Comparison of some blood parameters of rainbow trout (*Oncorhynchus mykiss*) living in running and still water

Coşkun O.F.[1*];Aydın D.[2];Duman F.[2]

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Abstract

The aim of this study was to compare some biochemical blood parameters of rainbow trout (*Oncorhynchus mykiss*) living in running or still waters. Some biochemical parameters of rainbow trout were examined, and the results were evaluated between sampling locations and according to seasonal changes. Healthy adult rainbow trout were caught from six different locations on the Bahçelik Dam Lake and the Zamantı River (Turkey). Blood samples were collected from the caudal vena cava and transferred to sample tubes. The blood serum samples were analyzed for 16 different biochemical parameters. In terms of the calculated blood parameters, no statistically significant differences were found between locations. However, seasonal changes affected the blood parameters significantly. alanine transaminase (ALT)was considerably higher in spring in fish caught from both the dam lake and the river. In the summer, aspartate transaminase (AST), Ca, urea, total protein and Fe were significantly higher than in other seasons. However, amylase was significantly lower in summer than in the other seasons. In the autumn, alkaline phosphatase (ALP) and triglyceride were significantly higher than in the other seasons. The results obtained from this study may be useful for research in fish biology and food safety.

Keywords: Bahçelik Dam Lake, Biochemical parameters, Zamantı River, *Oncorhynchus mykiss*.

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1-Department of Biology, Enstitute of Sciences, Erciyes University, 38039 Kayseri, Turkey.
2-Department of Biology, Faculty Sciences, Erciyes University, 38039 Kayseri, Turkey.
* Corresponding author's Email: ofcoskun1@hotmail.com

Introduction

Many environmental factors, such as water quality, pollution and diseases, may alter the physiological state of fish. Sensitive species may either migrate from a habitat or become locally extinct as a result of aquatic environmental pollution. In more resistant species, pollution causes the accumulation of a number of physiological, biochemical and genetic changes. Species in the upper trophic levels of the food chain may also accumulate pollutants in their bodies (Romanenko and Yevtushenko, 1985). Fish can be used to evaluate water quality and are bioindicators of environmental pollution (Borkovic *et al*., 2008). Fish blood parameters can be directly used to evaluate fish health (De Pedro *et al.,* 2005), and the chemical and physical properties of fish blood are highly sensitive to environmental change (Hughes and Nemcsok, 1988) and pollution (Yanik and Atamanalp, 2001). For example, factors such as disease and stress may cause significant changes in fish blood parameters (Cnaani *et al*., 2004; Chen *et al*., 2005). In addition, certain ecological factors, such as nutrition, may also lead to biochemical changes in blood parameters (Coz-Rakovac *et al*., 2005). Moreover, environmental factors, such as oxygen, water density, salinity, temperature and light, and physiological factors, such as age, species and reproductive state can all affect biochemical parameters in fish. In short, biochemical parameters function as an early indicator of stress in fish, and they can contribute to the determination of target organ toxicity.

There are many studies that have linked changes in fish blood parameters to environmental changes. However, these studies generally focus on cultured fish species (Lusková, 1997). Limited information is available regarding the biological response of fish in their natural environment, and information about the seasonal changes in the blood parameters of wild fish is sparse. According to Lusková (1997), data obtained from studies of fish in their natural environment are more realistic than laboratory studies (Lusková, 1997). Biochemical blood parameters in fish respond rapidly to any environmental change. When there is a reduction in a fish population, the nature of the change can be determined by analyzing the biochemical parameters of their blood, and appropriate protective measures can be implemented (Lusková, 1997).

Rainbow trout (*O. mykiss*) are the one of the most widely cultured freshwater fish species throughout the world as they grow rapidly, easily adapt to different environmental conditions (Talas and Gulhan, 2009) and have a high economic and nutritional value. Bahçelik Dam Lake is located on the Zamantı River, which joins the Goksu River to form the Seyhan River flowing into the Mediterranean Sea. There are several fish farms producing rainbow trout in the Bahçelik Dam Lake.

This study aims to a) analyse various biochemical blood parameters in healthy rainbow trout (*O. mykiss*) living in running and still water; b) determine whether season and sampling location influence blood parameters in fish; and c) compare the blood parameters of dam and river samples. It is anticipated that the findings will be used as a reference for other studies in aquaculture and aquatic ecology.

Materials and methods

Samples of healthy fish were caught from six designated locations (three dam locations and three river locations) on the Bahçelik Dam Lake and the Zamantı River. In each season (spring, summer, autumn and winter), three fish were caught from each location resulting in a total of 72 fish samples. At the time of sampling, water quality parameters were measured at all locations, including dissolved oxygen, pH, temperature and electrical conductivity.

Two mL of blood was drawn from the caudal vein of each fish shortly after collection, and the blood samples were stored in biochemical tubes without anticoagulant. The fish samples were evaluated after they were delivered to the laboratory, and blood samples were collected from fish that did not appear to have any infections or physical damage. Slowing or stopping blood circulation in fish has the ability to significantly increase protein levels (Hrubec and Smith, 1998), and therefore, blood samples were collected from fish in a short period of time. As a result of the potential haemolytic effects of anaesthetic treatments on the blood parameters, the fish were not anaesthetised (Smith and Hattingh, 1980). The tubes were centrifuged at 3,000 rpm for 15 minutes to obtain serum samples. The analysis of selected parameters of the serum samples was performed using a Roche Cobas 6000 auto-analyzer (IN, USA). The biochemical analysis of the blood samples was performed in the two-hour period following the blood collection. The following biochemical parameters were analyzed: glucose (GLC), cholesterol (CHOL), triglyceride (TG), total protein (TP), albumin (ALB), calcium (CA), urea, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), amylase, phosphorus (P), iron (Fe), potassium (K), sodium (Na) and chlorine (Cl).

For each blood parameter, the mean and standard deviation of triplicate measurements was calculated. The Kolmogorov-Smirnov test was used to determine whether the data was normally distributed, and Levene's test was used to test the homogeneity of the variances. In cases of heterogeneity, the data was re-evaluated after logarithmic transformation. One-way ANOVA was used to determine the significance of the differences between the groups, and a post-hoc Tukey's test was used to compare the mean values of each group. The statistical significance threshold was set at 95% (0.05). All statistical analyses were performed using SPSS 15.0 statistical software.

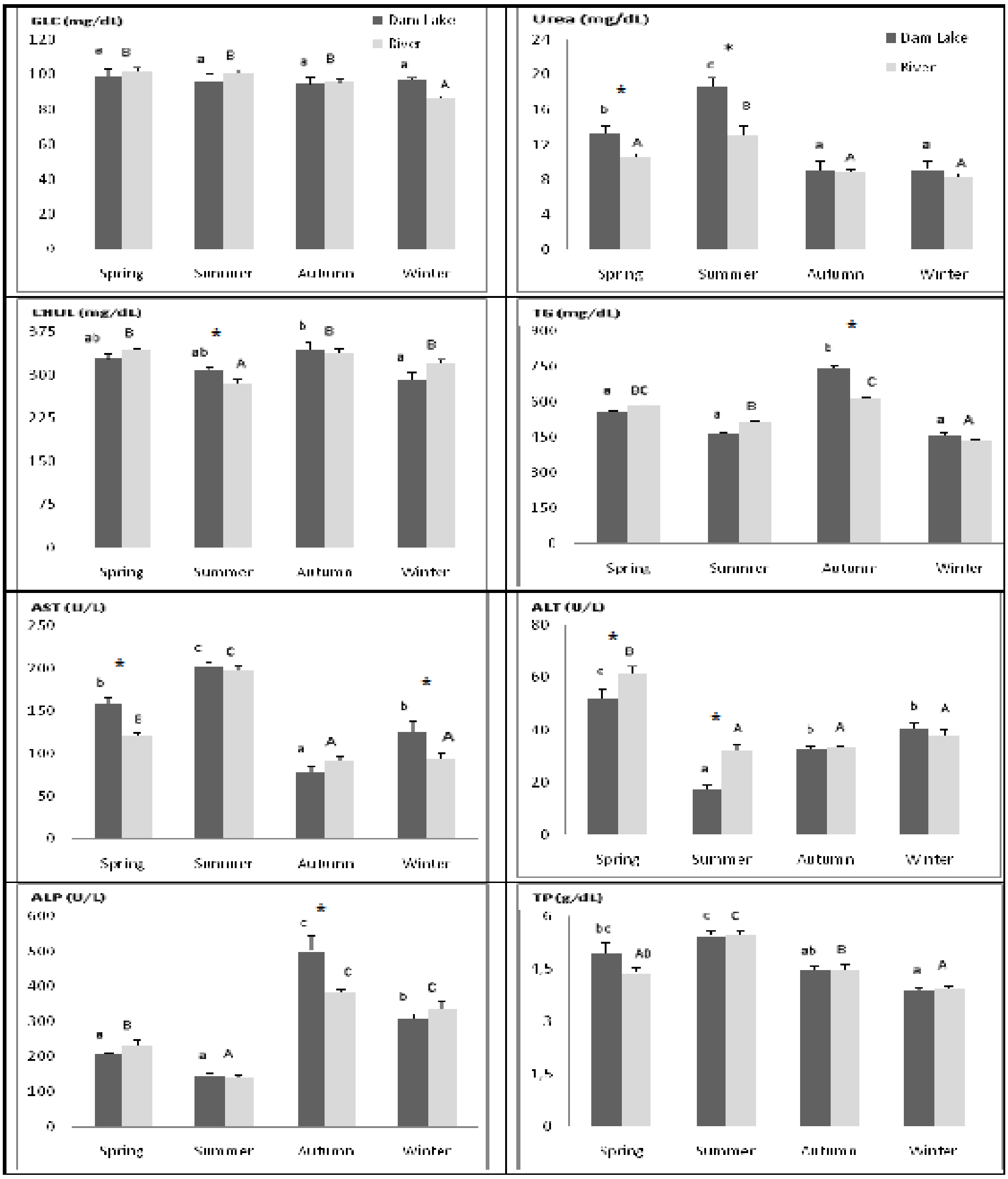

Figure 1: Seasonal values (Mean±S.E) of blood serum biochemical parameters (GLC, Urea, Chol, TG, AST, ALT,ALP and TP) of Rainbow Trout Fishes from Bahcelik Dam Lake And Zamantı River.

Different letters in the same location (Dam Lake and River) show the significant seasonal difference (*p*<0.05).

***shows the significant difference between Dam Lake and River samples in the same season.**

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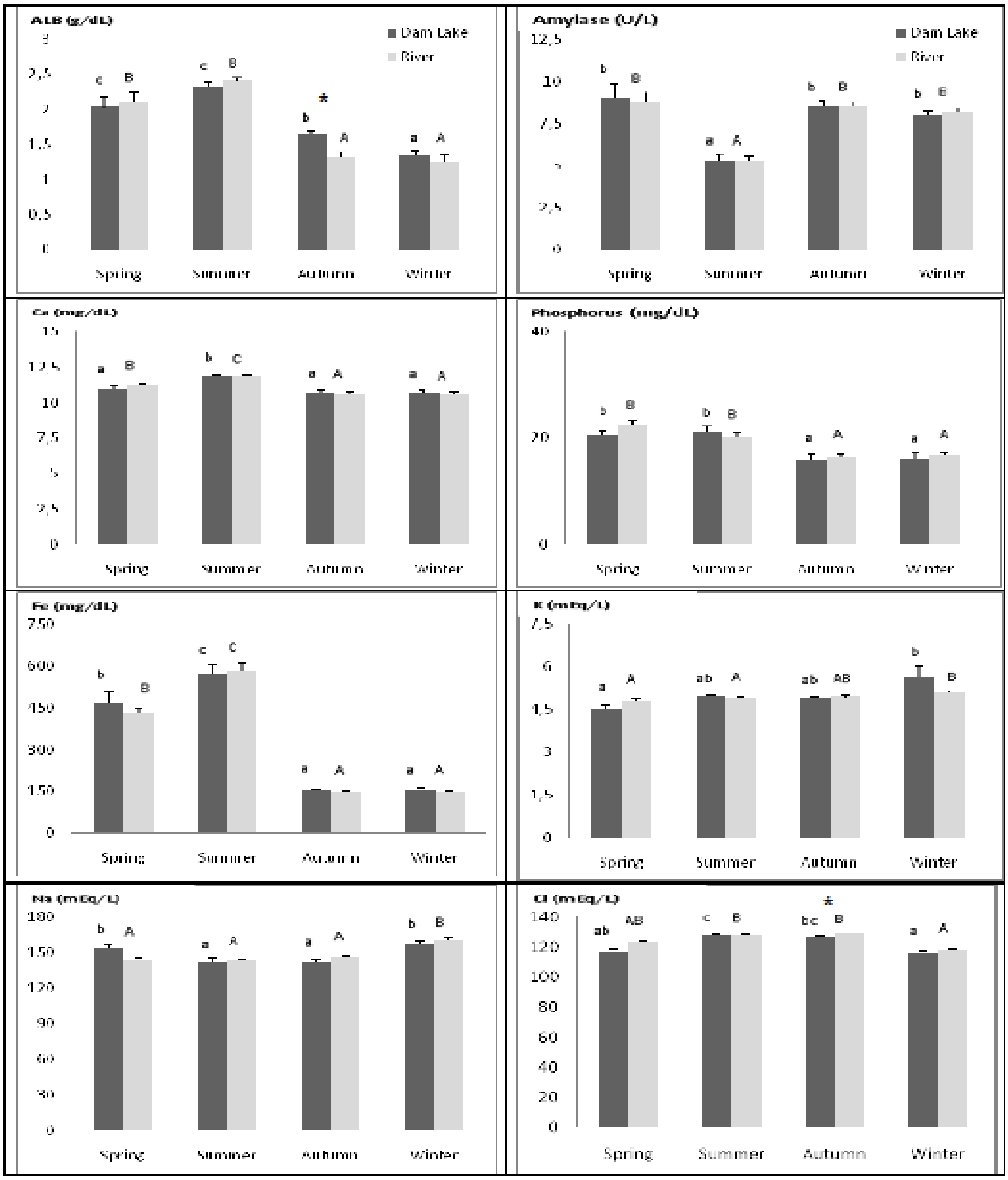

Figure 2: Seasonal values (±S.E) of blood serum biochemical parameters (ALB, Amylase, Ca, P, Fe, K, Na, Cl) of Rainbow Trout Fishes from Bahcelik Dam Lake And Zamantı River. Different letters in the same location (Dam Lake and River) show the si,gnificant seasonal difference (*p*<0.05).
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Results

Although there were no significant seasonal differences in the GLC and CHOL values in the trout collected from the dam (p>0.05) (Fig. 2), significant seasonal differences were observed in the other blood parameters (p<0.05). On the other hand, seasonal differences were observed in all the blood parameters of fish sampled from the river.

In the spring, ALT levels were highest in both the dam and river samples. Urea, AST, TP, CA and Fe values were significantly higher in the summer than in the other seasons, whereas amylase values were significantly lower. TG and ALP values were higher in the autumn than in the other seasons (p<0.05). Phosphorous levels were significantly higher in the spring and the summer than in the autumn and the winter. ALB levels were significantly lower in the autumn and winter than in spring and summer (p<0.05).

GLC values were not significantly different between seasons in the dam samples, whereas there was a significant reduction in GLC values in the river samples. While CHOL levels were not significantly different between seasons in the dam samples, CHOL levels were significantly lower in the summer in the river samples. The dam samples showed significantly higher Na levels in the spring and the winter, whereas the river samples showed significantly higher Na levels in the winter than in the other seasons.

The means of the blood parameters were compared between the dam and river samples. Urea and AST levels were highest in the spring in the dam samples compared to the river samples. On the other hand, ALT levels were higher in the river samples than in the dam samples. Urea and CHOL levels were highest in the summer in the dam samples compared to the river samples. The ALT levels in the summer in the river samples were again higher than in the dam samples. In the autumn, TG, ALP and ALB levels were higher in the dam samples than in the river samples. Cl levels in the river samples were higher than in the dam samples. In the winter, AST levels were higher in the dam samples than in the river samples.

Discussion

The blood physiology and biochemistry of fish are readily affected by environmental changes (Atamanalp *et al.*, 2002). In addition, the method of blood collection, and the age, nutrition and locality of the fish can also affect the blood parameters (Sakomoto *et al.*, 2001). In this study, the mean annual values of various blood parameter were different from those reported in *Leuciscus cephalus* (Seker *et al*., 2005), *Acipenser stellatus* (Shahsavani *et al*., 2010), *Synodontis membranacea* (Owolabi, 2011) and *Cyprinus carpio* **(**Kandemir *et al*., 2010) (Table 1 and 2). In the current study, there were no seasonal differences in the blood biochemistry of the fish samples between the sampling locations in the dam lake.

Table 1: Comparison of current study and earlier studies (GLC, Urea, CHOL, TG, AST, ALT, ALP, TP).

	GLC	Urea	CHOL	TG	AST	ALT	ALP	TP	References
***O.mykiss* (Dam Lake)**	96.44±2.21 mg/dL	12.49±0.72 mg/dL	316.84±6.19 mg/dL	553.83±25.24 mg/dL	139.96±8.86 U/L	35.63±2.4 U/L	289.99±25.24 U/L	4.69±1.13 g/dL	This Study
***O.mykiss* (River)**	96±1.42 mg/dL	10.21±0.43 mg/dL	321.11±4.77 mg/dL	537.25±14.87 mg/dL	125.89±7.6 U/L	41.19±2.22 U/L	271.87±17.6 U/L	4.56±1.11 g/dL	This Study
Leuciscus cephalus	-	-	293.98±89.27 mg/dL	117.8±14.07 mg/dL	325.2±22.93 U/L	-	-	6.07±2.657 mg/dL	(Seker *et al.*, 2005)
Acipenser stellatus	166.4±8.26 mg/dL	-	238.2±11.24 mg/dL	699.6±22.9 mg/dL	-	-	-	2.99±0.084 g/dL	(Shahsavani *et al.*, 2010)
Synodontis membranace	4.24±1.74 mmol/L	3.05±0.67 nmol/L	8.46±2.27 mmol/L	-	16.8±4.73 U/L	11.79±2.67 U/L	63.01±20.44 U/L	40.19±7.45 g/L	(Owolabi, O.D. 2011)
Cyprinus carpio	169.6±7.82 mg/dL	-	200±48.08 mg/dL	102±3.16 mg/dL	255.4±18.64 IU/L	34.2±1.64 IU/L	12.4±2.07 IU/L	3.28±0.32 g/dL	(Kandemir *et al.*, 2010)

Table 2: Comparison of current study and earlier studies (ALB, Ca, P, Fe, K, Na, Cl).

	ALB	Ca	P	Fe	K	Na	Cl	References
O.mykiss (Dam Lake)	1.84±0.07 g/dL	11.06±0.12 mg/dL	18.42±0.64 mg/dL	338.64±33.75 mg/dL	4.99±0.12 mEq/L	148.71±1.76 mEq/L	122.24±1.57 mEq/L	This Study
O.mykiss (River)	1.77±0.09 g/dL	11.09±0.1 mg/dL	18.97±0.51 mg/dL	329.92±32.41 mg/dL	4.92±0.03 mEq/L	148.72±1.63 mEq/L	124.5±1.14 mEq/L	This Study
Leuciscus cephalus	1.01±0.335 g/dL	18.49±3.86 mg/dL	12±2.83 mg/dL	17.41±1.83 mg/dL	-	-	-	(Seker *et al.*, 2005)
Acipenser stellatus	2.75±0.097 g/dL	8.29±0.28 mg/dL	12.39±0.26 mg/dL	-	2.75±0.1 mmol/L	149.2±1.92 mmol/L	-	(Shahsavani *et al.*, 2010)
Synodontis membranace	19.78±5.67 g/L	2.35±0.94 mmol/L	-	-	13.36±4.55 mmol/L	139.48±23.19 mmol/L	-	(Owolabi, O.D. 2011)
Cyprinus carpio	-	11.08±1.23 mmol/L	20.88±1.4 mmol/L	34.2±2.77 mmol/L	1.02±0.13 mmol/L	117.6±5.12 mmol/L	83±5.52 mmol/L	(Kandemir *et al.*, 2010)

This suggests that the dam lake is homogenous in terms of water chemistry and biology. Similarly, there were no seasonal differences in blood biochemistry of the fish samples between the sampling locations on the river.

GLC increases with the stress response in fish and may be considered as an indicator of stress (Percin and Konyalioglu, 2008). In the river samples, there was a significant reduction in the GLC levels in the winter. This may be due to low nutrient uptake or slower metabolism at lower temperatures (Smith *et al.*, 1987). Urea is an important waste product in fish, and high urea levels are an indicator of gill malfunction. Increases in water temperature may also stress fish. The increase in urea and AST levels in the warmer summer months in both the dam and river samples could be attributed to an increase in metabolism and pollution levels.

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The urea levels measured in the dam samples in the spring and the summer were significantly higher than in the river samples. This may be due to the exposure of the trout to stress factors in the spring and the summer.

Fish store total lipids primarily in the liver and the muscles (Kandemir and Polat, 2007). In most studies, lipid levels in fish are presented as CHOL and TG. During the reproductive period, feeding, heavy metals and toxic substances may alter CHOL levels (Lemaire *et al.,* 1991; Jeon *et al*., 1995). In the river samples, the reduction in CHOL levels in the summer may be attributed to low feeding activity. The TG level may differ with respect to the feeding status, and an increase in feeding activity in post-reproductive period may be the cause of elevated TG levels in the autumn in both the dam and the river. When the dam and river samples were compared, the CHOL levels in the summer and the TG and ALB levels in the autumn were highest in the dam samples.

Enzymes such as AST, ALT and ALP are used to monitor water pollution and to determine the effects of pollution on animals. ALT and AST are the major enzymes involved in amino acid metabolism. Elevated levels of these enzymes in fish are thought to be a result of injury to the liver or other organs (kidney and/or galls). Moreover, AST levels may increase as a result of certain viral fish diseases (Turnbull, 1999). ALP is affected by various factors including water chemistry, nutrient uptake and temperature (Sknoberg *et al*., 1997). When the mean blood parameters were compared, the AST levels in the dam samples in the spring and winter were found to be higher than in the river samples. This is thought to be the result of stress factors having a greater effect in closed systems.

TP may be used as an indicator of nutrition in fish (McCarthy *et al*., 1973), and ALB values are also related to nutritional state. TP and ALB levels in fish may differ with respect to reproductive period, nutritional state and habitat. In addition, toxic substances and diseases can also affect the levels of TP and ALB (Chen *et al*., 2003). The decrease in ALB levels in the autumn and the winter may be attributed to the slow metabolism of the fish. The increase in TP values in the summer, on the other hand, can be explained by changes in fish physiology that are caused by increased levels of organic substances due to elevated water temperatures.

Some blood electrolytes that are commonly analyzed in fish are Na, K, Cl, Ca and P. Ion regulation mechanisms are highly sensitive to all pollutants (Croke and McDonald, 2002). Ca is a component of bones and also regulates nerve and muscle functions. Toxic substances and diseases are able to alter Ca levels in the blood. In this study, Ca levels were significantly elevated in summer in both the dam and the river samples. Ca levels may increase before the reproductive period, and a similar increase in Ca levels was also observed in *Chalcalburnus tarichi* (Arabaci *et al.*, 2001), which is presumed to be related to the development of eggs in the ovaries (Handin *et al*., 2003).

Seasonal differences in blood parameters are thought to primarily result from variations in temperature, feeding and other factors. To use blood parameters as bioindicators in fish, standards should be determined for each fish species with respect to the environment they live in. Certain biochemical blood parameters of rainbow trout cultured in two different localities were determined in this study, and the aim of this study is to serve as a reference for future studies. It is suggested that these parameters are monitored in the inland waters where aquaculture operations are planned. In addition, the findings of this study may prove useful for future research in fish biology and food safety.

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