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The modulating role of salinity in herbicide toxicity to the marine nematode *Litoditis marina*

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Abstract
Estuarine ecosystems are highly dynamic, characterized by frequent fluctuations in salinity. These environments are also primary recipients of pesticide-laden runoff, such as those from the triazine group, which pose significant ecological risks. However, the interactive effects of these chemical and physical stressors are not yet fully understood. This study investigated the effects of salinity on atrazine toxicity. We used the estuarine nematode *Litoditis marina*, an ideal bioindicator species, in a fully crossed experiment. Mortality was assessed for 120 h across a range of salinity levels (15, 20, 25, and 30) and atrazine concentrations (0.2–20 mg/L). Atrazine lethality was significantly influenced by the exposure duration, salinity, and their interactions. Toxicity is inversely related to the salinity. Specifically, the LC_{50} at a salinity of 15 was 0.66 ± 0.01 mg/L, which is 10.4 times lower (i.e., more toxic) than the LC_{50} at a salinity of 30 (6.88 ± 0.64 mg/L). A similar pattern was observed for LC_{20}, which was 9.2 times lower at a salinity of 15 compared with that at 30. Reduced salinity dramatically increased atrazine toxicity in *L. marina*. These findings suggest that estuarine organisms are particularly vulnerable to pesticide pollution because inherent salinity fluctuations in their environment can intensify the toxic effects of contaminants. This highlights the importance of considering environmental variables in ecological risk assessment.

Keywords Atrazine · Ecotoxicology · Lethal concentration · Marine benthos · Mortality · Triazine

1 Introduction

Agricultural practices across the Americas (e.g., United States, Brazil, and Argentina) have led to extensive pesticide use, resulting in environmental pollution and adverse effects on organisms at different trophic levels [1, 2]. Agriculture is also a major cause of aquatic pollution, as numerous chemical products enter aquatic environments through runoff and leaching [3]. Among the most important agricultural pollutants are pesticides and fertilizer residues [4]. Pesticides belonging to the triazines group are used extensively on a large scale in many countries [5, 6]. Triazine herbicides are frequently sprayed on the leaves of sorghum, corn, coffee, cocoa, and sugarcane to control weeds [7, 8]. They can reach the soil

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through direct application, irrigation, and rainfall, ultimately contaminating rivers, seas, and estuaries and causing toxic effects at different trophic levels [8–10]. As lipophilic pesticides, triazine herbicides such as atrazine can bioaccumulate in fat-rich tissues, such as adipose tissue, thereby adversely affecting the health of the target organism [11].

Atrazine is characterized by a relatively low soil adsorption coefficient (mean Koc of 126.9 L/kg) [12], which facilitates its movement into groundwater through leaching. Additionally, it poses a significant risk of surface runoff, leading to contamination of freshwater, estuarine, and marine environments. Furthermore, its low solubility in water (33 mg/L at 25 °C) facilitates its transport into estuaries and bay areas via runoff [13, 14]. Highly biodiverse ecosystems such as estuaries are particularly vulnerable to this contaminant [15]. Atrazine, along with triazine pesticides, is still commonly found in water bodies, even in regions where its use has been banned for years, owing to its high persistence in the environment, particularly in sediments [12, 16]. As a result, organisms living in sediments may experience prolonged exposure to varying concentrations of atrazine [17].

The toxicity of a chemical compound depends on factors such as exposure time, concentration, organism susceptibility, chemical characteristics of the agent, and environmental abiotic factors such as salinity, temperature, pH, and dissolved oxygen content in water [18, 19]. Salinity is an abiotic factor that can alter the toxicity of contaminants to organisms. A study using nauplii of the copepod *Eurytemora affinis* as a model organism showed that triazine toxicity increased with decreasing salinity. After 96 h, the LC_{50} values were 0.5, 2.6, and 13.2 mg/L at salinities of 5, 15, and 25, respectively [20]. However, as planktonic organisms, their response may not reflect that of sediment-dwelling invertebrates, which have different exposure routes and physiological adaptations. Therefore, assessing the interactive effects of atrazine and salinity on a relevant benthic species is crucial to understand the real ecological risks in estuaries.

Various animals are used as model organisms for environmental pollution assessments, among which nematodes are particularly suitable due to their ease of laboratory cultivation, high abundance, diversity, and short generation times [21, 22]. The cosmopolitan nematode genus *Litoditis* is widely used in ecotoxicological laboratory experiments because it is easy to culture and handle and is sensitive to various contaminants [23, 24]. The Rhabditidae family, which includes *Litoditis marina* [25, 26] (Nematoda: Rhabditida), also contains *Caenorhabditis elegans*, which is considered the most commonly used metazoan model organism in modern experimental science [27]. The free-living nematode species complex *L. marina* consists of at least ten identified cryptic species [28], three of which (PmI, PmII, and PmIII) are frequently found in European coastal environments and marine algae deposits along the Belgian and Dutch coastlines [28, 29]. Its prevalence in estuarine sediments makes it an ecologically relevant model for studying the effects of contaminants that accumulate in this compartment.

Litoditis marina is a useful model for testing salinity-pollutant interactions because it inhabits estuarine regions characterized by variations in salinity due to tides, rainfall, ocean currents, evaporation, and climate [30–32]. The cryptic species *L. marina* PmII is particularly well-suited for this purpose due to its proven osmoregulatory ability [33]. While previous studies have indeed established that salinity can modulate the toxicity of various pollutants, including atrazine, in some aquatic organisms like copepods [20], a significant knowledge gap persists. Specifically, there is a lack of data investigating this interaction in benthic meiofauna, particularly in nematodes. As key players in nutrient cycling and as a foundational link in the benthic food web, nematodes are a valuable benthic component to use in investigating the ecotoxicology of estuarine systems. This is particularly problematic, given that triazine-based herbicides are known to accumulate in the sediments where these benthic organisms reside [12, 16]. The absence of specific toxicity data for nematodes under varying salinity conditions limits the accuracy of ecological risk assessments for these vital transitional ecosystems. Therefore, this study was designed to directly address this gap by providing a detailed assessment of how environmentally relevant variations in salinity modulate the lethal effects of atrazine on a representative estuarine nematode model species. We hypothesized that salinity would act as a mitigating factor, with higher salinity buffering the toxic effects of atrazine.

2 Materials and methods

2.1 Nematode culture

Laboratory stock cultures of *Litoditis marina* PmII were established from specimens collected from decomposing macroalgae off the Belgian coast near Blankenberge (51°19′14.0″ N, 3°08′22.7″ E). Initial cultures were xenic. The culture medium consisted of a mixture of Bacto-agar and nutrient agar (4 g Bacto-agar and 1 g nutrient agar (Difco™, BD, USA); B/N ratio of 4:1) at a final concentration of 1% prepared in artificial seawater (ASW) in a volume of 1 L with a salinity of 25

[34]. Salinity is reported throughout this manuscript as a dimensionless value on the Practical Salinity Scale of 1978 (PSS-78). The pH of the agar was kept between 7.5 and 8.0 using TRIS–HCl at a final concentration of 0.005 mol/L. After that, the nematode subcultures were prepared using agar medium, which was autoclaved to avoid contamination by fungi or bacteria. Liquid agar (12 mL) was poured into each polystyrene Petri dish (90 × 15 mm) achieving a level distribution of the medium. Once the agar had cooled down at room temperature, the nematodes were inoculated on the plates and a bacterial suspension (50 µL) of *Escherichia coli* K12 with a cell density of 3×10^9 cells/mL was added to the culture medium as food for the nematodes [35]. Finally, each Petri dish was sealed with ParaFilm and stored in an incubator at 18 ± 1 °C in the dark, following standard protocols for rhabditid nematodes (ISO 10872) to avoid phototoxicity as well as behavioral and physiological stress. Subcultures were prepared every 15 days by transferring five males and five females (adults) to each new Petri dish.

2.2 Preparation of triazine concentrations

In our toxicity tests, herbitrin 500 BR (ADAMA Br S/A) was used, which contains 500,000 mg/L of atrazine, which belongs to the triazine group, as the active ingredient. Serial dilutions were carried out in Falcon tubes, from which 0.1 mL of the original herbitrin solution was diluted in 49.9 mL of distilled water to prepare a stock concentration of 1,000 mg/L atrazine. After dilutions, the final atrazine concentrations in the Petri dishes for the lethality test ranged from 0.2 to 20 mg/L. These concentration ranges have also been investigated in other impact studies focusing on invertebrate model species [17, 24].

The correlation between the real and nominal concentrations of atrazine in water is very high, reaching 98% [36] to 99% [37]. This correlation consistently exceeded 90% across different salinities (up to 35) and triazine concentrations (up to 500 mg/L) within a 24-h period [38]. Seawater did not appear to reduce the solubility of atrazine, which is further supported by [39]. This pesticide is quite persistent, implying that its concentration decreases slowly over time, following a slow linear decline [40, 41], with a half-life exceeding a month [41], largely independent of salinity.

2.3 Assessing atrazine's lethal effects

We exposed the nematode *Litoditis marina* PmII to atrazine at different salinities using a fully crossed experimental design. We used atrazine final concentrations of 0.2, 1.0, 2.0, 10.0, and 20.0 mg/L. These levels were selected based on two criteria: (1) preliminary range-finding experiments to ensure they would span the full dose–response curve from no-effect to 100% mortality under the tested conditions, and (2) a review of previously published studies with other marine invertebrates to ensure environmental relevance and comparability [42].

The experiment was carried out in Petri dishes (60 × 15 mm) containing Bacto-agar (at a concentration of 1%), a standard methodology for ecotoxicological assays with benthic nematodes that simulates exposure via sediment pore water and ingestion [34], with buffered pH values (7.5–8.0) matching those of the stock culture medium of *L. marina*. After sterilizing Bacto-agar, a cholesterol solution (100 µg/L) was added, as nematodes cannot synthesize sterols when their only food source is bacteria [43]. For control treatments, the culture medium in the experimental plates consisted of 5 mL of sterile 1% Bacto-agar. In treatments combining the herbicide with different salinities, 0.1 mL of the respective atrazine solutions (Sect. 2.2) were thoroughly mixed with 4.9 mL of Bacto-agar in the experimental plates. Finally, each Petri dish was sealed with ParaFilm and stored in an incubator at 18 ± 1 °C in the dark, following standard protocols for rhabditid nematodes (ISO 10872), to avoid phototoxicity as well as behavioral and physiological stress. The different salinity values tested in this experiment (15, 20, 25, and 30) were obtained by preparing a series of dilutions of ASW with distilled water before Bacto-agar preparation. The salinity of 25 was selected as the control condition for the main experiment, as this is the standard salinity at which the nematode stock cultures are maintained.

Four replicates were prepared for each atrazine versus salinity treatment and control (only salinity variation without addition of atrazine). When the agar had solidified, the nematodes were inoculated; *E. coli* K12 was added to the plates as food to prevent mortality from starvation. Although it is uncommon to add food to lethality tests, nematodes primarily absorb pollutants through ingestion and through their cuticle [44, 45]. Ingestion in nematodes often requires a stimulus in the form of suitable food particles, which is why we chose to add food to the lethal-effect assays to ensure oral uptake would take place [46], which would also correspond with the natural situation of an environment in which food resources are available in addition to the exposure to the toxin.

Mobile adults (50 ± 2 individuals) of *L. marina* PmII were randomly collected from stock cultures, washed once in ASW with the respective salinities (15, 20, 25, and 30), and inoculated onto experimental plates. Live and dead

nematodes were quantified every 24 h for 5 days under a stereomicroscope. Immobile individuals viewed without peristaltic movement or pulsation of the pharyngeal butterfly valve were considered dead. An experimental duration of 5 days (120 h) was chosen to observe mortality during the gestational period, because this duration is the maximum experimental time possible before the F1 generation reaches adulthood, which would confound the mortality count of the initial cohort. Under the assay conditions in this experiment, *L. marina* PmII had a minimum development time from the appearance of first-stage juveniles to adults of approximately 5.8 d [33]. Therefore, there was no overlap of generations.

It should be noted that a formal positive control with a reference toxicant was not run concurrently with this specific experiment. However, the *Litoditis marina* cultures used in our study are maintained in a continuous testing program and are routinely assayed with other reference toxicants, consistently demonstrating predictable sensitivity. Based on this ongoing internal quality assurance, the clear, dose-dependent response to atrazine, and the low control mortality, we are confident about the validity of the results presented.

2.4 Data analysis

The concentration of atrazine that caused 20% and 50% lethality (LC_{20} and LC_{50}, respectively) after 120 h of experimentation was estimated following standard probit analysis methods [47, 48]. First, mortality values were corrected for control mortality using Abbott's formula [49]: Mc (%) = (%Mo – %Mt) × 100/(100 – %Mt), where Mc is the corrected mortality, Mo is the observed mortality in the treatment group, and Mt is the mortality in the control group. Subsequently, probit-transformed mortality values were regressed against the Log_{10} of atrazine concentrations. These calculations allowed descriptive estimates of the lethal concentration endpoints. The LC_{20} and LC_{50} values presented in the Results section represent the mean values (± standard deviation) of the experimental replicates.

To test for significant effects of salinity, exposure time, and their interaction on nematode mortality, a non-parametric permutational analysis of variance (PERMANOVA) was conducted using the PRIMER 6 software with the PERMANOVA + add-on. The analysis was based on a Euclidean distance matrix of the univariate mortality data. This approach provides a robust, non-parametric equivalent of a traditional ANOVA, making it ideal for ecological data that may not meet parametric assumptions, and is a valid method for analyzing univariate datasets [50]. The experimental design was modeled with 'Salinity' and 'Time' as fixed crossed factors, and 'Replicate' as a random factor nested within the interaction of Salinity and Time to account for the repeated-measures nature of the data. All *p*-values were obtained from 9,999 permutations; for terms with only few possible unique permutations, Monte Carlo *p*-values were used.

To visualize and predict the relationship between salinity, exposure time, and lethal concentrations, an exponential regression growth model was fitted to the LC_{20} and LC_{50} datasets for each time interval (24, 48, 72, 96, and 120 h) using Python (version 3.12.5). The regression plots illustrate the predicted toxicity values and their 95% confidence intervals across a continuous salinity range. Additionally, heatmap analysis was conducted to provide a comprehensive visualization of the LC_{20} and LC_{50} values as a function of both salinity and exposure time, allowing for a direct comparison of toxicity thresholds across all experimental conditions.

3 Results

3.1 Mortality in response to salinity treatments only

This section corresponds to the control test of the experiment, in which atrazine was not applied. Variation in salinity alone did not cause significant mortality in adult nematodes (Table 1) (Pseudo-F > 2.35; $p > 0.09$, Supplementary material 1). There was also no significant interaction effect between Time and Salinity (Pseudo-F > 5.88; $p > 0.99$) on nematode mortality. In the first two days of exposure (24 h and 48 h), there was no nematode mortality in any of these control treatments. However, from the third day (72 h) onwards, mortality began to occur in all treatments, with mean mortality values of 1% to 2% at 72 h. By the fifth day (120 h) of exposure, mean nematode mortality values were between 6% and 7.5%. Significant differences were observed among times in the last three days of the experiment (Supplementary material 2).

Table 1 Nematode mortality as a function of salinity and time, expressed as numbers of dead individuals, mean values ± standard deviation of four replicates per treatment, after exposure of *Litoditis marina* to different salinities

Salinity	24 h	48 h	72 h	96 h	120 h
15	0	0	1.00 ± 0.81	2.00 ± 0.81	3.50 ± 0.57
20	0	0	0.50 ± 0.57	1.50 ± 0.57	3.00 ± 0.81
25	0	0	1.00 ± 0.81	2.25 ± 0.95	3.75 ± 0.50
30	0	0	0.50 ± 0.57	1.75 ± 0.50	3.25 ± 0.50

3.2 Mortality when exposed to atrazine at different salinities

Mortality increased gradually over time for all salinity and pesticide combinations. At each salinity level, mortality also increased with increasing herbicide concentration at all sampling times (Fig. 1A, B, C, and D). At the end of the experiment, the lowest atrazine concentration (0.2 mg/L) reached a mortality of 38.5% at the lowest salinity (15)

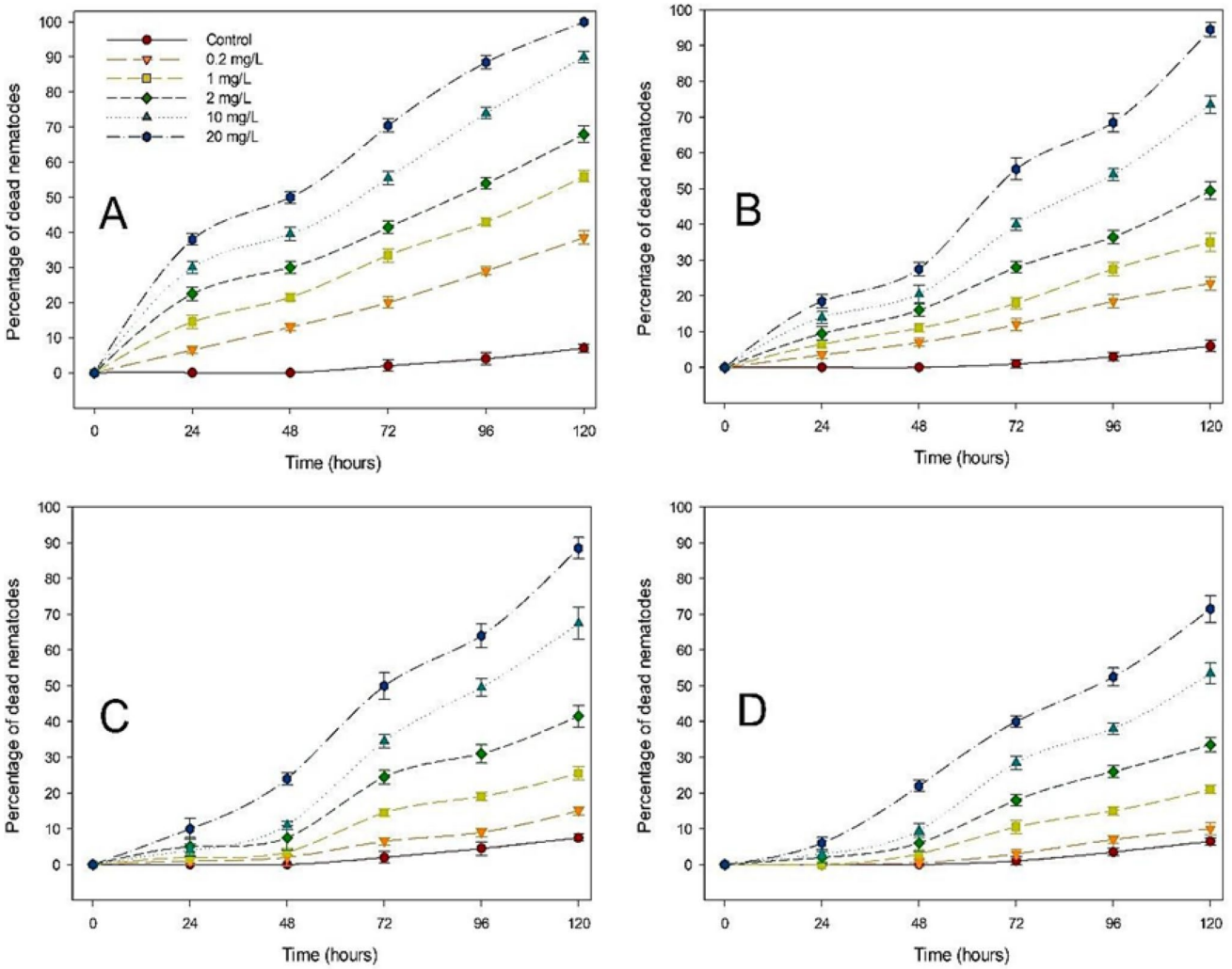

Fig. 1 Mortality of *Litoditis marina* (mean values ± standard deviation of four replicates per treatment) as a function of exposure time to five concentrations of atrazine and to the control (these expressed in the graph by the different colors) at different salinities: **A** 15; **B** 20; **C** 25 and **D** 30

(Fig. 1A), whereas mortality at the same atrazine concentration was less than 24% at all other salinities (p (MC) < 0.05) (Fig. 1B, C, and D) (see Supplementary materials 3, 4, 5, 6, and 7).

Mortality at the lowest atrazine concentration was 1.63, 2.56, and 3.85 times higher at a salinity of 15 than at salinities of 20, 25, and 30, respectively. At the highest concentration of atrazine (20 mg/L), the mortality at the end of the experiment also differed significantly among salinities (p (MC) < 0.05), with 100%, 94.5%, 88.5%, and 71.5% mortality at salinities of 15, 20, 25, and 30, respectively. Thus, the mortality values decreased with increasing salinity. Therefore, at salinity 15, our highest herbicide concentration caused 1.05, 1.12, and 1.39 times more nematode mortality than at salinities of 20, 25, and 30, respectively (p (MC) ≤ 0.003, Supplementary material 7).

3.3 Lethal effect concentrations of atrazine (LC_{20}, LC_{50}) at different salinities and exposure times

The lethal effect concentrations (LC_{20} and LC_{50}) of atrazine were significantly dependent on exposure time (Pseudo-F > 715; $p \leq 0.001$), on salinity (Pseudo-F > 246; $p \leq 0.001$), and on the interaction of these two factors (Pseudo-F > 51.79; $p \leq 0.001$) (Supplementary materials 8 and 9). LC_{20} also decreased significantly with decreasing salinity, regardless of exposure time (p (MC) = 0.0001) (Fig. 2). There were no significant differences between salinities of 25 and 30 at 48 h (p (MC) > 0.7) and 72 h (p (MC) > 0.05) of exposure (Fig. 2; Supplementary material 10).

LC_{20} and LC_{50} values decreased with increasing exposure time at all salinity levels. For instance, at a salinity of 15, the LC_{20} on the first day of exposure (24 h) was 2.00 ± 0.27 mg/L, but this value dropped significantly by > 2.5, > 5, > 7, and > 13-fold after 48, 72, 96, and 120 h, respectively (p (MC) ≤ 0.0003). Much the same response was observed for other salinities (p (MC) = 0.0001), except for a salinity of 25, where no significant difference was found between the LC_{20} values at 72 and 96 h (p (MC) > 0.1) (Fig. 2; Supplementary material 11).

Similar trends were observed for LC_{50} values (Fig. 3; Supplementary materials 12 and 13).

Our mathematical model demonstrated that at salinity levels up to 35, the LC_{20} for atrazine increased, exceeding 50 mg/L after 24 h of exposure in the nematode *Litoditis marina* PmII (Fig. 4). This trend was consistently observed across various time points, although the rates of increase differed. Notably, the slope of the mathematical fitting curve of the model decreased with increasing time, as shown in Fig. 4. The model demonstrates that the LC_{20} of the herbicide consistently decreases with prolonged exposure. This trend indicates that the toxic impact of atrazine becomes progressively more significant as the exposure duration increases.

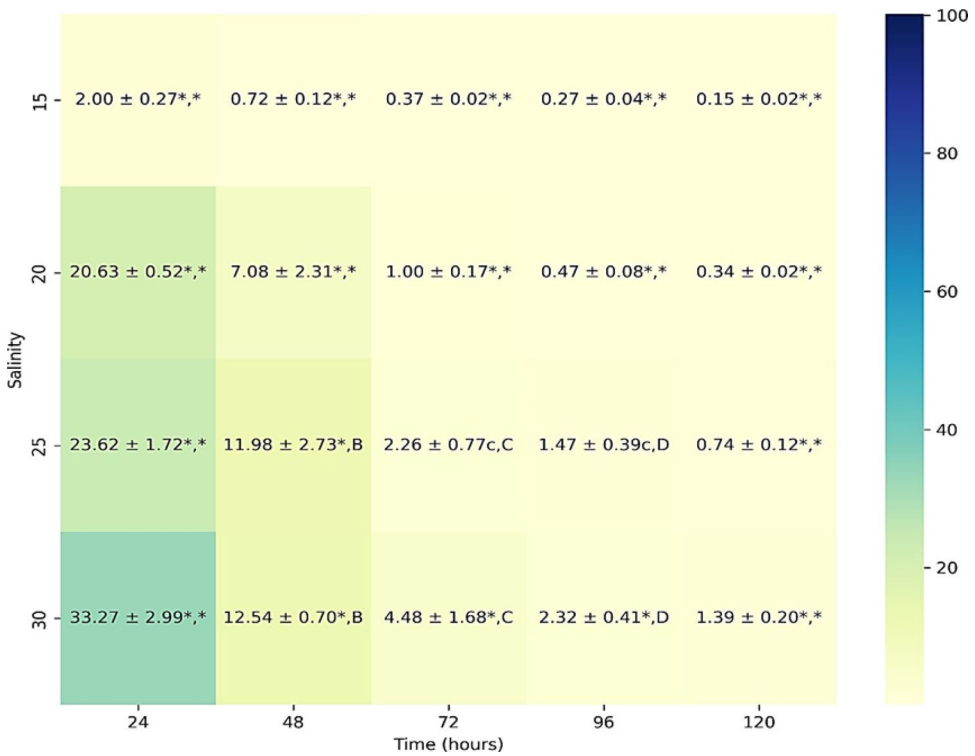

Fig. 2 Lethal concentration values of the herbicide atrazine that caused 20% mortality with color scale (LC_{20}, measured in mg/L) in *Litoditis marina* are presented as mean values ± standard deviations, based on four replicates per treatment. Significant differences as determined by the PERMANOVA main factor tests were indicated using lowercase letters for Salinity and uppercase letters for Time. An asterisk denotes a significant difference at $p < 0.05$ from all other treatments. Shared letters indicate no significant differences in comparisons among those factors with the same letter

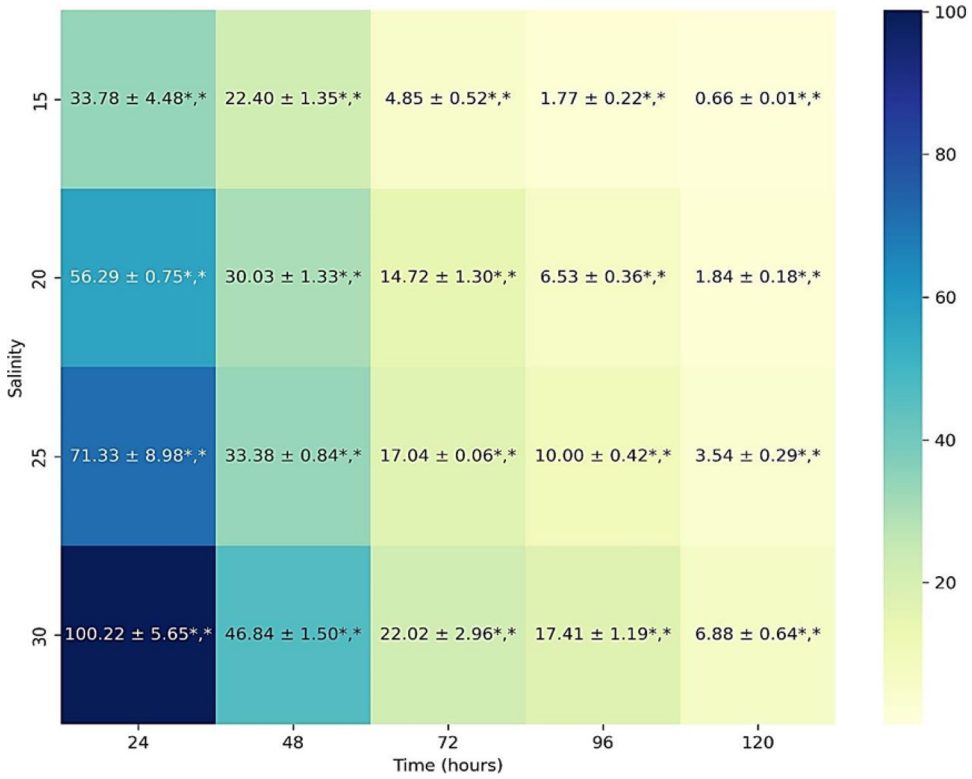

Fig. 3 Lethal concentration values of the herbicide atrazine that resulted in 50% mortality with color scale (LC_{50}, measured in mg/L) in *Litoditis marina* are presented as mean values ± standard deviations, based on four replicates per treatment. Significant differences as determined by the PERMANOVA main factor tests were indicated using lowercase letters for Salinity and uppercase letters for Time. An asterisk denotes a significant difference at $p < 0.05$. No shared letters were observed, as significant differences occurred among all factors

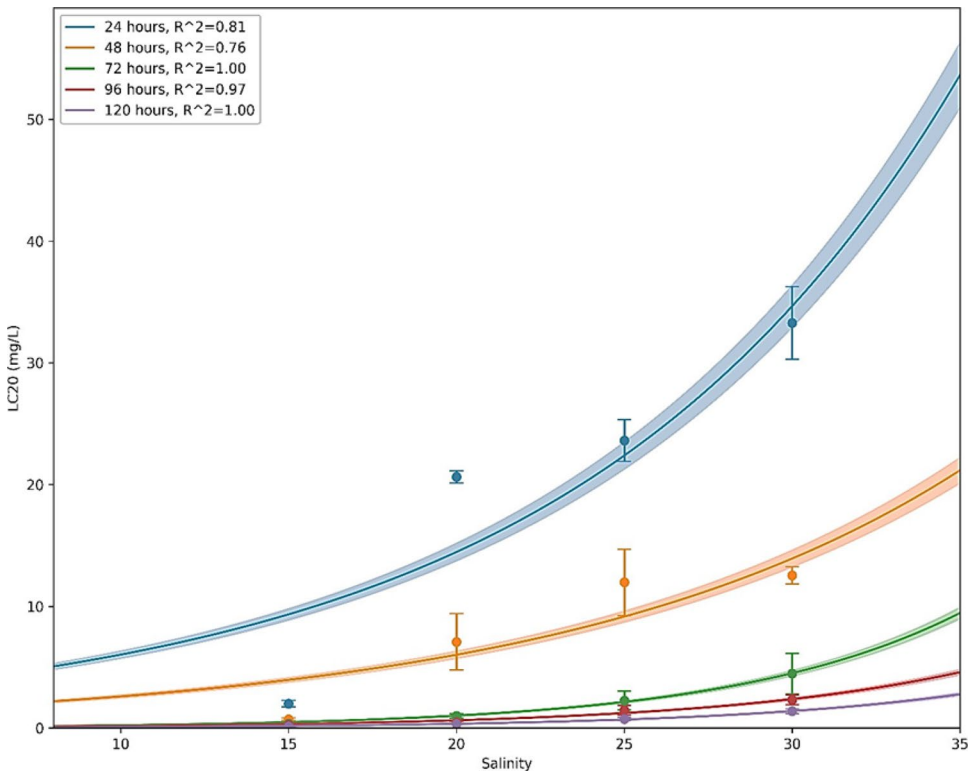

Fig. 4 Regression model demonstrating the lethal concentrations of the herbicide atrazine that caused mortality in 20% of the population (LC_{20}– mg/L) of *Litoditis marina*, across salinities ranging from 8 to 35, with exposure times from 24 to 120 h. The R^2 value indicates the goodness of fit for each regression line, while the points with error bars represent the standard deviations of the measurements. The shaded areas around the curves denote the confidence intervals of the fitted model

The exponential regression model showed that the LC_{50} for atrazine exceeded 140 mg/L after 24 h of exposure to a salinity of 35 (Fig. 5). The same arrangement was observed across various time points, although the rates of increase differed. Notably, the slope of the exponential fitting curve decreased over time (see Fig. 5 for details). These results were

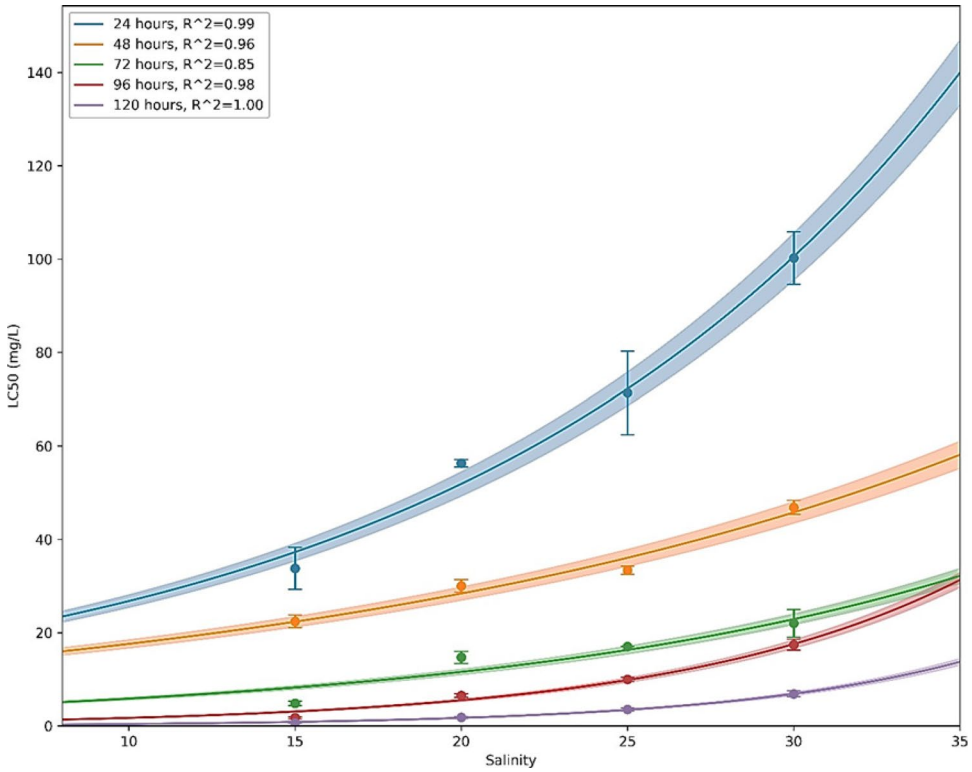

Fig. 5 Regression model demonstrating the lethal concentrations of the herbicide atrazine that caused mortality in 50% of the population (LC_{50}–mg/L) of *Litoditis marina*, across salinities ranging from 8 to 35, with exposure times from 24 to 120 h. The R^2 value indicates the goodness of fit for each regression line, while the points with error bars represent the standard deviations of the measurements. The shaded areas around the curves denote the confidence intervals of the fitted model

similar to our LC_{20} data, indicating that the greatest effects were observed at shorter exposure times. As a result, the LC_{50} of the pesticides decreased over the extended exposure periods.

4 Discussion

The present study suggests a clear antagonistic interaction between salinity and atrazine toxicity in the estuarine nematode *Litoditis marina*. Our central finding is that atrazine's toxic effect is inversely proportional to salinity, with the 120 h LC_{50} at a salinity of 15 being 10.4-fold lower than at a salinity of 30. This indicates that estuarine organisms face a heightened risk from pesticide exposure during periods of freshwater influx and in more upstream reaches, a critical factor for refining ecological risk assessments in transitional water bodies. To contextualize these findings, the lethal concentrations observed (e.g., 120 h LC_{50} ranging from 0.66 to 6.88 mg/L) are indeed higher than the chronic, average environmental concentrations of atrazine typically found in aquatic systems [51]. However, it is crucial to note that peak concentrations in agricultural runoff following application events can be orders of magnitude higher, occasionally entering the low mg/L range during acute discharge events [52]. Crucially, our results support the hypothesis that the 'safety margin' between environmental and lethal concentrations is not static but dynamically narrows under low-salinity conditions. Furthermore, atrazine's finding in estuarine sediments [53] can affect benthic organisms like *L. marina*, making the interaction with fluctuating salinity a critical and potentially underestimated risk factor in ecotoxicological models.

Salinity fluctuations in estuarine environments, which intensify owing to climate change, can alter the toxicity of aquatic pollutants [54, 55]. Factors such as exposure duration, acclimatization, species physiology, life stage, and specific pesticide characteristics influence how salinity alters pesticide toxicity [54]. Chemically, higher ionic concentrations can increase the octanol/water partition coefficient and decrease the solubility of the compounds in water. Biologically, different salinities alter organism physiology, emphasizing the need to understand the influence of salinity on contaminant toxicity for risk assessments [56, 57].

Studies have shown that changes in salinity do not have a major impact on the degradation of atrazine [38, 58], and its bioavailability is also not expected to be significantly affected by salinity [59]. Experiments by [60] demonstrated no significant loss of atrazine after 128 d at different concentrations and salinities. This suggests that the observed differences

in toxicity to organisms are more likely influenced by molecular associations or physiological absorption, rather than by changes in the pesticide's bioavailability [61].

Consistent with our findings, previous acute toxicity tests suggested a similar pattern of increased atrazine toxicity at lower salinities [20]. For instance, increasing concentrations of atrazine led to higher mortality rates in adult shrimp of the species *Metapenaeus affinis* at a low salinity of 4 [62]. Similarly, a study of *Cassiopea sp*. (Scyphozoa) juveniles exposed to atrazine under varying salinity levels indicated higher mortality at increased atrazine concentrations and lower salinities [63]. The results from our study with *Litoditis marina* (Fig. 1) corroborate these findings and extend this principle to benthic meiofauna, showing higher mortality at lower salinities (Fig. 1A) and lower mortality at higher salinities (Fig. 1D) while being exposed to atrazine. It is noteworthy that treatments involving salinity variation without atrazine did not result in significant mortality in *L. marina* adults (Table 1), aligning with findings of [30, 31], and with earlier observations that salinity variations within a broad meso to polyhaline range do not substantially affect the life history of this species [33].

Several mechanisms may explain this inverse relationship between salinity and toxicity. Salinity is known to influence the solubility of organic chemicals in marine ecosystems [39]. However, limited data exist on how salinity affects chemicals such as atrazine. One possibility is a physicochemical 'salting-out' effect, where the lipophilic atrazine becomes less soluble at higher salinities [11, 64, 65]. This could increase its partitioning into the lipids of the nematodes. Alternatively, and perhaps more importantly, lower salinity imposes osmotic stress on estuarine organisms, forcing them to expend energy on osmoregulation. At a biochemical level, this increased susceptibility under osmotic stress may be linked to an energetic trade-off. Maintaining osmotic balance in hyposaline water requires significant energy expenditure. This diversion of metabolic resources to osmoregulation could compromise the efficiency of cellular detoxification pathways, such as those mediated by cytochrome P450 monooxygenases and glutathione S-transferases (GSTs), which are essential for metabolizing xenobiotics like atrazine. A reduced capacity for detoxification would lead to higher intracellular accumulation of the parent compound and its toxic metabolites, thereby exacerbating cellular damage and increasing mortality.

Several studies have highlighted the lethal effects of atrazine on aquatic species, which vary with the concentration and exposure time [24, 66]. Copepods exposed to atrazine at a salinity of 30 exhibited an LC_{20} (96 h) of 2.65 mg/L for *Robertsonia propinqua* and 10.6 mg/L for *Quinquelaophonte sp*. [67]. In comparison, *Litoditis marina* appears to be more sensitive to atrazine (Fig. 2), which was unexpected because copepods are generally considered more sensitive to pollution and environmental disturbances than nematodes [68, 69]. *Litoditis marina* PmII in the present study was more sensitive to atrazine during the first 48 h of exposure than *L. marina* PmIII, which had a 48-h LC_{50} of 72.30 mg/L at a salinity of 25 [24]. In the same experiment conducted by [24], the LC_{50} values of atrazine in *L. marina* PmIII depended on exposure time. This was also observed in the present study, where at different salinities, the LC_{20} and LC_{50} values of the herbicide atrazine in *L. marina* PmII were time dependent (Figs. 2 and 3). This result was further evidenced by the continuous reduction in the slope over time in the regression models (Figs. 4 and 5). These regressions provided a good prediction of the LC_{20} and LC_{50} values in the marine range, as they have very high R^2 values.

Salinity plays a crucial role in the toxicity of various chemicals because of the factors related to chemical bioavailability and biological and physiological responses [70]. In a study by [20] assessing atrazine toxicity under different salinities in the copepod *Eurytemora affinis* nauplii, LC_{50} (96 h) values were 0.5 mg/L, 2.6 mg/L, and 13.2 mg/L at salinities of 5, 15, and 25, respectively. This greater lethality at lower salinities corroborates the findings of the current study, where at the end of the experiment (120 h), the LC_{50} of the herbicide atrazine for adult nematodes of *Litoditis marina* PmII, at salinity 15 was 10.4 times lower than the LC_{50} at salinity 30 (Fig. 3). These findings collectively suggest that high-salinity environments, such as seas, may help mitigate the toxicity of diverse types of contaminants by acting as natural buffers.

Atrazine is highly persistent in water, and its low solubility (33 mg/L) indicates that it is easily absorbed, reflecting its lipophilic nature [11, 14]. Our results suggest that salinities of 25 and 30 mitigated the toxic effects of atrazine on nematode survival (Fig. 1C and D), possibly by hindering absorption [38] or enhancing metabolic mitigation at high salinities [71]. Conversely, the interaction between low salinity and atrazine resulted in higher mortality in *L. marina* adults (Figs. 2 and 3). This differential response indicates that the regulatory effect of salinity on atrazine toxicity poses an environmental risk, particularly in rivers and estuaries, which often have low salinity and are near agricultural areas [42, 72]. Estuaries function as sinks for toxic agents, where pollutants can accumulate due to particle sedimentation and high ionic strength [67, 73, 74].

While this study provides robust data on acute lethal effects, we acknowledge that the 120 h (5-day) exposure period, while representing a full generation time of *L. marina* at 20 °C and thus approximating a lifetime exposure for this short-lived organism, does not fully capture the complexity of real-world environmental scenarios. Estuarine organisms are typically exposed to lower, sub-lethal concentrations of atrazine over multiple generations. Therefore, our acute LC_{50} data should be interpreted as representing worst-case, short-term scenarios, such as those following intense agricultural

runoff events. Despite the importance of estuarine environments, information on pesticide exposure and toxicity is lacking for many estuarine and marine species [56, 75], and fewer data are available on the possible effects of atrazine on invertebrates compared to vertebrate animals [76, 77]. Given that atrazine is relatively easily taken up by most aquatic organisms [78, 79] but can be quickly eliminated when they return to uncontaminated water [78], there is a critical need for studies addressing effects across multiple generations. Future research should therefore focus on investigating the transgenerational effects of sub-lethal concentrations of atrazine under the influence of fluctuating salinity on endpoints such as reproduction, growth, and underlying molecular mechanisms (e.g., osmoregulation and detoxification pathways) in *L. marina* and other estuarine invertebrates.

5 Conclusion

This study provides clear evidence of an antagonistic interaction between salinity and the acute toxicity of the herbicide atrazine on the estuarine nematode *Litoditis marina*. The central finding is that atrazine's lethal effect is inversely proportional to salinity, with the 120 h LC_{50} at salinity 15 being 10.4-fold lower than that observed at salinity 30. This underscores that the environmental risk posed by atrazine is not static, but dynamically amplified under low-salinity conditions. Given the increasing intensity of freshwater inputs in estuarine systems, these results suggest a critical need to incorporate salinity fluctuation as a key modifying factor in ecological risk assessments for transitional water bodies, particularly those receiving agricultural runoff.

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Data availability Data will be made available on request.

Declarations

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