

Short Communication

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TEFLUTHRIN-INDUCED MASS MORTALITY OF FARMED RAINBOW TROUT (*ONCORHYNCHUS* *MYKISS*) IN SERBIA: A CASE STUDY

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Abstract

We present the first documented case of mass mortality in farmed rainbow trout (*Oncorhynchus mykiss*) linked to tefluthrin contamination in a freshwater stream. In June 2013, a twelve-day pollution event occurred in the Rogačica River, Republic of Serbia, resulting in the death of approximately 160,000 trout fingerlings and yearlings, with a total biomass of 4,190 kg. Water samples were analyzed for pesticide residues using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with an Agilent 1260 Infinity LC system (Agilent Technologies, USA) coupled to a 6460 Triple Quad LC/MS mass spectrometer. Water analysis revealed tefluthrin concentrations of 2.04 and 0.52 µg/L, representing 9- to 34-fold exceedances of the reported 96 h LC50 for rainbow trout (0.06 µg/L). The contamination originated from washing pesticide-coated corn seed in a local distillery, representing a previously undocumented source of aquatic pesticide pollution. This case highlights the vulnerability of aquaculture to agricultural pesticides, the particular sensitivity of early life stages of salmonids to pyrethroids, and the need for improved regulatory and biosecurity measures to prevent similar events.

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Key words: *Oncorhynchus mykiss*, aquaculture, pesticide residues, pollution, pyrethroid insecticides, tefluthrin

TEFLUTRINOM IZAZVANA MASOVNA UGINUĆA KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*) U SRBIJI: STUDIJA SLUČAJA

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Kratak sadržaj

U radu je opisan prvi dokumentovani slučaj masovnog uginuća kalifornijske pastrmke (*Oncorhynchus mykiss*) u akvakulturi, povezan sa kontaminacijom površinskih voda teflutrinom. U junu 2013. godine došlo je do dvanaestodnevnog zagađenja u reci Rogačici (Srbija), što je rezultiralo uginućem oko 160.000 jedinki mlađi i jednogodišnjaka pastrmke, ukupne biomase od 4.190 kg. Uzorci vode su analizirani na prisustvo ostataka pesticida primenom tečne hromatografije u kombinaciji sa tandemskom masenom spektrometrijom (LC-MS/MS), korišćenjem sistema Agilent 1260 Infinity LC (Agilent Technologies, SAD) povezanog sa masenim spektrometrom 6460 Triple Quad LC/MS. Analiza vode potvrdila je koncentracije teflutrina od 2,04 i 0,52 µg/L, što je od 9 do 34 puta premašilo LC50 vrednost za kalifornijsku pastrmku (0,06 µg/L). Izvor kontaminacije bilo je pranje semena kukuruza obloženog pesticidom u lokalnoj destileriji, što predstavlja do sada nedokumentovan izvor unosa pesticida u vodene ekosisteme. Ovaj slučaj ukazuje na rizik kojem je akvakultura izložena usled neadekvatne upotrebe poljoprivrednih pesticida, izuzetnu osetljivost ranih razvojnih stadijuma salmonidnih vrsta na piretroide, kao i na potrebu za

unapređenjem regulatornih i biosigurnosnih mera radi prevencije sličnih događaja.

Ključne reči: *Oncorhynchus mykiss*, akvakultura, ostaci pesticida, zagađenje, piretroidni insekticidi, teflutrín

INTRODUCTION

Aquatic ecosystems and aquaculture operations are increasingly exposed to pesticides originating from agricultural practices. Among them, pyrethroids are widely applied because of their effectiveness against insect pests, yet they pose significant risks to non-target aquatic organisms, especially fish (Haya, 1989; Ullah et al., 2019). Rainbow trout (*Oncorhynchus mykiss*), one of the most widely farmed salmonids in Europe, is particularly sensitive to water quality deterioration and pesticide contamination (Velisek et al., 2006; Besser et al., 2020).

Previous studies have demonstrated that pyrethroids disrupt fish physiology, behavior, and survival, with early life stages being particularly susceptible (Werner and Moran, 2008; Richterova and Svobodova, 2012). Although pyrethroid toxicity is well documented, field-documented cases of large-scale fish mortality directly attributable to tefluthrin remain absent. Tefluthrin, a contact and vapor-phase pyrethroid insecticide, is considered one of the most acute toxic pyrethroids for vertebrates (EFSA, 2010; Soderlund et al., 2002). Recent experimental studies have provided further insights into its mode of action and toxic potential. For example, enantioselective effects of tefluthrin in zebrafish revealed differential developmental and physiological toxicities (Wang et al., 2023), while exposure during early life stages was shown to impair cardiac and skeletal development, increase oxidative stress, and induce apoptosis (Wu et al., 2025). Furthermore, pyrethroid-associated nephrotoxicity has been reported in catfish species under controlled conditions, highlighting the broader risks of this pesticide class for aquaculture species (Stilwell et al., 2024). Despite these findings, no aquaculture poisoning incidents involving tefluthrin have been officially reported to date.

We report the first confirmed case in Serbia of widespread rainbow trout mortality associated with tefluthrin contamination. The study details the extent of the fish losses, examines water residues, identifies the source of pollution, and highlights the implications of this previously unrecognized contamination pathway for aquaculture biosecurity and environmental management.

MATERIAL AND METHODS

The Rogačica River, a tributary of the Drina River in western Serbia, supports several small- to medium-scale rainbow trout farms (Figure 1.).

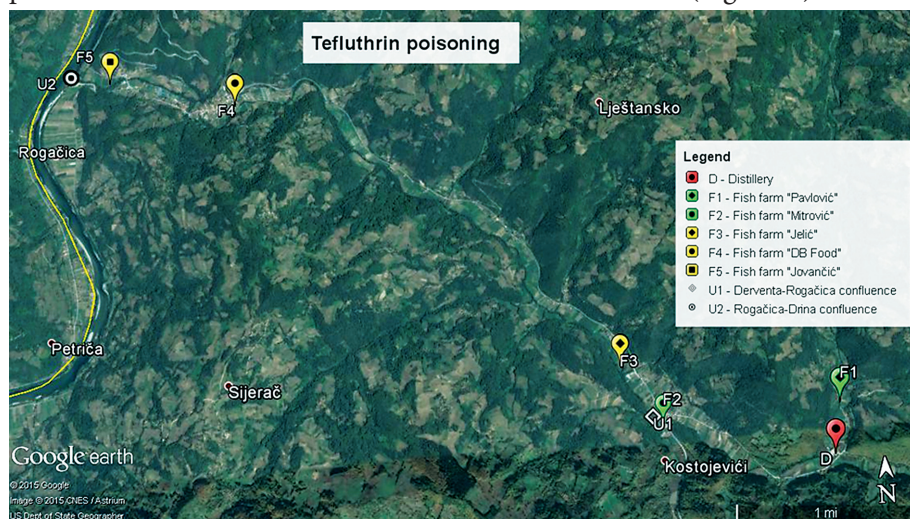


Figure 1. Location of trout farms (F1–F5) and the distillery (D) along the Rogačica River.

In June 2013, three downstream farms reported sudden fish mortality events, prompting an on-site investigation.

Mortality data were obtained through interviews with farm owners and verified by direct on-site observations. Dead fish were collected daily, weighed in bulk, and the number of individuals was estimated by dividing the total biomass by the mean body weight of representative size classes. To ensure accuracy, size-class distributions were determined from random subsamples, and estimates were cross-checked against farm records where available (Figure 2.).



Figure 2. Preparing dead fish for weighting at F4 fish farm on 21.6.2013.

Water samples were collected on 28 June 2013 from the pre-treatment precipitation facility and the initial rearing pond at the most severely affected farm (Figure 3.).



Figure 3. Water sampling location (R, S) at the F4 fish farm.

Samples were analyzed for pesticide residues using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with an Agilent 1260 Infinity LC system (Agilent Technologies, USA) coupled to a 6460 Triple Quad LC/MS mass spectrometer. Prior to analysis, samples were filtered through 0.45 µm membranes and subjected to solid-phase extraction (SPE) for pre-concentration and purification. Chromatographic separation was performed on a reverse-phase column, and compounds were detected using multiple reaction monitoring (MRM) in both positive and negative electrospray ionization modes. Quantification was achieved using external calibration with matrix-matched standards, while method validation included assessments of recovery (70 – 120%), repeatability (RSD < 20%), and limits of detection and quantification consistent with EU guidelines for pesticide residue analysis (European Commission, 2017; EFSA, 2010).

RESULTS AND DISCUSSION

The twelve-day pollution event began on 20 June 2013. At farm F4, white foam appeared on the water surface, followed by abnormal trout behavior: disorientation, surface swimming, reduced respiration, and lethargy, consistent with pyrethroid poisoning (Farag et al. 2021). Mortality occurred within 30–60 minutes, primarily affecting fingerlings (<100 g). Although some recovery was observed the following morning, repeated evening pollution episodes caused cumulative losses. Total mortality at farm F4 was 3,350 kg of rainbow trout (average 30 -100 g). Additional losses occurred at farm F3 (658 kg, mixed size classes) and farm F5 (182 kg, fry and fingerlings of 5–15 g). In total, approximately 160,000 fish (4,190 kg) died across the three farms, predominantly fry and fingerlings (Figure 4.).



Figure 4. Mortality of rainbow trout yearlings at the F4 fish farm on 21 June 2013

Necropsy of moribund and freshly dead rainbow trout revealed no significant pathomorphological changes, and no lesions indicative of infectious disease were observed, supporting chemical intoxication as the primary cause of mortality.

Water samples collected on 28 June 2013 confirmed tefluthrin residues at 2.04 µg/L (pre-treatment facility) and 0.52 µg/L (fish pool B14). These values exceeded the reported 96 h LC50 for rainbow trout (0.06 µg/L) by 34- and 9-fold, respectively (EFSA, 2010). Other pesticides (metalaxyl, metiram, imidacloprid, thiamethoxam) were detected but at concentrations well below their LC50 values, making tefluthrin the most likely cause of mortality (Table 1.).

Table 1. Results of water analysis from the Rogačica River in Serbia

Common name	Class of Pesticide	Purpose	LC50 value for rainbow trout	Water Analysis
Tefluthrin	Pyrethroid	Insecticide	0.06 µg/L (96 h; static)	pre-treatment: 2.04 µg/L B14: 0.52 µg/L
Carbofuran	Carbamate	Insecticide	0.3625 mg/L (96 h; semi-static)	Not detected
Thiram	Dithiocarbamate	Fungicide	0.046 mg/L (96 h)	Not detected
Metiram	Dithiocarbamate	Fungicide	0.33 mg/L (formulation; 96 h)	pre-treatment: 0.01 µg/L B14: 0
Metalaxyl	Phenylamide	Fungicide	> 100 mg/L (96 h)	pre-treatment: 0.02 µg/L B14: 0.01 µg/L
Imidacloprid	Neonicotinoid	Insecticide	211 mg/L (96 h)	pre-treatment: 0.04 µg/L B14: 0.09 µg/L
Clothianidin	Neonicotinoid	Insecticide	> 104.2 mg/L (96 h)	Not detected
Thiamethoxam	Neonicotinoid	Insecticide	> 125 mg/L (96 h)	pre-treatment: not detected B14: 0.02 µg/L

The official investigation identified the pollution source as a distillery upstream of the affected farms. Seed corn coated with tefluthrin had been washed prior to ethanol production, releasing contaminated wastewater directly into

the river. This contamination pathway is unprecedented; although pesticide runoff from fields is well known, this study provides the first evidence that industrial seed washing can directly cause mass fish mortality (Weston and Lydy, 2010; Méjanelle et al. 2020).

The observed mortality pattern, characterized by rapid onset, behavioral abnormalities, and a disproportionate impact on young trout, aligns with the established toxicological profile of pyrethroids, which affect the nervous system and are especially detrimental to early life stages (Ullah et al., 2019; Werner and Moran, 2008; Richterova and Svobodova, 2012). The disproportionate impact on fry and fingerlings supports previous laboratory findings showing that early life stages of rainbow trout are especially sensitive to pyrethroids (Haya, 1989). The loss of over 160,000 fish in less than two weeks highlights the economic and ecological risks of pesticide mismanagement. For farmers, such events result in severe financial damage, while for aquatic ecosystems, they emphasize the vulnerability of aquaculture operations located on small streams affected by upstream industrial or agricultural activities (Varela et al., 2023).

Pyrethroids are recognized for their persistence and potential to bioaccumulate. Even at sublethal levels, they can disrupt fish physiology and compromise ecosystem health (Hartz et al., 2019; Ullah et al., 2019). The exceptionally high concentrations observed in this case underscore the heightened vulnerability of aquaculture operations in small riverine systems to sudden contamination events. From a regulatory standpoint, this case highlights the need for stricter oversight of pesticide-treated seed handling. Industrial seed washing should be recognized as a high-risk activity. Recommended preventive measures include mandatory wastewater treatment at seed-processing facilities, regular monitoring of aquaculture water supplies in regions with intensive pesticide use, and incorporation of pesticide risk assessments into aquaculture biosecurity frameworks (Burch et al., 2025; Carvalho, 2017).

From the perspective of aquaculture operations, such events are situated within the broader framework of risks and uncertainties. A clear conceptual distinction between these terms is essential. Risk denotes situations in which the distribution of potential outcomes is either known a priori or can be statistically inferred from prior experience. Uncertainty, by contrast, arises when the probabilities of outcomes cannot be quantified (Knight, 1921). Importantly, risk assessments are often subjective, as probability estimates depend on the evaluator knowledge and perception (Hardaker, 2016). The incident under consideration could, in principle, be classified as a production risk. However, the poisoning was triggered by the presence of a substance that, under normal production conditions, would not be expected in corn used for alcohol manu-

facturing. This atypical causative factor indicates that the event could not have been reasonably foreseen. Consequently, it is more appropriately characterized as an uncertainty in trout production. Recognizing such distinctions is critical, as the management of uncertainty requires adaptive strategies that differ fundamentally from conventional risk-mitigation approaches in aquaculture biosecurity and farm management.

CONCLUSION

This study provides the first documented evidence of mass mortality of farmed rainbow trout caused by tefluthrin contamination. The incident resulted from washing pesticide-coated corn seed in a distillery, introducing toxic residues into a stream supplying multiple fish farms. Tefluthrin concentrations exceeded the LC_{50} for rainbow trout by more than an order of magnitude, causing the loss of approximately 160,000 fish, predominantly fingerlings. The findings highlight three critical points: (i) rainbow trout early life stages are highly susceptible to pyrethroids, (ii) unconventional agricultural practices such as seed washing can serve as acute source of water pollution, and (iii) aquaculture remains highly vulnerable to external environmental hazards despite internal biosecurity measures. To prevent similar incidents, strict regulations on pesticide-treated seed disposal, routine monitoring of aquaculture water supplies, and improved integration of environmental toxicology into aquaculture management are needed.

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Author's Contribution

SČ, PS, DB and VR made contributions to conception and design of the study, involved in data collection and drafting the manuscript. VR revised the manuscript critically and together with SČ prepared the final draft of the manuscript. All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

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