Sublethal Effects of Propiconazole On The Metabolism of Lambari Deuterodon Iguape (Eigenmann 1907), A Native Species From Brazil

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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. There was an increase and decrease in the metabolic rate at 0.01 and 0.1 μg L^{-1}, with a statistical difference from the control ratio for the two species. The same trend towards ammonia excretion was observed, initially increasing to a concentration of 0.01 μg L^{-1} and then decreasing as the concentration of propiconazole was increased. The averages of specific ammonia excretion in all concentrations were significantly different compared to the control. It was concluded that exposure to propiconazole increased the metabolic rates of *D. Iguape* and *D. rerio*, up to 0.05 μg L^{-1}, and decreased the rates when the concentration was doubled (0.1 μg L^{-1}); causing serious problems in fish metabolism. *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*.

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 carbofurans (Campos-Garcia et al. 2016; Ruíz-Hidalgo et al. 2016).

Propiconazole, (1 - [[2- (2,4-dichlorophenyl) -4-propyl-1,3-dioxolan-2-yl] methyl] -1H-1,2,4-triazole), 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 (*Mycospaerella 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. According to Baun et al. (1999), 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

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.

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 (24º12'25”S; 47º02’48”W). The *Danio rerio* zebra fish (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 (23º59'24”S; 46º18'23”W)

In the laboratory, a total of 120 fish of each species were kept for five days in independent 500-liter tanks, with constant aeration and daily water change (20%) to acclimate them to the conditions of the laboratory. The fresh water used for maintenance, passed

through three 2µm filters, two 1µm filters and one 0.5µm 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 hours before the experiments.

Tested substance

In this study, the main element, propiconazole (100 ng μL^{-1} in methanol, PESTANAL®, analytical standard - Empirical Formula $C_{15}H_{17}Cl_2N_3O_2$) (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 hours, with partial renewal (semi-static) and with twelve hours 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; 2.5 µg L^{-1}, in addition to a control treatment (without propiconazole). Mortality was recorded every two hours until the first 12 hours 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). 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 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 two hours. Five fish of each species for each concentration were subjected to measurement of oxygen consumption and ammonia excretion in each of the four concentrations (Barbieri and Ferreira 2011).

Before the beginning of the experiments, the animals were kept in respirometers with continuous water circulation for at least 60 minutes, 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 one hour (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 (Barbieri and Ferreira 2011). 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

The behavioral data were evaluated according to the means and standard deviations obtained by the statistical analysis of ANOVA (one-way), after checking the normal distributions and homoscedasticity (Levene test). The means and standard deviation of the routine metabolism data were submitted to the Shapiro-Wilk normality test and Levene's homoscedasticity test. Subsequently, the ANOVA test and Tukey's multiple comparison tests were applied. For both experiments, the differences were considered significant when $p < 0.05$.

Results

During the entire experiment, the average temperature was 24.7 ± 0.6ºC. 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 were exposed 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.

Medium 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 medium lethal concentration (LC50), were 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 were 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).

Table 1
Mortality percentage (%) of *Deuterodon iguape* lambari and zebrafish *Danio rerio* exposed to increasing concentrations of propiconazole (μg L^{-1}) for 96 h and the average Lethal Concentration (LC_{50} with 95% confidence interval) calculated by the Spearman-Karber analysis[1]

Time of exposure	**0.00**		**0.05**		**0.10**		**0.50**		**1.00**		**LC_{50} propiconazole (μg L^{-1})**	
	D. iguape	*D. rerio*	*D. iguape*	*D. rerio*	*D. iguape*	*D. rerio*	*D. iguape*	*D. rerio*	*D. iguape*	*D. rerio*	*D. iguape*	*D. rerio*
24h	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]	0.19 (0.13–0.28)[b]
48h	0.00	0.00	0.00	20.00	20.00	80.00	100.00	100.00	100.00	100.00	0.18 (0.14–0.23)[a]	0,08 (0.06–0.10)[a]
72h	0.00	0.00	13.33	53.33	80.00	100.00	100.00	100.00	100.00	100.00	0.07 (0.06–0.09)[a]	0.05 (0.04–0.06)[a]
96h	0.00	0.00	33.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.05 (0.04–0.06)[a]	0.03 (0.02–0.04)[a]

[1]Averages on lines with different letters indicate significant differences for the LC_{50} ($p < 0.05$)

The specific oxygen consumption for lambaris acclimated to a temperature of 25ºC decreased due to the increase in the concentration of propiconazole (Fig. 2).

At the concentration 0.1 μg L^{-1}, the specific oxygen consumption in relation to the exposure time decreased significantly (Fig. 2). There was a significant increase in the averages of specific oxygen consumption at concentrations 0.01 and 0.05 μg L^{-1}. For 0.1 μg L^{-1} there was a significant decrease. Using the ANOVA statistical test (Tukey, $p < 0.05$), it was found that the averages of specific oxygen consumption at concentrations 0.01 and 0.05 μg L^{-1} are not significantly different in relation to the time of exposure.

The excretion of ammonia for *D. iguape* acclimated to a temperature of 25ºC initially increased and then decreased 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 35% decrease in the metabolic rate compared to the control.

Using the ANOVA statistical test (Tukey, $p < 0.05$), it was found that the means of ammonia excretion at concentrations of 0.01 and 0.05 μg L^{-1} were significantly different in relation to the control and the 0.1 μg L^{-1} concentration (Fig. 3).

As for lambari *D. iguape*, the specific oxygen consumption for zebrafish *D. rerio* acclimated at a temperature of 25ºC also decreased due to the increase in the concentration of propiconazole (Fig. 4).

There was a significant increase in the means of specific oxygen consumption at a concentration of 0.01 μg L^{-1}. For 0.05 and 0.1 μg L^{-1} there was a significant decrease. Using the ANOVA statistical test (Tukey, $p < 0.05$), it was found that the averages of specific oxygen consumption at concentrations 0.05 and 0.1 μg L^{-1} are not significantly different in relation to the time of exposure (Fig. 4).

The results of ammonia excretion obtained from exposure to propiconazole showed a decrease in excretion rates for *D. rerio*, however at intermediate concentrations of propiconazole the excretion of ammonia peaked for *D. iguape*. The excretion of ammonia for *D. rerio* acclimated at a temperature of 25ºC initially increased and then decreased as the concentration of propiconazole increased (Fig. 5). The decrease in the averages of ammonia excretion at a concentration of 0.1 μg L^{-1} represented a decrease in the metabolic rate of around 60% in relation to the control.

Using the ANOVA statistical test (Tukey, $p < 0.05$), it was found that the averages of ammonia excretion at concentrations of 0.01, 0.05 and 0.1 μg L^{-1} were significantly different compared to the control (Fig. 5).

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. 2018).

Fish of the *Astyanax* and *Deuterodon* genera, popularly known as lambaris, 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 lambaris to study lethal and sublethal effects of pesticides (Erbe et al. 2010; 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 lambari *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 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 macrochiurus* 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, 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, 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.

Campos-Garcia et al. (2016), in studies conducted with tilapia (*Oreochromis 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 lambari *D. iguape* and zebrafish *Danio 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 lambaris and zebrafish exposed to propiconazole. Areechon 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.

Conclusion

The fish, *Deuterodon iguape*, proved to be a good bioindicator for studying polluted areas in environmental monitoring when compared to the zebrafish, *Danio rerio*. Both fish responded well to variations in propiconazole concentrations, through changes in oxygen consumption and ammonia excretion, when exposed to the contaminant.

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.

Declarations

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Competing interests

The authors declare that they have no competing interests in this section.

Availability of data and materials

The datasets used and/or analysed 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

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, was a contributor in writing the manuscript. LCB performed the statistical analysis, 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."

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.

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Figures

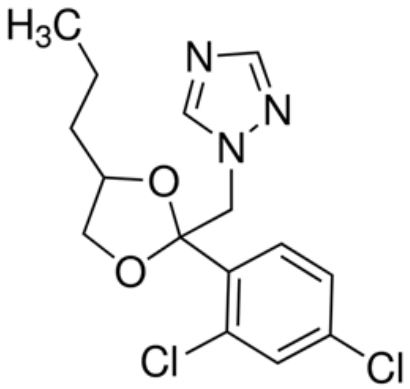

Figure 1

Chemical structure of Propiconazole (https://www.sigmaaldrich.com)

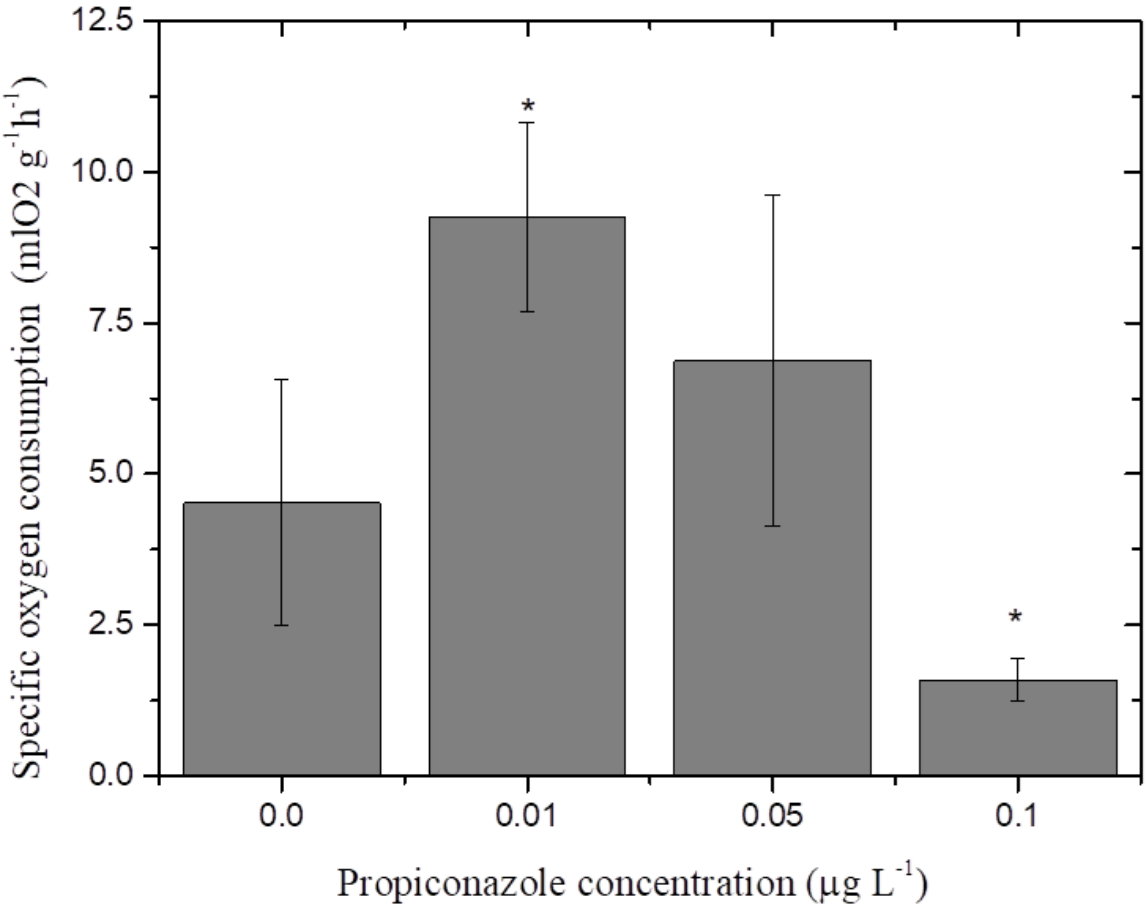

Figure 2

Specific oxygen consumption of Deuterodon iguape in relation to the concentration of propiconazole (µg L-1) and the time of exposure. The bars are the respective standard deviations and the asterisks represent the concentrations where there was a statistical difference in relation to the control

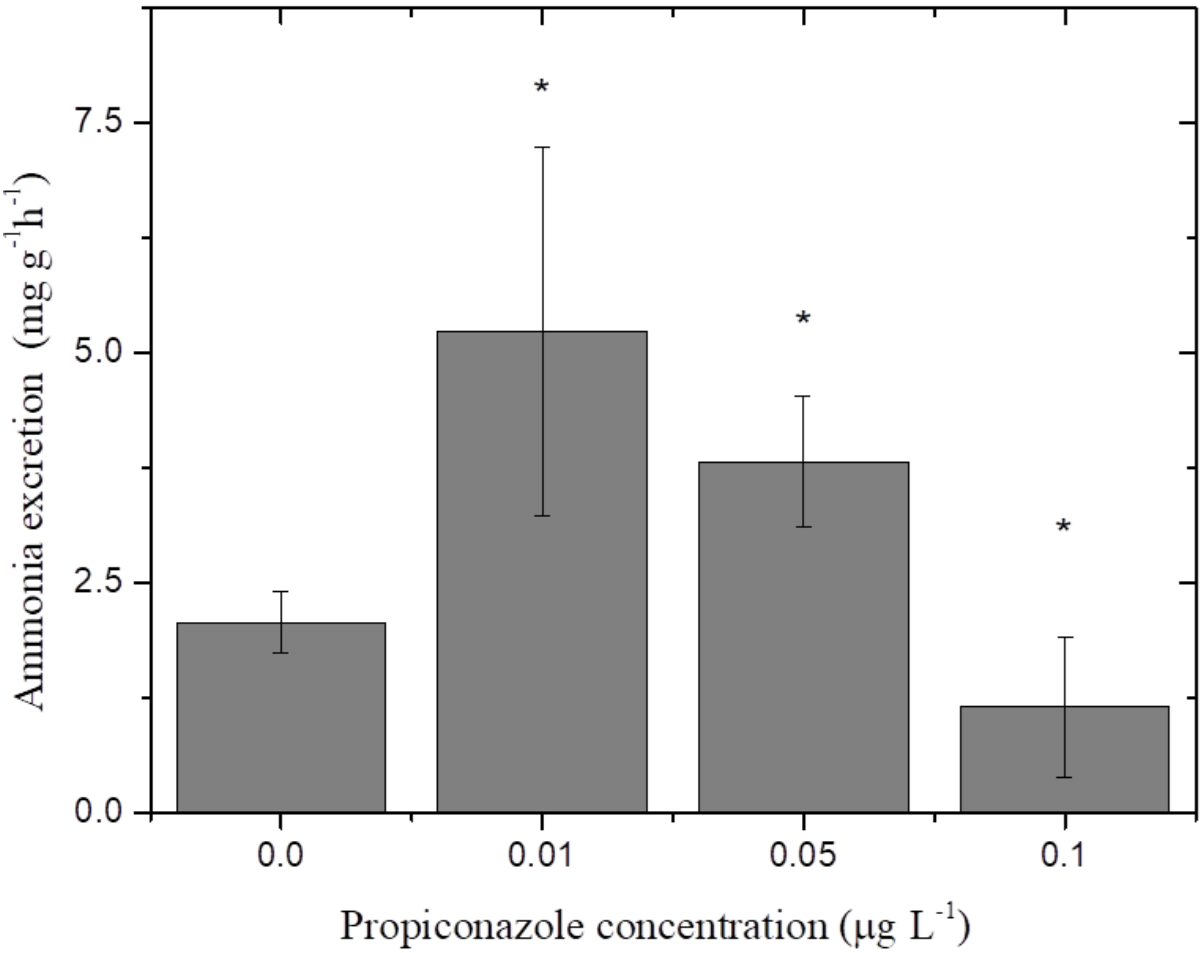

Figure 3

Excretion of ammonia from Deuterodon iguape in relation to the concentration of propiconazole (µg L-1). The bars are the respective standard deviations and the asterisks represent the concentrations where there was a statistical difference in relation to the control

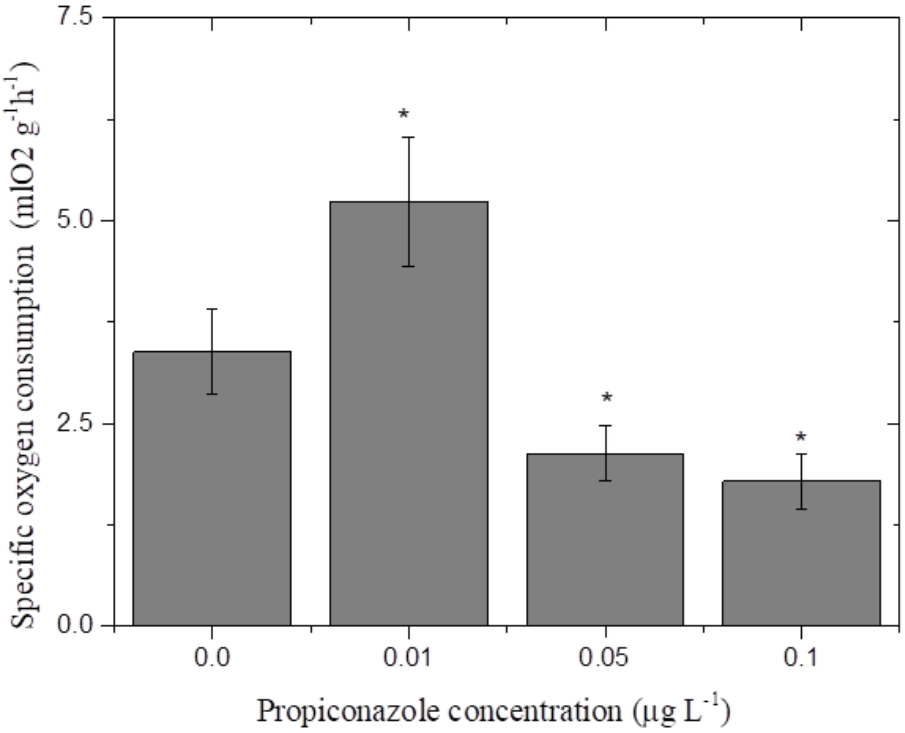

Figure 4

Specific oxygen consumption of zebrafish Danio rerio in relation to the concentration of propiconazole (µg L-1) and the time of exposure. The bars are the respective standard deviations and the asterisks represent the concentrations where there was a statistical difference in relation to the control

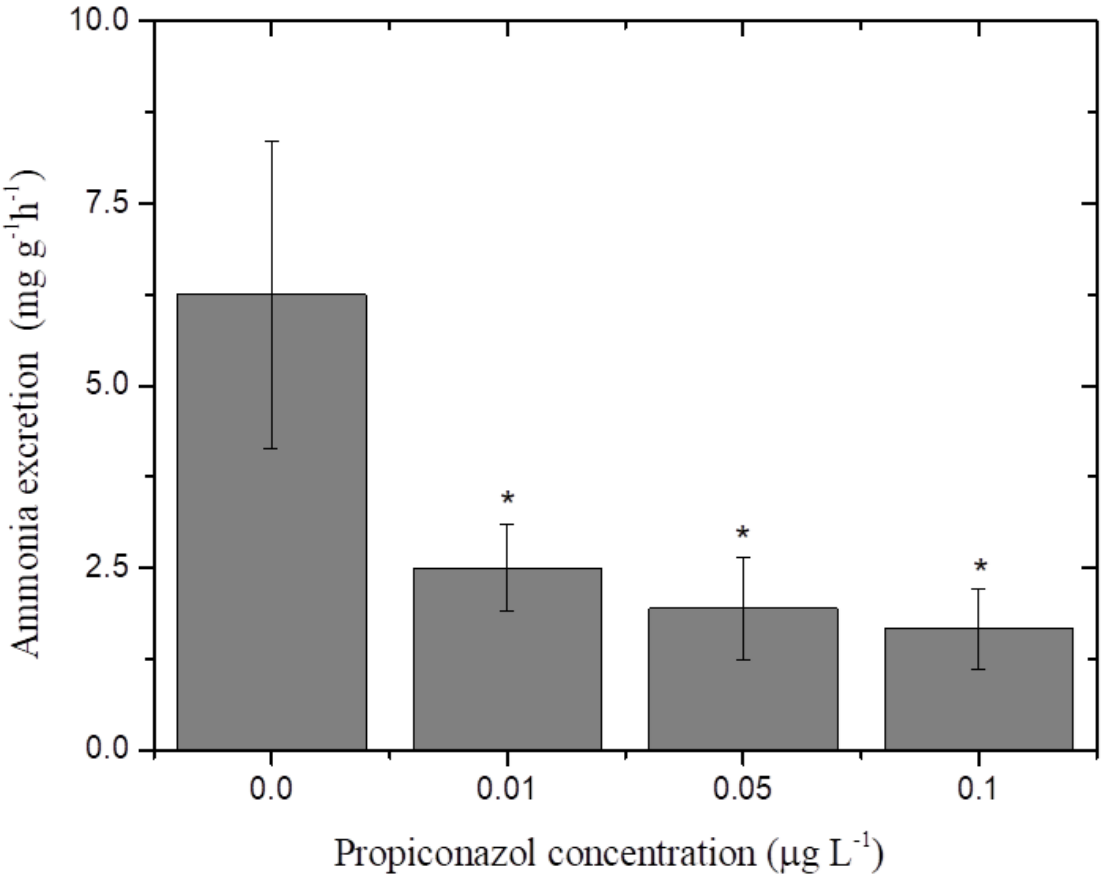

Figure 5

Ammonia excretion of zebrafish Danio rerio in relation to the concentration of propiconazole (µg L-1). The bars are the respective standard deviations and the asterisks represent the concentrations where there was a statistical difference in relation to the control