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Original paper

Histological alterations and oxidative stress parameters in common carp (*Cyprinus carpio* L. 1758) from blooming Hídvégi Pond, Hungary

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Summary. Accelerated eutrophication and climate change has led to more frequent blooming of cyanobacteria and the appearance of cyanotoxins worldwide. Because they are at the top of the aquatic food chain, fish can be exposed to cyanobacteria and their toxic metabolites. Thus, as one of the most complex organisms that inhabit aquatic ecosystems, fish can be good bioindicators of water pollution. The objective of this study was to assess several biomarkers in common carp (*Cyprinus carpio* L. 1758) from Hídvégi Pond (Hungary) sampled in June 2019: including histopathological changes of individual fish organs, biotransformation enzymes and oxidative stress parameters. An attempt was made to verify whether findings could be associated with previously reported cyanotoxin exposure. The results showed that DNA fragmentation was not observed; cytochrome P450 1A enzyme activity was higher in the testes, glutathione-S-transferase was significantly lower in the liver and ovaries, lipid peroxidation levels were high in all organs except the testes (with the highest level in the liver and gills); catalase and superoxide dismutase activity were significantly lower in the liver; total and Se-dependent glutathione peroxidase activity was significantly lower in the liver, while significantly higher activity was measured in the testes; glutathione reductase activity was increased in the liver and testes of *C. carpio* caught from Hídvégi Pond. Intense lipid peroxidation and changes in the activity of antioxidant enzymes indicate that fish from Hídvégi Pond were exposed to oxidative stress, potentially induced by cyanobacteria and/or their metabolites. Additionally, histopathological alterations were observed in the liver, kidneys and gills of fish, and specific damage to these organs could be directly linked to the action of hepatotoxic cyanotoxins. Therefore, it is likely that cyanobacteria and cyanotoxins have a detrimental effect on fish, as well as on other organisms including humans. Hence it is necessary to monitor cyanobacteria, cyanotoxins, and their effects on other organisms, and develop biomarkers capable of directly monitoring toxicity and pollution in the environment.

Keywords: biomarker, cyanobacteria, cyanotoxins, fish, histopathology.

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INTRODUCTION

Over the last few decades, environmental pollution has become an increasingly frequent topic of research and debate. The increased release of various toxic substances into the external environment has mostly been the result of numerous anthropogenic activities. Biomarkers, or rather biological responses, can be associated with the exposure to toxic substances (Peakall 1994; van der Oost et al. 2003). Several of the most commonly used biomarkers include genotoxicity (e.g. DNA fragmentation), biotransformation enzymes, parameters of oxidative stress, and morphological parameters (e.g. histopathology). Fish, as one of the most complex organisms that inhabit aquatic ecosystems, are considered to be good bioindicators since monitoring of various biomarkers can provide important information about the state of the aquatic environment (van der Oost et al. 2003).

Lake Balaton and Kis-Balaton are areas of great importance in Hungary, which in the past had frequent problems with water pollution and cyanobacterial blooming. Hídvégi Pond is a part of the Kis-Balaton Water Protection System (KBWPS), which was re-flooded in order to decrease cyanobacterial blooms and further deterioration of the water quality in Lake Balaton. During 2018, the success of this task was investigated by Marinović et al. (2021). Severe blooming of KBWPS and the presence of five microcystin (MC) congeners in water was noted, as well as MC- and saxitoxin (STX) synthetase-coding genes. Although MC accumulation in the tissues of different fish species caught from the lake was not found, histopathological analyses showed severe hepatic, kidney and gill damage. On the other hand, the water quality of Lake Balaton was much improved, indicating that the construction of the KBWPS did have a significant protective effect, and safeguards Lake Balaton from most effects of cyanobacterial blooms (Marinović et al. 2021).

Given that excessive blooming still occurs in KBWPS, further investigation was performed in June of 2019 in Hídvégi Pond, and new methods were employed to obtain more insight into the potential harmful effects on aquatic organisms. Therefore, the aim of this study was to determine the possible effects of cyanotoxins on fish tissues by: (1) assessing DNA damage (DNA double strand breaks); (2) determining the activity of biotransformation enzymes (ethoxyresorufin-O-deethylase- EROD activity and glutathione-S-transferase - GST); (3) analyzing parameters of oxidative stress (lipid peroxidation - LPO, as well as catalase activity - CAT, total superoxide dismutase - SOD, glutathione peroxidase - GPx and glutathione reductase - GR); and (4) histological analyses of various organs of common carp (*Cyprinus carpio* L. 1758) from the Hídvégi Pond.

MATERIALS AND METHODS

Sampling conducted in the study was approved by the National Office of Environment, Nature and Water Conservation: license OKTF-KP/8294-14/2016, for research in the protected areas of the Balaton catchment and by the Hungarian Ministry of Agriculture: license HHgF/122-1/2018, for collecting and sampling fish from natural populations.

Sampling

In June of 2019, at Hídvégi Pond (46°38.1′N; 17°9.8′E), which is part of Kis-Balaton (Hungary), fish were caught with a standard electrofishing device and sacrificed with a blow to the head. Four adult individuals of *C. carpio* (2 males/2 females; TL: 380 ± 26 mm) were sampled. Samples of liver, gills and gonads for molecular and biochemical analyses were snap frozen in liquid nitrogen on site and kept at -80 °C until further use. Samples of liver, kidney, gills, spleen, intestine, gonads (ovaries and testes) and muscles were collected and immediately fixed in 10% neutral-buffered formalin (NBF) for histopathological analyses for at least 24 hours at room temperature (RT 23 °C).

Six *C. carpio* adult individuals (3 males/3 females; TL: 320 ± 88 mm) from the regular broodstock of the Department of Aquaculture, Hungarian University of Agriculture and Life Sciences, Hungary, were used as a control. Fish were kept at 24 ± 1 °C, in a 12 h light/12 h dark cycle. They were fed twice a day with commercial feed (Aller Claria Float) rich in protein in concentrations dependent on fish size. Control samples for molecular, biochemical and histopathological analyses were collected, kept and processed in the same manner as the samples from Hídvégi Pond.

Molecular and biochemical analyses

Frozen fish organ samples (gills, liver, testis, ovaries) of *C. carpio* were homogenized in Tris buffer (10 mMTris-HCl containing 0.15 M KCl, 0.5 M sucrose, 1 mM EDTA, dithiothreitol (DTT) and 40 μg/mL aprotinin; pH 7.5) using a small bead mill (Tissue Lyser LT, Qiagen, Germantown, MD, USA). Subsamples of raw sample homogenates were frozen at -80 °C for analysis of LPO, DNA strand breaks and total protein content. Remaining subsamples were centrifuged at 12,000 ×*g* for 30 min at 4 °C. Supernatants were collected and aliquots were kept at -80 °C until enzyme activity measurements.

Protein concentration of the samples was determined in triplicate by the Bradford method (Bradford 1976), adapted to microplate format, using bovine serum albumin as standard. Absorbance was recorded at 595 nm after an incubation period of 10 min. Protein concentrations in samples

were calculated from a standard curve and used to express results from molecular and biochemical analyses. Analyses were performed in triplicate and evaluated using a Thermo Varioskan™ LUX multimode microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). The obtained results of all molecular and biochemical analyzes were statistically processed using the Mann-Whitney U test with a significance factor of $P < 0.05$.

DNA strand breaks were quantified by adaptation of the alkaline precipitation assay of Olive (1988). Fluorescence was measured at 360 nm excitation/450 nm emission wavelengths. Blanks contained identical constituents, with 25 µL homogenization buffer replacing the tissue homogenate. Salmon sperm DNA standard (Sigma-Aldrich) was used for DNA calibration and results were expressed as DNA strand breaks µg/mg protein.

EROD activity was determined according to Burke and Mayer (1974). The resultant 7-hydroxyresorufin was determined by fluorometry at an excitation wavelength of 520 nm and emission wavelength of 590 nm. Calibration was performed with serial dilutions of 7- hydroxyresorufin. Results were expressed as µM hydroxyresorufin/mg total protein.

GST activity was determined by the method of Habig et al. (1974) adapted to microplate format according to the following procedure: a solution of 100 mM glutathione (GSH) in phosphate buffer (pH = 6.5), and a second solution of 60 mM 1-chloro-2,4-dinitrobenzene (CDNB, $\varepsilon = 9.6\ mM^{-1}\ cm^{-1}$) in ethanol was prepared just before the assay. The reaction mixture consisted of phosphate buffer, GSH solution, and CDNB solution in a proportion of 4.95 mL (phosphate buffer): 0.9 mL (GSH): 0.15 mL (CDNB). In a microplate, 0.2 mL of the reaction mixture was added to 0.1 mL of the sample and GST activity was measured immediately every 20 s, at a wavelength of 340 nm, during the first 5 min. GST from equine liver was used as a positive control. Enzymatic activity was calculated from the slope of the absorbance curve and was expressed in Units (U) per mg of protein content (1 U being 1 µmol of substrate hydrolyzed/min).

LPO was evaluated based on the formation of malonaldehyde in tissue homogenates by the thiobarbituric acid method elaborated by Wills (1987). A 150 µL homogenate was mixed with 300 µL of 10% TCA containing 1 mM $FeSO_4$ and 150 µL of 0.67% thiobarbituric acid. The mixture was heated to 80 °C for 10 min, then precipitates were removed by centrifugation (10,000 ×*g* for 10 s). The supernatant was subjected to fluorescence measurements at 516 nm excitation/600 nm emission. Blanks and standards of tetramethoxypropane were prepared in homogenization buffer. Results were expressed as µmoles of thiobarbituric acid reactants (TBARS) per milligram of homogenate protein.

CAT activity was measured in triplicates following the method of Aebi (1984). The results were expressed as U/mg protein; a unit of CAT was defined as the amount of enzyme that catalyzed the dismutation of 1 mmol of H_2O_2/min.

SOD activity was measured in triplicates using the xanthine oxidase/cytochrome c method proposed by Crapo et al. (1978). The enzyme activity was calculated from the slope of the absorbance curve and was expressed in Units (U) per mg of protein (1 U causing 50% inhibition of the rate of cytochrome C reduction).

GPx activities were measured according to Paglia and Valentine (1967) modified by Lawrence and Burk (1976) and adapted to a 96-well microplate (Faria et al. 2009). GPx activity was expressed as U per mg of protein (a U corresponding to 1 µM NADPH hydrolyzed/min).

GR activity was also measured by the decrease of NADPH at 340 nm for 1 min and expressed as µM/mg protein per min^{-1} according to Carlberg and Mannervik (1975). The reaction medium contained 100 mM phosphate buffer (pH = 7.4), 0.1 mM NADPH and 60 µL supernatant.

Histopathological analyses

Tissue samples from several organs (liver, kidneys, gills, intestines, spleen, gonads and muscles) for histological analyses were collected and fixed in 10% NBF. Samples were processed by a standard histological procedure as described in our previous studies (Tokodi et al. 2020; Drobac Backović et al. 2021; Marinović et al. 2021). Gill and muscle samples were decalcified with 75% RDO - Rapid Decalcifier solution (Apex Engineering Products Corporation) prior to histological processing, which included dehydration of samples in graded ethanol series, clearing in xylol and subsequently embedding in paraffin wax blocks. Three sections (5-µm thin) per tissue per individual were cut and placed onto glass slides and stained with a standard hematoxylin and eosin (H&E) staining procedure. The sections were examined under a microscope (Nikon Eclipse 600) and photographed (QImaging Micro Publisher 3.0 digital camera).

RESULTS AND DISCUSSION

Molecular and biochemical changes in the fish tissues from Hídvégi Pond

DNA fragmentation

DNA fragmentation was not observed in any of the analyzed organs of fish caught from Hídvégi Pond in 2019 (Fig. 1).

Cyanotoxins, especially microcystin (MC), can cause genotoxicity by causing mutations or DNA fragmentation (Buratti et al. 2017). DNA damage due to the action of MC has been shown in mammals (Rao and Bhattacharya 1996;

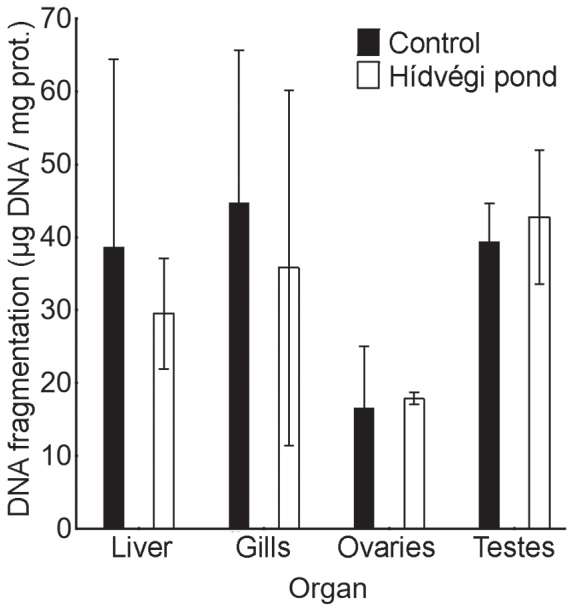

Fig. 1. Analysis of DNA damage in individual fish organs, in fish caught from Hídvégi Pond during 2019. DNA fragmentation. The lack of letters above the columns indicates the absence of statistically significant differences for a given organ (Mann-Whitney U test, $P < 0.05$).

Gupta et al. 2003; Rao et al. 2005; Gaudin et al. 2008), but also in fish (Tokodi et al. 2018). However, the absence of DNA damage may be due to low concentrations of cyanotoxins. Potential genotoxicity is most likely not a result of direct cyanotoxin action but occurs indirectly through the action of reactive oxygen species (ROS), by oxidative DNA lesion or inhibition of DNA-dependent protein kinase (Buratti et al. 2017).

Biotransformation enzyme activity

In fish caught from Hídvégi Pond in 2019, CYP1A (Cytochrome P450 1A) enzyme activity was only higher compared to controls in the testes, while no significant changes were observed in other organs (Fig. 2A). Regarding GST, significantly lower values were measured in the liver and ovaries of fish from Hídvégi Pond (Fig. 2B).

CYP1A enzyme is a phase I biotransformation enzyme. Increased activity of this enzyme was measured in the livers and gills of fish exposed to cyanotoxins (Garcia and Martinez 2012; Li et al. 2013; Isibor 2017). However, in the present study no changes in the activity of this enzyme were measured in these two organs. The main mechanism of MC biotransformation is through the action of phase II biotransformation enzymes, including GST, by creating conjugates with GSH. The reduced activity of GST enzyme measured in the liver and ovaries of fish from Hídvégi Pond coincides with other studies that examined the effects of cyanotoxins on fish (Guzmán-Guillén et al. 2013; Hou et al. 2015; Calado et al. 2017; Paulino et al. 2020). Differences in the activity of biotransformation enzymes can be a consequence of the action of cyanotoxins, and their inhibition or activation depends on the cyanotoxin itself.

Oxidative stress parameters

In fish caught from Hídvégi Pond during June 2019, all organs except the testes showed high levels of LPO (Fig. 3A). The highest levels were measured in the liver and gills. CAT activity (Fig. 3B) and SOD (Fig. 3C) were significantly lower in the liver, while no significant difference was measured in other organs. Regarding the activity of total (Fig. 3D) and Se-dependent GPx (Fig. 3E), significantly lower activity was

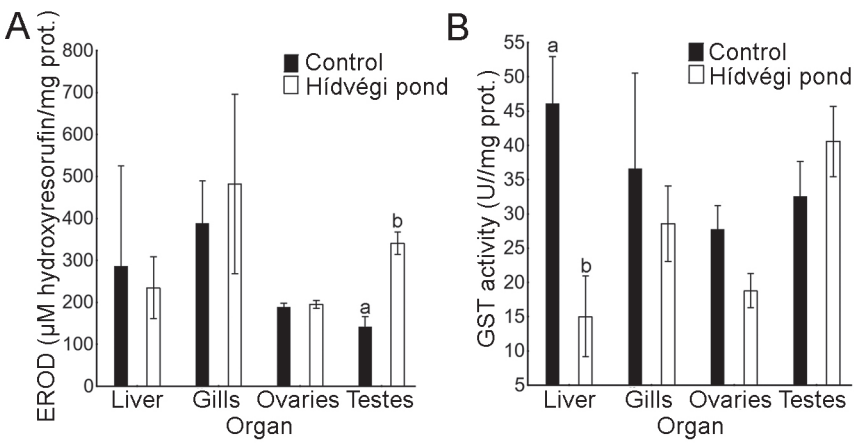

Fig. 2. Activity of CYP1A (EROD; (**A**) and GST (**B**) control fish and fish caught from Hídvégi Pond. Different letters above the columns indicate a statistically significant difference for a given organ (Mann-Whitney U test, $P < 0.05$), while the absence of letters indicates the absence of statistically significant differences for a given organ.

measured in the liver. GR activity showed no changes in any of the organs (Fig. 3F).

One of the main ways in which cyanotoxins cause damage to the cells and organisms is by causing increased production of free radicals and causing oxidative stress. When intensive ROS production occurs, these molecules begin to bind to proteins, lipids and nucleic acids and cause damage. LPO represents the binding of peroxide to lipids, most commonly membranes, where they cause imbalances in ion flux, permeability, and membrane structure (Martins et al. 2017). Elevated LPO levels often occur as a consequence of cyanotoxin action, and can be observed in the liver (Prieto et al. 2006, 2007; Atencio et al. 2008; Jiang et al. 2011; Lin et al. 2018), kidneys and gills (Prieto et al. 2006, 2007) of fish exposed to cyanotoxins, primarily MC. The significant increase in LPO measured in the present study coincides with the above-mentioned studies and indicates the presence of oxidative stress in the liver, gills and ovaries of fish caught from Hídvégi Pond.

SOD is an enzyme that actively scavenges superoxide ions and converts them into a molecule of oxygen and H_2O_2. The reduction of this enzyme in the liver coincides with some studies on fish exposure to cyanotoxins (Prieto et al. 2007; Atencio et al. 2008; Martins et al. 2017; Paulino et al. 2020), however, a number of studies also demonstrated an increase in the activity of this enzyme after exposure to cyanotoxins (Jos et al. 2005; Prieto et al. 2006; Chen et al. 2012; Garcia and Martinez 2012; Hou et al. 2015). The exact cause of such differences is not clear and may be the result of the action of different cyanotoxins in different concentrations, but it can also be specific to a given species of fish. There was no significant change in the activity of SOD enzyme in the gills and gonads, which indicates differences in the response of different organs to oxidative stress.

CAT and GPx are enzymes that metabolize H_2O_2 and lipid peroxides. In the present study, significantly lower activity of both enzymes in the liver was measured compared to controls. Similar observations were noted by Prieto et al. (2007), Atencio et al. (2008), Perendija et al. (2011), Chen et al. (2012) and Hou et al. (2015).

Intense LPO, as well as changes in the activity of antioxidant enzymes, indicate that fish caught from Hídvégi Pond had been exposed to oxidative stress. Since the most considerable changes occurred in the liver, this organ is probably more sensitive to oxidative stress compared to the gills and gonads. Changes in the activity of antioxidant enzymes similar to those observed in the present study often occur in fish exposed to cyanotoxins. However, the response of antioxidant enzymes to the action of cyanotoxins is not uniform. It is believed that the activity of these enzymes is

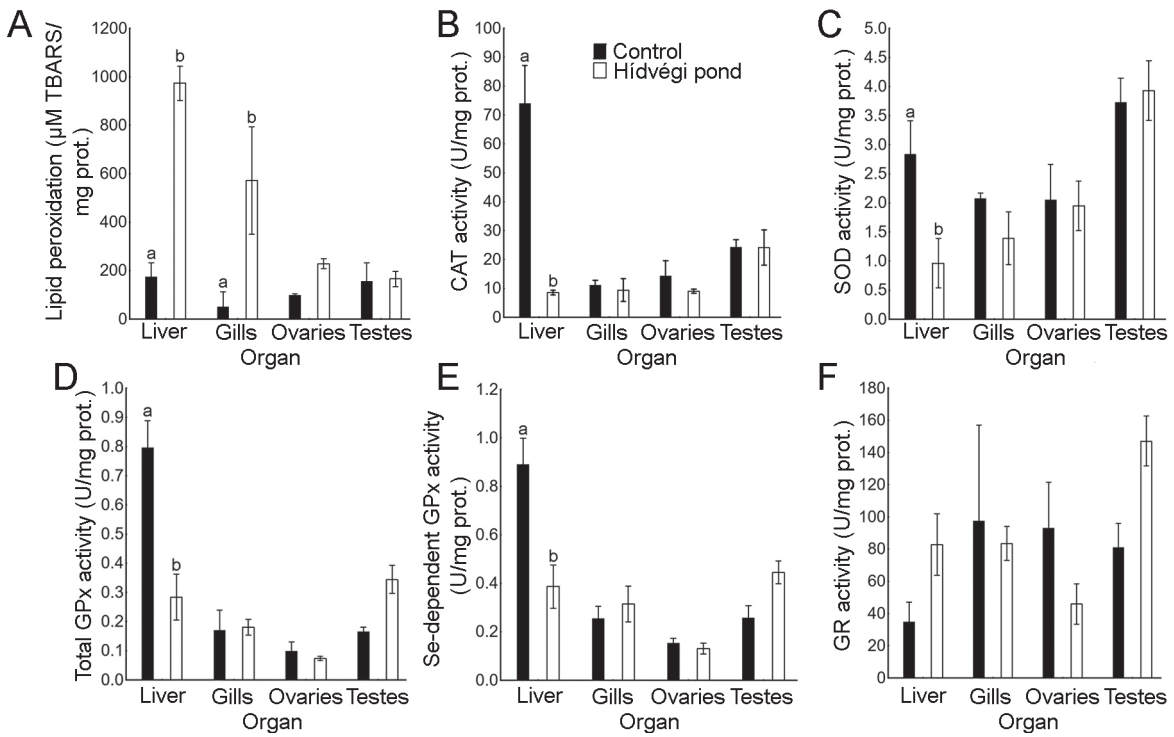

Fig. 3. LPO (**A**) and activity of CAT (**B**), SOD (**C**), total GPx (**D**), Se-dependent GPx (**E**) and GR (**F**) of control fish and fish caught from Hídvégi Pond. Different letters above the columns indicate a statistically significant difference for a given organ (Mann-Whitney U test, $P < 0.05$), while the absence of letters indicates the absence of statistically significant differences for a given organ.

most often inhibited during the acute action of higher concentrations of cyanotoxins, while long-term chronic exposure most often leads to the activation of these enzymes as a result of a defense system (Atencio et al. 2008). In the present study, inhibition of antioxidant enzyme activity was mainly observed, potentially indicating acute action of cyanobacterial metabolites that may cause oxidative stress. Indeed, some of the toxins could potentially inhibit the enzyme itself, or may affect its biosynthesis (Gagné 2014). Additionally, ROS could overwhelm antioxidant defenses, thereby lowering the concentrations measured; or on the other hand, toxins could potentially cause a high level of energetic stress, thereby limiting the production and/or synthesis of specific enzymes (Gagné 2014).

Histopathological alterations in fish tissue

All organs in control fish displayed normal histological structure. The most intense histopathological alterations were observed in the liver, kidneys and gills of fish from Hídvégi Pond, while no changes were found in the remaining tissues (intestines, muscles, spleen, ovaries and testes) that were sampled. The most pronounced liver alterations observed were intense vacuolization, loss of glycogen and clearing of the cytoplasm (Fig. 4 A, B). In some cases, mild alterations of the parenchymal structure were observed. This intense vacuolization led to a significant increase in hepatocyte size, i.e. hypertrophy. In some hepatocytes, the nuclei were condensed and seemed pyknotic. The predominant alteration observed in the kidneys was vacuolization of tubular cells, which in many instances led to complete detachment from the basal membrane (Fig. 4 C, D). Additionally, karyolysis was detected in the nuclei of tubular cells. In the gills, the most pronounced alterations were proliferation of the interlamellar epithelium, which in many cases led to complete fusions of the lamellae, as well as epithelial hypertrophy and lifting (Fig. 4 E, F). Other organs did not display any significant alterations.

During research conducted in 2018 on KBWPS, similar histopathological changes were observed, and the differences in the intensity of histopathological changes were similar to the differences in the intensity of cyanobacterial blooming (Marinović et al. 2021). Certain damage to the liver, kidneys and gills can be potentially linked to the action of cyanotoxins such as hepatotoxic MC or NOD (nodularin). Namely, these toxins bind to protein phosphatases (PP) through the ADDA group they possess, thus affecting certain very important processes within cells (Buratti et al. 2017). Modifications of the cytoskeleton and actin filaments are common after MC-induced inhibition of PP (Sun et al. 2014; Buratti et al. 2017), and the consequences of these modifications may be manifested as impaired liver parenchymal structure or morphological disturbances of hepatocytes or gill epithelial cells. Nuclear damage (hepatocyte nucleus pyknosis and renal tubule nucleus karyolysis) may be associated with the genotoxic activity of MC and decreased DNA-dependent protein kinase capacity due to MC action (Douglas et al. 2001; Buratti et al. 2017). Also, vacuolation of hepatocytes and renal tubule cells often occurs after the action of MC and NOD (Svirčev et al. 2015), however, this alteration is very unspecific, and can be caused by many pollutants, infections or even certain diets (Thoolen et al. 2010).

Histopathological changes in various tissues of fish caused by the action of cyanotoxins in laboratory conditions are summarized in the research of Svirčev et al. (2015). In short, the most intense changes were manifested in the liver and kidneys of fish, while damage to other organs was much milder. The most common liver changes were disruption of the liver parenchymal structure, rounding of hepatocytes, vacuolization, hypertrophy and pyknosis. As noted, these changes are a consequence of PP inhibition by MC. In the kidneys, the most commonly observed changes were dilatation of Bowman's capsule, atrophy of the glomeruli and vacuolization of tubular cells. Gills most often showed epithelial lifting, hypertrophy and hyperplasia of epithelial cells. These changes were also manifested in fish caught from Hídvégi Pond. Accordingly, it is possible to assume that cyanobacterial toxins had an impact on the occurrence of these changes.

Reported histopathological lesions in fish from Hídvégi Pond seem to be very mild and probably did not have an impact on fish health. Furthermore, most of the histopathological changes observed during these studies are of a reversible nature, and tissue recovery can be expected after the cessation of the irritant effect. This refers to the structural changes of the liver and kidney tissues, vacuolization, as well as the defense mechanisms of the gills. However, the results of this and previous research (Marinović et al. 2021) showed that the described changes persisted during all analyzed seasons in 2018 and in 2019, which may mean that the irritants that cause them are most likely constantly present in water and potentially could have a chronic effect on the fish from this location. Furthermore, since cyanobacteria in Hídvégi Pond and the whole KBWPS may be present throughout the year, it can be assumed that these organisms may be responsible for the manifested histopathological changes.

Influence of cyanobacterial blooms on certain fish biomarkers

Although cyanobacteria and cyanotoxin presence in Hídvégi Pond were not analyzed during this study, their presence was noted in our previous research conducted at the same location a year earlier (Marinović et al. 2021). In the KBWPS during 2018, in all investigated seasons (April,

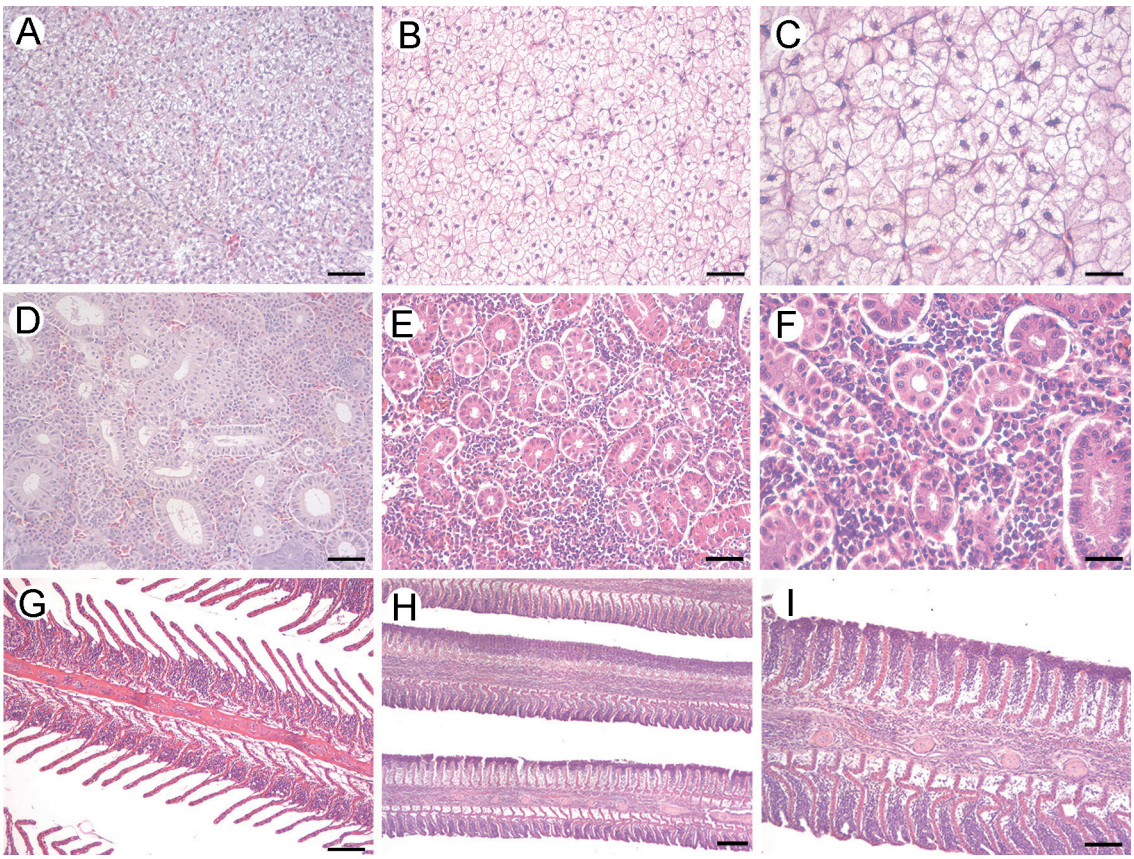

Fig. 4. Histopathological alterations in liver (**A-C**), kidneys (**D-F**) and gills (**G-I**) of fish from the Hídvégi Pond. (**A**) Control livers; (**B, C**) Livers of fish from the Hídvégi Pond displayed severe vacuolization which led to hepatocyte hypertrophy. (**D**) Control kidneys; (**E, F**) Kidneys of fish from the Hídvégi Pond also displayed vacuolization of tubular cells which led to their detachment from the basal lamina. (**G**) Control gills; (**H, I**) Gills of fish from the Hídvégi Pond showed proliferation of the interlamellar cell mass which led to complete fusions of secondary lamellae. H&E staining. (Scale bars: A, B, D, E = 50 μm; C, F = 25 μm; G, I = 100 μm; H = 200 μm.)

May, July and September) the number of cyanobacterial cells exceeded 10,000 cells/mL, which can be characterized as blooming (Falconer 1998), while the highest number of cells was recorded in July (13,991,700 cells/mL). *Aphanizomenon flos-aquae* Ralfs ex Bornet and Flahault was the most dominant species and *Dolichospermum spiroides* (Klebhan) Wacklin, L. Hoffmann and Komárek and *Microcystis aeruginosa* (Kützing) Kützing were extensive as well. In July and September, the presence of MC- (*mcyE*) and STX-coding (*sxtG*) genes was detected in biomass collected from KBWPS. Therefore, it was not surprising that five MC variants (MC-LR 1.29 μg/L, MC-YR and MC-LW 0.05 μg/L, MC-RR 0.02 μg/L, MC-LF 0.01 μg/L) were present in the water. Throughout the investigated period cyprinid fish from KBWPS displayed severe liver, kidney and gill alterations (Marinović et al. 2021).

During this investigation, several biomarkers of fish were monitored including histopathological changes of individual fish organs, biotransformation enzymes and oxidative stress parameters in fish from Hídvégi Pond sampled in June 2019. Cyanotoxins can cause changes in biomarkers at different levels of structural organization. In the present research, we established that fish from Hídvégi Pond caught in June 2019 were exposed to oxidative stress, and showed changes in the activity of biotransformation enzymes. Similar changes in biochemical markers have been observed in fish exposed to cyanotoxins (Jos et al. 2005; Prieto et al. 2006; Chen et al. 2012; Garcia and Martinez 2012; Hou et al. 2015). Additionally, histopathological alterations were observed in the liver, kidneys and gills, similarly to that reported in other studies of fish exposure to cyanotoxins in natural conditions (Carbis et al. 1996; Ernst et al. 2006; Li et al. 2007; Jiang et al. 2011; Svirčev et al. 2015; Drobac et al. 2016). Cyanobacterial toxins have different target organs, conditioned with specific carriers that transport them into the cells of a given organ. This phenomenon may also explain the fact that tissue damage is manifested predominantly in the liver, kidneys and gills, and not in other organs. Nonetheless, some authors have noted

changes in the intestines, muscles and gonads of exposed fish (Svirčev et al. 2015; Drobac et al. 2016).

Although the production of cyanotoxins is the most dangerous component of cyanobacterial blooms, it is not the only stress factor for fish. In addition to cyanotoxins, low oxygen concentrations, high pH, and high ammonia concentrations resulting from cyanobacterial blooms can also have very harmful effects on fish. Due to hypoxia, fish accelerate their rate of ventilation (Heath 1995), and there is a rearrangement within the gills where the interlamellar mass of cells retreats and there is an increase in respiratory area (Nilsson 2007), and thus increased absorption of toxic substances. On the other hand, one of the basic defense mechanisms of gills from toxic substances (including cyanotoxins) is hyperplasia of the interlamellar epithelium, detachment of the epithelium and consequent reduction of the respiratory surface (Lujić et al. 2015). If the action of toxic substances is continuous, and fish constantly maintain a minimal respiratory surface (fusion of lamellae), as observed during studies on KBWPS, acute hypoxia caused by the collapse of the cyanobacterial bloom can lead to mass fish mortality. Ammonia and high pH levels usually have a synergistic effect, since the concentration of the toxic non-ionized form of ammonia is higher at higher pH levels (Heath 1995). This form can easily diffuse through the gills, and thus increase the concentration in the blood, which can become toxic (Burdick et al. 2020b). Therefore, cyanobacterial blooms can have both direct and indirect effects on fish, even a subacute effect on fish, but that mortality can occur as a result of the synergistic actions of several stressors caused by cyanobacterial blooms (Jos et al. 2017). Ultimately, along with cyanobacteria and cyanotoxins, heavy metals, antibiotics, pesticides and others stressors may be present in the water, and cause similar damage to fish tissues.

Cyanobacterial blooms pose not only a danger and health risk to fish and organisms that inhabit blooming ecosystems, but to all organisms, including humans, that come into contact with such contaminated ecosystems (Chen et al. 2009; Drobac 2013; Svirčev et al. 2017). Therefore, because cyanobacterial blooms can have a detrimental effect on the environment, as well as human health, it is necessary to regularly monitor cyanobacteria, cyanotoxins, their effects on other organisms, and develop biomarkers that will directly indicate the levels of toxicity and pollution in the environment, so that potentially adverse consequences can be prevented in time.

CONCLUSIONS

In the present study we observed alterations in the activity of biotransformation and antioxidant enzymes, the presence of oxidative stress in the liver, gills and ovaries, as well as histological alterations in the livers, kidneys and gills of fish sampled from the blooming Hídvégi Pond compared to the control fish. On the other hand, molecular analyses displayed no significant DNA fragmentation in cells of any of the tested fish organs. Biomarkers tested in this study indicate that fish from the Hídvégi Pond are under severe environmental stress, possibly caused by cyanobacterial blooming. Even though low MC concentrations were previously detected (Marinović et al. 2021), the long-term presence of cyanobacteria could present a stress factor. However, biomarkers used in this study are not specific to cyanotoxin action and cannot decisively point to cyanobacerial blooms as a cause of the stress and observed damage. Nevertheless, this study demonstrates that utilization of different biomarkers at different levels of structural organization are important in decoding the effects of the environment on fish, and should be utilized to obtain the most holistic interpretation of the effects of environmental factors and cyanobacterial blooming on living organisms.

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