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**PROGRAMA INTEGRADO (UNESP, USP E UNICAMP) DE PÓS-GRADUAÇÃO
EM BIOENERGIA**

**ECOLOGICAL AND ECOTOXICOLOGICAL IMPACTS OF SUGARCANE
CULTURES ON AMPHIBIAN TADPOLE COMMUNITIES**

DAVID SÁNCHEZ DOMENE

Tese apresentada ao Instituto de Pesquisa em Bioenergia de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências.

Orientador(a):

Eduardo Alves de Almeida

Rio Claro – SP

Abril 2019

**UNIVERSIDADE ESTADUAL PAULISTA
“JULIO DE MESQUITA FILHO”
INSTITUTO DE PESQUISA EM
BIOENERGIA**

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David Sánchez Domene

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Comissão examinadora

Prof. Dr. Eduardo Alves de Almeida

FURB – Universidade Regional de Blumenau, Santa Catarina, Brasil

Dra. Juliane Silberschmidt Freitas

Pós-Doutoranda USP – Universidade de São Paulo, São Paulo, Brasil

Prof. Dr. Luís Olímpio Menta Giasson

FURB – Universidade Regional de Blumenau, Santa Catarina, Brasil

Prof. Dr. Camilo Dias Seabra Pereira

UNIFESP – Universidade Federal de São Paulo, São Paulo, Brasil

Prof. Dra. Maria Stela Maioli

UNESP – Universidade Estadual Paulista, São Paulo, Brasil

Conceito: *Aprovado*

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RESUMO

O Estado de São Paulo é responsável pela produção da metade da cana-de-açúcar no Brasil, e segue crescendo, sendo esse cultivo um dos principais responsáveis pelo desmatamento no interior do estado. A atual expansão da cana-de-açúcar está ocorrendo principalmente sob áreas de cultivos anuais e pastagens de pecuária extensiva. Embora possa parecer positivo, essa conversão de pastagens supõe um grande impacto sobre as poças de água construídas como bebedouros para o gado, as quais abrangem altas proporções de biodiversidade a sua vez. Nesse cenário, os anfíbios são especialmente vulneráveis, uma vez que a grande maioria das espécies de anuros, registrados no interior do estado, se reproduz nestas poças que estão sendo transformadas ou mesmo desaparecendo. Além disso, o cultivo intensivo de cana-de-açúcar exige o uso de agrotóxicos que devido as fortes precipitações sazonais provocam escoamento superficial a estes leitos aquáticos e sua subsequente contaminação por misturas complexas de pesticidas. Nesta dissertação de doutorado são apresentados os resultados de um estudo sobre os impactos ecológicos e ecotoxicológicos em girinos de diferentes espécies de anuros que habitam poças temporárias anexas aos cultivos de cana-de-açúcar. O estudo foi realizado na área agrícola circundante da cidade de São José do Rio Preto, uma das principais áreas de produção de cana-de-açúcar no noroeste paulista. A vegetação nativa dessa região tem sido continuamente desmatada desde o século XIX, e nas últimas décadas tem experimentado uma intensa expansão do cultivo de cana-de-açúcar, substituindo principalmente pastagens, o que torna essa região um local idôneo para pesquisa da anurofauna, a qual tem sido extensivamente estudada desde meados dos anos 60. Para compreender os impactos ecológicos e ecotoxicológicos nos girinos que habitam as poças temporais anexas aos cultivos de cana-de-açúcar, esta dissertação está formada por quatro capítulos nos quais são avaliados i) os impactos do cultivo da cana-de-açúcar sobre a estrutura das comunidades de anuros associadas a poças temporárias por meio do estudo dos efeitos das características locais das poças, da paisagem entorno a elas e da contaminação por pesticidas, ii) as malformações oculares encontradas em girinos coletados durante as amostragens de campo, iii) a confiabilidade do teste de desempenho de natação de escape como ferramenta para a detecção de comprometimento da mobilidade em organismos aquáticos expostos a pesticidas, e iv) os efeitos nas comunidades de água doce das misturas de pesticidas em um experimento microcosmos. Os resultados desses estudos vão desde a confirmação da importância das poças para a conservação de anfíbios, até a detecção de contaminação generalizada na região de São José do Rio Preto, bem como a apresentação dos primeiros casos de malformações anfíbias em paisagens agrícolas no Brasil, e a primeira linha de base de malformações para anfíbios na América do Sul.

Palavras-chave: Anfíbios, Cana-de-açúcar, Pesticidas, Mudança do uso do solo

ABSTRACT

São Paulo state cultivates half of the sugarcane in Brazil, being this culture among the main responsible for deforestation in the inland of the state. Current expansion of sugarcane is taking place mostly over annual croplands and extensive cattle ranching pastures. Although it may seem positive, this pasture conversion supposes a major impact on cattle ponds which support high proportions of biodiversity. In this scenario, amphibian populations are especially vulnerable since the vast majority of the anurans registered in the inland of the state breed in cattle ponds which are been transformed or even disappearing. In addition, intensive sugarcane culture demands the usage of pesticides which can enter ponds when heavy precipitation events cause surface run-off. Consequently, complex mixtures of pesticides occur in ponds near to cultures. In this doctoral dissertation are presented the results of a study of the ecological and ecotoxicological impacts on tadpoles of anurans species inhabiting temporary breeding ponds annex to sugarcane cultures. The study was conducted in the surrounding sugarcane-dominated agricultural area of the city of São José do Rio Preto, which stands out as an appropriate place for the research purpose, since it is part of São José do Rio Preto macro region, in the northwestern of São Paulo state, one of the major sugarcane production areas in Brazil, its native vegetation has been intensely deforested since the 19th century, in the last decades has experienced one of the greatest sugarcane expansions, mostly replacing cattle pastures, and its anurofauna has been extensively studied since mid-1960s. The dissertation includes four chapters in which are assessed i) the impacts of sugarcane cultivation on the anuran communities structure associated to temporary breeding ponds by assessing the effects of ponds characteristics, landscape and pesticides contamination, ii) eye malformation found in tadpoles collected during field samplings, iii) the reliability of the escape swimming performance test as a tool for the detection of motility impairment in aquatic organisms exposed to a pesticides, and iv) the effects on freshwater communities of pesticides mixtures in an outdoor microcosms experiment. The findings from these studies range from the confirmation of the importance of cattle ponds for amphibian conservation to the detection of widespread contamination in São José de Rio Preto region, as well as the introduction of the first report of amphibian malformations in agricultural landscapes in Brazil, as well as first malformation baseline for amphibians in South America.

Keywords: Amphibians, Sugarcane, Pesticides, Land-use-change

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GENERAL INTRODUCTION

In the early 70s, the report “Limits to Growth” alarmed about the global ecological constraints caused by the economic and population growth (Meadows et al. 2002), however much of the forecasts exposed by this report, as declines in food production, population level and energy availability, were overcome as a result of the research and technical development (RTD) of agricultural technologies, and its adoption by developing countries (Glaeser, 2011; Pingali and Raney, 2005). This RTD, named as “Green Revolution”, was based principally in the genetic improvement of crops, the mechanization of farming, the expansion of irrigation infrastructures, and the generalized use of synthetic fertilizers and pesticides, which doubled and tripled crop yields (Glaeser, 2011; Jordan, 2013). In fact, food production per capita was higher in the year 2000 than in 1970, despite having doubled population, and even though the arable land per person declined by 40 percent, from 0.43 ha in 1961/63 to 0.26 ha in 1997/99 (Meadows et al. 2002; FAO, 2003). Thus, Green Revolution driven intensification saved an estimated 18 to 27 million hectares of natural land from being brought into agricultural production (Stevenson *et al.* 2013). Nevertheless, it is scientific consensus that agriculture is the activity that has most modified the earth surface (Vitousek, 1997; World Bank 2001; Tilman *et al.* 2001; Foley, 2005). In 2016, agriculture used near to one-third of total land area, with India, United States of America, Russian Federation, China and Brazil alone representing 40 percent (FAO, 2018).

In Brazil, which holds the greatest diversity of amphibian species in the world (AmphibiaWeb, 2019), land use transformation is the main threat to amphibians (Eterovick *et al.* 2005; Silvano and Segalla, 2005). Amphibians endangerment is a global phenomenon, in fact the International Union for Conservation of Nature (IUCN) reports that approximately 40% of the world's amphibian species are endangered, making them the most vulnerable group among vertebrates (IUCN, 2019). Different studies have shown that amphibian population

declines are the reflect of complex interactions of impacts such as exotic species introduction (Fisher and Shaffer, 1996; Kiesecker *et al.* 2001), UV-radiation increase (Blaustein *et al.* 1994, 1997; Anzalone *et al.* 1998), climatic changes (Pounds, 2001), the emergence infectious diseases (Berger *et al.* 1998, 1999), the habitat loss and land-use changes (Stuart *et al.* 2004; Cushman, 2006), and the environmental contamination by anthropogenic pollutants (Blaustein *et al.* 2003).

In the last two decades, large areas in Brazil have been transformed by agribusiness because of the National Alcohol Program - Proálcool (Friberg, 2009, Lapola, 2010), which promoted sugarcane expansion to replace petroleum by sugar-derived ethanol in 1975. Further reinforcing this strategy, as a consequence of the Paris climate agreement within the United Nations Framework Convention on Climate Change (2015), the Brazilian government announced the “RenovaBio” program to boost participation of renewable fuels in its energy mix, which seeks an increase in ethanol production from 28 billion liters per year in 2015 to around 50 billion liters by 2030 (MME, 2017). Although in the last years has experienced a decline, Brazil remains the largest producer of sugar, and will continue to be the main producer by 2027, producing 34% of the world's sugarcane (OECD-FAO, 2018).

The state of São Paulo cultivates half of the sugarcane in Brasil (UNICA, 2018). Consequently, sugarcane is among the main responsible for deforestation in the inland of the state (Kronka, 2005), however, current deforestation rate by sugarcane expansion is a minor problem since it is taking place mostly over annual croplands and extensive cattle ranching pastures (Aguiar *et al.* 2009; Egeskog *et al.* 2014). Although it may seem positive, this pasture conversion is supposing a major impact on cattle ponds (Snodgrass *et al.* 2000; Beja and Alcazar 2003), which support higher proportions of biodiversity of aquatic organisms compared to larger freshwater systems (Santi *et al.* 2010; Biggs *et al.* 2014). In this scenario, amphibians populations are especially vulnerable since the vast majority of the anurans

registered in the inland of the state breed in cattle ponds (Da Silva *et al.* 2012), which are disappearing dramatically (Rodrigues *et al.* 2008; Joly *et al.* 2010), while the remaining ponds suffer the simplification of their vegetation (Da Silva *et al.* 2012), and pesticides contamination (Schiesari and Grillitsch, 2011). Intensive sugarcane culture demands the usage of pesticides such as herbicides, fungicides and insecticides, in order to prevent yield losses by pest infestations (Oerke and Dehne, 2004; Velasco *et al.* 2012). Heavy precipitation events, typical during the breeding season of amphibians in most inner São Paulo state, cause surface run-off which are the major route for pesticides entry into agricultural water bodies, (Leu *et al.* 2004; Taghavi *et al.* 2010; Stehle and Schulz, 2015). Consequently, complex mixtures of pesticides occur in water bodies near to cultures (Leu *et al.* 2004; Armas *et al.* 2005; Stehle and Schulz, 2015; Sánchez-Domene *et al.* 2018). Thus, whereas vegetation simplification reduces the number of microhabitats available to meet anuran species-specific requirements (Da Silva *et al.* 2012), pesticides contamination cause alterations in reproduction and development, malformations, biochemical disfunctions, immunosuppression, and mortality (*e.g.* Relyea 2005, 2008; Rohr *et al.* 2009; Mann *et al.* 2009; Shuman-Goodier and Propper, 2016; Freitas *et al.* 2017).

This doctoral dissertation presents a study of the ecological and ecotoxicological impacts on tadpoles of anurans species inhabiting temporary breeding ponds annex to sugarcane cultures. The study was done in the surrounding sugarcane-dominated agricultural area of the city of São José do Rio Preto, which stands out as an appropriate place for the research purpose, since it is part of São José do Rio Preto macro region, in the northwestern of São Paulo state, one of the major sugarcane production areas in Brazil, with around 894.736,80 hectares cultivated in 2018; involving 96 cities and a population of 1.5 million, approximately (IEA, 2019; SEADE, 2019). The native vegetation of this region has been intensely deforested since the 19th century, being replaced by pastures, croplands, and urban areas (Kronka *et al.* 1993).

Currently just a 4% of the native semi-deciduous Atlantic forests and Cerrado formations remains (Nalon *et al.* 2008), making this region the most deforested and fragmented in the state (Rodrigues *et al.* 2008). In the last decades, the region has experienced one of the greatest sugarcane expansions, mostly replacing cattle pastures (Aguiar *et al.* 2009). This, together with the extensive knowledge of its anurofauna, studied since mid-1960s (Provete *et al.* 2011), makes this region an appropriate place to study the impact of sugarcane expansion over the anurans associated to temporary breeding ponds.

The dissertation includes four chapters:

- 1. Effects of habitat characteristics and landscape on amphibian communities inhabiting a sugarcane dominated agroecosystem**, in which was studied the impact of sugarcane cultivation on the anuran communities structure associated to temporary breeding ponds by assessing the effects of local (ponds characteristics), landscape (land uses surrounding ponds), and contamination (pesticides in water) factors.
- 2. Eye malformation baseline in *Scinax fuscovarius* larvae populations that inhabit agroecosystem ponds in southern Brazil**, in which were studied rare eye malformations found in tadpoles collected during field samplings.
- 3. First vs. Best response analysis in escape swimming performance tests for ecotoxicology in tadpoles (*Boana lundii*)**, in which the reliability of the escape swimming performance test as a tool for the detection of motility impairment in aquatic organisms exposed to a pesticide was assessed.
- 4. Effects of pesticides mixtures occurring at tropical agroecosystems small water bodies on representative freshwater communities: a microcosm approach**, in which the effects on freshwater communities (phytoplankton-periphyton-tadpoles) of a field-based mixture of pesticides, at two concentrations, were assessed in an outdoor microcosms experiment.

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General conclusions

The main conclusions obtained after the work carried out during this research are listed below.

GENERAL CONCLUSIONS

1. Widespread pesticides contamination in ponds annex to sugarcane-dominated agroecosystems in northwest São Paulo have been detected. Traces of 10 different compounds were identified.
2. Species incidence and abundance of amphibian tadpoles in ponds from sugarcane-dominated agroecosystems are differently governed. While distance to forest fragments was the only factor driving the incidence, and also richness, of species, local pond variables, mostly those related to vegetation, were the only ones explaining their abundances.
3. The importance of maintaining ponds and native forest fragments, both endangered by sugarcane monoculture expansion, for successful amphibian conservation plans has been supported. In addition, the priority of preserving ponds with abundant stratified vegetation, with short and permeable connecting space with forest fragments, and located where runoff contamination is unlikely, has been argued.
4. First report of amphibian malformations in agricultural landscapes in Brazil, as well as first malformation baseline for amphibians in South America have been presented. This will be useful for future ecotoxicological surveys on amphibian populations inhabiting tropical agroecosystems.
5. We manage to prove that the two main analytical approaches used in escape swimming performance tests, first and best response analysis, can offer different results, thus leading to different conclusions when assessing the impact of toxicants over organisms.

6. The sole use of acetylcholinesterase as biomarker of exposure to organophosphate insecticides in tadpoles may be underestimating the effects of environmentally relevant concentrations given that escape's performance is also affected by non-cholinergic effects.

7. Although pesticides concentrations found in ponds from the sugarcane-dominated agroecosystems surrounding São José do Rio Preto did not show any dramatic effect neither on periphyton, phytoplankton, nor amphibians, the widespread contamination detected represents a potential danger for freshwater communities inhabiting agricultural ponds. Therefore, monitoring ponds annex to sugarcane plantations is recommended in order to favor early detections of dangerous concentrations of pesticides which could endanger freshwater communities inhabiting them.

8. Well ground water from the facilities of UNESP-Ibilce (São José do Rio Preto) were contaminated by at least 9 pesticides. This is added to the widespread pesticide contamination detected at ponds from the sugarcane-dominated agroecosystem surrounding São José do Rio Preto. These findings join to the allegations of widespread pesticides contamination in Brazil.