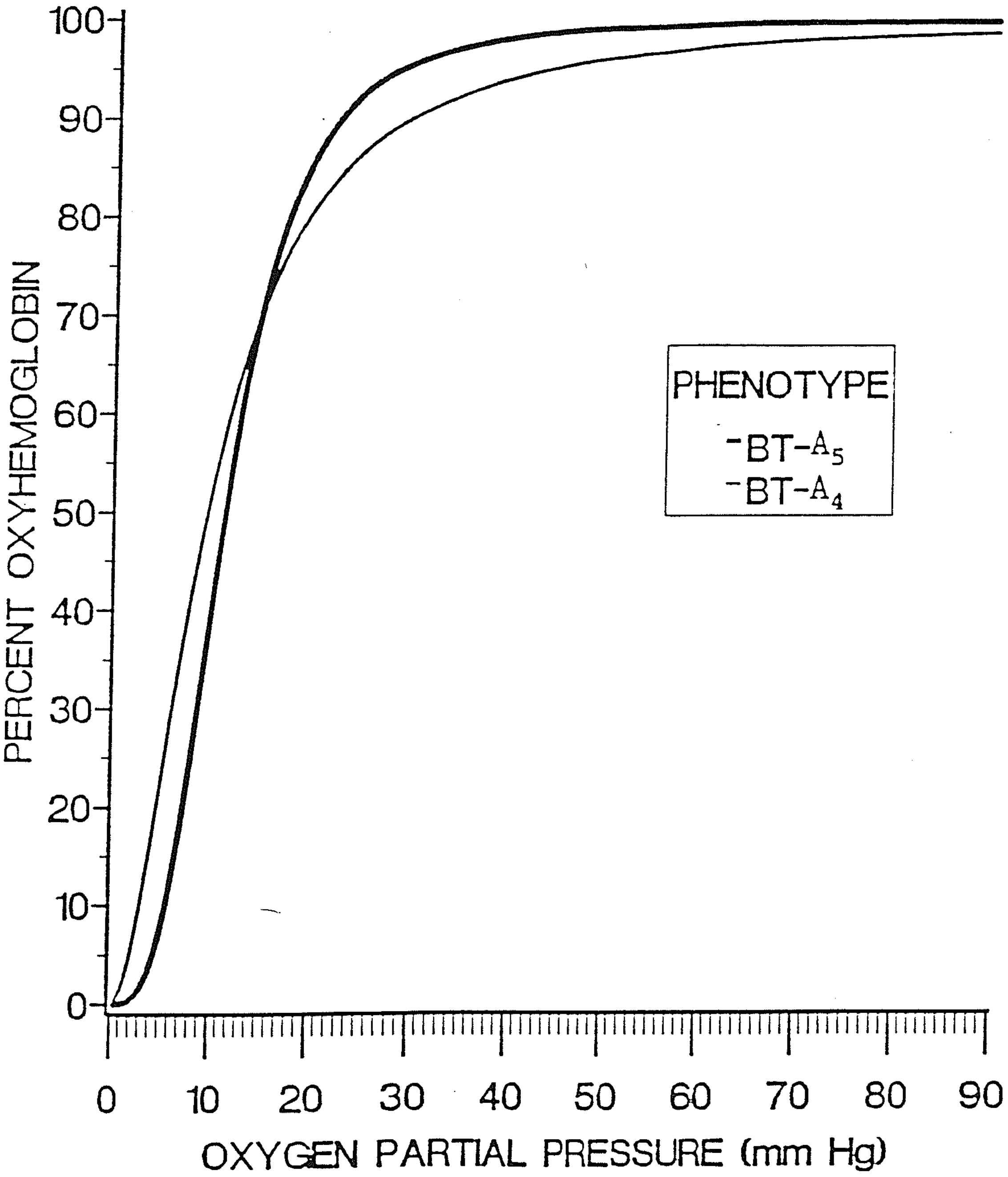

100
90
80
70
60
50
40
30
20
10
0
PERCENT OXYHEMOGLOBIN
0
10
20
30
40
50
60
70
80
90
OXYGEN PARTIAL PRESSURE (mm Hg)
PHENOTYPE
BT-A_5
BT-A_4

FIGURE 9. Effects of pH and temperatures on hemoglobin-oxygen affinities and heme-heme interaction of hemoglobins. Points represent the mean P_{50} values. Top ordinate n represents the heme-heme interaction. For details see text.

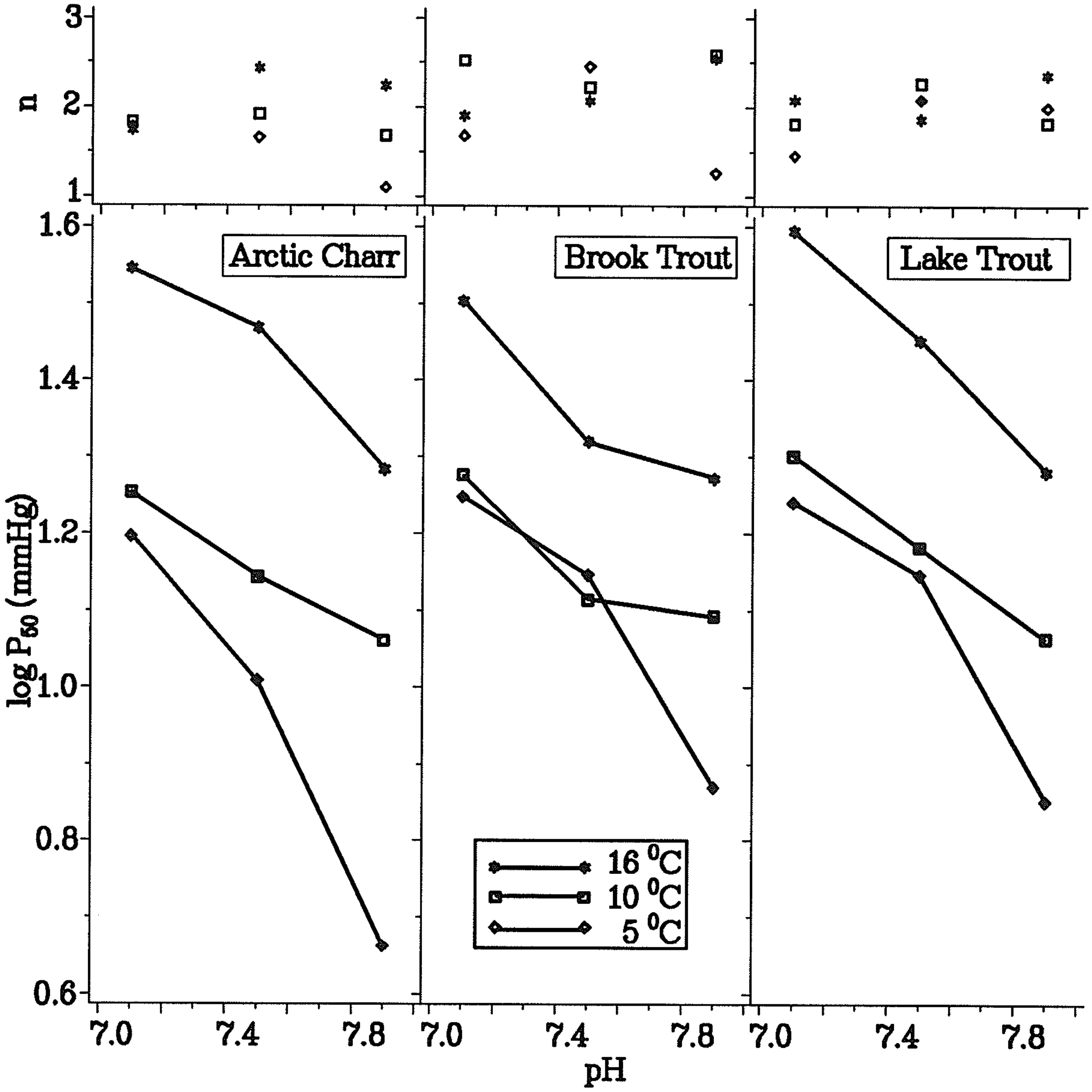

n
3
2
1
log P₅₀ (mmHg)
1.6
1.4
1.2
1.0
0.8
0.6
Arctic Charr
Brook Trout
Lake Trout
16 ⁰C
10 ⁰C
5 ⁰C
7.0
7.4
7.8
pH

Table 1

Estimate of Bohr shift (ϕ) of the hemoglobins of <u>Salvelinus</u> sp. at 5, 10, and 16°C over the 7.1-7.9 pH interval at [ATP] = 0.00.

	Water temperature		
Species	5^0C	10^0C	16^0C
Arctic Charr	-0.67	-0.24	-0.33
Brook Trout	-0.47	-0.23	-0.29
Lake Trout	-0.49	-0.30	-0.39

Table 2

The apparent heat of oxygenation (ΔH Kcal/mol) for *Salvelinus* sp. at [ATP] = 0.00 and at different pH levels over the temperature range of 5-16 °C.

Species	pH=7.1	pH=7.5	pH=7.9
Arctic Charr	-11.6	-15.3	-20.6
Brook Trout	-8.5	-5.8	-13.4
Lake Trout	-11.8	-10.2	-14.3

summarized in Table 3. The results of covariance analysis showed that the responses in oxygen affinity to pH were not significantly different among the three species (F = 0.14, P = 0.87), but the responses in P_{50} to temperature were significantly different (F = 5.11, P = 0.02).

Heme-heme interaction (n) of hemoglobins was relatively high and variable in all fish examined in this study (Appendix G and Fig. 9). In general, n was unrelated to pH and temperature and was similar among the three species (ANOVA; df = 24, t = 2.0639, P>0.05). The average n was 1.8 ± 0.34, 2.1 ± 0.41, and 2.0 ± 0.26 for Arctic charr, brook trout, and lake trout, respectively.

Effect of ATP on Hemoglobin-Oxygen Affinity

The oxygen affinities of hemoglobin solutions were highly sensitive to the changes of ATP concentrations as well as pH (Table 4). Oxygen affinity declined with increasing ATP concentration. The magnitude of this effect was greatest in lake trout and generally similar in Arctic charr and brook trout (Table 4). In all instances, the magnitude of the increase in P_{50} with increasing [ATP] was inversely proportional to pH. Furthermore, the proportional reduction in oxygen affinity ($\Delta \log P_{50}/\Delta \log[ATP]$) at pH 7.1 and 7.9 was similar in all species (F = 0.82, df = 11, P = 0.48 for pH = 7.1; F = 0.27, df = 11, P = 0.77 for pH = 7.9). At pH 7.5, however, the $\Delta \log P_{50}/\Delta \log[ATP]$ values for lake trout, brook trout, and Arctic charr were

Table 3

Multiple regression coefficients for the oxygen affinity (P_{50} mmHg) versus pH and water temperature (°C) in <u>Salvelinus</u> sp. measured at three pH values (7.1, 7.5, 7.9), three temperatures (5, 10, 16°C), and [ATP] = 2.50.

Sp.	N	Multiple reg. coefficients*			Standard errors			F value	PR > F	R^2
		a	b	c	a	b	c			
AC	9	11.2	-1.19	-0.12	3.26	0.43	0.28	24.3	0.002	0.94
BT	9	12.4	1.28	-0.21	1.74	0.23	0.15	49.8	0.0004	0.97
LT	9	11.2	-1.16	-0.05	1.30	0.17	0.11	135.2	0.0001	0.99

* $\log P_{50} = a + b\text{pH} + cT$

a = vertical-axis intercept

b = the slope of the response of P_{50} to pH

c = the slope of the response of P_{50} to temperature

Table 4

Effect of ATP on the oxygen equilibria of the hemoglobin solutions of Arctic charr, brook trout, and lake trout at temperature 10°C.

		pH=7.1		pH=7.5		pH=7.9	
Fish	ATP:Hb	P_{50}	ΔP_{50}*	P_{50}	ΔP_{50}	P_{50}	ΔP_{50}
Arctic Charr	0.00	17.9		13.9		11.5	
			27.1		13.2		5.3
	1.25	25.1		16.4		12.3	
			9.0		4.7		6.9
	2.50	28.1		17.4		13.4	
			3.7		3.9		4.0
	5.00	30.8		19.2		14.8	
	**	a=1.387±0.004 b=0.148±0.009		a=1.201±0.0007 b=0.114±0.0016		a=1.076±0.002 b=0.133±0.006	
Brook Trout	0.00	18.9		13.0		12.4	
			20.4		14.1		9.7
	1.25	24.4		15.5		14.0	
			10.2		7.4		5.5
	2.50	27.7		17.0		15.0	
			3.5		3.8		4.1
	5.00	30.2		18.7		16.6	
		a=1.375±0.007 b=0.153±0.017		a=1.177±0.0005 b=0.135±0.001		a=1.132±0.006 b=0.123±0.013	
Lake Trout	0.00	20.0		15.2		11.6	
			28.6		15.7		11.5
	1.25	28.6		18.5		13.4	
			11.2		10.1		7.4
	2.50	32.9		21.0		14.7	
			3.5		5.0		3.6
	5.00	35.9		23.8		16.1	
		a=1.444±0.009 b=0.164±0.022		a=1.250±0.0003 b=0.182±0.001		a=1.114±0.0003 b=0.132±0.0007	

* $$\Delta P_{50} = \frac{[\ln(P_{50})_2 - \ln(P_{50})_1] \times 100}{\Delta ATP}$$

** coefficients ± 1 standard error from least square regression; $\log P_{50} = a + b\log[ATP]$ for [ATP] = 1.25-5.00.

0.182 ± 0.001, 0.135 ± 0.001, and 0.114 ± 0.002, respectively. The results of the comparison of least squares means of $\log P_{50}$ indicated that the increase in P_{50} induced by ATP at pH 7.5 was significantly higher in lake trout than in brook trout and Arctic charr ($P = 0.02$ for lake trout versus brook trout; $P = 0.03$ for lake trout versus Arctic charr). The least square means derived from the model (Table 4) were 2.96, 2.81, and 2.77 for lake trout, Arctic charr, and brook trout, respectively.

Standard Metabolic Rate

The relationship of V_{O2} to fish mass (M) and water temperature (T) can be described by the equation:

$$\log V_{O2} = a + b\log M + cT$$

where V_{O2} is expressed in milligrams per hour, W in grams, T in degrees Celsius. The multiple regression equations and standard errors of the equation constants (a, b, and c) are summarized in Table 5. The models were highly significant in all cases ($P = 0.0001$) and appropriate for the data based on the plots of predicted values versus actual data (Figs. E1, E2, and E3; Appendix E). The interaction effect ($\log M \times T$) was not significant for any of the three species ($P = 0.39$, 0.89, and 0.11 for Arctic charr, lake trout, and brook trout, respectively). Thus, the response of the standard metabolic rate to a given change in temperature was independent of fish size.

Table 5

Multiple regression coefficients for the standard metabolic rate (mg $O_2.h^{-1}$) versus fish body mass (g) and water temperature (°C) in *Salvelinus* sp. measured over a temperature range of 5-16°C.

Sp.	N	Multiple reg. coefficients*			Standard errors			F value	PR > F
		a	b	c	a	b	c		
AC	54	-0.901	0.673^d	0.033^{f**}	0.121	0.068	0.005	101.8	0.0001
BT	49	-0.704	0.664^d	0.024^f	0.120	0.060	0.006	79.5	0.0001
LT	48	-1.417	0.895^e	0.039^g	0.067	0.037	0.003	598.9	0.0001

* $\log V_{o2} = a + b\log M + cT$
a = vertical-axis intercept
b = the slope of the double logarithmic relationship between metabolic rate and body mass
c = the slope of the response of metabolic rate to temperature

** coefficients with different superscripts are significantly different ($P < 0.05$).

Rate of oxygen consumption increased with water temperature and fish body mass in all species (Figs. 10, 11, and 12). The results of covariance analysis showed that the slopes (b) of the response in oxygen consumption to increases in mass were similar in Arctic charr and brook trout (b = 0.673 and 0.664, respectively; F = 0.02, df = 1, P = 0.88), but the slope (b = 0.895) was significantly higher in lake trout (F = 13.58, df = 1, P = 0.0004 for brook trout versus lake trout; F = 15.12, df = 1, P = 0.0002 for Arctic charr versus lake trout). The sensitivity of V_{o2} to temperature increased in order from brook trout, to Arctic charr, to lake trout with temperature exponent (c) equal to 0.024, 0.033, and 0.039. The c value for lake trout was significantly higher than that for Arctic charr (F = 3.25, df = 2, P = 0.043) and brook trout (F = 4.94, df = 2, P = 0.009). The increase in oxygen consumption with temperature as estimated by Q_{10} also exhibited the ranking: brook trout < Arctic charr < lake trout (Table 6).

Thus, the proportional increase in V_{o2} for a specific increase in either mass or temperature was greater in lake trout than in brook trout or Arctic charr (Figs. 13, 14 and 15).

FIGURE 10. The rate of standard oxygen consumption of Arctic charr in relation to body mass and water temperature.

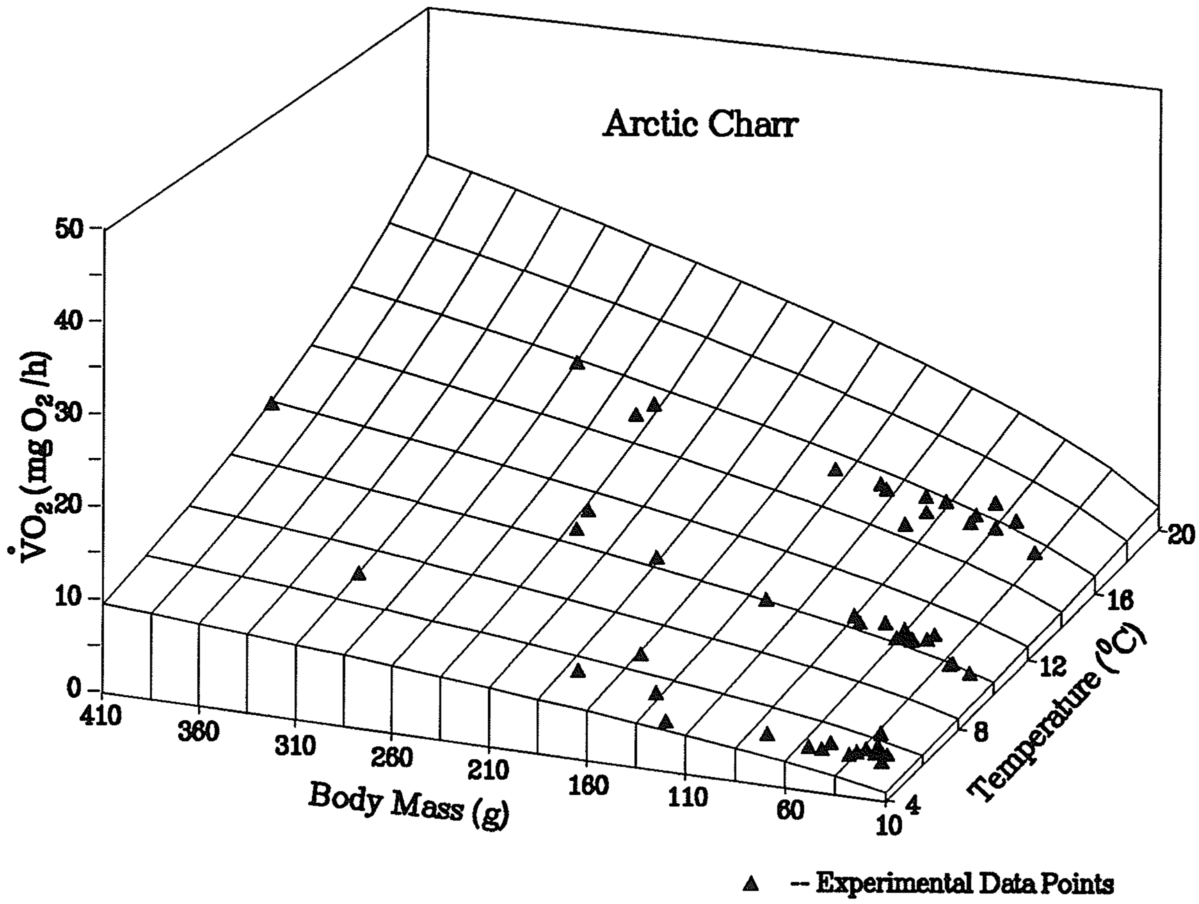

Arctic Charr
$\dot{V}O_2$ (mg O_2/h)
50
40
30
20
10
0
410
360
310
260
210
160
110
60
10
Body Mass (g)
4
8
12
16
20
Temperature (°C)
▲ -- Experimental Data Points

FIGURE 11. The rate of standard oxygen consumption of brook trout in relation to body mass and water temperature.

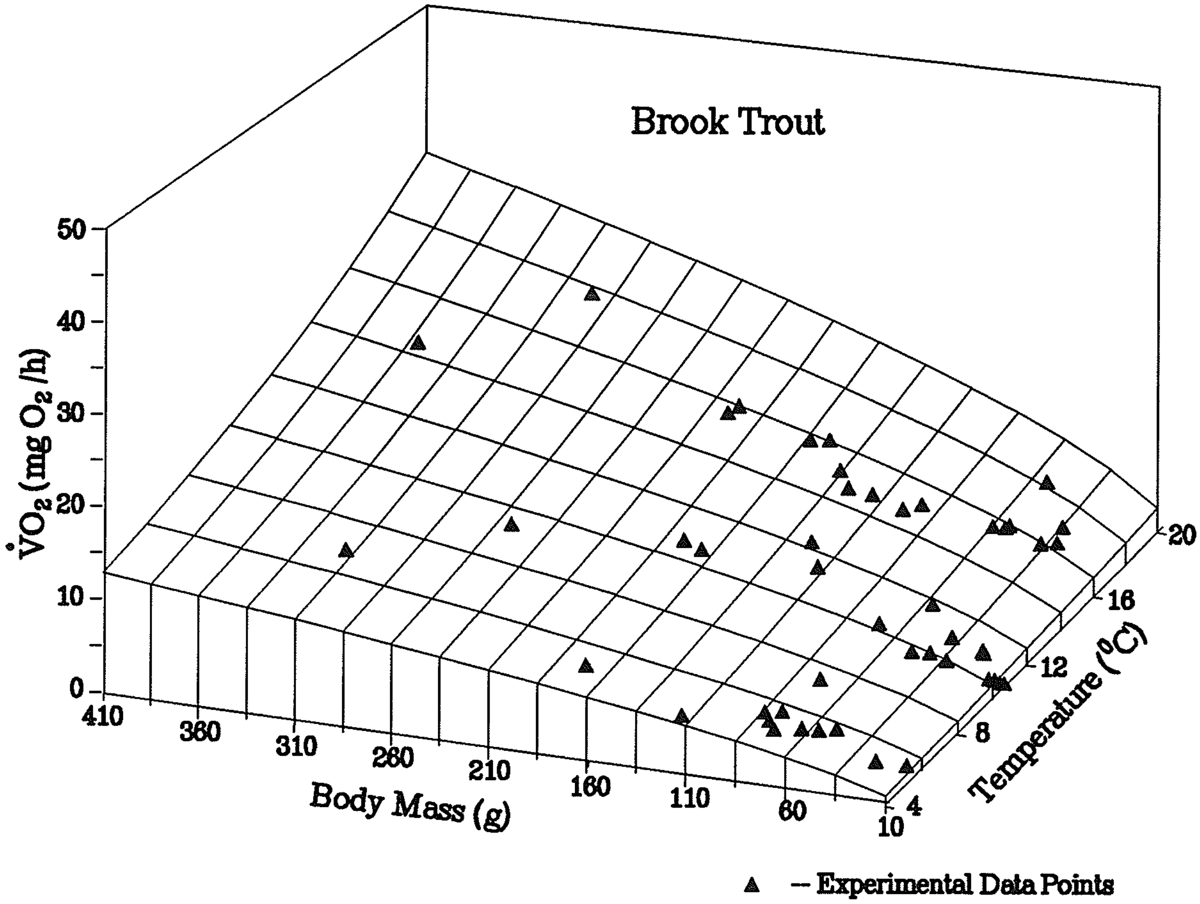

Brook Trout
50
40
30
20
10
0
$\dot{V}O_2$ (mg O_2/h)
410
360
310
260
210
160
110
60
10
Body Mass (g)
4
8
12
16
20
Temperature (°C)
▲ – Experimental Data Points

FIGURE 12. The rate of standard oxygen consumption of lake trout in relation to body mass and water temperature.

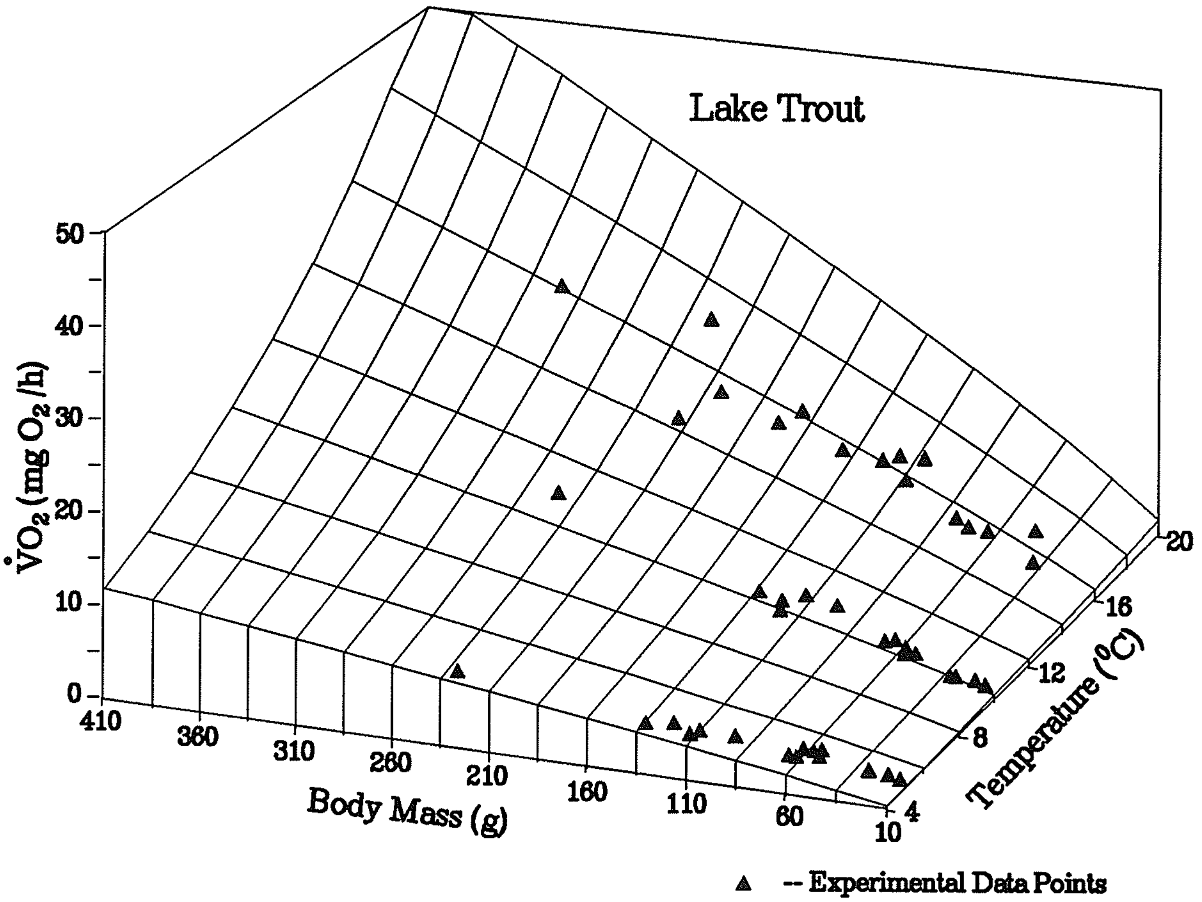

Lake Trout
$\dot{V}O_2$ (mg O_2/h)
0
10
20
30
40
50
Body Mass (g)
410
360
310
260
210
160
110
60
10
Temperature (°C)
4
8
12
16
20
▲ -- Experimental Data Points

Table 6

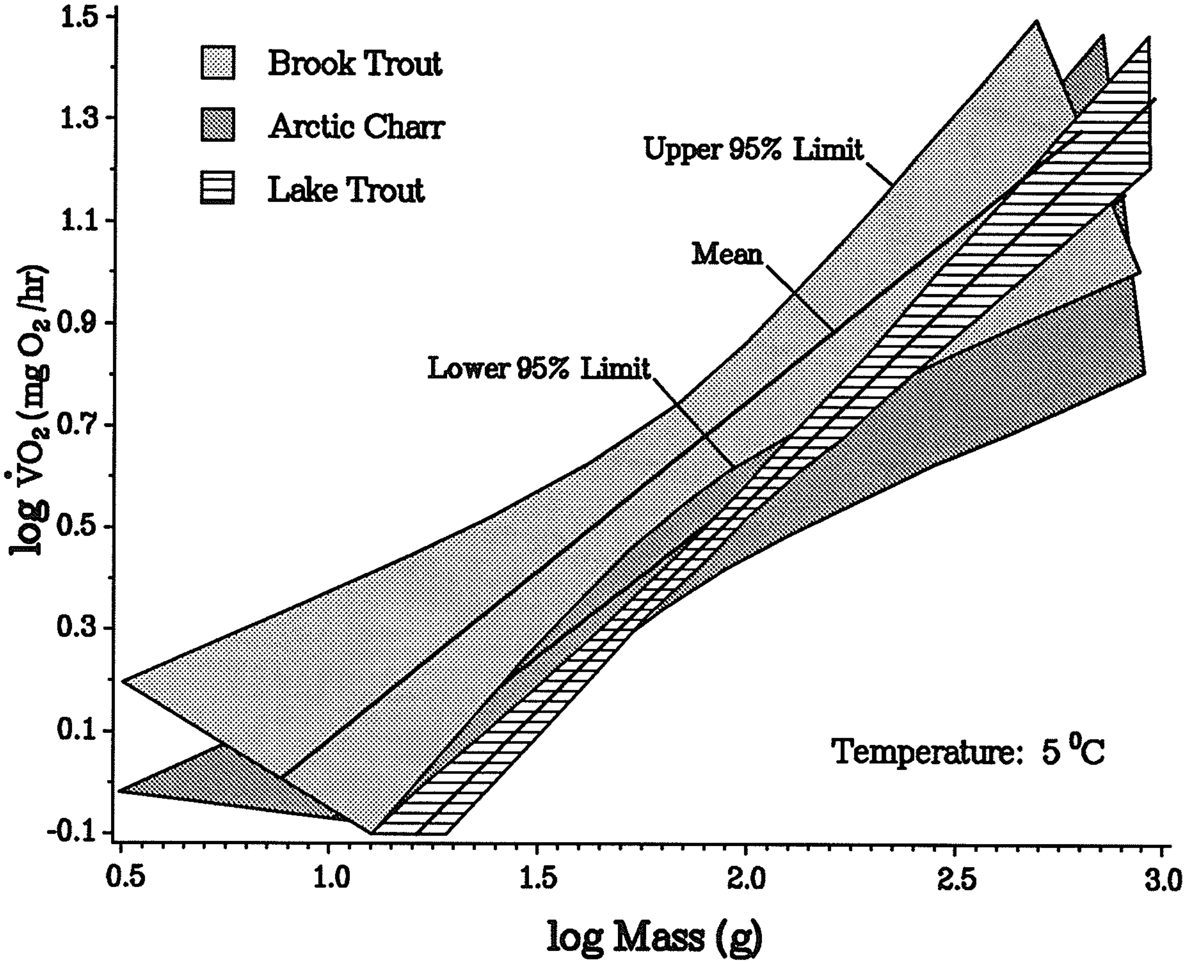
Estimates of Q_{10} for <u>Salvelinus</u> sp. at the temperature ranges of 5-15°C and 10-20°C.

		Oxygen consumption (Q)*				Q_{10}**
Fish	Weight (g)	5°C	10°C	15°C	20°C	
Arctic Charr	50	2.55	3.73	5.44	7.95	
	100	4.07	5.94	8.68	12.69	
	300	8.52	12.44	18.17	26.53	2.13
Brook Trout	50	3.52	4.66	6.17	8.17	
	100	5.57	7.38	9.77	12.94	
	300	11.56	15.31	20.27	26.85	1.75
Lake Trout	50	2.01	3.19	5.04	7.98	
	100	3.74	5.93	9.38	14.85	
	300	10.01	15.85	25.09	39.72	2.51

* The specific oxygen consumption (Q) were estimated using multiple regression equations at 5, 10, 15, and 20°C for each weight class.

** Q_{10}'s were derived as $[Q_{10(t2-t1)} = (Q_{t2} / Q_{t1})^{10/t2-t1}]$.

FIGURE 13. The relationship between standard metabolic rate and body mass for three _Salvelinus_ sp. at 5°C.



Brook Trout
Arctic Charr
Lake Trout
Upper 95% Limit
Mean
Lower 95% Limit
Temperature: 5 ⁰C
log $\dot{V}O_2$ (mg O_2/hr)
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0.5
1.0
1.5
2.0
2.5
3.0
log Mass (g)

FIGURE 14. The relationship between standard metabolic rate and body mass for three <u>Salvelinus</u> sp. at 10°C.

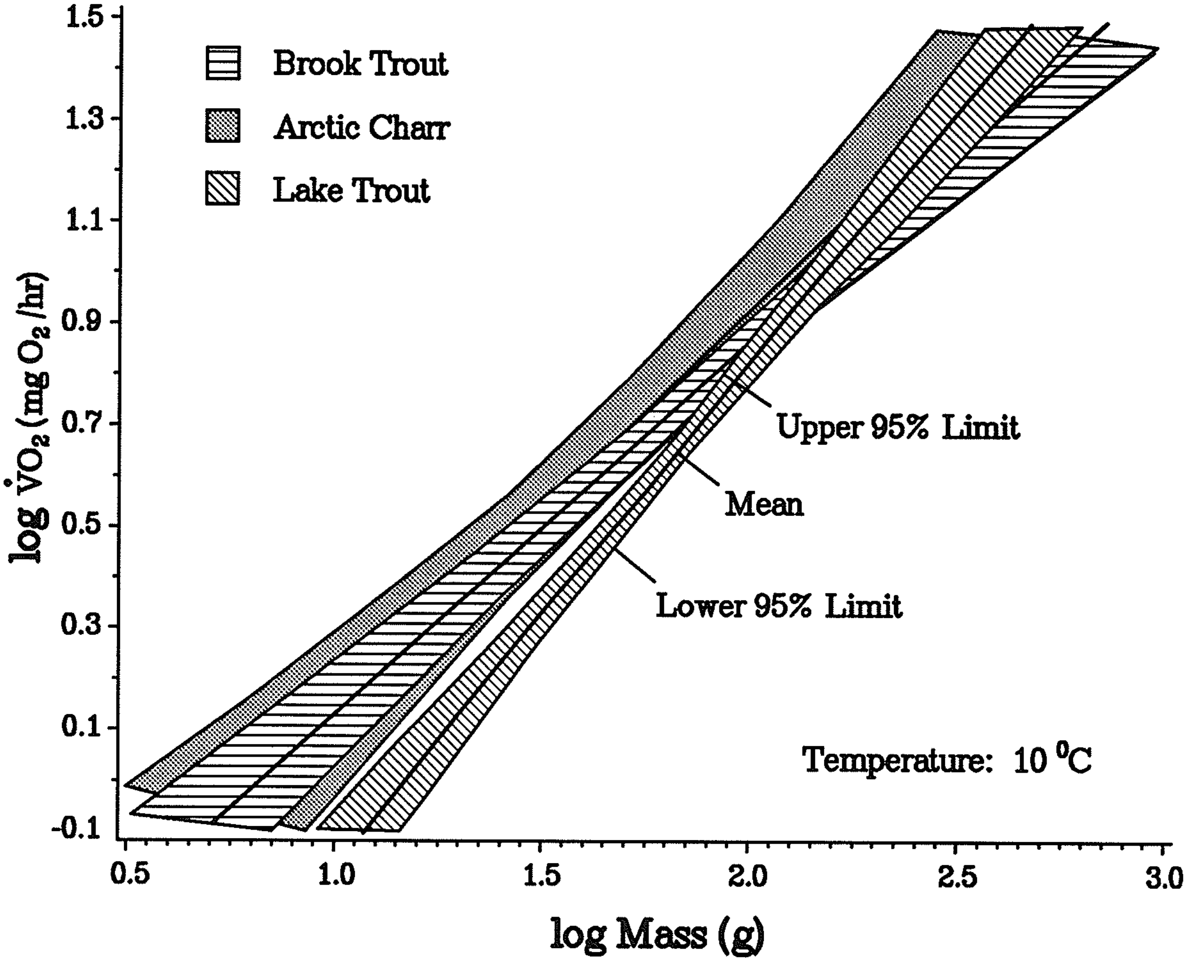
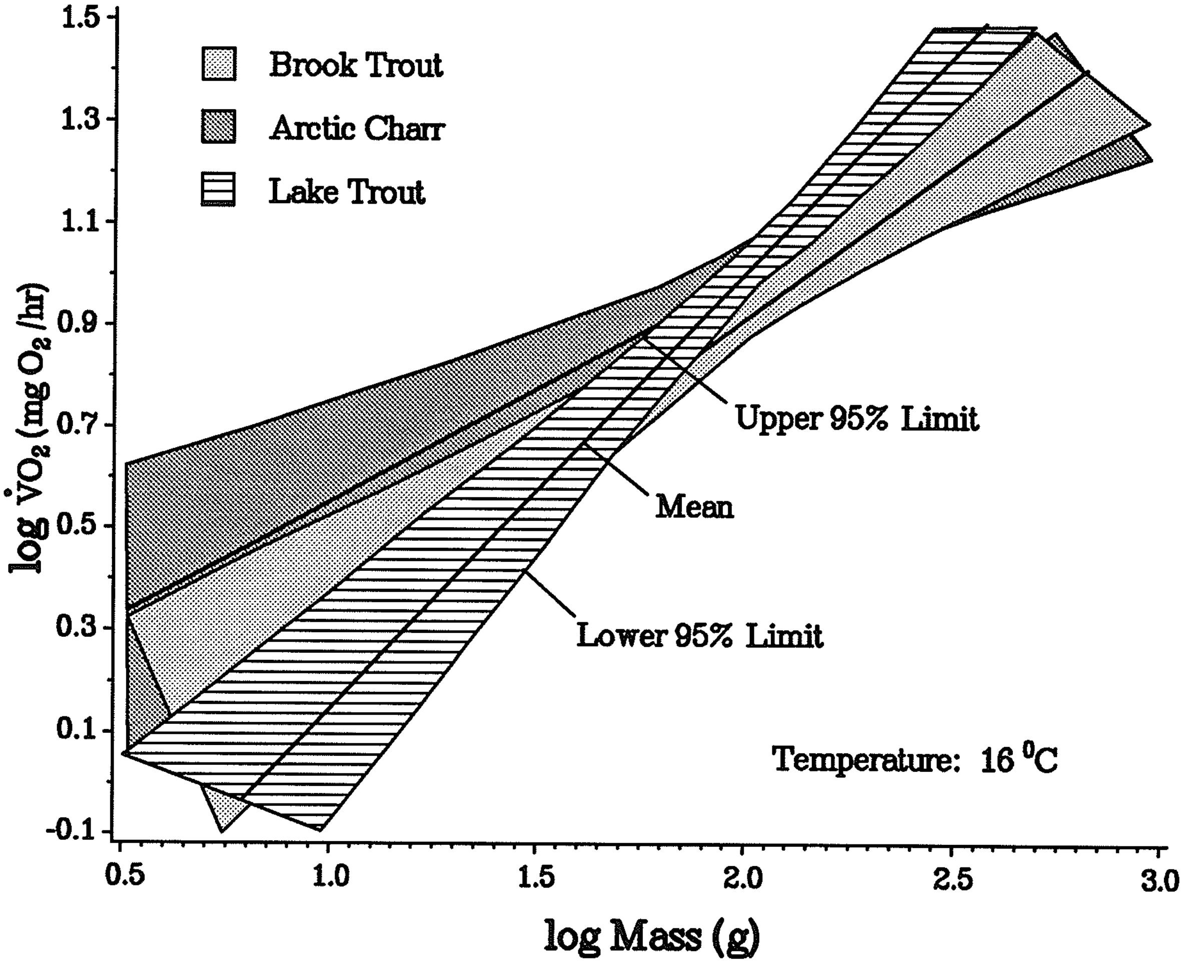

Brook Trout
Arctic Charr
Lake Trout
Upper 95% Limit
Mean
Lower 95% Limit
Temperature: 10 °C
log $\dot{V}O_2$ (mg O_2/hr)
log Mass (g)
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0.5
1.0
1.5
2.0
2.5
3.0

FIGURE 15. The relationship between standard metabolic rate and body mass for three _Salvelinus_ sp. at 16°C.


1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
log $\dot{V}O_2$ (mg O_2/hr)
Brook Trout
Arctic Charr
Lake Trout
Upper 95% Limit
Mean
Lower 95% Limit
Temperature: 16 °C
0.5
1.0
1.5
2.0
2.5
3.0
log Mass (g)

DISCUSSION

A total of 16 electrophoretically distinct hemoglobin isomorphs were observed among adult fish of the three members of Salvelinus. Three anodic and four cathodic components were common to all fish examined, although their relative concentrations were different within each species. Brook trout could be distinguished from Arctic charr and lake trout by the presence of seven versus five cathodic isomorphs in the latter two species. Lake trout and Arctic charr each exhibited five anodic and five cathodic components. These two species could be distinguished by the most anodic isomorph which accounted for 20% versus 4% of the total hemoglobin concentration in lake trout and Arctic charr, respectively. The electrophoretic hemoglobin patterns observed in the present study agree well with those observed in different stocks of brook trout, "amemasu", S. leucomaenis, and Dolly Varden, S. malma from Japan (Yamanaka et al., 1967).

In general, salmonids exhibit a high degree of hemoglobin polymorphism. Adult fish possess ten to sixteen isomorphs comprised of four to nine anodic and four to eight cathodic components (Yamanaka et al., 1965, 1967; Tsuyuki and Ronald, 1971; Giles and Vanstone, 1976; Harrington, 1985, 1986; Giles and Rystephanuk, 1989). The brook trout from the South Duck River exhibited two hemoglobin phenotypes which differed by the presence of a weak anodic component. Multiple hemoglobin phenotypes have been observed in several populations of salmonids including Arctic charr (Giles, 1991), lake trout (Giles, unpublished observations), brook trout (Yamanaka et al., 1967), and cutthroat trout (Braman et al., 1980). Although some variations in the relative concentration of specific hemoglobins have been shown to be related to differences in age and size (Wilkins, 1968, 1985; Koch, 1982), nutritional (Bergstrom and Koch, 1969) and environmental (Marinsky et al., 1990) factors, the fish in the present study were reared under identical conditions for at least two years and were all sexually mature adults. The final adult complement of hemoglobin isomorphs appears to be fully established at two years of age in Pacific salmon (Giles and Vanstone, 1976; Fyhn and Withler, 1991), Arctic charr (Giles and Rystephanuk, 1989), and Atlantic salmon (Koch, 1982). Therefore, the electrophoretic hemoglobin patterns observed in the present study probably reflect real differences in both the subunit structure and rate of synthesis of hemologous subunits among the three species of Salvelinus.

Comparisons of oxygen affinities are often complicated by differences in test conditions and by use of hemolyzated or whole blood. The intraerythrocytic pH of whole blood in unstressed salmonids is generally regulated in the range of 7.4 to 7.6 (Boutilier et al., 1988), and intraerythrocytic ATP:Hb molar ratios range from 0.8 to 1.3 (Giles and Randall, 1988; Boutilier et al., 1988). A summary of oxygen affinities, Bohr effects, and ΔH in whole blood and hemolyzates for various conditions of temperature is presented in Appendix H. The Bohr shift of hemoglobin from all species of Salvelinus ranged from -0.23 to -0.67 and was similar among the three species at each temperature. The magnitude of the Bohr shift changed with equilibration temperature and was -0.54, -0.26, and -0.34 at 5, 10, and 16°C, respectively. Estimates of the Bohr effect range from -0.52 to -0.64 for rainbow trout (Eddy, 1971; Iuchi, 1972; Weber et al., 1976; Vorger, 1985), and from -0.17 to -0.57 for coho salmon (Giles and Randall, 1988). Comparable estimates of the Bohr shift are not available from the literature for Arctic charr, lake trout, or brook trout.

The effect of ATP on oxygen affinity was significantly higher in lake trout than in brook trout or Arctic charr at pH 7.5. The magnitude of reduction in oxygen affinity by ATP was similar at pH 7.1 or pH 7.9 for all examined fish. The increase in P_{50} with increasing ATP declined with increasing pH for all three species. ATP and GTP are potent modifiers of oxygen affinity in many fish and appear to play a significant role in regulating oxygen transport under conditions of environmental hypoxia and temperature variation (Powers, 1974; 1980). In trout and salmon, however, the role of ATP is less clear. In rainbow trout, hypoxic conditions produce substantial decline in red cell ATP which is linearly related to an increase in blood oxygen affinity (Tetens and Lykkeboe, 1981), but in vitro studies of purified hemoglobins for this species indicate that ATP effects oxygen binding only at pH levels less than 7.2 (Pelster and Weber, 1990). Oxygen affinity of isolated hemoglobins of coho salmon were not sensitive to ATP at molar ratios up to 7.6:1 (Giles and Randall, 1988). Harrington (1986) reported that in king salmon, Oncorhynchus tshawytscha, only anodic hemoglobin isomorphs were sensitive to ATP and GTP while cathodic components were completely insensitive. The P_{50} of king salmon anodic hemoglobins increased by approximately 4.5 mm Hg as ATP:Hb was increased from 0 to 3.4 mol/mol at pH 7.3 and equilibration temperature 13°C. Under similar conditions (pH 7.5, temperature 10°C) an increase in ATP:Hb from 0 to 2.5 mol/mol resulted in an increase in P_{50} of 3.5, 4.0, and 5.8 mm Hg for hemolyzates from Arctic charr, brook trout, and lake trout, respectively. These effects are of sufficient magnitude to cause a large effect on oxygen uptake and delivery. For example, assuming a Po_2 at the tissues of 10 mm Hg 14, 18, and 17% more oxygen could be unloaded from hemoglobin in the presence of 2.5 versus 0.0 mole ATP per mole of hemoglobin in Arctic charr, brook trout, lake trout, respectively.

The apparent heat of oxygenation (ΔH) for the three species of Salvelinus ranged from -5.8 to -20.6 over a temperature range of 5 to 16°C. ΔH was similar at pH 7.1 and 7.5, but increased markedly at pH 7.9. In all cases ΔH for brook trout was lower than ΔH in lake trout or Arctic charr. These ΔH values are similar to that found in salmo irideus (Brunari et al., 1973), rainbow trout

(Vorger, 1986), and king salmo (Harrington, 1986), but are slightly higher than that in coho salmo (Giles and Randall, 1980) and sockeye salmo (Sauer and Harrington, 1988). The heme-heme interaction (n) in fish examined in this study revealed a high cooperativity between O_2-binding sites with n averaged 1.95 across all species. Heme-heme interaction was not related to species, pH, temperature, or ATP concentration in the present study. Reports of the effects of temperature and pH on hemoglobin subunit cooperativity are variable. Some species exhibit significant interaction while others exhibit either no or a weak interaction (Riggs, 1970).

In fish, SMR has been determined by extrapolating swimming speed-metabolic rate curves back to zero swimming speed (Brett, 1965). The measurements in the present study were designed to directly measure the SMR's in resting fish. The same measurement had been previously published (Graham,1949; Duthie, 1982; Giles, 1991). The SMR's determined in this study are comparable to the SMR's obtained by extrapolation to zero swimming speed for the three members of Salvelinus. The measured SMR versus predicted SMR (Job, 1955; Beamish, 1980) at 15°C for 100 g fish are 97.7 vs. 100, 86.8 vs. 80.0, and 93.8 vs. 46.4 mg $kg^{-1}h^{-1}$ for brook trout, Arctic charr, and lake trout, respectively. In the present study the effect of mass upon oxygen consumption in lake trout was substantially greater than in the other two species. This explains some of the disparity in the predicted vs. observed SMR of lake trout at 15°C. For a 100 g fish at 10°C, Giles (1991) reported a SMR of Labrador Arctic charr as 59.2 mg $kg^{-1}h^{-1}$ which is identical to the value of 59.4 mg $kg^{-1}h^{-1}$ obtained for the same strain of Arctic charr in this study. Although variations in SMR of fish may relate to differences in season (Fry, 1971) and the stage of development (Eccles, 1985), the SMR's of fish in the present study were measured at the same season with the same mass range for each species. The fluctuations in MR can be caused by introduction of fish to the respirometer chambers for the first 10-12 h (du Preez et al., 1986). The fish examined were acclimated to respirometers for at least 24 h. The time taken to complete the acclimation to respirometers is usually within in 12 h for different fish species (Morris and North, 1984; Talbot and Baird, 1985). The closed respirometers have some disadvantages such as the decreasing in oxygen tensions and increasing nitrogenous products during the course of test. However, the comparisons in SMR's among the three species in this study are still valid since all fish tested at the same situations. The reported Q_{10}'s for the SMR's of fishes are about 2 (Robinson et al., 1983). The Q_{10} values estimated from this study averaged 2.1 for all fish. The mass exponents (b) for the SMR's of Arctic charr, brook trout, and lake trout are 0.673, 0.664, and 0.895. These results are in the range of values found for salmonids. Rao (1971) obtained b values ranged from 0.7 to 0.9 for rainbow trout. Beamish (1964a) reported a slope of 0.88 for

Salmo trutta. Job (1955) and Giles (1991) found mass exponents ranging between 0.8 and 0.9 for brook trout and Arctic charr.

The fact that fish possess multiple hemoglobins may be an adaptive strategy to changing environments (Sharp, 1973; Powers et al., 1986). Harrington (1985) indicated that the electrophoretically distinct hemoglobins in species of salmonids appeared to provide an adaptive mechanism necessary for the life cycle of these fish. However, the correlation between the hemoglobin phenotypes and fish environments is complex and has not been sufficiently demonstrated (Perez and Rylander, 1985).

In a few instances, the electrophoretically distinguishable hemoglobin components in the blood of fish exhibit different physiological properties (Riggs, 1970; Riggs, 1979; Powers, 1980). The functional properties of the multiple hemoglobins in fish can be divided into two major categories. The type I hemoglobins have oxygen equilibrium curves which are strongly pH-and-temperature dependent. These hemoglobins typically have relatively low isoelectric points and are referred to as "anodic" hemoglobins based on their electrophoretic mobilities. The type II hemoglobins have oxygen equilibrium curves which are insensitive to both pH and temperature. The type II hemoglobins have relatively high isoelectric points and are designated as "cathodic" hemoglobins. Powers (1974) suggested that the cathodic hemoglobins might be important for hyperactive fish species and could ensure an adequate oxygen supply to the tissues during bursts of strenuous activity.

The anodic hemoglobins were thought to be involved in the delivery of oxygen against high oxygen pressures in the swimbladder or eye of fish (Blaxter and Tytler, 1978; Ingermann and Termilliger, 1982).

The lower proportion of cathodic components observed in lake trout relative to that of brook trout and Arctic charr suggests that the oxygen equilibria of lake trout would be more sensitive to temperature and/or pH. The hemoglobins of lake trout reported in the present study exhibited the highest sensitivities to variations in temperature, but no difference in sensitivity to pH was found for the species examined. The reason for this similarity in pH dependence of hemoglobins among the three species is unknown. Although the lowest values of Bohr effect and the apparent heat of oxygenation were obtained for brook trout hemoglobins, the oxygen binding characteristics of Arctic charr hemoglobins were generally similar to those of brook trout. Therefore, the quantitative differences in polymorphic hemoglobins in fish may relate to the differences in functional properties of hemoglobins.

The aquatic environment is extremely variable, particularly in relation to dissolved oxygen concentration and temperature. Numerous studies have demonstrated the adaptive plasticity of fish inhabiting diverse aquatic environments. The nature of this variability may be that each organism is endowed with basic physiological limitations which stringently define its ecological niche (Sharp, 1973). Although it is generally accepted that variations in the physical and chemical features of aquatic environments restrict the fish species that successfully occupy specific habitats, the physiological mechanisms through which the restrictive actions are exerted have not been properly studied yet.

Most fish are able to express some adaptive response in the oxygen transport functions of blood to variations in water temperature, dissolved oxygen, and pH (Powers et al., 1986). For example, fish inhabiting hypoxic environments have higher oxygen affinities and stronger Bohr effects. The high oxygen affinity and strong Bohr effect facilitate oxygen delivery to the tissues by assisting oxygenation of the blood at gills and increasing the P_{O2} gradient between blood and capillary (Riggs, 1970; Johansen et al., 1978; Giles and Randall, 1980; Giles, 1991). Most active fish living in well-aerated waters have low oxygen affinities and weak Bohr effects, which is an adaptive response to acidification of blood during strenuous activity (Riggs, 1970; Johansen et al., 1978; Powers, 1980; Randall, 1970).

The members of the genus Salvelinus examined in the present study exhibit substantial differences in their ecological distributions and temperature tolerances. These differences may be reflected in their respective respiratory and metabolic functions. Although at temperature below 15°C the SMR of all sized lake trout is not substantially different from that of Arctic charr or brook trout, the increase in oxygen required by large lake trout relative to Arctic charr or brook trout is more dramatic at temperature higher than 15°C. For a 300 g fish at 15°C, the SMR was 25% higher in lake trout than in either brook trout or Arctic charr. Since the oxygen equilibrium curves of lake trout hemoglobins reported in this study were the most sensitive to the changes of temperature, the large reduction in oxygen affinity at high temperature can hinder oxygen uptake in gills whereas more oxygen is required for metabolic functions. Therefore, this impediment between the oxygen transport and oxygen requirement may limit the ability of this species to select warmer thermal environments. The behavioural response of lake trout - avoiding high temperature by making extensive horizontal and vertical movements - suggests that fish strive to lower their metabolic rate to compensate the physiological restrictions of blood at high temperatures. By lowering temperature from 20 to 10°C, the metabolic energy cost in lake trout decreased as much as 2.5 fold for any given mass in this study.

The hemoglobins of brook trout had the lowest sensitivities to temperature and pH. Brook trout commonly inhabit environments in which the water temperature ranges from 0 to 24°C annually. The low temperature sensitivity of hemoglobins allows these fish to live in thermally fluctuating environments without experiencing changes in the oxygen transport properties of blood. Furthermore, the brook trout is an active fish and a vigorous swimmer. The strenuous activity of the fish, especially in emergency situations or under stress, can cause the acidification of the blood as lactic acid production is increased. The low pH sensitivity of hemoglobins is advantageous for brook trout. These results support the observation of Black et al. (1966). They showed that oxygen affinity and the Bohr effects were similar in field-acclimatized brook trout blood at summer and winter temperatures.

Arctic charr, like brook trout, are also active fish. A major difference between these two species is their ecological distribution. Arctic charr have the most northerly distribution, and as such, might be expected to show special physiological capabilities to withstand the extreme cold of northern waters. However, no such special physiological attributes have been observed in previous researches (reviewed by Johnson, 1980) or in the present study. McCauley (1958) investigated the maximum cruising performances of Arctic charr and brook trout at temperatures of 15°C and 20°C. He reported no differences between these two species. Although the oxygenation characteristics of hemoglobins in Arctic charr and brook trout were similar at 5-10°C and at all test ATP levels, the reduction in oxygen affinity at high temperature (16°C) in Arctic charr was larger than in brook trout. The P_{50} values at this temperature were 29.4 and 20.9 mmHg, respectively. The ΔH was also higher in Arctic charr at all test pH levels. These differences in temperature dependence of the oxygen equilibrium curves between two species reflect the differences in their environmental temperature range. A lower sensitivity of blood to temperature enables brook trout to adapt to higher upper environmental temperature than Arctic charr. At low temperature (5°C), Arctic charr hemoglobin had the largest Bohr shift among all the fish tested. The significance of this observation has not been established.

SUMMARY

In conclusion, the results of the present research supported the hypothesis of this study. The three species of Salvelinus differed in hemoglobin patterns, physiological characteristics of hemoglobins, and metabolic adjustment to temperature change. These differences among the three species were:

1) Although 7 electrophoretically distinct hemoglobins were common to all fish examined, species-specific differences in electrophoretic hemoglobin patterns of Salvelinus species were evident. Brook trout had two more cathodic components than Arctic charr and lake trout. Some hemoglobin components although found in all species, differed in their relative concentrations. The relative concentration of the total cathodic hemoglobins in lake trout was significantly lower than in Arctic charr and brook trout.

2) The hemoglobins of brook trout exhibited the lowest sensitivities to variations in temperature. The oxygen binding characteristics of Arctic charr were similar to those in brook trout, but higher P_{50} values and larger Bohr effect at high temperature were obtained for Arctic charr. The blood

of lake trout was most sensitive to the changes of temperature and ATP.

3) The effects of temperature and body mass on SMR were the greatest in lake trout (highest values for Q_{10} and body mass exponent). Similar effects of body mass on SMR were obtained for Arctic charr and brook trout. The Q_{10} value in Arctic charr, however, was higher than in brook trout.

4) Differences among the three species in blood-oxygen equilibrium characteristics and SMR adjustment to temperature change observed in this study are consistent with differences in environmental conditions preferred or tolerated by each species.

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Appendix A

Mean relative concentration and relative mobility of hemoglobin components.

	Arctic Charr (N=10)		Lake Trout (N=5)		Brook Trout A5 (N=6)		Brook Trout A4 (N=3)	
Hb*	Mean STD**	U95%*** L95%	Mean STD	U95% L95%	Mean STD	U95% L95%	Mean STD	U95% L95%
Concentrations								
A5	4.7 1.2	5.6 3.8	19.6 4.2	24.9 14.4	3.2 1.7	5.1 1.4	- -	- -
A4	16.8 1.8	18.1 15.5	29.9 3.7	34.6 25.3	12.9 1.7	14.7 11.2	15.8 4.4	24.6 6.9
A3	22.1 2.2	23.7 20.5	23.1 7.7	32.6 13.6	18.3 1.8	20.2 16.4	24.1 2.2	28.5 19.6
A2	15.4 1.2	16.2 14.5	6.2 0.8	7.1 5.2	15.8 1.1	16.9 14.7	17.5 2.5	22.6 12.5
A1	4.7 1.2	5.5 3.9	0.5 0.2	0.7 0.2	7.5 3.8	11.5 3.6	4.8 1.9	8.7 1.0
C1	4.4 1.4	5.5 3.4	1.3 0.3	1.6 1.0	1.3 0.4	1.6 0.9	1.6 0.3	2.2 1.0
C2	6.4 1.5	7.4 5.3	4.9 0.7	5.7 4.1	5.6 1.3	7.0 4.3	4.7 0.4	5.5 3.9
C3	12.4 1.1	13.2 11.5	7.1 1.1	8.5 5.7	4.6 1.2	5.8 3.4	2.5 0.4	3.3 1.6
C4	6.7 1.8	8.0 5.4	5.9 1.2	7.3 4.4	6.3 1.2	7.5 5.0	8.3 0.7	9.6 6.7
C5	6.5 1.7	7.7 5.3	1.5 0.9	2.6 0.4	11.0 1.4	12.5 9.5	8.1 1.3	10.8 5.4

Appendix A. (continued)

	Arctic Charr		Lake Trout		Brook Trout A5		Brook Trout A4	
	(N=10)		(N=5)		(N=6)		(N=3)	
	Mean	U95%	Mean	U95%	Mean	U95%	Mean	U95%
Hb	STD	L95%	STD	L95%	STD	L95%	STD	L95%
C6	-	-	-	-	8.1	9.2	7.5	15.8
					1.1	6.9	4.2	0.8
C7	-	-	-	-	6.0	8.5	5.2	7.4
					2.4	3.4	1.1	3.1
Mobilities								
A5	142.8	144.7	163.8	166.3	155.5	163.1	-	-
	2.6	140.9	2.0	161.3	7.2	148.0	-	-
A4	124.5	126.1	133.9	136.2	129.1	133.1	128.7	133.0
	2.3	122.8	1.9	131.6	3.8	125.1	2.1	124.5
A3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
A2	71.0	72.7	64.3	65.4	71.0	75.5	69.2	75.6
	2.5	69.2	0.9	63.2	4.3	66.5	3.2	62.8
A1	35.8	37.8	22.4	24.1	46.0	49.1	47.8	53.8
	2.7	33.9	1.4	20.6	3.0	42.9	3.0	41.7
C1	-43.0	-45.1	-18.9	-20.8	-28.6	-29.0	-28.4	-29.6
	2.9	-40.9	1.5	-17.0	0.3	-28.3	0.6	-27.2
C2	-69.7	-71.4	-48.1	-50.1	-46.6	-47.9	-45.0	-48.0
	2.4	-68.0	1.6	-46.0	1.2	-45.4	1.5	-42.1
C3	-100.0	-100.0	-75.7	-77.1	-72.0	-73.8	-68.4	-70.6
	0.0	-100.0	1.1	-74.2	1.8	-70.1	1.1	-66.1
C4	-119.9	-121.1	-100.0	-100.0	-85.3	-86.2	-84.6	-87.8
	1.7	-118.7	0.0	-100.0	0.8	-84.4	1.6	-81.4

Appendix A. (continued)

	Arctic Charr (N=10)		Lake Trout (N=5)		Brook Trout A5 (N=6)		Brook Trout A4 (N=3)	
	Mean	U95%	Mean	U95%	Mean	U95%	Mean	U95%
Hb	STD	L95%	STD	L95%	STD	L95%	STD	L95%
C5	-138.9	-140.4	-123.4	-124.7	-100.0	-100.0	-100.0	-100.0
	2.1	-137.4	1.0	-122.2	0.0	-100.0	0.0	-100.0
C6	-	-	-	-	-123.2	-125.5	-124.9	-136.7
					2.2	-120.9	5.9	-113.1
C7	-	-	-	-	-138.2	-140.9	-136.3	-146.1
					2.5	-135.5	4.9	-126.5

Total cathodic Hb concentration (mean±STD)

Arctic Charr	Lake Trout	Brook Trout A5	Brook Trout A4
36.34 ± 2.8	20.68 ± 3.7	41.64 ± 3.4	37.80 ± 4.6

* Hb = hemoglobin components
** STD = standard deviation
*** U95% = upper 95% confidence interval
L95% = lower 95% confidence interval

Appendix B

Comparison of measured oxygen content of fully oxygen saturated hemoglobin to theoretical value of oxygen content.

Species	Temp. (°C)	pH	Measured O_2 Content (ml O_2/dl Hb)	Theortical O_2 Content* (ml O_2/dl Hb)	%MetHb**
AC	10	7.5	6.0	6.1	1.6
AC	10	7.9	5.7	5.9	3.4
BT	10	7.9	5.5	5.8	5.2
BT	10	7.1	6.1	6.2	1.6
LT	10	7.1	5.1	5.3	3.8
LT	10	7.5	5.1	5.4	5.6
LT	10	7.9	5.1	5.4	5.6
AC	16	7.9	4.4	4.6	4.4
AC	16	7.1	4.7	5.0	6.0
AC	16	7.5	5.3	5.5	3.6
LT	16	7.5	4.8	5.0	4.0
LT	16	7.9	5.3	5.5	3.6
LT	16	7.1	4.8	5.1	5.9
BT	16	7.5	5.5	5.7	3.5
Mean(±1STD)					4.1(±1.43)

* theoretical oxyhemoglobin content

= (%satn X Hg X 0.00134)/100

Hgb = oxyhemoglobin concentration (mg/ml)

0.00134 = oxygen capacity (ml O_2/mg Hgb)

%satn = oxyhemoglobin percent saturation (mmHg)

$$** \ \%\text{MetHb} = \frac{(\text{Theortical } O_2 \text{ content} - \text{Measured } O_2 \text{ content})*100}{\text{Theortical } O_2 \text{ content}}$$

Appendix C

Calculated mean P_{50} values and 95% confidence interval for P_{50}.

Fish	Temperature (°C)	pH	ATP:Hb	P_{50}*	95% CI for P_{50} L95%	U95%
AC	5.0	7.1	0.00	15.7	14.7	16.7
AC	5.0	7.5	0.00	10.2	9.45	10.9
AC	5.0	7.9	0.00	4.63	4.46	4.80
BT	5.0	7.1	0.00	17.7	16.6	18.9
BT	5.0	7.5	0.00	14.0	12.1	16.3
BT	5.0	7.9	0.00	7.40	6.47	8.46
LT	5.0	7.1	0.00	17.5	15.1	20.2
LT	5.0	7.5	0.00	14.0	12.6	15.7
LT	5.0	7.9	0.00	7.10	5.86	8.61
AC	10.7	7.1	0.00	17.9	15.3	21.0
AC	10.7	7.5	0.00	13.9	13.1	14.9
AC	10.7	7.9	0.00	11.5	9.11	14.4
BT	10.7	7.1	0.00	19.0	16.8	21.5
BT	11.1	7.5	0.00	13.0	12.7	13.2
BT	10.8	7.9	0.00	12.4	6.13	25.2
LT	10.7	7.1	0.00	17.5	15.1	20.2
LT	10.7	7.5	0.00	14.0	12.6	15.7
LT	10.7	7.9	0.00	7.1	5.86	8.60

Appendix C. (continued)

Fish	Temperature (°C)	pH	ATP:Hb	P_{50}	95% CI for P_{50} L95%	95% CI for P_{50} U95%
AC	16.0	7.1	0.00	35.0	32.8	37.5
AC	16.0	7.1	1.25	46.0	42.9	49.3
AC	16.0	7.1	2.50	51.0	47.4	54.7
AC	16.0	7.1	5.00	55.2	51.4	59.1
AC	16.0	7.5	0.00	29.4	28.5	30.3
AC	16.0	7.5	1.25	36.2	34.6	37.8
AC	16.0	7.5	2.50	40.2	37.1	43.5
AC	16.0	7.5	5.00	44.9	41.0	49.2
AC	16.0	7.9	0.00	19.2	18.3	20.2
AC	16.0	7.9	1.25	22.0	20.8	23.3
AC	16.0	7.9	2.50	24.7	22.5	27.0
AC	16.0	7.9	5.00	27.3	24.3	30.8
BT	16.0	7.1	0.00	31.9	30.9	32.8
BT	16.0	7.1	1.25	41.3	38.9	43.8
BT	16.0	7.1	2.50	44.6	42.4	47.0
BT	16.0	7.1	5.00	47.9	43.8	52.4
BT	16.0	7.5	0.00	20.9	19.9	21.9
BT	16.0	7.5	1.25	24.8	22.5	27.4
BT	16.0	7.5	2.50	27.2	24.1	30.7

Appendix C. (continued)

Fish	Temperature (°C)	pH	ATP:Hb	P_{50}	95% CI for P_{50}	
					L95%	U95%
BT	16.0	7.5	5.00	29.1	27.2	31.2
BT	16.0	7.9	0.00	18.7	17.9	19.4
BT	16.0	7.9	1.25	21.5	20.4	22.7
BT	16.0	7.9	2.50	23.1	21.7	24.7
BT	16.0	7.9	5.00	25.5	24.7	26.3
LT	16.0	7.1	0.00	39.2	36.9	41.7
LT	16.0	7.1	1.25	45.9	40.9	51.5
LT	16.0	7.1	2.50	54.0	52.4	55.7
LT	16.0	7.1	5.00	59.9	55.7	64.4
LT	16.0	7.5	0.00	28.3	27.3	29.3
LT	16.0	7.5	1.25	33.7	32.2	35.1
LT	16.0	7.5	2.50	37.3	34.4	40.5
LT	16.0	7.5	5.00	40.6	37.3	44.2
LT	16.0	7.9	0.00	19.1	18.0	20.2
LT	16.0	7.9	1.25	22.5	21.4	23.6
LT	16.0	7.9	2.50	24.7	22.5	27.3
LT	16.0	7.9	5.00	28.6	23.2	35.1

* P_{50} values were determined from a least squares fit of the logarithem of the Hill appoximation: $\log[Y/(100 - Y)] = a + b\log P_{O2}$ (Appendix G).

Appendix D

Standard oxygen consumption data for Arctic charr, brook trout, and lake trout.

Fish	Temp. (°C)	Mass (g)	Length (mm)	Volume (l)	ΔPo_2(mean) (mmHg.h^{-1})	N	V_{O2} (mg.h^{-1})
AC	5.5	81.1	220	3.360	8.6	3	2.255
AC	5.5	61.0	196	3.346	5.7	3	1.488
AC	5.5	133.4	262	4.153	6.7	3	2.171
AC	5.5	137.6	246	4.131	15.5	3	4.996
AC	5.5	40.6	171	3.336	4.7	3	1.223
AC	5.5	54.4	195	3.344	5.5	3	1.435
AC	5.5	27.5	147	0.984	22.9	4	1.758
AC	5.5	27.9	145	0.996	22.0	3	1.710
AC	5.5	24.3	133	0.986	12.4	3	0.954
AC	5.5	21.7	136	0.988	23.3	3	1.796
AC	5.5	32.2	159	3.336	7.7	3	2.039
AC	5.5	146.2	251	4.146	27.2	2	8.950
AC	5.5	292.4	312	4.131	41.6	2	13.64
AC	5.5	178.2	274	4.153	19.1	3	6.295
AC	5.5	36.9	165	3.360	6.1	3	1.627
AC	5.5	49.9	182	3.336	8.3	3	2.196
AC	5.5	25.1	143	0.984	50.0	2	3.905
AC	5.5	26.3	151	0.996	32.1	2	2.537
AC	5.5	27.2	152	0.988	26.2	2	2.054
AC	10.1	50.8	163	3.366	20.3	3	4.825
AC	10.1	39.6	164	3.346	23.6	3	5.586
AC	10.1	54.7	177	3.353	24.1	3	5.709
AC	10.1	183.0	273	4.184	34.0	2	10.06
AC	10.1	218.9	282	4.146	48.4	2	14.19
AC	10.1	64.4	200	4.153	21.0	3	6.154
AC	10.1	31.7	159	3.336	10.7	4	2.525
AC	10.1	43.2	169	3.344	20.8	3	4.920
AC	10.1	77.7	206	3.344	25.3	3	5.885
AC	10.1	54.6	183	3.336	21.0	3	4.873
AC	10.1	51.4	175	3.336	20.0	3	4.641
AC	10.1	126.2	254	4.174	24.1	3	6.983
AC	10.1	59.0	186	3.346	20.2	3	4.690
AC	10.1	80.4	201	3.353	27.8	2	6.484
AC	10.1	388.0	341	4.184	74.1	2	21.57
AC	10.1	225.4	282	4.153	41.6	2	12.02
AC	10.1	30.4	153	3.335	11.7	4	2.714
AC	10.1	21.8	132	3.360	7.9	4	1.846

Appendix D. (continued)

Fish	Temp. (°C)	Mass (g)	Length (mm)	Volume (l)	ΔPo_2(mean) (mmHg.h^{-1})	N	V_{O2} (mg.h^{-1})
AC	16.0	49.8	169	3.353	34.6	6	7.245
AC	16.0	60.8	191	3.346	42.4	6	8.859
AC	16.0	73.7	203	3.344	30.1	6	6.285
AC	16.0	144.8	243	3.336	49.7	5	10.35
AC	16.0	108.5	248	4.153	20.0	6	5.187
AC	16.0	251.9	297	4.176	52.8	6	13.77
AC	16.0	120.5	242	4.184	35.8	6	9.353
AC	16.0	60.8	190	3.336	28.9	6	6.020
AC	16.0	40.1	172	3.335	18.6	6	3.874
AC	16.0	87.6	213	3.344	65.2	4	13.64
AC	16.0	86.6	210	3.353	39.7	5	8.330
AC	16.0	71.0	192	3.336	34.5	5	7.197
AC	16.0	97.1	205	3.336	41.3	5	8.621
AC	16.0	117.7	234	4.153	34.2	5	8.888
AC	16.0	284.1	311	4.176	72.0	2	18.81
AC	16.0	241.6	302	4.131	58.7	2	15.17
BT	5.5	82.9	194	3.336	19.4	2	5.126
BT	5.5	55.1	168	3.360	34.9	2	9.288
BT	5.5	173.9	252	4.146	22.7	2	7.454
BT	5.5	298.2	294	4.153	49.6	2	16.32
BT	5.5	46.8	163	3.336	16.5	2	4.360
BT	5.5	74.2	179	3.346	20.4	2	5.407
BT	5.5	55.8	166	3.335	15.0	2	3.962
BT	5.5	11.8	121	0.985	18.9	2	1.475
BT	5.5	78.3	191	3.335	12.9	3	3.420
BT	5.5	65.4	189	3.336	14.6	2	3.872
BT	5.5	80.8	192	3.346	16.0	3	4.256
BT	5.5	27.2	136	0.988	19.0	3	1.492
BT	5.5	125.0	223	3.360	13.2	2	3.526
BT	10.5	54.4		3.335	13.4	5	3.110
BT	10.5	71.4		3.336	24.0	3	5.571
BT	10.5	106.4		4.184	46.0	2	13.39
BT	10.5	163.5		4.174	38.1	3	11.07
BT	10.5	103.1		4.131	37.6	3	10.81
BT	10.5	172.6		4.153	40.8	4	11.79
BT	10.5	52.4		3.336	45.5	2	10.56
BT	10.5	58.7		3.344	40.1	2	9.331
BT	10.5	15.1		0.999	17.7	4	1.231
BT	10.5	9.7		0.984	15.0	3	1.027
BT	10.5	7.3		0.988	13.7	3	0.942
BT	10.5	12.1		0.987	17.4	3	1.195
BT	10.5	17.7		0.996	56.0	2	3.882

Appendix D. (continued)

Fish	Temp. (°C)	Mass (g)	Length (mm)	Volume (l)	ΔPo_2(mean) (mmHg.h^{-1})	N	V_{O2} (mg.h^{-1})
BT	10.5	36.7		3.336	11.2	6	2.600
BT	10.5	263.3		4.184	37.8	2	11.08
BT	10.5	33.9		3.344	22.0	2	5.155
BT	10.5	44.0		4.131	29.1	2	8.423
BT	10.5	45.3		3.336	16.5	3	3.226
BT	10.5	18.2		3.335	17.8	3	4.159
BT	10.5	129.7		4.153	63.1	2	18.36
BT	16.0	36.3	148	3.346	25.3	5	5.291
BT	16.0	33.3	148	3.336	58.4	5	12.18
BT	16.0	99.1	216	3.336	38.0	5	7.923
BT	16.0	61.6	179	3.344	31.4	5	6.563
BT	16.0	148.2	242	4.174	52.9	3	13.80
BT	16.0	202.6	269	4.131	59.8	3	15.44
BT	16.0	196.7	259	4.176	62.5	3	16.31
BT	16.0	276.2	297	4.153	103.7	2	26.92
BT	16.0	27.8	138	3.335	22.2	5	5.617
BT	16.0	55.2	175	3.360	31.2	4	6.552
BT	16.0	24.9	134	3.360	34.9	4	7.363
BT	16.0	52.9	173	3.353	32.4	4	6.822
BT	16.0	109.4	215	4.153	27.4	4	7.145
BT	16.0	138.2	214	4.176	33.3	3	8.732
BT	16.0	125.3	237	3.344	37.9	4	8.315
BT	16.0	142.4	228	4.184	36.8	4	10.59
BT	16.0	158.6	242	4.174	51.7	4	13.55
BT	16.0	370.9	319	4.131	73.5	2	19.07
LT	5.3	13.8	126	0.999	8.7	6	0.689
LT	5.3	19.7	132	0.988	13.2	4	1.033
LT	5.3	56.7	181	3.346	9.0	6	2.386
LT	5.3	62.1	185	3.336	9.4	6	2.484
LT	5.3	52.9	176	3.336	9.9	5	2.617
LT	5.3	29.5	151	3.360	4.3	6	1.145
LT	5.3	54.2	183	3.353	7.0	6	1.860
LT	5.3	29.2	148	3.344	4.0	6	1.060
LT	4.8	64.7	188	3.360	10.1	4	2.728
LT	4.8	61.1	186	3.353	9.6	5	2.588
LT	4.8	109.4	226	4.174	12.0	4	4.026
LT	4.8	136.9	233	4.184	11.9	4	4.002
LT	4.8	114.5	232	4.146	10.6	5	3.533
LT	4.8	91.6	216	3.346	14.5	4	3.900
LT	4.8	233.2	283	4.131	20.5	4	6.808
LT	4.8	122.5	229	4.153	1.1	5	4.373

Appendix D. (continued)

Fish	Temp. (°C)	Mass (g)	Length (mm)	Volume (l)	ΔPo_2(mean) (mmHg.h^{-1})	N	V_{O2} (mg.h^{-1})
LT	10.3	20.9		0.999	22.5	3	1.565
LT	10.3	56.5		3.336	14.5	3	3.367
LT	10.3	120.1		4.184	25.5	3	7.426
LT	10.3	51.5		3.335	15.3	4	3.552
LT	10.3	67.2		4.174	15.4	3	4.474
LT	10.3	91.6		4.131	26.9	3	7.735
LT	10.3	15.9		0.986	17.7	4	1.215
LT	10.3	131.8		4.153	27.9	3	8.065
LT	10.3	31.0		0.986	24.0	3	1.668
LT	10.3	107.7		4.174	28.3	4	8.325
LT	10.3	33.9		0.999	24.0	4	1.689
LT	10.3	56.4		3.335	17.4	5	4.090
LT	10.3	61.9		3.336	20.4	4	4.796
LT	10.3	57.4		4.184	11.5	6	3.391
LT	10.3	121.0		4.131	22.1	4	6.434
LT	10.3	237.0		4.153	54.2	3	15.86
LT	16.0	41.5	175	3.353	17.0	5	3.515
LT	16.0	40.1	170	3.336	33.9	5	6.974
LT	16.0	75.5	205	3.360	31.2	5	6.464
LT	16.0	98.7	232	3.336	65.2	3	13.41
LT	16.0	176.1	274	4.176	45.1	3	15.32
LT	16.0	141.8	249	4.184	51.1	3	13.18
LT	16.0	292.9	327	4.153	107.7	2	27.58
LT	16.0	111.8	233	3.344	64.4	4	13.28
LT	16.0	65.3	199	3.344	30.2	5	6.280
LT	16.0	108.3	238	3.346	51.7	4	10.76
LT	16.0	81.8	210	3.336	35.4	5	7.344
LT	16.0	229.4	286	4.174	55.5	3	14.41
LT	16.0	163.1	269	4.153	65.4	2	16.89
LT	16.0	206.6	296	4.176	68.8	3	17.87
LT	16.0	212.1	290	4.131	100.7	2	25.87
LT	16.0	120.6	242	4.184	48.6	4	12.65

Appendix E

Predicted values versus experimental data for standard oxygen consumptions at three different temperatures for Arctic charr (Fig. E1), brook trout (Fig. E2), and lake trout (Fig. E3).

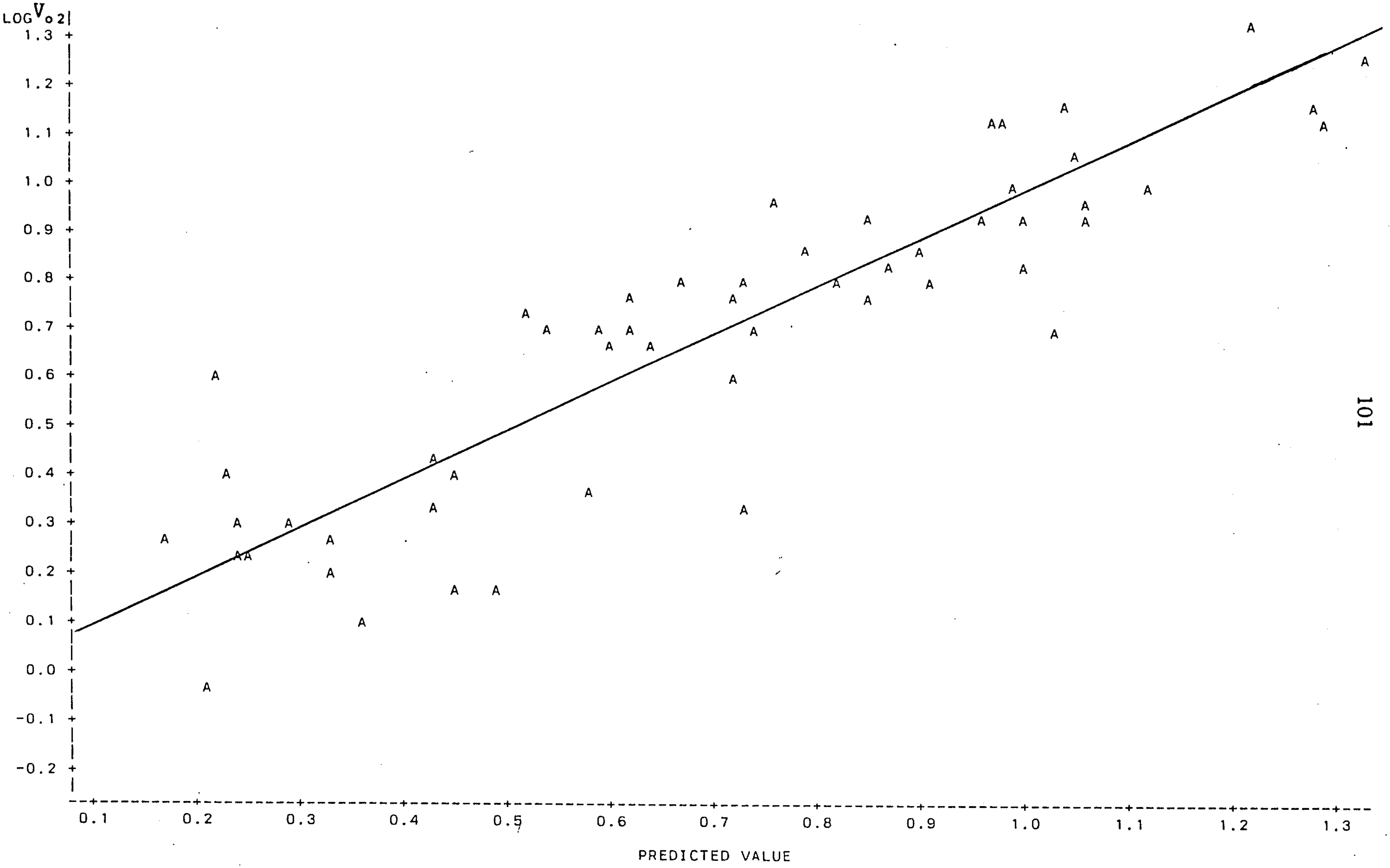

Figure E1: Predicted Values versus Experimental Data for Standard Oxygen Comsumption of Arctic Charr at Three Different Temperatures (A=1 OBS, B=2 OBS)

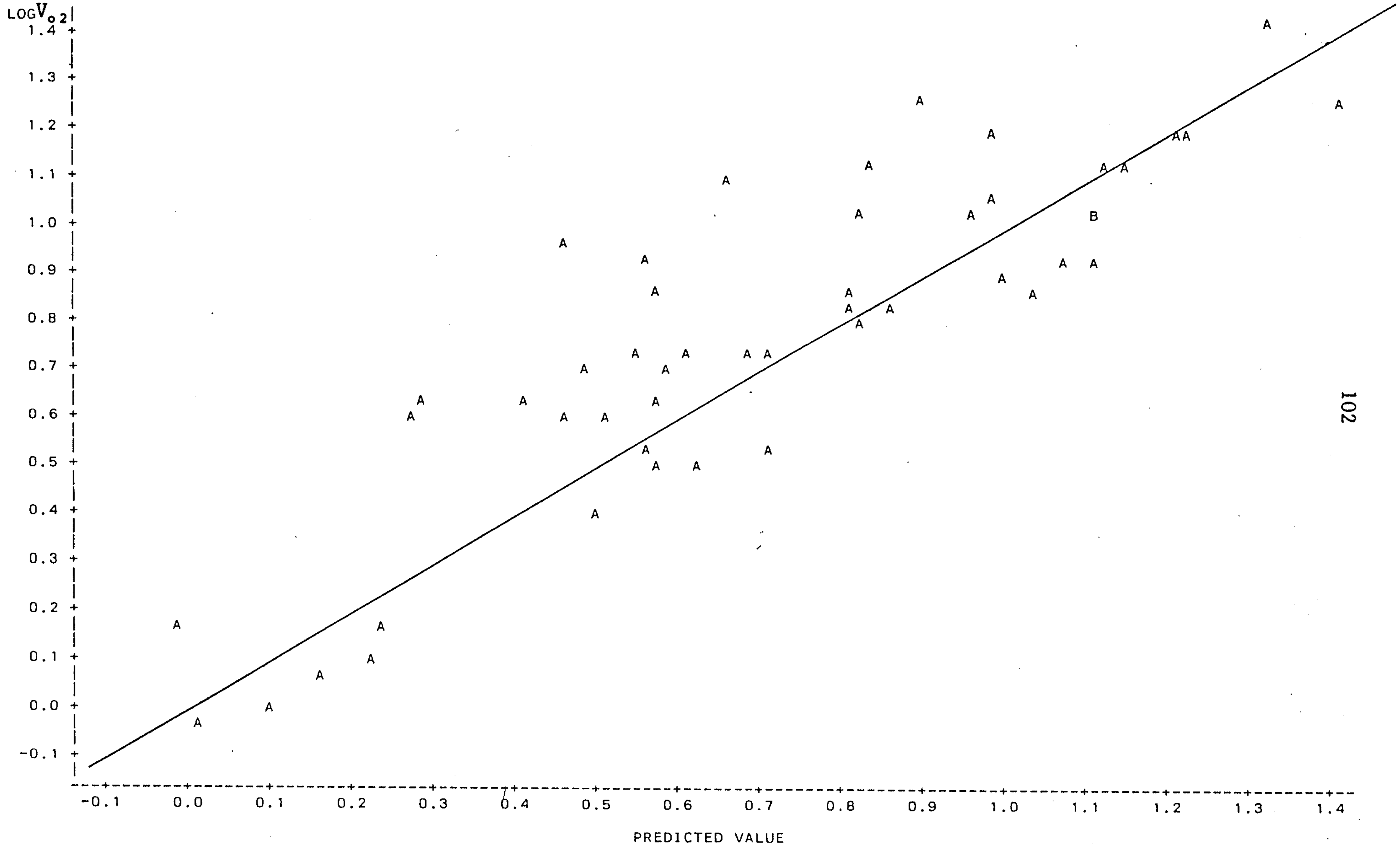

Figure E2 Predicted Values versus Experimental Data for Standard Oxygen Comsumption of Brook Trout at Three Different Temperatures (A=1 OBS, B=2 OBS)

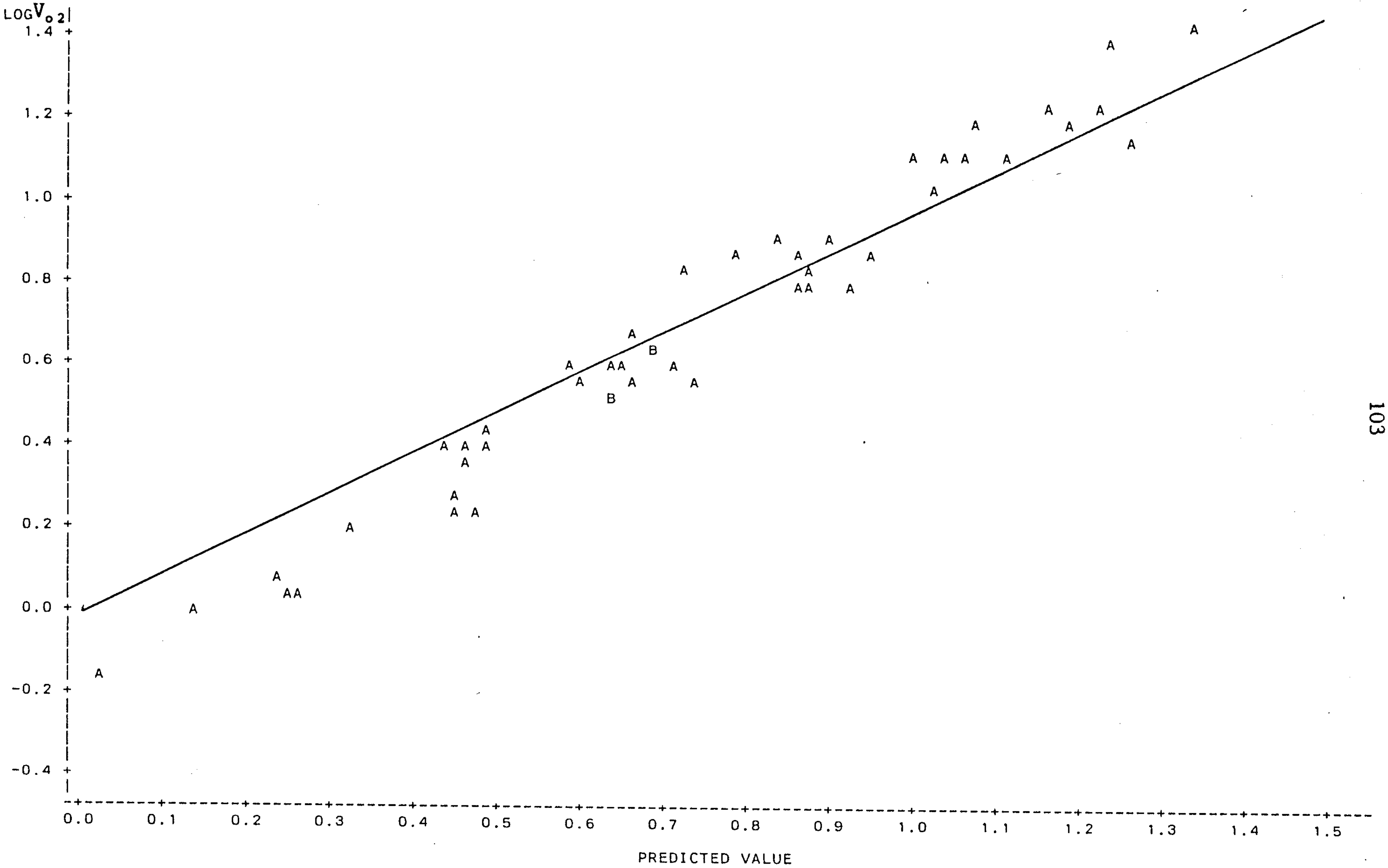

Figure E3: Predicted Values versus Experimental Data for Standard Oxygen Comsumption of Lake Trout at Three Different Temperatures (A=1 OBS, B=2 OBS)

Appendix F
Table F1

Regression coefficients for standard metabolic rate versus body mass of Salvelinus species at temperatures of 5, 10, and 16°C.

Fish	Temp. (°C)	N	Mass Range (g)	Equation* Constants		Standard Error		R^2	Signif. (P-value)
				a	b	a	b		
AC	5	19	21.7-292.4	-0.716	0.645	0.248	0.141	0.56	0.0003
	10	18	21.8-388.0	-0.602	0.736	0.104	0.055	0.92	0.0001
	16	17	40.1-284.1	-0.239	0.587	0.224	0.111	0.65	0.0001
BT	5	13	11.8-298	-0.574	0.663	0.269	0.144	0.65	0.0008
	10	18	7.3-263.3	-0.659	0.797	0.167	0.097	0.81	0.0001
	16	18	24.9-370.9	0.116	0.445	0.179	0.089	0.61	0.0001
LT	5	16	13.8-233.2	-1.093	0.823	0.079	0.043	0.97	0.0001
	10	16	15.9-237.0	-1.167	0.991	0.106	0.058	0.96	0.0001
	16	16	40.1-292.9	-0.707	0.858	0.204	0.098	0.85	0.0001

* $\log V_{O2} = a + b\log M$
V_{O2} = rate of oxygen consumption of the fish (mg O_2 /h)
a = vertical-axis intercept
b = slope of the regression
M = mass of the testing fish (g)

Appendix G

Experimental data for hemoglobin-oxygen equilibria of the three species of _Salvelinus_ and statistical analysis.

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
A. C.	16.0	7.5	0.00	4.00	21.3	17.7	29.41	2.433	0.996	Regression Output:		
A. C.	16.0	7.5	0.00	4.00	29.6	19.8				Constant		-3.573
A. C.	16.0	7.5	0.00	4.00	39.4	24.4				Std Err of Y Est		0.029
A. C.	16.0	7.5	0.00	4.00	51.2	30.5				R Squared		0.996
A. C.	16.0	7.5	0.00	4.00	64.0	37.8				No. of Observations		6.000
A. C.	16.0	7.5	0.00	4.00	80.7	52.3				Degrees of Freedom		4.000
										X Coefficient(s)	2.433	
										Std Err of Coef.	0.073	
A. C.	16.0	7.5	1.25	4.00	21.3	19.6	36.18	2.257	0.994	Regression Output:		
A. C.	16.0	7.5	1.25	4.00	29.3	25.5				Constant		-3.518
A. C.	16.0	7.5	1.25	4.00	39.2	29.3				Std Err of Y Est		0.038
A. C.	16.0	7.5	1.25	4.00	50.9	36.9				R Squared		0.994
A. C.	16.0	7.5	1.25	4.00	63.6	48.2				No. of Observations		6.000
A. C.	16.0	7.5	1.25	4.00	80.1	64.7				Degrees of Freedom		4.000
										X Coefficient(s)	2.257	
										Std Err of Coef.	0.089	
A. C.	16.0	7.5	2.50	4.00	21.1	22.1	40.21	2.446	0.976	Regression Output:		
A. C.	16.0	7.5	2.50	4.00	29.1	30.4				Constant		-3.924
A. C.	16.0	7.5	2.50	4.00	38.9	35.3				Std Err of Y Est		0.075
A. C.	16.0	7.5	2.50	4.00	50.9	38.0				R Squared		0.976
A. C.	16.0	7.5	2.50	4.00	63.4	51.2				No. of Observations		6.000
A. C.	16.0	7.5	2.50	4.00	79.9	69.0				Degrees of Freedom		4.000
										X Coefficient(s)	2.446	
										Std Err of Coef.	0.193	
A. C.	16.0	7.5	5.00	4.00	21.0	24.8	44.98	2.630	0.962	Regression Output:		
A. C.	16.0	7.5	5.00	4.00	28.8	35.4				Constant		-4.348
A. C.	16.0	7.5	5.00	4.00	38.7	40.4				Std Err of Y Est		0.093
A. C.	16.0	7.5	5.00	4.00	50.6	45.1				R Squared		0.962
A. C.	16.0	7.5	5.00	4.00	63.3	53.1				No. of Observations		6.000
A. C.	16.0	7.5	5.00	4.00	79.7	73.3				Degrees of Freedom		4.000
										X Coefficient(s)	2.630	
										Std Err of Coef.	0.262	
A. C.	16.0	7.1	0.00	4.10	16.6	14.5	35.06	1.742	0.995	Regression Output:		
A. C.	16.0	7.1	0.00	4.10	29.1	19.8				Constant		-2.691
A. C.	16.0	7.1	0.00	4.10	56.6	41.7				Std Err of Y Est		0.034
A. C.	16.0	7.1	0.00	4.10	63.6	47.7				R Squared		0.995
A. C.	16.0	7.1	0.00	4.10	61.7	46.6				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.742	
										Std Err of Coef.	0.072	
A. C.	16.0	7.1	1.25	4.10	16.5	17.5	46.04	1.616	0.995	Regression Output:		
A. C.	16.0	7.1	1.25	4.10	28.8	25.1				Constant		-2.688
A. C.	16.0	7.1	1.25	4.10	56.0	54.0				Std Err of Y Est		0.033
A. C.	16.0	7.1	1.25	4.10	62.7	65.9				R Squared		0.995
A. C.	16.0	7.1	1.25	4.10	61.1	58.7				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.616	
										Std Err of Coef.	0.064	
A. C.	16.0	7.1	2.50	4.10	16.4	19.7	51.00	1.652	0.995	Regression Output:		
A. C.	16.0	7.1	2.50	4.10	28.7	28.2				Constant		-2.822
A. C.	16.0	7.1	2.50	4.10	55.8	58.0				Std Err of Y Est		0.035
A. C.	16.0	7.1	2.50	4.10	62.3	72.9				R Squared		0.995
A. C.	16.0	7.1	2.50	4.10	60.8	64.3				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.652	
										Std Err of Coef.	0.070	
A. C.	16.0	7.1	5.00	4.10	16.3	21.8	55.21	1.711	0.995	Regression Output:		
A. C.	16.0	7.1	5.00	4.10	28.5	31.3				Constant		-2.980
A. C.	16.0	7.1	5.00	4.10	55.5	62.1				Std Err of Y Est		0.035
A. C.	16.0	7.1	5.00	4.10	62.1	77.8				R Squared		0.995
A. C.	16.0	7.1	5.00	4.10	60.6	68.3				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.711	
										Std Err of Coef.	0.072	
A. C.	16.0	7.9	0.00	4.15	28.7	12.6	19.25	2.240	0.993	Regression Output:		
A. C.	16.0	7.9	0.00	4.15	34.9	14.4				Constant		-2.877
A. C.	16.0	7.9	0.00	4.15	48.0	18.9				Std Err of Y Est		0.034
A. C.	16.0	7.9	0.00	4.15	75.9	31.1				R Squared		0.993
A. C.	16.0	7.9	0.00	4.15	53.2	21.3				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.240	
										Std Err of Coef.	0.111	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
A. C.	16.0	7.9	1.25	4.15	28.6	14.8	22.03	2.524	0.989	Regression Output:		
A. C.	16.0	7.9	1.25	4.15	34.7	17.5				Constant		-3.390
A. C.	16.0	7.9	1.25	4.15	47.8	22.3				Std Err of Y Est		0.042
A. C.	16.0	7.9	1.25	4.15	75.7	33.8				R Squared		0.989
	16.0	7.9	1.25	4.15	53.1	22.9				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.524	
										Std Err of Coef.	0.156	
A. C.	16.0	7.9	2.50	4.15	28.5	16.8	24.71	2.772	0.962	Regression Output:		
A. C.	16.0	7.9	2.50	4.15	34.5	21.2				Constant		-3.861
A. C.	16.0	7.9	2.50	4.15	47.7	24.8				Std Err of Y Est		0.078
A. C.	16.0	7.9	2.50	4.15	75.6	36.3				R Squared		0.962
A. C.	16.0	7.9	2.50	4.15	53.0	24.8				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.772	
										Std Err of Coef.	0.319	
A. C.	16.0	7.9	5.00	4.15	28.4	18.5	27.39	2.770	0.932	Regression Output:		
A. C.	16.0	7.9	5.00	4.15	34.3	23.6				Constant		-3.982
A. C.	16.0	7.9	5.00	4.15	47.4	28.5				Std Err of Y Est		0.104
A. C.	16.0	7.9	5.00	4.15	75.4	39.4				R Squared		0.932
A. C.	16.0	7.9	5.00	4.15	52.9	26.8				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.770	
										Std Err of Coef.	0.431	
L. T.	16.0	7.5	0.00	4.05	22.2	14.9	28.27	1.873	0.997	Regression Output:		
L. T.	16.0	7.5	0.00	4.05	34.5	19.7				Constant		-2.719
L. T.	16.0	7.5	0.00	4.05	48.6	27.2				Std Err of Y Est		0.025
L. T.	16.0	7.5	0.00	4.05	64.5	37.4				R Squared		0.997
L. T.	16.0	7.5	0.00	4.05	77.0	55.1				No. of Observations		6.000
L. T.	16.0	7.5	0.00	4.05	72.4	48.1				Degrees of Freedom		4.000
										X Coefficient(s)	1.873	
										Std Err of Coef.	0.050	
L. T.	16.0	7.5	1.25	4.05	21.9	19.7	33.64	2.174	0.994	Regression Output:		
L. T.	16.0	7.5	1.25	4.05	34.3	23.9				Constant		-3.319
L. T.	16.0	7.5	1.25	4.05	48.4	32.0				Std Err of Y Est		0.035
L. T.	16.0	7.5	1.25	4.05	64.2	43.3				R Squared		0.994
L. T.	16.0	7.5	1.25	4.05	76.8	58.8				No. of Observations		6.000
L. T.	16.0	7.5	1.25	4.05	72.1	53.4				Degrees of Freedom		4.000
										X Coefficient(s)	2.174	
										Std Err of Coef.	0.083	
L. T.	16.0	7.5	2.50	4.05	21.8	22.5	37.30	2.141	0.979	Regression Output:		
L. T.	16.0	7.5	2.50	4.05	34.2	26.4				Constant		-3.365
L. T.	16.0	7.5	2.50	4.05	48.3	33.8				Std Err of Y Est		0.067
L. T.	16.0	7.5	2.50	4.05	64.1	47.0				R Squared		0.979
L. T.	16.0	7.5	2.50	4.05	76.3	68.9				No. of Observations		6.000
L. T.	16.0	7.5	2.50	4.05	72.0	57.1				Degrees of Freedom		4.000
										X Coefficient(s)	2.141	
										Std Err of Coef.	0.156	
L. T.	16.0	7.5	5.00	4.05	21.6	25.4	40.58	2.261	0.975	Regression Output:		
L. T.	16.0	7.5	5.00	4.05	34.1	28.9				Constant		-3.637
L. T.	16.0	7.5	5.00	4.05	48.2	36.6				Std Err of Y Est		0.074
L. T.	16.0	7.5	5.00	4.05	63.9	50.5				R Squared		0.975
L. T.	16.0	7.5	5.00	4.05	76.2	72.2				No. of Observations		6.000
L. T.	16.0	7.5	5.00	4.05	71.8	60.6				Degrees of Freedom		4.000
										X Coefficient(s)	2.261	
										Std Err of Coef.	0.182	
L. T.	16.0	7.9	0.00	4.00	20.4	11.1	19.12	2.364	0.993	Regression Output:		
L. T.	16.0	7.9	0.00	4.00	25.8	12.1				Constant		-3.029
L. T.	16.0	7.9	0.00	4.00	37.7	15.2				Std Err of Y Est		0.035
L. T.	16.0	7.9	0.00	4.00	52.9	19.4				R Squared		0.993
L. T.	16.0	7.9	0.00	4.00	65.6	25.9				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.364	
										Std Err of Coef.	0.117	
L. T.	16.0	7.9	1.25	4.00	20.4	11.7	22.48	2.162	0.995	Regression Output:		
L. T.	16.0	7.9	1.25	4.00	25.7	14.2				Constant		-2.922
L. T.	16.0	7.9	1.25	4.00	37.5	18.0				Std Err of Y Est		0.029
L. T.	16.0	7.9	1.25	4.00	52.7	22.9				R Squared		0.995
L. T.	16.0	7.9	1.25	4.00	65.4	30.5				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.162	
										Std Err of Coef.	0.087	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
L. T.	16.0	7.9	2.50	4.00	20.3	12.7	24.84	2.223	0.981	Regression Output:		
L. T.	16.0	7.9	2.50	4.00	25.6	16.7				Constant		-3.101
L. T.	16.0	7.9	2.50	4.00	37.5	19.7				Std Err of Y Est		0.057
L. T.	16.0	7.9	2.50	4.00	52.6	25.9				R Squared		0.981
L. T.	16.0	7.9	2.50	4.00	65.3	32.4				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.223	
										Std Err of Coef.	0.178	
L. T.	16.0	7.9	5.00	4.00	20.2	14.5	29.14	2.311	0.898	Regression Output:		
L. T.	16.0	7.9	5.00	4.00	25.4	21.8				Constant		-3.385
L. T.	16.0	7.9	5.00	4.00	37.3	24.4				Std Err of Y Est		0.132
L. T.	16.0	7.9	5.00	4.00	52.5	28.9				R Squared		0.898
L. T.	16.0	7.9	5.00	4.00	65.2	35.7				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.311	
										Std Err of Coef.	0.449	
L. T.	16.0	7.1	0.00	4.05	14.0	16.9	39.29	2.083	0.994	Regression Output:		
L. T.	16.0	7.1	0.00	4.05	31.9	27.0				Constant		-3.320
L. T.	16.0	7.1	0.00	4.05	40.1	30.7				Std Err of Y Est		0.035
L. T.	16.0	7.1	0.00	4.05	65.6	54.3				R Squared		0.994
L. T.	16.0	7.1	0.00	4.05	51.0	41.0				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.083	
										Std Err of Coef.	0.090	
L. T.	16.0	7.1	1.25	4.05	13.9	20.7	46.13	2.377	0.975	Regression Output:		
L. T.	16.0	7.1	1.25	4.05	31.4	35.2				Constant		-3.955
L. T.	16.0	7.1	1.25	4.05	39.7	38.5				Std Err of Y Est		0.074
L. T.	16.0	7.1	1.25	4.05	65.5	55.6				R Squared		0.975
L. T.	16.0	7.1	1.25	4.05	50.6	49.6				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.377	
										Std Err of Coef.	0.221	
L. T.	16.0	7.1	2.50	4.05	13.8	22.7	54.05	2.114	0.999	Regression Output:		
L. T.	16.0	7.1	2.50	4.05	31.3	38.0				Constant		-3.663
L. T.	16.0	7.1	2.50	4.05	39.4	43.2				Std Err of Y Est		0.017
L. T.	16.0	7.1	2.50	4.05	64.7	72.7				R Squared		0.999
L. T.	16.0	7.1	2.50	4.05	50.4	53.9				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.114	
										Std Err of Coef.	0.045	
L. T.	16.0	7.1	5.00	4.05	13.6	26.4	60.00	2.248	0.991	Regression Output:		
L. T.	16.0	7.1	5.00	4.05	31.1	41.3				Constant		-3.997
L. T.	16.0	7.1	5.00	4.05	39.0	52.1				Std Err of Y Est		0.043
L. T.	16.0	7.1	5.00	4.05	64.4	78.4				R Squared		0.991
L. T.	16.0	7.1	5.00	4.05	50.2	57.6				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.248	
										Std Err of Coef.	0.121	
B. T.	16.0	7.5	0.00	4.00	25.8	13.0	20.86	2.066	0.995	Regression Output:		
B. T.	16.0	7.5	0.00	4.00	49.9	20.3				Constant		-2.725
B. T.	16.0	7.5	0.00	4.00	65.5	28.1				Std Err of Y Est		0.030
B. T.	16.0	7.5	0.00	4.00	77.2	38.6				R Squared		0.995
B. T.	16.0	7.5	0.00	4.00	40.5	16.9				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.066	
										Std Err of Coef.	0.082	
B. T.	16.0	7.5	1.25	4.00	25.7	15.7	24.81	1.974	0.981	Regression Output:		
B. T.	16.0	7.5	1.25	4.00	49.8	23.0				Constant		-2.753
B. T.	16.0	7.5	1.25	4.00	65.2	35.0				Std Err of Y Est		0.061
B. T.	16.0	7.5	1.25	4.00	76.9	46.3				R Squared		0.981
B. T.	16.0	7.5	1.25	4.00	40.4	19.4				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.974	
										Std Err of Coef.	0.158	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
B. T.	16.0	7.5	2.50	4.00	25.7	16.8	27.17	1.822	0.976	Regression Output:		
B. T.	16.0	7.5	2.50	4.00	49.7	26.2				Constant		-2.613
B. T.	16.0	7.5	2.50	4.00	65.0	38.7				Std Err of Y Est		0.068
B. T.	16.0	7.5	2.50	4.00	76.6	52.9				R Squared		0.976
B. T.	16.0	7.5	2.50	4.00	40.4	19.8				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.822	
										Std Err of Coef.	0.167	
B. T.	16.0	7.5	5.00	4.00	25.6	17.8	29.10	1.951	0.991	Regression Output:		
B. T.	16.0	7.5	5.00	4.00	49.6	27.8				Constant		-2.856
B. T.	16.0	7.5	5.00	4.00	64.9	40.8				Std Err of Y Est		0.041
B. T.	16.0	7.5	5.00	4.00	76.5	54.0				R Squared		0.991
B. T.	16.0	7.5	5.00	4.00	40.3	22.7				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.951	
										Std Err of Coef.	0.106	
B. T.	16.0	7.1	0.00	4.00	25.4	18.3	31.88	1.900	0.999	Regression Output:		
B. T.	16.0	7.1	0.00	4.00	46.6	29.6				Constant		-2.857
B. T.	16.0	7.1	0.00	4.00	59.2	37.9				Std Err of Y Est		0.016
B. T.	16.0	7.1	0.00	4.00	76.0	59.8				R Squared		0.999
B. T.	16.0	7.1	0.00	4.00	71.8	51.8				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.900	
										Std Err of Coef.	0.040	
B. T.	16.0	7.1	1.25	4.00	25.2	22.1	41.28	1.665	0.996	Regression Output:		
B. T.	16.0	7.1	1.25	4.00	46.3	36.2				Constant		-2.691
B. T.	16.0	7.1	1.25	4.00	56.5	51.1				Std Err of Y Est		0.029
B. T.	16.0	7.1	1.25	4.00	74.9	82.5				R Squared		0.996
B. T.	16.0	7.1	1.25	4.00	71.0	68.6				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.665	
										Std Err of Coef.	0.063	
B. T.	16.0	7.1	2.50	4.00	25.1	23.9	44.60	1.682	0.997	Regression Output:		
B. T.	16.0	7.1	2.50	4.00	46.2	36.8				Constant		-2.775
B. T.	16.0	7.1	2.50	4.00	56.3	55.4				Std Err of Y Est		0.025
B. T.	16.0	7.1	2.50	4.00	74.7	86.3				R Squared		0.997
B. T.	16.0	7.1	2.50	4.00	70.7	74.4				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.682	
										Std Err of Coef.	0.056	
B. T.	16.0	7.1	5.00	4.00	25.0	25.9	47.85	1.743	0.989	Regression Output:		
B. T.	16.0	7.1	5.00	4.00	46.0	41.2				Constant		-2.928
B. T.	16.0	7.1	5.00	4.00	57.9	62.4				Std Err of Y Est		0.046
B. T.	16.0	7.1	5.00	4.00	74.7	87.0				R Squared		0.989
B. T.	16.0	7.1	5.00	4.00	70.5	77.7				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.743	
										Std Err of Coef.	0.108	
B. T.	16.0	7.9	0.00	4.00	33.4	14.2	16.66	2.542	0.994	Regression Output:		
B. T.	16.0	7.9	0.00	4.00	56.8	21.8				Constant		-3.231
B. T.	16.0	7.9	0.00	4.00	75.0	29.3				Std Err of Y Est		0.027
B. T.	16.0	7.9	0.00	4.00	46.6	18.3				R Squared		0.994
B. T.	16.0	7.9	0.00	4.00	71.5	25.9				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.542	
										Std Err of Coef.	0.110	
B. T.	16.0	7.9	1.25	4.00	33.3	17.0	21.49	2.767	0.987	Regression Output:		
B. T.	16.0	7.9	1.25	4.00	56.7	24.9				Constant		-3.687
B. T.	16.0	7.9	1.25	4.00	74.8	32.7				Std Err of Y Est		0.041
B. T.	16.0	7.9	1.25	4.00	46.5	20.5				R Squared		0.987
B. T.	16.0	7.9	1.25	4.00	71.4	26.8				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.767	
										Std Err of Coef.	0.161	
B. T.	16.0	7.9	2.50	4.00	33.2	18.7	23.06	2.951	0.979	Regression Output:		
B. T.	16.0	7.9	2.50	4.00	56.6	26.6				Constant		-4.022
B. T.	16.0	7.9	2.50	4.00	74.7	34.2				Std Err of Y Est		0.053
B. T.	16.0	7.9	2.50	4.00	46.4	21.7				R Squared		0.979
B. T.	16.0	7.9	2.50	4.00	71.3	30.2				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.951	
										Std Err of Coef.	0.252	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
B. T.	16.0	7.9	2.50	4.00	33.2	18.7	23.06	2.951	0.979	Regression Output:		
B. T.	16.0	7.9	2.50	4.00	56.6	26.6				Constant		-4.022
B. T.	16.0	7.9	2.50	4.00	74.7	34.2				Std Err of Y Est		0.053
B. T.	16.0	7.9	2.50	4.00	48.4	21.7				R Squared		0.979
B. T.	16.0	7.9	2.50	4.00	71.3	30.2				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.951	
										Std Err of Coef.	0.232	
B. T.	16.0	7.9	5.00	4.00	33.1	20.7	25.45	3.272	0.994	Regression Output:		
B. T.	16.0	7.9	5.00	4.00	56.5	28.2				Constant		-4.599
B. T.	16.0	7.9	5.00	4.00	74.6	36.2				Std Err of Y Est		0.028
B. T.	16.0	7.9	5.00	4.00	48.3	24.8				R Squared		0.994
B. T.	16.0	7.9	5.00	4.00	71.2	32.8				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	3.272	
										Std Err of Coef.	0.144	
A. C.	10.7	7.5	0.00	4.35	28.7	8.8	13.92	1.924	0.997	Regression Output:		
A. C.	10.7	7.5	0.00	4.35	52.0	14.0				Constant		-2.201
A. C.	10.7	7.5	0.00	4.35	62.0	18.3				Std Err of Y Est		0.025
A. C.	10.7	7.5	0.00	4.35	75.6	25.1				R Squared		0.997
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.924	
										Std Err of Coef.	0.075	
A. C.	10.7	7.5	1.25	4.35	28.6	10.6	16.36	2.042	0.996	Regression Output:		
A. C.	10.7	7.5	1.25	4.35	51.6	16.3				Constant		-2.478
A. C.	10.7	7.5	1.25	4.35	61.7	21.1				Std Err of Y Est		0.029
A. C.	10.7	7.5	1.25	4.35	75.3	28.3				R Squared		0.996
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.0	
										Std Err of Coef.	0.082	
A. C.	10.7	7.5	2.50	4.35	28.6	10.8	17.40	1.872	0.995	Regression Output:		
A. C.	10.7	7.5	2.50	4.35	51.7	17.3				Constant		-2.323
A. C.	10.7	7.5	2.50	4.35	61.5	23.2				Std Err of Y Est		0.033
A. C.	10.7	7.5	2.50	4.35	75.1	31.1				R Squared		0.995
										No. of Observations		4.000
										Degrees of Free	ERR	0.000
										X Coefficient(s)	1.872	
										Std Err of Coef.	0.098	
A. C.	10.7	7.5	5.00	4.35	28.4	12.4	19.17	2.052	0.996	Regression Output:		
A. C.	10.7	7.5	5.00	4.35	51.5	19.0				Constant		-2.632
A. C.	10.7	7.5	5.00	4.35	61.4	24.6				Std Err of Y Est		0.030
A. C.	10.7	7.5	5.00	4.35	74.9	32.7				R Squared		0.996
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.052	
										Std Err of Coef.	0.096	
A. C.	10.7	7.1	0.00	4.00	17.8	7.8	17.90	1.836	0.994	Regression Output:		
A. C.	10.7	7.1	0.00	4.00	44.4	18.2				Constant		-2.300
A. C.	10.7	7.1	0.00	4.00	75.6	30.5				Std Err of Y Est		0.058
A. C.	10.7	7.1	0.00	4.00	82.4	43.9				R Squared		0.994
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.836	
										Std Err of Coef.	0.102	
A. C.	10.7	7.1	1.25	4.00	17.6	9.1	25.09	1.480	0.991	Regression Output:		
A. C.	10.7	7.1	1.25	4.00	43.8	21.0				Constant		-2.072
A. C.	10.7	7.1	1.25	4.00	73.8	45.1				Std Err of Y Est		0.068
A. C.	10.7	7.1	1.25	4.00	79.3	68.3				R Squared		0.991
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.480	
										Std Err of Coef.	0.101	
A. C.	10.7	7.1	2.50	4.00	17.3	11.1	28.07	1.603	0.990	Regression Output:		
A. C.	10.7	7.1	2.50	4.00	43.6	22.8				Constant		-2.322
A. C.	10.7	7.1	2.50	4.00	73.4	48.3				Std Err of Y Est		0.070
A. C.	10.7	7.1	2.50	4.00	79.1	70.3				R Squared		0.990
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.603	
										Std Err of Coef.	0.113	
A. C.	10.7	7.1	5.00	4.00	17.1	13.2	30.84	1.704	0.982	Regression Output:		
A. C.	10.7	7.1	5.00	4.00	43.4	24.1				Constant		-2.536
A. C.	10.7	7.1	5.00	4.00	73.1	50.6				Std Err of Y Est		0.095
A. C.	10.7	7.1	5.00	4.00	78.7	73.5				R Squared		0.982
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.704	
										Std Err of Coef.	0.165	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
A. C.	10.7	7.9	0.00	4.15	21.4	5.0	11.47	1.683	0.981	Regression Output:		
A. C.	10.7	7.9	0.00	4.15	37.8	9.3				Constant		-1.783
A. C.	10.7	7.9	0.00	4.15	55.2	13.9				Std Err of Y Est		0.078
A. C.	10.7	7.9	0.00	4.15	77.3	21.9				R Squared		0.981
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.683	
										Std Err of Coef.	0.165	
A. C.	10.7	7.9	1.25	4.15	21.4	4.9	12.29	1.609	0.932	Regression Output:		
A. C.	10.7	7.9	1.25	4.15	37.7	10.8				Constant		-1.753
A. C.	10.7	7.9	1.25	4.15	55.0	15.5				Std Err of Y Est		0.149
A. C.	10.7	7.9	1.25	4.15	77.3	22.0				R Squared		0.932
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.609	
										Std Err of Coef.	0.307	
A. C.	10.7	7.9	2.50	4.15	21.4	5.4	13.37	1.637	0.925	Regression Output:		
A. C.	10.7	7.9	2.50	4.15	37.6	11.8				Constant		-1.844
A. C.	10.7	7.9	2.50	4.15	54.9	16.9				Std Err of Y Est		0.156
A. C.	10.7	7.9	2.50	4.15	77.2	23.3				R Squared		0.925
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.637	
										Std Err of Coef.	0.329	
A. C.	10.7	7.9	5.00	4.15	21.3	6.8	14.80	1.855	0.970	Regression Output:		
A. C.	10.7	7.9	5.00	4.15	37.5	12.3				Constant		-2.171
A. C.	10.7	7.9	5.00	4.15	54.8	17.8				Std Err of Y Est		0.098
A. C.	10.7	7.9	5.00	4.15	77.0	25.7				R Squared		0.970
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.855	
										Std Err of Coef.	0.230	
L. T.	10.7	7.1	0.00	4.20	19.4	9.1	20.01	1.824	0.992	Regression Output:		
L. T.	10.7	7.1	0.00	4.20	41.6	17.7				Constant		-2.374
L. T.	10.7	7.1	0.00	4.20	62.4	24.4				Std Err of Y Est		0.058
L. T.	10.7	7.1	0.00	4.20	82.2	47.3				R Squared		0.992
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.824	
										Std Err of Coef.	0.113	
L. T.	10.7	7.1	1.25	4.20	18.9	13.8	28.65	1.882	0.983	Regression Output:		
L. T.	10.7	7.1	1.25	4.20	40.9	23.9				Constant		-2.742
L. T.	10.7	7.1	1.25	4.20	61.5	32.5				Std Err of Y Est		0.084
L. T.	10.7	7.1	1.25	4.20	80.3	64.4				R Squared		0.983
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.882	
										Std Err of Coef.	0.174	
L. T.	10.7	7.1	2.50	4.20	18.6	15.9	32.86	1.902	0.990	Regression Output:		
L. T.	10.7	7.1	2.50	4.20	40.6	26.2				Constant		-2.885
L. T.	10.7	7.1	2.50	4.20	60.8	38.4				Std Err of Y Est		0.063
L. T.	10.7	7.1	2.50	4.20	79.6	70.9				R Squared		0.990
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.902	
										Std Err of Coef.	0.134	
L. T.	10.7	7.1	5.00	4.20	18.4	17.6	35.88	2.010	0.997	Regression Output:		
L. T.	10.7	7.1	5.00	4.20	40.4	28.2				Constant		-3.126
L. T.	10.7	7.1	5.00	4.20	60.2	44.6				Std Err of Y Est		0.035
L. T.	10.7	7.1	5.00	4.20	79.6	71.1				R Squared		0.997
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.010	
										Std Err of Coef.	0.078	
L. T.	10.7	7.5	0.00	4.00	25.9	9.5	15.23	2.274	0.999	Regression Output:		
L. T.	10.7	7.5	0.00	4.00	41.7	12.9				Constant		-2.689
L. T.	10.7	7.5	0.00	4.00	58.4	18.4				Std Err of Y Est		0.033
L. T.	10.7	7.5	0.00	4.00	94.1	50.8				R Squared		0.999
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.274	
										Std Err of Coef.	0.060	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
L. T.	10.7	7.5	1.25	4.00	25.7	11.1	18.49	2.043	0.999	Regression Output:		
L. T.	10.7	7.5	1.25	4.00	41.4	15.2				Constant		-2.589
L. T.	10.7	7.5	1.25	4.00	58.0	22.1				Std Err of Y Est		0.022
L. T.	10.7	7.5	1.25	4.00	92.7	63.8				R Squared		0.999
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.043	
										Std Err of Coef.	0.038	
L. T.	10.7	7.5	2.50	4.00	25.5	13.2	21.04	2.032	0.995	Regression Output:		
L. T.	10.7	7.5	2.50	4.00	41.2	16.9				Constant		-2.688
L. T.	10.7	7.5	2.50	4.00	57.8	23.7				Std Err of Y Est		0.054
L. T.	10.7	7.5	2.50	4.00	91.9	70.9				R Squared		0.995
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.032	
										Std Err of Coef.	0.097	
L. T.	10.7	7.5	5.00	4.00	25.3	15.0	23.81	1.889	0.985	Regression Output:		
L. T.	10.7	7.5	5.00	4.00	41.0	18.7				Constant		-2.601
L. T.	10.7	7.5	5.00	4.00	57.6	25.4				Std Err of Y Est		0.093
L. T.	10.7	7.5	5.00	4.00	90.7	82.3				R Squared		0.985
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.889	
										Std Err of Coef.	0.164	
L. T.	10.7	7.9	0.00	4.00	29.6	7.6	11.62	1.825	0.980	Regression Output:		
L. T.	10.7	7.9	0.00	4.00	44.4	9.5				Constant		-1.944
L. T.	10.7	7.9	0.00	4.00	64.7	17.1				Std Err of Y Est		0.063
L. T.	10.7	7.9	0.00	4.00	72.5	19.2				R Squared		0.980
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.825	
										Std Err of Coef.	0.185	
L. T.	10.7	7.9	1.25	4.00	29.4	8.8	13.40	1.917	0.987	Regression Output:		
L. T.	10.7	7.9	1.25	4.00	44.2	11.2				Constant		-2.160
L. T.	10.7	7.9	1.25	4.00	64.5	19.1				Std Err of Y Est		0.049
L. T.	10.7	7.9	1.25	4.00	72.3	21.6				R Squared		0.987
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.917	
										Std Err of Coef.	0.152	
L. T.	10.7	7.9	2.50	4.00	29.3	9.7	14.72	1.946	0.983	Regression Output:		
L. T.	10.7	7.9	2.50	4.00	44.0	12.3				Constant		-2.272
L. T.	10.7	7.9	2.50	4.00	64.2	21.1				Std Err of Y Est		0.058
L. T.	10.7	7.9	2.50	4.00	72.1	23.1				R Squared		0.983
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.946	
										Std Err of Coef.	0.183	
L. T.	10.7	7.9	5.00	4.00	29.2	10.7	16.08	1.987	0.984	Regression Output:		
L. T.	10.7	7.9	5.00	4.00	43.9	13.4				Constant		-2.397
L. T.	10.7	7.9	5.00	4.00	64.1	22.6				Std Err of Y Est		0.055
L. T.	10.7	7.9	5.00	4.00	71.9	25.1				R Squared		0.984
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.987	
										Std Err of Coef.	0.178	
B. T.	10.7	7.1	0.00	4.10	29.1	13.2	18.92	2.517	0.987	Regression Output:		
B. T.	10.7	7.1	0.00	4.10	64.5	24.5				Constant		-3.214
B. T.	10.7	7.1	0.00	4.10	78.6	30.8				Std Err of Y Est		0.070
B. T.	10.7	7.1	0.00	4.10	85.7	41.8				R Squared		0.987
B. T.	10.7	7.1	0.00	4.10	91.0	44.4				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.517	
										Std Err of Coef.	0.164	
B. T.	10.7	7.1	1.25	4.10	28.9	15.5	24.44	1.934	0.992	Regression Output:		
B. T.	10.7	7.1	1.25	4.10	63.7	33.3				Constant		-2.684
B. T.	10.7	7.1	1.25	4.10	77.5	42.9				Std Err of Y Est		0.055
B. T.	10.7	7.1	1.25	4.10	84.0	60.4				R Squared		0.992
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.934	
										Std Err of Coef.	0.126	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
B. T.	10.7	7.1	2.50	4.10	28.6	17.9	27.68	2.007	0.993	Regression Output:		
B. T.	10.7	7.1	2.50	4.10	63.5	36.1				Constant		-2.895
B. T.	10.7	7.1	2.50	4.10	77.0	47.7				Std Err of Y Est		0.049
B. T.	10.7	7.1	2.50	4.10	83.6	65.4				R Squared		0.993
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.007	
										Std Err of Coef.	0.119	
B. T.	10.7	7.1	5.00	4.10	28.5	19.4	30.16	2.006	0.996	Regression Output:		
B. T.	10.7	7.1	5.00	4.10	63.2	38.7				Constant		-2.968
B. T.	10.7	7.1	5.00	4.10	76.6	52.6				Std Err of Y Est		0.036
B. T.	10.7	7.1	5.00	4.10	83.2	69.5				R Squared		0.996
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.006	
										Std Err of Coef.	0.087	
B. T.	11.1	7.5	0.00	4.05	24.9	7.9	12.98	2.220	1.000	Regression Output:		
B. T.	11.1	7.5	0.00	4.05	47.6	12.3				Constant		-2.472
B. T.	11.1	7.5	0.00	4.05	53.0	13.9				Std Err of Y Est		0.012
B. T.	11.1	7.5	0.00	4.05	78.1	22.8				R Squared		1.000
B. T.	11.1	7.5	0.00	4.05	84.6	28.1				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.220	
										Std Err of Coef.	0.028	
B. T.	11.1	7.5	1.25	4.05	24.8	9.0	15.51	2.138	0.998	Regression Output:		
B. T.	11.1	7.5	1.25	4.05	47.3	15.0				Constant		-2.545
B. T.	11.1	7.5	1.25	4.05	52.7	16.8				Std Err of Y Est		0.025
B. T.	11.1	7.5	1.25	4.05	77.6	27.6				R Squared		0.998
B. T.	11.1	7.5	1.25	4.05	84.0	33.2				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.138	
										Std Err of Coef.	0.055	
B. T.	11.1	7.5	2.50	4.05	24.7	10.3	16.98	2.249	0.998	Regression Output:		
B. T.	11.1	7.5	2.50	4.05	47.3	15.8				Constant		-2.768
B. T.	11.1	7.5	2.50	4.05	52.5	18.4				Std Err of Y Est		0.024
B. T.	11.1	7.5	2.50	4.05	77.5	29.3				R Squared		0.998
B. T.	11.1	7.5	2.50	4.05	83.9	35.1				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.249	
										Std Err of Coef.	0.057	
B. T.	11.1	7.5	5.00	4.05	24.6	11.4	18.69	2.323	0.991	Regression Output:		
B. T.	11.1	7.5	5.00	4.05	47.1	17.4				Constant		-2.954
B. T.	11.1	7.5	5.00	4.05	52.3	20.3				Std Err of Y Est		0.054
B. T.	11.1	7.5	5.00	4.05	77.1	33.1				R Squared		0.991
B. T.	11.1	7.5	5.00	4.05	83.8	35.7				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.323	
										Std Err of Coef.	0.131	
B. T.	10.7	7.7	0.00	4.25	29.1	9.5	15.38	1.836	0.999	Regression Output:		
B. T.	10.7	7.7	0.00	4.25	41.3	12.9				Constant		-2.180
B. T.	10.7	7.7	0.00	4.25	50.8	15.3				Std Err of Y Est		0.015
B. T.	10.7	7.7	0.00	4.25	73.9	27.3				R Squared		0.999
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.836	
										Std Err of Coef.	0.046	
B. T.	10.7	7.7	1.25	4.25	29.1	10.4	17.56	1.891	0.980	Regression Output:		
B. T.	10.7	7.7	1.25	4.25	41.1	15.1				Constant		-2.353
B. T.	10.7	7.7	1.25	4.25	50.4	18.9				Std Err of Y Est		0.061
B. T.	10.7	7.7	1.25	4.25	73.8	28.8				R Squared		0.980
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.891	
										Std Err of Coef.	0.191	
B. T.	10.7	7.7	2.50	4.25	28.9	11.6	19.37	1.972	0.973	Regression Output:		
B. T.	10.7	7.7	2.50	4.25	40.9	16.9				Constant		-2.538
B. T.	10.7	7.7	2.50	4.25	50.2	20.8				Std Err of Y Est		0.071
B. T.	10.7	7.7	2.50	4.25	73.6	30.7				R Squared		0.973
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.972	
										Std Err of Coef.	0.231	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
B. T.	10.7	7.7	5.00	4.25	28.2	19.2	26.60	2.638	0.632	Regression Output:		
B. T.	10.7	7.7	5.00	4.25	40.3	22.9				Constant		-3.759
B. T.	10.7	7.7	5.00	4.25	49.0	32.6				Std Err of Y Est		0.267
B. T.	10.7	7.7	5.00	4.25	73.6	30.7				R Squared		0.632
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.638	
										Std Err of Coef.	1.423	
B. T.	10.8	7.9	0.00	4.10	29.0	9.3	12.41	2.578	0.978	Regression Output:		
B. T.	10.8	7.9	0.00	4.10	48.2	11.2				Constant		-2.821
B. T.	10.8	7.9	0.00	4.10	80.6	21.9				Std Err of Y Est		0.108
										R Squared		0.978
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	2.578	
										Std Err of Coef.	0.389	
B. T.	10.8	7.9	1.25	4.10	28.9	10.0	14.04	2.459	0.995	Regression Output:		
B. T.	10.8	7.9	1.25	4.10	48.0	13.1				Constant		-2.822
B. T.	10.8	7.9	1.25	4.10	80.2	25.1				Std Err of Y Est		0.049
										R Squared		0.995
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	2.459	
										Std Err of Coef.	0.170	
B. T.	10.8	7.9	2.50	4.10	28.9	10.5	15.03	2.443	0.999	Regression Output:		
B. T.	10.8	7.9	2.50	4.10	47.9	14.3				Constant		-2.876
B. T.	10.8	7.9	2.50	4.10	80.1	26.7				Std Err of Y Est		0.020
										R Squared		0.999
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	2.443	
										Std Err of Coef.	0.068	
B. T.	10.8	7.9	5.00	4.10	28.7	12.3	16.60	2.592	0.982	Regression Output:		
B. T.	10.8	7.9	5.00	4.10	47.8	15.0				Constant		-3.163
B. T.	10.8	7.9	5.00	4.10	79.9	28.7				Std Err of Y Est		0.097
										R Squared		0.982
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	2.592	
										Std Err of Coef.	0.356	
L. T.	5.0	7.1	0.00	4.00	29.3	9.6	17.45	1.456	0.992	Regression Output:		
L. T.	5.0	7.1	0.00	4.00	48.9	16.5				Constant		-1.807
L. T.	5.0	7.1	0.00	4.00	74.2	34.2				Std Err of Y Est		0.042
L. T.	5.0	7.1	0.00	4.00	66.6	30.1				R Squared		0.992
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.456	
										Std Err of Coef.	0.095	
L. T.	5.0	7.1	1.25	4.00	28.9	13.8	23.50	1.754	0.996	Regression Output:		
L. T.	5.0	7.1	1.25	4.00	48.3	23.3				Constant		-2.404
L. T.	5.0	7.1	1.25	4.00	73.6	40.8				Std Err of Y Est		0.029
L. T.	5.0	7.1	1.25	4.00	66.1	35.1				R Squared		0.996
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.754	
										Std Err of Coef.	0.079	
L. T.	5.0	7.1	2.50	4.00	28.7	15.7	25.98	1.806	1.000	Regression Output:		
L. T.	5.0	7.1	2.50	4.00	48.1	24.9				Constant		-2.555
L. T.	5.0	7.1	2.50	4.00	73.2	45.1				Std Err of Y Est		0.004
L. T.	5.0	7.1	2.50	4.00	65.9	37.6				R Squared		1.000
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.806	
										Std Err of Coef.	0.011	
L. T.	5.0	7.1	5.00	4.00	28.7	16.3	27.91	1.780	0.992	Regression Output:		
L. T.	5.0	7.1	5.00	4.00	47.8	28.3				Constant		-2.574
L. T.	5.0	7.1	5.00	4.00	72.9	48.2				Std Err of Y Est		0.041
L. T.	5.0	7.1	5.00	4.00	65.7	39.1				R Squared		0.992
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.780	
										Std Err of Coef.	0.115	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
L. T.	5.0	7.5	0.00	4.10	35.0	10.8	14.02	2.094	0.993	Regression Output:		
L. T.	5.0	7.5	0.00	4.10	58.7	16.0				Constant		-2.401
L. T.	5.0	7.5	0.00	4.10	82.5	30.2				Std Err of Y Est		0.040
L. T.	5.0	7.5	0.00	4.10	65.6	18.6				R Squared		0.993
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.094	
										Std Err of Coef.	0.126	
L. T.	5.0	7.5	1.25	4.10	35.0	11.3	16.01	1.818	0.998	Regression Output:		
L. T.	5.0	7.5	1.25	4.10	58.4	19.9				Constant		-2.189
L. T.	5.0	7.5	1.25	4.10	81.9	36.7				Std Err of Y Est		0.022
L. T.	5.0	7.5	1.25	4.10	65.3	22.2				R Squared		0.998
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.818	
										Std Err of Coef.	0.059	
L. T.	5.0	7.5	2.50	4.10	34.8	13.4	18.57	1.964	0.996	Regression Output:		
L. T.	5.0	7.5	2.50	4.10	58.1	22.8				Constant		-2.492
L. T.	5.0	7.5	2.50	4.10	81.7	39.7				Std Err of Y Est		0.029
L. T.	5.0	7.5	2.50	4.10	65.1	24.8				R Squared		0.996
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.964	
										Std Err of Coef.	0.086	
L. T.	5.0	7.5	5.00	4.10	34.7	15.2	20.22	2.106	0.998	Regression Output:		
L. T.	5.0	7.5	5.00	4.10	58.1	23.1				Constant		-2.750
L. T.	5.0	7.5	5.00	4.10	81.6	41.5				Std Err of Y Est		0.020
L. T.	5.0	7.5	5.00	4.10	64.9	26.9				R Squared		0.998
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	2.106	
										Std Err of Coef.	0.065	
A. C.	5.0	7.9	0.00	4.00	36.8	2.8	4.62	1.101	1.000	Regression Output:		
A. C.	5.0	7.9	0.00	4.00	61.3	7.1				Constant		-0.733
A. C.	5.0	7.9	0.00	4.00	78.6	14.9				Std Err of Y Est		0.007
A. C.	5.0	7.9	0.00	4.00	50.2	4.7				R Squared		1.000
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.101	
										Std Err of Coef.	0.014	
A. C.	5.0	7.9	1.25	4.00	36.8	2.9	5.17	1.039	0.979	Regression Output:		
A. C.	5.0	7.9	1.25	4.00	61.1	9.2				Constant		-0.742
A. C.	5.0	7.9	1.25	4.00	78.5	16.2				Std Err of Y Est		0.059
A. C.	5.0	7.9	1.25	4.00	50.1	5.3				R Squared		0.979
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.039	
										Std Err of Coef.	0.107	
A. C.	5.0	7.9	2.50	4.00	36.8	3.4	6.05	1.084	0.956	Regression Output:		
A. C.	5.0	7.9	2.50	4.00	60.9	11.0				Constant		-0.847
A. C.	5.0	7.9	2.50	4.00	78.4	17.0				Std Err of Y Est		0.086
A. C.	5.0	7.9	2.50	4.00	50.0	6.3				R Squared		0.956
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.084	
										Std Err of Coef.	0.165	
A. C.	5.0	7.9	5.00	4.00	36.6	4.9	7.68	1.224	0.988	Regression Output:		
A. C.	5.0	7.9	5.00	4.00	60.8	12.1				Constant		-1.084
A. C.	5.0	7.9	5.00	4.00	78.0	20.6				Std Err of Y Est		0.045
A. C.	5.0	7.9	5.00	4.00	49.9	7.3				R Squared		0.988
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.224	
										Std Err of Coef.	0.095	
A. C.	5.0	7.5	0.00	4.00	31.6	6.5	10.16	1.658	0.994	Regression Output:		
A. C.	5.0	7.5	0.00	4.00	48.7	9.5				Constant		-1.669
A. C.	5.0	7.5	0.00	4.00	60.2	13.0				Std Err of Y Est		0.024
A. C.	5.0	7.5	0.00	4.00	62.1	14.0				R Squared		0.994
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.658	
										Std Err of Coef.	0.091	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
A. C.	5.0	7.5	1.25	4.00	31.4	8.6	13.96	1.580	0.996	Regression Output:		
A. C.	5.0	7.5	1.25	4.00	48.4	13.1				Constant		-1.809
A. C.	5.0	7.5	1.25	4.00	59.7	18.4				Std Err of Y Est		0.019
A. C.	5.0	7.5	1.25	4.00	61.7	18.6				R Squared		0.996
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.580	
										Std Err of Coef.	0.070	
A. C.	5.0	7.5	2.50	4.00	31.3	10.1	15.54	1.692	0.983	Regression Output:		
A. C.	5.0	7.5	2.50	4.00	48.3	14.0				Constant		-2.016
A. C.	5.0	7.5	2.50	4.00	58.6	19.6				Std Err of Y Est		0.040
A. C.	5.0	7.5	2.50	4.00	61.4	21.0				R Squared		0.983
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.692	
										Std Err of Coef.	0.159	
A. C.	5.0	7.5	5.00	4.00	31.2	11.1	17.55	1.753	0.996	Regression Output:		
A. C.	5.0	7.5	5.00	4.00	48.0	17.0				Constant		-2.182
A. C.	5.0	7.5	5.00	4.00	59.4	22.0				Std Err of Y Est		0.014
A. C.	5.0	7.5	5.00	4.00	61.3	22.4				R Squared		0.996
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.753	
										Std Err of Coef.	0.056	
A. C.	5.0	7.1	0.00	4.10	19.9	7.3	15.70	1.777	0.994	Regression Output:		
A. C.	5.0	7.1	0.00	4.10	41.8	12.8				Constant		-2.125
A. C.	5.0	7.1	0.00	4.10	56.0	17.2				Std Err of Y Est		0.034
A. C.	5.0	7.1	0.00	4.10	61.9	21.6				R Squared		0.994
A. C.	5.0	7.1	0.00	4.10	67.4	23.4				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.777	
										Std Err of Coef.	0.083	
A. C.	5.0	7.1	1.25	4.10	19.7	9.0	20.67	1.681	0.999	Regression Output:		
A. C.	5.0	7.1	1.25	4.10	41.5	16.9				Constant		-2.212
A. C.	5.0	7.1	1.25	4.10	55.4	22.9				Std Err of Y Est		0.014
A. C.	5.0	7.1	1.25	4.10	61.3	27.7				R Squared		0.999
A. C.	5.0	7.1	1.25	4.10	66.7	31.3				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.681	
										Std Err of Coef.	0.033	
A. C.	5.0	7.1	2.50	4.10	19.6	10.7	23.09	1.767	0.994	Regression Output:		
A. C.	5.0	7.1	2.50	4.10	41.3	18.2				Constant		-2.409
A. C.	5.0	7.1	2.50	4.10	55.2	25.0				Std Err of Y Est		0.032
A. C.	5.0	7.1	2.50	4.10	61.1	30.4				R Squared		0.994
A. C.	5.0	7.1	2.50	4.10	66.3	34.9				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.767	
										Std Err of Coef.	0.079	
A. C.	5.0	7.1	5.00	4.10	19.4	12.1	24.79	1.864	0.967	Regression Output:		
A. C.	5.0	7.1	5.00	4.10	41.2	19.5				Constant		-2.599
A. C.	5.0	7.1	5.00	4.10	55.1	26.2				Std Err of Y Est		0.048
A. C.	5.0	7.1	5.00	4.10	61.0	31.6				R Squared		0.967
A. C.	5.0	7.1	5.00	4.10	66.1	37.5				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	1.864	
										Std Err of Coef.	0.125	
B. T.	5.0	7.1	0.00	4.10	29.9	10.4	17.72	1.676	0.997	Regression Output:		
B. T.	5.0	7.1	0.00	4.10	39.0	14.0				Constant		-2.092
B. T.	5.0	7.1	0.00	4.10	56.9	21.1				Std Err of Y Est		0.022
B. T.	5.0	7.1	0.00	4.10	72.9	31.5				R Squared		0.997
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.676	
										Std Err of Coef.	0.061	
B. T.	5.0	7.1	1.25	4.10	29.7	13.1	24.05	1.517	0.989	Regression Output:		
B. T.	5.0	7.1	1.25	4.10	38.5	19.1				Constant		-2.095
B. T.	5.0	7.1	1.25	4.10	56.3	27.4				Std Err of Y Est		0.044
B. T.	5.0	7.1	1.25	4.10	71.6	44.2				R Squared		0.989
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.517	
										Std Err of Coef.	0.112	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
B. T.	5.0	7.1	2.50	4.10	29.4	16.1	26.56	1.756	0.982	Regression Output:		
B. T.	5.0	7.1	2.50	4.10	38.3	21.4				Constant		-2.501
B. T.	5.0	7.1	2.50	4.10	56.2	28.2				Std Err of Y Est		0.056
B. T.	5.0	7.1	2.50	4.10	71.4	46.1				R Squared		0.982
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.756	
										Std Err of Coef.	0.166	
B. T.	5.0	7.1	5.00	4.10	29.1	19.4	29.01	1.995	0.987	Regression Output:		
B. T.	5.0	7.1	5.00	4.10	38.2	22.3				Constant		-2.917
B. T.	5.0	7.1	5.00	4.10	56.0	31.0				Std Err of Y Est		0.049
B. T.	5.0	7.1	5.00	4.10	71.3	47.3				R Squared		0.987
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.995	
										Std Err of Coef.	0.165	
B. T.	5.0	7.5	0.00	4.20	29.5	9.5	13.98	2.448	0.977	Regression Output:		
B. T.	5.0	7.5	0.00	4.20	44.1	12.1				Constant		-2.804
B. T.	5.0	7.5	0.00	4.20	57.0	16.2				Std Err of Y Est		0.101
B. T.	5.0	7.5	0.00	4.20	69.2	22.1				R Squared		0.977
B. T.	5.0	7.5	0.00	4.20	93.2	37.4				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.448	
										Std Err of Coef.	0.218	
B. T.	5.0	7.5	1.25	4.20	29.2	12.3	18.18	2.366	0.995	Regression Output:		
B. T.	5.0	7.5	1.25	4.20	43.7	16.1				Constant		-2.981
B. T.	5.0	7.5	1.25	4.20	56.6	20.4				Std Err of Y Est		0.044
B. T.	5.0	7.5	1.25	4.20	68.7	27.0				R Squared		0.995
B. T.	5.0	7.5	1.25	4.20	92.0	49.5				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.366	
										Std Err of Coef.	0.095	
B. T.	5.0	7.5	2.50	4.20	29.2	12.9	19.06	2.343	0.981	Regression Output:		
B. T.	5.0	7.5	2.50	4.20	43.7	16.5				Constant		-2.999
B. T.	5.0	7.5	2.50	4.20	56.6	21.1				Std Err of Y Est		0.087
B. T.	5.0	7.5	2.50	4.20	68.4	30.1				R Squared		0.981
B. T.	5.0	7.5	2.50	4.20	92.0	50.4				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.343	
										Std Err of Coef.	0.189	
B. T.	5.0	7.5	5.00	4.20	29.0	14.7	20.65	2.493	0.996	Regression Output:		
B. T.	5.0	7.5	5.00	4.20	43.5	18.5				Constant		-3.278
B. T.	5.0	7.5	5.00	4.20	56.5	21.9				Std Err of Y Est		0.038
B. T.	5.0	7.5	5.00	4.20	68.5	29.2				R Squared		0.996
B. T.	5.0	7.5	5.00	4.20	91.7	53.8				No. of Observations		5.000
										Degrees of Freedom		3.000
										X Coefficient(s)	2.493	
										Std Err of Coef.	0.088	
B. T.	5.0	7.9	0.00	4.10	70.8	14.9	7.39	1.259	0.999	Regression Output:		
B. T.	5.0	7.9	0.00	4.10	57.9	9.6				Constant		-1.094
B. T.	5.0	7.9	0.00	4.10	51.5	7.7				Std Err of Y Est		0.006
										R Squared		0.999
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	1.259	
										Std Err of Coef.	0.031	
B. T.	5.0	7.9	1.25	4.10	70.6	16.6	7.63	1.098	0.985	Regression Output:		
B. T.	5.0	7.9	1.25	4.10	57.8	10.7				Constant		-0.969
B. T.	5.0	7.9	1.25	4.10	51.5	7.8				Std Err of Y Est		0.031
										R Squared		0.985
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	1.098	
										Std Err of Coef.	0.135	

FISH	TEMP (C)	pH	ATP:Hb (mol/mol)	[Hb] (g/dL)	% Sat.	Po2 (mm Hg)	P50 (mm Hg)	n	R*R	Regression Analysis: log(Y/(100-Y)) = a + blogPo2		
B. T.	5.0	7.9	2.50	4.10	70.5	18.4	8.10	1.048	0.996	Regression Output:		
B. T.	5.0	7.9	2.50	4.10	57.7	11.2				Constant		-0.952
B. T.	5.0	7.9	2.50	4.10	51.4	8.4				Std Err of Y Est		0.015
										R Squared		0.996
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	1.048	
										Std Err of Coef.	0.063	
B. T.	5.0	7.9	5.00	4.10	70.4	19.0	10.35	1.397	0.990	Regression Output:		
B. T.	5.0	7.9	5.00	4.10	57.5	13.3				Constant		-1.418
B. T.	5.0	7.9	5.00	4.10	51.2	10.5				Std Err of Y Est		0.025
										R Squared		0.990
										No. of Observations		3.000
										Degrees of Freedom		1.000
										X Coefficient(s)	1.397	
										Std Err of Coef.	0.138	
L.T.	5.0	7.9	0.00	4.15	26.9	4.1	7.11	1.988	0.970	Regression Output:		
L.T.	5.0	7.9	0.00	4.15	66.7	10.3				Constant		-1.693
L.T.	5.0	7.9	0.00	4.00	64.2	8.9				Std Err of Y Est		0.078
L.T.	5.0	7.9	0.00	4.00	36.4	5.9				R Squared		0.970
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.988	
										Std Err of Coef.	0.248	
L.T.	5.0	7.9	1.25	4.15	26.8	4.3	8.38	1.586	0.993	Regression Output:		
L.T.	5.0	7.9	1.25	4.15	66.6	12.5				Constant		-1.464
L.T.	5.0	7.9	1.25	4.00	63.8	12.2				Std Err of Y Est		0.036
L.T.	5.0	7.9	1.25	4.00	36.3	6.2				R Squared		0.993
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.586	
										Std Err of Coef.	0.091	
L.T.	5.0	7.9	2.50	4.15	26.8	5.0	9.85	1.584	0.991	Regression Output:		
L.T.	5.0	7.9	2.50	4.15	66.4	15.1				Constant		-1.574
L.T.	5.0	7.9	2.50	4.00	63.7	13.7				Std Err of Y Est		0.042
L.T.	5.0	7.9	2.50	4.00	36.2	7.4				R Squared		0.991
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.584	
										Std Err of Coef.	0.108	
L.T.	5.0	7.9	5.00	4.15	26.7	5.6	11.57	1.575	0.953	Regression Output:		
L.T.	5.0	7.9	5.00	4.15	66.2	16.7				Constant		-1.675
L.T.	5.0	7.9	5.00	4.00	63.5	16.1				Std Err of Y Est		0.096
L.T.	5.0	7.9	5.00	4.00	36.0	9.5				R Squared		0.953
										No. of Observations		4.000
										Degrees of Freedom		2.000
										X Coefficient(s)	1.575	
										Std Err of Coef.	0.248	

Appendix H

A summary of the oxygenation characteristics of the blood of various salmonids

Fish	Temp. (°C)	pH	P_{CO_2} (mmHg)	[ATP] (mol/mol)	WB/S*	P_{50} (mmHg)	$\Delta \log P_{50}/\Delta pH$	ΔH (Kcal/mol)	Reference
Arctic charr	5	7.5		2.5	S	15.5			Present Study
	11	7.5		2.5	S	17.4			
	15	7.5		2.5	S	40.2			
	5	7.1-7.9			S		-0.67		
	10	7.1-7.9			S		-0.24		
	16	7.1-7.9			S		-0.33		
	5-16	7.1			S			-11.6	
	5-16	7.5			S			-15.3	
	5-16	7.9			S			-20.6	
	10		2.5		WB	19.4			Giles, 1991
	10		0.25		WB	12.4			
Brook trout	5	7.5		2.5	S	19.1			Present Study
	11	7.5		2.5	S	17.0			
	16	7.5		2.5	S	27.2			
	5	7.1-7.9			S		-0.47		
	10	7.1-7.9			S		-0.23		
	16	7.1-7.9			S		-0.29		
	5-16	7.1			S			-8.5	
	5-16	7.5			S			-5.8	
	5-16	7.9			S			-13.4	
	0		0-1		WB	7.0			Black, 1966
	5		0-1		WB	5.0			
	15		0-1		WB	12.0			
	20		0-1		WB	17.0			
	3		10		WB	7.0			Power, 1980
	15		10		WB	18.0			
Lake trout	5	7.5		2.5	S	18.6			Present Study
	11	7.5		2.5	S	21.0			
	15	7.5		2.5	S	37.3			
	5	7.1-7.9			S		-0.49		
	10	7.1-7.9			S		-0.30		
	16	7.1-7.9			S		-0.39		
	5-16	7.1			S			-11.8	
	5-16	7.5			S			-10.2	
	5-16	7.9			S			-14.3	

Appendix H. (continued)

Fish	Temp. (°C)	pH	Pco_2 (mmHg)	[ATP] (mol/mol)	WB/S*	P_{50} (mmHg)	$\Delta logP_{50}/\Delta pH$	ΔH (Kcal/mol)	Reference
Coho salmo	9.8	7.4			S	17.9			Giles & Randall, 1980
	9.8		3.4		WB	15.6			
	5		3.4		WB	9.8			
	10		3.4		WB	12.5			
	14.8		3.4		WB	14.8			
	10	7-8.2			S		-0.17		
	10	7-8.2			S		-0.57		
	5-10	7.9			S			-20.7	
	10-15				S			-20.8	
Rainbow trout	10	6.5-8					-0.64		Lucki,1972
	10	6.5-8					-0.60		Vorger,1985
	10	6.5-8					-0.57		Eddy,1971
	15	6.5-8					-0.52		Weber,1976
		7.8			WB			-4.9	Vorger, 1986
		8.4			WB			-8.9	
		7.8			S			-10.5	
		8.4			S			-14.2	
Sockeye salmo		7-7.7			S		-0.93		Sauer & Harrington, 1988
		7.4			S			-4.4	
King salmo		7-8			HbA**			-9.6	Harrington, 1986
		7-8			HbC			-6.8	
Salmo irideus		<8.9			Hb			-14.5	Brunori et al., 1973

* WB = whole blood; S = hemoglobin solution;

** HbA = anodic hemoglobin; HbC = cathodic hemoglobin;