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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The gonadal health status of Cyprinidae fish species collected from the river impacted by anthropogenic activities

Antropojenik aktivitelerden etkilenen nehirden toplanan Cyprinidae familyasına ait balık türlerinin gonad sağlığı durumları

Hatice Dane[1] ● Turgay Şişman[2*]

[1]Department of Biology, Science Faculty, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0001-7623-7037
[2]Department of Molecular Biology and Genetics, Science Faculty, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0002-1098-1741

*Corresponding author: tsisman@atauni.edu.tr
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Abstract: Many freshwater ecosystems are contaminated with heavy metals released by municipal wastewater, cultivation and factory wastewater. The aim of the study was to evaluate the negative impact of metal pollution on the fish reproductive system. It was performed using the gonadal histopathology, hepatosomatic index (HSI), and gonadosomatic index (GSI) of three fish species, *Capoeta damascina*, *Squalius semae* and *Alburnus sellal* inhabiting in Karasu River (Erzurum, Turkey) contaminated by various anthropogenic activities. The highest GSI values were determined for each sex according to the seasons, and lower GSI values were observed in polluted stations in all three species. It was observed that HSI values in fish varied significantly from station to station. In addition, an increase in ovarian and testicular lesions (degeneration in oocyte and spermatocytes, atresia, congestion, infiltration, edema, vascular hypertrophy, fibrosis) was detected in the polluted areas. The results clearly showed that the metal pollution in the river adversely affected the reproductive system of the fish species living in the river.

Keywords: Freshwater fish, Karasu River, gonad histology, GSI, HSI, water pollution, histopathology

Öz: Birçok tatlı su ekosistemi, belediye atık suları, tarım ve fabrika atık sularında bulunan ağır metallerle kirlenmektedir. Bu çalışmanın amacı, metal kirliliğinin balıkların üreme sistemi üzerindeki olumsuz etkilerini değerlendirmektir. Bunun için çeşitli antropojenik faaliyetlerle kirlenen Karasu Nehri'nde (Erzurum) yaşayan üç balık türünde (*Capoeta damascina*, *Squalius semae* ve *Alburnus sellal*) gonadal histopatoloji, hepatosomatik indeks (HSI) ve gonadosomatik indeks (GSI) analizleri yapıldı. Mevsimlere göre en yüksek GSI değerleri her cinsiyet için belirlenmiş ve her üç türde de kirli istasyonlarda düşük GSI değerleri gözlenmiştir. HSI değerleri ise istasyonlar arası önemli oranda değişkenlik göstermiştir. Ayrıca özellikle kirli bölge balıklarının yumurtalık ve testislerinde tespit edilen patolojik lezyonlarda (oosit ve spermatositlerde dejenerasyon, atrezi, konjesyon, infiltrasyon, ödem, vasküler hipertrofi, fibrosis gibi) belirgin artışların olduğu saptanmıştır. Sonuçlar nehirdeki metal kirliliğinin nehirde yaşayan balık türlerinin üreme sistemini olumsuz etkilediğini açıkça göstermiştir.

Anahtar kelimeler: Tatlısu balığı, Karasu Nehri, gonad histolojisi, GSI, HSI, su kirliliği, histopatoloji

INTRODUCTION

Pollutants merged in both terrestrial and aquatic environments due to anthropogenic activities have a negative impact on both human and other organisms. It is known that anthropogenic chemical production has increased in recent years. This production reaches up to 400 million tons annually (Gavrilescu et al., 2015). This increase causes a rise in the amounts of substances merging in aquatic system. There is not enough information on the potential environmental risks of these substances as of now (Naidu et al., 2016). Mixing of unwanted substances in aquatic systems results in an unbalance regarding physical, chemical, and biological properties of water ecologically (Yadav et al., 2018). Among these substances, industrial wastes cause water pollution, thereby posing a threat to both aquatic plants and animals (Gupta et al., 2015). This pollution leads to a constant decrease in water flora and fauna, especially in fish population. All aquatic species, including fish, either absorb pollutants directly from water or take them through food chain (Łuczyńska et al., 2018). Fish, being the most important species used in the estimation of pollutant levels in water, provide some advantages in terms of identifying natural properties of aquatic systems and evaluating habitat changes (Martinez-Haro et al., 2015). In addition, due to being in most of the aquatic systems exposed to pollutants, their effects on the structure of food chain, and their ecological suitability, fish are considered as a main bioindicator in evaluating the quality of aquatic ecosystems (Corredor-Santamaría et al., 2019).

Exposure to pollutants may cause an exposure to acute or chronic toxicity in fish organs, especially gonads. This leads to a deficiency in reproduction. The health of the fish reproductive process is an important indicator that the organism is self-sustainable (Zulfahmi et al., 2018). Pesticides, heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, alkylphenolic compounds, phthalates, and endocrine disrupting chemicals may damage the reproductive physiology of fish populations (Agbohessi et al., 2015). In some studies, it

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was reported that gonadal abnormalities, decreased gonadosomatic index values, varied hormone levels, delay in plasma vitellogenin levels and gametogenesis occurred in fish which were found in areas with wastewater containing agricultural and industrial chemicals (Gilroy et al., 2012; Kaptaner, 2015). However, there is not enough information on the ecological risks caused by reproductive disruption following exposure to toxic substances in freshwater fish species in Turkey and the subject needs further research.

Capoeta damascina (Güldenstädt, 1773), *Squalius semae* (Linnaeus, 1758) and *Alburnus sellal* (Heckel, 1843) species used in this study belong to Cyprinidae family. These species are widespread in Euphrates and Tigris River systems in the Eastern and Southeastern Anatolia regions in Turkey (Geldiay and Balık, 2009). Wastewater in Erzurum province, agricultural activities in Karasu Basin and industrial wastes pollute the surface water of the Karasu River (Sönmez et al., 2012; Anonymous, 2016; Aydoğan et al., 2017; Dane and Şişman, 2017). In fact, previous reports have shown that the surface water and sediment of the river are particularly contaminated with metals (Dane and Şişman 2020a, 2020b). In this study, it was aimed to evaluate histopathologically how the fish species found in the natural fauna of selected locations are affected in terms of gonadal health due to metal pollution.

MATERIALS AND METHODS

Karasu River is unfortunately polluted by discharge of domestic and industrial (cement, paint, and plaster factories) wastes. Wastewater caused by industrial facilities located in the organized industrial zone in Erzurum is connected to municipality's sewage, and the sewage waste is discharged into Karasu River (Anonymous, 2016). In the study, four different stations were selected on the river. The stations were chosen by paying attention to the pollution load of the river and the regions where the fish species were observed. The first station was Dumlu (1st station 40° 05' 36.1" N 41° 22' 49.0" E). Other stations were as follows: Ilıca (2nd station 39° 57' 10.6" N 41° 04' 15.2" E), Aşkale 1 (3rd station 39° 54' 52.7" N 40° 40' 29.4" E) and Aşkale 2 (4th station 39° 56' 15.1" N 40° 37' 25.9" E) (Figure 1).

Dumlu station was taken as the reference region. Livestock and pasture farming are carried out around it and there is a settlement with a population of 1413. There are two factories around the Ilıca (2nd) and agricultural activities are carried out. In addition, the sewage water of the city is given to the river from here. Aşkale stations (3rd and 4th) are the last areas where the river leaves the Erzurum plain and where all the wastes of the city mix, and there are paint and cement factories nearby. Average heavy metal levels and physico-chemical parameters of the surface waters of the sampling stations are shown in Table 1 (Dane and Şişman, 2020a).

Species belonging to the natural fauna of the river, *Capoeta damascina*, *Squalius semae* and *Alburnus sellal* were caught from the stations using nets in May, June, July, August, and September (2015-2016). A total of 158 mature fish were caught from the river, including 54 *C. damascina* (28 ♀♀ 26 ♂♂), 52 *A. sellal* (29 ♀♀ 23 ♂♂) and 52 *S. semae* (24 ♀♀ 28 ♂♂). Permissions required for the study were taken from the relevant authorities before the study was initiated. The fish were brought to the laboratory alive and taken to intensely air-conditioned aquariums. No morphological abnormalities were observed in the fish. The mean total lengths and weights of the fish were 20.79 ± 3.42 cm and 89.08 ± 21.32 g (*C. damascina*), 17.83 ± 3.96 cm and 44.22 ± 5.37 g (*A. sellal*), 16.07 ± 2.03 cm and 48.09 ± 9.26 g (*S. semae*).

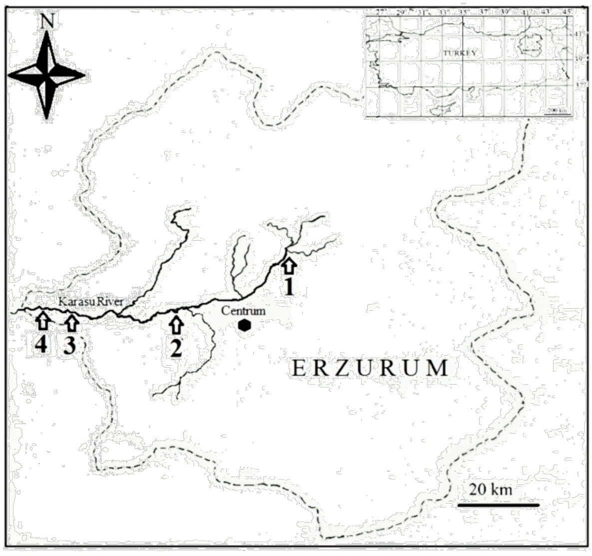

Figure 1. General view of the sampling stations

The hepatosomatic index (HSI) is expressed as the ratio of liver weight to body weight, and provides information about the health status of the fish and the quality of water. The fish were decapitated and the liver were removed. Hence the total organ weight of liver was taken for HSI. HSI was calculated according to the following formula: HSI=(Liver weight (g) / Fish weight (g) × 100 (Sadekarpawar and Parikh, 2013).

The gonadosomatic index (GSI) represents the ratio of fish gonad weight to body weight and used in identifying gonadal development in fish. For GSI calculation, fish were euthanized using MS 222 (75 mg/L). After measuring fish weight, the abdomen was cut from the front of the anus to the bottom of the gill, and organs were taken out the body. After measuring gonadal weight, the following formula was used in GSI calculation. GSI = (Gonadal weight) / (Total body weight) x 100 (Sadekarpawar and Parikh, 2013).

Fifteen fish from each station were used in histological analysis. Gonad samples were fixed in 10% neutral buffered formalin for up to 48 hours and subjected to a standard histological procedure. Parrafin embedded samples were cut with microtome with 5µm thickness. Slides were stained with hematoxylin and eosin (H&E) (Gautier, 2011) and microscopically analyzed with Leica DM750 light microscope and LASEZ software programme. The 10 sections of the gonads of each fish were analyzed. Twenty fields per section of tissue were observed.

Table 1. Current data of the mean metal levels and physico-chemical parameters of surface water samples of the stations (mg/L), and the water quality classes of the river water according to Turkish Water Pollution Control Regulation, and maximum acceptable limits of metal for river waters according to United Nations Economic Commission for Europe (UNECE, 1994), (Dane and Şişman, 2020a)

Metals	1. Station	2. Station	3. Station	4. Station	TS IV	UNECE 1994
Cr	8.55 ± 1.5^{a,b}	6.7 ± 1.2^{a,b}	12.1 ± 2.1^{a,b,*}	13.1 ± 1.8^{a,b,*}	>0.2	0.016
Mn	5.5 ± 1.1^{a}	5.7 ± 1.3^{a}	9.3 ± 1.5^{a,*}	7.7 ± 1.4^{a,*}	>3.0	
Fe	4.1 ± 0.9	5.5 ± 1.1^{a,*}	8.4 ± 1.3^{a,*}	7.8 ± 1.6^{a,*}	>5.0	
Co	0.7 ± 0.1^{a}	0.8 ± 0.1	1.26 ± 0.2^{a,*}	1.2 ± 0.1^{a,*}	>0.2	
Ni	0.8 ± 0.1^{a}	0.74 ± 0.1^{a}	1.17 ± 0.1^{a}	1.01 ± 0.1^{a}	>0.2	1.400
Cu	0.51 ± 0.1^{a,b}	0.57 ± 0.1^{a,b}	0.88 ± 0.1^{a,b,*}	0.82 ± 0.1^{a,b,*}	>0.2	0.018
Zn	0.42 ± 0.1^{b}	0.46 ± 0.1^{b}	0.66 ± 0.1^{b}	0.61 ± 0.1^{b}	>2	0.120
As	1.14 ± 0.1^{a,b}	1.27 ± 0.1^{a,b}	1.55 ± 0.2^{a,b,*}	1.46 ± 0.2^{a,b,*}	>0.1	0.360
Se	0.11 ± 0.01^{a}	0.20 ± 0.01^{a,*}	0.24 ± 0.01^{a*}	0.35 ± 0.05^{a,*}	>0.02	
Pb	0.29 ± 0.01^{a,b}	0.69 ± 0.1^{a,b,*}	2.55 ± 0.5^{a,b,*}	2.96 ± 0.6^{a,b,*}	>0.05	0.082
Physico-chemical parameters						
Temperature (ºC)	21±4.1	23±4.8*	25±5.7*	25±5.5*	>30	-
pH	7.81±1.5	7.74±1.6	8.03±1.2	8.06±1.4	9.0	-
Dissolved oxygen (mg/L)	7.62±1.0	6.32±1.5	5.60±1.1*	4.31±0.8*	<3	-

Values are expressed as mean ± standard errors. Asterisk shows statistical difference compared to reference station (1st Station). TS IV; Turkish Standard IV means the surface water are polluted. The letter "a" indicates that metal concentration exceeds the TS IV value, and the letter "b" indicates that metal concentration exceeds the UNECE limit.

Histological damage detected in gonads was assessed via the Degree of Tissue Change (DTC). Damages detected in tissues are grouped as the following according to DTC parameter: 0 (no abnormality), 1 (low abnormality frequency), 2 (medium abnormality frequency), and 3 (high abnormality frequency) (Abdel-Moneim et al., 2012). Abnormalities in gonads were classified according to the damage phase in DTC calculation. These phases are as follows: 1st Phase: Tissue has a normal function. 2nd Phase: Normal function of the tissue is disrupted at medium severity. 3rd Phase: An irreversible damage has occurred in the tissue (Bernet et al., 1999). DTC values were found using the formula: $DTC = (1 \times \Sigma I) + (10 \times \Sigma II) + (100 \times \Sigma III)$. In the formula, ΣI, ΣII and ΣIII indicate the total number of abnormalities observed in the phases respectively. After finding the DTC value for each fish, the mean index was calculated for each station. This index was analyzed according to following criteria. DTC: between 0-10: organ functions are normal; between 11-20: mild organ damage; between 21-50: medium-level organ damage; between 51-100: severe organ damage; above 100: irreversible organ damage (Poleksic and Mitrovic-Tutundzic, 1994).

The general evaluation of GSI and HSI were performed by ANOVA. Duncan test was used for multiple comparisons in variance analysis. The data were interpreted by considering $p < 0.05$ significance level.

RESULTS

Mean HSI values of the species caught according to stations are given in Table 2. The highest HSI values for *C. damascina* and *S. semae* were recorded in the 3rd station, The highest value for *A. sellal* was obtained in the 4th station. When a comparison was made between the stations in terms of HSI values, it was determined that the HSI values were significantly higher in the other three stations compared to the 1st station ($p < 0.05$).

Table 2. HSI values of the species according to stations

Stations	*Capoeta damascina*	*Alburnus sellal*	*Squalius semae*
1. Station	0.34 ± 0.06^{c}	0.40 ± 0.07^{c}	0.46 ± 0.06^{d}
2. Station	0.63 ± 0.07^{b}	0.95 ± 0.08^{b}	0.90 ± 0.07^{c}
3. Station	1.34 ± 0.03^{a}	1.29 ± 0.04^{a}	1.70 ± 0.10^{a}
4. Station	1.11 ± 0.06^{a}	1.43 ± 0.08^{a}	1.38 ± 0.08^{b}

Data are presented as mean ± SD. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$)

The spawning time of the fish caught in the Karasu River was determined by analyzing the gonadosomatic index (GSI) of the male and female individuals. In Table 3, the mean GSI values of female and male individuals are presented for three species. The highest GSI values were recorded in June, whereas the lowest values were recorded in August for *C. damascina*. On the other hand, the highest GSI value was found in *A. sellal* females in June, males in May, and the lowest in both sexes in August. The mean highest value for both female and male individuals of *S. semae* was recorded in May, whereas the lowest value was recorded in July. When the GSI values of fish were compared, the highest average index among the species was observed in *A. sellal*. When the values of the female and male individuals were compared, it was found that the GSI values of the female individuals were higher than the males in all three species. According to the data, the reproductive period for the species was found to be May and June.

The change in mean GSI values in these species by station is given in Table 4. When GSI values of species were analyzed by station, the lowest value for *C. damascina* was recorded in the 3rd station, and the highest value was recorded in the 1st station. The highest mean GSI value for *A. sellal* was obtained in the 1st station, and the lowest GSI value was recorded in the 4th station. The lowest mean value for *S. semae* was recorded in the 3rd station, and the highest mean value was recorded in the 1st station.

Table 3. GSI values of the species by months

Period	*Capoeta damascina*		*Alburnus sellal*		*Squalius semae*	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
May	3.86 ± 1.08^{b}	2.52 ± 0.33^{b}	5.04 ± 0.25^{b}	4.75 ± 0.58^{a}	4.72 ± 0.65^{a}	3.24 ± 0.16^{a}
June	5.87 ± 1.01^{a}	3.85 ± 1.05^{a}	7.65 ± 1.99^{a}	3.81 ± 0.35^{b}	3.65 ± 0.11^{b}	2.59 ± 0.12^{b}
July	1.24 ± 0.03^{d}	0.98 ± 0.03^{d}	2.56 ± 0.35^{c}	2.02 ± 0.22^{c}	0.82 ± 0.05^{e}	0.25 ± 0.08^{e}
August	1.04 ± 0.05^{e}	0.39 ± 0.02^{e}	0.92 ± 0.07^{e}	0.87 ± 0.01^{e}	1.06 ± 0.03^{d}	0.96 ± 0.07^{c}
September	1.36 ± 0.02^{c}	1.14 ± 0.02^{c}	1.26 ± 0.03^{d}	1.12 ± 0.02^{d}	1.20 ± 0.02^{c}	0.81 ± 0.04^{d}

Data are presented as mean ± SD. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$)

Table 4. GSI values of the species according to stations

Stations	*Capoeta damascina*	*Alburnus sellal*	*Squalius semae*
1. Station	5.90 ± 0.20^{a}	6.13 ± 0.94^{a}	5.58 ± 1.04^{a}
2. Station	5.56 ± 0.10^{a}	4.28 ± 0.51^{b}	4.03 ± 0.23^{b}
3. Station	3.34 ± 0.15^{b}	3.41 ± 0.18^{c}	3.01 ± 0.05^{c}
4. Station	4.12 ± 0.13^{b}	3.06 ± 0.12^{d}	3.27 ± 0.12^{c}

Data are presented as mean ± SD. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$)

According to these data, it was concluded that mean GSI values were remarkably low, especially in the 3rd and 4th stations, for all three species ($p < 0.05$). When the current state of the surface waters of the stations where the fish samples were taken, the physico-chemical parameters were within the allowable limit values, while the metal levels (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br and Pb) were higher at the third and fourth stations compared to the other stations (Table 1). On the histological slides of fish caught from Station 1, follicles containing multiple oocysts at different developmental stages (chromatin nucleolar, perinucleolar, cortical alveolar, vitellogenical phase, and maturation stage) were observed (Figure 2).

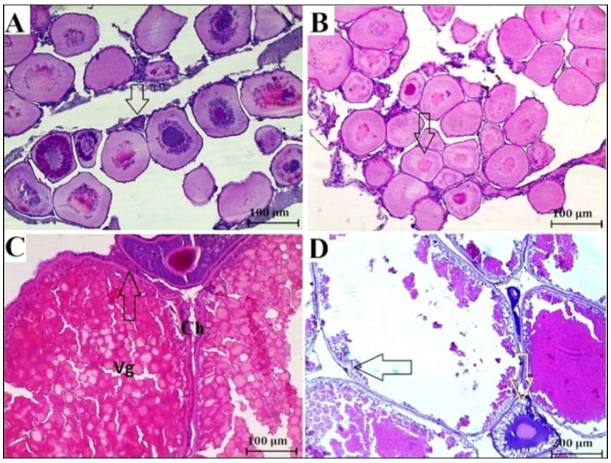

Figure 2. Normal ovarian histology of fish from the Karasu River. A) Chromatin nucleolar oocyte (arrow), (*C. damascina*). B) Perinucleolar oocyte (arrow). C) Vitellogenic oocyte (arrow), Vg: vitellus granule, Ch: chorion (*C. damascina*). D) Cortical alveolar oocyte (yellow arrow), and mature oocyte (black arrow: animal invagination) (*A. sellal*). H&E

When the ovaries were evaluated according to the stations, histopathological damage was observed at varying rates in all three species, especially from the last two stations. In the section in which oocystes were placed belonging the chromatin nucleolar phase in the fish analyzed, congestion in blood vessels, melanomacrophage, mononuclear cell infiltration, and edema were observed (Figure 3A). In addition to the frequently observed enlargement of the follicular epithelium of the cortical alveolar oocyte (Cao) (Figure 3B), mononuclear cell infiltration damage (Figures 3C and 4A), and a severe case of fibrosis (Figure 3C) were detected. Cao shows the cell membrane of the oocyte. The abnormality showed that the vacuoles borders in the cell and the cytoplasm were mixed together in some places. Disorder of cytoplasm and vacuole in the Cao (Figure 3D), proteinaceous fluid and edema in the interstitial space (Figure 4A), as well as atresia (Figure 4B) were identified. Other pathologies were degeneration and malformation in oocytes (Figure 4C) and degeneration in the cytoplasm of the transformed cortical alveolar oocyte (Figure 4D).

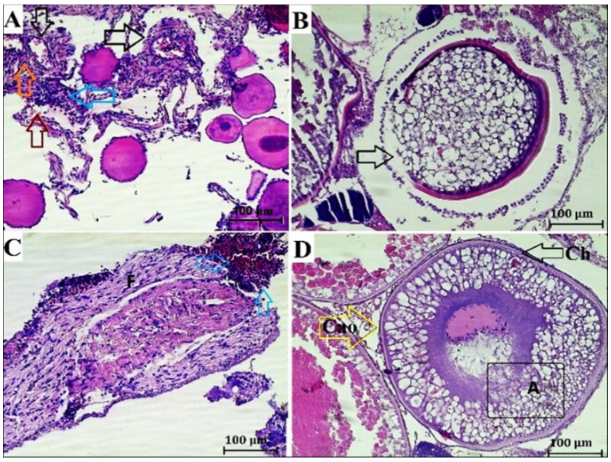

Figure 3. The ovarian pathologies detected in fish species of Karasu River. A) Congestion (black arrows), melanomacrophage (orange arrow), infiltration (blue arrow), edema (red arrow), (*C. damascina*). B) Enlargement of the follicular epithelium (arrow), (*A. sellal*). C) Infiltration (blue arrows) and fibrosis (F), (*C. damascina*). D) Cytoplasm and vacuoles disorder (square), Cao; cortical alveolar oocyte, Ch; chorion, A; alveol (*A. sellal*). H & E

In Table 5, the histological damages detected in the ovary tissues of fish species according to the stations were given with

DTC frequencies. Accordingly, it was concluded that there was no difference between the species in terms of the type of histopathological abnormality, and the frequency of histopathological damage from the 1st station to the others increased even more for all three species.

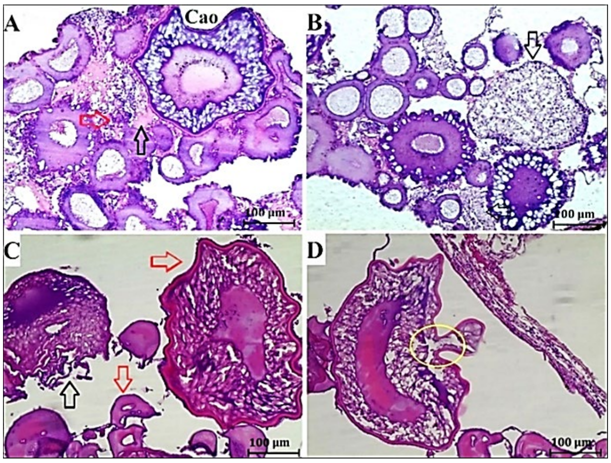

Figure 4. The ovarian pathologies detected in fish species of Karasu River. A) Edema and interstitial proteinaceous fluid (black arrow), infiltration (red arrow), Cao; cortical alveolar oocyte, (*A. sellal*). B) Atresia (arrow), (*A. sellal*). C) Degeneration in the oocyte (black arrow) and oocyte deformity (red arrows), (*S. semae*). D) Degeneration in the oocyte cytoplasm of the deformed cortical alveolar oocyte (yellow circle), (*S. semae*). H & E

When the testicular tissue of the fish caught from Station 1 was examined, a normal structure consisting of numerous seminiferous tubules and interstitial spaces between the tubules, curved, oval or round shaped and of different sizes, containing germ cells at different developmental stages was observed. Secondary spermatocytes that were dark stained in mature testis were observed as clusters in an area close to the tubule lumen. Being the smallest cells, spermatids that were coloured darker was on the lumen. In many sections, it was found that sperms filled the inside of tubules completely (Figure 5).

On the other hand, histological damages were detected at varying frequencies in the testicles of fish caught from other stations. In fish testes analyzed, especially vascular hypertrophy (Figure 6A) and severe congestion (Figures 6A and 6B) were remarkable. In addition, degenerations in the efferent duct and seminiferous tubules (Figure 7A), proteinaceous fluid and inflammatory infiltrate (Figure 7A), edema (Figure 7A and 7B), hypertrophy and degeneration in spermatocytes (Figure 8A), separation in the interstitial area (Figure 6B, 7A, and 8B), and fibrous formations (Figure 6B, 7A, and 8B) were observed. While the lümen of the efferent duct should be smooth, a part of the lumen surface was fragmented in polluted area fish testis (Figure 7A).

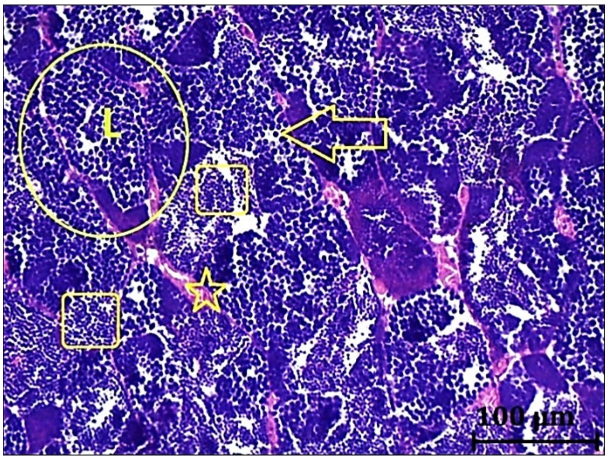

Figure 5. Normal testis histology of fish from the Karasu River (*C. damascina*). Blood vessel (star), spermatids (squares), secondary spermatocyte (arrow), lumen (L), and seminiferous tubule (circle). H & E

In many testes sections, the borders of epithelium surrounding tubules could not be seen clearly, and tissue integrity was disrupted (Figure 8A). Major degeneration was also noted in the seminiferous tubule epithel (Figure 8B). Congestion in tissues with abnormalities has been observed as a common pathology (Figures 8A and 8B).

Table 5. Histological abnormalities and DTC frequencies detected in the ovaries of the species according to the stations

Stations		1. St			2. St			3. St			4. St		
Fish species		C	A	S	C	A	S	C	A	S	C	A	S
Ovarium pathologies	DTC level												
Atresia	I	1	1	1	1	1	1	1	2	2	1	2	1
Infiltration	I	0	0	0	1	0	1	2	2	2	1	2	2
Proteinaceous fluid	I	0	0	0	0	0	1	1	2	2	1	2	2
Congestion	I	0	0	0	0	0	0	2	1	2	2	2	2
Melanomacrophage center	I	1	1	1	1	1	1	2	1	3	1	3	3
Oocyte deformity	I	0	0	0	0	0	0	1	0	1	0	1	1
Edema	I	0	0	0	0	0	1	1	1	2	1	1	1
Enlargement of the follicular epithelium	II	0	0	0	0	0	0	1	2	2	0	1	1
Degeneration in the oocyte cytoplasm	II	0	0	0	0	1	0	0	1	2	1	1	1
Degeneration of oocytes	II	0	0	0	0	0	1	1	1	2	1	1	1
Fibrosis	III	0	0	0	0	0	0	1	0	1	0	1	0

0: no alteration, 1: mild and focal, 2: moderate and multi-focal, 3: severe and diffuse alterations. St; Station, C; *Capoeta damascina*, A; *Alburnus sellal*, S; *Squalius semae.*

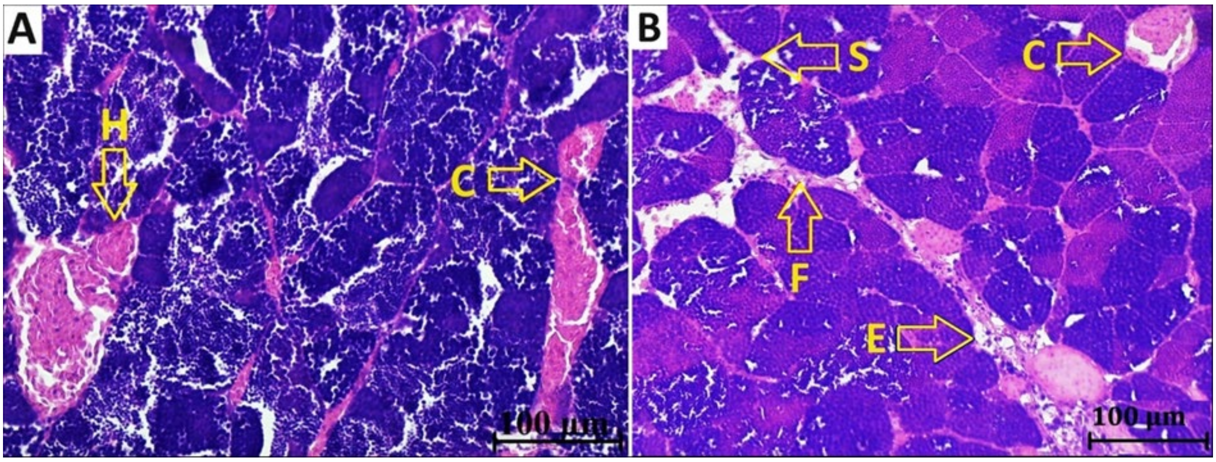

Figure 6. The testis pathologies detected in fish species of Karasu River. A) Vascular hypertrophy (H) and congestion (C), (*C. damascina*).B) Congestion (C), fibrosis (F), separation in the interstitial area (S) and edema (E). H & E

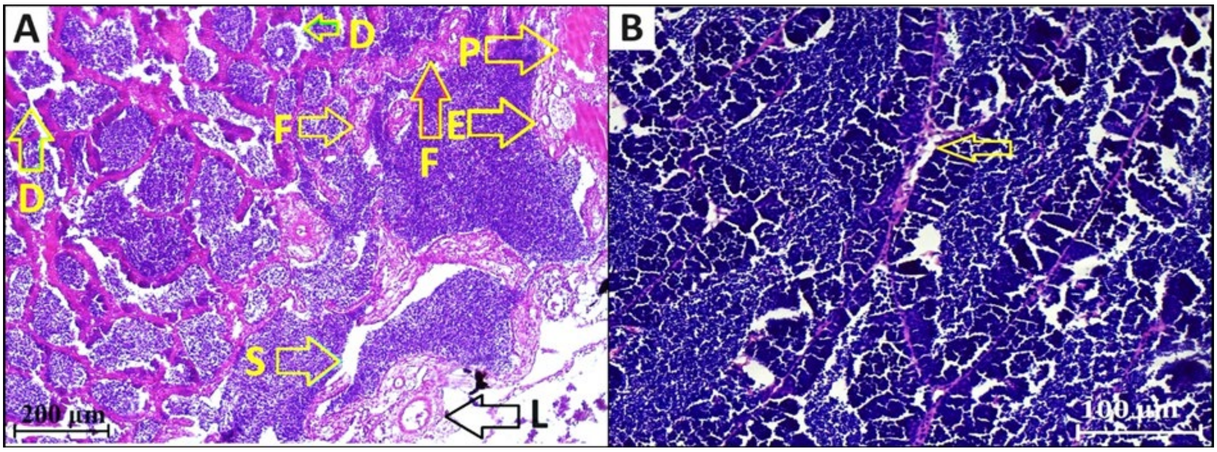

Figure 7. The testis pathologies detected in fish species of Karasu River. A) Proteinaceous fluid and inflammatory infiltrate (P), degeneration of seminiferous tubule (D), fibrosis (F), edema (E), separation in the interstitial area (S), lumen degeneration in the efferent duct (L), (*A. sellal*). B) Edema (yellow arrow) (*A. sellal*). H & E

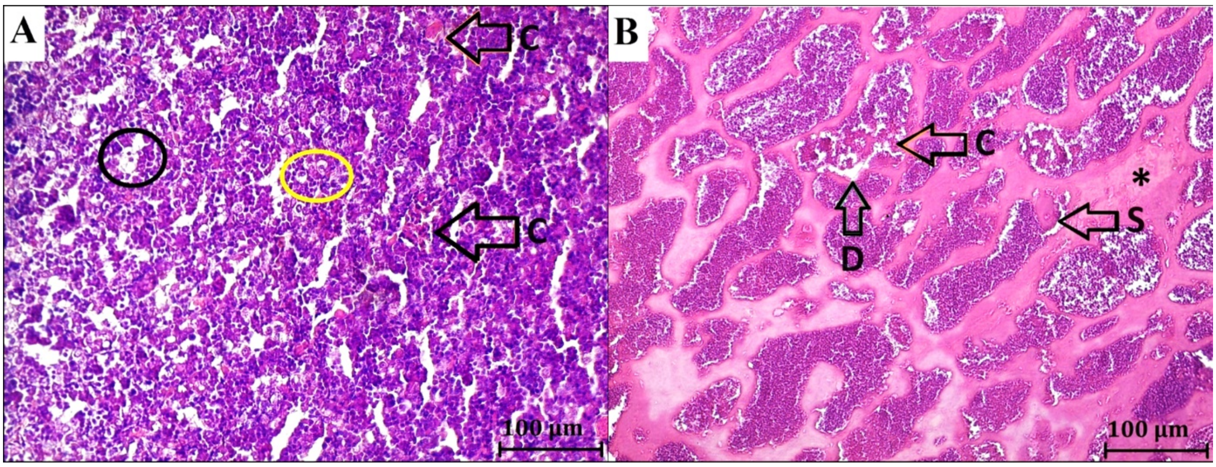

Figure 8. The testis pathologies detected in *S. semae*. A) Degeneration in primary spermatocyte (black circle), hypertrophy in primary spermatocyte (yellow circle), congestion (C), and undetermined seminiferous tubule boundaries (general view). B) Degeneration of the seminiferous tubule epithelium (D), congestion (C), separation in the interstitial area (S), and severe fibrosis (asterisk). H & E

The histological damages detected in the testes of fish species according to stations were given with DTC frequencies in Table 6. Accordingly, it was concluded that there was no difference among fish species in terms of the type of histopathological damage.

Similarly, in all three species, a significant increase was observed in the frequency of the damages at stations 3 and 4. Mean DTC values of fish gonads are given in Table 7. The DTC values demonstrated that gonads had a normal function in the 1st station in all three species, whereas gonads had mild or medium-level damage in other stations. DTC values for *C. damascina* revealed that gonads had mild damage in the 4th station, medium-level damage in the 3rd station, and had normal structure in the 2nd station. DTC values for *A. mossulensis* revealed that the organ had medium-level damage in the 3rd and 4th stations, and mild damage in the 2nd station. DTC values for *S. semae* revealed that gonads had medium-level damage in the 3rd and 4th stations, and mild damage in the 2nd station.

When DTC values for these species were compared, it was seen that the highest values belonged to *S. semae*. While the highest DTC values for *C. damascina* and *S. semae* were recorded in the 3rd station, the highest value for *A. sellal* was recorded in the 4th station. It was found that the differences in DTC values were statistically significant ($p < 0.05$).

Table 6. Histological abnormalities and DTC frequencies detected in the testes of the species according to the stations

Station		**1. St**			**2. St**			**3. St**			**4. St**		
Fish species		**C**	**A**	**S**	**C**	**A**	**S**	**C**	**A**	**S**	**C**	**A**	**S**
Testis pathologies	**DTC level**												
Proteinaceous fluid and inflammatory infiltrate	I	0	0	0	0	1	1	2	1	3	1	2	2
Separation in the interstitial area	I	0	0	0	0	0	1	2	1	2	1	1	1
Congestion	I	1	1	1	1	1	1	2	2	3	2	2	2
Edema	I	0	0	0	0	0	0	1	1	2	1	2	1
Hypertrophy in spermatocyte	I	0	0	0	0	0	0	1	1	2	1	1	1
Deformity of the seminiferous tubule	I	0	0	0	0	0	1	1	2	2	1	1	1
Undetermined seminiferous tubule boundaries	I	0	0	0	1	1	1	2	2	3	2	2	3
Vascular hypertrophy	I	0	0	0	1	1	1	1	2	2	1	1	2
Lumen degeneration of efferent duct	II	0	0	0	0	0	0	1	1	1	1	1	1
Degeneration in spermatocyte	II	0	0	0	0	0	0	1	1	1	1	1	1
Degeneration of seminiferous tubule	II	1	1	1	1	1	1	3	2	3	2	3	3
Fibrosis	III	0	0	0	0	0	0	1	0	1	0	1	1

0: no alteration, 1: mild and focal, 2: moderate and multi-focal, 3: severe and diffuse alterations. St; Station, C; *Capoeta damascina*, A; *Alburnus sellal*, S; *Squalius semae.*

Table 7. Average DTC values for three fish species from the stations

Stations	***Capoeta damascina***	***Alburnus sellal***	***Squalius semae***
1. Station	5.85 ± 0.22^{d}	8.03 ± 0.31^{d}	8.07 ± 0.35^{d}
2. Station	8.61 ± 0.32^{c}	16.02 ± 0.25^{c}	18.20 ± 0.14^{c}
3. Station	28.60 ± 0.34^{a}	23.16 ± 0.27^{b}	47.63 ± 0.25^{a}
4. Station	18.54 ± 0.19^{b}	40.10 ± 0.24^{a}	39.50 ± 0.36^{b}

Data are presented as mean ± standard deviation. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$).

DISCUSSION

Heavy metal pollution is considered to be a serious problem especially in aquatic systems (Joshi, 2011). The reason for the pollution is the increasing industrial, agricultural and mining activities. These activities are increasing day by day (Ashraf et al., 2012). The Karasu River takes all the pollutants of the Karasu Basin and the pollution in the river unfortunately continues. In previous studies, it was reported that the metals analyzed in the water and sediment of the river were above the reported standard values (Sönmez et al., 2012; Aydoğan et al., 2017; Dane and Şişman, 2017), and that the surface waters were extremely polluted water of 4th class quality. In addition, in 2015-2016, concentrations of 6 elements each (V, Mn, Fe, Co, Ni, Sr) in 3rd station and (Ti, Cr, Cu, Zn, As, Pb) in 4th station in water samples were found to be higher compared to the others, and the abiotic parameters (temperature, pH, and dissoved oxygen) did not exceed the standard (Dane and Şişman, 2020a, 2020b). Therefore, it is highly probable that the low GSI values and high gonad histopathologies detected in the fish caught from the 3rd and 4th stations in the current study are the effects of heavy metals in the water.

The GSI value was analyzed on a monthly basis in order to identify gonad development in the fish. In Karasu River, the highest mean GSI value was recorded in June and the lowest value in August for *C. damascina* female and male population. Similarly, Sen et al. (2008) reported that the spawning period for *C. damascina* was between the first week of June and second week of July, while the highest GSI value was recorded in late May for females, and in early June for males, and the lowest values were recorded in July. For *A. sellal*, the highest GSI value was recorded in June for females, and in May for males, while the lowest value was recorded in August. In many studies, it was reported that the highest GSI values of the species were in June, as it was the case in our study (Uçkun and Gökçe, 2015). In Karasu River, the highest mean GSI value was recorded in May and the lowest value in July for *S. semae*. The spawning period for *S. semae* was also reported to be in May and July in Karakaya Dam Lake and Muş Karasu Stream (Kalkan et al., 2005; Sen and Saygin, 2008). GSI is also an indicator of reproductive success being sensitive to various chemical pollutants. Monitoring pollutants which have negative effects on germ cells development and spawning of fish is necessary for preserving their genetic distribution in their region and ecological processes (Corsi et al., 2003). The comparison of GSI values between the stations in the study revealed that the mean GSI values were significantly lower in the 3rd and 4th stations compared to the 1st station for all three species. At the same time, this finding showed that these stations are the most polluted locations of the river. Similarly, low GSI values were reported at stations with the highest pollution density in different fish species (Ribeiro et al., 2013; Kaptaner, 2015). Sadekarpawar and Parikh (2013) reported that pollution in freshwater resources negatively affects the gonads of *Oreochromis mossambicus* based on GSI values and histopathological analysis results.

Aquatic pollution causes an increase in HSI value. HSI is accepted as a good indicator for chemical pollution and provides information on the fish health (Pyle et al., 2005). In the current study, significant differences between HSI values and stations were determined. There are some studies that support this situation. Authman (2011) reported that HSI value in fish exposed to high-dose Al was higher than control group. Monsefrad et al. (2012) reported that some heavy metals were determined in the liver and muscle of fish caughted the Caspian Sea, and the metals increased the fish liver weight and there was a positive correlation between Cd levels and HSI values.

Histopathological abnormalities detected in gonads of the species analyzed in the study has revealed that metal pollution causes serious damages for all three species. In the study, the increase in the frequency of ovarian histopathology in polluted stations was remarkable. The atresia process observed in the study may occur as a normal physiological mechanism in the fish ovary. On the other hand, it is known that atresia is an important response to water pollution. Atresia observed especially in previtellogenical oocyte is an important indicator of a pathological case (Kaptaner, 2015). Hyperplasia and hypertrophy in granulosa cells taking part in the atresia process is a typical sign of atresia (İşisağ Üçüncü and Çakıcı, 2009). The increase in melanomacrophage centers storing xenobiotic substances and containing pigment in ovary in terms of number and size reflects the stress conditions surrounding fish such as chemical pollution. Blazer (2002) reported that severe fibrosis detected in ovaries could be a chronic tissue response to damages caused by pollutants. Studies investigating the effects of heavy metal pollution on the ovaries have reported deformation in oocytes and a decrease in the number of oocytes (Ambani, 2015; Khillare et al., 2017). On histopathological changes in oocysts, which is one of the damages detected in this study, Berois et al. (2011) noted that oocyte sheaths, and particularly zona radiata, are useful bioindicators to identify changes in the aquatic environment. Later, membrane damage detected in primary oocysts at later stages was accepted as an epithelial response to pollution.

Testes, an important part of male reproductive system, is one of the most affected tissues by inner and outer environment changes. Hormones, defects in growth factors or chemical substances may pose serious problems to testis. It was reported that mercury, a xenobiotic metal, caused proliferation in the interstitial tissue of mature individuals' testes in *Rasbora dandia* as well as a decrease in GSI and spermatozoa, thereby decreasing reproductive success (Rajan and Kuzhivelil, 2015). Bashir et al. (2022) reported that there were histopathological abnormalities such as testes degeneration, generalized tissue degeneration produced by fragmentation and detachment of basement membrane, necrosis, and fibrosis in the testis of the fish collected from a metal-polluted river. Another study also demonstrated that environmental pollutants affect estradiol and testosterone levels in fish (Hayati et al. 2022). Panti et al. (2017) demonstrated that pollutants also affect the cell and tissue structure of fish testes resulting in changes in the size of the seminiferous tubules. Many other studies have shown that there are serious changes (various inflammations, degenerative lesions, increase in melanomacrophage centers, disintegration in the seminiferous borders, accumulation of proteinaceous water in the interstitial area of the ovaries, follicular atresia) in fish gonads exposed to various chemicals in their habitats (Feist et al., 2015; Torres-Martinez et al., 2017). In light of all these studies on gonads and our findings, it could be easily said that irreversible histopathological damages in fish may alter the reproductive success and population balances of the species in the future.

CONCLUSION

The findings of the present study clearly showed that the reproductive system of the natural fish species of Karasu River was affected by the pollution. Although species are histologically affected, they can still survive despite the pollution on the river. However, if pollution continues in this way, it is obvious that reproductive health of the fish species will be disrupted. Since the reproductive success will decrease due to pollution, there is a possibility that a distinct decrease will be seen in these populations in the near future. Therefore, further studies both on the field and in the laboratory are needed in order to identify pollutants and mixtures in the river and to analyze how specific changes in fish are related to reproductive health and population effects.

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AUTHORSHIP CONTRIBUTIONS

Hatice Dane performed experimental parts. Turgay Şişman purposed the research idea, analyzed histological slides, designed the study protocol, wrote the manuscript draft and prepared the final version of the paper, and supervised the whole research. All authors approved the submission and publication of this manuscript.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

The legal permissions was obtained from Atatürk University Animal Experiments Local Ethics Committee (ATA36643897/25.09.2013).

DATA AVAILABILITY

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

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