Sublethal effects of propiconazole on the metabolism of lambari Deuterodon iguape (Eigenmann 1907), a native species from Brazil

Marcelo Barbosa Henriques · Karina Fernandes Oliveira Rezende · Leonardo Castilho-Barros · Edison Barbieri

Received: 21 January 2021 / Accepted: 14 May 2021 / Published online: 18 June 2021 © The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract The objective of this study was to analyze the sublethal effects of propiconazole on Deuterodon iguape, a native fish common in Brazil, which has potential for aquaculture and use as a bioindicator. The hypothesis was to test whether D. iguape has a metabolism similar to Danio rerio so that its use in bioassays may be validated. Lethal concentration (LC50) and metabolic rates were studied in fish exposed to propiconazole. Specific oxygen consumption and ammonia excretion for D. iguape and D. rerio increased by 0.01 µg L⁻¹ and then decreased as the propiconazole concentration increased. The decrease in the averages of specific oxygen consumption at the concentration of 0.1 µg L⁻¹ represented a reduction in the metabolic rate compared to the control of 71% for D. iguape and 40% D. rerio. For the ammonia excretion, at the same concentration, there was a reduction of 68.7% and 45.4% for D. iguape and D. rerio, respectively. When comparing ammonia excretion of the two species for each concentration of propiconazole, there was a significant difference ($p < 0.05$) in relation to the control and for the highest concentration (0.1 µg L⁻¹). As for specific oxygen consumption, there was a statistically significant difference only for the concentration of 0.1 µg L⁻¹. D. iguape proved to be a good and useful bioindicator for ichthyologists or ecologists in studies of moderate pesticide contamination in freshwater aquatic environments, as its metabolic response was similar to D. rerio.

Keywords Ammonia excretion · Danio rerio · LC 50 · Oxygen consumption · Propiconazole · Triazole fungicide

Introduction

Most water bodies in Brazil, and in the world, are contaminated by some type of chemical pollutant (Lopes et al. 2017). Agricultural pesticides are used excessively and beyond what is really necessary to control pests of agricultural crops. About 90% of what is applied is lost in the environment, and the biological response in terms of pest control is not reached (Barrera-Méndez et al. 2019). These losses occur due to factors such as application techniques, physical and chemical properties of pesticides, and environmental
conditions (Ghormade et al. 2011). The impact of pesticides on the quality of groundwater has been a relevant and discussed subject worldwide (Lanchote et al. 2000). Less than 0.1% of the amount of pesticides applied to crops reaches the target organisms, while the other 99.9% has the potential to move to other environmental compartments, such as the surface and groundwater (Sabik et al. 2000).

Toxic compounds such as pesticides can affect aquatic organisms by compromising their behavioral, nutritional, and physiological status (Van der Oost et al. 2003). For this reason, studies of the behavior and metabolism of fish and shrimp can assist in monitoring the environmental quality where these organisms are present. Using behavior and metabolism as a biomarker, it is possible to analyze the general physical state of these animals when in contact with certain toxic substances (Arias et al. 2007). Behavior analyses, specific oxygen consumption, and ammonia excretion can provide answers about the general health of aquatic organisms when under stress in the environment (Adams 1990).

An organism’s metabolic rate is a useful and sensitive indication of its energy consumption. Therefore, in aerobic organisms, quantifying the rate of oxygen consumption can be directly associated with the amount of energy released from the oxidation of the food substrate. Based on the amount of oxygen consumed by an animal over a period of time, it is possible to calculate the energy spent during the same period to maintain its vital processes (Barbieri et al. 2019).

The evaluation of oxygen consumption and ammonia excretion in fish was used, for example, to study the toxic effects caused by: nanoparticles (Rezende et al. 2018), ammonium chloride (Barbieri and Doi 2012), metallic trace elements (Barbieri 2007; Martinez et al. 2013; Ferrarini et al. 2016), and carbofuran (Campos-Garcia et al. 2016; Ruiz-Hidalgo et al. 2016).

Propiconazole is a broad-spectrum fungicide used to control fungal diseases in agricultural crops (Satapute and Kaliwal 2018). It has an action interfering with the ergosterol biosynthesis and inhibiting steroid demethylation (Ouadah-Boussouf and Babin 2016). Compared to other fungicides, propiconazole is difficult to degrade in the environment and exhibits relatively high acute toxicity, which can contaminate soil, water, and indirectly fauna, flora (Friberg et al. 2003; Garrison et al. 2011), and mainly a wide range of aquatic organisms (Cobas et al. 2016). These compounds, even in low concentrations, affect the structure and function of natural communities, causing damage ranging from molecular levels to that of entire populations, proving that intensive agricultural practices are highly impactful to the environment and are directly related to the reduction of biodiversity (Barbieri and Ferreira 2011).

Kronvang et al. (2003) detected up to 130 ng g$^{-1}$ of propiconazole in the sediment of streams in a Danish plain. Kahle et al. (2008) found it in concentrations of up to 27 ng L$^{-1}$ in effluents of lakes and wastewater treatment plants in Switzerland. Kreuger (1998) observed propiconazole at maximum concentrations of 20 µg L$^{-1}$ in the water of agricultural basins in the south from Sweden. In Brazil, the impacts related to the chronic and environmental toxicity of the application of pesticides were ignored or considered irrelevant for many years (Barbieri and Ferreira 2011). In addition to the possible exposure of these fungicides to humans and wildlife through soil sediment and residual water, their stereoselective transformation forming new compounds is more harmful to flora and fauna and is also concerning (Garrison et al. 2011). Due to their high mobility, particles of <2 µm can be important carriers of propiconazole, causing water pollution (Wu et al. 2003).

There is a risk of contamination in the food chain, which can affect humans through the consumption of contaminated fish. In the meantime, there is also the danger of contamination at sublethal levels which can affect the predator–prey relationship, eating habits, reproductive success, and the general metabolism of fish (Arias et al. 2007).

Banana farming is one of the most important agricultural activities in Brazil. The crop ranks second in volume of fruit produced, with approximately 6.75 million tons per year (Statista 2018), second only to oranges (Hanada et al. 2015). Due to phytosanitary problems, mainly leaf diseases such as the black sigatoka (Mycosphaerella fijiensis) and the yellow sigatoka (Mycosphaerella musicola), the crop can display a low productivity. These diseases are mainly controlled with the use of propiconazole, which acts on a wide spectrum of diseases caused by ascomycetes, basidiomycetes, and deuteromycetes (Garrison et al. 2011).
In Brazil, the use of exotic species for toxicity tests is mainly due to the scarcity of studies on the biology and sensitivity of allochthon species that could be used as test organisms. Therefore, there is an urgent need for studies in order to find native species of different trophic levels that are considered important to the environment, and can be standardized as test organisms, since the exotic species used have no ecological relevance (Zagatto and Bertoletti 2010). In the present study, lambari *D. iguape* was chosen because it is a native species of the Atlantic forest, easily produced in captivity and has a wide distribution in this habitat (Henriques et al. 2018). According to Baun et al. (2000), there is not a single species of organism that represents the effects caused by a pollutant in a given ecosystem. Therefore, there is a need to use several species of test organisms in order to represent the different levels in the trophic chain, increasing, thus, the probability of more comprehensive and reliable responses, involving organisms of different sensitivities. The lambari *Deuterodon iguape* is a small-sized Characiform native to the Atlantic Forest watershed (Fonseca et al. 2017). It is an endemic species of small rivers and streams in the tropical and subtropical forest region. It also has wide market possibilities, since recent studies have discovered, in addition to selling for human consumption, its use as live bait in recreational fishing (Henriques et al. 2019).

The zebrafish, *Danio rerio*, is the model fish most used in bioassays on genetics, neurophysiology, and biomedicine (Amsterdam and Hopkins 2006; Teng et al. 2019). Other important aspect in zebrafish is the transparent embryo for an easy teratogenesis assessment (Keshari et al. 2016). It has several attributes that make it particularly efficient for experimental manipulation. It is a small, robust, and very prolific fish, which can be kept easily and at a low cost in the laboratory (Spence et al. 2008). The great advantage of its use as a model organism is that, as a vertebrate, it is more comparable to humans than model species of invertebrates, such as *Drosophila melanogaster* (Barbazuk et al. 2000), and is more susceptible to genetic and embryological manipulation than model species of mammals, such as rats, in which these procedures are more complicated and expensive (Spence et al. 2008).

The hypothesis tested in this study was that the Atlantic forest lambari *D. iguape* has a metabolism similar to zebrafish *D. rerio* so that its use in bioassays can also be validated and mainly be used as a bioindicator species of aquatic environment conditions. This study aimed to verify the toxicity and sublethal effects of propiconazole on the Atlantic Forest lambari *D. iguape* and zebrafish *D. rerio*, through the evaluation of its metabolic rate.

**Material and methods**

For the lambari *Deuterodon iguape*, young individuals were used (average total length: 3.0 ± 0.32 cm) produced in the municipal fish farm of Guanhanhã, in Peruíbe, SP (24°12′25″S; 47°02′48″W). The *Danio rerio* zebrafish (average total length: 2.6 ± 0.21 cm) used in the experiment was produced at the Mariculture Reference Laboratory Unit of the Fisheries Institute, in Santos (23°59′24″S; 46°18′23″W).

In the laboratory, a total of 120 fish of each species were kept for 5 days in independent 500-L tanks, with constant aeration and daily water change (20%) to acclimate them to the conditions of the laboratory. The freshwater used for maintenance, passed through three 2-µm filters, two 1-µm filters, and one 0.5-µm filter arranged sequentially. The fish were fed with extruded commercial feed, 2.0 mm with 36% crude protein (CP), 7% ether extract (EE), and 4% crude fiber (CF), placed in the tanks, in the proportion of 2% of the live weight. Feeding was suspended 24 h before the experiments.

**Tested substance**

In this study, the main element, propiconazole, (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl] methyl]-1H-1,2,4-triazole), (100 ng μL⁻¹ in methanol, PESTANAL®, analytical standard—Empirical Formula C₁₅H₁₇Cl₂N₃O₂) (Fig. 1), used as an active ingredient in the composition of the commercial pesticide formula TILT, one of the most used pesticides by Brazilian banana growers.

Determining the LC50 for fish subjected to propiconazole

The experiment for determining the average lethal concentration, which represents the concentration
calculated to cause 50% mortality in a tested population over a given period (LC50) (Rand and Petrocelli 1985), was short term, up to 96 h, with partial renewal (semi-static) and with 12 h of light cycle. *D. iguape* and *D. rerio* were placed in 20-L aquariums at 25 °C. For each treatment, 20 fish of each species (5 for each replica) were exposed to propiconazole concentrations of 0.1; 0.5; 1; and 2.5 µg L⁻¹, in addition to a control treatment (without propiconazole). Mortality was recorded every 2 h until the first 12 h and later at 24, 36, 48, 72, and 96 h. For each treatment, the percentage of survival was plotted against the exposure period (Barbieri 2007).

To obtain the propiconazole desired concentration, the necessary volume of the main substance (1 mg propiconazole mL⁻¹) was calculated for volume aquarium and set with the help of a micropipette at the end of the acclimation. Water chemical analysis to confirm exposure concentrations of propiconazole was performed using an HPLC system (1200 series, Agilent Technologies, CA, USA) coupled to a 6130 quadrupole mass spectrometer with a G1978B multimode ion source [electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)].

The acute lethal effects of low concentrations of propiconazole in both species were analyzed by determining the average lethal concentration (LC50). The LC50 was calculated using the trimmed Spearman-Karber statistical method (with Abbott correction), proposed by Hamilton et al. (1978).

The pH, dissolved oxygen, and the concentration of ammonia, nitrite, and nitrate were monitored at the beginning and at the end of each test, to verify the water quality conditions of the experiment. For this measurement, pHmeters, ATAGO-S/Mill refractometer, Winkler’s method for dissolved oxygen (Winkler 1888), and Koroleff’s (1970) colorimetric method for ammonia were used.

Routine metabolism

The two species of fish studied were similar in size, *D. iguape* (3.0 ± 0.32 cm) and *D. rerio* (2.6 ± 0.21 cm), but regardless, we examined both the consumption of oxygen and the excretion of specific ammonia, that is, per unit weight. Consumption and excretion were divided by the animal’s weight, specific consumption = consumption g⁻¹ and specific excretion = excretion g⁻¹.

Both fish species acclimated to a temperature of 25 °C were exposed to concentrations of 0.0, 0.01, 0.05, and 0.1 µ L⁻¹ of propiconazole for a period of 2 h. Five fish of each species for each concentration were subjected to measurement of oxygen consumption and ammonia excretion in each of the four concentrations with three replicates (Barbieri and Ferreira 2011).

Before the beginning of the experiments, the animals were kept in respirometers with continuous water circulation for at least 60 min, to alleviate the stress resulting from handling. Then, the water supply was suspended, and the respirometers were closed so that the fish consumed the oxygen present in a known volume of water, for a period of 1 h (Barbieri and Doi 2012). The respirometers were protected by a shield to isolate the animals from possible movements in the laboratory. The difference between oxygen concentrations, determined at the beginning and at the end of confinement, represented the animal’s consumption during the period; the same was applied for ammonia. To minimize the effect of the lack of oxygen on metabolism, the duration of the experiments was regulated in such a way that the oxygen concentration at the end of the experiments was always greater than 70% of its initial concentration.

![Chemical structure of propiconazole](https://www.sigma-aldrich.com)
Dissolved oxygen was determined using the Winkler method (1888) and ammonia concentration using the Nessler method (standard methods for the examination of water and wastewater).

Statistical analysis

Behavioral data were assessed according to species considering ammonia excretion and specific oxygen consumption in relation to the different concentrations of propiconazole tested. In addition, the species were compared separately according to ammonia excretion values and specific oxygen consumption for each propiconazole concentration. Initially, normality was analyzed based on the Shapiro–Wilk test and the homogeneity of variances by the Levene test. For the species model as a function of ammonia excretion and specific oxygen consumption, for each concentration, the differences were analyzed based on the Student’s T test. As for the model in which each species was analyzed separately in relation to the different concentrations, the data were submitted to analysis of variance (ANOVA) and to the post-hoc Tukey test. For both experiments, the differences were considered significant when $p < 0.05$.

Results

During the entire experiment, the average temperature was $24.7 \pm 0.6 \, ^{\circ}C$. The water parameters pH, ammonium, nitrite, and nitrate did not significantly differ among the experimental units ($p < 0.05$) and continued within the range considered as acceptable for tropical fish species.

The acute toxicity of propiconazole to *D. iguape* and *D. rerio* exposed to different concentrations of this pesticide for periods of up to 96 h, expressed as LC50, is shown in Table 1. These results showed that propiconazole produced higher toxicities in both species of fish.

Mortality

The percent mortality of *D. iguape* exposed to propiconazole at each 24-h interval is shown in Table 1. No deaths of control animals were observed. The higher the concentration of pesticide the fish was exposed

| Time of exposure | LC50 propiconazole (µg L⁻¹) | D. iguape | D. rerio | D. iguape | D. rerio | D. iguape | D. rerio | D. iguape | D. rerio | D. iguape | D. rerio |
|-----------------|-----------------------------|-----------|----------|-----------|----------|-----------|----------|-----------|----------|-----------|----------|
| 24 h            | 0.00                        | 0.00      | 0.00     | 0.00      | 0.00     | 33.33     | 26.66    | 80.00     | 100.00   | 100.00    | 0.58 (0.43–0.78) a |
| 48 h            | 0.00                        | 0.00      | 0.00     | 20.00     | 20.00    | 80.00     | 100.00   | 100.00    | 100.00   | 100.00    | 0.38 (0.24–0.52) a |
| 72 h            | 0.00                        | 0.00      | 0.00     | 13.33     | 13.33    | 53.33     | 80.00    | 100.00    | 100.00   | 100.00    | 0.07 (0.05–0.09) a |
| 96 h            | 0.00                        | 0.00      | 0.00     | 33.33     | 33.33    | 100.00    | 100.00   | 100.00    | 100.00   | 100.00    | 0.05 (0.04–0.06) a |

1 Averages on lines with different letters indicate significant differences for the LC50 ($p < 0.05$).
to, the higher the mortality observed. After being exposed to propiconazole, death was first observed at a concentration of 0.05 µg L⁻¹ in the first 72 h. Mortality rates of 100% were observed after a 24-h exposure period at concentrations of 1.0 µg L⁻¹ and were also 100% after 96 h at a concentration of 0.1 µg L⁻¹. Only 26.66% average mortality was observed during the first 24 h at 0.05 µg L⁻¹, while 100% mortality rates during the first 48 h.

Zebrafish exposed to propiconazole at each 24-h interval is shown in Table 1. No deaths of control animals were observed. Mortality rates of 100% were observed after a 24-h exposure period at concentrations of 1.0 µg L⁻¹ and were also 100% after 96 h at a concentration of 0.05 µg L⁻¹. Only 33.33% average mortality was observed during the first 24 h at 0.10 µg L⁻¹, while 100% mortality rates during the first 72 h.

Lethal concentration

The acute toxicity of propiconazole to D. iguape exposed to different concentrations of this pesticide for periods of up to 96 h, expressed as the lethal concentration (LC50), is 0.58 µg L⁻¹, 0.18 µg L⁻¹, 0.07 µg L⁻¹, and 0.05 µg L⁻¹ for the 24-, 48-, 72-, and 96-h exposures, respectively (Table 1). The lethal concentration calculated by Spearman-Karber analysis for zebrafish is 0.19 µg L⁻¹, 0.08 µg L⁻¹, 0.05 µg L⁻¹, and 0.03 µg L⁻¹ for the 24-, 48-, 72-, and 96-h exposures, respectively (Table 1).

Routine metabolism

The specific oxygen consumption for D. iguape acclimated at a temperature of 25 °C, initially increased at a concentration of 0.01 µg L⁻¹ and then decreased due to the increase in the concentration of propiconazole (Fig. 2). At the concentration of 0.1 µg L⁻¹, the specific oxygen consumption in relation to the exposure time decreases significantly in comparison with the control (Fig. 2). The decrease in the averages of specific oxygen consumption at a concentration of 0.1 µg L⁻¹ represented a 71% decrease in the metabolic rate compared to the control.

There was a significant increase in the averages of specific oxygen consumption at a concentration of 0.01 µg L⁻¹. Using the ANOVA statistical test (Tukey, p < 0.05), it was found that the average of specific oxygen consumption at a concentration of 0.05 µg L⁻¹ was not significantly different in relation to the time of exposure.

The specific oxygen consumption for D. rerio acclimated at a temperature of 25 °C also increases in the concentration of 0.01 µg L⁻¹ and then decreases due to the increase in the concentration of propiconazole (Fig. 2). There was a significant increase in the averages of specific oxygen consumption at a concentration of 0.01 µg L⁻¹. For 0.05 and 0.1 µg L⁻¹ concentrations, there were significant decreases. The decrease in the averages of specific oxygen consumption at a concentration of 0.1 µg L⁻¹ represented a 40% decrease in the metabolic rate compared to the control. Using the ANOVA statistical test (Tukey, p < 0.05), it is found that the averages of specific oxygen consumption at concentrations 0.05 and 0.1 µg L⁻¹ were significantly different in relation to the time of exposure (Fig. 2).

The excretion of ammonia for D. iguape acclimated to a temperature of 25 °C initially increases and then decreases as the concentration of propiconazole increased (Fig. 3). The decrease in the averages of ammonia excretion at a concentration of 0.1 µg L⁻¹
represented a 68.7% decrease in the metabolic rate compared to the control.

Using the ANOVA statistical test (Tukey, \( p < 0.05 \)), it is found that the mean of ammonia excretion at a concentration of 0.1 \( \mu g \ L^{-1} \) was significantly different in relation to the control (Fig. 3).

The results of ammonia excretion obtained from exposure to propiconazole showed a decrease in excretion rates for \( D. \) rerio. Using the ANOVA statistical test (Tukey, \( p < 0.05 \)), it is found that the mean of ammonia excretion at a concentration of 0.1 \( \mu g \ L^{-1} \) was significantly different in relation to the control (Fig. 3). The decrease in the averages of ammonia excretion at a concentration of 0.1 \( \mu g \ L^{-1} \) represented a decrease in the metabolic rate of around 45.4% in relation to the control.

When comparing the two species for each concentration of propiconazole tested, it is observed that for the excretion of ammonia, there was a significant difference (\( p < 0.05 \)) in the controls and for the highest concentration applied (0.1 \( \mu g \ L^{-1} \)). As for the specific oxygen consumption, there is only a statistically significant difference for the concentration of 0.1 \( \mu g \ L^{-1} \) (Table 2).

**Discussion**

Fish are excellent biological models to be used in the environmental monitoring of polluted and unpolluted aquatic environments (Damato and Barbieri, 2012). In addition, they can be found in most aquatic environments, playing an important ecological role in food chains (Cort and Ghisi 2014; Barbieri et al. 2019).

Fish of the Astyanax and Deuterodon genera, popularly known as \( D. \) iguape, have excellent potential as a bioindicator because they are very common, small, omnivorous specimens with considerable economic value and are beginning to be used in several studies for biomonitoring and bioassays in Brazil (Cort and Ghisi 2014).

In the present study, it was observed that \( D. \) iguape was a good biological model, responding well as a bioindicator, corroborating with other studies that also used \( D. \) iguape to study lethal and sublethal effects of pesticides (Erbe et al. 2010; 2012).

**Table 2** Comparison of ammonia excretion and specific oxygen consumption between Deuterodon iguape and Danio rerio in relation to the concentrations of propiconazole (\( \mu g \ L^{-1} \))

| Factor                  | Propiconazole concentration (\( \mu g \ L^{-1} \)) | t-statistic | df | \( p \)-value | \( p \)-signif |
|-------------------------|---------------------------------------------------|-------------|----|---------------|---------------|
| Ammonia excretion       | 0.00                                              | 6.565       | 5  | 0.006         | Ns            |
|                         | 0.01                                              | 6.565       | 5  | 0.006         | Ns            |
|                         | 0.05                                              | 6.565       | 5  | 0.006         | Ns            |
|                         | 0.10                                              | 6.565       | 5  | 0.006         | Ns            |
| Oxygen consumption      | 0.00                                              | 6.565       | 5  | 0.006         | Ns            |
|                         | 0.01                                              | 6.565       | 5  | 0.006         | Ns            |
|                         | 0.05                                              | 6.565       | 5  | 0.006         | Ns            |
|                         | 0.10                                              | 6.565       | 5  | 0.006         | Ns            |

\( df \) the degrees of freedom for the t-statistic
Bueno-Krawczyk et al. 2015; Galvan et al. 2016) and effects of other pollutants such as gasoline (Galvan et al. 2016) and carbofuran (Barbieri et al. 2019). Therefore, due to its availability and mainly its sensitivity to small changes in the aquatic environment that resulted in measurable changes, this fish can be used in Brazil as a biological model in biomonitoring studies and bioassays with pesticides.

According to Arias et al. (2007), in recent years, aquatic biota is constantly exposed to a large number of toxic substances released daily in open environments, without proper treatment, from different sources of emission. Among the pollutants present in the water are fungicides, such as propiconazole. These fungicides can cause mortality and alterations in the metabolism of fish, as observed in the results obtained in this study with tests carried out with D. iguape and zebrafish D. rerio, examining toxicity (LC50), specific oxygen consumption, and ammonia excretion. It is common for fish and other aquatic organisms to be subject to receiving water contaminated by pesticides because they are close to vegetable cultivation fields treated with these substances (Hernández-Moreno et al. 2011; Cobas et al. 2016).

The toxicity tests with D. rerio are recommended because it is an organism used worldwide as a standard to establish LC50-96 h for pesticides and other pollutants. The propiconazole LC50 was compared between D. rerio and D. iguape in order to recognize possible differences in sensitivity between species. According to the LC50-96 h value calculated in this study, the acute toxicity of propiconazole for D. iguape was similar to that observed for D. rerio, with values of 0.05 (0.04–0.06) and 0.03 (0.02–0.04) µg L⁻¹, respectively. In this case, we found no significant difference in sensitivity between D. iguape and D. rerio. However, the toxicity of propiconazole was approximately three times greater for D. rerio than for D. iguape, when the exposure period was 24 h. This result leads to the supposition that D. iguape is more tolerant to the fungicide in the first 24 h of exposure.

The toxicity of propiconazole to fish has not been well documented, especially for D. iguape and D. rerio. For example, Hemalatha et al. (2016) obtained the 96-h LC50 of propiconazole for the fish Labeo rohita at 8.9 µL L⁻¹, a toxicity value much higher than those recorded in the present study where the LC50 for D. iguape and D. rerio were 0.05 µg L⁻¹ and 0.03 µg L⁻¹, respectively. Wilfriel (2005) recorded 96-h LC50 values of propiconazole for various fish between 5.3 and 6.8 mg L⁻¹ (Oncorhynchus mykiss 5.3 mg L⁻¹, Cyprinus carpio 6.8 mg L⁻¹, and Lepomis macrochirus 6.4 mg L⁻¹).

Hernández-Moreno et al. (2011) argue that there is a great variability in the results of LC50 found for different species, and even within the same species, therefore, comparisons of results should be interpreted with caution to avoid erroneous conclusions possibly due to the applied test, the testing conditions, and stage of life of the exposed organisms, among other factors. However, the fact is that concentrations found in the environment of 12.90 mg L⁻¹ can be very harmful to fish (Teng et al. 2019).

In the tests carried out with lambari and zebrafish, propiconazole, when used alone, demonstrated a significant effect in reducing the specific consumption of oxygen and the excretion of ammonia in the highest concentrations tested.

The decrease in specific oxygen consumption is closely associated with a decrease in metabolism (Barbieri et al. 2019), observed during the experiments through the low consumption of individuals exposed to higher concentrations of propiconazole compared to those exposed to lower concentrations.

This study demonstrates the action of propiconazole as a potentially toxic substance for the metabolic functions of D. iguape and D. rerio fish. It was observed that the specific oxygen consumption and ammonia excretion decreased at the highest concentration (0.1 µg L⁻¹) for both fish. This physiological response to the presence of xenobiotics is directly associated with changes in metabolism and occurs due to the fish’s attempt to maintain its homeostasis (Barbieri et al. 2019). Atypical situations can stimulate protein synthesis not directly related to growth, such as stress and thermal shock, toxicity to metals, toxicity to pesticides, deprivation of nutrients, and metabolic disorders, among others (Mommsent, 1998; Damato and Barbieri 2012).

According to Rand and Petrocelli (1985), fish can absorb pesticides directly from the water, and the gills are the main absorbing organ. The decrease in specific oxygen consumption is closely associated with a decrease in metabolism, a fact observed during experiments carried out through the low mobility of individuals exposed to higher concentrations of pesticides (Campos-Garcia et al. 2016). According to Vargas et al. (1991), xenobiotics affect the breathing
processes of organisms by inducing them to use other sources of energy, which can be used for detoxification reactions and stabilization of metabolic patterns, which may explain the reduction in specific oxygen consumption to the extent where the concentration of propiconazole was increased.

The exposure of Nile tilapia Oreochromis niloticus to Folidol 600, an organophosphate insecticide whose active ingredient is methyl parathion, widely used in aquaculture tanks to eliminate predator insect larvae, promoted not only a significant reduction in oxygen consumption and an increase in ammonia excretion, but also an increase in hematological parameters (hematocrit and hemoglobin concentration) and an inhibition of cholinesterase activity (AChE, BChE, and PChE), as the concentration and exposure time increased. Lesions of the branchial tissue as well as the loss of ability to maintain homeostasis were identified as possible causes of these alterations. Both at low and high concentrations of the pesticide, cholinesterase activity was inhibited, which may reflect the fish’s attempt to compensate to stay alive (Barbieri and Ferreira 2011).

Campos-Garcia et al. (2016), in studies conducted with tilapia (O. niloticus), obtained an increase in specific oxygen consumption in individuals subjected to high concentration of carbofuran carbonate, which resulted in an increase in the metabolic rates of fish. A similar result was recorded by Barbieri et al. (2019) studying the effects of carbofuran on lambari Astyanax ribeirae.

In the tests for ammonia excretion performed with D. iguape and D. rerio, there was a statistically significant decrease in relation to the control when subjected to the presence of the fungicide, propiconazole, at the highest concentrations, demonstrating changes in the excretion of both fish. Barbieri and Ferreira (2011) identified changes in the excretion of ammonia in toxicity studies carried out with tilapia, O. niloticus, exposed to different concentrations of Folidol 600, which were similar to the results obtained in this study for D. iguape and zebrafish exposed to propiconazole. Arechon and Plumb (1990) and Heath et al. (1993) proposed that this response probably occurs due to a possible lesion in the branchial tissue, resulting in internal hypoxia and stimulation of erythropoiesis.

According to Mommsen (1998), atypical situations can stimulate protein synthesis that are not directly related to growth, such as stress from heat shock, nutrient deprivation, metabolic disorders, metal toxicity, viral infection, and others.

In freshwater fish, the final residues of protein metabolism are excreted mainly in the form of ammonia, and the mechanisms of this excretion are through gills and kidneys, and even in some fish species, the skin can perform this function (Bombardelli and Hayashi 2005). The results of ammonia excretion obtained from exposure to propiconazole showed a decrease in excretion rates; this fact suggests a decrease in protein metabolism as a mechanism to maintain the energy balance of fish submitted to propiconazole.

The fish Geophagus brasiliensis exposed to the herbicide 2,4-D (dichlorophenoxyacetic acid), a pesticide commonly used in pastures and in the control of aquatic macrophytes, showed a significant reduction in the consumption of dissolved oxygen in the water and an increase in ammonia excretion, probably related to the accumulation and metabolism of the contaminant in their lysosomes as well as other metabolic changes (Barbieri, 2008). Perhaps the same may have occurred with D. iguape and D. rerio, which had altered oxygen consumption and ammonia excretion, with a statistical difference between them only at the highest concentration (0.1 µg L⁻¹). This result may indicate a difference in sensitivity between the two species, although in relation to the control, the species at this concentration were statistically different.

Gills play an important role in transporting gases for respiration and in regulating the osmotic and ionic balance of fish (Tabassum et al. 2016). The gills are composed of a single layer of vulnerable delicate tissues that can be easily damaged by any xenobiotic dissolved or suspended in the media in which the organism lives (Guzmán-Guillén et al. 2015). The changes in oxygen consumption as well as in the ammonia excretion recorded in the present study may be related to the histopathological lesions observed by Tabassum et al. (2016), such as edema, hypertrophy of epithelial necrosis, bulging binge fusion of gill lamellae, and other degenerative changes in the vascularization of the gills that occur when fish are exposed to xenobiotics (Melo et al. 2015). In summary, propiconazole proved to be toxic to D. iguape and D. rerio causing significant changes in metabolism and toxicity in both species of fish.
In view of the increase and the diverse uses of pesticides in Brazil, their ecological effects must always be studied and evaluated. The risks of toxicity of pesticides to fish are fundamental mainly because they are consumed by human populations. The data obtained here from acute toxicity can be useful to evaluate the negative short-term results on *D. iguape*, a fish commonly used to feed riverside communities. These results suggest that metabolic responses can be used as potential biomarkers to monitor residual pesticides present in aquatic environments and provide useful parameters to assess the physiological effects in fish. This implies that the results can serve as parameters for taking necessary precautions in the application of pesticides to avoid problems related to the life cycle of fish and other aquatic organisms. Moreover, these results will be able to provide indices for further studies considering the environmental risk assessment. However, the application of these findings needs to be complemented with more detailed histological studies before they can be established as special biomarkers for monitoring the aquatic environment.

**Conclusion**

*Deuterodon iguape* and *Danio rerio* responded well to exposure of varying propiconazole concentrations, as observed through changes in oxygen consumption and ammonia excretion.

In environmental monitoring, fish are excellent biological models for comparing polluted and unpolluted areas. In addition, these animals can be found in almost any aquatic environment, playing an important ecological role in the food chain. *D. iguape* was found to be a good biological model, and that specific oxygen consumption and specific ammonia excretion were good physiological biomarkers for exposure to propiconazole.

Much remains to be studied about the mechanisms of interactions between propiconazole and other environmental factors such as pH, temperature, and hardness in the aquatic environment, and how fish are affected when they come into contact with this pesticide. Therefore, further studies are needed to better understand the potential environmental risks of exposure to propiconazole and especially the toxicokinetic and toxicodynamic characteristics of this xenobiotic in the environment.

**Authors’ contributions** MBH analyzed and interpreted data, was a major contributor in writing the manuscript, and was the author who submitted the manuscript. KFOR analyzed and interpreted data and was a contributor in writing the manuscript. LCB performed the statistical analysis and was a contributor in writing the manuscript. EB analyzed and interpreted data and was an important contributor in writing the manuscript. All authors read and approved the final manuscript.

**Funding** This study was financially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)—São Paulo Research Foundation (process 2018/19747–2) and the National Council for Scientific and Technological Development (CNPq, Brazil, for the productivity research grant, process no. 302705/2020–1).

**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code availability (software application or custom code)** Not applicable in this section.

**Declarations**

**Ethics approval and consent to participate** This study followed the ethical principles for animal experimentation adopted by the Brazilian School of Animal Experimentation (COBEA) and received authorization (no.14/2018) from the Ethics Committee on Animal Experimentation of the Fisheries Institute, São Paulo, Brazil.

**Consent for publication** Not applicable in this section.

**Competing interests** The authors declare that have no competing interests.

**References**

Adams SM (1990) Status and use of biological indicators for evaluating the effects of stress on fish. Am Fish Soc Symposium 8:1–8

Amsterdam A, Hopkins N (2006) Mutagenesis strategies in zebrafish for identifying genes involved in development and disease. Trends Genet 22:473–478. https://doi.org/10.1016/j.tig.2006.06.011

Areechon N, Plumb JA (1990) Sublethal effects of malathion on Chanell a catfish *IctalurusPunctatus*. B Environ Contam Tox 44:435–442. https://doi.org/10.1007/BF01701226
Arias ARL, Buss DF, Albuquerque C, Inácio AF, Freire MM, Egler M, Mugnai R, Baptista DF (2007) Utilização de bioindicadores na avaliação de impacto e no monitoramento da contaminação de rios e córregos por agrotóxicos. Cien Saude Colet 12:61–72. https://doi.org/10.1590/S1413-81322007000100011

Barbazuk WB, Korf I, Kadavi C, Heyen J, Tata S, Wun E, Bedell JA, McPherson JA, Johnson SL (2000) The syntenic relationship of the zebrafish and human genomes. Genome Res 10:1351–1358. https://doi.org/10.1101/gr.144700

Barbieri E (2007) Use of metabolism and swimming activity to evaluate the sublethal toxicity of surfactant (LAS-C12) on Mugil platanus. Braz Arch Biol Technol 50:101–112. https://doi.org/10.1590/S1516-89132007001000012

Barbieri E (2008) Effect of 2,4-D herbicide (2,4-dichlorophenoxyacetic acid) on oxygen consumption and ammonia excretion of juveniles of Geophagus brasiliensis (Quoy & Gaimard, 1824) (Osteichthyes, Cichlidae). Ecotoxicology 18:55–60. https://doi.org/10.1007/s10646-008-0256-3

Barbieri E, Doi SA (2012) Acute toxicity of ammonia on juvenile Cobia (Rachycentroncanadum, Linnaeus, 1766) according to the salinity. Aquacult Int 20:373–382. https://doi.org/10.1007/s10499-014-9467-3

Barbieri E, Ferrarini AMT, Rezende KFO, Martinez DST, Alves OL (2019) Effects of multiwalled carbon nanotubes and carbofuran on metabolism in Astyanax ribeirensis, a native species. Fish Physiol Biochem 45:417–426. https://doi.org/10.1007/s10695-018-0573-2

Barrera-Méndez F, Miranda-Sánchez D, Sánchez-Rangel D, Bonilla-Landa I, Rodríguez-Haas B, Monribot-Villanueva JB, Olivares-Romero JL (2019) Propiconazole nanocapsulation in biodegradable polymers to obtain pesticide controlled delivery systems. J Mex Chem Soc 63:50–60. https://doi.org/10.29356/jmcs.v63i1.564

Baun A, Jensen SD, Bjerg PL, Christensen TH, Nyholm N (2000) Toxicity of organic chemical pollution in groundwater downgradient of a landfill (Grindsted, Denmark). Environ Toxicol Chem 34:1647–1652. https://doi.org/10.1016/s0278-1121(00)01156-0

Bueno-Krawczyk ACD, Guiolosi IC, Piancini LDS, Azevedo JC, Ramsdorf WA, Guimarães ATB, Cestari MM, Silva de Assis HC (2015) Multibiomarker in fish to evaluate a river used to water public supply. Chemosphere 135:257–264. https://doi.org/10.1016/j.chemosphere.2015.04.064

Campos-Garcia J, Martinez DST, Rezende KFO, Da Silva JRMC, Alves OL, Barbieri E (2016) Histopathological alterations in the gills of Nile tilapia exposed to carbofuran and multiwalled carbon nanotubes. Ecotox Environ Safe 133:481–488. https://doi.org/10.1016/j.ecoenv.2016.07.041

Cobas M, Meijide J, Sanromán M, Pazos M (2016) Chestnut shells to mitigate pesticide contamination. J Taiwan Inst Chem Eng 61:166–173. https://doi.org/10.1016/j.tice.2015.11.026

Cort CCWD, Ghisi NC (2014) Uso de alterações morfológicas nucleares em Astyanax spp. para avaliação da contaminação aquática. Mundo Saúde 38:31–39. https://doi.org/10.15343/0103-7809.2014801031039

Damato M, Barbieri E (2012) Study on the acute toxicity and metabolic changes caused by cadmium exposure on the fish Hyphessobrycon callistus used as an indicator of environmental health. Mundo Saúde 36:574–581. https://doi.org/10.15343/0103-7809.2012364574580

Ferrarini AMT, Rezende KFO, Barbieri E (2016) Use of swimming capacity to evaluate the effect of mercury on Pocelia vivipara (Poceliídeos) according to salinity and temperature. J Mar Biolo Oceanogr 5:1–5. https://doi.org/10.4172/2324-8661.1000163

Erbe MCL, Ramsdorf WA, Vicari T, Cestari MM (2010) Toxicity evaluation of water samples collected near a hospital waste landfill through bioassays of genotoxicity piscine micronucleus test and comet assay in fish Astyanax and ecotoxicity Vibrio fischeri and Daphnia magna. Ecotoxicology 20:320–328. https://doi.org/10.1007/s10646-010-0581-1

Fonseca T, Costa-Pierce BA, Valenti WC (2017) Lambiri aquaculture as a means for the sustainable development of rural communities in Brazil. Rev Fish Sci Aquac 25:316–330. https://doi.org/10.1007/s12588-017-0026-0

Friberg N, Lindstrøm M, Kronvang B, Larsen SE (2003) Macroriververtebrate/sediment relationships along a pesticide gradient in Danish streams. Hydrobiologia 494:103–110. https://doi.org/10.1023/A:1023014.97817.801030

Galvan GL, Lirola JR, Felisbino K, Vicari T, Yamamoto CI, Cestari MM (2016) Genetic and hematologic endpoints in Astyanax altiparanae (Characidae) after exposure and recovery to water-soluble fraction of gasoline (WSFG). B Environ Contam Tox 97:63–70. https://doi.org/10.1007/s00128-015-2544-6

Garrison AW, Avants JK, Miller RD (2011) Loss of propiconazole and its four stereoisomers from the water phase of two soil-water slurries as measured by capillary electrophoresis. Int J Environ Res Public Health 8:3453–3467. https://doi.org/10.3390/ijerph8083453

Ghormade V, Deshpande MV, Paknikar KM (2011) Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnol Adv 29:792–803. https://doi.org/10.1016/j.biotechadv.2011.06.007

Guzmán-Guillén R, Prieto Ortega AL, Gutiérrez-Praena D, Moreno IM, Movano R, Blanco A, Cameán AM (2015) Vitamin E pretreatment prevents histopathological effects in tilapia (Oreochromis niloticus) acutely exposed to cylindrosporin. Environ Toxicol 32:1469–1485. https://doi.org/10.1002/tox.22152

Hamilton MA, Russo RC, Thurston RV (1978) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 12:417. https://doi.org/10.1021/es60140a017

Hanada R, Gasparotto L, Moreira A (2015) Avaliação da sensibilidade de Mycosphaerella fijiensis oriundos de plântanos
Henriques MB, Fagundes L, Petesse ML, Silva NJR, Rezende KFO, Barbieri E (2018) Lambari fish *Deuterodon iguape* Eigenmann 1907 as an alternative to live bait for estuarine recreational fishing. Fish Manag Ecol 25:400–407. https://doi.org/10.1111/fme.12308

Henriques MB, Carneiro JS, Fagundes L, Castilho-Barros L, Barbieri E (2019) Economic feasibility for the production of live baits of lambari (*Deuterodon iguape*) in recirculation system. Bol Inst Pesca 45:e516. https://doi.org/10.20950/1678-2305.2019.45.4.516

Hernández-Moreno D, Pérez-López M, Soler F, Gravato C, Martinez DST, Alves OL, Barbieri E (2013) Carbon nanotubes enhanced the lead toxicity on the freshwater fish *Labeo rohita*. Biocatal Agric Biotechnol 8:321–327. https://doi.org/10.1016/j.bcab.2016.10.009

Kreuger J (1998) Pesticides in stream water within an agricultural catchment in southern Sweden, 1990–1996. Sci Total Environ 216:227–251. https://doi.org/10.1016/s0048-9697(98)00155-7

Keshari V, Adeeb B, Simmons AE, Simmons TW, Diep CQ (2016) Zebrafish as a model to assess the teratogenic potential of nitrite. J Vis Exp 108:e53615. https://doi.org/10.3791/53615

Koroleff F (1970) Direct determination of ammonia in natural waters as indophenol blue. Information on techniques and methods for seawater analysis (and laboratory report). J Cons Int Explor Mer 3:19–22

Kreuger J (1998) Pesticides in stream water within an agricultural catchment in southern Sweden, 1990–1996. Sci Total Environ 216:227–251. https://doi.org/10.1016/s0048-9697(98)00155-7

Kronvang B, Laubel A, Larsen SE, Friberg N (2003) Pesticides and heavy metals in Danish streamed sediment. Hydrobiologia 494:93–101. https://doi.org/10.1023/A:1025441610434

Lanchote VF, Bonato PS, Cerdeira AL, Santos NA, Carvalho D, Gomes MA (2000) HPLC screening and GC-MS confirmation of triazine herbicide residues in drinking water from sugar cane area in Brazil. Water Air Soil Pollut 118:329–337. https://doi.org/10.1023/A:1005147405509

Lopes FP, Pereira BF, Alves RMS, Valim BJT, Figueiredo FAT, Pitol DL, Caetano FH (2017) Ultramorphological changes in gill rakers of *Astonaxan altiparanae* (Characidae) kept in contaminated environments. Fish Physiol Biochem 43:1033–1041. https://doi.org/10.1007/s10169-017-0350-7

Martinez DST, Alves OL, Barbieri E (2013) Carbon nanotubes enhanced the lead toxicity on the freshwater fish. J Phys Conf Ser 429:012043. https://doi.org/10.1088/1742-6596/429/1/012043

Melo KM, Oliveira R, Grisolia CK, Domingues I, Pieczarka JC, de Souza FJ, Nagamachi CY (2015) Short-term exposure to low doses of rotenone induces developmental, biochemical, behavioral, and histological changes in fish. Environ Sci Pollut Res Int 22:13926–13938. https://doi.org/10.1007/s11356-015-4596-2

Mommens TP (1998) Growth and metabolism. In: Evans D (ed) The Physiology of Fishes, Second edition. CRC Press, Boca Raton, pp 65–100

Ouadah-Boussouf N, Babin PJ (2016) Pharmacological evaluation of the mechanisms involved in increased adiposity in zebrafish triggered by the environmental contaminant tributylin. Toxicol Appl Pharmacol 294:32–42. https://doi.org/10.1016/j.taap.2016.01.014

Rand GM, Petrocelli SR (1985) Fundamentals of aquatic toxicology: methods and applications. United States: p. 335–373

Rezende KFO, Bergami E, Alves KVB, Corsi I, Barbieri E (2018) Titanium dioxide nanoparticles alters routine metabolism and causes histopathological alterations in *Oreochromis niloticus*. Bol Inst Pesca 44:e343. https://doi.org/10.20950/1678-2305.2018.343

Ruiz-Hidalgo K, Masis-Mora M, Barbieri E, Carazo-Rojas E, Rodríguez-Rodriguez CE (2016) Ecotoxicological analysis during the removal of carbofuran in fungal bioaugmented matrices. Chemosphere 144:864–871. https://doi.org/10.1016/j.chemosphere.2015.09.056

Sabik H, Jeannot R, Rondeau B (2000) Multiresidue methods using solid-phase, extraction techniques for monitoring priority pesticides, including triazines and degradation products, in ground and surface waters. J Chromatogr A 885:217–236. https://doi.org/10.1016/S0021-9673(99)01084-5

Satapute PP, Kaliwal BB (2018) *Burkholderia* Sp. strain BBK_9: a potent agent for propiconazole degradation. In: Bidoia ED, Montagnolli RN (eds) Toxicity and Biological Degradation Testing, Methods in Pharmacology and Toxicology. Humana Press, New York, pp 87–108

Spence R, Gerlach G, Lawrence C, Smith C (2008) The behavior and ecology of the zebrafish, *Danio rerio*. Biol Rev Camb Philos Soc 83:13–34. https://doi.org/10.1111/j.1469-185X.2007.00030.x

Statista (2018) Banana production in Brazil. https://www.statista.com/statistics/987946/banana-production-volume-brazil/. Accessed 26 Dec 2020

Tabassum H, Dawood AQ, Sharma P, Khan J, Raisuddin S, Parvez S (2016) Multi-organ toxicological impact of fungicide propiconazole on biochemical and histological profile of freshwater fish *Channa punctata* Bloch. Ecol Indic 63:359–365. https://doi.org/10.1016/j.ecolind.2015.11.052
Vargas VMF, Guidobono RR, Henriques JAP (1991) Genotoxicity of plant extracts. Mem Inst Oswaldo Cruz 86:67–70. https://doi.org/10.1590/S0074-0276199100600017

Wilfriel P (2005) Directory of microbicides for the protection of materials: a handbook. Ed. Wilfried Paulus, Springer

Winkler LW (1888) Methods for measurement of dissolved oxygen. Ber Dtsch Chem Ges 21:2843–2854. https://doi.org/10.1002/cber.188802102122

Wu QL, Riise G, Kretzschmar R (2003) Size distribution of organic matter and associated propiconazole in agricultural runoff material. J Environ Qual 32:2200–2206. https://doi.org/10.2134/jeq2003.2200

Zagatto PA, Bertoletti E (2010) Ecotoxicologia Aquática - Princípios e Aplicações, 2 ed. Editora Rima, São Paulo, pp 1–13

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.