http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(3), 174-181 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.3.03

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Seasonal changes in antioxidant enzyme activities of *Garra rufa* (Heckel, 1843) in Göynük Stream (Bingöl, Türkiye)

Göynük Çayı'nda (Bingöl, Türkiye) *Garra rufa*'nın (Heckel, 1843) antioksidan enzim aktivitelerindeki mevsimsel değişimleri

Muammer Kırıcı[1*] ● Nurgül Şen Özdemir[2] ● Fatma Caf[3] ● Mustafa Koyun[4]

[1]Bingöl University,Food Agriculture and Livestock Vocational School, Department of Veterinary Health, Bingöl, Türkiye https://orcid.org/0000-0003-1888-4388
[2]Bingöl University,Food Agriculture and Livestock Vocational School, Department of Veterinary Health, Bingöl, Türkiye https://orcid.org/0000-0001-6070-9765
[3]Bingöl University,Food Agriculture and Livestock Vocational School, Department of Veterinary Health, Bingöl, Türkiye https://orcid.org/0000-0002-0363-4848
[4]Bilecik Şeyh Edebali University, Faculty of Science, Department of Molecular Biology and Genetics, Bilecik, Türkiye https://orcid.org/0000-0002-9047-0430

**Corresponding author: muammerkirici@hotmail.com*
Received date: 06.03.2023 *Accepted date: 10.07.2023*

How to cite this paper:
Kırıcı, M., Özdemir, N.Ş., Caf, F., & Koyun, M. (2023). Seasonal changes in antioxidant enzyme activities of *Garra rufa* (Heckel, 1843) in Göynük Stream (Bingöl, Türkiye). *Ege Journal of Fisheries and Aquatic Sciences, 40*(3), 174-181. https://doi.org/10.12714/egejfas.40.3.03

Abstract: In this study, seasonal variations of antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD)) and malondialdehyde (MDA) levels in gill, kidney, muscle and liver tissues of *Garra rufa* (Heckel, 1843) caught from Göynük Stream, one of the most important branch of Murat River, were investigated. Fish samples were caught from two stations (Garip and Ilıcalar), which are determined regularly every month, and brought to the laboratory. The levels of biomarkers in tissues were determined by spectrophotometric methods. It was determined that the difference between the parameters in the studied tissues was statistically significant ($P < 0.05$) between the two stations, but the difference between the stations in the liver tissue in all seasons in GR enzyme was not statistically significant. However, it was determined that the differences between the seasons were significant in both stations. GR and G6PD enzyme activities were found to be lower than other enzyme activities among the enzyme groups studied, but CAT and SOD enzyme activities were found to be higher than the other enzymes., enzyme activities in muscle tissue are lower than activities in the other tissues among tissues.

Keywords: Doctor fish, *Garra rufa*, Murat River, oxidative stress, seasonal changes

Öz: Bu çalışmada, Murat Nehri'nin en önemli kollarından biri olan Göynük Çayı'ndan yakalanan *Garra rufa* (Heckel, 1843) balıklarının solungaç, böbrek, kas ve karaciğer dokularında mevsimlere bağlı olarak antioksidan enzim aktivitelerindeki seviyelerindeki değişimler incelendi. Bu amaç doğrultusunda balıklar, her ay düzenli olarak belirlenen iki istasyondan (Garip ve Ilıcalar) yakalanarak laboratuvara getirildi. Dokularda enzim aktiviteleri ve MDA seviyesi spektrofotometrik yöntemlerle belirlendi. Çalışma sonucunda, genel olarak iki istasyon arasında tüm dokularda parametreler arasındaki fark istatistiki olarak önemli bulunmuştur ($p < 0.05$). Ancak karaciğer dokusunda GR enziminde tüm mevsimlerde istasyonlar arasındaki fark istatistiki olarak önemli olmadığı belirlendi. Bununla beraber, her iki istasyonda da genel olarak mevsimler arasındaki farklılıkların önemli olduğu gözlemlendi. Çalışmada, GR ve G6PD enzim aktiviteleri diğer enzim aktiviteleri ile kıyaslandığında düşükken, CAT ve SOD aktiviteleri ise diğerlerinden yüksektir. Dokular arasında ise kas dokusundaki enzim aktiviteleri, diğer dokulardaki aktivitelerden daha düşüktür.

Anahtar Kelime: Doktor balık, *Garra rufa*, Murat Nehri, oksidatif stres, mevsimsel değişiklikler

INTRODUCTION

Garra rufa (Heckel, 1843) is a member of the Cyprinidae family that lives at the bottom of streams and rivers, clinging to underwater rocks and stones with its sticky organ under its mouth. Its mouth structure is sticky and crescent-shaped, because it feeds on zooplankton and phytoplankton. The adhesive organ consists of four parts: the anterior fold with the disc fringing, the posterior fold, the numb part, and the posterior free part of the disc (Grassberger and Hoch, 2006; Teimori et al., 2011). *G. rufa* has no teeth and they eat by breaking the dead skin with their mouth movements and offer micro-massage with their movements. *G. rufa* is a fish species that lives in Sivas thermal springs at high temperatures and is used in the treatment of many diseases. Especially, a successful result is achieved when used on psoriasis and various skin diseases (Karahan, 2007). In the literature, it has been determined that Garra species are good for many skin diseases such as eczema, pus-wound-acne and psoriasis (Duman, 2010; Karaaslan, 2010).

Many different kinds of biomarkers have been used all around the world to assess the impact of pollutants on aquatic life. They track biological reactions such as the induction of biotransformations (Wong et al., 2000), the induction of protein levels (Linde et al., 2001), the suppression of enzyme activity (Peebua et al., 2007), the integrity of DNA (Ergene et al., 2007), etc. Therefore, these biomarkers offer a technique for definitive early warning signs of water pollution for fish species (Strmac and Braunbeck, 2000). Fish are useful bioindicators because of their abundance, richness, and relatively simple

identification. They are ideal for assessing regional and global environmental changes because they are less vulnerable to natural microenvironmental changes caused by lesser organisms (Gadzala-Kopciuch et al., 2004). In fact, changes in environmental conditions due to seasonal variations are likely to have an impact on enzyme activity. Therefore, it can be difficult to interpret biomarker data. Also, different levels of an enzyme biomarker may represent natural fluctuations in a species' annual physiological cycle, rather than exposure to chemical pollution (Robillard et al., 2003; Kırıcı et al., 2022a). Seasonallly, environmental conditions and metabolic activities have an effect on the responses of enzymatic activity. Therefore, knowing how biomarkers naturally evolve can help interpret field findings and distinguish between the beginning of a biological disturbance and natural variability (Figure 1) (Bocchetti and Regoli, 2006; Barda et al., 2014).

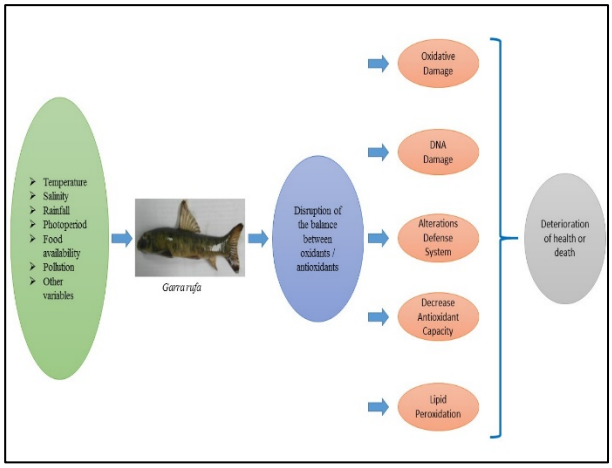

Figure 1. A schematic representation of the damages caused by different factors in *Garra rufa* in aquatic resources

MATERIALS and METHODS

Study area and stations

This study was carried out at Garip and Ilıcalar stations on Göynük Stream. Göynük Stream is one of the branches of the Murat River, which flows through the Genç District of Bingöl Province (Figure 2). These locations were chosen considering that there would be no problem in the supply of fish throughout the year with the observations made in the region. Additionally, Ilıcalar Station is a location with hot springs, thus the temperature values there are higher than seasonal averages. Garip Station, located within the borders of Genç district, has generally cold water. It is known that *Garra rufa* frequently travels to warm waters. Hence, Ilıcalar and Garip stations were chosen according to the migration requirements of the species. Gill nets and cast nets with different mesh sizes were used in the fish samplings.

Taking tissue samples from fish

In the study, 221 *G. rufa* (average weight: 11.26 ± 4.82 g; length: 9.7 ± 3.16 cm) fish were caught in one year. The caught fish were brought to the laboratory in tanks with air stones in the water taken from their natural habitat. The fish were transported as carefully as possible and kept away from stress factors. The fish were anesthetized in the laboratory with anesthetic matter (50 ppm, benzocaine) and tissues were removed rapidly. The abdominal region was opened by cutting the fish with the non-pointed part of the scissors from the anus region. The kidney, liver, gill, and gonad tissues used in the study were carefully removed. The kidney, liver, gill, and gonad tissues of each fish were placed in separate tubes. Until the time of the study, the tissues were stored at -80 °C (Kırıcı et al., 2017).

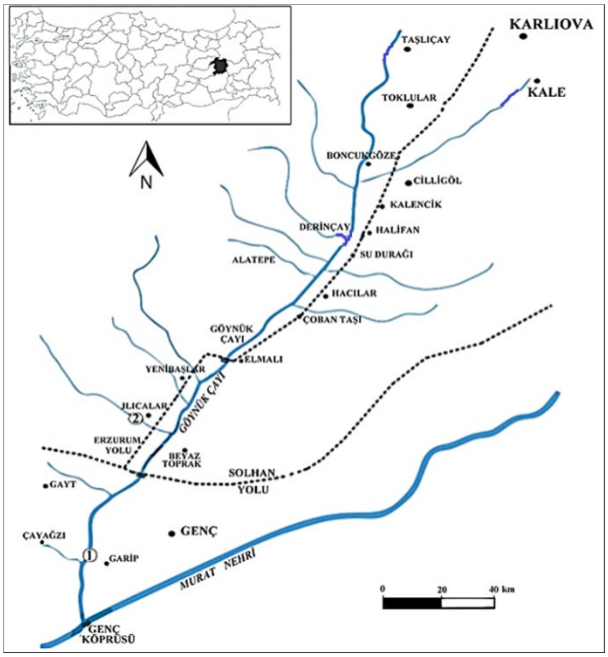

Figure 2. Stations (Garip (1) (38°47'13.2"N 40°32'56.9"E); Ilıcalar (2) (38°58'57.7"N 40°41'04.4"E) (Modified from Koyun et al., 2018; Kırıcı et al., 2022a)

Preparation of homogenate

Frozen tissues taken from -80 °C were expected to thaw at +4 °C. After the melting process, 0.9% NaCl was used to remove the blood from the tissues in the tube they were in. The kidney, gonad, liver and gill tissues of each fish were weighed separately and homogenized. Each tissue sample was cut into small pieces with the help of scissors. This fragmented tissue sample was placed in a porcelain mortar that had been pre-cooled to -80 °C. Liquid nitrogen was then added and the mixture was crushed to a paste-like consistency. The crushed tissue sample was taken into a tube and 3 times its weight of KH_2PO_4 buffer solution was added on it. Then the tissue sample was centrifuged at +4 °C at 13000 rpm for two hours. Supernatant existing on the upper section was carefully taken with a dropper. Following this, the precipitated part was removed. The supernatant was used to determine enzyme activity (Beutler, 1971).

Determination of lipid peroxidation

Malondialdehyde is a lipid peroxidation product and is a good stress marker. The MDA level was determined by reading

on a spectrophotometer (Shimadzu UV/VIS-1201) at a maximum of 532 nm. For this, 200 µl of sample from each extracted tissue was taken into the tube and 800 µl of phosphate buffer was added. It was suspended with 25 µl of BHT to stop oxidation. Then µl of 30% TCA was added. Samples were voxed, mixed in vortex and kept at -20 ºC for 2 hours. Then centrifugation was carried out at 2000 rpm for 15 minutes. 1 ml sample was taken from the upper phase and 75 µl of EDTA Na_2H_2O and 250 µl of TBA were transferred on it, respectively. After vortexing, it was kept in a water bath with a temperature of 90 ºC for about 15 minutes. Readings were taken when the samples were at room temperature (Slater, 1984).

Measurement of levels of antioxidant enzymes

G6PD activities were determined by measuring the change in absorption at 340 nm at 37 ºC, as described by Beutler. For the determination of the activity of the enzyme, NADPH formed at the end of the reaction is taken into account. Because the activity of the G6PD enzyme, which catalyzes the production of 6 phosphoglucanolac from glucose-6-phosphate, is directly proportional to the decreasing amount of NADP in this process (Beutler, 1975).

In the measurement of the activity of the GR enzyme, it is measured by the maximum absorbance of the reacting NADPH at 340 nm. In the reaction catalyzed by the GR enzyme, it causes a decrease in NADPH. Enzyme activity was determined by monitoring this decrease spectrophotometrically at 340 nm (Carlberg and Mannervik, 1985).

SOD activity, Sun et al. (1988) using the method suggested. The nitroblue tetrazolium (NBT) is reduced via the xanthine-xanthineoxidase system, which generates superoxide, as the foundation of this technique. SOD activity was measured in units per gram of tissue protein (U/g).

GPx activity was investigated using Beutler (1975) method. In the presence of hydrogen peroxide, GPx catalyzes the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). When hydrogen peroxide is in the environment, GR and NADPH work together to convert the GSSG produced by GPx into GSH. By measuring the decrease in absorbance at 340 nm caused by the oxidation of NADPH to $NADP^+$.

CAT determination was made according to the Aebi (1983) method. In summary; When the sample is added to the H_2O_2 solution, which is prepared in phosphate buffer (pH 7.4) and gives approximately 0.500 absorbance at 240 nm. In the meantime, the decrease in absorbance was followed on the monitor and the activity was calculated from the slope (Aebi, 1983).

Determination of protein in fish tissue

Lowry et al. (1951) performed spectrophotometrically reported tissue protein determination. This approach relies on complexes of protein peptide bonds with copper ions forming in an alkaline media. The blue-violet hue created by the copper-peptide complexes and the foline reagent reaction is read at 750 nm using a blind versus spectrophotometer.

Statistics

Using the SPSS 23.0 computer program, it was evaluated whether there was a significant difference between the stations and seasons in the changes of enzyme activities in fish tissues, according to One-Way ANOVA and Duncan test. Data were given as mean ± standard error. P<0.05 value was considered statistically significant.

RESULTS

MDA level, a marker of oxidative stress, in kidney tissue was found to be higher in Garip Station tissue values in autumn and spring seasons compared to Ilıcalar Station, and the difference was statistically significant. However, kidney tissue MDA level did not show a statistically significant difference between stations in the summer and winter seasons. Significant differences were detected between stations in all seasons in the enzyme levels of SOD, CAT and GR, which are markers of oxidative stress. In G6PD kidney tissue level activity, the difference between stations in autumn was statistically significant, but the difference between summer, winter and spring seasons was not statistically significant. In GPx activity in autumn, winter and spring seasons a statistically significant difference was found (P<0.05) (Table 1).

Table 1. Levels of gill tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	3.81 ± 0.71^{a,*}	0.11 ± 0.05^{a}
	Autumn	1.26 ± 0.22^{b,*}	0.24 ± 0.07^{a}
	Winter	0.95 ± 0.18^{b}	0.84 ± 0.78^{b}
	Spring	1.97 ± 0.29^{b,*}	0.27 ± 0.11^{a}
SOD	Summer	34.46 ± 3.30^{a,*}	0.33 ± 0.01
	Autumn	15.71 ± 1.19^{b,*}	0.17 ± 0.03
	Winter	19.91 ± 1.24^{b,*}	0.21 ± 0.03
	Spring	11.60 ± 2.00^{b,*}	0.14 ± 0.02
CAT	Summer	470.1 ± 23.0^{a,*}	23.70 ± 1.30^{a}
	Autumn	308.4 ± 19.4^{b,*}	36.47 ± 2.40^{b}
	Winter	289.0 ± 16.9^{b,*}	48.39 ± 2.90^{c}
	Spring	395.1 ± 17.4^{c,*}	27.20 ± 1.10^{a}
GR	Summer	1.08 ± 0.16^{a,*}	4.47 ± 0.97^{a}
	Autumn	5.01 ± 1.73^{b,*}	2.81 ± 0.39^{b}
	Winter	2.18 ± 0.76^{a}	2.34 ± 0.35^{b}
	Spring	2.75 ± 0.32^{a}	3.24 ± 1.76^{b}
G6PD	Summer	1.10 ± 0.29	1.82 ± 0.40^{a}
	Autumn	0.84 ± 0.18*	5.67 ± 0.82^{b}
	Winter	1.04 ± 0.22	2.45 ± 0.54^{a}
	Spring	0.97 ± 0.13	1.64 ± 0.32^{a}
GPx	Summer	17.00 ± 3.11^{a}	19.18 ± 1.93^{a}
	Autumn	12.47 ± 2.18^{b,*}	38.36 ± 3.02^{b}
	Winter	8.33 ± 1.47^{c,*}	24.17 ± 2.35^{a}
	Spring	9.03 ± 1.80^{c,*}	23.13 ± 2.80^{a}

*P<0.05 when compared with values at Göynük
a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P < 0.05)

Statistically significant differences were found between stations in MDA level in spring, summer and autumn seasons in gill tissue. SOD and CAT enzyme activities were found to be

significantly different between Garip and Ilıcalar stations in all seasons. Significant differences were found in GR activity in summer and autumn seasons. A statistically significant difference was found between stations in GPx activity in all seasons except summer. In the gill tissue, there was no statistical difference between SOD activities at Garip Station and G6PD activities at Ilıcalar Station in all the seasons (P<0.05) (Table 2).

A statistically significant difference was found between stations in MDA levels of liver tissue in all the seasons except for spring. There are statistically significant differences between the stations in SOD, CAT and G6PD activities in all seasons. On the other hand, no significant difference was detected between Garip and Ilıcalar stations in GR activity of liver tissue. No significant difference was detected in autumn and winter months, while a statistically significant difference was detected between stations in GPx activity in summer and spring seasons. There was no significant difference between the GR activities at Garip Station and G6PD activities at Ilıcalar Station between seasons (P<0.05) (Table 3).

A statistically significant difference was found between the stations in all seasons in MDA level, SOD, CAT, G6PD and GPx activities of gonad tissue. Only, there was a statistically significant difference between stations in GR activity in the summer season. No significant difference could be detected in G6PD activities between the seasons, in Ilıcalar Station. Significant statistical differences were detected in MDA levels and enzyme activities at other stations (P<0.05) (Table 4).

Table 2. Levels of gill tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	3.81 ± 0.71^{a,*}	0.11 ± 0.05^{a}
	Autumn	1.26 ± 0.22^{b,*}	0.24 ± 0.07^{a}
	Winter	0.95 ± 0.18^{b}	0.84 ± 0.78^{b}
	Spring	1.97 ± 0.29^{b,*}	0.27 ± 0.11^{a}
SOD	Summer	34.46 ± 3.30^{a,*}	0.33 ± 0.01
	Autumn	15.71 ± 1.19^{b,*}	0.17 ± 0.03
	Winter	19.91 ± 1.24^{b,*}	0.21 ± 0.03
	Spring	11.60 ± 2.00^{b,*}	0.14 ± 0.02
CAT	Summer	470.1 ± 23.0^{a,*}	23.70 ± 1.30^{a}
	Autumn	308.4 ± 19.4^{b,*}	36.47 ± 2.40^{b}
	Winter	289.0 ± 16.9^{b,*}	48.39 ± 2.90^{c}
	Spring	395.1 ± 17.4^{c,*}	27.20 ± 1.10^{a}
GR	Summer	1.08 ± 0.16^{a,*}	4.47 ± 0.97^{a}
	Autumn	5.01 ± 1.73^{b,*}	2.81 ± 0.39^{b}
	Winter	2.18 ± 0.76^{a}	2.34 ± 0.35^{b}
	Spring	2.75 ± 0.32^{a}	3.24 ± 1.76^{b}
G6PD	Summer	1.10 ± 0.29	1.82 ± 0.40^{a}
	Autumn	0.84 ± 0.18*	5.67 ± 0.82^{b}
	Winter	1.04 ± 0.22	2.45 ± 0.54^{a}
	Spring	0.97 ± 0.13	1.64 ± 0.32^{a}
GPx	Summer	17.00 ± 3.11^{a}	19.18 ± 1.93^{a}
	Autumn	12.47 ± 2.18^{b,*}	38.36 ± 3.02^{b}
	Winter	8.33 ± 1.47^{c,*}	24.17 ± 2.35^{a}
	Spring	9.03 ± 1.80^{c,*}	23.13 ± 2.80^{a}

*P<0.05 when compared with values at Göynük
a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P < 0.05)

Table 3. Levels of liver tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	8.95 ± 2.58^{a,*}	4.65 ± 0.73^{a}
	Autumn	10.11 ± 2.98^{a,*}	15.98 ± 1.53^{b}
	Winter	4.38 ± 1.16^{b,*}	10.88 ± 1.99^{c}
	Spring	5.55 ± 1.37^{b}	7.36 ± 1.09^{d}
SOD	Summer	48.36 ± 2.26^{a,*}	7,11 ± 1.45^{a}
	Autumn	82.42 ± 2.38^{b,*}	8.30 ± 2.47^{a}
	Winter	40.79 ± 2.17^{a,*}	6.90 ± 1.08^{a}
	Spring	84.07 ± 2.26^{b,*}	4.07 ± 1.27b
CAT	Summer	54.46 ± 3.30^{a,*}	224.35 ± 34.82^{a}
	Autumn	31.55 ± 2.99^{b,*}	241.84 ± 27.75^{a}
	Winter	28.18 ± 1.43^{b,*}	325.73 ± 48.20^{b}
	Spring	40.92 ± 3.09^{c,*}	198.03 ± 17.39^{a}
GR	Summer	3.70 ± 1.67^{a}	2.47 ± 0.27
	Autumn	3.80 ± 1.86^{a}	2.91 ± 0.39
	Winter	1.56 ± 0.66^{b}	3.04 ± 0.35
	Spring	1.95 ± 0.75^{b}	3.45 ± 0.34
G6PD	Summer	0.79 ± 0.03*	16.70 ± 5.21^{a}
	Autumn	0.60 ± 0.01*	21.67 ± 6.01^{b}
	Winter	0.71 ± 0.02*	16.35 ± 4.07^{a}
	Spring	1.02 ± 0.02*	15.19 ± 2.18^{a}
GPx	Summer	4.93 ± 1.08^{a,*}	8.39 ± 2.72^{a}
	Autumn	8.64 ± 2.13^{b}	9.06 ± 2.81^{a}
	Winter	5.61 ± 1.98^{a}	7.28 ± 2.02^{b}
	Spring	2.76 ± 0.83^{c,*}	9.64 ± 1.94^{a}

*P<0.05 when compared with values at Göynük
a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P < 0.05)

Table 4. Levels of gonad tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	4.88 ± 0.49^{a,*}	0.26 ± 0.03^{a}
	Autumn	7.13 ± 0.51^{b,*}	0.08 ± 0.02^{b}
	Winter	4.42 ± 0.47^{a,*}	0.04 ± 0.02^{b}
	Spring	3.99 ± 0.25^{a,*}	0.05 ± 0.01^{b}
SOD	Summer	6.21 ± 0.78^{a,*}	13.36 ± 1.87^{a}
	Autumn	9.37 ± 1.96^{b,*}	14.21 ± 2.00^{a}
	Winter	5.93 ± 1.24^{a,*}	21.08 ± 2.96^{b}
	Spring	6.23 ± 0.81^{a,*}	14.89 ± 2.01^{a}
CAT	Summer	29.42 ± 4.58^{a,*}	8.18 ± 1.32^{a}
	Autumn	11.16 ± 2.73^{b,*}	19.11 ± 4.47^{b}
	Winter	19.48 ± 3.47^{b,*}	7.88 ± 1.55^{a}
	Spring	19.92 ± 3.18^{b,*}	8.34 ± 2.35^{a}
GR	Summer	0.30 ± 0.09^{a,*}	4.2 ± 0.47^{a}
	Autumn	0.69 ± 0.12^{b}	0.9 ± 0.04^{b}
	Winter	0.41 ± 0.09^{a}	1.3 ± 0.09^{b}
	Spring	0.19 ± 0.06^{a}	0.8 ± 0.03^{b}
G6PD	Summer	0.19 ± 0.11*	5.4 ± 0.88^{a}
	Autumn	0.23 ± 0.09*	8.6 ± 2.19^{b}
	Winter	0.15 ± 0.01*	5.8 ± 0.93^{a}
	Spring	0.10 ± 0.01*	5.0 ± 0.91^{a}
GPx	Summer	4.28 ± 0.25^{a,*}	0.11 ± 0.02^{a}
	Autumn	8.20 ± 0.23^{b,*}	0.13 ± 0.07^{a}
	Winter	3.41 ± 0.15^{a,*}	0.94 ± 0.48^{b}
	Spring	6.28 ± 0.25^{a,b,*}	0.18 ± 0.09^{a}

*P<0.05 when compared with values at Göynük
a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P < 0.05)

DISCUSSION

The Murat River, the largest branch of the Euphrates River, is one of Türkiye's most important water resources, passing through the borders of Bingöl province. Murat River is a 722 km long in

Eastern Anatolia, formed by the merging of branches originating from Aladağ and Muratbaşı Mountain in the north of Van Lake, and flowing to the Keban Dam by moving westward, passing through the north of Bingöl province Genç district. Its total length in Bingol Province is 96 km and it is the most important water source of Bingöl Province. Today, water resources are heavily threatened by various ecological problems such as natural pollution, pesticides and household wastes (Kırıcı et al., 2016a).

In ecotoxicology; biomarkers are used in describe of interactions between a biological system and a chemical, physical or biological environmental pollutant. Inhibition or induction of biomarkers is a useful approach to determine the effects and potential effects of xenobiotics on living organisms *in vivo* (RendÓn-von Osten et al., 2005; Taysı et al., 2021a). Fish can be used as biomonitors to test for pollution and determine the ecological health of the aquatic environment because they are highly sensitive to it. Resistance of aquatic organisms to contamination; it is affected by many factors, including its phylogenetic location, ecological and biological characteristics of the living thing, physiological conditions and the presence of effective detox mechanisms (Hotard and Zou, 2008). In this study, it was aimed to correlate the effects of different seasonal changes in the aquatic ecosystem on fish with changes in biomarker response and to identify reliable biomarkers for monitoring. *G. rufa* fish, a species belonging to the Cyprinidae family, naturally distributed in Türkiye, Syria, Iran, Iraq and Jordan, can be used as a biomarker for the detection of seasonal biomarker changes and the pollution of rivers. In this study, *G. rufa* was chosen as a marker organism due to the ease of sampling, high adaptability to the region, and high ecological and economic importance of *G. rufa* from Göynük Stream on the Murat River. Most of the enzymatic activities in poikilothermic species vary with the environmental temperature. In fact, the level of many enzyme activities is not directly dependent on ambient temperature, but on physiological activity, which is tightly correlated with water temperature. Variations in biotic parameters such as sex, size, age, gonadal maturity or hunger are known to affect biomarkers and complicate the interpretation of the environmental significance of the markers (Forget et al., 2003).

Oxidative stress is a result of an imbalance between the generation and elimination of reactive oxygen species (ROS) in aquatic species, which can be brought on by a variety of anthropogenic and natural stimuli (xenobiotics) (Halliwell and Gutteridge, 2007; Yonar et al., 2016). An excellent cleaning potential against oxidative stress is provided by antioxidant defenses. Enzymatic and non-enzymatic antioxidant defense components are frequently used as biomarkers in environmental monitoring investigations (Sheehan and Power, 1999). Indicators of xenobiotic exposure and/or consequences, biomarkers are cellular, biochemical, molecular, or physiological alterations assessed in an organism's cells, body fluids, tissues, or organs (Lam and Gray, 2003; Kırıcı et al., 2015; Kırıcı et al., 2016b; Taysı et al., 2021b). A promising approach for biomonitoring aquatic systems is the evaluation of the enzyme biomarkers superoxide dismutase (SOD) and catalase (CAT), glutathione (GSH) content, and malondialdehyde (MDA) production activities (Livingstone, 2001; Yonar et al., 2011; Ispir et al., 2017). In a number of marine and freshwater animals, antioxidants including CAT, SOD, and GSH have been proposed as indicators of pollutant or seasonally related oxidative stress, and their activation represents a response to pollution (Borkovic et al., 2005; Yonar et al., 2012; Topal et al., 2014). However, environmental variables such as temperature, dissolved oxygen and food availability are known to influence oxidative stress responses through their effects on metabolism and reproduction (Sheehan and Power, 1999; Kırıcı et al., 2022b). The results obtained in this study show that seasonal factors have a significant effect on the antioxidant defense system. External factors such as temperature (Verlecar et al., 2007), salinity (Prevodnik et al., 2007), pH (Lima et al., 2007), nutrient source (Khessiba et al., 2005), and reproductive cycle (Filho et al., 2001), seasonal changes can be effective from complex interactions between endogenous factors.

Danabas et al. (2015) investigated some oxidative stress parameters (SOD, CAT and GPx activities and MDA levels) determined in the gill tissues of *Capoeta umbla* caught in different seasons from 10 sampling areas in Uzuncayir Dam Lake. They indicated that changes in SOD and CAT activities for the four seasons were found to be statistically significant ($p < 0.05$). The highest SOD enzyme activity was found in September 2011 in region 6, while the lowest in region 2 was found in March 2012. The highest CAT enzyme activity was found in the 3rd region in March 2012, while the lowest value was determined in the 4th region in September 2011. GPx activities were statistically insignificant in regions 2, 6 and 9 according to the four seasons ($p > 0.05$). The lowest GPx activity was found in September 2011 at region 8. Changes in MDA levels between seasons were statistically significant ($p < 0.05$). The highest MDA level was detected in zone 1 in September 2011, while the lowest was detected in zone 5 in March 2011. In this study, while the highest activity in kidney tissue was measured in CAT activity in Garip station in autumn season, the lowest activity was measured in GR activity in Garip station in winter. In the gill tissue, CAT activity was measured as the highest activity in the summer at Ilıcalar station, while the MDA level was measured as the lowest in the Garip station in the summer. In liver tissue, the highest CAT activity was measured at Garip station in winter, while the lowest was measured in G6PD activity in autumn at Ilıcalar station. In the gonad tissue, the highest CAT activity was measured at Ilıcalar station in summer, while the lowest MDA level was measured at Garip station in winter.

In a study evaluating the liver antioxidant enzyme activities of *Salmo trutta caspius, Salmo trutta labrax* and *Salmo trutta macrostigma* species, it was reported that these enzymes were

not affected by seasonal temperature changes, but there were changes in antioxidant enzyme activities in autumn. It has been stated that this oxidative enzyme change, which is an indicator of oxidative stress, may be related to pre-reproduction. It was emphasized that the rapid decrease in temperature, heavy rainfall and increase in daylight could trigger stress in fish. In addition, the same research team generally determined the liver SOD, GPx, CAT, G6PD, GR and GST enzyme activities in 3 different species (*Salmo trutta caspius, Salmo trutta labrax* and *Salmo trutta macrostigma*) found higher (Aras et al., 2009). They determined that GPx activities in all 3 fish, liver and gill tissues were higher than other enzyme activities studied in all seasons. In this study, CAT activity was generally found to be higher than other enzyme activities in all tissues. Especially, CAT activity was highest in the gill tissue at Ilıcalar Station in summer. In a study conducted in Munzur Alası of Munzur Stream, it was reported that the activities of glutathione peroxidase, catalase, superoxide dismutase and superoxide dismutase antioxidant enzymes in the muscle, liver and gill tissues of *Salmo* sp. increase in summer months. In addition, it was reported that liver tissue superoxide dismutase activity was statistically significant between seasons, and muscle, gill and liver tissues glutathione peroxidase activity was statistically significant in summer (Can et al., 2017).

CONCLUSIONS

As a result of this study, it was observed that antioxidant enzyme activities and MDA levels changed according to the seasons. Although the levels of oxidative stress parameters vary according to the seasons, these parameters can also be modified by various factors such as sex, sexual maturity, reproductive parameters and pollutants. The number of studies similar to this study should be done more by increasing the parameters.

ACKNOWLEDGEMENTS AND FUNDING

This study was supported by the Research Fund (BAP-ZF.2016.00.006) of Bingöl University (Türkiye).

AUTHORSHIP CONTRIBUTIONS

All authors took part in designing the research, collecting and writing the manuscript. Muammer Kırıcı analysed all data of the study statistically and writing the manuscript. Muammer Kırıcı and Mustafa Koyun prepared their field studies and references. Nurgül Şen Özdemir undertook the editing and application of the article. Muammer Kırıcı and Fatma Caf have edited the graphics and figures of the article. All authors took part in a part of the article. All authors approved the submission and publication of this manuscript.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest on this manuscript.

ETHICAL STATEMENT

The research was approved by Bingöl University Animal Experiments Local Ethics Committee in terms of sampling and use of experimental animals with decision number 06/5 at the meeting held on 13.10.2016. All researchers declare that all trials were conducted in accordance with ethical values.

DATA AVAILABILITY

The data supporting the conclusions of this paper are available in the main paper.

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