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CONCEPT NOTE

Chironomus sancticaroli (Diptera, Chironomidae) as a Sensitive Test Species: Can We Rely on Its Use After Repeated Generations, Under Laboratory Conditions?

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Abstract
In ecotoxicological assays, previously selected and standardized organism tests are exposed to an environmental sample. Some species of the *Chironomus* genus have been extensively used in ecotoxicological assays. Among these, *Chironomus tentans* is usually utilized in the USA and *Chironomus sancticaroli* in Brazil. We conducted ecotoxicological bioassays to compare a population of *C. sancticaroli*, kept for 6 years under laboratory conditions, with a sylvatic population of the same species, collected in the field. The aim was to test the hypothesis that populations of *C. sancticaroli*, maintained in the laboratory for long periods, could have a different response to stressors/substances. We analyzed the responses of *C. sancticaroli* for potassium chloride, zinc chloride, potassium dichromate, linear alkylbenzene sulphonate (LAS) and caffeine. The results showed no significant differences between the two populations in the analyses and seems to indicate the possible use of *C. sancticaroli* from populations kept in the laboratory for long periods.

Keywords Chironomidae · Ecotoxicology · Population genetics · *Chironomus*

Human beings have impacted aquatic ecosystems in several ways, leading to negative consequences (Marotta et al. 2008; Guimarães et al. 2014). Recently, so-called emerging pollutants have received increasing attention, which include pharmaceuticals, natural and synthetic hormones, caffeine and microplastics (Ghiselli and Jardim 2007; Gaffney and Valentine 2011; Di Gregorio et al. 2014; Francini et al. 2018). Similarly, an increase in agricultural activities has caused deforestation, together with the growth of fertilizers in aquatic systems, which have potentially damaging metals in their composition (Roy et al. 2016; Ferreira-Junior et al. 2017; Corbi et al. 2018). Aquatic ecotoxicology has been an effective research field for evaluating environmental impacts on aquatic biota by applying toxicity tests to different aquatic organisms (Fonseca and Rocha 2004; You et al. 2004; Nowak et al. 2009; Ribeiro et al. 2012; Cortez et al. 2012; Novelli et al. 2012; Corbi et al. 2015; Cui et al. 2018; Dornfeld et al. 2018). In these tests, organisms are exposed to concentrations of a substance and the resultant toxic effects are registered and quantified. Among the variety of species used in ecotoxicological assays, benthic organisms are indicated for toxicity tests that use sediments as a study matrix, because these organisms are in direct contact with them or in interstitial waters. Insect larvae have been particularly appropriate as test species, since they spend most of the critical period of their development in water, where they are exposed to chemicals through water and sediment (Di Veroli et al. 2014). Among these groups, *Chironomus* species are the most widely used standard test organisms

Editor's Note: Since this paper does not fall fully within the Aims and Scope of BECT, due to the lack of confirmation of nominal concentrations analytically, it was decided that it should be accepted as a "Concept Note" since it contained some appealing data for our readership. Interestingly, both populations of *C. sancticaroli*, laboratory reared and field collected, exposed to various concentrations of inorganic (e.g. zinc chloride) and organic (e.g. caffeine) compounds showed similar dose response. These data further verify the usefulness of laboratory-based cultures for aquatic toxicity bioassays to reflect the response in natural populations.

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in both water and sediment testing (Jeyasingham and Ling 2000; Dornfeld et al. 2018). In Brazil, some populations of the insect *Chironomussancticaroli* have been extensively utilized in studies which involve the rearing and determination of sediment toxicity in aquatic ecosystems (Strixino and Strixino 1981; Fonseca and Rocha 2004; Printes et al. 2011; Geromel-Costa et al. 2018; Dornfeld et al. 2018). Overall, populations of this species are kept in laboratory conditions over long periods of time to be used in ecotoxicological assays, which can decrease its genetic variability and thus, can alter its sensitivity to different stressors/substances. We test the hypothesis that *C. sancticaroli* populations, reared in laboratory conditions for long periods of time, can exhibit different responses to substances/stressors when compared to sylvatic populations of the same species. We performed several laboratory assays comparing produced effects on a population of *C. sancticaroli* reared in the laboratory for 6 years, and responses of a sylvatic population of the same species, collected recently (3 months) in the same place, in the field.

Materials and Methods

The species *C. sancticaroli*, was described by Strixino and Strixino (1981), from specimens collected in the City of São Carlos, SP (Fig. 1). The midge *C. sancticaroli* were chosen as test organisms for toxicity testing due to their wide distribution throughout Brazil, relative sensitivity to contaminants and ease of culture under laboratory conditions.

The *C. sancticaroli* populations were kept in plastic trays (38 cm length × 33 cm width × 6 cm height) covered by nylon cages in order to prevent the escape of adult mosquitoes, according to Fonseca and Rocha (2004) and the Organization for Economy Co-Operation and Development, OECD (2011). The food source was placed weekly on the trays, consisting of flocculated fish food Tetramin®, at a proportion of 0.04 mg L^{-1}. The experiments were conducted under the following conditions: conductivity between 25 and 55 µS cm^{-1}, hardness between 12 and 16 mg L^{-1} for $CaCO_3$ and pH between 6.5 and 7.5.

Sylvatic and laboratory *C. sancticaroli* were collected from a stabilization lake, located at a chicken slaughterhouse, in São Carlos, São Paulo, Brazil. The larvae of this species live commonly in these places, with high levels of

Fig. 1 Life cycle of *Chironomus sancticaroli* – (*1*) eggs (*2*) larvae eclosion (*3*) first instar larvae (*4*) larvae from fourth instar (*5*) pupa, and (*6*) adult female, adult male

organic matter. All lakes are free from other anthropogenic impacts, as industrial or mining activities.

The eggs from the sylvatic adults which emerged in the laboratory were acclimated in laboratory rearing trays. We performed acute tests with *C. sancticaroli* using organisms from field collections and from laboratory rearing to make a comparison of sensitivity. Increasing concentrations of potassium chloride (KCl), zinc chloride ($ZnCl_2$), potassium dichromate ($K_2Cr_2O_7$), linear alkylbenzene sulphonate (LAS – $C_{12}H_{25}C_6H_4SO_3Na$) and caffeine ($C_8H_{10}N_4O_2$) were also tested. According to the OECD (2011), the test solutions were prepared by dilution of a stock solution, dissolving the test substance directly in the dilution water (dechlorinated tap water) and the final dilutions were made using 1000 mL volumetric flasks. The tests were developed under the same rearing conditions of temperature and photoperiod. The experiments were performed in triplicate in glass vessels of 500 mL capacity, filled with 250 mL of the test solutions, made with dechlorinated tap water, 50 g of sand and 4 ± 1 mg of triturated fish food Tetramin®. We placed six larvae at III or IV instar in each vessel. The vessels were not aerated or fed through the test period, which lasted 96 h. The control tests were performed as the same way for the experiments and containing only dechlorinated tap water, sand and food source. After 96 h, we counted the number of living larvae. The larvae were placed in 1.5 mL Eppendorf tubes and conserved in isopropyl alcohol. The nominal concentrations of stressors/substances were not confirmed analytically.

We calculated the LC_{50} for sensitivity tests using KCl as a reference substance. The results were analyzed using the "drc" package (Ritz and Streibig 2005) in "R" software (R Core Team 2014) to obtain the LC_{50} value for potassium chloride. Afterwards, we applied a Kruskal–Wallis analysis to the results in order to assess possible significant differences between the populations tested. We adopted the *p*-value of 0.05, to analyze the significance. For this analysis, we used the PAST software (version 1.68) (Hammer et al. 2000).

Results and Discussion

In general, the results showed no significant differences between the two populations in the analyses. The greatest differences between the responses were those from sensitivity tests using KCl, as a reference substance. From the results of this experiment, the *C. sancticaroli* larvae from the field population are less sensitive to KCl when compared to those from laboratory rearing (Fig. 2). The field larvae mortality was low, even in the highest concentration of KCl. On the other hand, the Kruskal–Wallis analysis did not show a significant difference between these two populations (p value > 0.05). The LC_{50} calculation for this experiment showed a value of 5.62 mg L^{-1} for laboratory-reared larvae, which is considered within the ideal sensitivity for the species (between 2.6 and 6.4 mg L^{-1}) (Morais et al. 2014). The experiments testing the effects of zinc chloride also showed similar results. Thus, it can be observed in Fig. 2 that both populations exhibited similar mortality rates, when exposed to different concentrations of this compound. Likewise, the statistical analysis did not indicate significant differences. Additionally, the outcomes from the other experiments (potassium dichromate, LAS and caffeine) followed the same pattern to those previously described (Fig. 2).

However, for the species used in ecotoxicological assays in Brazil, there is a doubt concerning the reliability of its populations, reared for long periods of time in the laboratory. For studies using *C. sancticaroli*, sensitivity tests over a reference substance are performed in order to verify the viability for adopting the population. These sensitivity experiments, usually using KCl, are crucial to validate laboratory research. In addition to KCl, zinc chloride and copper chloride are also used (Zagatto and Bertoletti 2008; OECD 2011). The results of the KCl experiments showed that the laboratory larvae, despite being kept for long periods of time in controlled conditions, respond well to this substance in the sensitivity experiments.

Variety, contrary to less stress tolerant genotypes, may increase frequencies of alleles that provide a selective advantage under stressed conditions as pointed out by Nowak et al. (2009). Contaminants might lead to amplified mortality, reduced fertility and consequently decrease the number of individuals that contribute to the next generation, as pointed out by Nowak et al. (2007) studying the impact of tributyltin in the genetic variation in *Chironomus riparius* larvae species. Our results, comparing a population of *C. sancticaroli*, kept for 6 years under laboratory conditions, with a sylvatic population of the same species, collected in the field, seems to indicate reliability of using *C. sancticaroli* from populations kept in the laboratory for long periods.

Overall, this study can contribute to environmental analysis using *C. sancticaroli* and might collaborate with other researches in the ecotoxicology field. In this work, we sought to find a possible resistance in the natural (sylvatic) population of *C. sancticaroli* when compared to a population raised in the laboratory for long periods. Our findings attempt, where possible, to determine how stressors can affect the dynamics of the two populations of the species in ecotoxicological studies, although the nominal concentrations of stressors/substances were not confirmed analytically. This improved understanding may dispel some doubts about the potential use of laboratory-created species for extensive periods.

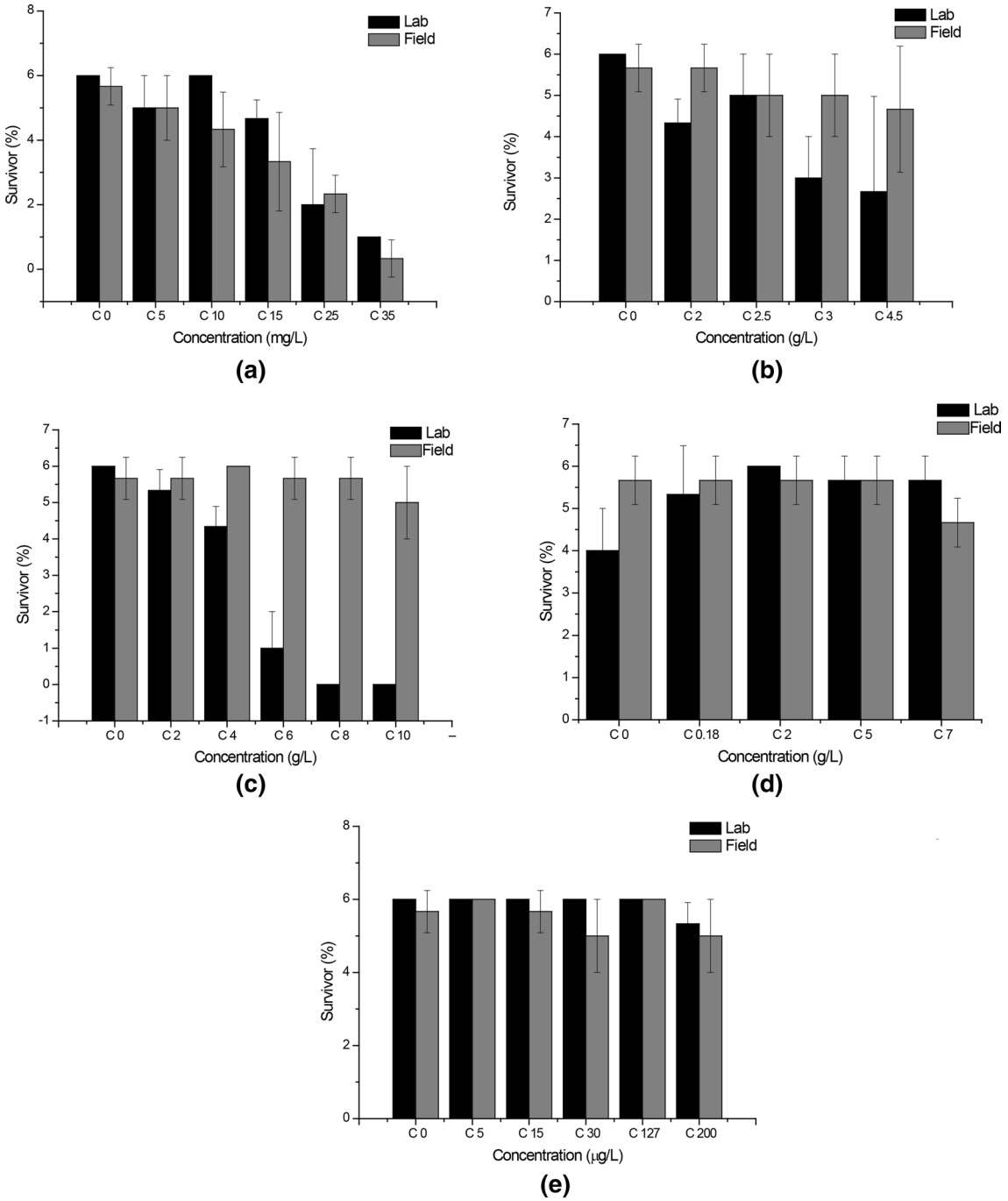

Fig. 2 Mean values and standard deviation of *Chironomus sancticaroli* populations survivor, in acute toxicity tests: **a** LAS, **b** potassium dichromate, **c** KCl, **d** zinc and **e** caffeine. The Kruskal–Wallis analysis did not show any significant differences between these two populations (p value > 0.05). The experiments were performed in triplicate

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