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Effects of pyrimethanil fungicide on Chironomidae community structure

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Abstract: The use of agrochemicals in agriculture may impact aquatic ecosystems, particularly influencing the stream insect communities. Among aquatic insects, the family Chironomidae is the most abundant and species-diverse insect group found in freshwater ecosystems. However, in the southern hemisphere, studies with Chironomidae are still sparse, compared to Europe and North America. The present study evaluates the responses of Chironomidae species (Insecta: Diptera) to pyrimethanil fungicide in a mesocosm experiment. Water contamination and chironomid community were monitored over 10 months. After five months of monitoring, the pyrimethanil fungicide was completely degraded and there was a statistically significant increase in the Margalef Richness and Shannon-Wiener Index (H') in the control units when compared with the contaminated mesocosms (p = 0.003). Our results point out that the utilization of agrochemicals can be a harmful factor influencing negatively the Chironomidae populations. This finding has key implications for insect conservation strategies and ecological management environments.

Key words: agrochemicals, aquatic insects, Chironomidae, agriculture, aquatic pollution.

INTRODUCTION

Agrochemicals pollution from farming exerts a toxic impact in the soil in the adjacent areas and on freshwater macroinvertebrates, frequently causing loss of sensitive insect orders such as Plecoptera (stoneflies), Ephemeroptera (mayflies) and Trichoptera (caddisflies) (Karouna-Renier & Sparling 2001, Crisci-Bispo et al. 2007, Corbi et al. 2013). The occurrence of agrochemicals in aquatic systems continues to create major risks to non-target aquatic species (Liu et al. 2013, Shinn et al. 2015). Ecological disturbance caused by agrochemicals can potentially impact the microalgae community and therefore the system's primary productivity (Ferraz et al. 2004). Species richness is similarly influenced by these anthropogenic disturbances, which may lead to losses of taxa and cause spatial discontinuities in expected gradients (Bojsen & Jacobsen 2003, Okano et al. 2017). Numerous freshwater biomonitoring plans use the macroinvertebrate community, particularly aquatic insects, as indicators of pollution, habitat modification, and water quality (Rosenberg 1992, Cranston 1995, Roque et al. 2000, Roy et al. 2003, Bonada et al. 2006, Carter et al. 2006, Hauer & Resh 2006, Corbi & Trivinho-Strixino 2008, 2017, Ferrington 2008, Corbi et al. 2011, Molozzi et al. 2012, Nicacio & Juen 2015, Vanacker et al. 2018, Dodds & Whiles 2020).

In most freshwater benthic communities, the Chironomidae is the most abundant and diverse insect group in freshwater systems (Rosenberg 1992, Cranston 1995, Roque et al. 2010, Corbi & Trivinho-Strixino 2017). Chironomids are one of the most ubiquitous and species rich families of aquatic insects (Ferrington 2008, Merritt et al. 2008) and have received consideration by researchers worldwide due to their abilities as biological indicators of environmental conditions (Pinder 1986). A variety of chironomids have known tolerances to environmental gradients, which can be used to infer impacted ecosystems (Courtney & Merritt 2008, Tang et al. 2009, Roque et al. 2010, Eggermont & Heiri 2012, Corbi & Trivinho-Strixino 2017, Corbi et al. 2019). The status of chironomid responses to fluctuations in the aquatic systems could permit their utilization to monitor streams, lakes and ponds (Rosenberg 1992). However, as pointed out by Nicacio & Juen (2015), in the southern hemisphere, studies with Chironomidae are still sparse, compared to Europe and North America.

In recent years, the expansion of agricultural activities has resulted in increased use of pesticides and fertilizers (Corbi et al. 2006, 2018). Among these substances, pyrimethanil – N-(4,6-dimethylpyrimidin-2-il)-anilin fungicide is widely used in several types of agricultural crops around the world, as sugarcane and citrus (Verdisson et al. 2001, Anfossi et al. 2006, Seeland et al. 2013). The pyrimethanil (4,6-dimethyl-N-phenyl-2-pyrimidinamine) is one of the fungicides most widely used in monocultures and has been detected in aquatic ecosystems (Shinn et al. 2015, Araújo et al. 2015, Müller et al. 2019). According to EFSA (European Food Safety Authority 2006), pyrimethanil is a substance with minimal risk to human health, has poor water solubility and short degradation time, is not degraded by hydrolysis or photolysis in the aquatic environment. This fungicide can penetrate the plant cuticle where it is applied, acting to inhibit the secretion of fungal enzymes, which ultimately reduces dispersion of the fungus (EFSA 2006).

Among the recent studies and research relating to this agrochemical, Seeland et al. (2012), evaluated the effects of pyrimethanil in aquatic macroinvertebrates through ecotoxicological tests and showed that thermal and multigenerational effects should be considered when evaluating the ecotoxicity of pesticides and concluded that acute pyrimethanil -toxicity on *Chironomus riparius* increased with high temperature. Müller et al. (2012), evaluated the environmental risk of pyrimethanil in the context of climate changes, in the *C. riparius*, and they demonstrated that not only the impact of climate change, but also low concentrations of pesticides may pose a reasonable risk for aquatic insects in future. Shinn et al. (2015) studied the effects of pyrimethanil in the growth of algae *Selenastrum capricornutum* and showed that the presence of pyrimethanil in an aquatic system can cause immediate impact if it reaches concentrations close to 1.0 mg L^{-1} and Araújo et al. (2014), studied avoidance of fish (*Danio rerio*) in different concentrations of this fungicide. In this context, Araújo et al. (2015), also demonstrates that pyrimethanil is potentially toxic for many aquatic species, affecting survival, reproduction, feeding, growth, and that it can disturb the environmental quality; Baglieri et al. (2016) analyzed the possible use of *Chlorella vulgaris* and *Scenedesmus quadricauda* microalgae to degrade pyrimethanil fungicide in water; Colombo et al. (2017) concluded that the pyrimethanil fungicide applications, in agriculture cultivation, may lead to a decrease in *Chironomus sancticaroli* genetic diversity and Müller et al. (2019), studied the impact of pyrimethanil in the aquatic primary producers and shows that the growth of key structural macroalgae and macrophytes was affected by the fungicide. However, even with these studies, little is known about the relationship and influence of pyrimethanil fungicide in the structure and dynamics of the Chironomidae community.

The present study aimed to evaluate, through experimental field units, the effects of pyrimethanil fungicide on Chironomidae (Insecta: Diptera) community structure. The present study hypothesizes that the application of the fungicide pyrimethanil in agricultural areas, causes changes in the Chironomidae community structure of adjacent aquatic environment.

MATERIALS AND METHODS

Mesocosms and water monitoring

The present study was conducted at the Center for Water Resources and Applied Ecology (CRHEA), School of Engineering of São Carlos (EESC), University of São Paulo (USP), Brazil.

The mesocosm arrangement was composed of two treatments: non-contaminated controls and pyrimethanil treated tanks. Each mesocosm system consisted of 1500 L cylindrical tanks with 1.43/1.75 m (bottom/top) diameter and 0.83 m height, separated by 2 meters each one. All mesocosms had a layer of natural sediment and were filled with water pumped from the Lobo Reservoir (Itirapina, SP, Brazil) 22°10’0.5” (S) and 47°54”10.51” (O) (Shinn et al. 2015). Throughout this same period (October 2012) the water source was known to be non-toxic to the fish *Danio rerio* and tadpoles of *Leptodactylus latrans*, *Lithobates catesbeianus* and Cladocera *Ceriodaphnia silvestrii* (Shinn et al. 2015). In order to avoid allochthonous material, the mesocosms were allocated 15 centimeters above the ground level. Water was removed from the Lobo Dam using a suction pump. To minimize the risk of overflows due to precipitation, holes were drilled at the edge of each mesocosm, in order to allow surplus water to escape. Subsequently, specimens of aquatic macrophytes rooted in the sediment at the same volume in each mesocosms.

The commercial formulation Mythos®, which contains 300 $g.L^{-1}$ of pyrimethanil as active ingredient, was dissolved in distilled water and added to the treated mesocosms to a final nominal pyrimethanil concentration of 1 $mg.L^{-1}$, which corresponds to the chronic LOEC (lowest observed effect concentration) for *Daphnia magna* species reproduction (Seeland et al. 2012). The mesocosms were organized in two groups, each with three replicates. Units 1, 3 and 4 served as treatments and the second group served as controls (units 2, 5 and 6). The mesocosms contamination occurred in October 20th, 2012 (Figure 1). The use of the commercial formulation instead of pure active ingredient was preferred as we consider it the most environmentally relevant approach to be tested in outdoor mesocosm systems (Shinn et al. 2015).

Physical and chemical variables, such pH, temperature, conductivity and dissolved oxygen, were measured with the aid of a multiparameter water quality probe (Horiba® U10). Water monitoring was performed monthly over the period between October 2012 and August 2013. Pyrimethanil concentrations were confirmed and determined by high-performance liquid chromatography – (HPLC) according to Müller et al. (2012). The analyses for pyrimethanil determination concentrations were carried out at the Chemistry Institute of the University of São Paulo (IQSC/USP).

Chironomidae sampling

Chironomidae fauna were collected monthly, with a core sampler, during October 2012 and August 2013. Following the sampling procedures, the material collected was stored in 70% alcohol. Chironomidae were identified to morphospecies using appropriate keys (Trivinho-Strixino & Strixino 1995, Trivinho-Strixino 2011). The individuals collected were quantified to determine the chironomids community structure present in each mesocosm. After characterizing the aquatic community, the Margalef richness and Shannon-Wiener diversity index (H') were assessed.

Statistical Analysis

The Margalef Richness Index and Shannon-Wiener Diversity Index, which assessed differences between control and treatment mesocosms, were calculated using the PAST (Paleontological statistics) software, version 3.14 (Hammer et al. 2001). We also applied a t-tests (α = 0.05), to analyze the significative differences in these indices between contaminated and control units. The comparison between the water variables was performed using t-tests (α = 0.05), with the aid of software R Project, version 3.3.1. (R Core Team 2016).

RESULTS

Water analyses and pyrimethanil concentration

The analysis related to the water variables showed no statistically significant differences between controls and treatment mesocosms. At the beginning of the monitoring, the pyrimethanil concentrations found in the three treatment mesocosms showed similar values to 1.40 (0.06) mg.L^{-1}. The complete degradation of pyrimethanil occurred in the fifth month of monitoring (Figure 1). The p-values for pH, temperature, dissolved oxygen and conductivity were all higher than 0.05.

Chironomidae community

In total, 1263 individuals from two Chironomidae subfamilies were collected: Chironominae and Tanypodinae. Seven different genera from eight distinct species were identified. The individuals and their occurrence and distribution in the mesocosms are listed in Table I and Figure 2.

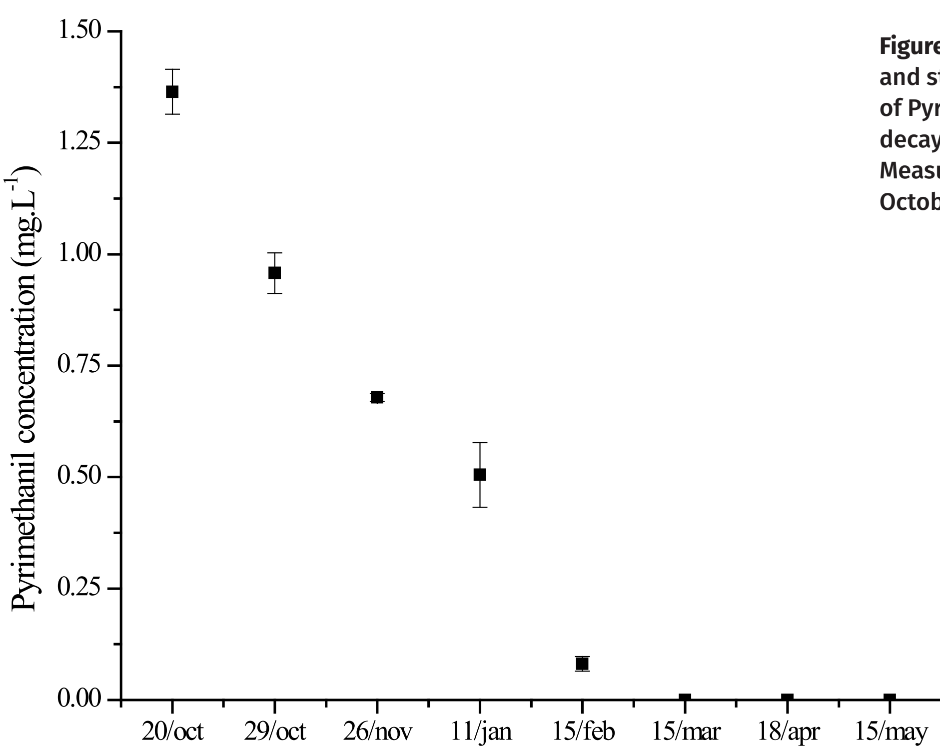

Figure 1. **Mean concentrations and standard deviation of Pyrimethanil fungicide decay in treated mesocosms. Measures obtained from October 2012 to May 2013.**

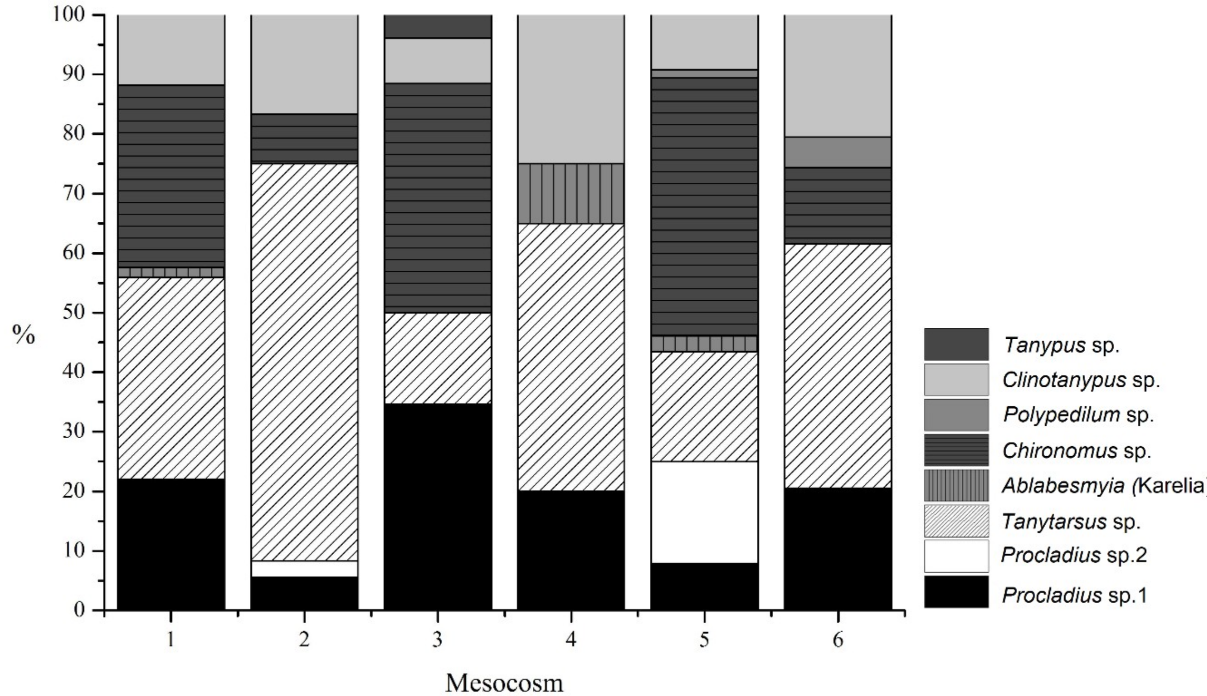

Figure 2. Chironomid species distribution (%) in the six mesocosms. Mesocosms 1, 3 and 4, with Pyrimethanil fungicide and mesocosms 2, 5 and 6, without Pyrimethanil.

Table I. Chironomidae abundance in the treated and control mesocosms, during the experiments.

Sample dates	Oct./12		Nov./12		Jan./13		Feb./13		Mar./13		Apr./13		May/13		Jun./13		Jul./13	
Taxa/Mesocosms*	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**
Procladius sp. 1	4	1	3	2	0	0	0	4	5	1	2	1	2	1	5	0	0	3
Procladius sp. 2	0	2	0	0	0	1	0	1	0	0	0	4	0	4	0	2	0	1
Tanytarsus sp.	7	4	0	3	2	4	3	9	1	6	12	16	0	16	1	4	1	3
Ablabesmyia (Karelia)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1
Chironomus sp.	0	2	0	1	0	0	2	17	7	13	0	3	11	3	5	0	2	2
Polypedilum sp.	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Clinotanypus sp.	0	0	0	0	0	0	0	1	0	1	1	3	1	3	6	3	1	2
Tanypus sp.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Margalef richness and Shannon-Wiener diversity (H') in the treatment mesocosms were low at the beginning of the experiment when compared with the values of control mesocosms. After the complete pyrimethanil degradation, the metrics of treatment mesocosms began to increase with values like those obtained in the control's units. The Margalef richness and Shannon-Wiener diversity (H') values in mesocosms are presented in Figure 3 and Figure 4, respectively.

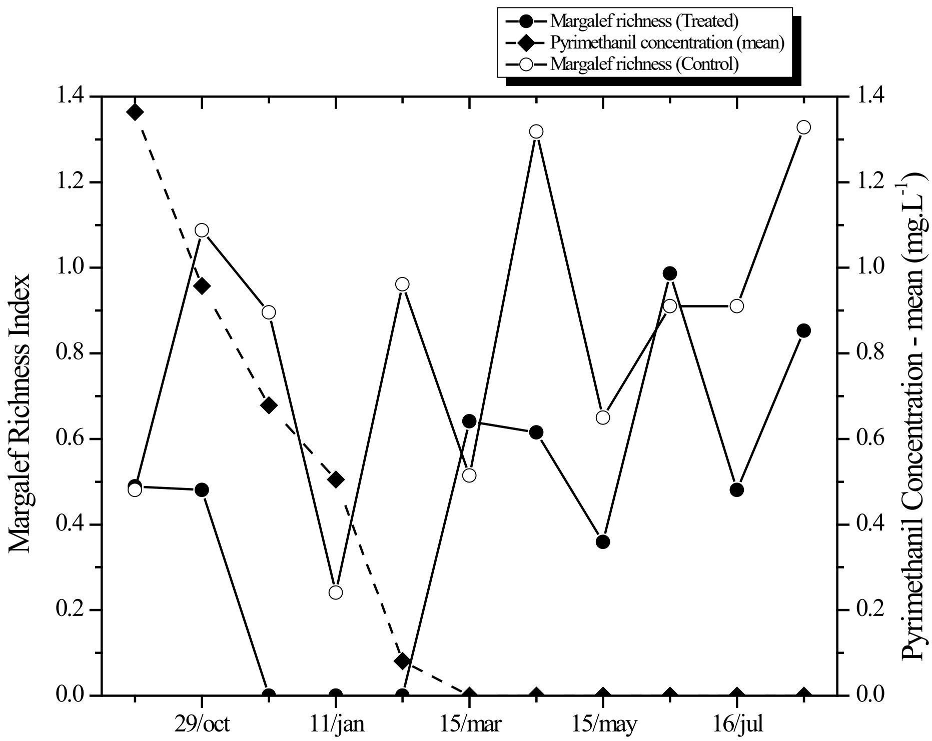

Figure 3. Margalef Richness index mean values in mesocosms and Pyrimethanil concentration mean. Measures obtained from October 2012 to July 2013.

The t-test, applied to diversity and species richness indexes between the treatment and control mesocosms, showed significant differences (p = 0.003) among the units.

DISCUSSION

The present study hypothesizes that the application of the fungicide pyrimethanil in agricultural areas, causes changes in the Chironomidae community of adjacent aquatic environments. The understanding of anthropogenic stressors effect, such as pyrimethanil fungicide, in the Chironomidae organism's distribution and abundance is fundamental to the evaluation of impacts on aquatic environments (Carter et al. 2006), especially in streams adjacent to agricultural crops. Other studies also pointed to the loss of Chironomidae diversity and richness species in areas with agriculture impacts in Neotropical streams (Corbi & Trivinho-Strixino 2006, Kleine et al. 2011, Corbi et al. 2018).

The low species richness observed in the six mesocosms and great homogeneity can be explained in several ways. According to Bojsen & Jacobsen (2003), water bodies located in open areas without riparian vegetation, have characteristics favoring the increase of periphyton biomass, due to the high incidence of light and the absence of organic matter, providing the presence of scrapers groups. Despite the low Chironomidae richness observed in the six mesocosms, the units without contamination presented higher richness. Caquet et al. (2000) showed in their experiments with mesocosms simulating lentic environments, that the spontaneous colonization of insects can require several months to occur. On the other hand, in the following months of pyrimethanil degradation, there was continuous recovery of the Chironomidae community.

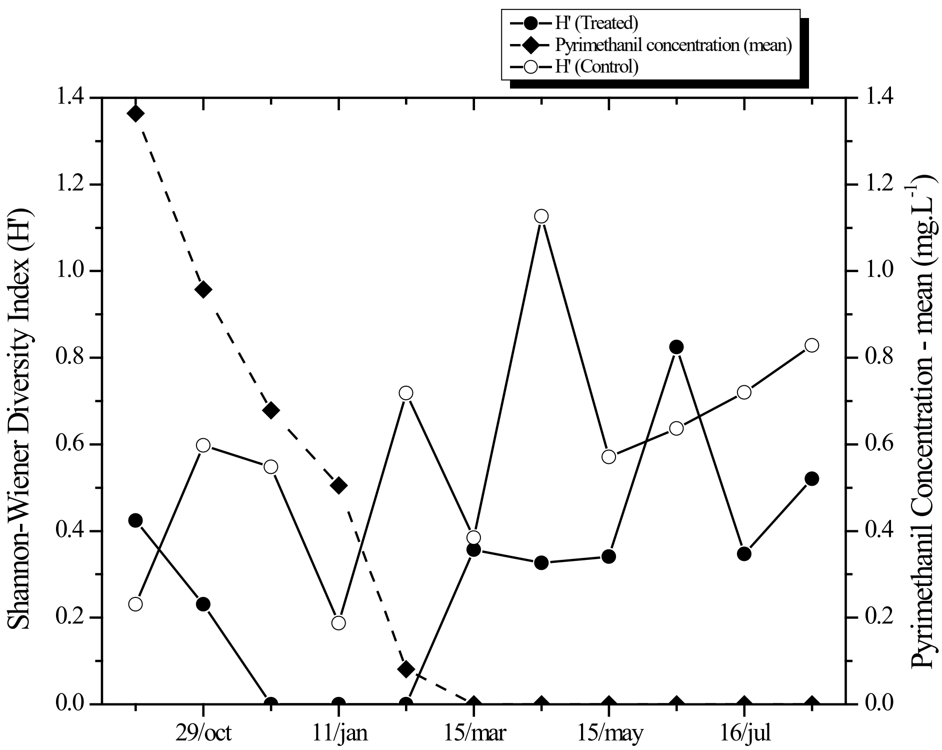

Figure 4. Shannon-Wiener diversity index mean values in mesocosms and Pyrimethanil concentration mean. Measures obtained from October 2012 to July 2013.

The environmental monitoring conducted in the six mesocosms, linked to methodologies and approaches of ecotoxicology, can provide insights into the mechanisms that act on the Chironomidae community dynamics and structure, especially when evaluating the effects of a harmful substance on groups of great environment importance (Ferrington 2008). This study showed a decrease in the chironomid community in experiments contaminated with pyrimethanil fungicide. The loss of Chironomidae diversity in aquatic environments can result in loss of different functions in these environments, such as the reduction fish species and nutrient cycling in this environment as suggested by several researchers (Cranston 1995, Trivinho-Strixino & Strixino 1995, Roque et al. 2000, Ferrington 2008). A study conducted by Colombo et al. (2017), showed that pyrimethanil fungicide can reduce the genetic diversity of *Chironomus sancticaroli* species (Diptera: Chironomidae) exposed to different pyrimethanil concentrations. In this study, the authors concluded that the pyrimethanil applications in agriculture cultivation, may lead to a decrease in aquatic biota genetic diversity. This condition may begin the disappearance of species in the long term-exposure, common situations in Brazilian agriculture and would have significant consequences for conservation plans and ecological managing.

The high temperature observed in the tropical regions can also be determinant to the low Chironomidae diversity in the pyrimethanil contaminated sites. Similar observations were made by Seeland et al. (2012), who found that pyrimethanil -toxicity on *Chironomus riparius* increased with elevated temperature. Similarly, Seeland et al. (2013) also stated that the life-stage specific temperature-dependent ecotoxicity of pyrimethanil demonstrate the complexity of pesticide-temperature interactions, especially considering global climate change predictions.

This research demonstrates the negative effects of pyrimethanil fungicide, which may contaminate streams adjacent to agricultural areas, via runoff process. In Brazil, it's also knows that the application of agrotoxics, as Pyrimethanil, together with the problem of the devastation of riparian vegetation, has led to different impacts on the water resources of the neighboring areas. The present study contributes to the evaluation of dynamics of the Chironomidae community structure in relation to contamination by Pyrimethanil, extensively used in the agriculture fields around the world. Our results point out that the utilization of agrochemicals can be a harmful factor influencing the Chironomidae populations. This finding has important implications for insect's conservation strategies and ecological management environments.

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