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Effects on enzyme activity and DNA integrity in rainbow trout *Oncorhynchus mykiss* exposed to fish farm effluents

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ABSTRACT: Fish farm effluents are known to affect water quality and freshwater ecosystems, potentially harming non-target organisms and ecosystem processes. We studied the effect of fish farm effluents at different concentrations (3.125–100% v/v) on catalase (CAT) and glutathione *S*-transferase (GST) activity as well as the DNA integrity of *Oncorhynchus mykiss* fry over 24–120 h. Biochemical responses and DNA damage analysis were conducted to assess the impact. We found that fish farm effluent had higher conductivity, nitrate, nitrite, and total dissolved solids concentrations downstream compared to upstream of the farm. Interestingly, no antibiotics were detected in the effluent. CAT activity significantly increased in the fish liver at concentrations of 12.5, 50, and 100% of the effluent after 72 h. In the gills, a significant increase was observed at concentrations ranging from 6.25 to 100% of the effluent after both 24 and 72 h. GST activity increased significantly in the liver at a concentration of 100% of the effluent after 72 h and in the gills at concentrations of 25, 50, and 100% after 24 h, with a decrease noted at higher concentrations. DNA damage assessment revealed significant DNA strand breaks in blood cells at concentrations of 12.5, 25, 50, and 100% of the effluent after 120 h of exposure. The results demonstrate that fish farm effluents can induce oxidative stress, causing damage to DNA integrity in blood cells. Our findings emphasize the potential ecological risks posed by fish farm effluents to aquatic organisms.

KEY WORDS: Fish farm effluents · Oxidative stress · DNA damage · Chemical products · Rainbow trout

1. INTRODUCTION

Surface water bodies are frequently impacted and contaminated by toxic effluent discharges originating from various anthropogenic sources (Ruiz-Zarzuela et al. 2009, Tello et al. 2010), including effluents discharged from the continental aquaculture industry. Salmon-farming production involves the use of flow-through and recirculating systems that release effluents containing high levels of nutrients from unconsumed

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feed and excretion compounds, thereby influencing the water quality of the receiving waterbody (Le Moal et al. 2019). Intensive aquaculture systems are characterized by a high density of fish per unit volume of water, which increases the susceptibility of these organisms to diseases caused by viruses, bacteria, fungi, and parasites. To combat these diseases, therapeutic treatments are employed, including the use of antibiotics such as florfenicol (FLO) and oxytetracycline (OTC), which are incorporated into the feed, as well as antiparasitics such as bronopol, formalin, and sodium chloride, which are applied through immersion baths (Aaen et al. 2015, Alvarado-Flores et al. 2021). The remnants of these products are subsequently discharged into the aquatic environment due to the inefficiency of the water-treatment systems (Gros et al. 2010, Quesada et al. 2013). In Chile, wastewater generated by land-based aquaculture is treated with primary treatment (solid waste removal) only, and therefore it may be categorized as a biological stressor for the receiving stream (Nimptsch et al. 2015, Stojanović et al. 2017).

Many environmental pollutants, also known as xenobiotics (i.e. chemical substances that are not naturally produced by organisms), have the ability to persist in aquatic environments (Kümmerer 2009, Puckowski et al. 2016) and interact with organic matter, leading to synergistic effects (Kopinke et al. 1995, Bervoets et al. 1996, Madureira et al. 2010) and detrimental consequences for aquatic ecosystems (Ferreira et al. 2007, Reda et al. 2013, Antunes et al. 2016). Therefore, a simplistic characterization of water quality based only on physical and chemical parameters does not provide a comprehensive assessment of the potential biological effects in these ecosystems. The routine effluent monitoring that is performed is based on government regulations for water quality, which establish maximum allowable limits for variables such as pH, dissolved oxygen concentration, biochemical oxygen demand, and total dissolved solids (TDS), among others. However, these regulations do not incorporate the detection of specific contaminants such as antibiotics, disinfectants, and antifungals that in mixture can have chronic toxicological effects on aquatic species (Boyd 2003, El-Gohary et al. 2020, Ahmad et al. 2021).

Oxidative stress is recognized as one of the key indicators of detoxification processes, such as the biotransformation of xenobiotics to increase their hydrophilicity for easier elimination from the organism. Exposure to xenobiotics can lead to the generation of intermediate chemical species, including reactive oxygen species (ROS), which have the potential to disrupt the integrity of biological membranes (Silvestre 2020, Gonçalves et al. 2021). To counteract the detrimental effects of increased ROS within an organism, antioxidant defense mechanisms are activated as a detoxification response, which involves the upregulation of several enzymes including superoxide dismutase (SOD), glutathione peroxidase, and catalase (CAT), which help neutralize the ROS (Chinnadurai et al. 2022). Additionally, an increase in the activity of glutathione *S*-transferase (GST) may be observed, as it plays a role in the conjugation of lipid peroxides generated during oxidative stress (Singhal et al. 2015). However, if the antioxidant defenses are overwhelmed or insufficient, oxidative stress can result in damage to macromolecules, including genetic material, or even trigger apoptosis (Solé & Livingstone 2005, Burkina et al. 2015, Ribalta et al. 2015).

Fish species exhibit high sensitivity to xenobiotic exposure, due to their ability to absorb these chemical substances through their gills and tissues. Xenobiotics can bioaccumulate within fish cells and interact at the molecular level with various cellular components (Singh 2000, Braz-Mota et al. 2015, Poley et al. 2018). The comet assay is a rapid and highly sensitive technique employed to detect low levels of DNA damage caused by xenobiotics (Jiang et al. 2023). This assay relies on the susceptibility of damaged sites to alkaline conditions (pH > 13) during electrophoresis, resulting in the visualization of DNA fragmentation in individual cells, which represents single-stranded DNA breaks, double-stranded breaks, and alkali-labile sites (de Lapuente et al. 2015). Although the comet assay has been extensively utilized to assess DNA damage in various fish species exposed to industrial and domestic wastewater, studies on the genotoxic potential of fish farm effluents remain limited (Klobučar et al. 2010, Hallare et al. 2016, Hussain et al. 2018).

Ecotoxicological evaluation with complete effluents measuring response in terms of oxidative stress and genotoxic potential can provide important information for environmental regulation and management. Therefore, the main objective of the present study is to assess the impact of a fish farm effluent on CAT and GST activity as well as DNA integrity of rainbow trout *Oncorhynchus mykiss* erythrocytes to confirm its potential prooxidant and genotoxic effects.

2. MATERIALS AND METHODS

2.1. Sampling site

Water samples were collected from the effluent of a mixed-type fish farm that utilizes a combination of a flow-through system and a recirculating aquaculture

system (RAS). The effluent flows into the Molco Alto River, 15 km south-east of Villarrica city, La Araucanía region, in Chilean Patagonia (39° 20′ 14″ S, 72° 5′ 44″ W) (Fig. 1). This fish farm accounts for an estimated annual production of 471 t of *Salmo salar* fingerlings and smolts, which are feed *ad libitum* with extruded commercial pellets that have low phosphorus content and high digestibility. Sample collections were carried out in summer, given that the use of pharmaceutical products and disinfectants increases in this season and therefore results in greater waste concentrations in the effluent. Water sampling was carried out via individual time-weighted composites, which represent the average effluent characteristics during the compositing period. The samples were collected directly from the effluent before its discharge into the river, through the manual collection of discrete portions of equal volume at regular time intervals. These samples were stored in a 1000 l fiberglass tank. To carry out tests, approximately 83.3 l was collected every 1 h, for 12 h and for 4 d until the collection of 4000 l was complete. Samples were taken to the Aquaculture Experimental Unit of the Catholic University of Temuco and stored in a 5 m^3 tank under dark conditions in a cold chamber at 4°C (United States Environmental Protection Agency 2017). Physical–chemical analysis and determination of antibiotics was carried out 1 d after sampling, and the exposure test was carried out 2 d after the effluent was obtained.

2.2. Effluent characterization

2.2.1. Nutrient determination and physical–chemical parameters

For the effluent samples, the following parameters were measured (see Table 1). Ammonium and total phosphorus were determined according to the methods recommended by the Chilean standards NCh 2313/16 2010 and NCh 2313/15: 2009, respectively. Phosphate, nitrates, nitrites, and total nitrogen were analyzed in accordance with 4500-P-E, 4500-NO3 E, 4500-NO2 B, and 4500 C-N Standard Methods (American Public Health Association 1992), respectively. Conductivity, TDS, pH, and temperature were measured using a waterproof multiparameter instrument (Hanna Instruments, model HI9829).

2.2.2. Antibiotics determination

OTC and FLO were analyzed according to Shama (2016), using high-performance liquid chromatography (Shimadzu, Prominence) with a diode array detector. For OTC determination, separation was carried out using a C18 column (250 mm length, 4.6 × 100 mm, 2.7 µm particle diameter) and 10 mM oxalic acid (A), 0.1% trifluoroacetic acid (B), and acetonitrile (C) (55:25:20) (Hassan 2016) as the mobile phase. The flow

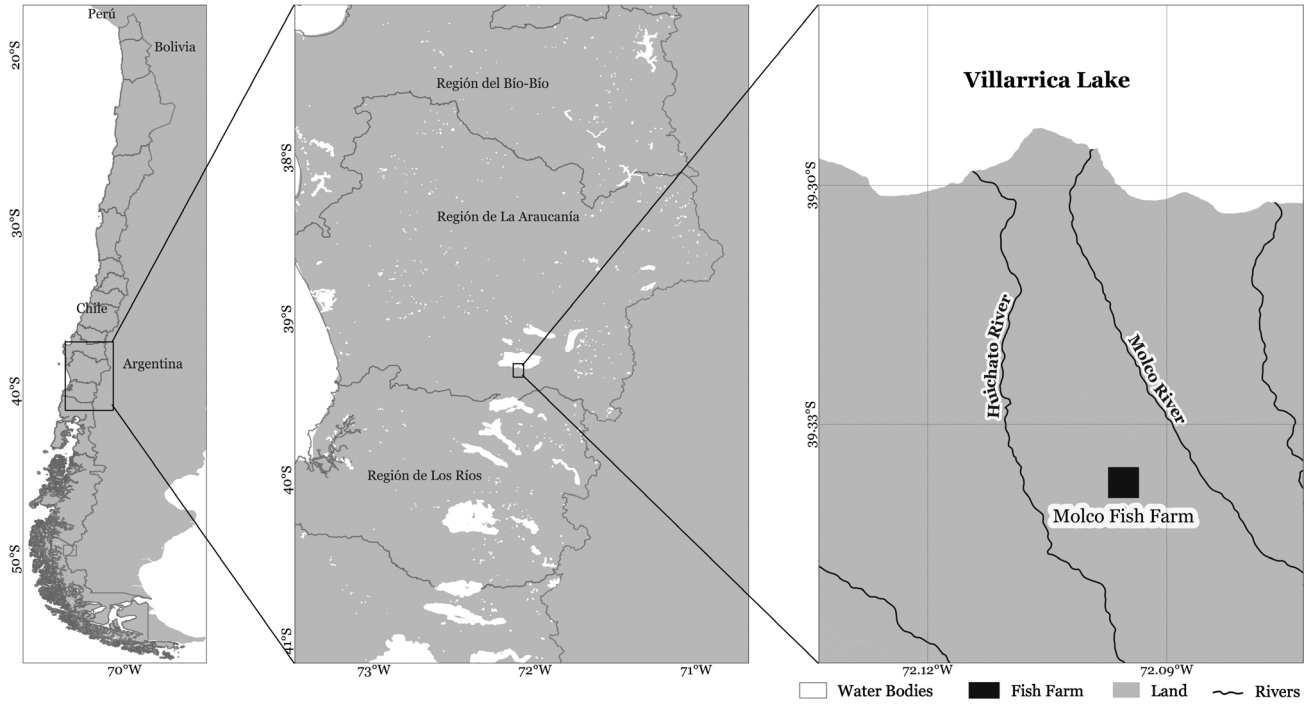

Fig. 1. Study area map located in Villarrica city, La Araucanía region, Chile

rate was 1.0 ml min^{-1} and the injection volume was 100 µl. Detection and quantification at a wavelength of 365 nm were 4.0 and 13.2 ng ml^{-1}, respectively. For FLO determination, separation was carried out using a C18 column of 150 mm in length, with 0.1% trifluoroacetic acid and acetonitrile as the mobile phase. The flow rate was 1.5 ml min^{-1} and injection column was 100 µl. Detection and quantification at 223 nm were 160 and 440 ng ml^{-1}, respectively.

2.3. Animal testing

Fingerlings of *Oncorhynchus mykiss* (Family Salmonidae, Order Salmoniformes) were used to evaluate oxidative stress and genotoxicity. All-female monosex specimens were obtained from the Huililco fish farm population (39° 14′ 18″ S, 71° 50′ 30″ W), located in Pucón, in La Araucanía region. Fish were transported in a 1000 l fiberglass tank at a temperature of 9.8°C with constant oxygenation to the experimental culture unit at the Catholic University of Temuco. In this facility, fish selection and separation were carried out through visual inspection, and individuals with malformations and eroded fins were excluded. Additionally, a uniform selection based on size and weight was performed to achieve a condition factor equal to or greater than 1, indicative of the good health status of the specimens. Average weight and length were recorded as 33.3 ± 2.09 g and 14.7 ± 1.05 cm, respectively. Fish were acclimated for 2 wk under controlled photoperiod conditions (12 h light:12 h dark) at a temperature of 14.4°C. During this period, specimens were fed as proposed by Rodehutscord & Pfeffer (1999), using commercial pellets that were suspended 48 h prior to the experiment assay.

2.4. Exposure conditions

Treatments consisted of a series of effluent dilutions (Fig. 2), as proposed by the United States Environmental Protection Agency (2000) in the Whole Effluent Toxicity method to assess the combined effects of all constituents of a complex effluent. This procedure allows the determination of the environmental exposure of aquatic life in an effluent when chemical, physical, and biological characteristics of the discharges are unknown. Additionally, main parameters were measured to provide a more comprehensive and realistic scenario of the potential effects of discharges on fish. The dilution series comprised 100, 50, 25, 12.5, 6.25, and 3.125% of the effluent, which takes into account the water quality changes as the discharge dilutes and mixes with the receiving water. Dilutions were prepared with the incoming water of the fish farm. Water was previously treated on a rotatory filter for suspended particles and with UV rays to control microorganisms. In addition, a negative control (NC) was incorporated using the same water, and a positive control (PC) was incorporated using a non-lethal concentration of copper sulfate (2.2 µg l^{-1}) for the *O. mykiss* individuals (ECHA 2019). Treatments and controls were carried out in triplicate using a system of 24 static tanks holding 45 l of water; 7 rainbow trout individuals were added to each tank. The water in each tank was renewed by 40% daily. Samples were taken after 24, 72, and 120 h, in which CAT and GST were measured using one fish from each tank on each occasion. Additionally, after 120 h, DNA damage was evaluated using another specimen. Fish were randomly selected.

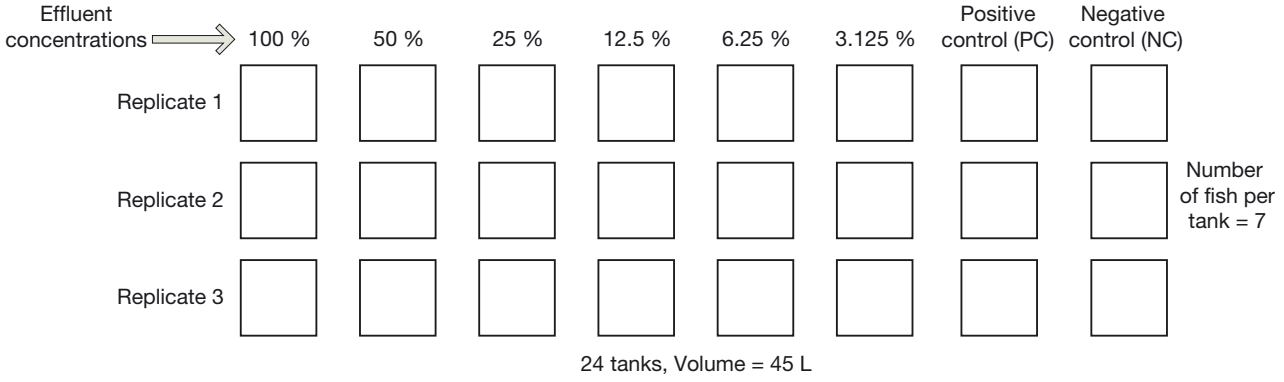

Fig. 2. Experimental design, consisting of 3 replicate treatments with effluent concentrations ranging from 3.125–100% as well as positive and negative controls (see Section 2.4). A total of 7 individual *Oncorhynchus mykiss* were added to each treatment tank

2.5. Collection of biological samples

The biological samples of liver, gills, and blood were chosen due to their significance in responding to environmental factors, offering specific insights into the health status of the fish. For the analysis of enzymatic activity, the fish were euthanized with benzocaine (AVMA 2020) after 24, 72, and 120 h of exposure. Liver and gill samples were promptly excised; tissues were rinsed with a 0.9% NaCl saline solution and maintained at −80°C. For the comet assay, fish exposed for 120 h were anesthetized, and blood samples were collected from the caudal vein and subsequently transferred into tubes coated with 5% ethylenediaminetetraacetic acid (EDTA) (Oliveira et al. 2008). The samples were stored in microtubes at 4°C, and the assay was conducted on the same sampling day. The proposed methodology was approved by the Research Ethics Committee of the Universidad Católica de Temuco.

2.6. Enzymatic activity

Enzyme extraction was carried out in accordance with Ogunji et al. (2007). Defrosted liver and gill samples were homogenized by adding 0.1 M potassium phosphate buffer (pH 6.5) containing 1.4 mM dithioerythritol, 20% glycerol, and 1 mM EDTA. Membranes were discarded by centrifugation at 10 000 × *g* for 10 min at 4°C and cytosolic proteins were concentrated by ammonium sulphate precipitation, centrifugation, and resuspension of the pellet in 1 ml of 20 mM sodium phosphate buffer (pH 7.0), followed by desalting via Sephadex columns. Protein extracts were stored at −80°C prior to enzyme activity assays.

GST activity was measured in the soluble (cytosolic) fraction according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate and analyzed at a wavelength of 340 nm. CAT activity was measured at 240 nm as described by Claiborne (1985) following the consumption of hydrogen peroxide (H_2O_2). Specific enzymatic activity was expressed in nkatal mg^{-1} protein, where 1 katal is defined as 1 mol substrate turnover per second. Total protein content was assessed in accordance with the Bradford method (Bradford 1976) using bovine serum albumin as standard, and absorbance was measured at 595 nm.

2.7. Comet assay on blood cells

The comet assay was performed with minor modifications as previously described by Dhawan et al. (2003). For this assay, 20 µl of the samples were diluted in 100 µl of PBS and then 30 µl was extracted and resuspended in 75 µl of 1% low melting point agarose, layered onto microscope slides precoated with 1% normal melting agarose (dried at room temperature). Two gels were mounted on each slide and covered with a coverslip. Coverslips were removed immediately after agarose solidification and slides were immersed in a cold fresh lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% DMSO, pH 10) for 24 h at 4°C. Slides were placed for 30 min in a horizontal gel electrophoresis tank filled with a cold electrophoresis buffer (10 N NaOH, 200 mM EDTA, pH 10) to allow DNA unwinding. Electrophoresis was carried out in the same buffer for 30 min at 25 V and 300 mA. After electrophoresis, slides were neutralized with 0.4 mM Tris (pH 7.5). Slides were subsequently stained with 20 µl of DAPI (1 µl ml^{-1}) per gel. Images were examined at 400× magnification using a fluorescence microscope (Nikon Eclipse, E200) coupled with a CMOS digital camera. A total of 100 randomly selected cells (50 cells on each one of the 2 replicate slides) were analyzed per treatment. Tail DNA percentage was used to measure DNA damage, given its extended and recommended use for comet-data analysis (Lovell & Omori 2008), and computed using the open-source Comet Score 1.5 Image Analysis System (TriTek Corp).

2.8. Statistical analysis

Data on enzymatic activity are expressed as means and their respective standard errors. The comparison of the results was carried out using ANOVA, with prior assessment of normality and homogeneity assumptions using the Shapiro-Wilk and Levene tests, respectively. Post hoc comparisons were made using a Tukey test (Zar 1999). The DNA integrity assessment results were associated with non-parametric data and therefore were analyzed using the Kruskal-Wallis test, with a Dunn's test employed for multiple comparisons. The construction of the databases and the management of the information was carried out using XLSTAT 2019.

3. RESULTS

3.1. Effluent characterization

The concentration of nutrients, antibiotics, and physical−chemical parameters are summarized in

Table 1. Effluent physico-chemical characteristics and concentration of antibiotics. Values in the 'Effluent' column were obtained in a single measurement; values in the 'Upstream' column were obtained from the fish farm's Environmental Surveillance Report for the same sampling period. Values in the 'Maximum allowable limit' column are according to the Chilean emissions regulations D.S. MINSEGPRES No. 90/2000. NSS: not specified in the standard

Parameter	Effluent	Upstream	Maximum allowable limit	Units
Ammonia	1.285	1.00	NSS	mg l^{-1}
Phosphate	1.305	–	NSS	mg l^{-1}
Total phosphorus	0.653	<0.2	10	mg l^{-1}
Nitrates	1.535	<0.10	NSS	mg l^{-1}
Nitrites	0.0385	<0.01	NSS	mg l^{-1}
Total nitrogen	2.53	1.6	50	mg l^{-1}
Conductivity	111	53.5	NSS	µS cm^{-1}
Total dissolved solids	56	34	NSS	ppm
pH	8.3	6.9	6.0–8.5	–
Oxytetracycline	–		NSS	–
Florfenicol	–		NSS	–

Table 1. Nutrient concentrations in the effluent were found to be below the maximum limit allowed by the Chilean emissions regulations (MINSEGPRES 2000), while OTC and FLO detection was negative. However, conductivity (σ) and TDS increased in the effluent as compared to the water entering the fish farm, which presented a σ of 53.5 µS cm^{-1} and a TDS concentration of 34 mg l^{-1}.

3.2. Enzymatic activities

CAT activity in the liver and gills of fish exposed to different concentrations of the effluent is shown in Fig. 3. After 72 h of exposure, an

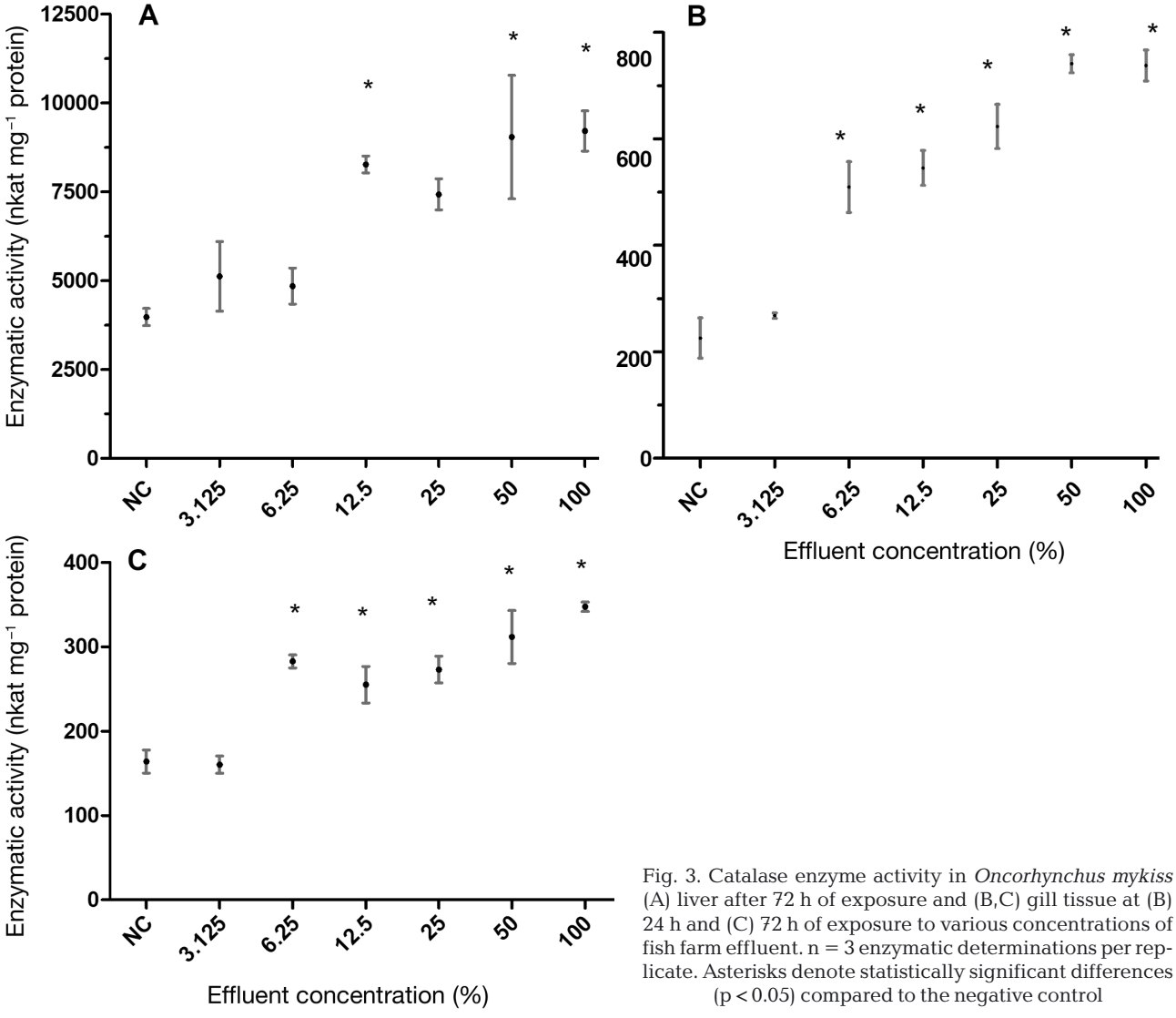

Fig. 3. Catalase enzyme activity in *Oncorhynchus mykiss* (A) liver after 72 h of exposure and (B,C) gill tissue at (B) 24 h and (C) 72 h of exposure to various concentrations of fish farm effluent. n = 3 enzymatic determinations per replicate. Asterisks denote statistically significant differences ($p < 0.05$) compared to the negative control

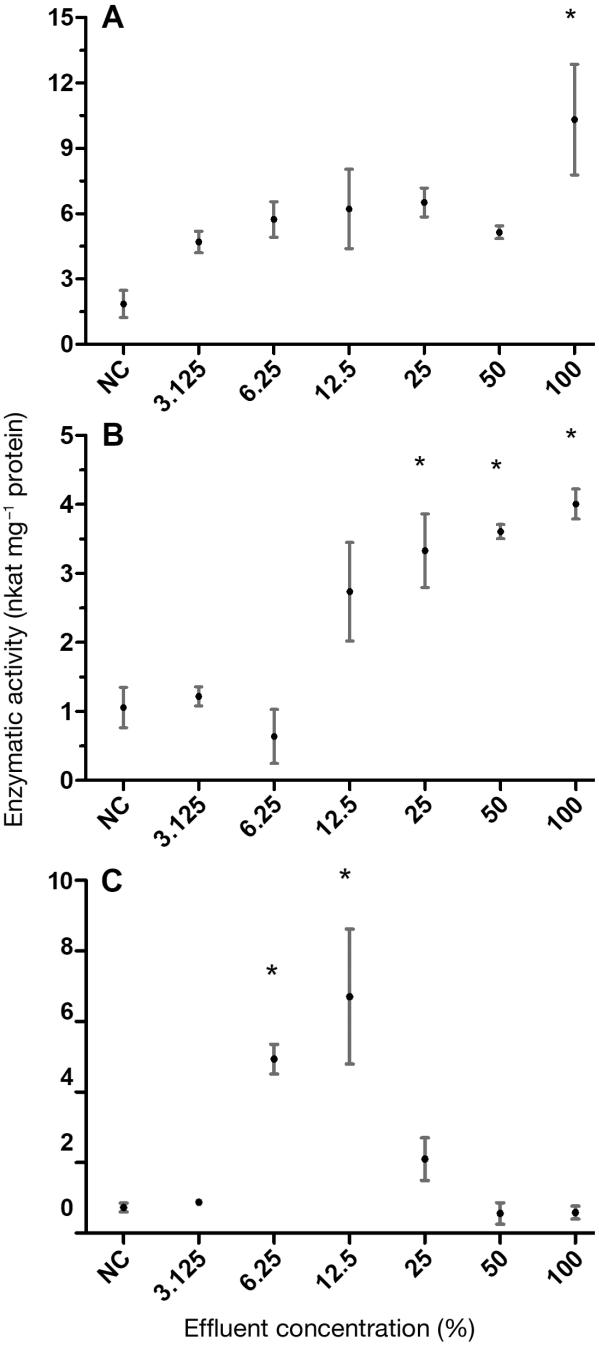

Fig. 4. Glutathione *S*-transferase enzymatic activity in *Oncorhynchus mykiss* (A) liver at 72 h of exposure and (B,C) gill at (B) 24 h and (C) 72 h of exposure to various concentrations of the fish farm effluent. n = 3 enzymatic determinations per replicate. Asterisks denote statistically significant differences ($p < 0.05$) compared to the negative control

increase in CAT activity was detected in the liver with significant differences compared to the NC ($F_{6,14}$ = 6.71, p = 0.0017) at concentrations of 12.5, 50, and 100% of the effluent. Similarly, an increase in CAT activity was observed in the gills at 24 h ($F_{6,14}$ = 39.27, p = 0.0001) and 72 h ($F_{6,14}$ = 16.80, p = 0.0001) at concentrations of 6.25, 12.5, 25, 50, and 100% effluent with significant differences from the NC. As shown in Fig. 4A, an increase in GST activity was observed in the liver at 72 h of exposure at a concentration of 100% of the effluent ($F_{6,14}$ = 3.84, p = 0.0178). In the gills, significant differences were observed at 25, 50, and 100% effluent at 24 h (Fig. 4B) ($F_{6,14}$ = 11.85, p = 0.0001), and at 6.25 and 12.5% effluent at 72 h of exposure (Fig. 4C) ($F_{6,14}$ = 9.89, p = 0.0002). By contrast, GST activity was inhibited at the higher concentrations. An increase in CAT activity was observed in the liver at 24 and 120 h as well as in the gills at 120 h. Additionally, an increase in GST activity was noted in the liver at 24 and 120 h, while in the gills, there was an inhibition of GST activity at 120 h. However, no significant differences were found in these results.

3.3. Genotoxic evaluation

DNA damage in blood cells after 120 h of exposure is shown in Fig. 5. We observed that DNA damage increased with increasing concentration of the effluent, resulting in greater DNA migration to the electrophoretic chamber at the highest effluent concentration (Fig. 6). Fish blood cells showed significant differences (Kruskal-Wallis H = 1388, $p < 0.0001$) in tail DNA percentages compared to NC values at 12.5, 25, 50, and 100% effluent concentration. The PC showed a marked genotoxic response, which confirms the validity of the NC results.

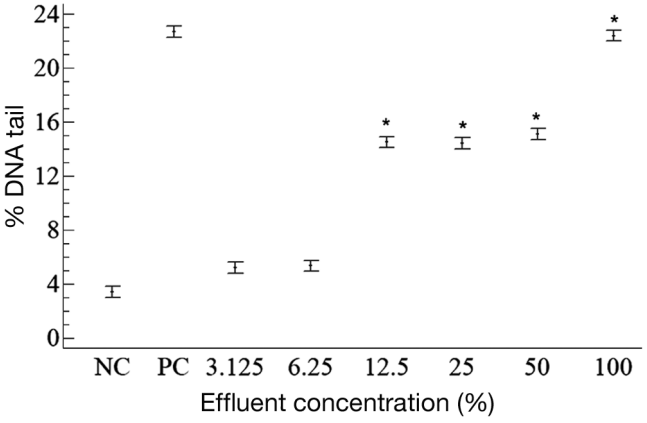

Fig. 5. Percentage of DNA damage in *Oncorhynchus mykiss* red blood cells. Data are mean ± SD. Asterisks denote significant differences ($p < 0.05$) from negative control (n = 6)

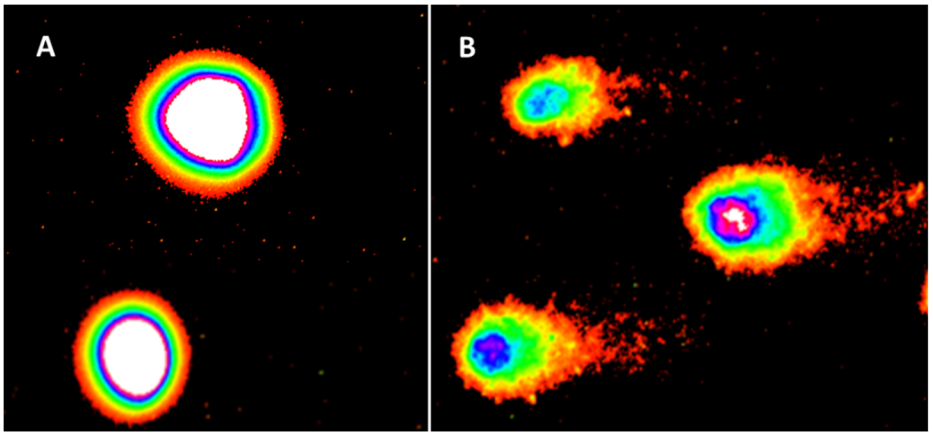

Fig. 6. TriTekComet Score representative image of *Oncorhynchus mykiss* red blood cells exposed to (A) negative control and (B) 100% effluent, indicating significant DNA damage

4. DISCUSSION

In the present study, we observed genotoxic effects of fish farm effluents on *Oncorhynchus mykiss*, reaching significant genetic damage at 12, 25, 50, and 100% v/v of effluents. Furthermore, oxidative stress was confirmed through the analysis of CAT and GST enzymes in fish tissues. The characterization of fish farm effluents showed negative results for the antibiotics FLO and OTC, although a slight increase in σ and TDS was detected. However, due to the high usage and discharges of various types of chemical residues and drugs in fish farms, we cannot rule out the possibility that other, unanalyzed compounds in the effluents might have been responsible for both the genotoxicity and oxidative stress in the *O. mykiss* model.

The fish were affected by the effluent from the fish farm, as evidenced by the activity of CAT. This enzyme represents the primary line of defense against oxidative stress in the antioxidant system, and an increase in its activity contributes to the removal of ROS induced by cells exposed to contamination. CAT activity response was similar in both liver and gill tissues, implying the presence of high peroxide concentrations at 24 and 72 h, at which point a significant increase in CAT activity was observed. This signifies the high sensitivity and rapid response of CAT in reducing O_2- and H_2O_2, as well as the intense metabolic activity of the organ (Rao 2006). However, in the gills, an increase in activity was observed at lower concentrations (6.25%) of the effluent compared to that in the liver. These distinctions might be associated with the exposure route, mode of entry, or absorption and bioaccumulation of xenobiotics in different organs (Gravato et al. 2006). The gill epithelium serves as the primary site for the transfer of xenobiotics present in the water. Xenobiotics are absorbed by the gills and enter the circulatory flow, resulting in tissue changes and reduced oxygen exchange in gill lamellae (Yang et al. 2000). Nevertheless, this organ has a higher renewal rate compared to other organs due to cortisol acting as a compensatory defense mechanism in response to stress (Wendelaar-Bonga 1997), which prevents the absorption of xenobiotics by the gills, restores homeostasis, and thus withstands oxidative changes (Jin et al. 2010, Paulino et al. 2012). However, this barrier was likely inhibited after 72 h of exposure, showing significant differences at lower concentrations. Furthermore, a dose-dependent response was observed, indicating the activation of the phase II biotransformation and defense system (Reynaud et al. 2008, Burkina et al. 2015).

GST activity also suggests the presence of prooxidant compounds in the effluent (Ahmad et al. 2006). This enzyme catalyzes the conjugation of glutathione (GSH) with compounds that have reactive electrophilic groups, such as xenobiotics, allowing for the protection and repair of nucleophilic groups in proteins and nucleic acids oxidized by ROS (Carvalho et al. 2012). However, in this study, GST activity in the gills was inhibited at 72 h at concentrations of 25, 50, and 100% of the effluent (Fig. 2C). This could be related to the loss of compensatory mechanisms in prolonged exposures (Wiegand et al. 2000, Ballesteros et al. 2009, Xing et al. 2012) due to severe oxidative stresses resulting from electrolyte imbalance (Lavado et al. 2013). This imbalance may be associated with the presence of salt ions in the effluent, as confirmed through σ measurements, which, under natural conditions, typically range from 20 to 70 µS cm^{-1} in the streams of southern Chile (Rivera et al. 2004).

In fish farms, 5% NaCl (sodium chloride) is employed as a disinfectant agent for the control of para-

sites in fish, with an annual application of 20–30 t of salt (Zaror et al. 2004, Tello et al. 2010, Eissa et al. 2017), which increases the σ of freshwater from 50 up to >2000 µS cm^{-1} downstream of the discharged effluents (Table 2) (Nimptsch et al. 2015). In the present study, the σ was 111 µS cm^{-1}, which is roughly equivalent to 20–36.9 mg l^{-1} of salt (Encina-Montoya et al. 2020). Osmotic equilibrium relies on biochemical and molecular mechanisms. In this regard, some authors have reported an increase in antioxidant activity in fish exposed to different salinity levels, such as an increase in GST in the liver and gene transcription (Maryoung et al. 2015, Cañedo-Argüelles et al. 2016, Kefford et al. 2016, Amiri et al. 2018). They have also noted an increase in the activity of heat shock proteins (hsp70) and osmotic stress proteins (osp54) in the gill tissue of *Salmo salar* and *Perca flavescens* (Smith et al. 1999) and changes in the drift rate of benthic macroinvertebrates (Encina-Montoya et al. 2020). Therefore, changes in water salinity may be associated with the generation of ROS that induce oxidative stress (Eissa et al. 2017). Moreover, GST activity can be influenced by various factors. These range from the direct action of effluent contents on the enzyme system and substrate reduction (i.e. GSH) to the presence of defective regulatory mechanisms in GST genes. This latter observation suggests that metabolites remain in the organisms, triggering an increase in ROS. Additionally, the intensity and duration of applied stress along with the susceptibility of the exposed organism, as well as the levels of GSH in cells (a GST cofactor), maintained by the activity of glutathione reductase, may also exert influence on said activity (Islam et al. 2019). However, ROS inducers promote rapid cellular protection by GSH, leading to a decrease in its content, which can further oxidize to its reduced form, favoring its decrease. Additionally, GSH is synthesized in the liver and is then transferred to other tissues, potentially causing a decrease of this enzyme in the gills (Cheung et al. 2001, Srikanth et al. 2013, Kaur 2017). Similarly, it has been reported that GST activity is higher in the liver than in the gills, as confirmed in this study (Antunes et al. 2016). This can be attributed to the fact that the gills are in direct contact with the water, and therefore they are exposed to higher concentrations of xenobiotics than the liver. This is related to the 'no observed effect concentration' (NOEC) values of GST obtained in the gills, which correspond to 3.125 and 12.5% of the effluent at 24 and 72 h of exposure, respectively. By contrast, in the liver, values of 6.25 and 50% of the effluent at 24 and 72 h of exposure, respectively, were obtained. The NOEC values of CAT in the gills correspond to 3.125% of the effluent at 24 and 72 h and in the liver, the NOEC was 6.25% at 72 h. This indicates that the toxicity of the effluent in terms of enzymatic response is higher in the gills than in the liver, and the latter only shows a significant effect after 24 h of exposure.

ROS can directly cause DNA strand breakage, while xenobiotics have a potentiating effect on DNA molecular alterations that are susceptible to rapid repair processes (e.g. formation of photoproducts, adducts, and base modifications) (Barbosa et al. 2010). However, some authors have highlighted the potential impact of prolonged exposure to genotoxic agents on the efficacy of DNA repair mechanisms (San Juan-Reyes et al. 2015, Du et al. 2016). This assertion was substantiated in the present study with the observation of substantial damage to blood cells exposed to concentrations ranging from 12.5 to 100% of the effluent after 120 h of exposure. The highest concentration of the effluent had an effect similar to that caused by copper sulfate, which was used as a PC, causing significant DNA damage in the fish blood cells and validating the experimental design of the study. It should be noted that this compound is a potentially toxic algaecide, antifungal, and antiparasitic agent, capable of inducing ROS and interacting in the redox cycle, causing mutations and DNA damage in aquatic organisms (Oliveira et al. 2008, Chairi et al. 2010).

In Chile, fish farm effluents comprise a complex mixture of chemicals, unconsumed food, and metabolic waste.

Table 2. Temporal behavior of conductivity and Florfenicol and iodine concentrations during the summer season from 2013 to 2023. Data were extracted from Nimptsch et al. (2015) and the fish farm's Environmental Surveillance Program, prepared by Emissions Control. ND: not detected

Year	Conductivity (µS cm^{-1})		Florfenicol (mg l^{-1})		Iodine (mg l^{-1})	
	Upstream	Discharge	Upstream	Discharge	Upstream	Discharge
2013	51.5	790	ND	ND	<3	<3
2014	52.1	1070	ND	ND	<0.050	<0.050
2015	51	610	ND	ND	<3	<3
2016	54.7	2944	ND	ND	<1.0	<1.0
2017	39	87	ND	18	<3	<3
2018	53.5	62.5	<0.024	<0.024	<5	<5
2019	58.8	147	<0.024	<0.024	<5	<5
2020	53.2	126	<0.048	<0.048	<5	<5
2021	50.4	169	<0.024	<0.024	<5	<5
2022	49.4	12 9	<0.024	<0.024	<5	<5
2023	47.8	171	<0.024	<0.024	<5	<5

Identifying and quantifying the chemical constituents in these effluents is challenging; however, their quantification was achieved using a predictive exposure model (fugacity model) (Alvarado-Flores et al. 2021). Consequently, the presence and potential synergistic effects of pharmaceuticals in the effluent cannot be disregarded. These compounds have the potential to bioaccumulate in aquatic organisms depending on their physicochemical properties. Their impact on the antioxidant metabolism and genotoxicity in fish and invertebrate species has been extensively elucidated, with notable examples including amoxicillin (Orozco-Hernández et al. 2019), OTC, FLO (Botelho et al. 2015), benzalkonium chloride (Antunes et al. 2016, Lavorgna et al. 2016), formalin (Mert et al. 2015, Resendes et al. 2018), glutaraldehyde (Christen et al. 2017), emamectin benzoate (Olsvik et al. 2008), and phenol (Avilez et al. 2008) as well as the additive effect of some antimicrobials (Jerbi et al. 2011). Although OTC and FLO were not detected in this study, it is likely that these compounds underwent degradation through processes such as photolysis and hydrolysis, as documented in Xuan et al. (2010). FLO has been previously reported in fish farm effluents, along with the presence of iodine, as detailed in Table 2.

The evaluated fish farm used a mixed configuration consisting of a continuous flow-through system that expels substantial volumetric flow rates and an RAS that discharges approximately 6% of the total system volume daily, releasing a concentrated waste stream into the freshwater environment (Van Rijn 2013, Bregnballe 2022). Water treatment practices within fish farms involve the application of various flocculants and coagulants, with acrylamide and ferric chloride being the most commonly used compounds (Rishel & Ebeling 2006, Liu et al. 2013, Igwegbe & Onukwuli 2019), and the discharge of waste products from these substances is also possible. Acrylamide exhibits high solubility and mobility, facilitating its presence in the environment. It elicits genotoxic effects on aquatic organisms, enhances enzyme activity, and exerts chronic effects on invertebrates, thus posing an ecological risk to species exposed to environmentally relevant concentrations (Sotero-Santos et al. 2007, Pourrezaei et al. 2011). Additionally, acrylamide further amplifies metal concentrations in water and the toxicity of sludges (Renault et al. 2009, Xiong et al. 2018). Furthermore, ferric chloride acts as a prooxidant chemical and can provoke detrimental effects in fish, such as reproductive toxicity and neurotoxicity, involving oxidative stress mechanisms (Pereira et al. 2020). Based on these considerations, it is crucial to assess the presence of flocculants and coagulants in effluent samples for future investigations.

The detection and quantification of effluent constituents plays a crucial role in elucidating their contribution to the overall environmental impact. The continuous discharge of organic matter and fish excretion products contrasts with the intermittent release of chemical compounds, which presents challenges in terms of accurate measurement (Burridge et al. 2010, Tello et al. 2010, Antunes et al. 2016). Additionally, the absence of restrictive regulations regarding the use of chemical products, coupled with the lack of official declarations regarding industry quantities, further complicates the assessment of their ecological effects.

The results suggest that oxidative stress is one of the mechanisms underlying the genotoxic potential of the effluent. Furthermore, the results of the enzymatic activity analysis yield valuable insights into the potentially toxic effects of the effluent on *O. mykiss*. However, we recommend including the measurement of the activity of other enzymes, such as SOD or cytochrome P-450, which play a fundamental role in defense against oxidative stress. This would enhance our understanding of the physiological responses and potential risks associated with exposure to varying concentrations of fish farm effluents. Moreover, the use of rainbow trout individuals of both sexes is recommended to ensure that the results are more representative and applicable to the biological reality of this species in natural populations.

5. CONCLUSIONS

To assess the prooxidant and genotoxic potential of a fish farm effluent, we investigated the impact of different concentrations of the effluent on CAT and GST activities in liver and gill samples as well as DNA damage to blood cells of *O. mykiss*. The analysis of differences between treatments revealed that the effluent induces CAT and GST activities and DNA damage, which may be an important mechanism underlying potential chronic effects in fish. However, further research is required to explore other biochemical mechanisms to provide a comprehensive understanding of oxidative stress in aquatic organisms exposed to fish farm effluents.

The findings of this study underscore the significance of monitoring and conducting a thorough analysis of xenobiotics in aquatic environments affected by fish farm discharges, aiming to establish causality in the obtained results. Further research is needed to explore the potential biological impacts of the aqua-

culture industry on freshwater ecosystems, for which genotoxicological studies prove to be a valuable tool in assessing biological risks. These studies enable the early detection of adverse effects in aquatic organisms, and the resulting information can be used to develop effective management strategies and preserve the health of watercourses.

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