Asian Journal of Chemistry Vol. 20, No. 3 (2008), 2264-2274

Investigation on Relations of Blood Chemistry Parameters in Bluefish (*Pomatomos saltatrix* L. 1758) using Multidimensional Scaling Technique

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The relationships among various blood chemistry parameters of Bluefish (*Pomatomus saltatrix* L. 1758) were investigated by multi-dimensional scaling technique (MDS). The results of the MSD technique showed that blood chemistry parameters were affected by seasonal changes, total number of parameters in the model and the interactions between the parameters. The relationships among various parameters were more apparent in winter with a coefficient of 87.8 %, which was followed by fall with 70.5 % and spring with 69.9 %. The results of the statistical analysis also indicated that direct bilirubin, total bilirubin, albumin, uric acid, total protein and low-density lipoproteins parameters of winter Bluefish were associated or similar with each other. The blood proteins did not seem to be related to any of the blood minerals except for K in winter. It was found that the relationships among Cl, K, Na and creatine and between uric acid and cholesterol were high in the fall caught Bluefish. The relationships among the blood parameters of the spring caught Bluefish suggested that high-density lipoproteins, total proteins and cholesterol parameters were associated with each other. It is concluded that MDS may be a valuable tool to study relationships and interactions between various blood chemistry parameters and exogenous factors.

Key Words: Bluefish (*Pomatpmus saltatrix* L. 1758), Serum blood, Multidimensional scaling, Blood biochemistry parameters, Seasonal changes.

INTRODUCTION

Blood parameters are biological indicators of physiological status in fish. They are affected by various factors, such as age, length, weight, sex, maturity, feeding habit, season, temperature, pH, infectional diseases, toxic factors, medication, capture technique and stress that is caused by handling and transportation[1-14]. Increased use of blood chemistry parameters has recently led to the use of various statistical analysis methods to obtain information from a given set of data. However, the requirement for monitoring

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multiple parameters to assess their potential effects on fish has often resulted in difficulties to establish meaningful relationships due to interactions between various parameters and exogenous factors that can influence blood chemistry. A potential solution to this problem is the use of multivariate analysis methods. Multivariate analytical techniques have become relatively common as the tools of data evaluation in biology to obtain meaningful information that is hidden in a set of data. By using these techniques, it is also possible to present the data in an easy and understandable format[15-17].

The aim of this study was to explore the potential use of multidimensional scaling technique (MDS) in investigating the relations among various blood parameters of bluefish (*Pomatamus saltatrix* L.1758). MDS provides a visual representation of the pattern of similarities and/or dissimilarities among a set of variables. Briefly, MDS plots the variables on a map such that variables that are very similar to each other are placed near each other whereas the ones that are very different from each other are placed away from each other on the map. The points that are close to each other in the map indicate relationships between the pairs as well as similarity of behaviour with respect to the remaining variables or objects[18].

In this study, Bluefish was used as a model species to establish relationships between various blood chemistry parameters using MDS technique. Bluefish is an economically important species in Turkey. It is available year round, particularly during winter and spring running from the Black Sea down to the Aegean Sea. The data presented here also lays the basis for reference values of blood chemistry parameters for this species.

EXPERIMENTAL

This study was carried out between September 2002 and May 2003 in the Dardanelles (Canakkale), which is located between the Aegean Sea and the Sea of Marmara in Turkey. Fish were captured by professional anglers from three stations (Fig. 1). 30 Bluefish were captured in each season. Blood samples were taken immediately after capture. The fish were then weighed to the nearest 0.01 g and measured (total length) to the nearest mm. The samples were collected from the caudal vessel using a 5 mL plastic syringe, which was placed in polypropylene tubes and transferred in an ice box. Samples were analyzed within 2 h following sampling. The analyses were carried out by an enzymatic auto analyzer.

Biochemical analyses: The blood samples were centrifuged at 4000 rpm for 10 min and the extracted serum samples were analyzed by auto analyzer (ILab 900 and 1800) using a method similar to that described by Bricknel *et al.*[19]. Biochemical measurements were carried out for urea, creatine (cre), uric acid (ua), total protein (tp), albumin (alb), globulin (glb),

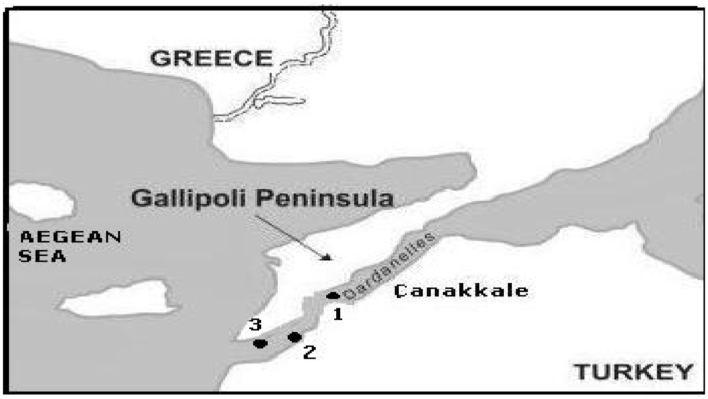

Fig. 1. Map of the dardanelles (1, 2 and 3 represent sampling stations)

total bilirubin (tbil), direct bilirubin (dbil), indirect bilirubin (ibil), cholesterol (chol), tryglyceride (tg), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), amilaz, sodium, potassium, chloride, phosphorus, iron and calcium.

TABLE-1
CHEMICAL PROPERTIES OF WATER

Months	NO_2 (mg/L)	NO_3 (mg/L)	NH_4 (mg/L)	Temp. (ºC)	Salinity (%)
January	0.0020	0.200	0.023	9.5	22.0
February	0.0032	0.106	0.080	8.5	23.0
March	0.0021	0.041	0.031	10.0	21.0
April	0.0105	0.109	0.009	17.5	23.0
May	0.0015	0.145	0.045	14.0	21.0
September	0.0013	0.192	0.002	23.0	23.0
October	0.0009	0.177	0.002	17.5	22.0
November	0.0008	0.158	0.003	15.5	21.0
December	0.0007	0.108	0.002	11.0	22.0

Statistical method: Multidimensional scaling technique (MDS) was used to investigate the relationships among the blood chemistry parameters of Bluefishes. MDS is a technique for finding a configuration of points in low dimensional space. Essentially, the purpose of MDS is to provide a visual representation of the pattern of proximities (*i.e.*, similarities or distances) among a set of objects. MDS plots the objects on a map such that objects that are very similar to each other are placed near each other on the map and objects that are different from each other, are placed far away from each other on the map. Briefly, the points that are close to each other in the map indicate relationships between the pairs as well as similarity of behaviour with respect to the remaining variables or objects. MDS is primarily concerned with the representation of objects as a configuration of points, usually in two-dimensional maps, in such a way that maximizes the fit between the proximity measure of each pair of variables (or objects) and the distances between all of them in the map. The points located in the graphs reproduce distances between each pair, controlled by the distance of each variable (object) with each one of the remaining variables (objects). According to distances, configuration distances are computed by helping of follow linear regression equation:

$$d_{ij} = a + bd_{ij} + e \quad (1)$$

where a: is the constant, b: regression coefficient or slope and e: is the residual term.

The degree of correspondence between the distances among points implied by MDS map and the matrix input by the user is measured (inversely) by a stress function. The general form of these functions is as follows:

$$\text{Stress} = \sqrt{\frac{\sum\sum\left(f(X_{ij}) - d_{ij}\right)^2}{\text{Scale}}} \quad (2)$$

where, d_{ij} refers to the euclidean distance, across all dimensions, between points i and j on the map, $f(X_{ij})$ is some function of the input data and scale refers to a constant scaling factor, used to keep stress values between 0 and 1. When the MDS map perfectly reproduces the input data, $f(X_{ij}) - d_{ij}$ is for all i and j, so stress is zero. Thus, the smaller the stress, the better the representation. Kruskal[20] suggests the stress be informally interpreted according to the following guidelines.

TABLE-2
STRESS COEFFICIENTS FOR GOODNESS OF FIT

Stress	Goodness of fit
≤ 0.20	Poor
0.100-<0.200	Fair
0.050-<0.100	Good
0.025-<0.050	Excellent
0.000-<0.025	Perfect

Generally, classical MDS employs euclidean distance to model dissimilarity. That is, the distance dij between points i and j is defined as

$$d_{ij} = \sqrt{\sum\left(X_{ia} - X_{ja}\right)^2}$$

where X_i specifies the position (coordinate) of point i on dimension a.

RESULTS AND DISCUSSION

Descriptive statistics of blood chemistry parameters are presented in Table-3. Multidimensional scaling analysis using these blood chemistry parameters provided good separation of the parameters in terms of creating the relationships (Figs. 2-7).

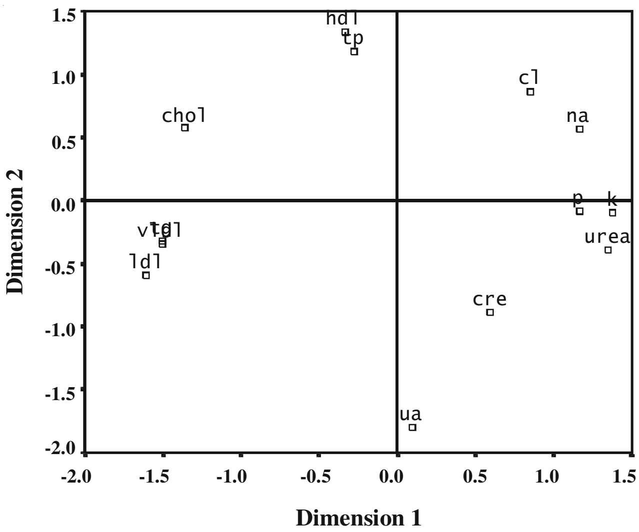

Fig. 2. Derived Stimulus Configuration Euclidean Distance Model (Spring)

Data in Fig. 6, which was plotted to investigate the relationships among blood chemistry parameters of the winter caught Bluefish indicates that dbil, tbil, alb, ua, tp and LDL parameters were associated or similar with each other. Fe, Cl, P, AST, Na, ALT and Ca also followed a similar pattern. Chol, urea, tg, cre, glb, K and VLDL behave in a way akin to those above. ALP and Amilaz activities were, on the other hand, likely related with each other. A similar relation was also present for LDH, HDL and ibil.

In general, blood proteins did not seem to be related to any of the blood minerals except for K. The results of the analysis implied that the blood minerals are especially associated with AST and ALT enzymes. Bluefish with high Fe, Cl, P, Na and Ca concentrations seemed to have high ALT and AST enzyme activities. These results suggest that blood proteins are effective on blood lipids, while blood minerals are effective on blood enzymes. It is probable that the effect of blood minerals on ALT and AST enzymes or *vice versa* is more pronounced. R^2 value of the blood parameters of winter Bluefish was found to be 87.8 %. R^2 value, which was generated in this way is an indicator in demonstrating that much of the variation (87.8 %) in blood parameters could be explained *via* $d_{ij} = a + b\delta_{ij} + e$.

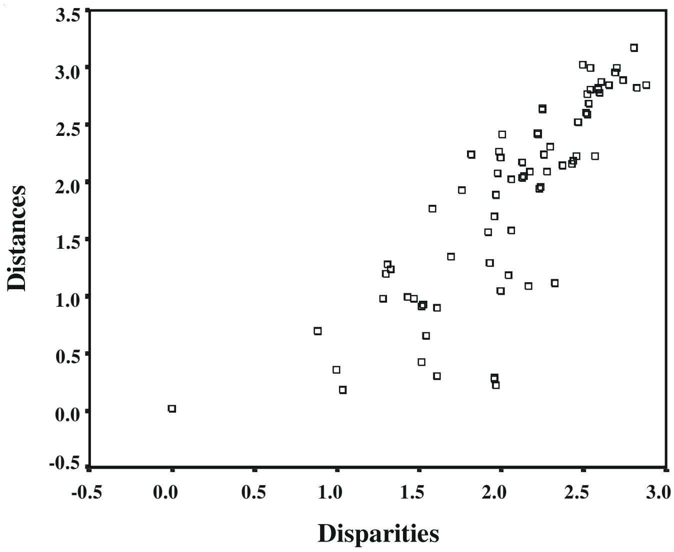

Fig. 3. Scatterplot of linear fit euclidean distance model (Spring)

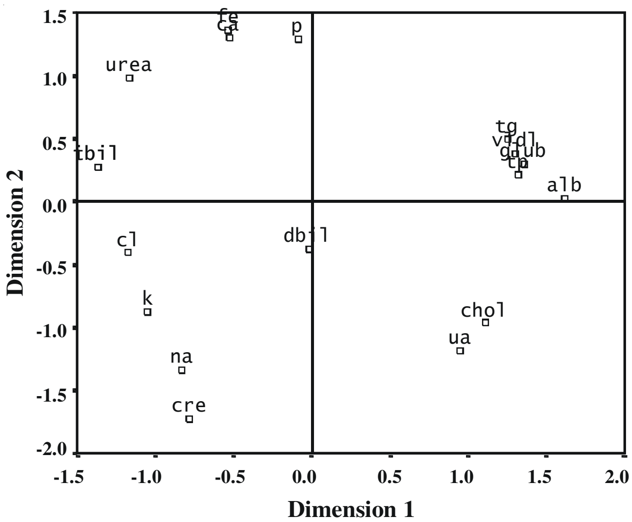

Fig. 4. Derived stimulus configuration euclidean distance model (Fall)

The stress coefficient, which is another indicator of goodness of fit, was calculated as 0.0026. Thus, we conclude that MDS analysis technique is a suitable technique for investigating the relationships among the blood chemistry parameters. This can also be seen in the examination of Fig. 7.

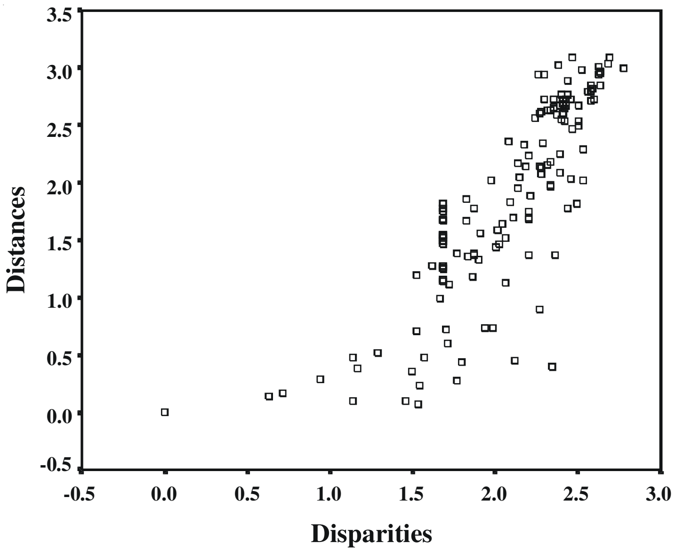

Fig. 5. Scatterplot of linear fit euclidean distance model (Fall)

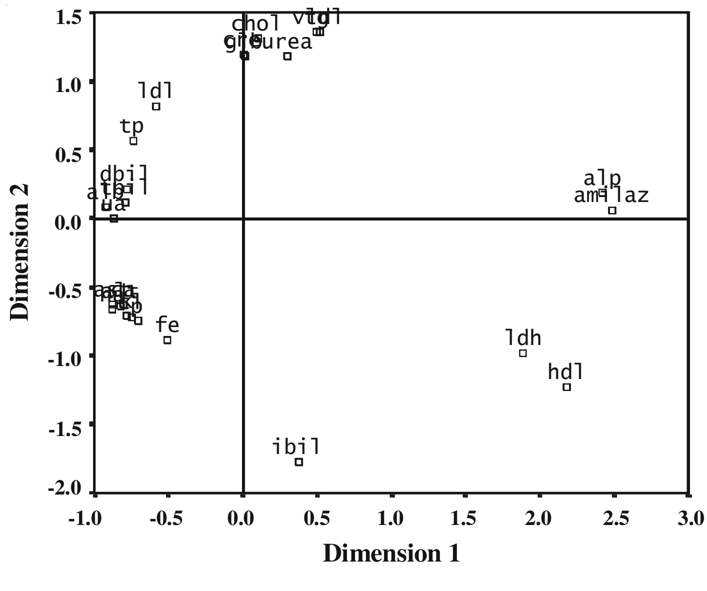

Fig. 6. Derived Stimulus Configuration Euclidean Distance Model (Winter)

Data which was plotted to investigate the relationships among the blood chemistry parameters of the fall caught Bluefish suggested that the relationships among Cl, K, Na and cre are high (Fig. 4). Similarly the relationship between ua and chol are also high. Tg, VLDL, alb, tp and glb followed

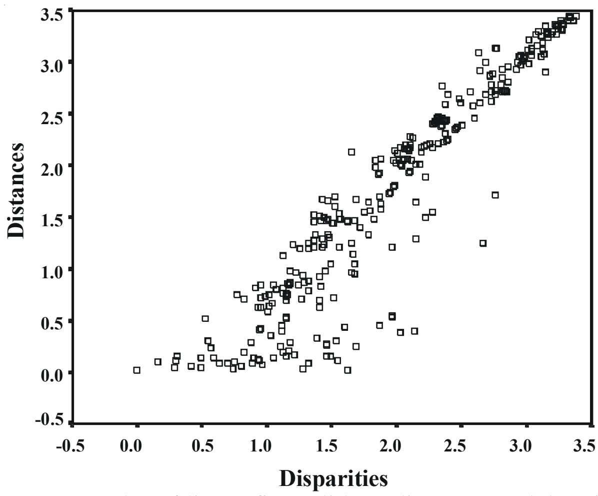

Fig. 7. Scatterplot of linear fit euclidean distance model (Winter)

a similar pattern to those above. Likewise, ibil, tbil, urea, Fe, Ca and P seemed to bear similarities. On the other hand, the relationships between dbil and other blood parameters are not high.

R^2 value of the blood parameters of the fall caught Bluefish was found be 70.5 %. The stress coefficient was calculated as 0.097. When R^2, the stress coefficient and Fig. 5 are evaluated altogether, the relationships among blood parameters (goodness of fit value) was found to be lower compared to that of the winter caught Bluefishes.

The examination of Fig. 2, which was plotted to investigate the relationships among the blood parameters of the spring caught Bluefish suggested that HDL, tp and chol parameters were associated with each other. Urea, ua, P, K and cre followed a similar pattern. LDL, tg and VLDL and Ca and Cl behaved in a way similar to those above. R^2 value of the blood parameters of the spring caught Bluefish was found 69.9 %. The stress coefficient which is an indicator of the goodness of fit, was calculated as 0.20. When R^2, the stress coefficient and Fig. 3 are evaluated altogether, the goodness of fit value was found to be lower compared to those of the fall and the winter caught Bluefish.

The results of the present study demostrate that MDS technique is suitable for investigating the relationships among the blood chemistry parameters of Bluefish year around. The visual interpretation of the blood parameters is straight-forward and provides quick and easy identification onconflicting and correlating parameters and their interactions with each other. A major disadvantage of other multivariate analysis techniques, such as Discrimenant Analysis, Cluster Analysis, Factor Analysis or Principal Component Analysis, is the requirement for a set of data to be multivariate normal with linear relationships. In contrast, MDS does not impose such restrictions and can be applied to similarities or dissimilarities.

TABLE-3
DESCRIPTIVE STATISTICS OF BLOOD PARAMETERS BY SEASONS

Season	Spring		Fall		Winter	
Blood proteins	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$
Urea	6.44	0.76	12.66	0.40	8.25	0.92
Creatine (mg/dl)	0.56	0.05	0.22	0.04	0.58	0.05
Uric acid (mg/dl)	1.25	0.24	4.46	0.24	2.62	0.21
Total protein (g/dl)	2.99	0.14	4.90	0.12	4.74	0.16
Albumin (g/dl)	*	*	1.64	0.04	1.43	0.07
Globulin (g/dl)	*	*	3.41	0.09	3.15	0.13
Total bilirubin (mg/dl)	*	*	0.12	0.01	0.50	0.07
Direct bilirubin (mg/dl)	*	*	0.10	0.00	0.54	0.08
Indirect bilirubin (mg/dl)	*	*	0.02	0.01	0.00	0.00
Blood Lipids	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$
Cholesterol (mg/dl)	267.10	13.46	426.86	9.53	276.12	9.60
Triglycerides (mg/dl)	202.67	21.00	506.00	28.61	178.92	20.50
HDL (mg/dl)	132.93	8.26	*	*	161.63	5.88
VLDL (mg/dl)	40.27	4.05	97.53	7.10	36.18	4.06
LDL (mg/dl)	92.86	8.42	*	*	84.70	9.59
Blood Enzymes	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$
AST (IU/L)	*	*	*	*	195.13	20.89
ALT (IU/L)	*	*	*	*	35.80	4.02
LDH (IU/L)	*	*	*	*	315.07	32.53
ALP (IU/L)	*	*	*	*	10.50	0.43
Amilaz (U/L)	*	*	*	*	156.54	6.11
Blood Minerals	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$	$\overline{X}$	$S_{\overline{X}}$
Sodium (mmol/L)	303.08	14.61	266.53	7.10	309.96	8.53
Potassium (mmol/L)	38.09	3.88	24.19	2.20	33.76	1.64
Chlorine (mmol/L)	176.76	6.44	194.80	1.63	183.88	5.96
Phosphorus (mg/dl)	12.845	1.23	7.56	1.09	21.11	1.53
Iron (mg/dl)	*	*	76.04	4.08	23.42	4.20
Calcium (mg/dl)	*	*	1.90	0.11	1.00	0.16

*Not determined.

In accordance with previous studies[7,17,21], the present study points out that the blood chemistry parameters vary according to seasonal changes, the number of blood parameters in the model and interactions among blood parameters. For example, while the blood proteins had no effects on blood minerals concentrations of the winter caught bluefish (except K^+), they affected mineral concentrations of the fall caught bluefish. In addition, as the season progressed (winter to spring), the relationships among the blood parameters became less apparent. This could be due to the effect of season or lack of enzyme activity during winter since the relations between different parameters can be influenced by environmental factors such as seasonal changes, the number of blood parameters examined, or the interrelations between the different blood parameters, or by the relationships between other parameters which are included in the model.

The results of present study are similar to those of others with respect to total protein[22]. However, the amount of albumin differs. The difference between these two studies could be attributed to the difference in fish species used in the studies. Jayaram and Beamish[23] and McKim *et al.*[24] reported that the level of blood plasma total protein of *Salvelinus namaycush*, a cold water fish species like Bluefish, decreased when the water temperature increased indicating the influence of water temperature on the physiological status of this animal. The results of present study also suggested that there was high a correlation between triglycerides and cholesterol concentrations of the winter caught Bluefish (Fig. 6). The present results support the findings of Karagül *et al.*[18].

Jeon *et al.*[25] pointed out that triglycerides, cholesterol and lipid components are affected by fish species. In their study, the albumin concentration for cold water species varied between 1.2 and 1.9 gd L^{-1}. Means for cholesterol concentrations of *Oncorhynchus kisutc* and *Oplegnathus fasciatus* fishes were 300 gd L^{-1}. Shimma *et al.*[3] reported that the total protein and cholesterol levels of the winter caught *Oncorhynchus mykiss* were 4.0 ± 1.2 and 280.1 mgd L^{-1}, respectively. The total protein and cholesterol levels of this species, which was caught in fall were 4.8 ± 0.7 and 342 ± 80 mgd L^{-1}, respectively. In present study, the albumin level was found to vary between 1.4370-1.6467 gd L^{-1}. On the other hand, the cholesterol levels of the spring, fall and winter caught Bluefish (*P. saltatrix*, L.1758) were found to be 267.1034 ± 13.4673, 426.86 ± 9.53 and 276.12 ± 9.6 mgd L^{-1}, respectively. Therefore, cholesterol levels in Bluefish change depending on the season.

The high concentrations of serum K^+ in bluefish may have been due to capture method. Substantial increases in serum K^+ levels were reported in other fish species as a result of heavy exercise, intracellular acidification and acute stress[26].

In conclusion, the present study demonstrates that MDS technique can, successfully, be used to investigate relations between various blood parameters in fish. The present findings are similar to those of others with some minor differences which may be a factor of fish species, number of blood chemistry parameters, differences among environmental factors or the application of different statistical analysis techniques. Further research is required to investigate the effect of other factors on the blood chemistry parameters.

The results of this study have important implications for the biology of other fish species. Multidimensional scaling method can be used to investigate relationships in other research areas such as aquaculture and population dynamics where multiple parameters are involved.

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(*Received*: 13 June 2007; *Accepted*: 1 December 2007) AJC-6103