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According to Canonical Correlation, the Evaluation of Bluefish (*Pomatomus saltatrix*) Blood Chemistry

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Abstract: Blood chemistry parameters can provide essential information on the physiological status of the animal and therefore allow accurate evaluations of the general health status. Canonical correlation analysis is a fundamental statistical tool. The goal of canonical correlation analysis is to evaluate the relative contribution of each variable to the derived canonical functions in order to explain nature of the relationships. CCA was used to determining, whether the blood protein parameters are related in any way to the blood lipids, enzymes, minerals. However, a linear association between predictor variables (blood proteins) and dependent variables (lipids, enzymes and minerals) were determining. These analyses results shown that canonical correlation analysis can be using prediction of relationships from blood proteins with other blood chemistry parameters.

Key words: Bluefish, *pomatomus saltatrix*, canonical correlation, blood chemistry, animals, enzymes

INTRODUCTION

The evaluation of blood chemistry parameters in animals is a routine and important tool in clinical practices. This simple technique can provide essential information on the physiological status of the animal and therefore, allow accurate evaluations of the general health status. However, the predictive value is compromised by the lack of reliable normal databases and available reference laboratories to properly analyze these samples (Berg and Bremset, 1998). The inference between these chemical measurements to certain diseases is mostly borrowed from experience in mammalian systems and only a few blood chemistry parameters have been confirmed experimentally, primarily in major fish species. In addition to our limited knowledge of blood chemistry of fish, the issue is further compounded by reports involving different sampling protocols as well as which parameters are determined. Moreover, many other factors including environmental (temperature, photoperiod, stock density, salinity) and physiological (reproductive cycle, age, gender, nutrition) have been reported to impact on blood parameters of fish. All of these have contributed to a limited use of blood chemistry parameters as a tool in fish health management.

It is well known that intensive fish culture is often accompanied by increased incidences of pathologies. Many studies have demonstrated the usefulness of hematology and blood biochemistry in the assessment of fish health and as a biomarker of exposure to pollution (Bricknell *et al*., 1999). Previous information on hematology and blood biochemistry in fish is fragmentary (Collazos *et al*., 1998; Craig, 1977; De Pedro *et al*., 1998). Exposures to environmental seasonal cycles in light, temperature and food availability are likely to affect blood and body composition. Indeed, seasonal changes in body composition, hematology and blood biochemistry have been described in several fish species (Groff and Zinkl, 1999; Handy and Depledge, 1999; Itazawa, 1957; Johnson and Wichern, 1988; Jonsson and Jonsson, 1998; Knoph and Masoval, 1996) but little is known to date on seasonal variations in body composition, hematology and blood biochemistry in fish.

Canonical Correlation Analysis (CCA) is a fundamental statistical tool. Canonical variables are linear combinations of the original quantitative measurements that contain the highest possible multiple correlation with each group and that summarize among-class variation (Leonard and McCormick, 1999).

The goal of CCA is to evaluate the relative contribution of each variable to the derived canonical functions in order to explain nature of the relationship(s). Consider the following two equations:

$$U_m = a_{m1}X_1 + a_{m2}X_2 + a_{mp}X_p \quad (1)$$

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$$V_m = b_{m1}Y_1 + b_{m2}Y_2 + ... + b_{mp}Y_q \quad (2)$$

Equation 1 and 2 gives the new variables U_m and V_m, which are a linear combination of the X (pre-slaughter) and Y (after slaughter) variables respectively. Let C_m be the correlation between U_m and V_m.

The objective of canonical correlation is to estimate a_{m1}, a_{m2}... a_{mp} and b_{m1}, b_{m2}... b_{mq} such that C_m is maximum. Equation 1 and 2 are the canonical equations, U_m and V_m are the canonical varieties and C_m is the canonical correlation (Master *et al*., 1990).

In the study, CCA was used to determine whether the blood protein parameters are related in any way to the blood lipids, enzymes, minerals. From canonical correlation, a linear association between predictor variables (blood proteins) and dependent variables (lipids, enzymes and minerals) were determined.

MATERIALS AND METHODS

Sample collection was monthly performing 3 different areas between December and February on Dardanelles (Fig. 1).

In each collection was caught 30 Bluefish (*Pomatomus saltatrix*) caught, weighed and measured. Mean Bluefish sizes were measured 30.62±0.20 cm and 349.89±8.26 g. Some chemical properties of sea water were shown in Table 1.

Immediately after capture fish were cleaned to prevent mucus contamination and blood samples were collected.

Blood was sampled from the caudal vein using a gauge needle and 5 mL syringe and than later transported laboratory. Chemical analyses were conducted using an enzymatic auto analyzer (Svoboda *et al*., 2001; Val *et al*., 1998; Rowley *et al*., 1988).

Blood samples were centrifuged for 10 min at 6.67×10^{-8} g and the extracted serums were analyzed using ILab 900 and 1800 auto analyzer (Xia, 2008).

Biochemical parameters analyzed Urea (U), Creatine (CR), Uric Acid (UA), Total Protein (TP), Albumin (ALB), Globulin (GLB), Total Bilirubin (TBL), Direct Bilirubin (DBL), Indirect Bilirubin (IBL), Cholesterol (CHL), Triglycerides (TRG), High-Density Lipoproteins (HDL), Low-Density Lipoproteins (LDL), Very Low-Density Lipoproteins (VLDL), Alanine Amino Transferase (ALT), Aspartate amino Transferase (AST), Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), Amylase (AMY), Sodium (Na), Potassium (K), Chloride (Cl), Phosphorus (P), Iron (Fe) and Calcium (Ca). Statistical analyses were performed with SAS PROC CANCORR.

Table 1: Chemical properties of sea water on Dardanelles

Months	NO_2	NO_3	NH_4	Temperature (°C)	Salinity (%)
December	0.0007	0.108	0.002	11	22
January	0.0020	0.200	0.023	9.5	22
February	0.0032	0.106	0.08	8.5	23

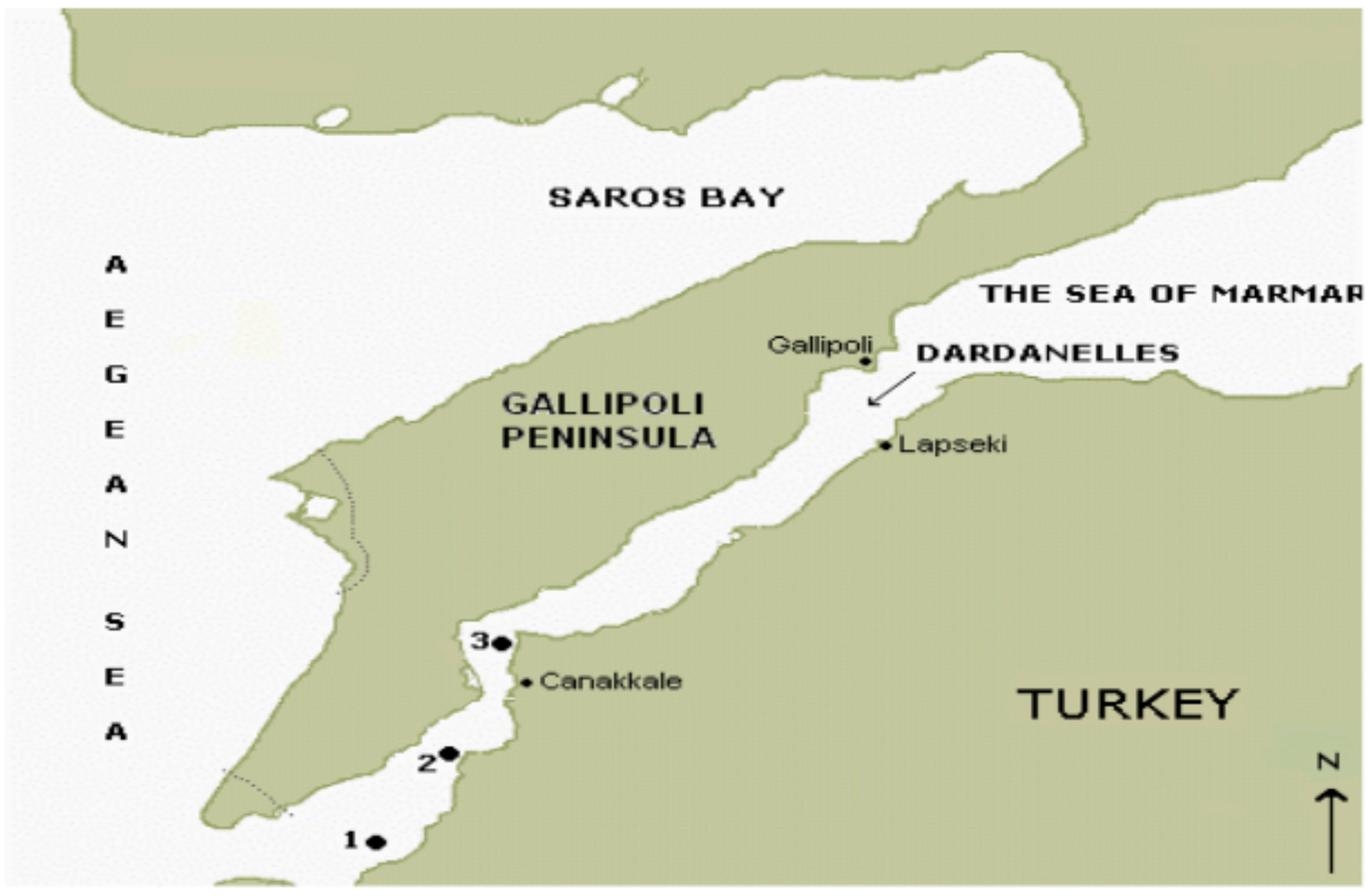

Fig. 1: Map of Dardanelles (1, 2, 3: represent stations where Bluefish caught)

RESULTS

Table 2 shows the correlations among the blood proteins and lipids and enzymes (original variables). The correlations between the blood proteins and lipids parameters are moderate, the largest being 0.87 between TP and LDL and followed by TBL and CHL (0.82) and TBL and LDL (0.80).

The correlations between the blood proteins and enzymes are also moderate, the largest being 0.77 between DBL and ALP and 0.75 between IBL and alp. The correlations between the blood proteins and minerals are small, the largest being 0.54 between UA and Na, DBL and Na and DBL and Na (Table 3). Table 4 displays the correlations of blood lipids with enzymes and minerals. The correlations between the blood lipids and enzymes are moderate, the largest being -0.90 between alt and Na followed by the correlation between ALP and Na (0.82). On the other hand, the correlations between the blood lipids and minerals are small, the largest being -0.57 between HDL and Na.

The correlations among the enzymes and minerals are moderate, the largest being -0.90 between alt and Na followed by the correlation between ALP and Na (Table 5). It was shown that the 0.997 calculated canonical correlation between blood proteins and blood lipids was significant ($p<0.01$). Relationship among the blood protein parameters of bluefish were expressed as following and canonical correlation was shown in Table 6.

Table 2: Correlations among the blood proteins and lipids and enzymes

	Lipid/enzymes									
Proteins	CHL	TRG	HDL	VLDL	LDL	AST	ALP	LDH	ALT	AMY
U	0.17	0.30	-0.43*	0.30	0.33	0.52*	0.38	-0.13	-0.37	-0.25
CR	0.15	-0.02	-0.37	-0.01	0.09	0.06	0.07	-0.41	-0.11	-0.44*
UA	0.55**	-0.07	-0.32	-0.07	0.61*	0.38	0.50*	-0.22	-0.40*	-0.33
TP	0.72**	0.53**	-0.58**	0.52**	0.87**	0.29	0.43*	-0.55*	-0.26	-0.30
ALB	0.40*	0.36	-0.59**	0.36	0.62**	0.44*	0.50*	-0.58*	-0.33	0.50*
TBL	0.82**	0.59**	-0.32	0.58**	0.80**	0.05	-0.01	-0.27	0.01	-0.08
DBL	0.08	0.02	-0.47*	0.02	0.23	0.53*	0.77**	-0.43	-0.38	-0.53*
IBL	0.11	0.32	-0.55**	0.32	0.32	0.57*	0.75**	-0.49	-0.42*	-0.53*

Table 3: Pearson-moment correlations among the blood proteins and minerals

	Minerals					
Proteins	Na	K	Cl	P	Fe	Ca
U	0.31	0.19	0.20	0.07	-0.04	0.25
CR	0.26	0.25	0.10	-0.10	0.07	0.17
UA	0.54**	0.37	0.41*	0.37	0.15	0.49
TP	0.44*	0.30	0.33	0.40*	0.43*	0.48*
ALB	0.48*	0.26	0.20	0.29	0.34	0.46*
TBL	0.05	0.19	0.25	0.30	0.42*	0.29
DBL	0.54**	0.03	0.23	0.25	0.11	0.42*
İBL	0.54**	0.09	0.27	0.29	0.24	0.48*

Table 4: Pearson-moment correlations among the blood lipids and enzymes and minerals

	Enzyme/minerals										
Lipids	AST	ALP	LDH	ALT	AMY	Na	K	Cl	P	Fe	Ca
CHL	-0.27	-0.17	-0.39	0.18	0.12	-0.07	0.14	0.14	0.15	0.28	0.10
TRG	0.13	-0.03	-0.33	-0.05	0.05	-0.03	0.10	0.06	0.18	0.31	0.16
HDL	-0.64**	-0.60**	0.41	0.53**	0.42*	-0.57*	-0.33	-0.25	-0.28	-0.19	-0.41*
VLDL	0.14	-0.01	-0.33	-0.04	0.04	-0.03	0.09	0.06	0.17	0.30	0.15
LDL	0.17	0.18	-0.43	-0.24	-0.12	0.33	0.43*	0.37	0.39	0.38	0.41*

Table 5: Pearson-moment correlations among the blood enzymes and minerals

	Minerals					
Enzyme	Na	K	Cl	P	Fe	Ca
AST	0.69**	0.55**	0.39	0.40	-0.02	0.43*
ALP	0.82**	0.30	0.33	0.31	-0.09	0.49*
LDH	-0.39	-0.25	0.04	0.01	0.04	-0.14
ALT	-0.90**	-0.70**	-0.65*	*-0.52**	-0.25	-0.66**
AMY	-0.68**	-0.25	-0.24	-0.10	0.11	-0.35

*p<0.05,**p<0.01

Table 6: Canonical Correlation Coefficients, R^2 and p-values

C.Cr.	R^2	p-values	Canonical varieties
Proteins: Lipids			
0.997	0.994	0.00	V_1=0.4788X1+0.4495X2+0.3802X3+1.5272X4-0.1300X5-1.1109X6+0.2057X7-0.8064X8-0.5642X9
			W_1=0.2397Y1-18.8105Y2-0.9316Y3+18.3536Y4+0.3796
Proteins: Enzymes			
0.998	0.996	0.00	V_1=-8.784X1-4.109X2+12.32X3-21.33X4+17.9X5+1.56X6+0X7+0X8+0X9
			W_1=-0.457Y1+1.20Y2+1.22Y3-0.481Y4+0.252Y5
Proteins: Minerals			
0.989	0.979	0.00	V_1=0.3168X1+0.3618X2-0.4957X3+1.4799X4-1.087X5+0.2438X6-0.1714X7-0.5541X8+0.1853X9
			W_1=-1.0305Y1+1.1741Y2+1.0265Y3-0.2967Y4+0.2515Y5-0.6448Y6
Lipids: Enzymes			
0.246	0.06	0.55	V_1=-0.58X1-21.52X2+4.45X3+22.86X4+3.26X5
			W_1=-1.35Y1+1.91Y2-0.61Y3-0.06Y4+0.45Y5
Lipids: Minerals			
0.955	0.914	0.00	V_1=-0.69X1-2.46X2+0.14X3+2.57X4+1.56X5
			W_1=0.23Y1+0.83Y2-0.46Y3+0.13Y4+0.25Y5+0.22Y6
Enzymes: Minerals			
0.76	0.578	0.096	V_1=-2.01X1+0.82X2-0.95X3+0.81X4-1.67X5
			W_1=-0.13Y1+1.87Y2-14.01Y3+0.29Y4+4.05Y5+8.92Y6

$$V_1 = 0.4788U+0.4495CR+0.3802UA+1.5272TP-0.1300ALB-1.1109GLB+0.2057TBL-0.8064DBL-0.5642IBL$$

While relationship among the blood lipid parameters were expressed as following equation:

$$W_1 = 0.2397CHL-18.8105TRG-0.9316HDL+18.3536VLDL+ 0.3796LDL$$

DISCUSSION

Evaluation of the relationship between these parameters the above equations was sufficient. R^2 value of the canonical correlation is 99.4%. When coefficients of the V_1 and W_1 equations were examined, it was seen that while CHL, VLDL and LDL lipids were affected as positively from U, CR, UA, TP and TBL, while TRG and HDL were negatively affected. It can be said that TP with a contribution of 1.5272 is the most determinative protein in the increase in CHL, VLDL and LDL lipids. On the other hand, the cholesterol, VLDL and LDL lipids were affected as negatively from ALB, GLB, DBL and IBL proteins. These results suggested that, those bluefish with the higher U, CR, UA, TP and TBL tend to also have higher CHL, VLDL and LDL, while they tend to low TRG and LDH. Likewise, those bluefish with the higher ALB, GLB, DBL and IBL also have TRG and HDL.

It was shown that the 0.998 calculated canonical correlation between blood proteins and blood enzyme was significant ($p<0.01$). R^2 value of the canonical correlation is 99.6%. When coefficients of the V_1 and W_1 canonical varieties were examined, it was seen that while ALT, LDH and AMY enzymes were affected as positively from UA, ALB and GLB, AST and ALP were negatively affected. On the other hand, TBL, DBL and IBL had no effect on any of the blood enzymes measured.

These results suggested that those bluefish with the higher UA, ALB and GLB tend to also have higher ALT, LDH and AMY, while they tend to low AST and ALP. Likewise, those bluefish with the higher U, CR and TP also have AST and ALP. It can be show that UA with a contribution of 12.32 is the most determinative protein in the increase in ALT, LDH and AMY enzymes, while UA with a contribution of 21.33 is the most determinative protein in the increase in AST and ALP enzymes.

It was shown that the 0.989 calculated canonical correlation between blood proteins and blood minerals was significant ($p<0.01$). R^2 value of the canonical correlation is 97.9%. When coefficients of the V_1 and W_1 canonical varieties were examined, it was seen that while Na, P and Ca enzymes were affected negatively from U, CR, TP, GLB and IBL, K, Cl and Fe were positively affected. These results suggested that those Bluefish with the higher U, CR, TP, GLB and IBL tend to also have lower Na, P and Ca while they tend to higher K, Cl and Fe minerals. At the same time, those bluefish with the higher UA, ALB, TBL and DBL also have Na, P and Ca minerals. It can be show that ALB with a contribution of 1.087 is the most determinative protein in the increase in Na, P and Ca minerals.

Canonical correlation coefficients for lipids-enzymes and enzymes-minerals were 0.246 ($p = 0.55$) and 0.76 ($p = 0.096$), respectively. However, the canonical correlation coefficient for lipids-minerals was 0.955 ($p<0.01$). R^2 value of the canonical correlation is 91.4%. When coefficients of the V_1 and W_1 canonical varieties were examined, it was seen that while Na, K, P, Fe and Ca

minerals were affected as negatively from CHL and trig or vice versa, Cl was positively affected. These results suggested that those bluefish with the higher CHL and TRG tend to also have lower Na, K, P, Fe and Ca minerals, while they tend to have higher Cl. At the same time, those Bluefish with the higher HDL, VLDL and LDL also have Na, K, P, Fe and Ca minerals. It can be said that VLDL with a contribution of 2.57 is the most determinative lipids in the increase in Na, K, P, Fe and Ca minerals.

CONCLUSION

As results of these analyses, CCA was used to determining whether the blood protein parameters are related in any way to the blood lipids, enzymes, minerals. However, a linear association between predictor variables (blood proteins) and dependent variables (lipids, enzymes and minerals) were determining.

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