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Changes in the metabolic processes, cytomorphology, and histology of the fish *Carassius gibelio* exposed to 2,4,6-trinitrotoluene

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The environmental impact of explosive weapons in Ukraine occurs during hostilities and will take place in the post-war period due to decomposition of unexploded ordnance and release of toxic compounds. Of this, 2,4,6-trinitrotoluene (TNT) is the primary ingredient, however, its effect on the freshwater hydrobionts has not been sufficiently studied. We aimed to establish in the model experiment the biochemical and histological changes in the tissue of *Carassius gibelio*, which may serve as biomarkers of exposure and the effect of TNT, and to predict the functional status of fish in polluted post-war waters. High induction of GST activity and LPO processes was revealed in the liver, gills, and muscles of the fish both under acute (8 hours at concentration 35 mg/L) and chronic (21 days at concentration 5 mg/L) TNT action, and assessed as the biomarkers of toxic exposure. The modulation of redox balance and detoxification intensity in the fish body can be considered as a biochemical adaptation of *C. gibelio* to long-term TNT action at low concentration. The changes in liver cells and nuclei morphometric indices, and the histopathological changes in the hepatocytes and gill structure were assessed as the biomarkers of TNT's toxic effects on *C. gibelio*. The lipoid dystrophy of hepatocytes and hypertrophy of the gill epithelium reflected the toxicant-induced metabolic modulation and can be considered as a morpho-physiological adaptation of the fish to the chronic TNT action at low concentration. However, such abnormalities as increase in nucleus area/cell area ratio, binucleation, and karyolysis in hepatocytes, as well as the lamellae distortion and dilation of the lamellar apical tips in the gills indicated irreversible changes that reduce the vitality of the fish and decrease the possibility of *C. gibelio*'s complete adaptation even to low-dose TNT exposure. The obtained results highlight the need to study the natural water ecosystems of Ukraine contaminated with explosives to assess the current condition and survival prospects of the hydrobionts.

Keywords: crucian carp; TNT; GST; MDA; liver; gills; muscles; histopathology; biomarkers.

Introduction

The widespread use of explosive weapons due to Russia's war in Ukraine has already caused enormous damage to the environment, including aquatic ecosystems (Alpatova et al., 2022; Pereira et al., 2022; Koziy, 2025). In particular, the ecological state of the territories of the Southern Ukrainian regions was assessed as catastrophic due to damage to wetlands and river habitats and the number of pollutants detected in water samples, including heavy metals and hydrocarbons. However, the negative impact of hostilities can also be felt in the post-military period, as a decomposition of munitions that enter the environment releases heavy metals and toxic explosive compounds, the effects of which can last for decades (Lotufo et al., 2016; Shumilova et al., 2023).

The primary ingredient in almost every munition is the secondary explosive 2,4,6-trinitrotoluene (TNT), which is a thermally and chemically stable nitroaromatic energetic compound with a low melting point (Strehse et al., 2017; Schillinger et al., 2020), as well as with the established ability to accumulate in a number of living organisms and exert toxic effects (Gledhill et al., 2019). According to the results of recent studies, the harmful effects of explosives on the environment do not have a statute of limitations. In particular, TNT, its metabolites and other munition compounds were found in in almost all organisms collected in the Baltic Sea near World War II munitions dumpsites (Appel et al., 2018; Koske et al., 2020; Beck et al., 2022).

The danger of TNT to biota is unquestionable because it is a strong oxidizing agent, whose intracellular metabolism is generally characterized by redox cycling and generation of noxious reactive molecules (Adomako-Bonsu et al., 2024). Living organisms produce the reactive oxygen species (ROS) as the by-products that occur during the metabolic processes, and must control a balance between pro-oxidant and antioxidant processes using the antioxidant systems (Topić Popović et al., 2023). The influence of xenobiotics can cause a shift in the redox balance of cell membranes towards the excessive production of reactive oxygen species, such as hydroxyl and hydroperoxyl radicals (Gaschler & Stockwell, 2017; Hu et al., 2021). Next, these compounds can interact with cell polyunsaturated fatty acids, triggering the formation of lipid radicals, as well as lipid peroxide radicals or lipid hydroperoxides, which can damage biomolecules, including proteins and unsaturated lipids in cell membranes (Tsikas, 2017). The sensitivity of lipids to the action of oxidants leads to growth of lipid peroxidation (LPO) intensity in the cells, which characterizes oxidative stress and is accompanied by the formation of various oxidation products, in particular malondialdehyde (MDA), which serve as a measurable marker of lipid peroxidation (Garsia et al., 2020; Mas-Bargues et al., 2021).

The cellular defense system of living organisms operates with different nonenzymatic and enzymatic protective mechanisms, including biotransformation enzymes. Among the latter, glutathione S-transferases (GSTs) are a superfamily of enzymes catalyzing the glutathione conjugation with various reactive electrophiles, including endogenous and xenobiotic substrates (Dobritzsch et al., 2020). Conjugation with glutathione provides the phase-II metabolization which detoxifies various compounds (Oyewole et al., 2025), because the resulting conjugates are usually very polar substances that are excreted from the organism (Aksoy et al., 2016). The wide variety of GST isoforms provides detoxification activity against ROS produced both by inorganic substances, such as heavy metals, and by organic pollutants (Yılmaz & Çomaklı, 2023). Therefore, glutathione S-transferase is a well-known biomarker of environmental pollution which can detect both chronic exposure to multiple pollutants and acute and short-term hazardous pollution (Bocedi et al., 2023).

When studying the impact of xenobiotics on fish, histopathological biomarkers allow us to assess the state of fish organs, including gills, kidney and liver, that perform vital physiological functions and the biotransformation of xenobiotics in the body of fish. Among them, the liver is the main organ for the biotransformation of organic xenobiotics (Topić Popović et al., 2023), so changes in its structure can be significant for the assessment of fish health (Carvalho et al., 2022). Histopathological changes in fish liver under pollutants action most often affect lipid metabolism (Wagenaar & Barnhoorn, 2018; Wang et al., 2019). According to Lang et al. (2017), liver histological parameters represent a higher level of response in exposed fish, which is generally indicative of irreversible damage.

A number of studies have examined the toxicity of 2,4,6-trinitrotoluene in aquatic biota, however, the vast majority of studies concerned marine species of different trophic levels (Koske et al., 2019; Beck et al., 2022), in particular accumulation and biotransformation of TNT by blue mussels *Mytilus edulis* (Ballentine et al., 2015). Therefore, it is extremely important to study the effects of 2,4,6-trinitrotoluene and other explosives on a wider range of freshwater living organisms, including their ability to biodegrade the explosives (Khromykh et al., 2023).

Previously, we found the destructive cell abnormalities in *C. gibelio* erythrocytes due to exposure to 2,4,6-trinitrotoluene, including size and shape changes of red blood cells, decreased hemoglobin content, and the erythrocyte cytoplasm vacuolization, as well as nuclear displacement and impaired cell division (Sharamok et al., 2024). Abnormalities of erythrocytes that appeared in the blood of *C. gibelio* under the influence of TNT were also detected as the effects of various toxicants on fish organisms, and are among the earliest biomarkers of toxicity (Kaur & Kaur, 2015). Concurrently, the effects of TNT on other fish tissues and cellular structure remains insufficiently researched. The goal of this study was to reveal the biochemical and histological changes in the fish gills, liver, and muscles under the chronic and acute action of TNT in order to assess the functional state and survival prospects of fish in polluted post-war waters.

Materials and methods

The two-year-old fish of Prussian carp (*Carassius gibelio* Bloch) served as the test object, since this species is widely distributed in water bodies, being a commonly consumed freshwater fish. TNT toxicity was studied in a model experiment carried out in September 2024 in the Biology Research Institute of Oles Honchar Dnipro National University (Dnipro, Ukraine). Before the experiments, the fish with a weight of 23.1 ± 0.8 g and a length of 18.1 ± 0.6 cm, provided by the Scientific Research Institute "Aquarium", were kept in aerated settled tap water for 10 days, receiving food both during acclimatization and in the experiment.

Aqueous medium contamination was simulated by the addition of 2,4,6-trinitrotoluene, prepared in analytical quantities as described (Khromykh et al., 2023), to the aquariums for chronic (5 mg/L during 21 days) and acute (35 mg/L during eight hours) experiments. TNT concentration for the chronic experiment was chosen according to threshold values of toxicity for various marine fish from 0.8 to 7.6 mg/L (Koske et al., 2019). The given TNT concentration during the chronic experiment was maintained by weekly updating of the aquarium water contamination. Samples of the fish gill, liver, and muscle were obtained by anatomical dissection, and the tissues were processed according to generally accepted methods (Koziy, 2011). To obtain histological preparations, paraffin blocks were prepared, and sections were made using a microtome, and stained with hematoxylin-eosin. Photo fixation of the histological preparations was done at a magnification of 400× using a digital camera Sciencelab T500 5.17M mounted on a Ulab XY-B2TLED 78 microscope.

To determine the morphometric parameters and to reveal the pathological changes of fish hepatocytes, 10 fields of view were examined on 10 histological preparations for each variant of the experiment. For hepatocytes and their nuclei, the length (L, μm), width (W, μm), and area (S, μm^2) were determined. Pathological liver cells are expressed as a percentage of total cells.

This research adhered to bioethical standards according to the Regulations on the Ethics Committee (Bioethics) in Ukraine (2012).

Lipid peroxidation (LPO) in fish tissues was evaluated by measuring malondialdehyde (MDA) content at 532 nm after reaction with thiobarbituric acid, according to Garsia et al. (2020), and expressed as nmol MDA/g tissue.

Glutathione-S-transferase (GST) activity in gill, liver, and muscle tissues was determined at 340 nm using the method of Habig and Jakoby (1981) with 2,4-dinitrochlorobenzene (DNCB) as a substrate. Activity is expressed as μM DNCB/min/mg tissue (U/mg tissue).

All bioassays were carried out in quintuple replication. Experimental results were analyzed using one-way ANOVA and presented as mean ± SD (standard deviation). Additionally, the multiple comparison of samples by Tukey's test, and multivariate analysis were used. For the last analysis, the hepatocyte cytomorphological data matrix was formed, where the columns represented the samples, and the rows described the cell and nucleus parameters. Exploratory factor analysis was conducted using principal component factor analysis with Varimax rotation, employing a factor loading threshold of > 0.70. All differences of means were statistically significant at $P < 0.05$.

Results

The course of lipid peroxidation processes and enzyme protection in the tissues of *C. gibelio* changed significantly under both chronic (21 days at concentration 5 mg/L) and acute (8 hours at concentration 35 mg/L) exposure to 2,4,6-trinitrotoluene. During the chronic TNT exposure, both activity of glutathione S-transferase and malondialdehyde content in the liver, gill and muscle tissues of fish were increased (Fig. 1).

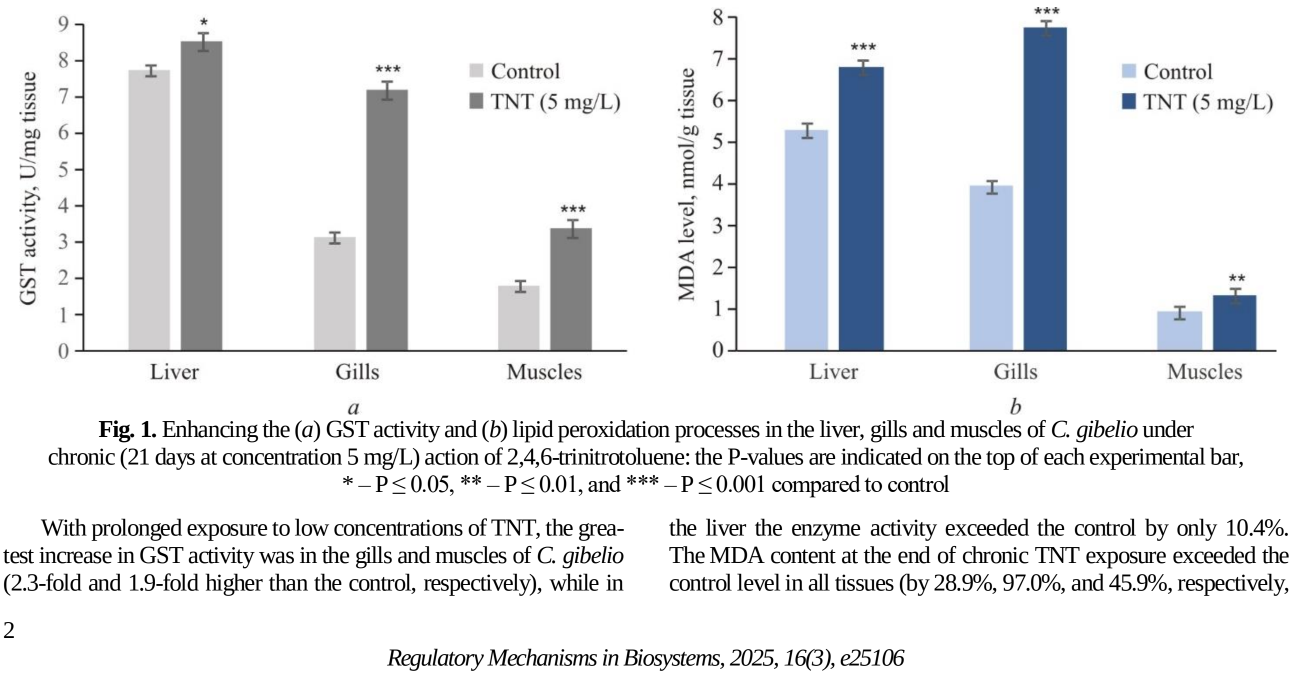

Fig. 1. Enhancing the (*a*) GST activity and (*b*) lipid peroxidation processes in the liver, gills and muscles of *C. gibelio* under chronic (21 days at concentration 5 mg/L) action of 2,4,6-trinitrotoluene: the P-values are indicated on the top of each experimental bar, * – $P \leq 0.05$, ** – $P \leq 0.01$, and *** – $P \leq 0.001$ compared to control

With prolonged exposure to low concentrations of TNT, the greatest increase in GST activity was in the gills and muscles of *C. gibelio* (2.3-fold and 1.9-fold higher than the control, respectively), while in the liver the enzyme activity exceeded the control by only 10.4%. The MDA content at the end of chronic TNT exposure exceeded the control level in all tissues (by 28.9%, 97.0%, and 45.9%, respectively, in the liver, gills, and muscles). In the acute experiment, 2,4,6-trinitrotoluene action on *C. gibelio* caused the fluctuations in rate of lipid peroxidation as well as in GST activity level in the liver, gill and muscle tissues (Table 1).

Table 1
The changes in GST activity (U/mg) and MDA content (nmol/g) in the tissues of *C. gibelio* during the acute (8 hours at concentration 35 mg/L) action of 2,4,6-trinitrotoluene (x ± SD, n = 5)

Exposure time, h	GST activity			MDA content		
	liver	gills	muscles	liver	gills	muscles
0 (control)	6.43 ± 0.10^{a}	3.58 ± 0.05^{a}	1.40 ± 0.03^{a}	4.68 ± 0.08^{a}	3.82 ± 0.06^{a}	1.11 ± 0.04^{a}
2	6.24 ± 0.07^{a}	3.48 ± 0.06^{a}	1.39 ± 0.02^{a}	5.17 ± 0.07^{b}	10.35 ± 0.12^{b}	2.28 ± 0.09^{b}
4	6.96 ± 0.16^{b}	3.69 ± 0.07^{b}	1.51 ± 0.03^{b}	5.29 ± 0.06^{b}	4.25 ± 0.07^{c}	1.65 ± 0.05^{c}
6	7.17 ± 0.16^{b}	4.16 ± 0.11^{c}	2.67 ± 0.06^{c}	4.04 ± 0.11^{c}	7.25 ± 0.09^{d}	2.35 ± 0.08^{db}
8	5.51 ± 0.07^{c}	3.22 ± 0.05^{d}	1.48 ± 0.03^{db}	4.82 ± 0.10^{da}	8.33 ± 0.18^{e}	3.03 ± 0.07^{e}

Note: different letters within a column indicate significant differences in mean values by Tukey's test ($P \leq 0.05$).

Acute exposure to 2,4,6-trinitrotoluene slightly decreased GST activity in the liver, gills, and muscles within two hours, after which the enzyme was activated in all tissues. The most prominent increase in GST activity was in six hours (11.2%, 16.2%, and 90.7% higher than control in the liver, gills, and muscles, respectively). However, during 8 hours of exposure. the enzyme activity significantly decreased in the liver and gills (to 85.7% and 89.9% of the control), remaining higher than the control in the muscles.

Changes in lipid peroxidation processes in *C. gibelio* fish body during the acute experiment were revealed already after the second hour of exposure to TNT as MDA content growth in the liver, gills, and muscles (respectively, 110.6%, 271.1%, and 205.1% of the control). Further, the MDA content in the fish tissues varied widely, and after 8 hours of the experiment slightly exceeded the control in the liver, while in the gills and muscles it reached, respectively, 218.1% and 273.0% of the control.

Cytomorphological parameters of the fish hepatocytes and cell nuclei were greatly affected by both the chronic and acute influence of 2,4,6-trinitrotoluene on *C. gibelio* (Table 2).

The cell area of *C. gibelio* hepatocytes increased under the acute treatment, reaching 46.5% above the control, while it declined to 91.3% of control during chronic TNT action. The similar trends were revealed for the alteration of cell width and cell length in the experimental conditions: slight decrease in both indicators with chronic exposure, but exaggeration of control level by 20% with acute TNT exposure. The morphometric parameters of cell nuclei were significantly altered by both chronic and acute 2,4,6-trinitrotoluene action. The chronic treatment caused the greatest increase in all parameters. including nucleus area (86.7% above control), width (39.2% above control), and length (30.8% above control level), while the effect of acute TNT exposure was lower (respectively, 59.9%, 25.8%, and 6.3% above control level for nucleus area, width, and length). Ratio nucleus area/ cell area grew slightly during the acute TNT action, while it exceeded the control by two times under chronic exposure.

Table 2
Changes of area (S, μm^2), width (W, μm), and length (L, μm) of the *C. gibelio* hepatocytes and nuclei under the action of 2,4,6-trinitrotoluene (x ± SD, n = 100)

Treatment option	Cell			Nucleus			Nucleus area / cell area
	S	W	L	S	W	L	
Control	328 ± 54.6^{a}	17.04 ± 1.79^{a}	23.89 ± 1.60^{a}	11.88 ± 0.62^{a}	3.65 ± 0.17^{a}	4.45 ± 0.22^{a}	0.036 ± 0.009^{a}
TNT (5 mg/L, 21 days)	300 ± 18.6^{a}	16.07 ± 1.22^{a}	23.46 ± 1.31^{a}	22.18 ± 0.88^{b}	5.08 ± 0.17^{b}	5.82 ± 0.19^{b}	0.074 ± 0.014^{b}
TNT (35 mg/L, 8 hours)	480 ± 57.0^{b}	20.44 ± 1.64^{b}	28.83 ± 1.31^{b}	18.99 ± 1.69^{c}	4.59 ± 0.24^{c}	5.62 ± 0.28^{c}	0.040 ± 0.009^{a}

Note: see Table 1.

Multivariate analysis of the morphometric data matrix of *C. gibelio* hepatocytes and their nuclei in control and experimental conditions revealed three main factors (loading was ≥ 0.70), whose influence most determined the data structure. with the corresponding loading of 33.64%, 20.30%, and 16.89% (Table 3).

Table 3
Multivariate analysis factors of morphometric data matrix of *C. gibelio* hepatocytes alteration under TNT action

Factor	Eigenvalue	Total, %	Cumulative eigenvalue	Cumulative, %
1	6.06	33.64	6.06	33.64
2	3.65	20.30	9.71	53.94
3	3.04	16.89	12.75	70.84

The two-dimensional scatter diagram (scatter plot 2D) represents the geometric interpretation of the structure of the *C. gibelio* hepatocytes' morphological data, formed by the influence of two main factors with highest loading (Fig. 2).

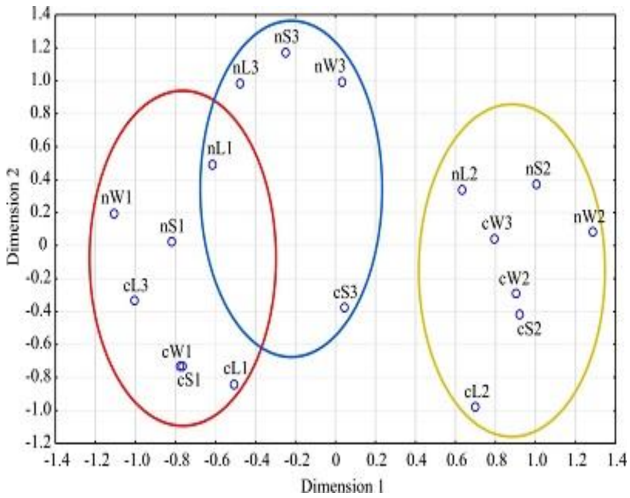

Fig. 2. Geometric structure of factor analysis of *C. gibelio* hepatocytes' cytomorphology in multidimensional scaling: (1) control, (2) acute, and (3) chronic TNT impact on (c) cell and (n) nucleus length (L), width (W), and area (S)

The first significant factor (with a loading of 33.64%) most determined the differences in the morphometric parameters of the nuclei of *C. gibelio* hepatocytes relative to the control condition, especially the area of the nuclei under both treatment conditions and the width of the nuclei under chronic exposure to TNT, as well as the differences in cell length and cell area under the acute effect of TNT. The second factor (loading 20.3%) had the greatest impact on the differences in cell width and hepatocyte nuclear area in both variants of exposure to TNT. Histopathological effects of the *C. gibelio* exposure to 2,4,6-trinitrotoluene were observed in the liver and gills under the acute and chronic influence of toxicant. In the liver cells, TNT action caused the alterations of the characteristics that applied to both the hepatocytes and nuclei (Fig. 3).

Several cell abnormalities of hepatocytes' structure were revealed in the liver of both control *C. gibelio* and those exposed to 2,4,6-trinitrotoluene, however, the frequency of pathological changes of the cells and nuclei increased under the influence of the toxicant (Table 4).

Chronic exposure of the *C. gibelio* to TNT caused the most striking increase in the number and variability of hepatocyte pathologies, including lipoid cell dystrophy absent in control and acute exposure, binucleation (70.2% above the control), karyolysis (three thousand times higher than the control level), while the frequency of nucleus shift did not differ significantly from the control. Under acute exposure, binucleation and karyolysis exhibited, respectively, 150% and 953.3% of control value, and nucleus shift in hepatocytes reached 125.5% of control. The composition of the gill lamellae of *C. gibelio* fish exposed to 2,4,6-trinitrotoluene underwent pathological changes under both acute and chronic treatment (Fig. 4).

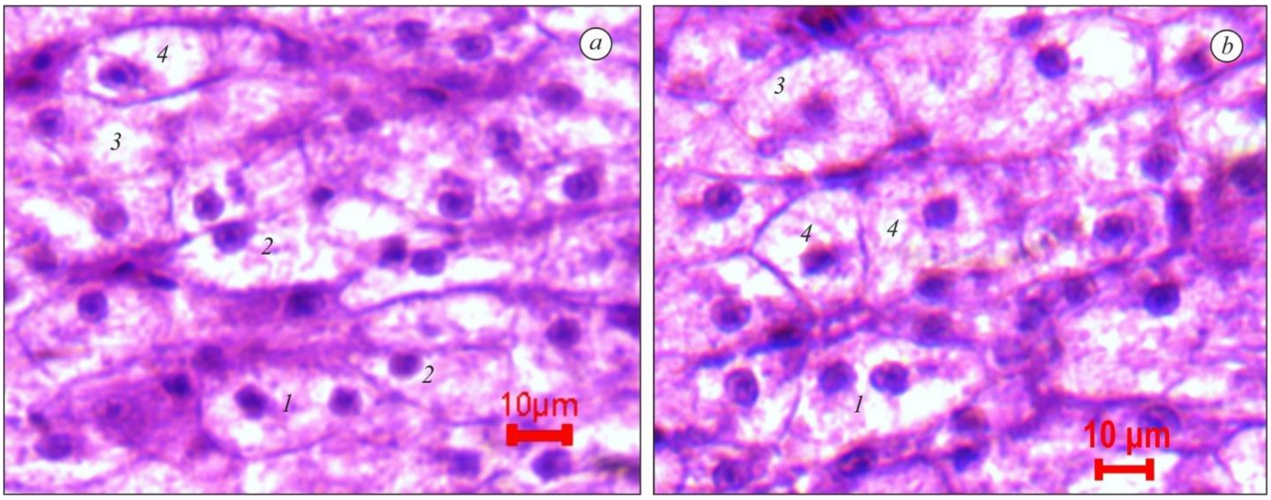

Fig. 3. Histopathological changes in the *C. gibelio* hepatocytes and nuclei due to 2,4,6-trinitrotoluene chronic (21 days at concentration 5 mg/L) and acute (8 hours at concentration 35 mg/L) action (*a*, *b*): *1* – binucleation, *2* – nucleus shift, *3* – karyolysis, *4* – lipid vacuolation of cells

Table 4
Cell pathologies (% of the total cell number) in the liver of *C. gibelio* fish exposed to TNT (x ± SD, n = 100)

Treatment option	Abnormal forms of hepatocytes			
	nucleus shift	binucleation	karyolysis	lipoid cell dystrophy
Control	16.14 ± 0.49[a]	0.84 ± 0.03[a]	0.30 ± 0.01[a]	not found
TNT (5 mg/L, 21 days)	16.31 ± 0.43[a]	1.43 ± 0.05[b]	9.04 ± 0.49[b]	44.28 ± 2.33
TNT (35 mg/L, 8 hours)	20.26 ± 0.54[b]	1.26 ± 0.04[c]	2.86 ± 0.15[c]	not found

Note: see Table 1.

The gills of *C. gibelio* fish treated with 2,4,6-trinitrotoluene were characterized by both the strengthening histopathological signs also found in control samples, and the emergence of toxicant-induced abnormalities, such as thickening of the apical tips of the lamellae (Table 5). Exacerbation of the hypertrophy of lamellae epithelium in the *C. gibelio* gills was revealed under both the short-term and long-term TNT action (17 and almost 7 times higher than control, respectively). Higher dilation of the lamellar apical tips was revealed due to chronic exposure to 2,4,6-trinitrotoluene. Both the acute and chronic TNT influence caused the growth of the level of distorted gill lamellae (18% and 16% above control, respectively), while the significant increase in the necrosis frequency (33.4% above the control) was found only due to acute TNT action.

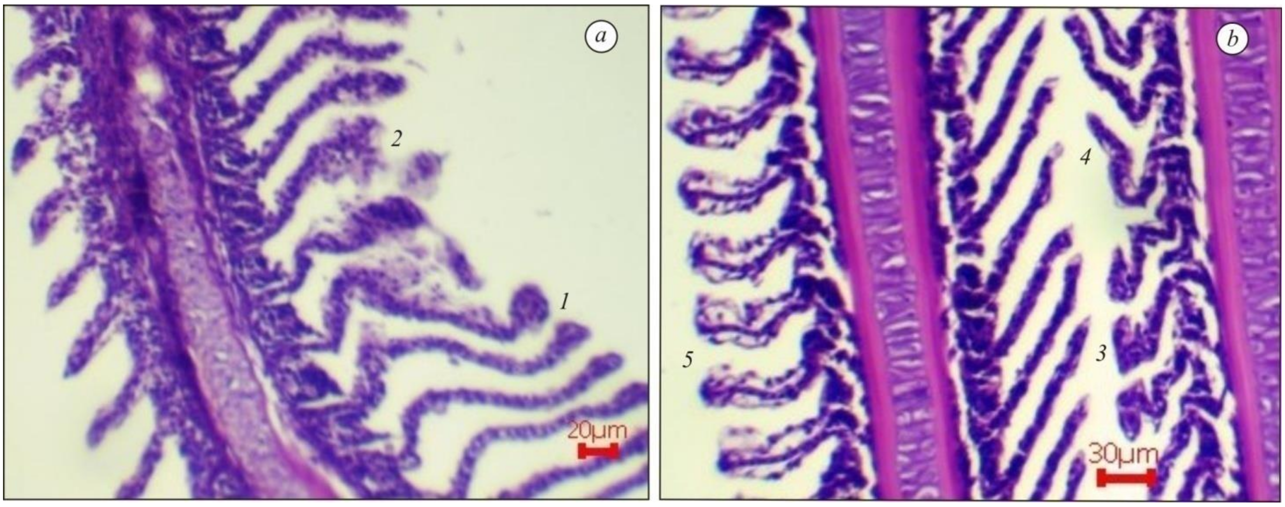

Fig. 4. Histopathological changes in the *C. gibelio* gill lamellae due to TNT chronic (21 days at concentration 5 mg/L) and acute (8 hours at concentration 35 mg/L) action, (*a*): *1* – dilation of the lamellar apical tips, *2* – necrosis, (*b*): *3* – epithelial hyperplasia, *4* – distortion of the lamellae, *5* – detachment of the epithelium

Table 5
Ffrequency of lamellae pathologies (% of the total cell number) in gills of C. gibelio exposed to TNT (x ± SD, n = 100)

Treatment option	Abnormal forms of lamellae			
	epithelial hypertrophy	dilation of the lamellar apical tips	distortion of the lamellae	necrosis
Control	1.30 ± 0.04[a]	not found	31.60 ± 1.01[a]	8.17 ± 0.33[a]
TNT (5 mg/L, 21 days)	8.53 ± 0.31[b]	5.68 ± 0.17[a]	36.95 ± 1.11[b]	8.23 ± 0.27[a]
TNT (35 mg/L, 8 hours)	21.82 ± 0.68[c]	3.98 ± 0.14[b]	37.32 ± 1.08[b]	10.90 ± 0.35[b]

Note: different letters within a column indicate significant differences in mean values by Tukey's test (P ≤ 0.05).

Discussion

Different fish species have been used as the experimental models to assess the health of polluted aquatic ecosystems and serve as indicators of aquatic contamination, including the effects of heavy metals (Hossain et al., 2021; Yılmaz et al., 2023), pesticides (Özaslan et al., 2018; Ogueji et al., 2023), various chlorophenolic compounds (Mukherjee et al., 2022), and polycyclic aromatic hydrocarbons (Santana et al., 2018). The influence of explosives, including 2,4,6-trinitrotoluene, on the marine hydrobionts was the goal of the recent studies conducted in the waters near munitions dumpsites in the Baltic Sea (Koske et al., 2019; Koske et al., 2020), while the inhabitants of freshwater bodies have received insufficient attention. In our experiment, *C. gibelio* fish exposed to short-term and long-term TNT influence showed the alteration in lipid peroxidation level and the defense enzyme activity, as well as histopathological changes in the hepatocytes and gills, which were assessed in accordance with (Kostić et al., 2017; Topić Popović et al., 2023) as biomarkers of exposure and biomarker of effect, respectively.

The dynamic changes in lipid peroxidation intensity in the liver, gills, and muscles of *C. gibelio* during acute TNT exposure suggest the activation of mechanisms mitigating the oxidative stress. However, at the end of the short-term experiment, LPO levels exceeded the control levels in all tissues, especially in the gill and muscle. During chronic exposure to low TNT concentration, greatly increased MDA content in all *C. gibelio* tissues characterized the fish adaptation. However, the elevated LPO intensity in fish muscle suggests possible TNT or metabolite accumulation, making the fish unsuitable for consumption. Similar findings have been reported for accumulation of various synthetic organic contaminants in the tissues of the Brazilian guitarfish *Pseudobatos horkelii* with their predominant concentration in the liver, while the accumulation in the muscles indicated a long-term effect of pollutants (de Souza-Leal et al., 2025). Generally, LPO products' level has an important informative character, specifically, the malondialdehyde level is used as a marker of membrane phospholipid oxidation through lipid peroxidation (Arojojoye & Adeosun, 2016). Significantly increased malondialdehyde levels in the gill, liver, and muscle of Indian major carp *Catla catla* were assessed as the enzymatic biomarker of triclosan impact (Hemalatha et al., 2019). Increase in LPO level at 62% and 100% was shown, respectively, in the liver and gill of the matrinxa *Brycon amazonicus* exposed to sub-lethal concentration (20% of LC_{50}) of cypermethrin-based insecticide, and was assessed as the oxidative stress (de Moraes et al., 2018).

The intracellular defense mechanisms of fish use detoxifying enzymes, including a multifunctional glutathione S-transferase superfamily, which is the potent tool against various contaminating agents (Bocedi et al., 2023) and one of the intensely investigated biomarkers of the aquatic contamination and fish detoxifying ability. In our study, chronic TNT exposure activated glutathione S-transferase in the *C. gibelio* liver, gills, and muscles. Acute exposure initially decreased GST activity in all tissues, followed by an increase peaking at the sixth hour; subsequently, GST activity sharply declined, reflecting phases of the enzymatic protection compensation and decompensation. Therefore, the increased GST activity in *C. gibelio* tissues during long-term exposure to low TNT concentrations indicates fish adaptation, suggesting the exposure stress was partially or completely overcome. At the same time, the dynamics of enzyme activity under acute stress indicated the exhaustion of the defense mechanism, in the fish liver especially. The dependence of the *C. gibelio* GST activity on the TNT concentration and duration of exposure is consistent with the data of other authors on the effects of various toxicants. Recent studies reported the purified enzyme characteristics of the glutathione S-transferases from muscle (Aksoy et al., 2016) and kidney tissues (Yılmaz & Çomaklı, 2023) of the pearl mullet (*Chalcalburnus tarichii* Pallas), as well as the inhibition action (IC_{50}) of some heavy metals and pesticides on the enzyme activity. Increased glutathione-S-transferase activity was established in the liver of juvenile *Prochilodus lineatus* exposed to sub- chronic action of titanium dioxide nanoparticles (14 days), indicating the essential role of glutathione S-transferase in metabolizing nanoparticles and ROS in liver cells (Carmo et al., 2019). Study on accumulation of the human pharmaceutical active compounds in the fish species from two sampling areas with different degrees of urbanization revealed the significantly enhanced GST activity in the fish muscle and liver (Rojo et al., 2021). On the contrary, the inhibition of glutathione S-transferase activity in the liver of *Chalcalburnus tarichii* Pallas was caused by the pesticides 2,4-D, glyphosate, dichlorvos, and some others (Özaslan et al., 2018). It is indicative that the high increase in GST activity along with LPO intensity reduction were revealed in the liver of the freshwater fish *Rhamdia quelen* exposed to diclofenac, while activity of the antioxidant enzymes catalase and superoxide dismutase was reduced (Guiloski et al., 2017).

Cytomorphological changes in the *C. gibelio* hepatocytes were associated with the increase in LPO intensity and GST activity in the liver due to TNT influence. Since the liver plays a central role in fish physiology, regulating lipid synthesis and catabolism, lipid homeostasis requires maintaining the balance between lipogenesis (fatty acid synthesis) and fat catabolism (lipolysis) (Carvalho et al., 2021). Changes in the morphometric indices of *C. gibelio* hepatocytes were the greatest with the acute exposure to TNT, while chronic action most affected the parameters of nuclei and the ratio nucleus area to cell area. Histopathological changes such as nucleus shift, binucleation, and karyolysis occurred both with acute and chronic TNT action, indicating the gross structural violations in *C. gibelio* liver cells; in particular, a sharp increase in karyolysis (the process of nucleus dissolving) level during the chronic exposure may indicate intense cell death of the necrotic type. At the same time, the lipoid dystrophy of *C. gibelio* hepatocytes was found only due to chronic TNT influence, indicating the persistent damage of 2,4,6-trinitrotoluene to the cytoplasmic structures. Lipoid cell dystrophy is typical of fish exposed to toxic substances and is considered a hallmark of toxicant-induced metabolic disorder; thereby our findings are consistent with the results of other studies regarding the structural alterations of fish liver cells under toxic influence. For example, according to Topić Popović et al. (2023), changes in fish hepatocytes are non-specific histopathological biomarkers indicating the state of fish, and can be manifested as hypertrophy and hyperplasia of cells, as well as the accumulation of a large amount of fat in fish liver, i.e. fatty liver. Such changes occur as a result of toxicant-induced alteration of nutrient metabolism in the liver (Weinrauch et al., 2021), in particular, fat deposition is the result of an imbalance between lipogenesis and lipolysis in liver tissue (Wang et al., 2019). This condition is considered pathological because it disrupts the fish's metabolic use of nutrients and energy, and therefore fish health and performance (Carvalho et al., 2021). A similar conclusion was made by Carmo et al. (2019) regarding the observed cell hypertrophy, disruption of the hepatic cords' location, and degenerative morphological changes in the liver of Neotropical detritivorous fish under the influence of titanium dioxide nanoparticles. Histopathological changes in the liver of *Clarias gariepinus* and *Cyprinus carpio* from polluted freshwater ecosystems mostly manifested as lipidosis (lipid accumulation), as well as steatosis when lipid vacuoles in hepatocytes displace and compress the nucleus (Wagenaar & Barnhoorn, 2018).

Gills of fish are recognized as a primary target organ of dissolved pollutants, because of their direct contact with the external medium through large surface (Sabullah et al., 2015; Curcio et al., 2022). The gills of *C. gibelio* exposed to 2,4,6-trinitrotoluene showed several morphological alterations compared to control (hypertrophy, hyperplasia and detachment of the epithelium, distortion of the lamellae, and dilation of the lamellar apical tips), assessed as histopathological changes in the composition of the gill lamellae. Epithelial hypertrophy (increase in cell volume) and hyperplasia (an increase in the number of cells, or proliferation) in lamellae were reported as the primary toxic effect of lead (Macirella et al., 2019), heavy metals complex (Fonseca et al., 2017), combined water pollution (Kostić et al., 2017), and potent neurotoxic pesticide emamectin benzoate (Ogueji et al., 2023). These two processes are closely related and partly develop as concomitants of each other, but hyperplasia is observed only if the cell population is capable of DNA synthesis, which enables mitosis. Next, the epithelial hyperplasia causes gill lamellae to fuse with each other, thicken their distal ends, decrease in size, and distort the lamellae; all these violations lead to the transformation of the gill apparatus into a structureless mass. The gill apparatus of fish plays fundamental roles in many physiological functions, including gas exchange, osmoregulation, excretion, water balance, and acid-base regulation (Curcio et al., 2022; Mahapatra et al., 2022). Therefore, histopathological changes in the gills of *C. gibelio*, detected under both acute and chronic influence of 2,4,6-trinitrotoluene, should be considered as threatening challenges for the health of the fish, which will have adverse consequences for the preservation of their population.

The results of a chronic experiment can be used for an approximate forecast of the adaptive capabilities of *C. gibelio* to contamination of natural water bodies with explosive substances. That is, during the long-term exposure of *C. gibelio* to TNT low concentration, induction of the glutathione S-transferase activity and lipid peroxidation processes took place in the fish tissues, and a constant prooxidants/antioxidants ratio was formed in the liver, gills, and muscles, which can be considered as a biochemical adaptation; in turn, the lipoid dystrophy of hepatocytes and hyperplasia of lamellar epithelium in the gills can characterize the morpho-physiological fish adaptation. At the same time, the chronic effect of the toxicant caused drastic changes in the hepatocyte's nuclei, including an increase in morphometric parameters, karyolysis and binucleation, as well as epithetlial detachment and necrosis in the gill lamellae. Such signs indicate the irreversible negative processes that can reduce the vitality of the fish organism and denies (at least calls into question) the possibility that *C. gibelio* can complete adaptation to low-dose contamination of their habitat with 2,4,6-trinitrotoluene. Our findings are consistent with the results of recent research showing that low doses of toxic compounds in water do not guarantee the absence of negative effects on aquatic organisms. For example, even the environmentally relevant concentrations of the anti-inflammatory drug diclofenac affected the antioxidant enzymes activity and LPO processes in the liver and testis of the freshwater South American catfish (Guiloski et al., 2017). Ecologically relevant concentrations of lead caused the modulation of redox status and conspicuous pathological changes in the gills of *Danio rerio*, including osmoregulatory dysfunctions (Curcio et al., 2022).

Given the scale and duration of hostilities in Ukraine, the continuous use of explosives, and the lack of current data on water contamination, accurate assessments and forecasts of the health of hydrobionts are impossible. Therefore, model studies are needed to understand the marker signs of the toxic effects of TNT and other pollutants, characterize species-specific responses of freshwater hydrobionts, and indicate the potential consequences of toxicant action on reproduction and the functional state of the subsequent generations of aquatic organisms.

Conclusion

The toxicity of 2,4,6-trinitrotoluene on *C. gibelio* was studied in a model experiment using the long-term (21 days at concentration 5 mg/L) and short-term (8 hours at concentration 35 mg/L) exposure. In the fish liver, gill, and muscle tissues, high induction of glutathione S-transferase activity and the intensification of lipid peroxidation were revealed under both the acute and chronic TNT action, and assessed as the biomarkers of toxic exposure. Under the chronic exposure to TNT, high accumulation of LPO products and activation of GST were found in all the *C. gibelio* tissues, especially in the gills, which are in direct contact with pollutants dissolved in water. Such trend indicated a significant redox balance modulation and a high detoxification intensity in the fish body cells, and can be considered as a biochemical adaptation of the *C. gibelio* to long-term action of 2,4,6-trinitrotoluene at low concentration. In the acute exposure, LPO intensity increased in the fish gills, liver, and muscles, while GST activity in all tissues decreased after eight hours indicating exhaustion of the defense mechanism in the liver especially.

The alteration of morphometric indices of the fish liver cells and nuclei, as well as the histopathological changes in the hepatocytes and gills' structure were assessed as the biomarkers of toxic effect on the organism of *C. gibelio* under the acute and chronic influence of TNT. Such changes as lipoid dystrophy of hepatocytes and hyperplasia of lamellar epithelium in the gills are the hallmarks of toxicant-induced metabolic modulation and can be considered as a morpho-physiological adaptation to the chronic action of TNT at low concentration.

Changes in the cytomorphological parameters of the hepatocytes of *C. gibelio* were the greatest with acute TNT exposure, while chronic action most affected the nuclei indices and the ratio of nucleus area to cell area. Histopathological changes in hepatocytes, such as nucleus shift, binucleation, and karyolysis occurred both with acute and chronic TNT effects, indicating the gross structural violations in *C. gibelio* liver cells; in particular, a sharp increase in karyolysis level during chronic exposure may indicate intense cell death of the necrotic type.

Histopathological changes in the gills of *C. gibelio* due to short-term and long-term TNT exposure included such structural disorders as hypertrophy, hyperplasia and detachment of the epithelium, distortion of the lamellae and dilation of the lamellar apical tips, and necrosis in the gill lamellae. If epithelial hypertrophy and hyperplasia signal early toxic effects on fish gills, then the lamellar distortion and apical tip dilation indicate severe damage. These irreversible changes can reduce the vitality of the fish and preclude complete adaptation of *C. gibelio* to contamination of water with explosives, even to low-dose TNT exposure. The structural and metabolic changes in the liver, gills, and muscles of *C. gibelio* indicate strong oxidative stress and pathological disorganization of the fish tissues under short-term and long-term exposure to TNT in a model polluted water body. The obtained results clearly demonstrate the need for research into the state of Ukraine's natural water ecosystems contaminated with 2,4,6-trinitrotoluene and other explosive substances.

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The authors declare no conflicts of interest.

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