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# Ecological consequences when organisms avoid a contaminated environment: A study evaluating the toxicity of fipronil

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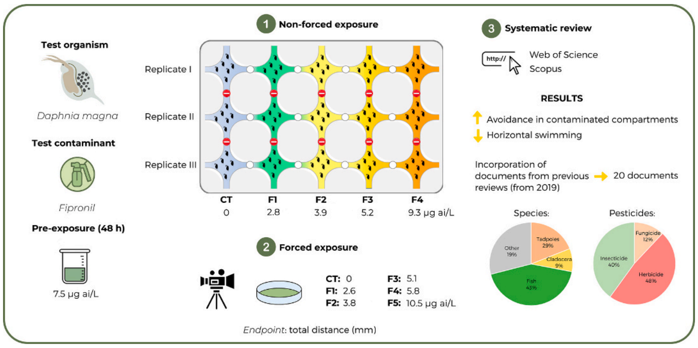
## HIGHLIGHTS

- Effects of fipronil on avoidance and swimming behaviors were studied for *D. magna*.
- A pre-exposure was carried out to evaluate effects in a landscaped environment.
- Organisms traveled shorter distances when the contamination gradient increased.
- Organisms were able to select the less contaminated sites after 48 h pre-exposure.
- The non-forced exposure may be used to assess contamination-driven habitat selection.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The ability of aquatic organisms to sense the surrounding environment chemically and interpret these signals correctly is crucial to their survival and ecological niche. This study applied the Heterogenous Multi-Habitat Assay System - HeMHAS to evaluate the avoidance potential of *Daphnia magna* to detect fipronil-contaminated habitats in a connected landscape after a short (48 h), previous, forced exposure to an environmentally relevant concentration of the same insecticide. The swimming of daphnids was also analyzed by recording the total distance covered. *D. magna* preferred areas with less contamination, although the effect of fipronil on their swimming ability (a decrease) was observed for all the concentrations tested. The application of non-forced multi-compartment exposure methodologies is a recent trend and is ecologically relevant as it is based on how contamination can really produce changes in an organism's habitat selection. Finally, we consider the

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importance of more non-forced exposure approaches where Stress Ecology can be aggregated to improve systemic understanding of the risk that contaminants pose to aquatic ecosystems from a broader landscape perspective.

## 1. Introduction

Chemical pollution originates from different human activities and threatens ecosystems, human health, and wildlife worldwide (Fuller et al., 2022). Due to population growth and the high demand for food production, the intensification of agriculture has been considered a driver of changes to ecosystems that consequently cause global environmental problems (Steffen et al., 2015; Molotofs et al., 2018). The application of pesticides and fertilizers is intrinsic to intensive agriculture, such chemicals then reach aquatic systems through drift, leaching, or runoff, thus damaging these ecosystems (Schiesari et al., 2023; Kumar et al., 2021). As a result, sublethal concentrations of these compounds present in aquatic environments can induce energy costs for organisms. Exposure to sublethal concentrations of pollutants acts as a factor for the decline of biodiversity, it can interrupt and change some biological processes such as: gene expression (Salesa et al., 2022), biochemical mechanisms (Freitas et al., 2022), physiological functions (Moreira et al., 2020), and reproduction (Silva et al., 2021).

Given this, the search for more comprehensive and targeted ecological risk assessment is becoming increasingly important and, therefore, classical methodologies and new strategies may be used complementarily to provide more realistic exposure scenarios (Blasco et al., 2020). Despite physiological mechanisms that allow a population to remain in contaminated environments, the cost of doing so may decrease populations' resistance to additional future stressors (López-Valcárcel et al., 2023). Thus, it is important to evaluate the effects of stressors that act consecutively on previously impacted populations because that is a way to identify how the previous exposure to contamination changes the mechanisms and responses that organisms may employ to face the toxicity (Coutellec and Barata, 2013; Venâncio et al., 2023).

*Daphnia* is a well-known ecotoxicological model organism employed to assess the ecological risk of pesticides and other contaminants using mostly standardized chronic and acute toxicity tests (Brock and Van-Wijngaarden, 2012) and more daring strategies, such as multigenerational (Araújo et al., 2019), landscape (Moreira et al., 2023) and swimming behavior (Vera-Herrera et al., 2022) approaches. For example, it has been shown that a previous history of pesticide disturbances experienced by *D. magna* alters its ability to cope with subsequent stressors (López-Valcárcel et al., 2020, 2023).

The insecticide fipronil was selected as it was shown in a previous study that *D. magna*, with no previous exposure to contamination, was not able to avoid fipronil contamination when placed in a contamination gradient (Moreira et al., 2023). Ecologically, the loss of connectivity in environments could make populations more susceptible to toxicity; however, even if populations maintain this capacity to detect and avoid contamination, they could suffer other critical effects related to a population's isolation and loss of connectivity as contamination can limit the areas that are explored. Thus, using the HeMHAS - Heterogenous Multi-Habitat Assay System, we sought to understand whether a previous exposure of daphnids to fipronil could alter their avoidance responses to this insecticide. Therefore, our objectives were: i) to evaluate the ability of *D. magna* to detect and avoid habitats contaminated by fipronil gradients in a spatially connected landscape after previously being exposed to the insecticide, and ii) to evaluate the role of exposure to sublethal concentrations of fipronil on the swimming capacity of *D. magna*. In addition, we discuss our results taking into consideration existing literature on behavioral ecotoxicology (i.e., avoidance) and the toxic role of pesticide contamination from three perspectives: i) changes in behavioral and regulatory homeostasis based on the toxicity data available (past trend), ii) the recent trend to test the repellency of contaminants, that is related to the changes imposed on organisms' habitat selection processes by contamination (present trend), and iii) the expected increase in ecological relevance due to integrating the non-forced multi-compartment exposure methodologies with ecotoxicological studies (future trend).

## 2. Materials and methods

### 2.1. Culture conditions and test organisms

The cultures were maintained, according to USEPA-United States Environmental Protection Agency (2022), in one-liter glass beakers with 20 to 30 ind/L at 16 h: 8 h (light: dark) and 20 ± 2 °C, with pH: 8.5 ± 0.6 and dissolved oxygen (D.O.) $> 6$ mg/L (YSI-556 Multiparametric probe) and with an irradiance of $\approx 200$ μmol photons $m^{-2}$ $s^{-1}$. In accordance with Álvarez-Manzaneda et al. (2017), the culture water used was commercial mineral water with $<4$ g P/L, with chemical composition of bicarbonates ($HCO_{3-}$) 202 mg/L; sulphates ($SO_{4-}$) 57 mg/L; chlorine (Cl): 15 mg/L; calcium (Ca): 64 mg/L; magnesium (Mg): 16 mg/L; sodium (Na): 9 mg/L and silica ($SiO_2$): 4 mg/L; conductivity 0.23 mS $cm^{-1}$) and enriched with vitamins: thiamine (75 mg/L), vitamin B12 (2 mg/L), biotin (0,75 mg/L) and sodium selenite 158 (2 mg/L) following Díaz Báez et al. (2009).

The microalgae *Scenedesmus* sp. ($5 \times 10^4$ cells/mL) was provided ad libitum as food every second day of the week. Neonates were obtained from cultures (third to fifth brood $\leq$24 h) and used as test-organisms (OECD 211, 2012; OECD 202, 2000). The same strain of *D. magna* was used and cultivated under the same conditions as the study by Moreira et al. (2023).

### 2.2. Chemical and stock solution

Stock solution of 1.6 mg/L of fipronil administered as commercial formulations (Regent® 800 WG, purchased from BASF, Brazil) were used for all the behavioral tests with *D. magna*. Culture water was used to dilute the concentrations.

### 2.3. Chemical analysis of the pesticide

The samples were collected from each treatment at the beginning and at the end of the experiments for both the avoidance and swimming behavior experiments. In accordance with Goulart et al. (2020), the compound was quantified by liquid chromatography coupled with mass spectrometry (LC-MS/MS). The samples were initially diluted in methanol (1:1) and then filtered in a PTFE syringe filter with a diameter of 13 mm and pore size of 0.22 μm. The instrumental quantification limit (LOQ) was determined by the signal-to-noise ratio (SNR) method using an SNR of 10:1 and the different concentrations of the insecticide were determined by using external calibration curves. The LOQ for fipronil was 0.1 μg/L, and its linear working range was 0.1 to 100 μg/L.

### 2.4. HeMHAS - heterogeneous multi-habitat assay system

The HeMHAS consists of a total of 15 compartments of 320 mL each (Araújo et al., 2018) (Fig. S1). It is a two-dimensional (non-forced exposure) system that simulates many different contamination scenarios. All compartments (15) were used in the present study, due to the number of concentrations tested. HeMHAS simulates an environment with patches of contamination as it has connectivity between compartments, and such connections can be either open or closed.

### 2.5. *Avoidance tests: Habitat selection*

All the organisms used in the avoidance experiments were pre-exposed to 5.0 μg of a.i./L of fipronil for 48 h in 1-liter flasks. This concentration is in the range commonly recorded in many aquatic ecosystems, including Brazilian ones (Marchesan et al., 2010; CETESB, 2018).

The gradients of fipronil used in the experiments were prepared with four real concentrations: 2.8 ± 0.06 (F1), 3.9 ± 0.04 (F2), 5.17 ± 0.02 (F3), and 9.3 ± 2.81(F4) μg active ingredient - a.i./L, in addition to the compartment without initial contamination - culture water only (Table S1). The different concentrations were placed in the compartments with the connection doors between them closed to maintain the concentration gradient. Then, 6 individuals of *D. magna* were placed in each compartment (320 mL). As the fipronil gradient avoidance experiments were performed with 4 concentrations, plus a control for each compound, 30 daphnids were used per replicate and 3 replicates were tested (Fig. 1). A fully non-contaminated experiment (control) was performed, with three replicates, to verify that the daphnids could move freely between the compartments but did not display a tendency to move to certain compartments in the absence of contamination. Each compartment of the HeMHAS was filled with 320 mL of culture water and ten daphnids were placed in each compartment. The daphnids used were between 6 and 8 days old (previously exposed to fipronil for 48 h) and the number of organisms in each compartment was registered after 24 h in the control and fipronil experiments.

### 2.6. *Analysis of swimming behavior*

Experiments were performed to assess any effects on the motility of *D. magna*. *D. magna* neonates (6–24 h) were randomly selected from the stock culture and kept in glass beakers of 1000 mL containing the culture medium for 96 h, which was renewed every 48 h, and fed as described to culture the organisms to analyze swimming behavior. Subsequently, 96-h-old organisms were exposed (forced exposure) to the same concentrations used in the multicompartment system (HeMHAS), as described in the previous section. The values (mean and standard deviation) of the initial and final concentrations were: 2.6 ± 0.01 (F1), 3.85 ± 0.04 (F2), 5.13 ± 0.5 (F3; the same used in pre-exposure), 5.85 ± 0.08 (F4; one slightly higher than the pre-exposure) and 10.5 ± 0.15 (F5) μg a.i./L (Table S2). The organisms were also exposed to the control treatment (CT).

Five daphnids were exposed individually per treatment for 48 h in plastic cups of 200 mL containing 100 mL of the test solution. The exposure of the organisms to 20 ± 2 °C, under continuous white light, was carried out under the same conditions as the avoidance experiments. At the end of the 48 h-exposure, their swimming behavior was studied by image analysis. Video analyses were performed to record the horizontal motility of each individual from each treatment ($n = 10$) using 6-well culture plates filled with 4 mL of culture water and, after 1 min acclimatization, its movement was recorded for 1 min. The videos were shot in Full 1080p, 60 fps using a 48 MP resolution camera (size 4:3). The videos were analyzed using the Kinovea (2023) v. 0.9.5 software (https://www.kinovea.org/). This software was calibrated, using the measurements of the well, to measure total distance (mm) as the endpoint of the swimming behavior.

### 2.7. *Statistical analysis*

Firstly, the distribution of organisms (%) in each compartment was calculated in the non-forced exposure system for the avoidance responses and the total distance (mm) for swimming behavior. Then, the median and the standard error (SE) among the three and ten replicates were calculated for the avoidance responses and swimming behavior, respectively.

The selection of a given compartment by the organisms in the avoidance experiments was not considered independent as organisms might be influenced by adjacent compartments. The avoidance responses underwent analysis using General Linear Models, in which a negative binomial distribution converged with our results considering count data (Gallucci, 2019). For the swimming behavior, normality (Shapiro-Wilk test) and homogeneity of data (Levene test) were verified and differences between treatments were assessed by one-way analysis of variance (ANOVA). The ANOVA was followed by Tukey's post-hoc test to discriminate the statistically significant differences among the treatments. All the analyses were carried out with a confidence interval of 95 % ($p < 0.05$) in the Jamovi software (Jamovi, 2022).

## 3. Results

### 3.1. *Validation of tests and chemical analyses*

The integrated values [mean ± standard deviation (SD)] initial and final (after 24 h) concentrations of fipronil determined in the avoidance

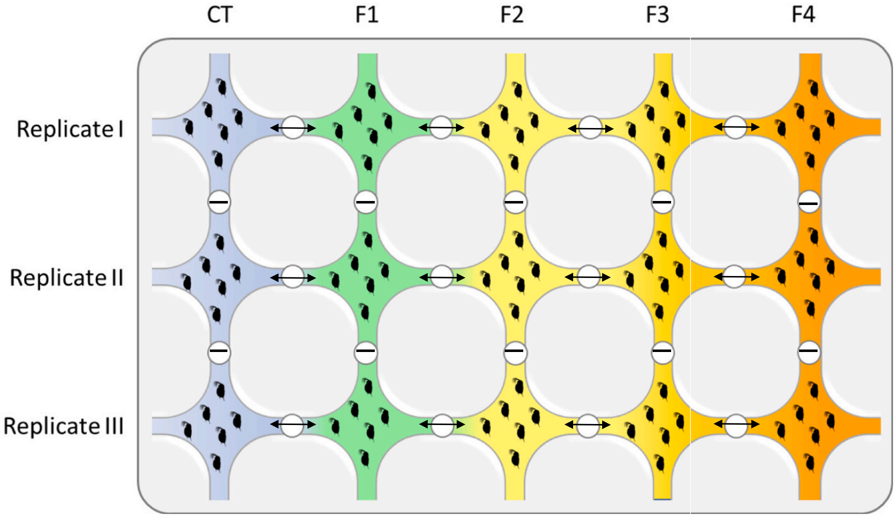

**Fig. 1.** Scheme of the experimental design for the avoidance experiments. The concentration of fipronil (F) increases horizontally. Then, *D. magna* (6 individuals) were inserted initially into each compartment (320 mL). Connections among the concentrations are open (↔) while among the replicates they are closed (—).

experiments were: 2.8 ± 0.06. (F1), 3.9 ± 0.04 (F2), 5.17 ± 0.02 (F3), and 9.3 ± 2.81(F4) μg a.i./L. For the control, the final value (mean ± SD) was 2.4 ± 0.02 μg a.i./L. The quantified value (mean ± SD) for the pre-exposure concentration was 5.13 ± 0.05 μg a.i./L. The values are also presented in Table S1.

Regarding the concentrations (mean ± SD) of fipronil determined at the beginning and end of the experiments for swimming analysis, they are, respectively, presented in Table S2. For each compartment, the following were quantified: 2.6 ± 0.01 (F1), 3.85 ± 0.04 (F2), 5.13 ± 0.5 (F3; the same used in pre-exposure), 5.85 ± 0.08 (F4; one slightly higher than the pre-exposure) and 10.5 ± 0.15 (F5) μg a.i./L (Table S2); with regard to the control, the value was less than the quantification limit.

The distribution of the daphnids in the control tests for avoidance showed no preference or avoidance for any area of the experimental system; therefore, the distribution of daphnids was random ($F_{4,15}$ = 1.79, $p$ = 0. 208).

### 3.2. Avoidance responses to fipronil

The average percentage of organisms obtained from the fipronil gradients for D. magna are shown in Fig. 2. It may be observed that the different fipronil concentrations influenced the percentage of organisms in each compartment, with a clear majority preference for the uncontaminated compartment (Fig. 2). Upon considering the avoidance response, the treatment F1 did not exhibit significant differences from the control ($p > 0.05$), whereas F2, F3, and F4 did ($p < 0.05$).

### 3.3. Swimming behavior

Regarding horizontal swimming behavior, *D. magna* exposed to fipronil showed lower values of the variable analyzed, total distance (data in mm): F1 = 190.12 ± 16.87; F2 = 168.11 ± 14.77; F3 = 152.86 ± 16.99; F4 = 117.62 ± 8.97 and F5 = 110.38 ± 6.18 mm when compared to the control (253.93 ± 17.27 mm), (one-way ANOVA tests, $F_{5,59}$ = 13.822, $p$ = 0.001) (Fig. 3).

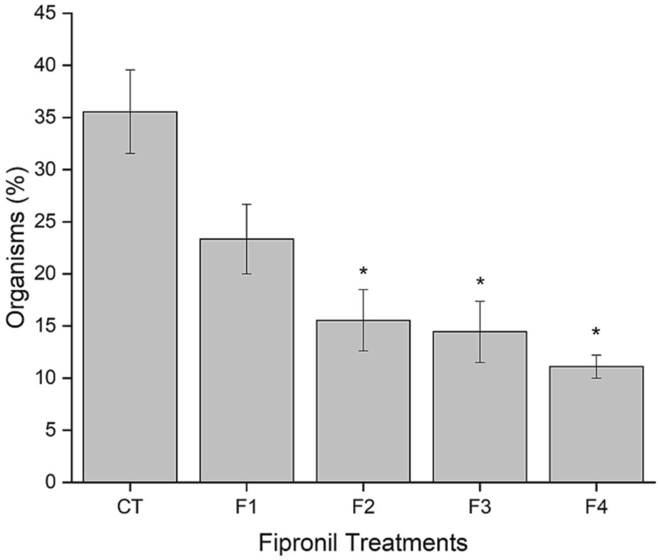

**Fig. 2.** Distribution (in %; with mean values ± SE of the three replicates) of daphnids along a concentration gradient of Regent® 800 WG (a.i. fipronil), after 24 h in HeMHAS. Fipronil treatments were: 0 (CT), 2.8 (F1), 3.9 (F2), 5.17 (F3) and 9.3 μg ai/L (F4). All daphnids, except those of the control, were previously exposed to 7.5 ai/L of fipronil for 48 h.

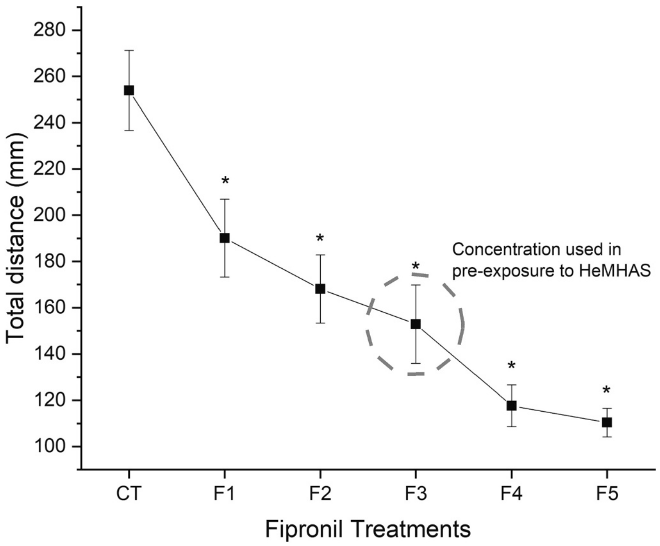

**Fig. 3.** Swimming behavior of *D. magna* after 48 h of exposure with respect to total distance (mm). Bars represent the mean ± SE ($n = 10$) for each treatment. Fipronil treatments were: 0 (CT), 2.6 (F1), 3.85 (F2), 5.13 (F3), 5.85 (F4) and 10.5 μg ai/L (F5). Asterisks indicate statistically significant differences ($p < 0.05$) between treatments.

## 4. Discussion

### 4.1. Behavioral ecotoxicology approaches

According to the results presented previously, a prior sublethal exposure to fipronil led *D. magna* to prefer less contaminated habitats when they were exposed to a fipronil gradient. Comparing our results to a previous study published by Moreira et al. (2023), a clear effect of the pre-exposure can be observed: while those authors did not observe avoidance response by *D. magna* exposed to a fipronil gradient, we observed that a previous sublethal exposure increased the perception and avoidance of the gradient of fipronil contamination by the organisms. Similarly, a previous study with *D. magna* using sublethal concentrations of the insecticide dimethoate showed that a previous exposure to contamination made the daphnids more vulnerable to other stressors applied subsequently (López-Valcárcel et al., 2020). This indicates that a population can remain in a contaminated environment, potentially making the population vulnerable to new stressors, which it might be exposed to in the future (Barbosa et al., 2017). Given this, it is important to evaluate any history of exposure to a contaminant (even if sublethal) to identify any future effects on the parameters evaluated without this previous exposure and also in combination with other stressors (Venâncio et al., 2023; Verheyen et al., 2022). According to Aulsebrook et al. (2020), after exposure to subsequent stressors, the cost of sublethal exposure could take the form of a change in energy allocation, such as the expenditure of energy in detoxification. An allocation of energy to detoxification by organisms, thereby altering their sensitivity when dealing with stress (Campos et al., 2019), has been observed previously for aquatic invertebrates exposed to fipronil (Demirci et al., 2018), such as *Chironomus riparius* (Park, 2016; Monteiro et al., 2019).

Another point to be considered is the difference between the forced (focused on toxic effects) and non-forced (focused on habitat selection response) approaches. When the toxic effects on a population that was previously exposed to contamination are evaluated, what is being analyzed is how the previous exposure affected the capacity of the organisms to physiologically face the new exposure to contamination. In this circumstance, the damage produced by the previous exposure can reduce or prevent future detoxification processes, making the organisms more susceptible to contamination, due to the higher energy expenditure, such as previously discussed (Campos et al., 2019; Aulsebrook et al., 2020; López-Valcárcel et al., 2020). However, when the habitat selection response in a non-forced approach is used to assess the previous exposure, the process is related to the organisms' ability to sense their environment (Venâncio et al., 2023). Although the previous exposure might have affected their ability to perceive the risk and avoidance could not be observed, sometimes organisms need some time exposed to the contaminant to then be able to select the environment with least toxicity. This delay in avoiding contamination, where the organism may not react to the first exposure, has been observed in zebrafish exposed to glyphosate (Mena et al., 2022). Therefore, if we compare the more intense avoidance response to fipronil in the current study (for the organisms that were previously exposed to fipronil) with the study by Moreira et al. (2023), we may posit a hypothesis. In the former case, the population may have responded more significantly as it had a greater ability to recognize the contaminant, while in the latter case, concerning a population that was not previously exposed to the contaminant and then did not avoid the fipronil, this could be attributed to the lack of ability to recognize the risk to which the organisms were exposed.

Analysis of swimming behavior has also been considered an endpoint to detect the sublethal effects of pollutants on aquatic animals (Kane et al., 2004; Eissa et al., 2010). It is known that pesticides can cause abnormal swimming behavior or impaired swimming in different organisms of aquatic fauna (Bridi et al., 2017; Moreira et al., 2019; Freitas et al., 2019). The results of the present study indicated that the total distance covered by *D. magna*, compared to the control, was significantly altered (decreased) when exposed to all concentrations of fipronil tested. The insecticide acts by disrupting the gamma-aminobutyric acid (GABA) receptor, resulting in hyperexcitation and consequent insect mortality (Tingle et al., 2003; Das et al., 2006). Its neurotoxic mode of action in non-target organisms has been pointed out in the literature, even for other aquatic invertebrates (Pinto et al., 2021; Silva et al., 2021). Furthermore, deficiencies in swimming capacity imply reductions in foraging and, consequently, in the energy available for growth and reproduction.

The exposure of animals to pesticides, including fipronil, has caused changes in swimming behavior for some species of both invertebrates and vertebrates. For example, the fish *Pimephales promelas* had its swimming performance reduced (Beggel et al., 2010) when exposed to fipronil at concentrations >142 μg/L. When video monitoring the behavior of *D. magna* after exposure to sublethal concentrations of glyphosate, Hansen and Roslev (2016) detected a decrease in acceleration, displacement, and swimming speed. Vera-Herrera et al. (2022) observed that swimming behavior increased in *D. magna* exposed to a mixture of pesticides (chlorpyrifos and terbutylazine), even at the highest concentration. As indicated by those authors, this response is not necessarily beneficial, as it is resultant of a frenetic movement due to an over-excitement, which could lead to a higher energetic expense with negative metabolic consequences.

### 4.2. *Ecological implications of using non-forced exposure approaches: past, present and future*

No limits for the presence of fipronil in surface waters in Brazil (CONAMA, 2005) have been established yet. Concentration ranges from 6 to 465 μg/L in the southeastern region of Brazil (CETESB-Companhia Ambiental do Estado de São Paulo, 2018), and from 0.05 to 26 μg/L in the southern region (Marchesan et al., 2010) have already been detected. In addition, concentrations ranging from 0.001 to 0.06 μg/L, 0.0008 to 0.014 μg/L and 0.001 to 6.41 μg/L for fipronil have been reported for surface water bodies in China, the European Union and the United States, respectively (Ensminger et al., 2013; Fang et al., 2019). Thus, the results obtained in the present study have significant ecological relevance because the effects were observed at concentrations that have already actually occurred in natural aquatic environments.

Various different approaches to exposure to several pesticides were extracted from the literature available. Table S3 shows an update on the review from Moreira-Santos et al. (2019) and the complete systematic review in Table S4 with information concerning studies about the effects of pesticides on spatial avoidance assays, highlighting: the pesticides studied, the test-organisms, period, methodological approach, and the main responses. The literature indicates that while the Y-shaped maze was widely utilized in the past, wherein the control of the flow of water and contaminant solution into each arm of the maze was ensured, nowadays, more recent studies have leaned towards employing a non-forced exposure in a multi-compartmentalized system. This represents an advance from the assessment of an individual binary selection towards the collective (population) response in a gradient of stressors. This shift highlights the growing preference for more ecologically relevant experimental setups that may, potentially, answer specific questions concerning the behavior of test-organisms when exposed to stressors (Blasco et al., 2020).

The Y-shaped maze provides a two-choice scenario, while the application of HeMHAS in such experiments offers a valuable advantage by providing a comprehensive two-dimensional overview of the organisms' behavior in response to contaminant gradients. The number of compartments used for the studies in the literature review varied depending on the specific focus of the research. For instance, employing 6 or 7 compartments proved to be effective to examine the gradient response to a single contaminant. On the other hand, using 20 compartments proved suitable for studying mixtures of two pesticides (Moreira et al., 2022; Moreira et al., 2023). This is an environmentally relevant approach considering the prevalence of co-contaminations and multiple stressors in real-world scenarios. Our study was the first to investigate the effects of a prior contamination on the avoidance behavior of daphnids regarding pesticides.

Considering this need for more complex and realistic behavioral ecotoxicological approaches, Jacquin et al. (2020) proposed an interesting scheme. Focusing on fish, they described how environmental stressors of different natures act at different levels within an organism, altering connected processes beginning with the biochemistry and physiology, and influencing behavior and cognition and culminating in an individual's fitness that finally impacts on a population's stability. In this scheme, behavior plays a crucial role by conditioning exposure to the triggering stressors, which may be related to either remaining in a contaminated environment, or fleeing from it. The work by Vera-Herrera et al. (2022), for example, addressed the effect of neurotoxicity on the distribution of daphnia in a pesticide gradient, thereby including a mechanistic approach to the assessment of a relevant behavioral response.

Another interesting feature related to the complexity of environmental stress, and well represented by chemical pollution, is the interaction between pollutants. This point is important regarding behavioral assessments as the interaction of stressors can produce completely different responses when compared to individual exposures, as observed in relation to zebrafish confronted by mixtures of pesticides (Mena et al., 2022). Such differences can occur because some pollutants in a mixture can mask another factor of stress, or they can distract or mislead an organism, thus preventing an appropriate behavioral response related with habitat selection (Dominoni et al., 2020). Studies by Tierney et al. (2010) and Tierney and Pyle (2023) have addressed the effect of pollutants that damage the sensory (olfactory) organs of fish, as well as the interference of contaminants in the routes of migratory species. Hence, the process of perception could be another fruitful target of future research related to behavioral responses associated with habitat selection and the distribution of populations. The avoidance response relies on sensing the environment and responding rapidly to the detection of the pollutant that should spare the organisms from suffering major physiological consequences (Moreira-Santos et al., 2019; Araújo et al., 2020). From this perspective, the integration of metabolic processes is related to exposure and reflected in behavior; in turn, the physiological processes are related to the perception of the environment. Therefore, studies evaluating the effects of combined stress factors should be undertaken in the field of behavioral ecotoxicology.

The availability of tools for behavioral assessments has increased in the last few decades. This advance has allowed data about the ecological relevance of these approaches to be collected, which supports the inclusion of behavioral ecotoxicology within the process of ecological risk assessment for chemical pollutants (Hellou, 2011; Ford et al., 2021). However, some adjustments have been suggested regarding the standardization of the methods and the quantification of responses that are essential for regulatory purposes (Ford et al., 2021). In this regard, the spatial avoidance method applied and reviewed here accomplishes the goal of being suitably controlled and easily standardizable. Furthermore, the information produced by using such methods, which are employed to analyze organisms' habitat selection, have clear ecological implications and relevance as they relate directly to the distribution of populations in the context of environmentally realistic contamination.

Finally, these novel approaches bring a complementary perspective to ecotoxicology, which includes not only the chemical heterogeneity of contamination throughout a landscape but it also adds other environmental factors (either biotic or abiotic ones) that may be used to assess how the habitat selection processes are disturbed by contaminants (Araújo et al., 2020; Salvatierra et al., 2022). Furthermore, broadening the experimental approach to include multiple endpoints and stressors by increasing the number of studies with simultaneous exposures to sequential and integrated analysis of their effects may be a very informative path for future studies.

## 5. Conclusions

We have shown that after a previous exposure to a sublethal concentration of fipronil, *D. magna* was able to detect a gradient of fipronil and avoid potentially toxic concentrations, and the forced exposure affected its swimming capacity. Therefore, it is necessary to study the history of the impact of any particular contamination to better assess how sublethal concentrations of pesticides may change the sensitivity of aquatic organisms to other stressors and the future consequences that this may have at the ecosystem level. Applying open gradient spatial avoidance methods constitutes a versatile tool for behavioral ecotoxicology. They are easily standardizable, allow the simultaneous integration of different variables, and provide ecologically relevant results leading to more realistic ecotoxicology assessments.

## CRediT authorship contribution statement

**Raquel A. Moreira:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **María Pilar González:** Writing – review & editing, Investigation. **Mariana A. Dias:** Methodology, Investigation. **Allan P. Ogura:** Writing – review & editing, Methodology, Formal analysis. **Freylan Mena:** Writing – review & editing. **Cassiana C. Montagner:** Resources, Methodology. **Evaldo L.G. Espíndola:** Writing – review & editing, Project administration, Funding acquisition. **Julián Blasco:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Gema Parra:** Writing – review & editing, Resources, Methodology, Investigation. **Cristiano V.M. Araújo:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171480.

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