AN ABSTRACT OF THE THESIS OF

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presented on June 29, 1998

Title: Effects of stress on blood viscosity and other blood parameters in striped bass, *Morone saxatilis* (Walbaum)

Abstract approved: David K. Saunders

The culture of striped bass, *Morone saxatilis,* for commercial and recreational purposes is a popular form of aquaculture in the United States. However, midsummer mortalities, which could be due to high temperatures and oxygen depletion, have been reported. Cultured fish are exposed to stressful factors such as temperature, transport, and hypoxia. The objective of this study was to investigate the effects of stress on blood viscosity and other blood parameters in striped bass. Thirty-three adult striped bass, weighing between 1040-1800 g, were divided into five groups: control, hypoxia, simulated transport, diseased (henneguya), and temperature. Red blood cell count, white blood cell count, hematocrit, hemoglobin concentration, mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), mean cell hemoglobin (MCH), and plasma protein concentration were determined. Viscosity measurements were determined at 10 different shear rates. At packed cell volumes of 30% and 40%, the simulated transport group had a significant decrease in apparent viscosity as compared to controls at higher shear rates (37.5 s^{-1}, 75 s^{-1}, 150 s^{-1}), whereas the hypoxic group showed a significant increase in apparent viscosity as compared to controls at higher shear rates (37.5 s^{-1}, 75 s^{-1}, 150 s^{-1}). The diseased group showed a significant increase in relative viscosity as compared to controls at higher shear rates. The temperature group at 24°C

and 27°C showed a significant increase in apparent viscosity when compared to the controls at higher shear rates. The stress of transporting striped bass in well-aerated water had little impact on apparent blood viscosity. However, hypoxia could be a major cause of midsummer mortalities in striped bass because increased apparent viscosity could lead to decreased blood flow in the systemic circulation, thereby decreasing oxygen delivery to tissues. This research was conducted with financial assistance from the Emporia State University Research and Creativity Committee.

EFFECTS OF STRESS ON BLOOD VISCOSITY AND OTHER BLOOD PARAMETERS IN STRIPED BASS, *MORONE SAXATILIS* (WALBAUM)

A Thesis

Submitted to

The Division of Biological Sciences

EMPORIA STATE UNIVERSITY

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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July, 1998

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ACKNOWLEDGEMENTS

I would like to take this opportunity to thank the faculty and graduate students of the Division of Biological Sciences for their overwhelming support throughout my study at Emporia State University. Also I would like to extend words of thanks to Milford Fish Hatchery staff under the management of Tommie Crawford, for providing me with fish specimens for my project. I thank Roger Ferguson, Jessica Filosa, Jeff Witters, and Kham Noam for their help in this project.

A special word of thanks goes to Dr. Saunders, my major advisor, for his patience, support, and advice throughout my study. Also I would like to thank my graduate committee members, Dr. Edds, Dr. Robbins, and Dr. Sievert for their criticism and encouragement. I would like to thank Dr. Smalley for showing me how to run a radioimmunoassay for cortisol determinations. I would further like to thank the African-American Institute for their financial support and giving me the opportunity to study in the United States. Lastly, I would like to thank the Emporia State University Research and Creativity Committee for their financial assistance for this project.

I would like to thank my late parents, Enoch and Alita Lebelo, for their guidance and teaching me the value of an education. Furthermore, I would like to dedicate this degree to my family for the encouragement and support they gave me throughout my study.

PREFACE

This thesis was written in the style required by the *Journal of Fish Biology*.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The striped bass, *Morone saxatilis*, is native to estuaries and coastal zones of the Atlantic ocean and Gulf of Mexico, and has been introduced to the Pacific Coast and into the freshwater habitats of inland waters. Striped bass are voracious predators feeding on a wide variety of fishes and invertebrates such as lobsters, shrimps, and crabs. Its spawning time varies from April to July, depending upon temperature, water quality, food availability, and latitude. Stocked populations have increased dramatically in many freshwater reservoirs in the southern and central United States when this anadromous species was recognized as capable of existing in freshwater. In the 1940s, propagation, stocking, and management of striped bass in inland waters followed the discovery of a reproducing land-locked population in Santee-Cooper Reservoir in South Carolina (Scruggs, 1957).

The culture of striped bass for commercial and recreational purposes is currently a popular form of aquaculture in the USA, and the trend appears to be one that will continue for a long time. This species is favored mainly because of its large size, ease of raising in hatcheries (especially in spring and winter), and its firm, well-flavored flesh. The largest striped bass caught to date was in April 1989 and weighed 22.0 kg with a total length of 110.5 cm. Furthermore, the striped bass can breed successfully with white bass (*Morone chrysops*) to produce a hybrid called a wiper (*Morone saxatilis* X *Morone chrysops).* The first hybrid was reared in 1965 and currently its culture in the United States is gaining overwhelming support. The hybrid is resistant to different forms of stress, such as high temperatures and low dissolved oxygen levels, and thus accounts for

the genetic successes of the species and aquacultural practices in fish industry (Sublette *et al.*, 1990; Harrel, 1992).

Generally, cultured fish are reared under conditions that are rarely optimal relative to their natural habitats, particularly in intensive culture systems where water quality can be poor, with drastic temperature changes, oxygen depletion, overcrowding, insufficient food supply, and insufficient light conditions. In addition, common aquacultural procedures such as handling, stocking, disease treatment, confinement, transport, and overexercise usually cause a variable degree of trauma to the fish. These procedures often result in mortalities that can occur immediately from severe treatment, or later, resulting from osmoregulatory imbalances or infectious diseases, thus affecting the success of stocking programs. Different methods, such as pre-transport starvation, chilled shipping water, anesthetic treatments, and salt additives, have been applied to reduce the adverse effects of fish transportation; however marked species differences exist in the efficacy of these treatments (Robertson *et al.*, 1988).

Considerable attention has been given to the possible detrimental effects of stress on fish and fish populations resulting from various aquacultural practices. Despite the extensive research, a good definition of biological stress remains elusive. However, a common notion implicit in most definitions of stress is that stress represents a response by fish to a stimulus and this response somehow alters the homeostatic state of the fish (Barton & Iwama, 1991). As such, stress has been defined as any type of stimulus acting on a biological system and the response thereof (Robertson *et al.*, 1987). When a fish is exposed to any type of stressor, physical or chemical, stress responses are elicited that are generally categorized into primary, secondary, and tertiary effects, depending on the

severity of the stimulus. These reactions have evolved as adaptive mechanisms that enable the animal to reestablish the homeostatic environment after exposure to adverse stimuli (Wedemeyer *et al.*, 1990).

Primary responses result from stimulation of the hypothalamus-pituitary interrenal axis with its corresponding chain of hormone production: corticotropin releasing hormone, then adrenocorticotropic releasing hormone, and subsequent oversecretion of corticosteroids from the interrenal cells. As in other veterbrates, cortisol is the primary adaptive stress hormone secreted in fishes to combat stressful effects. Love (1980) reported that increased cortisol levels in plasma led to a decrease in ATP or GTP in most teleost fishes. The decrease in these energy-rich compounds in red blood cells increased the oxygen-binding affinity of hemoglobin. In addition, catecholamines are secreted by the chromaffin cells, which are located in the posterior cardinal veins as a part of the primary response. Secondary responses are usually defined as the immediate actions and effects of both catecholamines and cortisol at the blood and tissue levels, including increases in cardiac output, oxygen uptake, mobilization of energy substrates, and disturbance of osmoregulatory mechanisms. Tertiary responses are signified by inhibition of growth, reproduction, and immune response and reduced capacity to tolerate subsequent or additional stressors (Robertson *et al.*, 1987; Bollard *et al.*, 1993; Wendelaar Bonga, 1997).

Despite the general and often remarkable success of stocking and managing striped bass in freshwater reservoirs, there have been some puzzling inconsistencies. For example, warm surface layers, coupled with decreased dissolved oxygen, together with a scarcity of prey species in the preferred temperature-oxygen range can produce stress to

this fish species, and coupled with overfishing can lead to population decline. At several freshwater locations, midsummer mortalities have been reported among the large adults. According to Matthews (1985) in his survey of 80 freshwater reservoirs in the United States, most of the mortalities occurred in fish with a mass greater than 5 kg. During summer stratification, the upper waters in the epilimnion become too hot for adult striped bass, whereas deep, cold waters lack adequate dissolved oxygen. The population decline could be resulting from thermal stress and low dissolved oxygen, decreased fecundity of adults (from overcrowding at a thin layer of the stratified water column consisting of cool waters), and increased susceptibility to disease. Coutant (1985) reported that this species has an optimal temperature range of 18-25°C and requires dissolved oxygen (DO) concentrations above 2-3 ppm. However, Moyle (1976) indicated that the species could survive temperatures as high as 35°C, although they are under stress once temperature exceeds 25°C. Therefore, to augment the stocking programs of this fish species, all those factors should be carefully monitored (Sublette *et al.*, 1990; Tomelleri & Eberle, 1990).

Fish blood, like that of other vertebrates, consists of two portions: formed elements and plasma. The formed elements consist of nucleated red blood cells, reticulocytes, white blood cells, and thrombocytes. Blood is a heterogenous biological fluid that transports oxygen, carbon dioxide, hormones, waste products, and nutrients in the body. Oxygen binds to hemoglobin, a blood pigment, located in the red blood cells, and is taken to tissues for metabolic needs. Oxygen delivery to tissues remains an important concept to study because the maximum rate at which oxygen can be made available to metabolizing tissues establishes a strong upper limit for metabolic rate. Blood parameters such as hematocrit, hemoglobin concentration, red blood cell count,

white blood cell count, and derived hematological indices such as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC), are useful in the diagnosis and assessment of fish health status (Barham *et al.*, 1980). In general, conditions such as ambient temperature, hypoxia, handling, and exercise change the hematocrit in fish to a substantial extent because the circulatory system of a fish is in close association with the external environment. Changes in the blood physiology can be used as indicators of the systemic response to such stimuli. These changes can be increased by the release of catecholamines during the primary stages of stress, which can mobilize red blood cells from the spleen or induce red blood cell swelling as a result of fluid shift into the intracellular compartment. Stress alters the homeostatic conditions of the fish in different ways, and consequently, the study of hematological and rheological responses to stress, which can lead to the improvement of oxygen transport to tissues is of particular significance (Pages *et al.*, 1995; SØrensen & Weber, 1995).

Blood is regarded as a non-Newtonian fluid because its viscosity depends on flow velocity or shear rate. The flow of blood within the vascular system depends on the rheological properties of its constituents and the interactions among these components. Blood viscosity can be defined as the ratio of shear stress to shear rate and is expressed as $dyn \cdot s/cm^2$ or centipoise, ($1 dyn \cdot s/cm^2 = 0.1 Pa \cdot s$). Shear stress is the frictional force per unit area required to move a fluid plane over a neighboring plane. Shear rate is the gradient of velocity between adjacent fluid planes during flow ($cm/cm \cdot s = s^{-1}$). Shear rates can be approximated for given blood vessels using a formula derived for the Newtonian fluid:

$$4V / r \qquad \text{(equation 1)}$$

where V equals mean blood flow velocity ($cm \cdot s^{-1}$) and r is the blood vessel radius (cm) (Graham & Fletcher, 1983).

Blood viscosity in general depends on the following: temperature, hematocrit, shear rate, cell aggregation, cell deformability, cell size and shape, presence of a nucleus, plasma proteins, and internal fluid of the red blood cell (Table I). At low shear rates cell aggregation becomes the controlling factor, whereas at higher shear rates, red blood cell deformability primarily affects blood flow. Red blood cell aggregation is mostly influenced by the plasma protein concentration (PPC) in the whole blood (Chien, 1975). Serum albumin contributes little to red blood cell aggregation; however fibrinogen and the globulin fractions (α, β, ϕ, γ) have been known to have a substantial impact on aggregation. In addition, the plasma-red blood cell interaction could play a significant role in altering aggregation because the negative charge on the surface of the red blood cells from sialic acid tends to interact with positively-charged macromolecules in the plasma (Chien, 1975; Karp, 1996).

Red blood cell deformability can be affected by a number of factors, such as membrane flexibility, ATP levels, cortisol and catecholamine levels, and the viscosity of the internal fluid. Soyten (1981) indicated that osmotic pressure, cholesterol content, and oxygen tension further influence the deformability of red blood cells. The membrane flexibility is determined by the red blood cell surface-volume relation and the tensile property of the membrane, which in turn depends on the chemical composition and the metabolic state of the membrane. Karp (1996) reported that the fluidity of the

Table I. Factors affecting blood viscosity and their potential effects on blood flow.

Factor	Effect on blood viscosity	Effect on blood flow	Reference
↑ hematocrit	↑ blood viscosity	↓ blood flow	Chien, *et al.*, 1971
↓ temperature	↑ blood viscosity	↓ blood flow	Chien, *et al.*, 1971
↓ shear rate	↑ blood viscosity	↓ blood flow	Chien, *et al.*, 1971
↑ cell aggregation	↑ blood viscosity	↓ blood flow	Chien, *et al.*, 1971
↓ cell deformability	↑ blood viscosity	↓ blood flow	Chien, *et al.*, 1971
↑ plasma protein concentrations	↑ blood viscosity	↓ blood flow	Chien, *et al.*, 1971

bilayer membrane depends on lipid composition and cholesterol content. Short fatty acid chains and unsaturated fatty acids increase fluidity whereas long fatty acids chains and saturated fatty acids decrease fluidity of the bilayer membrane. Cholesterol prevents stacking together of fatty acids and thus the fluidity is increased. In addition, the red blood cell membrane has viscous and elastic components, characteristic of resistance to deformation, which are mainly determined by the protein's skeleton that underlies the lipid membrane of the cell (Chien, 1975; Graham & Fletcher, 1983; Fletcher & Haedrich, 1987).

Increased red blood cell deformability at higher shear rates allows for decreased blood viscosity in microvessels, particularly the capillaries, that have a diameter smaller than the dimension of the red blood cells (Lecklin *et al.*, 1995). In addition, the deformed red blood cells tend to orient themselves in such a way that the axes are aligned with the flow, resulting in a decrease in viscosity (Chien, 1975).

Blood viscosity is considered to be a major contributing factor affecting total resistance to blood flow and consequently, the rate of oxygen delivery to the tissues. The Poiseuille-Hagen equation is regarded as the most fundamental component in evaluating the physiological cardivascular function:

$$Q = \frac{\Delta P \pi r^4}{8 \eta l} \qquad \text{(equation 2)}$$

where Q is blood flow, ΔP is the pressure difference, r is the radius of the vessel, η is the blood viscosity, and l is the length of the vessel. The concept of a compromise between oxygen carrying-capacity and blood viscosity led to the optimal hematocrit theory in which oxygen transport capacity is related to hemoglobin concentration [Hb] and viscosity through the following relationship:

$$\text{Oxygen transport-capacity (OTC)} = \frac{1.3\,[Hb]}{\eta} \qquad \text{(equation 3)}$$

where 1.3 is the volume of oxygen bound per gram of hemoglobin, and η is the blood viscosity in Pa.s (Wells & Baldwin, 1990; Wells & Weber, 1991). The size of erythrocytes and the concentration of hemoglobin within them appear to be important for matching OTC. Increasing the number of erythrocytes in the blood and the hemoglobin they contain is an obvious way of raising oxygen-carrying capacity. This mechanism is limited by the work that must be performed by the heart in pumping a viscous suspension of erythrocytes to metabolizing tissues where oxygen is exchanged for carbon dioxide. Moreover, blood viscosity increases at low shear rates in the peripheral circulation, further impeding oxygen delivery to tissues (Wells & Baldwin, 1990).

From the standpoint of an increasing world demand for an inexpensive and easily obtainable source of dietary protein, aquaculture of striped bass and other freshwater fish can help alleviate protein deficiencies in many parts of the world. As for any intensive food-producing enterprise, effective and intensive stocking programs, disease treatments, and stress management in aquaculture are of prime importance in maintaining the efficiency of operation and enhancing fish production. However, there have been few viscometric studies on fish, especially those studies aimed at evaluating the possible hemorheological effects of stress. In this study, I hypothesized that stress has an effect on blood viscosity and other related blood parameters in striped bass and that ultimately blood flow is altered. To test this hypothesis I investigated the effects of different stressful factors common in intensive aquacultural systems, and evaluated their potential effects on blood parameters. Understanding the effects of these factors could help fish culturists to reduce mortalities that occur when fish are exposed to stress.

MATERIALS AND METHODS

MAINTANANCE OF ANIMALS

Thirty-three adult striped bass (*Morone saxatilis*), were obtained from Milford Fish Hatchery, (Junction City, KS), weighing between 1040-1800 g. Fish were fed a diet of trout chow daily, consisting of about three percent of their body weight. Water temperature ranged from 23-28°C during experimental periods (June-August, 1997 and May 1998). Fish were divided into five groups: control (healthy fish), simulated transportation, hypoxia, diseased, and temperature. For the simulated transportation group, fish were placed in a transporting tank with well-aerated water for 24 hours. For hypoxic simulation, fish were placed in a 1000 L tank, and water flow was shut down to allow dissolved oxygen levels to drop to sub-optimal production levels for four hours. Fish were sampled when the dissolved oxygen was 4 ppm. At the Milford Fish Hatchery, summer oxygen levels are generally around 8-10 ppm. The diseased group consisted of fish infected with henneguya disease (proliferative gill disease or Hamburger gill disease) for one week. Clinically, fish with henneguya develop numerous white cysts on their skin and gills. Cysts on the gills (both intralammelar and interlammelar) can cause extensive granulomatous inflammation and hyperplasia of the gill surface, leading to serious respiratory problems (Moeller, 1996). Seven fish were randomly assigned to each of the five groups, except in hypoxic group where one fish died during experimental preparation and only six fish were sampled. For the temperature group six fish were exposed to a temperature of 27°C for four hours at a dissolved oxygen concentration of 5.5 ppm.

BLOOD SAMPLING

Fish were anesthetized with 62.5 mg/L solution of tricaine methanesulphonate (MS 222). Blood samples were taken *via* cardiac puncture using a heparinized syringe, and 6-8 ml of blood from each fish were placed into a separate labeled vacutainer containing heparin. All blood samples were immediately placed on ice. Hematocrit (Hct) was determined on freshly-obtained samples using the microcapillary hematocrit method. No correction was made for trapped plasma. The samples were transported on ice to our lab at Emporia State University for further analysis.

VISCOSITY MEASUREMENTS

Individual blood samples were separated and placed into three Eppendorf tubes. Each tube was centrifuged at low speeds for approximately 30 seconds. Some of the plasma from the first tube was added to the second tube to create hematocrits above and below that determined from the original sample. The hematocrit in the third tube represented the normal hematocrit and was not altered. All tubes were kept in an ice bath until viscosity measurements could be made. Viscosity measurements were made using a Wells-Brookfield cone/plate viscometer (Model DV-II+, Brookfield Engineering Lab, Stoughton, MA), with a CP-40 cone using 0.5 ml of sample. The viscometer was calibrated with distilled water or standard oil of known viscosity before data recording. Viscosity measurements of the control and temperature groups were made at two different temperatures (24°C and 27°C), and in all other groups the measurements were made at 24°C. The temperature of the sample cup was kept constant with an external water bath. Viscosity determinations were made over a range of ten different shear rates

corresponding with the rotational speed of the cone, and the results were reported in centipoise (cP). The shear rates were 3.75 s^{-1}, 7.5 s^{-1}, 15 s^{-1}, 18.8 s^{-1}, 30 s^{-1}, 37.5 s^{-1}, 75 s^{-1}, 150 s^{-1}, 375 s^{-1}, and 750 s^{-1}. No evidence of lysis was observed at any stage of the procedure. A linear regression equation was calculated for each shear rate using the three different hematocrits of each fish. The mean r^2 value for these regressions was 0.96. From the regression equation, apparent viscosity values were predicted for different hematocrits. Plots were made for the log viscosity *versus* hematocrit using Lotus 1,2,3 spreadsheet software. Plasma viscosity was also determined from each fish at ten different shear rates at a constant temperature of 24°C for all experimental groups and 24°C and 27°C for the control and temperature groups. Relative viscosity values at different shear rates were obtained by using the following formula:

$$\frac{\text{Apparent viscosity}}{\text{Plasma viscosity}} \qquad \text{(equation 4)}$$

Shear dependence (SD) of all samples was calculated in order to estimate the influence of red blood cell aggregation and red blood cell deformability on shear thinning. Shear dependence was calculated using the following equations (Graham *et al.*, 1985):

Shear dependence due to red blood cell aggregation ($SDRBC_a$)

$$SDRBC_a = \frac{(\eta_{3.75} - \eta_{30.})}{\eta_{3.75}} \qquad \text{(equation 5)}$$

Shear dependence due to red blood cell deformability ($SDRBC_d$)

$$SDRBCd = \frac{(\eta_{37.5} - \eta_{150})}{\eta_{37.5}} \qquad \text{(equation 6)}$$

Deformability of red blood cells was estimated using the following equation (Dintenfass, 1968):

$$\eta_r = (1- HT)^{-2.5} \qquad \text{(equation 7)}$$

where η_r is the relative viscosity, H= hematocrit and T = Taylor's factor. The equation can be rearranged and written as follows:

$$T = (1- (\mu s/\mu o)^{-0.4}) / H \qquad \text{(equation 8)}$$

where μs = whole blood viscosity, μo = plasma viscosity, H = hematocrit, and T = Taylor's factor. An increase in Taylor's factor signifies a decrease in red blood cell deformability (Aarts *et al.*, 1984).

HEMATOLOGY

Hematological parameters were determined within 24 hours. Red blood cell counts (RBCC) were determined by diluting fresh blood in a standard red blood cell pipette (1:200) with 0.9% NaCl saline solution (Dacie and Lewis, 1984). The diluted blood was placed on a hemocytometer and cells were counted using the method described by Hesser (1960). White blood cell counts (WBCC) were determined by diluting fresh blood with Shaw's fluid (1:20) in a standard white blood cell pipette (Shaw, 1930) and the same procedure was followed as for the red blood cell count (Shaw, 1930; Hesser, 1960).

Hemoglobin measurements were done using cyanomethemoglobin method (Sigma Chemicals, St. Louis, MO). Mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell hemoglobin (MCH), were calculated using the following equations: MCHC = Hb / Hct, MCV = Hct / RBCC and MCH=Hb / RBCC (Wickham *et al.*, 1990).

After viscosity determinations, the plasma was frozen for later plasma protein and hormone determinations. Plasma protein concentrations (PPC) were determined with the BioRad method using bovine serum albumin as a standard (Bradford, 1976). Plasma samples were read against a standard curve obtained from bovine serum albumin. All measurements were made in duplicate and averaged.

STATISTICAL ANALYSIS

Means and standard deviations were determined for all the hematological and hemorheological parameters. A one-way analysis of variance (ANOVA) with a Student-Newman-Keuls (SNK) multiple range test were used to compare means of apparent, relative, and plasma viscosity measurements. The same tests were used to compare means of MCHC, MCH, MCV, RBCC, WBCC, shear dependence due to red blood cell aggregation and shear dependence due to red blood cell deformability, Taylor's factor, and plasma protein concentration. Student 's t-test was used to compare apparent viscosity means of the control and temperature groups at different shear rates and hematocrits. Differences were considered significant at $P \leq 0.05$ (Zar, 1996).

RESULTS

Results obtained in this study are shown in Figures 1 through 13 and in Tables II through V. Figures 1-3 show mean values of apparent viscosity for whole blood vs. packed cell volume for the control, hypoxic, simulated transport, and diseased groups at a temperature of 24°C. Apparent blood viscosity increased with an increase in hematocrit in all the groups at all shear rates. No significant differences were observed between groups at lower shear rates (3.75 s^{-1}, 7.5 s^{-1}, 15 s^{-1}, 30 s^{-1}, and 37.5 s^{-1}) at hematocrits of 10-40%. At shear rates of 75 s^{-1}, and 150 s^{-1}, the hypoxic group showed a significantly-higher apparent viscosity as compared to controls at hematocrits of 30-70%. Figures 4 and 5 show mean values of apparent viscosity *vs.* shear rate for the control, hypoxic, simulated transport, and diseased groups at packed cell volumes of 30% and 40%, respectively, at a temperature of 24°C. No significant difference in apparent viscosity was observed at a shear rate of 3.75 s^{-1}, however significant differences between the hypoxic group and controls at higher shear rates were observed. The transport group showed a significantly lower apparent viscosity at a shear rate of 3.75 s^{-1} at a hematocrit of 30% and 40%. Figure 6 shows the mean values of plasma viscosity *vs.* shear rate at 24°C. The plasma viscosity observed for the hypoxic group was significantly higher as compared to controls at all shear rates. Figures 7-9 show the relative viscosity *vs.* packed cell volume at shear rates of 3.75 s^{-1}, 75 s^{-1}, and 150 s^{-1}. The diseased group showed a significantly higher relative viscosity when compared to the control group at higher shear rates and hematocrits of 50% and 70%. Figure 10 shows the oxygen transport capacity (OTC) *vs.* shear rate at original hematocrits. The diseased group consistently showed a

Figure 1. Apparent viscosity *vs.* packed cell volume (PCV) at a shear rate of 3.75 s^{-1} for control, hypoxic, simulated transport, and diseased groups at 24°C. (Means ± SD).

▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

Apparent Viscosity vs. Packed Cell Volume
SR=3.75 (per second)

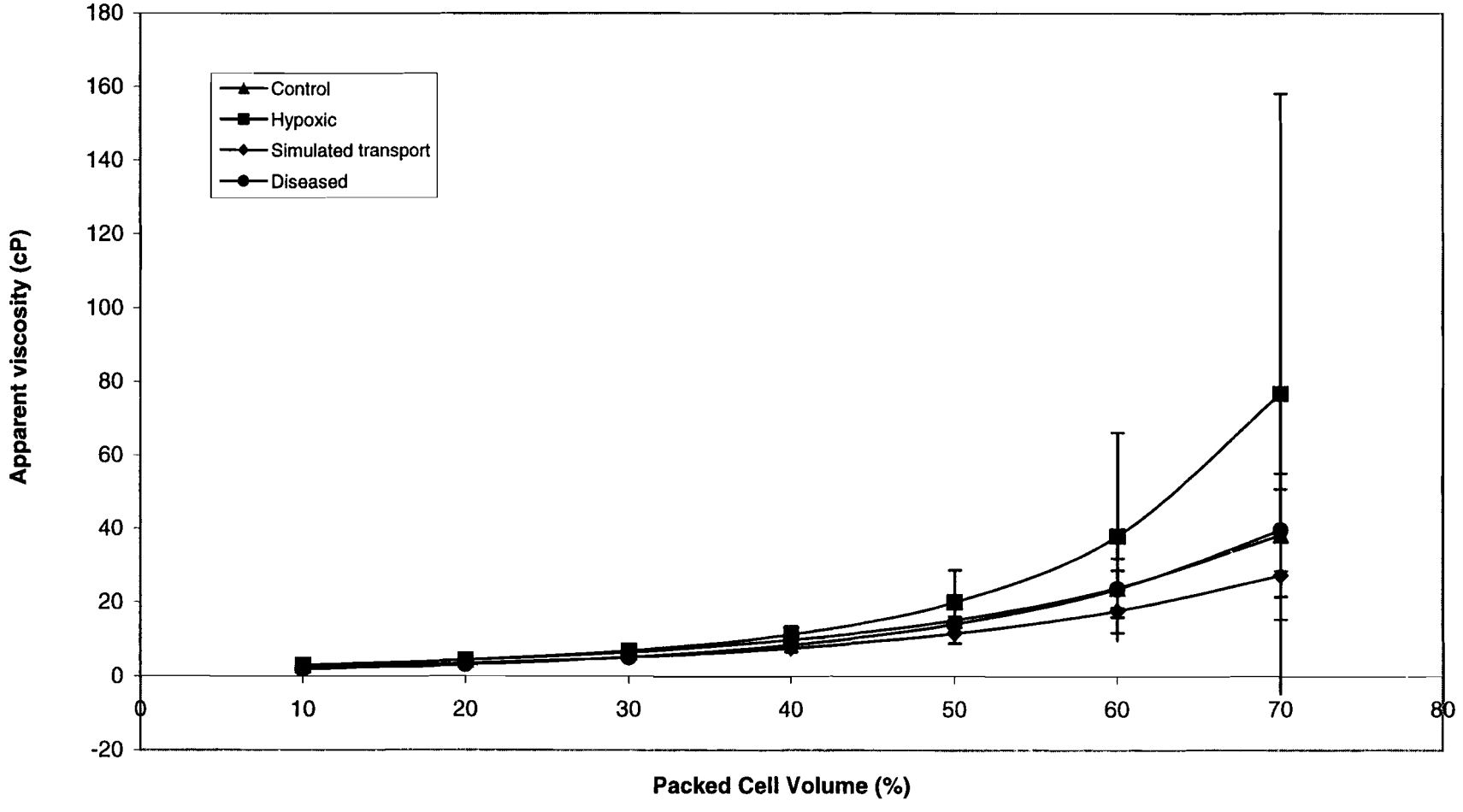


Figure 2. Apparent viscosity *vs.* packed cell volume (PCV) at a shear rate of 75 s^{-1} for control, hypoxic, simulated transport, and diseased groups at 24°C. (Means ± SD). ▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

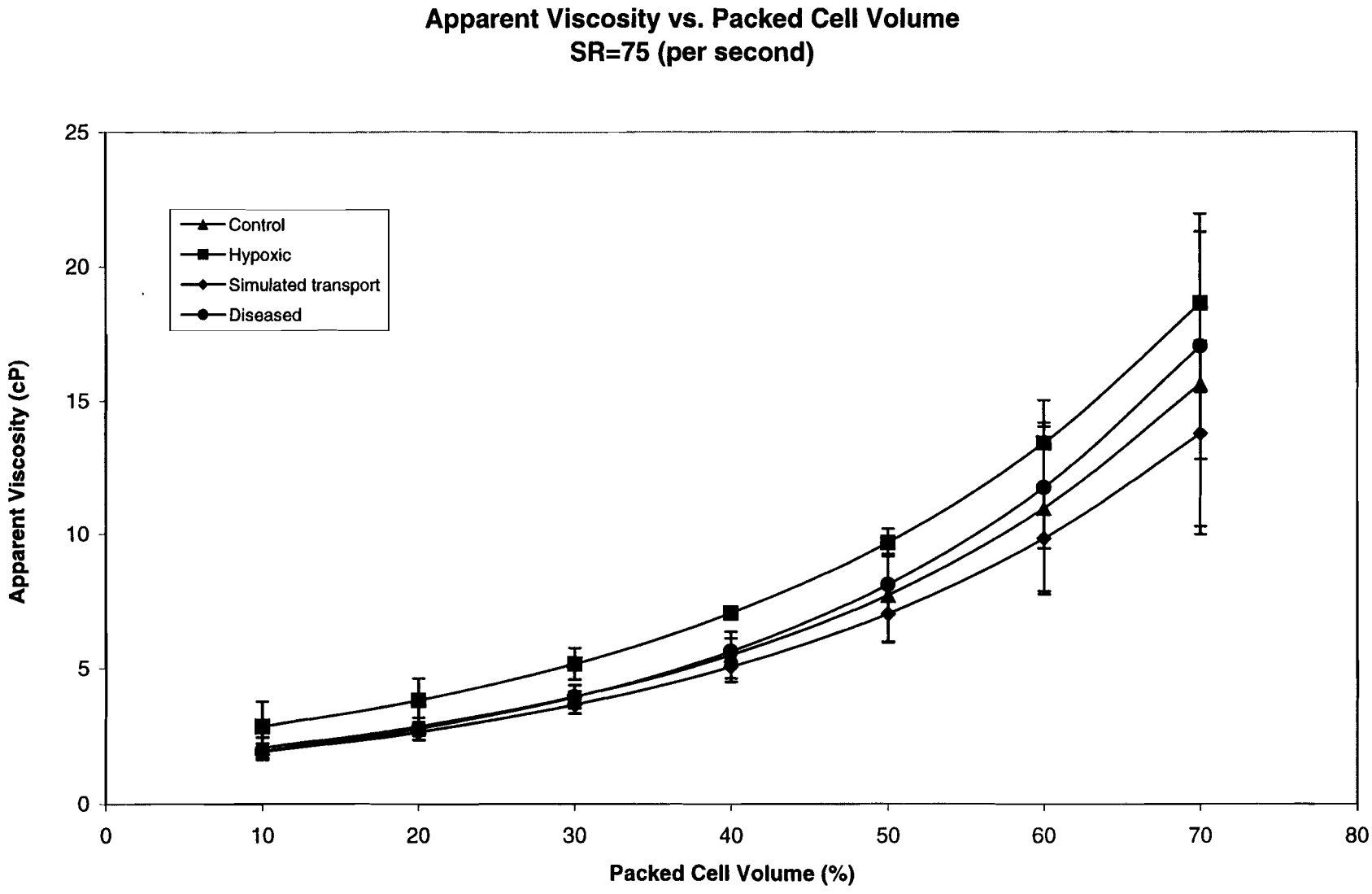

Apparent Viscosity vs. Packed Cell Volume
SR=75 (per second)
Control
Hypoxic
Simulated transport
Diseased
Apparent Viscosity (cP)
Packed Cell Volume (%)
0
5
10
15
20
25
0
10
20
30
40
50
60
70
80

Figure 3. Apparent viscosity *vs.* packed cell volume (PCV) at a shear rate of 150 s^{-1} for control, hypoxic, simulated transport, and diseased groups at 24°C. (Means ± SD). ▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

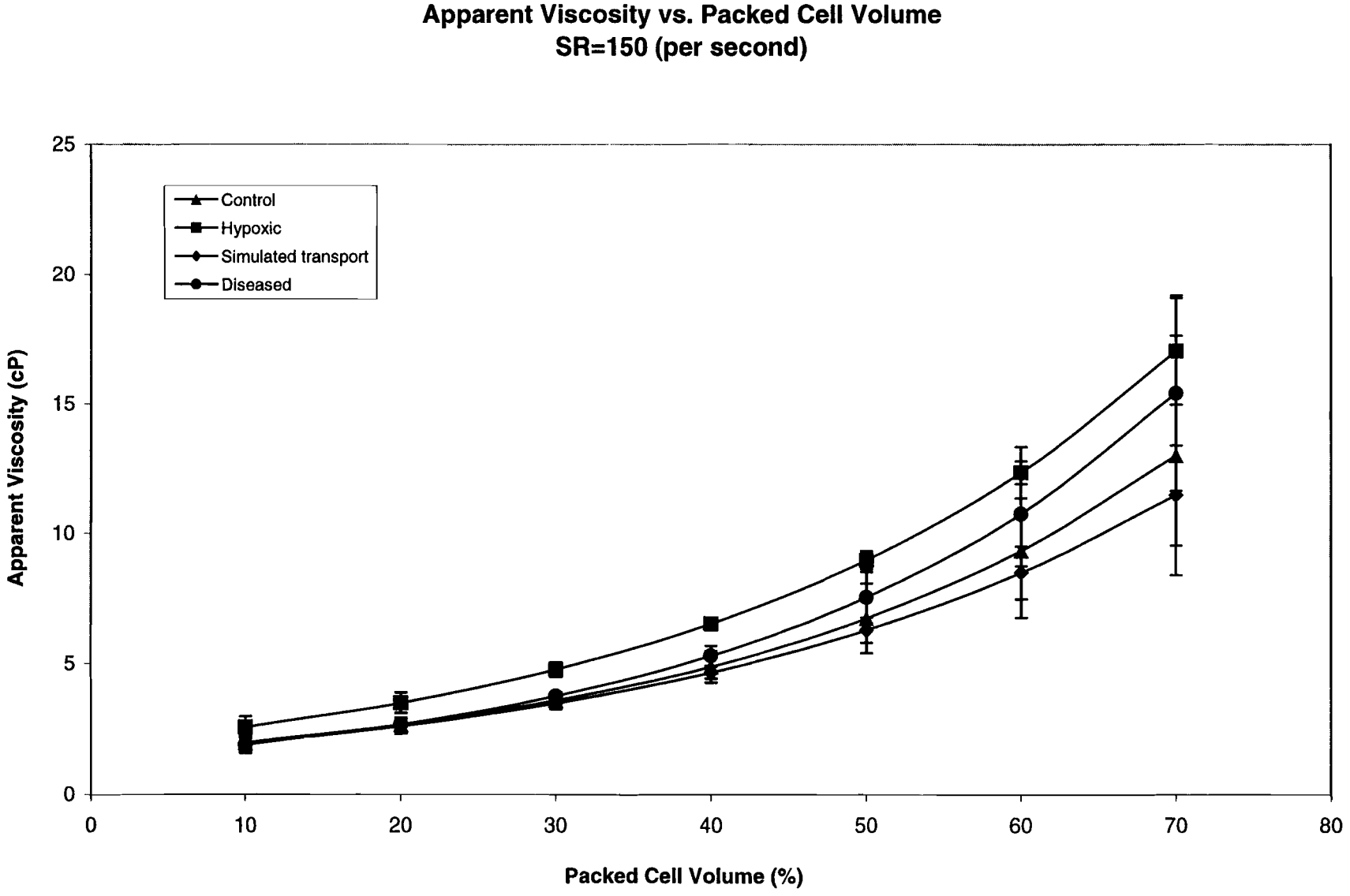

Apparent Viscosity vs. Packed Cell Volume
SR=150 (per second)
Control
Hypoxic
Simulated transport
Diseased
Apparent Viscosity (cP)
Packed Cell Volume (%)
0
5
10
15
20
25
0
10
20
30
40
50
60
70
80

Figure 4. Apparent viscosity *vs.* shear rate at a packed cell volume (PCV) of 30% for control, hypoxic, simulated transport, and diseased groups at 24°C. (Means ± SD). ▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

Apparent Viscosity *vs.* Shear Rate
PCV 30%

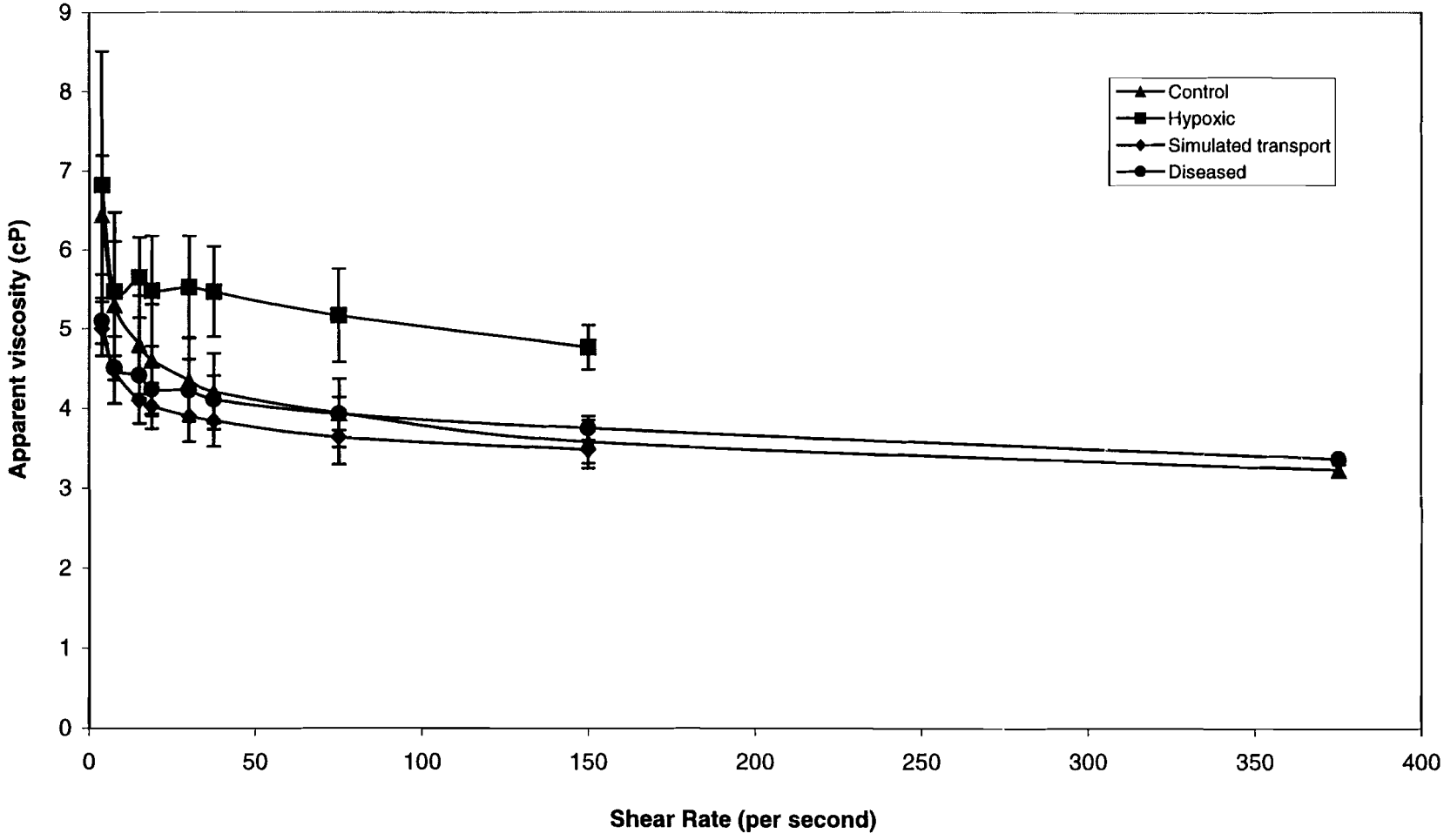


Figure 5. Apparent viscosity *vs.* shear rate at a packed cell volume (PCV) of 40 % for control, hypoxic, simulated transport, and diseased groups at 24°C. (Means ± SD). ▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

Apparent Viscosity *vs.* Shear Rate
PCV 40%

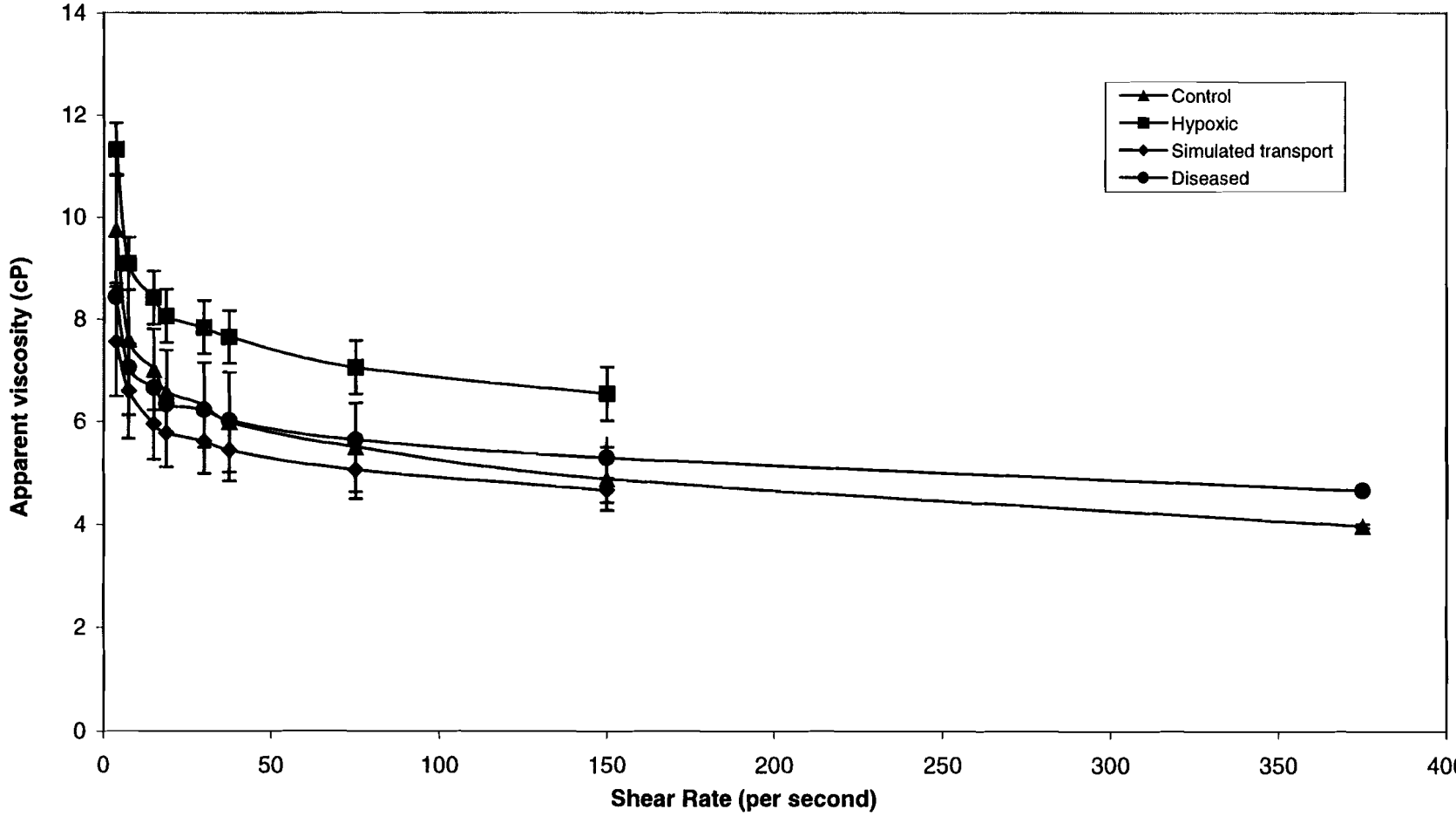


Figure 6. Plasma viscosity *vs.* shear rate for control, hypoxic, simulated transport, and diseased groups at 24°C. (Means ± SD). ▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

Plasma Viscosity *vs*. Shear Rate

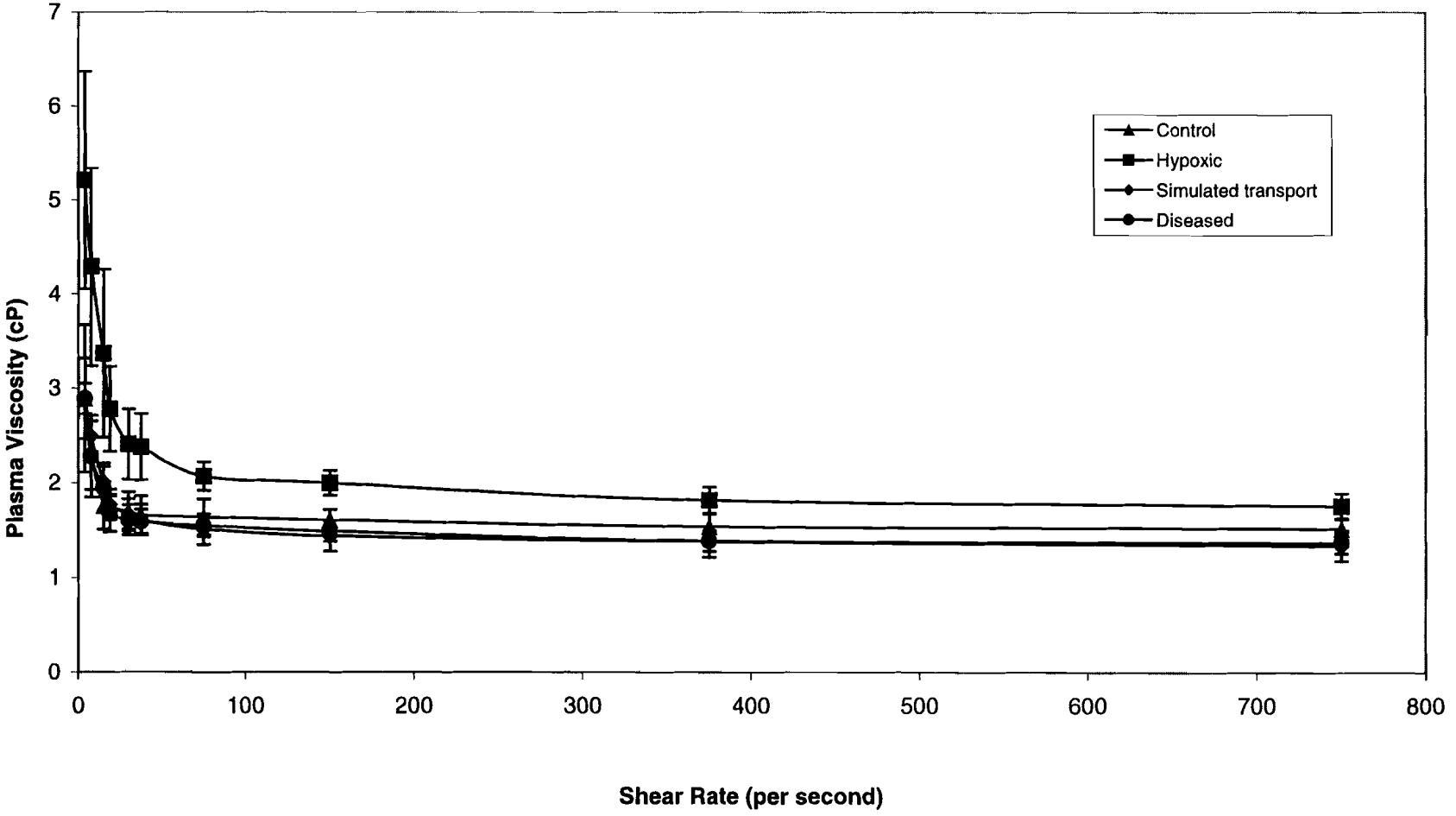


Figure 7. Relative viscosity *vs.* packed cell volume (PCV) at a shear rate of 3.75 s^{-1} for control, hypoxic, simulated transport, and diseased groups. (Means ± SD).

▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

Relative Viscosity vs. Packed Cell Volume
SR=3.75 (per second)

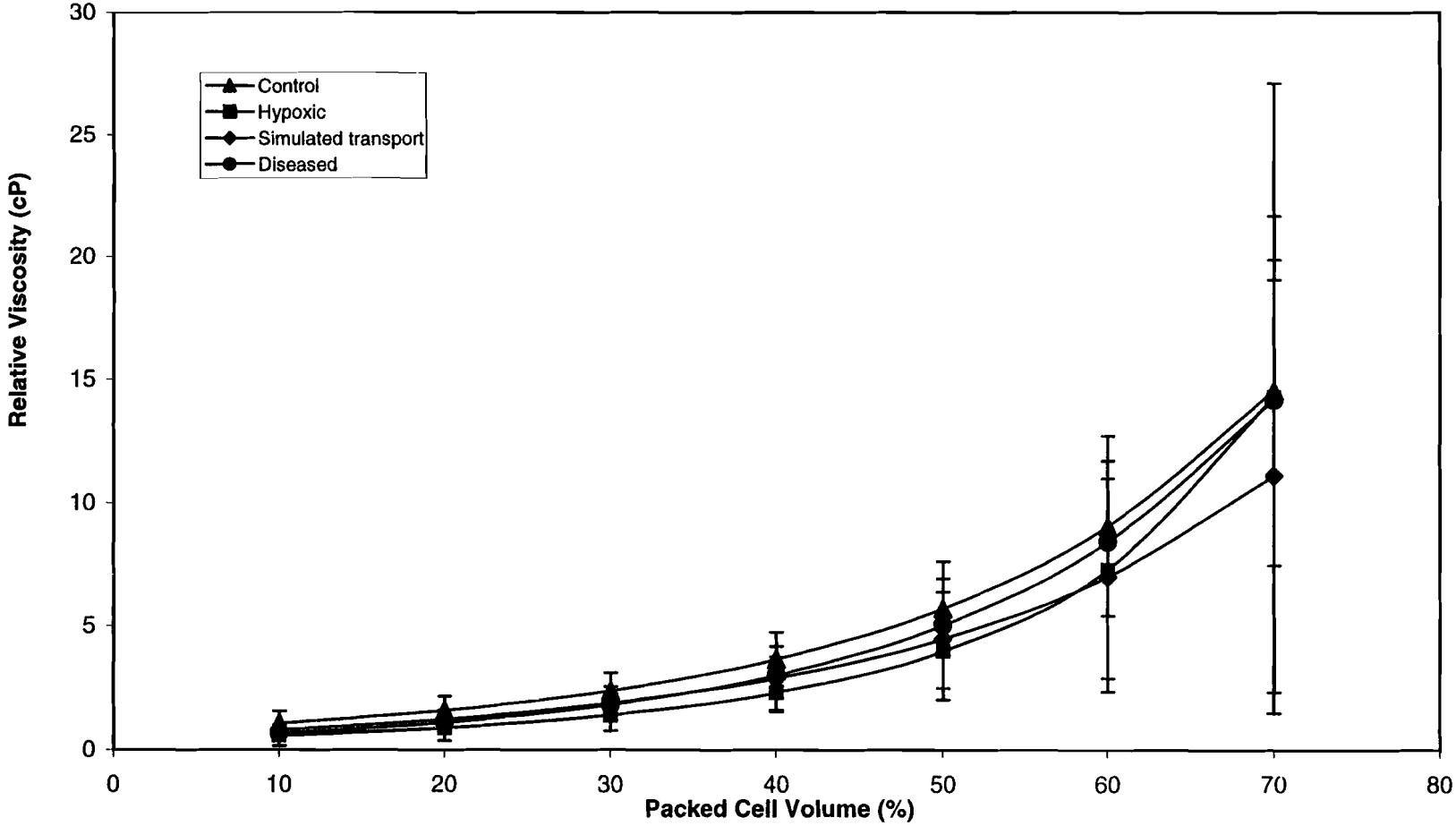


Figure 8. Relative viscosity *vs.* packed cell volume (PCV) at a shear rate of 75 s^{-1} for control, hypoxic, simulated transport, and diseased groups. (Means ± SD). ▲, control; ■, hypoxic; ◆, transport; and ●, diseased.

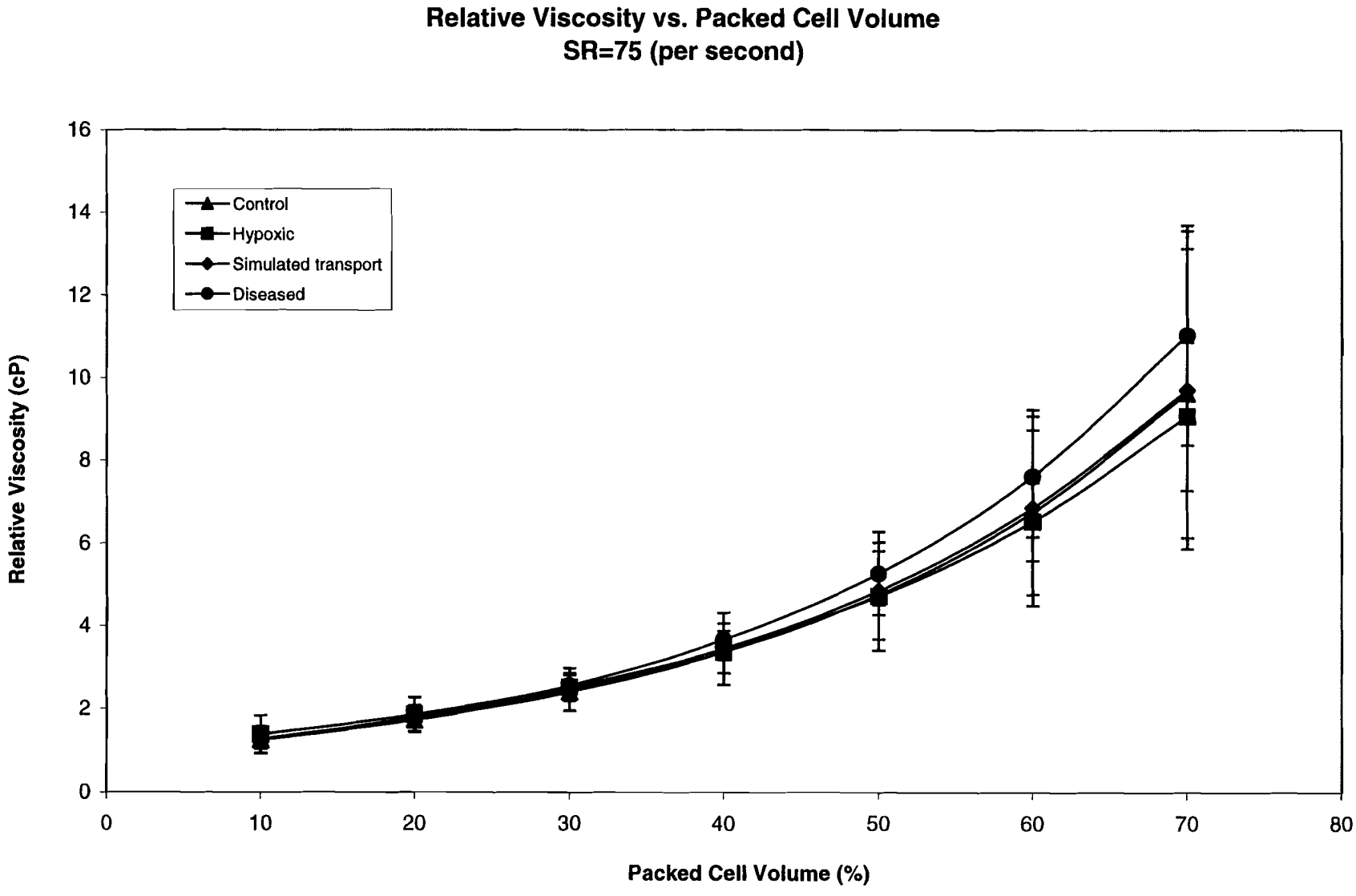

Relative Viscosity vs. Packed Cell Volume
SR=75 (per second)
Control
Hypoxic
Simulated transport
Diseased
Relative Viscosity (cP)
Packed Cell Volume (%)
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
70
80

Figure 9. Relative viscosity *vs.* packed cell volume (PCV) at a shear rate of 150 s^{-1} for control, hypoxic, simulated transport, and diseased groups. (Means ± SD).

▲, control; ■, hypoxic; ◆, transport; and ●, diseased

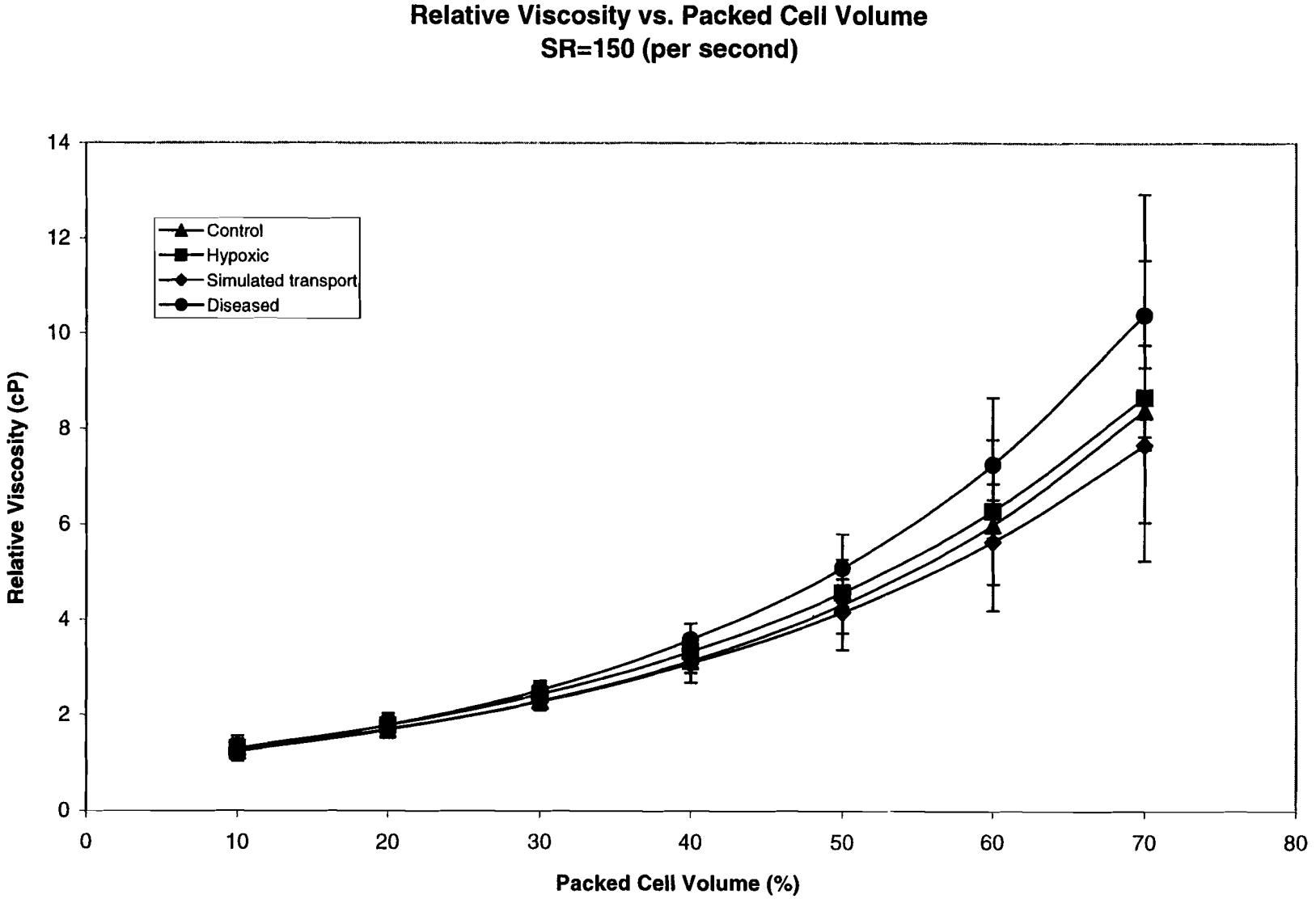
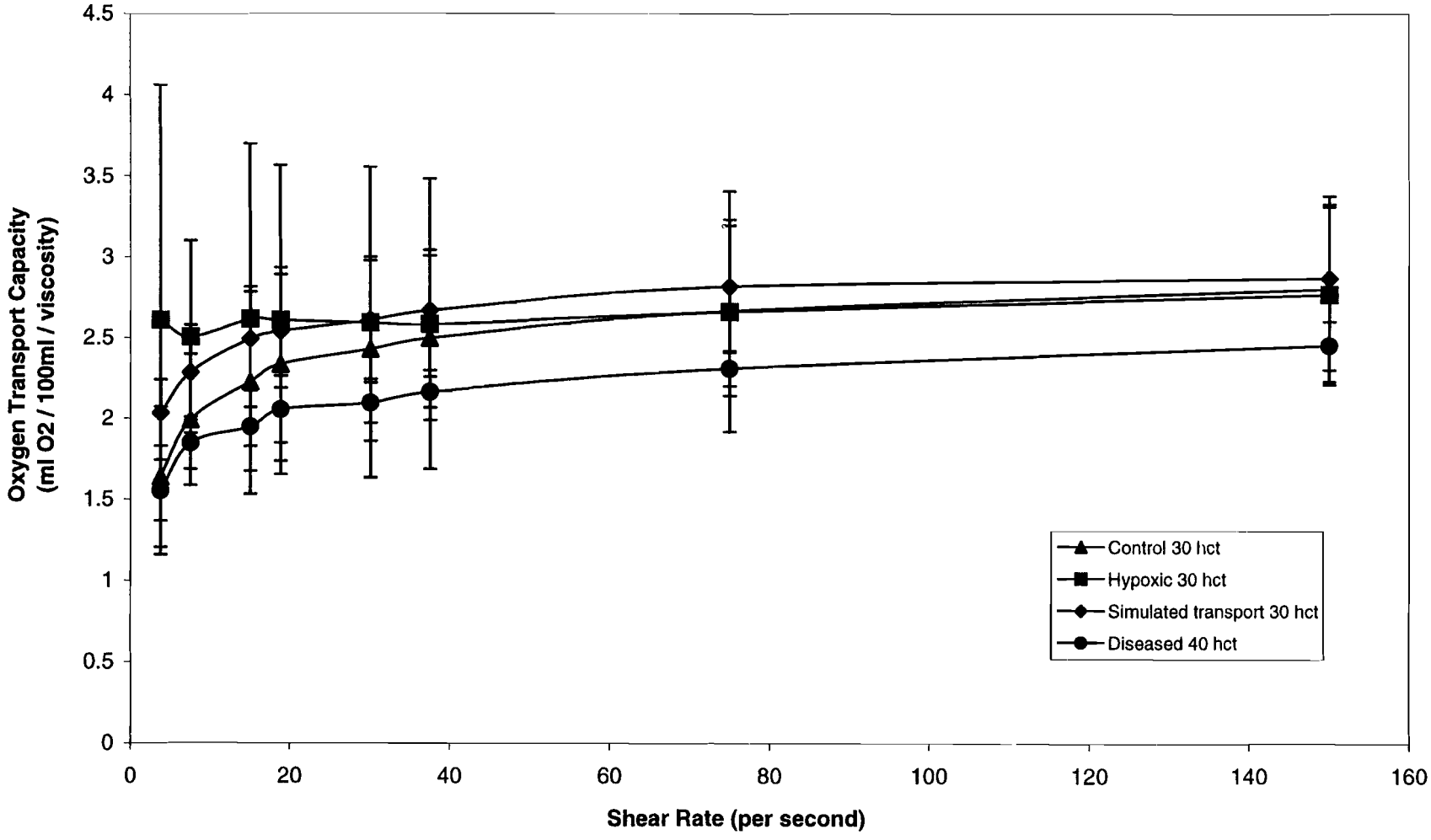

Relative Viscosity vs. Packed Cell Volume
SR=150 (per second)
Control
Hypoxic
Simulated transport
Diseased
Relative Viscosity (cP)
Packed Cell Volume (%)
0
2
4
6
8
10
12
14
0
10
20
30
40
50
60
70
80

Figure 10. Oxygen transport capacity (OTC) *vs.* shear rate for control, hypoxic, and simulated transport groups at a hematocrit of 30% and diseased group at a hematocrit of 40% at 24°C. (Means ± SD). ▲, control; ■, hypoxic; ◆, transport; and ●, diseased

Oxygen transport capacity vs. Shear Rate



lower OTC at a hematocrit of 40% as compared to the controls at a hematocrit of 30%, but the difference was not significant.

Figures 11-13 show apparent viscosity *vs.* packed cell volume for the control and temperature groups. The temperature group at 24°C and 27°C showed a significantly higher apparent viscosity at a shear rate of 150 s^{-1} and hematocrit of 70% when compared to the control group at the same temperature.

Table II shows shear dependence due to aggregation and deformability. No significant difference in shear dependence due to red blood cell aggregation or deformability was found among any of the groups. Table III shows mean Taylor's factor values for all the groups at a shear rate of 150 s^{-1} and at a hematocrit of 70%. No significance difference was observed among the groups but there was a clear trend of an increase in Taylor's factor values in hypoxic, diseased , and temperature (24°C and 27°C) groups.

Table IV shows mean values for hematocrit, hemoglobin concentration, red blood cell count, white blood cell count, derived indices, and plasma protein concentration for each group. Fish in the diseased group had significantly-higher hematocrits and hemoglobin values as compared to controls. The hypoxic group had an increase in MCHC and a decrease in MCV but values were not significant when compared to the controls. The temperature group showed a significantly-lower red blood cell count and a significantly-higher MCV when compared to the control group. The white blood cell count observed in the hypoxic, transport, and diseased groups were significantly higher as compared to controls. The hypoxic group showed a significantly-higher plasma protein

Figure 11. Apparent viscosity *vs.* packed cell volume (PCV) at a shear rate of 3.75 s^{-1} for control and temperature groups at 24°C and 27°C. (Means ± SD). ◆, temperature 24°C; ■, temperature 27°C; ▲, control 24°C; ×, control 27°C .

Apparent Viscosity vs.Packed Cell Volume
SR=3.75 (per second)

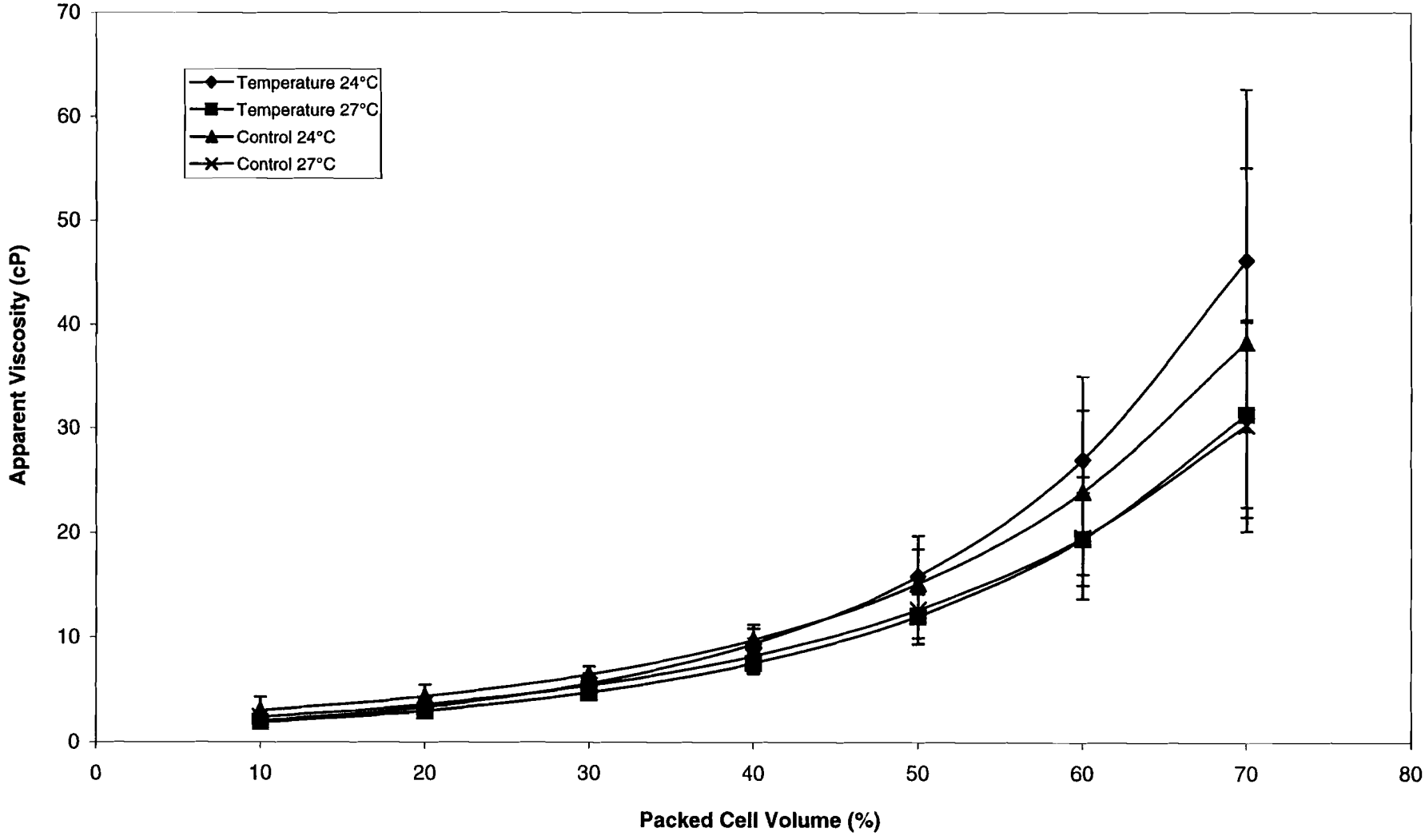


Figure 12. Apparent viscosity *vs.* packed cell volume (PCV) at a shear rate of 75 s^{-1} for control and temperature groups at 24°C and 27°C. (Means ± SD). ◆, temperature 24°C; ■, temperature 27°C; ▲, control 24°C; ×, control 27°C .

Apparent Viscosity vs. Packed Cell Volume
SR=75 (per second)

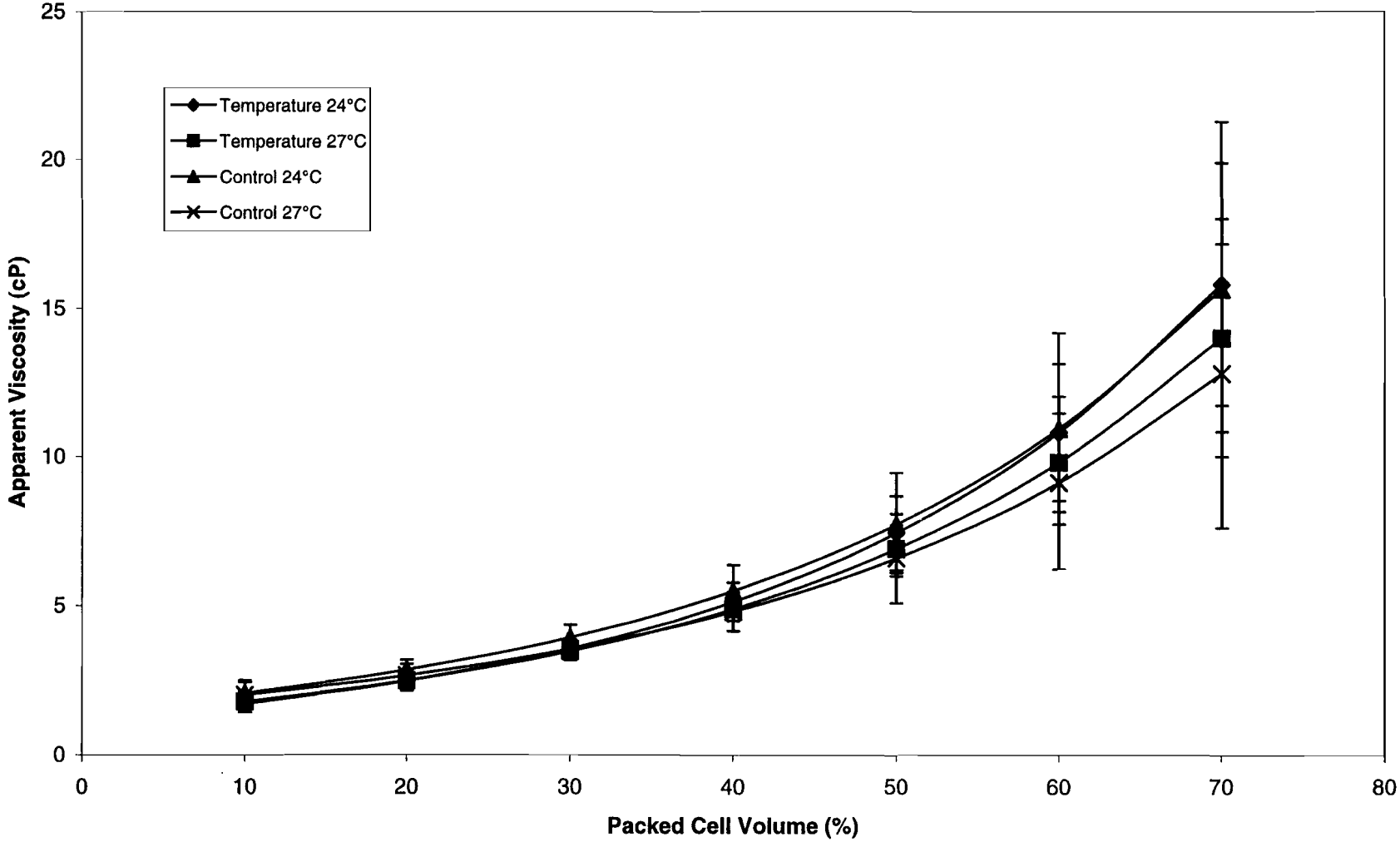


Figure 13. Apparent viscosity *vs.* packed cell volume (PCV) at a shear rate of 150 s^{-1} for control and temperature groups at 24°C and 27°C. (Means ± SD). ◆, temperature 24°C; ■, temperature 27°C; ▲, control 24°C; ×, control 27°C .

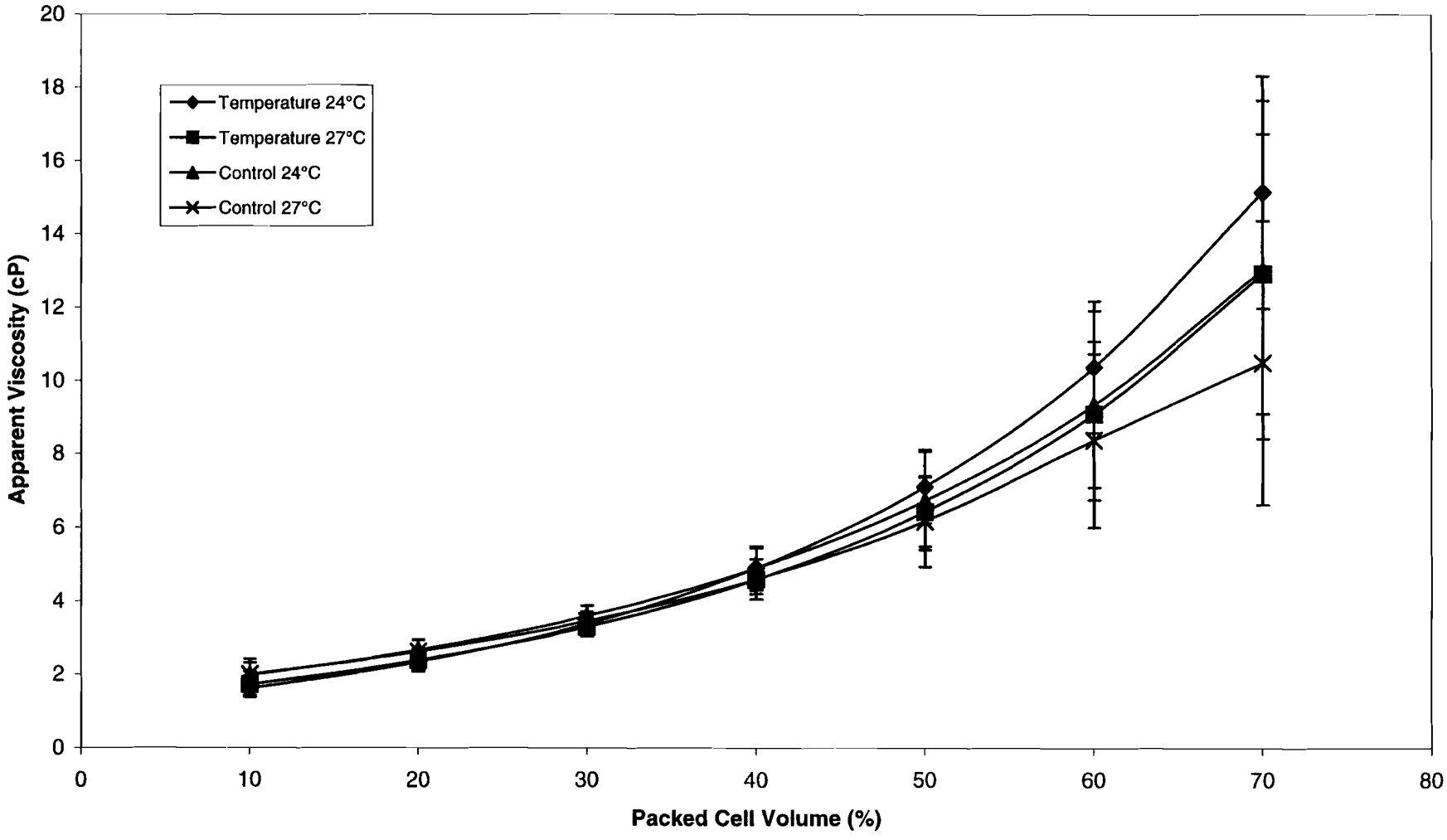

Apparent Viscosity vs. Packed Cell Volume
SR=150 (per second)
Temperature 24°C
Temperature 27°C
Control 24°C
Control 27°C
Apparent Viscosity (cP)
Packed Cell Volume (%)
0
2
4
6
8
10
12
14
16
18
20
0
10
20
30
40
50
60
70
80

Table II. Shear dependence values due to both cell aggregation and cell deformability at a hematocrit of 30%. (Means ± SD).

Shear Dependence due to aggregation at a hematocrit of 30%.

Treated Group	Temperature (°C)	Means ± SD
Control	24°C	0.315 ± 0.110
Control	27°C	0.272 ± 0.098
Hypoxic	24°C	0.114 ± 0.377
Simulated transport	24°C	0.214 ± 0.069
Diseased	24°C	0.165 ± 0.112
Temperature	24°C	0.287 ± 0.121
Temperature	27°C	0.206 ± 0.332

P = 0.322

Shear Dependence due to deformability at a hematocrit of 30%.

Treated Group	Temperature (°C)	Means ± SD
Control	24°C	0.114 ± 0.045
Control	27°C	0.063 ± 0.029
Hypoxic	24°C	0.108 ± 0.070
Simulated transport	24°C	0.070 ± 0.182
Diseased	24°C	0.085 ± 0.057
Temperature	24°C	0.108 ± 0.175
Temperature	27°C	0.098 ± 0.026

P = 0.080

Table III. Mean Taylor 's factor values at a shear rate of 150 s^{-1} and hematocrit of 70% (Means ± SD).

Taylor's factor at a shear rate = 150 s^{-1}

Hematocrit (Hct) = 70%

Treated Group	Temperature (°C)	Taylor's factor (Means ± SD)
Control	24°C	0.791 ± 0.1112
Control	27°C	0.736 ± 0.182
Hypoxic	24°C	0.823 ± 0.036
Simulated transport	24°C	0.790 ± 0.055
Diseased	24°C	0.867 ± 0.076
Temperature	24°C	0.856 ± 0.057
Temperature	27°C	0.812 ± 0.079

P = 0.267

Table IV. Hematological parameters, MCH, MCHC, MCV, and plasma protein concentration for all groups.

Variable	Control $n = 7$	Hypoxic $n = 6$	Transport $n = 7$	Diseased $n = 7$	Temperature $n = 6$
Hct (%)	31.07 ± 5.17	32.00 ± 3.71	29.85 ± 3.53	38.70 ± 4.09*	31.33 ± 1.21
[Hb] (gm/dL)	7.94 ± 1.61	9.11 ± 0.74	7.80 ± 1.12	10.05 ± 1.18*	7.92 ± 0.85
RBCC $(10^6)/mm^3$	3.20 ± 0.35	4.06 ± 0.84	3.50 ± 0.50	3.80 ± 0.56	2.36 ± 0.26*
WBCC$(10^5)/mm^3$	2.20 ± 0.79	4.40 ± 0.77*	4.40 ± 0.24*	4.90 ± 0.79*	2.80 ± 0.10
MCH(10^{-6})pg	2.50 ± 0.43	2.40 ± 0.56	2.30 ± 0.39	2.70 ± 0.30	3.34 ± 0.20
MCV$(10^{-6})\mu m^3$	9.70 ± 1.10	8.2 ± 1.50	8.50 ± 5.60	10.00 ± 1.40	14.58 ± 3.48*
MCHC (g%)	25.60 ± 3.29	28.38 ± 3.06	26.48 ± 3.12	25.92 ± 1.51	25.29 ± 2.69
PPC (g/dL)	4.93 ± 0.65	5.72 ± 0.34*	3.89 ± 0.49*	3.69 ± 0.84*	3.71 ± 0.30*

* = significantly different as compared to controls ($P \leq 0.05$)

concentration as compared to controls, whereas the plasma protein concentration observed in the diseased group was significantly lower as compared to controls.

DISCUSSION

The objectives of this study were to investigate the effects of stress on blood viscosity and other blood parameters and to evaluate the potential role of the altered blood viscosity as a possible cause of fish mortalities during summer. Little information is available on blood viscosity and related hemorheological factors in striped bass, and as a result, the data obtained in this study are compared with data reported for other fish species (Table V). Fishes, like all other vertebrates, have a cardiovascular system with a heart as the main pump in line with the branchial and systemic capillary beds connected to veins and arteries (Moyle & Cech, 1996). Understanding the propulsion of blood through the circulation to nourish tissues with oxygen, remains an important aspect to evaluate adaptive responses of fish, especially when they are exposed to stressful factors. Because the oxygen demand for fish varies with stage of life history, environmental conditions, and other physical forces (especially those practiced in aquacultural programs), changes in the number of red blood cells and their properties should be studied (Moyle & Cech, 1996).

HEMATOLOGICAL VARIABLES:

Observations in this study found that hematological and hemorheological parameters of adult striped bass were altered by different stressful situations. Hematological values (Hct, Hb, RBCC, and WBCC) observed for the control group in

this study were comparable to those previously reported by Tisa *et al.*, (1983); Hunn *et al.*, (1992); Cech *et al.*, (1996), for unstressed striped bass (Table V).

There was a significant increase in hematocrit in the diseased group as compared to the control group. The proliferative gill disease (henneguya) causes inflammation and hyperplasia of the gills, and ultimately impairs oxygen uptake. The increase could be due to the prolonged parasitic infection that caused insufficient uptake of oxygen, the fish body then responded by releasing red blood cells from the spleen. An increased number of red blood cells in the system increases blood oxygen-carrying capacity and improves oxygen delivery to the tissues (Soldatov, 1996). However, Barham *et al.*, (1980), reported a decrease in hematocrit in rainbow trout *Oncorhynchus mykiss,* that were infected with species of *Aeromonas* and *Streptococcus* bacteria. These bacteria infect kidneys, spleen, and liver, and thus likely did not affect oxygen uptake. The diseased group showed a significantly-increased hemoglobin concentration as compared to the control group. The increase in hemoglobin observed in this group was likely associated with the significant increase in hematocrit. There was a significant increase in white blood cell numbers in the diseased group. This increase in white blood cells could be due to pathological responses of the fish as observed in other teleosts. The values obtained in this study for plasma protein concentrations in diseased fish concur with those reported by Barham *et al.*, (1980) for rainbow trout *O. mykiss* (Table III). Fibrinogen, prothrombin, albumin, alpha, beta, and gamma globulins have been shown to lower during pathological conditions (Barham *et al.*, 1980). Pages *et al.*, (1995) observed the same trend in gilthead seabream *Sparus aurata,* and suggested that reduction could result

Table V. Hematological parameters obtained in this study compared to those obtained from previous studies for a variety of fish species.

Species	Hct (%)	[Hb] (gm/dL)	RBCC (10^6) /mm^3	MCH (10^{-6}) pg	MCV (10^{-6}) μm^3	MCHC (g%)	PPC (g/dL)	References
Striped bass (unstressed)	31.07	7.94	3.20	2.50	9.70	25.60	4.93	Present study
Striped bass (hypoxic)	32.00	9.11	4.06	2.40	8.20	28.38	5.72	Present study
Striped bass (simulated transport)	29.85	7.80	3.50	2.30	8.50	26.48	3.89	Present study
Striped bass (diseased)	38.70	10.05	3.80	2.70	10.00	25.92	3.69	Present study
Striped bass (temperature)	31.33	7.92	2.36	3.34	14.58	25.29	3.71	Present study
Gilthead seabream (exercise)	32.10	7.49	2.81	2.67[a]	11.42[b]	23.40	N/A	Pages *et al.*, 1995
Striped bass (unstressed)	30.00-50.00	N/A	N/A	N/A	N/A	N/A	4.10-16.00	Hunn *et al.*, 1992
Striped bass (unstressed)	25.00-41.00	6.00-12.00	N/A	N/A	N/A	N/A	3.10-6.00	Tisa *et al.*, 1983
Rainbow trout (healthy)	38.39	5.44	1.08	5.40[a]	35.54[b]	21.58	4.33	Barham *et al.*, 1980
Rainbow trout (diseased)	27.05	3.32	0.82	4.05[a]	32.99[b]	20.62	3.10	Barham *et al.*, 1980

N/A = Not available; a= calculated by dividing [Hb] by RBCC and b = calculated by dividing mean Hct by mean RBCC.

from increased protein catabolism during stress or possibly from globulin degradation associated with immunogical responses.

The hypoxic group showed a slight increase in hematocrit, but the increase was not significant when compared to controls. The slight increase could be due to red blood cell swelling, loss of plasma fluid, or splenic release of new red blood cells as has been observed in most vertebrates exposed to hypoxia (Pinder & Smits, 1993). This was in contrast to the findings by Soldatov (1996) in flounder *Pleuronectes flescus luscus,* exposed to hypoxia for 24 hours. Hypoxia enhanced the proliferation of red blood cells in the pronephros of the kidneys, and thus increased the hematocrit. In rainbow trout *O. mykiss*, it was shown that cannulated fish exposed to acute hypoxia (1 hour) had an increase in hematocrit; however a decrease in hematocrit was evidenced after chronic hypoxia (2 weeks). Previous researchers reported rapid and persistant swelling of red blood cells in fish when exposed to hypoxia, perhaps leading to changes in hematocrit (Marinsky *et al.*, 1989). The reason for the insignificant increase in hematocrit in hypoxic striped bass in this study may be due to the duration of hypoxic exposure (four hours). The hypoxic group showed an increase in MCHC but was not significant, perhaps due to greater number of smaller cells as might be suggested by the low MCV. The hypoxic group showed a significant increase in plasma protein concentrations. This could be a result of loss of plasma fluid when animals were exposed to the hypoxic conditions. The same response has been observed in bullfrogs *Rana catesbeiana* during hypoxia (Pinder & Smits, 1993).

No change in hematocrit was observed in the temperature group, but red blood cell count was significantly decreased. It seems reasonable to speculate that cells were

removed by the spleen and not replaced with new ones. The increased MCV would suggest that the remaining cells in circulation increased in volume as has been previously reported (Murad *et al.*, 1990). The cardiovascular systems of ectotherms respond to increasing temperature by increasing heart rate and blood pressure, hence increasing cardiac output (Langille & Crisp, 1980). This likely increases blood flow to gills where intake of water takes place, and thus might account for the swelling of red blood cells that was evidenced by a significant increase in MCV.

HEMORHEOLOGY:

Viscosity measurements observed in this study demonstrated that the hypoxic group had a significant increase in apparent viscosity at higher shear rates (shear rate = 30 s^{-1}, 37.5 s^{-1}, 75 s^{-1}, 150 s^{-1}) at hematocrits of 30-70%. Chien *et al.*, (1971) indicated that at higher shear rates, the only contributing factors for determining whole blood viscosity are red blood cell deformability and their orientation during flow. In fish red blood cells, the presence and size of the nucleus plays a significant role in determining red blood cell deformability. Therefore, the increased apparent viscosity observed in this study for the hypoxic group could be due to decreased deformability of red blood cells, which also could be associated with the significant increase in MCHC. The increased MCHC might decrease the red blood cell deformability by increasing intracellular viscosity (Viscor *et al.*, 1984). Additionally, the decreased deformability of red blood cells in the hypoxic group could result from depletion of energy rich compounds or increases in plasma protein concentration.

Brauner & Wang (1997) reported that an environmental hypoxia induced a reduction in the nucleoside triphosphates (NTP): hemoglobin ratio. The reduction in this

ratio during hypoxia could result from a decrease in the rate of glycolysis and from the inhibition of phosphofructokinase. Furthermore, reduction in intraerythrocytic levels of ATP and GTP, which are regarded as main nucleoside triphosphates in the fish erythrocytes, have been extensively reported in fish exposed to hypoxia. Cortisol and catecholamines, regarded as stress indicators, have been correlated with the decrease in red blood cell ATP concentration (Love, 1980; Val *et al.*, 1995). The decrease in red cell ATP levels could be due to reduced oxidative phosphorylation caused by reduced oxygen supply (Greany & Powers, 1978). Such decreases in ATP can also lead to a decrease in red blood cell deformability and cause an increase in apparent viscosity (SØrensen & Weber, 1995).

ATP is thought to act as a chelating agent to reduce the Ca^{2+} concentration in the membrane. The possible increase in membrane rigidity following ATP depletion can be reversed by introduction of EDTA or Mg^{2+}(Chien, 1975). The Ca^{2+}- Mg^{2+}- ATP relationship might act on the inner membrane surface to regulate the physicochemical state of the membrane proteins, with Ca^{2+} favoring gel formation and ATP and Mg^{2+} moving the equilibrium toward the sol state. A second possibility for the Ca^{2+} induced membrane rigidity is the stimulation of a Ca^{2+}-dependent contractile protein (Chien, 1975). The secretion of catecholamines during hypoxia increases red blood cell oxygen consumption, resulting in increased ATP use and might further reduce ATP availability (Love, 1980).

The levels of catecholamines were not determined in this study but their effects on red blood cells are well documented. Nikkinmaa & Huestis (1984) reported the adrenergic swelling of red blood cells due to increased extracellular K^+ and

catecholamines. This beta-adrenergic stimulation of red blood cell function appears to help the arterial oxygen content of the striped bass especially in severe oxygen depletion (Nikinmaa *et al.*, 1984). Catecholamines stimulate an increase in red blood cell deformability (Chiocchia & Motias, 1989). They augment two factors that are likely involved in altering red blood cell deformability, that is, cellular cyclic AMP and cell volume, both of which are increased during hypoxic conditions. Cyclic AMP-dependent phosphorylation seemingly controls the functional roles of certain peripheral proteins on the red blood cell membrane skeleton and thus influences red blood cell deformability. However, if cyclic AMP increases but the cells do not swell, then the cells become more rigid and the blood viscosity is increased, which then slows down blood flow to tissues (Chiocchia & Motias, 1989). This might explain the higher relative viscosity of the hypoxic group because they failed to show cell swelling.

The hypoxic group showed a significantly higher plasma viscosity when compared to controls. This could result from the observed increased plasma protein concentration. Soivio & Tuurala (1981) reported that when rainbow trout were exposed to hypoxia, there was an increase in plasma removal because of the high capillary permeability in fish. This might lead to an increase in plasma protein concentration as plasma proteins typically cannot pass through capillaries.

The diseased group showed a significant increase in relative viscosity when compared to the controls at shear rates of 75 $s^{-1}$and 150 s^{-1} and would suggest a decreased red blood cell deformability in these animals. The decrease in cell deformability could suggest that the diseased fish were also exposed to decreased oxygen supply to their tissues. However, no significant difference was observed in

shear dependence due to red blood cell aggregation or deformability. The Taylor's factor values did not show any significant difference but trends were observed where the hypoxic, diseased, and temperature (24°C and 27°C) groups showed higher values. Lack of statistical significant difference could be because these formulae are not powerful at shear rates of 150 s^{-1} and below.

The transport group showed a significantly lower apparent viscosity when compared to controls at a shear rate of 3.75 s^{-1} and a hematocrit of 30%. The significantly-lower apparent viscosity could be due to increased oxygen availability in the water. Soyten (1981) reported that an increase in oxygen levels tended to increase the deformability of red blood cells. The relative viscosity observed in this group was low but not significant, but would suggest possible increased red blood cell deformability in the transport group. Plasma viscosity measurements observed in the transport group did not show any differences from the control group. This finding would further suggest the idea that transport stress does not significantly alter these hemorheological parameters especially when the water is well-aerated.

Apparent viscosity in the temperature group decreased with an increase in temperature. The results concur with findings by Guard & Murrish (1975), on blood obtained from Antarctic birds and mammals. There was a significant increase in apparent viscosity at a shear rate of 150 s^{-1} and a hematocrit of 70% when compared to controls at a temperature of 24°C. This could imply that the cells from the temperature group were less deformable when compared to those from the control group. In addition, a significantly-higher apparent viscosity was observed in the temperature group at a shear rate of 150 s^{-1} and a hematocrit of 70% when compared to controls at a

temperature of 27°C. Such increases in apparent viscosity could be due to decreased dissolved oxygen levels, which were associated with an increase in temperature, thereby leading to a decrease in cell deformability.

The oxygen transport capacity observed in this study concurs with findings by Wells & Baldwin (1990), in which they found that oxygen-carrying capacity could be optimized at the expense of the flow properties of blood. The diseased group showed a lowered oxygen transport capacity compared to controls irrespective of the increased hematocrit observed (38.79% *vs.* controls at 31.07%). The transport group showed an increase in oxygen transport capacity when compared to controls despite having no difference in hematocrit (29.86% *vs.* controls at 31.07%). This was likely the result of a decreased blood viscosity in the transport group. Suprisingly, the hypoxic group did not show any significant changes in OTC, despite an increased blood viscosity and was probably because of the increased hemoglobin concentration that occurred in this group.

This study suggests that hypoxia could be one of the major factors that contribute to the midsummer mortalities in the striped bass as evidenced by the increased apparent viscosity in the hypoxic group. Increased apparent blood viscosity could lead to decreased blood flow in the systemic circulation, which could further impair oxygen delivery to the already oxygen-deprived tissues. Simulated transportation of this species of fish in well-aerated water did not significantly alter hemorheological parameters. Increased temperature did show changes in other blood parameters such as red blood cell count, MCV, and plasma proteins, but showed no changes in hematocrit. However, a significant increase in apparent viscosity at high shear rates and at a hematocrit of 70%

could suggest that the increased temperature also has an influence in increasing blood viscosity hence lowering the blood flow to tissues.

Further detailed studies are necessary in the future to elucidate: 1) why increased plasma protein concentration occurred in the hypoxic group, 2) why decreased red blood cell numbers occurred in the temperature group, and 3) the physiological role of oxygen on red blood cell deformability.

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Effects of stress on blood viscosity and other blood parameters in striped bass, *Morone saxatilis* (Walbaum)
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Date Received