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Oxidative Stress Biomarkers in *Carassius gibelio* from Lakes of Varying Ecological Quality

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Abstract

The Water Framework Directive 2000/60/EC requires the assessment of the ecological quality in all surface waters using biological indices, yet the effective application of these indices often demands extensive and long-term monitoring data. Oxidative stress biomarkers offer a promising complementary approach, as they can detect early biochemical responses of organisms to environmental degradation. In this study, we evaluated the suitability of two oxidative stress biomarkers - malondialdehyde (MDA) levels and DNA damage - in the gonads of a freshwater fish species, the Prussian carp *Carassius gibelio* (Bloch, 1782) as indicators of ecological condition in lakes of differing environmental quality. Fish were sampled from four lakes representing a gradient of physicochemical and ecological status. Both MDA concentrations and DNA damage showed significant ($p < 0.05$) differences among lakes. However, only DNA damage in the gonads was significantly ($p < 0.05$) associated with lake ecological quality as determined by the Greek Lake Fish Index (GLFI), with higher biomarker responses observed in lakes of poorer status. These findings demonstrate that oxidative stress biomarkers in *C. gibelio* reflect variations in lake ecological quality and may serve as sensitive, early-warning tools for biomonitoring and pollution assessment in freshwater ecosystems.

Keywords: Greece; gonads; DNA damage; MDA; biomonitoring; WFD; ecological quality indices

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1. Introduction

The world's aquatic ecosystems are increasingly threatened by human activities (Dudgeon et al., 2006; Reid et al., 2019), which alter their physical and chemical conditions and exacerbate pollution processes (Søndergaard & Jeppesen, 2007). Aquatic organisms inhabiting these ecosystems are consequently exposed to environmental changes that may adversely affect their physiology (Kaloyianni et al., 2019) and survival. Among these organisms, fish are particularly responsive to environmental conditions and are widely regarded as reliable indicators of aquatic ecosystem degradation (Pinna et al., 2023). Accordingly, fish play a crucial role in ecotoxicological studies and biomonitoring surveys (Chovanec et al., 2003; Naigaga et al., 2011; Kaloyianni et al., 2019) and are commonly used as sentinel organisms across multiple levels of biological organization, ranging from community-level assessments to cellular-level approaches (Chovanec et al., 2003).

At the cellular level, pollutants can disrupt homeostasis in fish (Livingstone, 2001; López-López et al., 2011; Kaloyianni et al., 2019). A primary mechanism underlying this disruption is the induction of oxidative stress in fish tissues, resulting from the excessive production of reactive oxygen species (ROS) (López-López et al., 2011). Elevated ROS levels can trigger lipid peroxidation and lead to significant DNA damage (Juan et al., 2021). In this context, the assessment of oxidative stress–related biomarker responses in bioindicator species have been widely recommended. Biomarkers are measurable indicators of biological responses to environmental changes and provide valuable insights into the effects of pollutants on living organisms (Kaloyianni et al., 2019; Gemusse et al., 2021).

Although biomarkers can serve as crucial tools in environmental assessment, the established approach adopted within the European Union is the implementation of the European Water Framework Directive 2000/60/EC (WFD). WFD has promoted the biomonitoring of surface waters (inland, transitional, coastal) across Europe and requires all EU Member States to achieve at least good ecological status or potential for their surface water bodies by the year 2027. The assessment of ecological status is based on the condition of biological quality elements (BQEs), such as phytoplankton, aquatic macrophytes, phytobenthos, benthic macroinvertebrates and fish. Specifically, in accordance with WFD requirements, the ecological status of surface water bodies should be evaluated using biological indices that rely on well-defined pressure–response relationships. Moreover, these indices assess ecological quality/status as a deviation from reference conditions, defined as the absence of significant human disturbance, and express results on a five-class color-coded scale ranging from 0 (bad quality/status) to 1 (high quality/status).

Following this concept, the use of biomarkers could support the implementation of the WFD, as they may function as early warning systems for environmental degradation (Lomartire et al. 2021). *Carassius gibelio*, a widely distributed freshwater fish (Froese & Pauly, 2025), has been successfully used in ecotoxicological studies and has been proposed as a reliable bioindicator organism (Simonyan et al., 2016; Kaloyianni et al., 2019; Bobori et al., 2020; Mijošek et al., 2021). Consequently, the assessment of biomarker responses in such a widespread species could provide valuable insights into ecosystem health, particularly when focusing on specific tissues that play crucial role in species population persistence. Gonads, the reproductive organs of fish, are lipid-rich tissues that are highly responsive to pollution (Corsi et al., 2003); however, they remain relatively underutilized as target tissues in ecotoxicological research. To date, only a limited number of studies have investigated oxidative stress biomarkers in fish gonads (e.g., Kaptaner, 2015; Tyor & Pahwa, 2018; El-Shenawy et al., 2021; Pahwa et al., 2022).

The present study aimed to investigate the responses of sensitive biochemical and genotoxic biomarkers related to oxidative stress - Malondialdehyde (MDA) levels and DNA damage - in the gonads of the freshwater fish, the Prussian carp *Carassius gibelio* (Bloch, 1782), a successful invasive species in Greek lakes (Perdikaris et al., 2012). In detail, we focused on assessing and comparing the ecotoxicological responses of fish gonads in relation to the physicochemical characteristics and ecological quality status of four studied lakes, testing the hypothesis that differences in lake ecological quality would be reflected in variations in biomarker responses. To our knowledge, this is the first study to examine oxidative stress biomarkers in the gonads of *C. gibelio* in Greek lakes, and also the first attempt to correlate biochemical and genotoxic biomarkers with environmental variables and further to lakes ecological quality.

2. Materials and Methods

2.1. Study Area

The study included four natural lakes located in Northern Greece, namely Doirani, Vegoritida, Volvi, Petron (Figure 1). Among them, Lake Doirani is transboundary and shared with the Republic of North Macedonia. The studied lakes vary in their limnological and physicochemical characteristics (Table 1), as well as their ecological quality classification based on different national ecological quality indices (Table 2).

Figure 1. Map of the studied lakes.

Table 1. Morphometric and physicochemical characteristics (mean ± standard error, minimum-maximum values) of the studied lakes. Data acquired from: [a]River Basins Management Plans of the Special Secretariat for Waters, Ministry of Environment, Greece, [b]Zervas et al. (2021) and [c]on line available data for the years 2020-2022 of the Greek Biotope/Wetland Centre (EKBY). DO: Dissolved Oxygen, EC: Electrical Conductivity, TP: Total Phosphorus, NO_3: Nitrates, TSS: Total Suspended Solids, BOD_5: Biochemical Oxygen Demand.

Lake	Area[a] (km^2)	Mean depth[b] (m)	Secchi depth[c] (m)	pH[c]	DO[c] (mg/L)	EC[c] (μS/cm)	TP[c] (mg/L)	NO_3[c] (mg/L)	TSS[c] (mg/L)	BOD_5[c] (mg/L)
			Mean (± standard error) (minimum-maximum values)							
Doirani	38.87	4	1.48 (±0.14) (0.8-4.0)	8.90 (±0.04) (8.53-9.26)	10.16 (±0.28) (7.33-13.01)	686.45 (±14.98) (583-965.9)	0.044 (±0.004) (0.019-0.085)	0.47 (±0.08) (0.27-2.1)	7.93 (±0.66) (1.76-14.89)	4.52 (±0.49) (0.8-8.5)
Petron	12.36	3	0.34 (±0.04) (0.20-0.70)	8.91 (±0.09) (8.2-9.55)	1051 (±0.69) (7.65-18.39)	979.82 (±32.88) (737.7-1162)	0.066 (±0.006) (0.022-0.127)	1.41 (±0.59) (0.27-7.1)	29.51 (±5.26) (10.4-96.7)	5.4 (±0.86) (7.65-18.39)
Vegoritida	53.96	25	3.14 (±0.28) (1.10-5.20)	8.84 (±0.06) (8.11-9.16)	9.66 (±0.29) (7.66-13.26)	629.27 (±14.54) (510.2-727.3)	0.042 (±0.005) (0.016-0.091)	0.75 (±0.21) (0.27-3.6)	2.26 (±0.32) (0.35-5.67)	2.92 (±0.5) (0.3-7.3)
Volvi	72.07	13	1.62 (±0.08) (1.1-2.5)	8.94 (±0.05) (8.5-9.35)	9.64 (±0.46) (6.3-13.5)	923.87 (±23.15) (773.3-1069)	0.060 (±0.007) (0.023-0.132)	1.48 (±0.57) (0.27-7.0)	3.94 (±0.34) (0.69-6.75)	4.7 (±0.7) (6.3-13.5)

Table 2. Ecological quality classification of the studied lakes assessed by different Greek indices covering the last decade. EQR: Ecological Quality Ratio, [a]GLFI: Greek Lake Fish Index (Petriki et al., 2017), [b]GLBiI: Greek Lake Benthic macroinvertebrate Index (Ntislidou et al., 2018), [c]HeLPhy: Hellenic Lake Phytoplankton (Tsiaoussi et al., 2017), [d]HeLM: Hellenic Lake Macrophytes (Zervas et al., 2018), [e]HelLBI: Hellenic Lake Littoral Benthic macroinvertebrate Index (Mavromati et al., 2021).

Lake	Quality class (EQR) per Index				
	GLFI[a]	GLBiI[b]	HelPhy[c]	HeLM[d]	HelLBI[e]
Doirani	High	Good	Moderate	Good	Moderate

	(0.81)	(0.69)	(0.57)	(0.77)	(0.47)
Petron	Moderate (0.52)	Moderate (0.54)	Good (0.63)	-	Moderate (0.55)
Vegoritida	Good (0.66)	Moderate (0.54)	Good (0.64)	Good (0.62)	Good (0.69)
Volvi	Moderate (0.58)	Moderate (0.41)	Moderate (0.46)	Good (0.73)	Moderate (0.44)

2.2. Fish Species Selection and Sample Procedure

A total of 32 adult *C. gibelio* individuals (n=8 from each lake) were provided by local professional fishermen in January of 2023. The species was selected based on its abundance and frequent occurrence in commercial catches of the selected lakes, as well as its previous use in ecotoxicological studies (Kaloyianni et al. 2019). *C. gibelio* is common in shallow eutrophic lakes and is sold in local markets for human consumption, with some quantities exported to EU countries (Leonardos 2015). Specimens were immediately placed on dry-ice and transferred to the laboratory where they were measured for total length (to the nearest 1 mm) and weighed (to the nearest 0.1 g). Then dissected for gonad tissue collection. Samples were stored at -80^{o}C until further analyses. Fish treatment was conducted in accordance with local guidelines for the care of animals, complying with the Official Journal of the Greek Government No. 106/30 April 2013 on the protection of animals used for scientific purposes.

2.3. Biochemical and Genotoxic Indicators

2.3.1. Lipid Peroxidation Quantification, MDA Levels Measurement

Gonad samples were analyzed following the method described by Niehaus and Samuelsson (1968). This experimental procedure is based on the formation of lipid peroxyl radicals and hydroperoxides as the result of the reaction of free radicals or non-radical species with polyunsaturated fatty acids (PUFAs). Results are expressed as nmol MDA per mg protein since one of the terminal products of lipid peroxidation is MDA (malondialdehyde), a reactive aldehyde which forms an adduct with two thiobarbituric acid molecules producing a pink color compound (TBARS). The concentration of TBARS was detected spectrophotometrically at 535 nm as the absorbance is proportional to MDA concentration (ε 1.5 * 10^5 L/ mol cm) (Wills, 1969). The quantification of the total protein concentration was conducted using bovine serum albumin as a standard solution according to the Bradford method (Bradford,1976). The results are expressed as nmol MDA/mg protein. The procedure followed was described in detail in Dimitriadi et al. (2021).

2.3.2. Alkaline Single-Cell Gel Electrophoresis (Comet Assay)

Gonad samples were analyzed following the comet assay protocol as modified by Dailianis et al. (2005). The procedure followed was described in detail in Dimitriadi et al. (2021). In brief, gonad cells were treated with collagenase, embedded in agarose, underwent cell lysis and electrophoresis under alkaline conditions. Finally, after ethidium bromide staining, the presence of comets was examined under an inverted fluorescence microscope. Four slides per sample were measured as technical replicates. One hundred cells were randomly selected and scored from each slide using the software Tritek CometScoreTM 2.0 (Tritek Corporation, Wilmington, DE, USA). The findings are expressed as a percentage of DNA in the tail (%DNA in tail) (Figure 2). %DNA in tail and Olive moment in positive control data (1 μM H_2O_2) were 28.3 ± 5.2 and 40 ± 6.3, respectively.

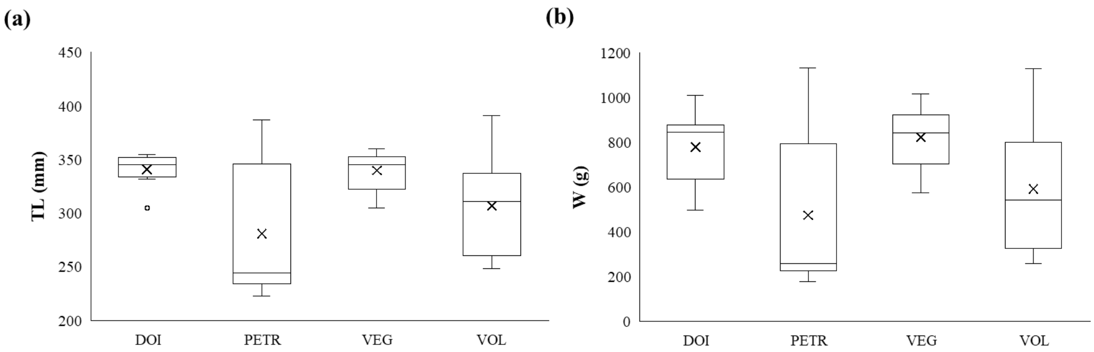

Figure 2. Box plots of (a) total length (TL, mm) and (b) weights (W, g) of *Carassius gibelio* from lakes Doirani, Petron, Vegoritida and Volvi. The lower and upper boundaries of the boxes represent the 25th and 75th percentiles, respectively. The lines (whiskers) indicate the maximum and minimum values of the measurements. The interior line of the box represents the median, while the dot indicates the mean and the circles the outlier values.

2.4. Data Acquire and Statistical Analysis

Morphometric data for the studied lakes were obtained from the Greek River Basins Management Plans (2nd Revision 2024) of the Special Secretariat for Waters of the Greek Ministry of Environment (https://wfdver.ypeka.gr/el/home-gr/), assessed on August 2025, and Zervas et al. (2021). The lakes' physicochemical characteristics were derived from the publicly available data of the Greek Biotope/Wetland Centre (EKBY, https://wfd.ekby.gr/apotelesmata/biologika-physikochimika-dedomena/) and assessed on August 2025. The latter correspond to the years 2020-2022, a period reflecting the presence of the sampled fish individuals in the lakes.

All data were first explored for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. As the majority of the biomarker, total length, and weight data deviated from normality, non-parametric statistical tests were applied throughout the study. Thus, the independent Kruskal-Wallis tests were performed to test whether fish total length, weight and biomarker values differed significantly across lakes ($p < 0.05$). When significant differences were detected, pairwise comparisons were contacted using Mann-Whitney U tests with Bonferroni correction to identify specific differences between lakes for each biomarker ($p < 0.05$). Relationships of biomarker responses with environmental variables were assessed using the non-parametric Spearman's rank correlation. Moreover, to examine the association between biomarker responses and lake ecological quality, fish biomarker values were regressed against the corresponding lake EQR values, as estimated using various ecological indices. All statistical analyses were conducted using IBM SPSS ver. 29 (SPSS, Inc. Chicago, USA) with significance level set at $p < 0.05$.

3. Results

3.1. Fish Morphometrics

Fish mean total length (TL ± standard error) was 341 ± 5.7 mm for specimens from Lake Doirani, 281 ± 22.6 mm for specimens from Lake Petron, 340 ± 6.8 mm for specimens from Lake Vegoritida, 307 ± 17 mm for specimens from lake Volvi. Accordingly, fish weight averaged 765 ± 66.9 g for specimens from Lake Doirani, 399 ± 132.8 g for Lake Petron, 858 ± 41.3 g for Lake Vegoritida and 562 ± 118.9 g for Lake Volvi. The distribution of fish total lengths and weights (Figure 2) did not differ significantly among lakes (Kruskal-Wallis, $p > 0.05$). These measurements indicate that the sampled individuals were predominantly 2–3 years old (Zhelev et al., 2015). This age class corresponds to the period 2020–2022, reflecting the time during which the sampled fish were present in the lakes and, therefore, their period of exposure to environmental stressors.

3.2. Oxidative Stress Biomarkers

MDA values differed significantly across lakes (Kruskal-Wallis test, $p < 0.01$; Table 3, Figure 3). Mean MDA levels were higher in the gonads of fish collected from Lake Petron compared to those from the other studied lakes (Table 3). More specifically, mean MDA levels were significantly higher (approximately sixfold) in fish from Lake Petron than in specimens from Lake Doirani (Mann-Whitney, $p < 0.01$) where the lowest mean MDA value was observed, as well as those from Lake Vegoritida (Mann-Whitney test, $p < 0.01$).

Mean DNA damage values also differed significantly among lakes (Kruskal–Wallis test, $p < 0.01$), following the same pattern observed for MDA. Gonads of fish collected from Lake Petron exhibited a higher degree of DNA fragmentation compared to those from the other studied lakes (Table 3, Figure 3). Specifically, mean DNA damage values were 1.3-fold higher in fish from Lake Petron than in those from Lake Doirani (Mann–Whitney test, $p < 0.01$). In addition, mean DNA damage values in samples from Lake Volvi were significantly higher than those from Lake Doirani (Mann–Whitney test, $p < 0.05$), while specimens from Lake Vegoritida and Lake Volvi exhibited mean DNA damage values that were 1.1- and 1.2-fold higher, respectively, compared to samples from Lake Doirani.

Overall, both biomarkers followed a consistent trend, with gonadal samples from Lake Petron exhibiting the highest biomarker values, whereas those from Lake Doirani showed the lowest.

Table 3. Mean MDA (nmol/mg protein) and %DNA in tail values. Standard error, minimum and maximum values are also provided.

	Lake			
Parameter	**Doirani**	**Petron**	**Vegoritida**	**Volvi**
	Mean (± standard error), minimum - maximum values			
MDA (nmol/mg protein)	2.31 (±0.28) 1.67-2.97	14.70 (±0.81) 10.39-17.81	4.97 (±0.64) 2.97-7.35	5.42 (±1) 3.91-9.19
%DNA in tail	15.1 (±0.54) 13.14-18.35	19.68 (±1.15) 16.08-24.81	16.19 (±0.53) 13.41-18.73	18.44 (±0.34) 16.69-19.82

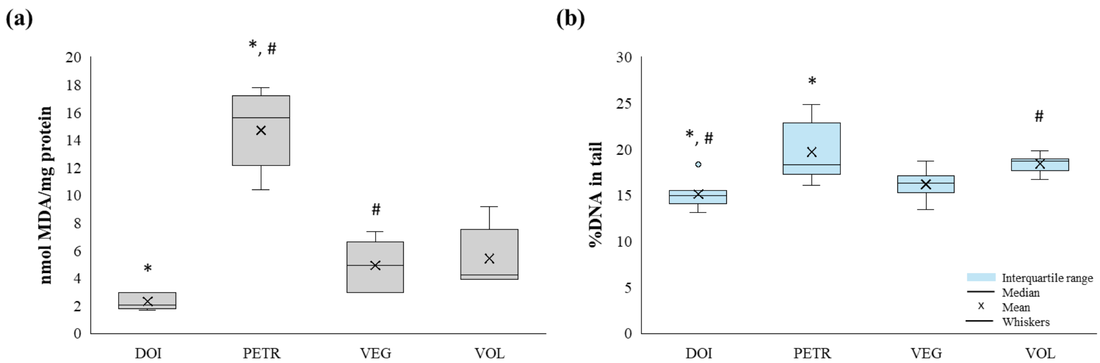

Figure 3. Boxplots of (a) MDA levels and (b) %DNA in tail estimated in the gonads of *Carassius gibelio* in lakes Doirani (DOI), Petron (PETR), Vegoritida (VEG) and Volvi (VOL). The lower and upper boundaries of the boxes represent the 25th and 75th percentiles, respectively. The lines (whiskers) indicate the maximum and minimum values of the measurements. The interior line of the box represents the median, while the dot indicates the mean and the circles the outlier values. * and # indicate statistically significant differences (Mann-Whitney pairwise comparisons, p-value<0.05) between biomarker values across lakes.

3.3. Biomarker- Environmental Parameter Relationships

MDA levels and DNA damage showed no significant correlation ($p > 0.05$) with the examined morphological and environmental lake parameters (Table 4). However, a significant interrelationship ($p < 0.01$) was observed between MDA levels and DNA damage (Table 4).

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Table 4. Spearman correlation analysis of environmental parameters and the estimated mean MDA (nmol/mg protein) and %DNA in tail values. ** statistically significant correlations (p < 0.01).

	Area	**Mean depth**	**Secchi depth**	**pH**	**DO**	**EC**	**TP**	**NO3**	**BOD**	**TSS**	**Chla**	**Mean MDA**	**Mean %DNA in tail**
Area	1	0.80	0.80	0.20	***-1*****	-0.40	-0.40	0.40	-0.40	-0.80	-0.80	-0.20	-0.20
Mean depth		1	***1*****	-0.40	-0.80	-0.80	-0.80	0.01	-0.80	***-1*****	***-1*****	-0.40	-0.40
Secchi depth			1	-0.40	-0.80	-0.80	-0.80	0.01	-0.80	***-1*****	***-1*****	-0.40	-0.40
pH				1	-0.20	0.80	0.80	0.80	0.80	0.40	0.40	0.60	0.60
DO					1	0.40	0.40	-0.40	0.40	0.80	0.80	0.20	0.20
EC						1	***1*****	0.60	***1*****	0.80	0.80	0.80	0.80
TP							1	0.60	***1*****	0.80	0.80	0.80	0.80
NO3								1	0.60	0.01	0.01	0.80	0.80
BOD									1	0.80	0.80	0.80	0.80
TSS										1	***1*****	0.40	0.40
Chla											1	0.40	0.40
Mean MDA												1	***1*****
Mean %DNA in tail													1

3.4. Biomarker-ecological quality relationships

Biomarker values were regressed against lake water ecological quality, as assessed by various biological indices (Table 2). Lipid peroxidation levels (MDA) did not show significant relationships with any of the tested ecological quality indices. In contrast, mean %DNA in tail exhibited a significant negative association only with the fish-based ecological quality index (GLFI; $R^2 = 0.915$, $p < 0.05$). Specifically, DNA damage increased as GLFI values decreased (Figure 4). Overall, genotoxic biomarkers appeared to be more sensitive to variations in lake ecological quality than lipid peroxidation. This negative correlation is more obvious in Figure 5 where the mean values of the two biomarkers are presented in relation to the EQR values of the lakes.

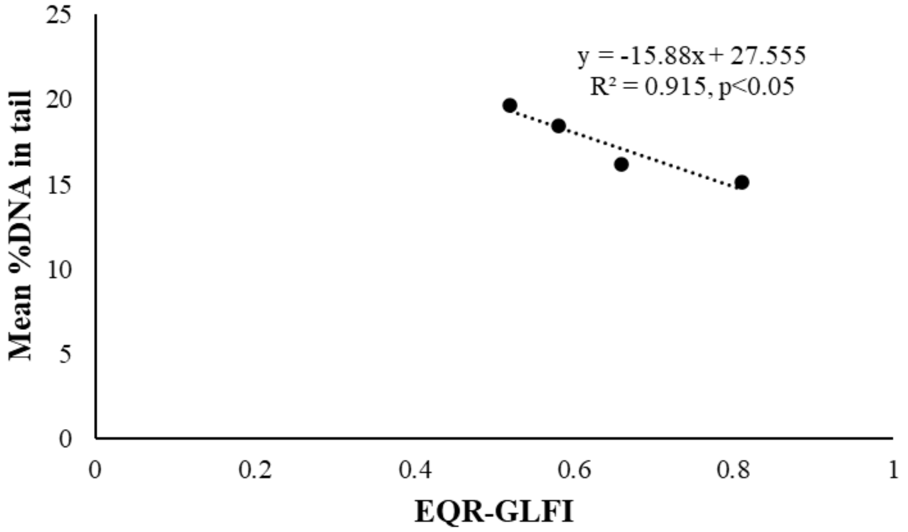

Figure 4. Linear regression of mean %DNA in tail values against the ecological quality ratio (EQR) of each of the studied lake as defined by the Greek Lake Fish Index (GLFI).

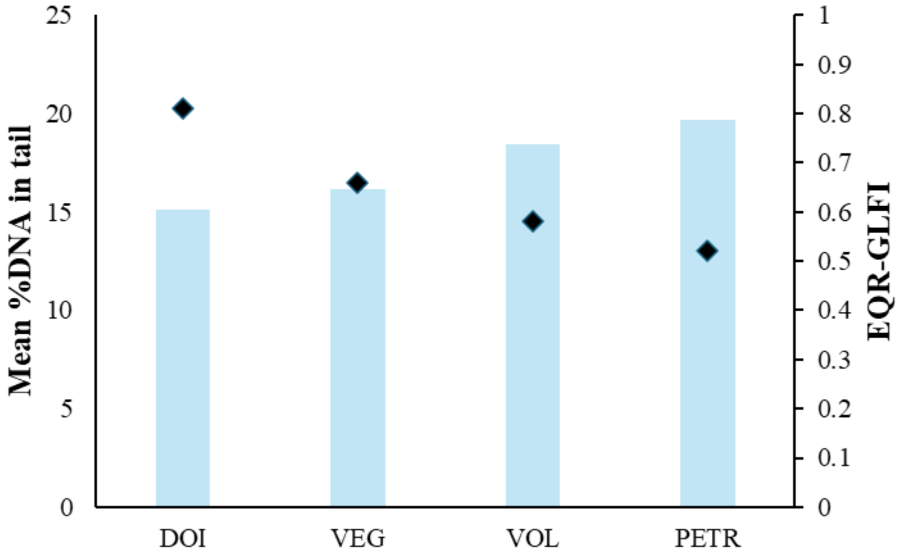

Figure 5. Mean %DNA in tail values (columns) and EQR values (dots) as assessed by the Greek Lake Fish Index (GLFI). DOI, VEG, VOL, PETR for Lakes Doirani, Vegoritida, Volvi and Petron, respectively.

4. Discussion

There is a growing body of research employing biochemical and molecular biomarkers in fish as effective tools for environmental biomonitoring of aquatic ecosystems (Santana et al., 2018; Kaloyianni et al., 2019; Bobori et al., 2020; Lombardero et al., 2024). Among these, malondialdehyde (MDA), a marker of lipid peroxidation, and DNA damage, a genotoxicity indicator, have been widely proposed as sensitive biomarkers responding to a variety of environmental stressors and pollutants in fish (Kaloyianni et al., 2019; 2020; 2021).

In the present study, these two biomarkers were assessed in fish gonads collected from four lakes, with the aim of exploring potential associations between biomarker responses and the ecological quality of lake waters. Most ecotoxicological studies investigating oxidative stress and genotoxicity in fish have focused on tissues such as gills, liver, spleen, heart, and muscle (Farombi et al., 2007; Oliva et al., 2012; Orso et al., 2023), whereas fish gonads have been used comparatively rarely (Kroon et al., 2017). Gonadal tissue was selected in this study due to its high content of polyunsaturated fatty acids (PUFAs) (Ezenwosu et al., 2021), which renders it particularly susceptible to oxidative damage and pollutant-induced stress (Santos et al., 2013; Kaptaner et al., 2016; Tyor & Pahwa, 2018; El-Shenawy et al., 2021).

Elevated lipid peroxidation and DNA damage in fish gonads have been consistently reported in contaminated environments (Gagnaire et al., 2017; Barros et al., 2017; Tyor & Pahwa, 2017; Bhat et al., 2023). MDA, in particular, has been proposed as a reliable biomarker against heavy metal contamination in the gonads of *Oreochromis niloticus* (El-Shenawy et al., 2021). Kaptaner (2015) reported an increase in lipid peroxidation and histological lesions in the gonads of *Alburnus tarichi* from impacted lake systems, followed by reduction in gonadosomatic index. Moreover, Kaptaner et al. (2016) have linked histological abnormalities to increased oxidative stress responses, suggesting that water pollution in lake waters may affect the reproductive health of fish. In addition, Tyor & Pahwa (2018), observed oxidative stress-related increases of MDA in testes of *Clarias gariepinus* from contaminated areas of a riverine system. Also, Pahwa et al. (2022) reported a significant decline in antioxidant enzyme activity in the ovaries of *Clarias gariepinus* from the polluted areas. Finally, in vitro exposure experiments have demonstrated that pollutants can induce increased MDA levels in fish gonads (Zhang et al., 2016; Sumi & Chitra, 2019; Sha et al., 2021; Kutluyer et al., 2022; Sutha et al., 2022)

With respect to genotoxicity, both field and laboratory studies have demonstrated that environmental contaminants can induce DNA damage in fish gonads (Faßbender & Braunbek, 2013; Liu et al., 2014; Beaton et al., 2019). Santos et al. (2013) demonstrated a link between sperm DNA integrity and reproductive impairment in Gasterosteus aculeatus. In general, genotoxicity is suggested to disrupt reproductive processes through mechanisms such as gamete loss via cell death, increased embryonic mortality, and harmful heritable mutations, all of which can affect recruitment rates and population dynamics (Anderson & Wild, 1994; Santos et al., 2013). In parallel, high oxidative stress in gonads may be indicative of impaired reproductive capacity (Samarin et al., 2019). According to Devaux et al. (2011), DNA damage in the sperm cells of Salmo trutta and Salvelinus alpinus is associated with decreased reproductive rates. Additionally, environmental contaminants have been shown to reduce the motility and fertilization ability of fish spermatozoa that result to a high level of infertility (Ezenwosu et al., 2021; Hatef et al., 2013). In line with this notion, it's plausible to speculate that the lower biomarker levels observed in fish gonads from Lake Doirani may indicate reduced oxidative stress and, consequently, a higher potential for reproductive health compared to fish from the other studied lakes. In contrast, the elevated MDA and DNA damage levels observed in the gonads of fish from lake Petron – a shallower and more eutrophic lake - may suggest impaired reproductive performance, as pollutant-induced alterations and lesions in gonadal tissue can lead to reduced fertility (Ezenwosu et al., 2021).

Our results revealed clear differences in biomarker levels among the studied lakes. Specifically, fish from Lake Doirani exhibited the lowest mean levels of both MDA and DNA damage, suggesting comparatively lower oxidative and genotoxic stress in this system. In contrast, fish collected from Lake Petron showed markedly elevated levels of both biomarkers, indicating increased oxidative stress in gonadal tissue, as reflected by enhanced lipid peroxidation and DNA fragmentation. These findings are consistent with the observed environmental status of the lakes: Lake Petron is characterized by higher concentrations of total phosphorus and total suspended solids, as well as lower Secchi disc depth, indicative of greater eutrophication pressure. Furthermore, Lake Petron is classified as having moderate ecological quality based on the Greek Lake Fish Index (GLFI), exhibiting the lowest ecological quality ratio (EQR) among the studied lakes. The GLFI is an ecological quality index designed to reflect the response of lake fish communities to eutrophication, particularly total phosphorus levels, by incorporating metrics such as the relative contribution of omnivorous fish species to total catch (Petriki et al., 2017). Although no direct relationships were detected between the studied biomarkers and individual environmental parameters, higher GLFI values were generally associated with lower MDA levels and reduced DNA damage. This pattern supports a negative relationship between oxidative/genotoxic stress in fish gonads and overall ecological quality. Conversely, the low biomarker levels observed in Lake Doirani are in agreement with its classification as having high ecological quality according to GLFI, suggesting that the biomarker responses measured here reflect differences in lake ecological condition.

In general, environmental parameters linked to eutrophication and anthropogenic pressure appear to play an important role in shaping oxidative stress responses in fish tissues. Previous studies have shown that eutrophication can elevate oxidative stress while suppressing antioxidant defenses (Stoliar & Luschack, 2012). Furthermore, the presence of high chlorophyll levels in lake systems correlates with increased MDA values, as observed in the gills of *Liza aurata* (Pereira et al., 2010). MDA levels were also increased in the liver of *Cyprinus carpio* and *Hypophthalmichthys nobilis* from eutrophic lakes affected by cyanobacterial blooms (Moutou et al., 2012; Sun et al., 2013). In addition to eutrophication, elevated levels of BOD_5 and TSS have been associated to increased MDA levels in fish tissues (Pandey et al., 2003; Morais et al., 2016). Moreover, low dissolved oxygen levels and highwater turbidity contribute to elevated MDA and DNA damage in fish from rivers under anthropogenic stress (Barros et al., 2017; Rubio-Vargas et al., 2024). According to Castro et al. (2020), increased lipid peroxidation and DNA damage in the sperm cells of *Colossoma macropomum*, due to elevated water temperature, high pH, and hypoxia, may adversely affect reproduction and reduce sperm quality. These observations support the interpretation that the biomarker patterns observed in the present study may be related to differences in water quality and trophic status among the lakes.

Overall, our research enhances the comprehension of the correlation between biomarkers associated with oxidative stress and lake water ecological quality, as assessed by fish-based indices, in accordance with the WFD. While community-level biological metrics remain central to ecological status assessment under the WFD, the present findings suggest that MDA levels and DNA damage in fish gonads may provide complementary, early-warning signals of ecosystem disturbance. Following this concept, community metrics and ecotoxicological approaches seem to be complementary (Martinez-Haro et al., 2022; Novais et al., 2023), reinforcing the need to use combined approaches of different disciplines to achieve the best and early evaluation of the ecosystem health and ecological water quality. Moreover, the integration of ecotoxicological biomarkers with biological indices may enhance the sensitivity and timeliness of lake water quality assessment. Further research is required to validate these biomarkers across broader spatial and temporal scales and to support their potential incorporation into harmonized ecological assessment and classification schemes.

5. Conclusions

The current study suggests that measuring MDA levels and DNA damage in fish gonads may reveal variations in environmental conditions and differences in ecological quality across different lake water bodies. Notably, significant correlations were observed between DNA damage and the Greek Lake Fish Index (GLFI). Higher levels of oxidative stress biomarkers were associated with lower ecological quality, indicating that these biomarkers could be useful as early indicators of water quality. However, additional research is needed to confirm these results and to assess the broader applicability of biomarkers in evaluating ecological quality and biomonitoring in freshwater ecosystems.

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