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Effects of the insecticide imidacloprid on aquatic invertebrate communities of the Ecuadorian Amazon☆

Marcela Cabrera[a,b], Mariana V. Capparelli[c], H. Mauricio Ortega-Andrade[d], Evencio Joel Medina-Villamizar[e], Andreu Rico[a,*]

[a] Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, 46980 Paterna, Spain
[b] Grupo de Investigación de Recursos Hídricos y Acuáticos, Facultad de Ciencias de la Vida, Universidad Regional Amazónica Ikiam, Tena, Ecuador
[c] Instituto de Ciencias del Mar y Limnología-Estación El Carmen, Universidad Nacional Autónoma de México, Ciudad del Carmen 24157, Mexico
[d] Grupo de Investigación en Biogeografía y Ecología Espacial, Facultad de Ciencias de la Vida, Universidad Regional Amazónica Ikiam, Tena, Ecuador
[e] School of Industrial, Computer and Aeronautical Engineering, University of León, Campus de Vegazana, 24071, León, Spain

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ABSTRACT

Imidacloprid is a neonicotinoid insecticide that has received particular attention due to its widespread use and potential adverse effects for aquatic and terrestrial ecosystems. Its toxicity to aquatic organisms has been evaluated in central and southern Europe as well as in (sub-)tropical regions of Africa and Asia, showing high toxic potential for some aquatic insects and zooplankton taxa. However, its toxicity to aquatic organisms representative of tropical regions of Latin America has never been evaluated. To fill this knowledge gap, we carried out a mesocosm experiment to assess the short- and long-term effects of imidacloprid on freshwater invertebrate communities representative of the Ecuadorian Amazon. A mesocosm experiment was conducted with five weekly applications of imidacloprid at four nominal concentrations (0.01 μg/L, 0.1 μg/L, 1 μg/L and 10 μg/L). Toxic effects were evaluated on zooplankton and macroinvertebrate populations and communities, as well as on water quality parameters for 70 days. Given the climatic conditions prevailing in the study area, characterized by a high solar radiation and abundant rainfall that resulted in mesocosm overflow, there was a rapid dissipation of the test compound from the water column (half-life: 4 days). The macroinvertebrate taxa *Callibaetis pictus* (Ephemeroptera), *Chironomus* sp. (Diptera), and the zooplankton taxon *Macrocyclops* sp., showed population declines caused by the imidacloprid treatment, with a 21-d Time Weighted Average No Observed Effect Concentrations (21-d TWA NOEC) of 0.46 μg/L, except for *C. pictus* which presented a 21-d TWA NOEC of 0.05 μg/L. In general terms, the sensitivity of these taxa to imidacloprid was greater than that reported for surrogate taxa in temperate zones and similar to that reported in other (sub-)tropical regions. These results confirm the high sensitivity of tropical aquatic invertebrates to this compound and suggest the need to establish regulations for the control of imidacloprid contamination in Amazonian freshwater ecosystems.

1. Introduction

The intensification of agricultural production practices in tropical countries of Latin America has led to an increase on the use of pesticides necessary for pest control (Carriquiriborde et al., 2014; Lewis et al., 2016). Ecuador, for example, went from using 6 thousand tons of pesticides in 2013 to 19 thousand tons in 2021 (FAO, 2023), partly driven by a sharp increase in crops destined for export (bananas, cut flowers, and cocoa) which have replaced traditional agriculture (Andrade-Rivas et al., 2023). The combination of intensive pesticide use and the heavy precipitation events characteristic of the region contribute to the transport of pesticide residues from soil and vegetation to aquatic ecosystems (Ramírez-Morales et al., 2021; Cabrera et al., 2023). Several studies have reported the presence of pesticides (e.g. carbamates, neonicotinoids, triazines and organophosphates) in surface waters of Ecuador (Andrée et al., 2021; Cabrera et al., 2023; Deknock et al., 2019; Villegas et al., 2021) and other tropical freshwater ecosystems of Latin America (Echeverría-Sáenz et al., 2021; Merga & Van den Brink, 2021).

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* Corresponding author.
E-mail address: andreu.rico@uv.es (A. Rico).

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Of these pesticides, imidacloprid was one of those that generated the greatest concerns, being found in concentrations of up to 3 μg/L in the Amazonian region (Cabrera et al., 2023).

Imidacloprid is a neonicotinoid insecticide patented in 1985 (Tomizawa & Casida, 2005) which has been registered in more than 120 countries for over 140 crop uses (Jeschke et al., 2011). Imidacloprid has a systemic action against piercing-sucking insects. Additionally, it has proven to be highly effective in controlling fleas on cats and dogs (Tomizawa & Casida, 2005). Imidacloprid operates by blocking the nicotinic neuronal pathway in the insect nervous system, causing insect excitation, paralysis and ultimately death at effective doses (Chen et al., 2014; Jeschke et al., 2011; Tomizawa & Casida, 2005). The European Union banned the outdoor use of imidacloprid in 2018, due to is potential impact on pollinators (EU Regulation, 2018/783). The U.S. EPA and New York State Department of Environmental Conservation have restricted the use of imidacloprid products labelled for widespread outdoor and foliar application, and seed treatment (US EPA, 2020). However, Ecuador and many other countries of tropical regions have not implemented any restrictions on the use of imidacloprid. For example, Ecuador has recently authorized 99 formulations with the active ingredient imidacloprid alone or in combination with other insecticides or fungicides for different types of applications (MAGAP, 2023).

Several studies have shown that imidacloprid poses high toxic potential to non-target aquatic insects, affecting development, survival, and emergence. The most sensitive taxa to imidacloprid are Ephemeroptera and some Diptera larvae (Colombo et al., 2013; Merga & Van den Brink, 2021; Montaño-Campaz et al., 2023; Raby et al., 2018a; Raby et al., 2018b; Rico et al., 2018; Sumon et al., 2018); although other studies have pointed at Odonata (Hayasaka et al., 2019; Jinguji et al., 2013), and zooplankton such as cyclopoid and rotifer taxa (e.g. *Keratella* sp.) as highly sensitive to this compound (Dimitri et al., 2021; Rico et al., 2018; Sumon et al., 2018). Studies published within the last few years show that subtropical and tropical aquatic ecosystems may be more sensitive to imidacloprid as compared to temperate ones (Merga & Van den Brink, 2021; Rico et al., 2018; Sumon et al., 2018). This increased sensitivity can be attributed to various factors, being higher temperature one of the most prominent ones. Mangold-Döring et al. (2022) found that the LC50 values of imidacloprid to *Gammarus pulex* significantly decreased with increasing water temperature. One of the reasons for this is the greater uptake of imidacloprid with increasing water temperature (Camp & Buchwalter, 2016). In addition, temperature increases the rate of imidacloprid biotransformation and therefore accelerates the generation of the metabolite imidacloprid-olefin, which has been found to be more harmful than the parent compound, increasing the individual effects of imidacloprid exposure over time (Huang et al., 2023; Huang et al., 2021; Mangold-Döring et al., 2022). Other factors that may affect the vulnerability of tropical aquatic ecosystems to imidacloprid are related to differences in species composition and species interactions. For example, tropical aquatic insects usually have shorter generation times, which may result in larger energy expenditure and lower detoxification capacity, increasing toxicant sensitivity. At the same time, such ecological traits confer opportunities for faster recolonisation from un-exposed sites and population recovery (Liess et al., 2008; Rico & Van Den Brink, 2015). Previous experiments have shown that imidacloprid contamination in freshwater mesocosms exposed to high temperatures may induce cyanobacterial blooms. These cyanobacteria blooms could represent a significant public health and environmental concern due to their potential toxicity and impact on aquatic ecosystems, including altered nutrient dynamics and significant changes on the structure of aquatic communities (Dimitri et al., 2021).

So far, the ecotoxicological effects of imidacloprid to aquatic populations and communities has been assessed in micro- and mesocosm experiments performed under temperate conditions (Ratte & Memmert, 2003; Colombo et al., 2013; Hayasaka et al., 2012 & Mohr et al., 2012) and under (sub-)tropical conditions of Asia (Sumon et al., 2018 & Dimitri et al., 2021) and Africa (Merga & Van den Brink, 2021), while there have been no assessments performed in tropical regions of Latin America. As pointed out above, differences in temperature, but also in community composition, may yield to differences in the sensitivity and recovery capacity of aquatic ecosystems to imidacloprid across different climatic and biogeographic regions. Therefore, this study aimed to evaluate the long-term effects of imidacloprid on freshwater invertebrate communities representative of the Ecuadorian Amazon and to compare these results with the outcomes of similar studies performed in other parts of the world.

2. Materials and methods

2.1. Experimental design

The experiment was conducted at the outdoor mesocosm station of the Universidad Regional Amazónica Ikiam (Napo, Ecuador) between May and August of 2023. The mesocosms were made of cylindrical polyvinyl chloride tanks (diameter: 115 cm; height:108 cm) covered on the contour with 60% polyshade mesh to prevent overheating (Fig. S1). The mesocosms were filled up with 5 cm of sediment collected from the Colonso river and 400 L of well water. The water was previously analysed to ensure that imidacloprid was not present. Each mesocosm had two pebble baskets with leaves and two stone traps for the sampling of macroinvertebrates. The mesocosms were inoculated with macroinvertebrate species collected from unpolluted water bodies close to the Colonso Chalupas Biological Reserve, and 500 mL of concentrated plankton collected from the same sampling locations as a source of phytoplankton and zooplankton (further details are provided in the Supplementary Material, Text S1). Then, the mesocosms were left for 4 weeks to allow the establishment of the biological communities prior to the start of the experiment. During this period, 20% of the water was exchanged between the mesocosms every two weeks in order to homogenize the structure of the aquatic communities. Nitrogen (1.4 mg/L of N, as NH_4NO_3) and phosphorus (0.18 mg/L of P, as KH_2PO_4) were added to the mesocosms every three weeks following Merga & Van den Brink (2021) and Rico et al. (2014) to stimulate phytoplankton growth.

The experiment was conducted with 15 mesocosms randomly distributed within five different treatments, with three replicates per treatment. Imidacloprid was applied once per week at the dose of 0 μg/L (Control), 0.01 μg/L, 0.1 μg/L, 1 μg/L and 10 μg/L during 5 weeks (starting May 5, 2023). After the application period, the imidacloprid concentrations, the water quality parameters, and the response of the biological communities were evaluated for seventy days since the first imidacloprid application. Additionally, 25 L of water was removed weekly from each mesocosm to maintain the initial volume (400 L) and prevent excess overflow. The methods used for the imidacloprid analysis, the water quality analysis, and the analysis of the biological populations and communities are described in the following sections.

2.2. Imidacloprid application, sampling and analysis

Imidacloprid was purchased from Sigma-Aldrich in powder form with purity ≥98% (Pestanal, analytical standard). Concentrated solutions were prepared from a stock solution of imidacloprid (250 mg/L) prepared in Milli-Q water. The concentrated solutions were poured over the water column of the mesocosms on day 0, 7, 14, 21 and 28 relative to the first application and mixed with the water using a wooden stick. Water samples from the mesocosms were collected in 500 mL amber glass bottles. Samples were taken 1 h after each application in the 0.1, 1 and 10 μg/L treatments and on days 7, 14, 21, 28 before to imidacloprid dosing. Additionally, samples of the 1 and 10 μg/L treatments were collected on days 29, 30, 31 and 32 to evaluate the dissipation of imidacloprid in the mesocosms after the last application. Samples from the controls and the 0.01 μg/L treatment were taken on days 0 and 21 before and after the imidacloprid application.

The imidacloprid samples from the 1 μg/L (after application) and the

10 μg/L treatments were directly injected into an UPLC-Qtof/MS (Waters Model Xevo G2 QTOF) for quantification, while the samples from the controls and the 0.01 μg/L, 0.1 μg/L, and 1 μg/L (before application) treatments were subjected to solid phase extraction (SPE). For this, Oasis HLB cartridges (Waters, 6 cc, 200 mg) were preconditioned with 6 mL of MeOH and 6 mL of MilliQ Water. Next, the cartridges were rinsed with 10 mL of MilliQ water and dried for 5 min under full vacuum (10 bar) to eliminate residual water. Then, 250 mL of the water sample were passed through the SPE cartridges and analytes were eluted with 6 mL of methanol. The extracts were concentrated with nitrogen for approximately 2 h, reconstituted with 1 mL of acetonitrile:water (10:90, v/v) and vortex stirred for 1 min. The reconstituted samples were transferred to an amber glass vial for analysis, using the same equipment as described above. Operation parameters for analytical equipment are shown in Table S1, while the retention times (Tr), instrumental detection and quantification limits (LOD, LOQ) and methodological limits (MDL, MQL) of the validation at a FC250 pre-concentration factor for imidacloprid are provided in Table S2.

2.3. Water quality parameters

Water pH, temperature (T), electric conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) were measured at 8 h a.m. and 5 h p.m. on days −7, 0, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 relative to the first imidacloprid application. Measurements were performed using a WTW multiparameter at a water depth of 30 cm.

Water samples (500 mL) were collected from each mesocosm for the analysis of ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO_4^{3-}), and chlorophyll-a. These samples were taken on day −7, 14, 28, 42, 56 y 70 relative to the first application. NO_3^-, NO_2^-, PO_4^{3-} and NH_4^+ were analysed in the laboratory using an ion chromatograph equipped (Prominence Shimadzu, Japan) according to Pfaff (1996) and Thomas et al. (2002). For the analysis of the chlorophyll-a, 200 mL of the microcosm water was filtered through a Whatman GF/C glass-fibre filter (mesh size: 0.45 μm) and measured using ultraviolet–visible (UV-VIS) spectrophotometer (UV-3600 PLUS Shimadzu, Japan) according to APHA, (2012).

2.4. Zooplankton and macroinvertebrates

Zooplankton and macroinvertebrate samples were collected on days −7, 14, 28, 42, 56 and 70 relative to the first imidacloprid application. Details regarding their sampling and identification are provided in the Supplementary Material (Text S1).

2.5. Data analyses

The half-life (DT50) of imidacloprid in the mesocosms was calculated by Ln (2) divided by the dissipation coefficients (k), which were calculated assuming first order kinetics. Time-weighted average concentrations (TWAC) of imidacloprid in the mesocosms were calculated with the equations described by Roessink et al. (2013).

To effects of imidacloprid on the zooplankton and the macroinvertebrate communities were analysed using the Principal Response Curve (PRC) method (Van den Brink & Braak, 1999) with the CANOCO software version 5.0 (Braak et al., 2012). The PRC diagram represents the temporal differences in species composition between treatments and the control (C_{dt}), and the affinity of each taxon with the PRC (b_k), so that the species with the highest b_k values show a population decline, and the species with negative b_k values a population increase related to the chemical concentration (for further details see Van den Brink & Braak, 1999). To assess the statistical significance of the treatment effects, a Monte Carlo permutation test employing redundancy analysis (RDA) was conducted on each sampling day. Calculated p-values <0.05 indicate significant effects of the treatment on the community composition for a given sampling day. NOECs (No-observed-effect-concentrations) at the community level were calculated using the Williams test (Van Den Brink et al., 1996) with the sample scores obtained by a Principal Component Analysis (PCA) for each individual sampling day.

NOECs and their respective Minimum Detectable Difference (MDD) values were calculated to assess the effect of imidacloprid on water quality parameters, abundance of zooplankton and macroinvertebrate taxa. The MDD provides a power analysis for the concentration-response relationships underlying the calculated NOECs. It is defined as the difference between the means of a treatment and the control that must exist to detect statistically significant effects. The MDDs mainly depend on the number of replicates and the variance of the measured endpoint. The lower the MDD value, the higher the statistical robustness of the calculated NOEC. On the other hand, MDDs >100% indicate that the power of the statistical test used to derive the NOEC is too low to demonstrate treatment-related effects (Brock et al., 2015). The effects of the treatment on the sampled populations (i.e., NOEC calculation) and the corresponding MDDs were calculated for each sampling day, including the sampling prior to the application of imidacloprid (day −7) to determine any possible statistically significant differences that were randomly occurring and were not related to the imposed treatments. The NOECs and MDDs were calculated with the Community Analysis computer program version 4.3.05 (Hommen et al., 1994), and using the Williams test (Williams 1971, 1972). Prior to the analyses, the zooplankton and macroinvertebrate abundance data were ln (ax + 1) transformed as described in Van den Brink et al. (2000).

The taxa sampled in the mesocosms were classified according to their MDD values into three categories and the effects were classified into the classes proposed by Brock et al. (2015). For details see the Supplementary Material (Text S3).

3. Results and discussion

3.1. Imidacloprid concentrations and dissipation in the test mesocosms

The measured concentrations of imidacloprid in the treatments 1 h after the first application were between 93% and 113 % the intended concentrations (Table S3). After 7 days of exposure, the concentrations of imidacloprid in the water of the mesocosms decreased to approximately 80% (Fig. 1; Table S4). No imidacloprid residues were found in the controls during the experiment. The calculated DT50 for imidacloprid in the experiment was, on average, 4.0 ± 1.9 days (Table S3). Merga & Van den Brink (2021) reported a DT50 ≥ 10 days under tropical climatic conditions in Ethiopia (temperature range of 16.6–20.9 °C). Sumon et al. (2018) reported a DT50 of approximately 7–10 days with a mean temperature of 28 °C under sub-tropical conditions in Bangladesh. Rico et al. (2018) reported a DT50 of approximately 10 days under Mediterranean climatic conditions (temperature range of 16–25 °C), and Duchet et al. (2023) reported a DT50 of 15 days in temperate climate conditions of the USA. This means that the dissipation of imidacloprid in our study was about 2–4 times faster than the dissipation rates shown in other mesocosm studies (including studies in the tropical region). This could be influenced by the forced and unforced overflow of the mesocosms, which eliminated at least 1/8 of the imidacloprid concentration per week, and the environmental conditions during the experiment, which were: average ambient temperature: 25 °C (max 36 °C; min 18 °C); average precipitation: 2.1 mm/day (max 21 mm/day; min 0 mm/day); and average solar radiation: 233 W/m^2 (max 736 W/m^2; min 58 W/m^2) (Fig. S2). Lu et al. (2015) argued that the dissipation of imidacloprid from water occurs mainly by photolysis, and the high ambient temperature favour this process. Solar radiation was probably the most important cause explaining the differences between the dissipation rate observed in our study and other studies performed in tropical and subtropical regions, where mesocosms were covered with greenhouse plastic sheets to prevent overheating. Due to the rapid dissipation rate of imidacloprid in our mesocosms, the TWAC concentrations of the different treatments are a more suitable measure to assess

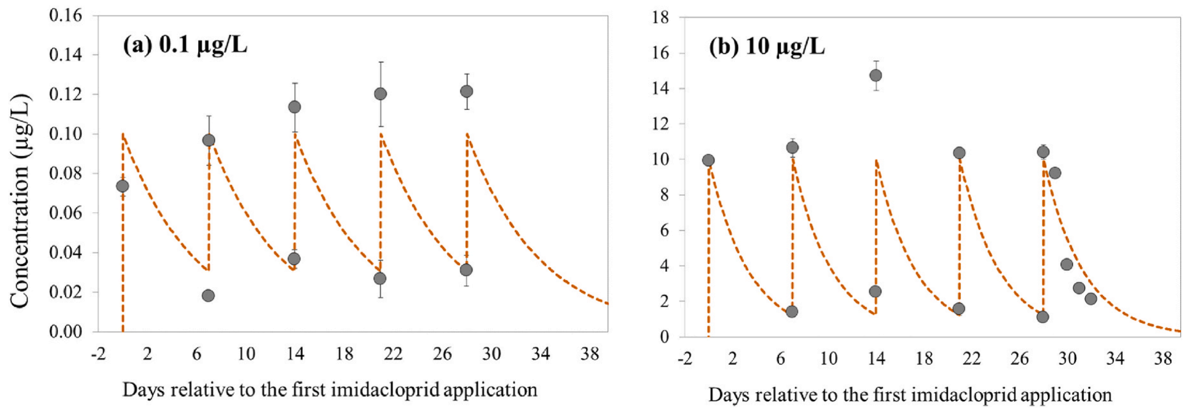

Fig. 1. Measured concentrations of imidacloprid in the 0.1 µg/L treatment (a) and in the 10 µg/L treatment (b). Data are expressed as mean ± standard deviation (dots). The dashed line shows the predicted imidacloprid concentration based on the calculated dissipation rate constant for each treatment assuming first order kinetics.

chronic exposure. The TWACs ranged between 0.007 and 5.86 µg/L for the first 21 days of experiment (which allow comparison with other studies), and between 0.003 and 0.6 for the whole experimental period (Table S3).

3.2. Indirect effects of imidacloprid on water quality parameters

Mean water temperature in the mesocosms was 24 °C (max 25 °C; min 22 °C) in the morning and 27 °C (max 29 °C; min 24 °C) in the afternoon. The mean dissolved oxygen concentration was 7 mg/L (max 9 mg/L; min 4 mg/L) in the morning and 9 mg/L (max 11 mg/L; min 6 mg/L) in the afternoon (92%–103% saturation). The mean pH was 7 (max 9; min 4) in the morning and 9 (max 11; min 6) in the afternoon, and the mean electric conductivity was 14 µS/cm (max 17 µS/cm; min 11 µS/cm) in the morning and 18 µS/cm (27 µS/cm; min 12 µS/cm) in the afternoon (Fig. S3). The mean concentration of nutrients was 0.1 mg/L (max 0.85 mg/L; min < LOD mg/L) of NH_4^+, 1.4 mg/L (max 3.4 mg/L; <LOD min 6 mg/L) of NO_3^-, and 0.09 mg/L (max 0.22 mg/L; min < LOD mg/L) of PO_4^{-3} over the time of investigation.

Generally, the Williams test indicated no significant treatment-related effects on the evaluated water quality parameters. However, the imidacloprid application resulted in a significant increase in pH over time (NOEC <0.01 µg/L; Table S5). Higher pH is often associated with higher phytoplankton biomass, due to a decreased impact of grazing (Dimitri et al., 2021; Silva et al., 2014). Similar effects have been reported in other studies performed with neonicotinoids under temperate, sub-tropical and Mediterranean conditions (Dimitri et al., 2021; Duchet et al., 2023; Rico et al., 2018). However, although the chlorophyll-a concentration in our experiment varied from 0.6 to 20 µg/L, no significant differences were observed with respect to the control, probably due to variation between replicates. In other studies in temperate, and (sub)-tropical areas, water quality parameters did not show consistent treatment-related effects (Kreutzweiser et al., 2007; Merga & Van den Brink, 2021; Sumon et al., 2018).

3.3. Effects of imidacloprid on zooplankton

A total of 13 different zooplankton taxa were identified in the mesocosms, distributed in Copepoda (*Macrocyclops* sp.), Cladocera (*Moina* sp.), and Rotifera (*Plationus, Lecane lunaris, Lecane bulla, Lecane luna, Polyarthra* sp., *Polyarthra vulgaris, Ascomorpha* sp., *Anuraeopsis fissa, Lepadella* sp., *Trichocerca* sp., *Euclanis* sp*)*. The PRC analysis only showed

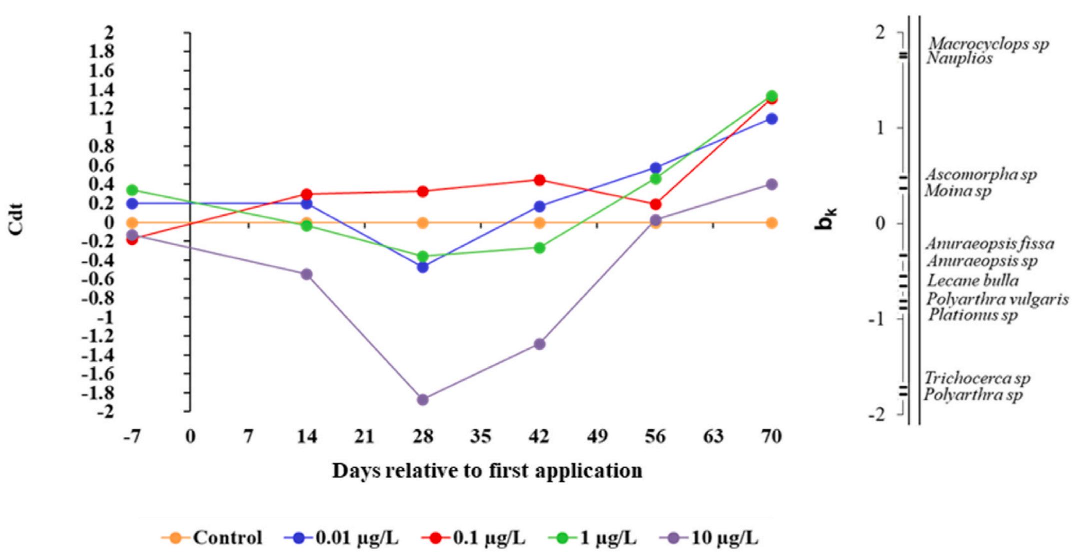

Fig. 2. Principal Response Curve (PRC) showing the effects of imidacloprid on the zooplankton community throughout the experimental period. Differences between the treated zooplankton community and the control at various sampling dates are indicated by the sample weights (C_{dt}). The species weight (b_k) reflects the affinity of each taxon with the PRC. Taxa with bk values between 0.1 and −0.1 are not shown. Of all variance 26 % could be attributed to sampling day, and 26 % to the imidacloprid treatment, out of which 29 % is displayed in the first PRC (Monte Carlo p-value = 0.06).

marginally significant effects of imidacloprid on the zooplankton community (Monte Carlo p-value = 0.06; Fig. 2). The PRC analysis indicated that adult *Macrocyclops* and its naupliar stages (nauplii) had an abundance decline caused by imidacloprid (b_k values > 1.5), while some Rotifera (*Trichocerca* sp., *Polyarthra* sp.) showed a population increase related to imidacloprid (b_k values < −1.5). The RDA analysis indicated significant effects of imidacloprid on the zooplankton community on days 28 and 42, with calculated p-values of 0.02 and 0.004, respectively. The calculated zooplankton community NOEC for these sampling days based on the Williams test performed with the PCA sample scores was 1 μg/L (nominal concentration). On day 56 and 70 the RDA indicated non-significant effects suggesting that at the end of the experimental period the zooplankton community had recovered.

The results of the Williams test performed to assess the effects of imidacloprid on individual zooplankton taxa are shown in Table 1. Significant effects on individual abundance were found for *Macrocyclops* (adults and nauplii), *Moina* sp, *Polyarthra* sp., *Anuraeopsis* sp., and *Euchanis* sp. For most of them, population declines were quantitatively restricted and occurred in isolated sampling days (Effect class 2; Table S6; Fig. 3). These corresponded to NOECs of 1 μg/L (21-d TWACs of 0.46 μg/L), except for *Euchlanis* sp. that showed a NOEC of 0.1 μg/L (21-d TWACs of 0.05 μg/L). *Macrocyclops* adults showed a pronounced short-term effect during the exposure period that was followed by recovery towards the end of the experiment (Effect class 3 A; Fig. 3), with a NOEC of 1 μg/L (21-d TWACs of 0.46 μg/L), however it should be noted that statistically significant differences between the highest treatment level and the control were already present before to the application of imidacloprid (Table 1). Some Rotifera taxa (*Lecane bulla, Polyarthra vulgaris, Ascomorpha* sp. *Anuraeopsis* sp., *Trichocerca* sp.) showed restricted population abundance increases in isolated sampling days with calculated NOECs that ranged from <0.01 μg/L to 1 μg/L (21-d TWACs of 0.007–0.46 μg/L; Effect class 2 or 4 A).

Cyclopoids have been shown to be amongst the most sensitive freshwater zooplankton taxa to imidacloprid and other neonicotinoid insecticides (Dimitri et al., 2021; Rico et al., 2018; Sumon et al., 2018). In our study, the threshold concentration for the decline of nauplii (21-d TWACs of 0.46 μg/L) was larger than that reported by Merga & Van den Brink (2021) for the same stages, <0.06 μg/L (21-d TWAC), and Sumon et al. (2018), 0.02 μg/L (21-d TWAC), in other tropical regions, and slightly lower than that reported by Rico et al. (2018), 2.8 μg/L (21-d TWAC), for the Mediterranean region (Table 2). On the other hand, the calculated NOEC for *Macrocyclops* sp. adults (21-d TWACs of 0.46 μg/L) was in the order of magnitude of that reported by other studies performed in (sub-)tropical (Sumon et al., 2018; 21-d TWAC 0.23 μg/L) or Mediterranean regions (Rico et al., 2018; 21-d TWAC 0.52 μg/L), and an order of magnitude lower than that reported for Cyclopoids in the temperate region of Europe (21-d TWAC 3.6 μg/L; Ratte & Memmert, 2003). The effects found on Rotifera in this study pointed towards a high sensitivity of this taxonomic group in the Amazon region, as shown in other regions of the world (Sumon et al., 2018; Rico et al., 2018; Dimitri et al., 2021; Merga & van den Brink, 2021). In addition, some treatment-related increases occurring during or nearly after the imidacloprid application may be related to the grazing and competition release created by the decrease of larger zooplankton (*Macrocyclops* sp.)

Table 1
Population and community NOECs for zooplankton and macroinvertebrates. Only taxa in categories 1 and 2 are displayed together with their corresponding NOECs (μg/L), and the MDD values between brackets (%). Arrows indicate a population abundance increase (↑) or a decrease (↓) related to the chemical treatment. > indicates that there were no statistically significant effects, so that the NOEC should be higher than 10 μg/L; - indicates that the taxa were absent that sampling day; n.c. indicates that the MDD could not be calculated due to the absence of individuals in the controls. Calculations done on day −7 refer to statistically significant differences prior to the application of the test compound, and therefore should not be considered as true NOECs. *The RDA indicated non-significant effects.

Class/Order/Taxa	Cat.	Sampling days					
		−7	14	28	42	56	70
Zooplankton							
Community		>	10	1	1	1*	>
COPEPODA							
Cyclopoida							
Macrocyclops sp	1	1 (66) ↓	1 (73) ↓	1 (86) ↓	>	1 (85) ↓	>
Nauplii	1	>	>	1 (88) ↓	>	>	>
CLADOCERA							
Moina sp	1	1 (93) ↓	>	>	1 (84) ↓	>	>
ROTIFERA							
Plationus	2	>	>	>	>	>	>
Lecane bulla	2	>	>	>	0.1 (n.c.) ↑	>	-
Polyarthra sp.	1	>	>	>	>	1 (98) ↓	>
Polyarthra vulgaris	2	-	-	-	1 (n.c.) ↑	>	<0.01 (91) ↑
Ascomorpha sp.	1	>	1 (95) ↑	>	>	>	>
Anuraeopsis sp.	2	-	>	<0.01 (n.c.) ↑	>	0.1 (97) ↓	>
Trichocerca sp.	2	-	>	1 (n.c.) ↑	>	>	>
Euchlanis sp.	2	-	-	-	>	>	0.1 (98) ↓
Macroinvertebrates							
Community		>	1	1	1	1	0.1
INSECTA							
Ephemeroptera							
Baetidae/*Callibaetis pictus.*	1	>	1 (69) ↓	1 (69) ↓	1 (70) ↓	1 (68) ↓	0.1 (88) ↓
Caenidae/*Caenis* sp.	2	>	>	1 (90) ↓	>	>	>
Odonata							
Libellulidae/*Pantala flavescens*	1	>	>	1 (85) ↓	>	>	>
Diptera							
Chironomidae/*Chironomus* sp.	1	>	1 (57) ↓	1 (79) ↓	>	<0.01 (63)	1 (64) ↑
Culicidae	2	>	1 (97) ↓	>	1 (81) ↓	>	>
Trichoptera							
Hydropsychidae/*Diplectrona* sp.		>	–	–	–	–	–
Hemiptera							
Naucoridae	2	–	<0.01 (84) ↓	>	–	–	–
GASTROPODA							
Architaenioglossa							
Ampullarriidae/*Pomace* sp.	2	>	>	1 (97) ↓	>	>	>

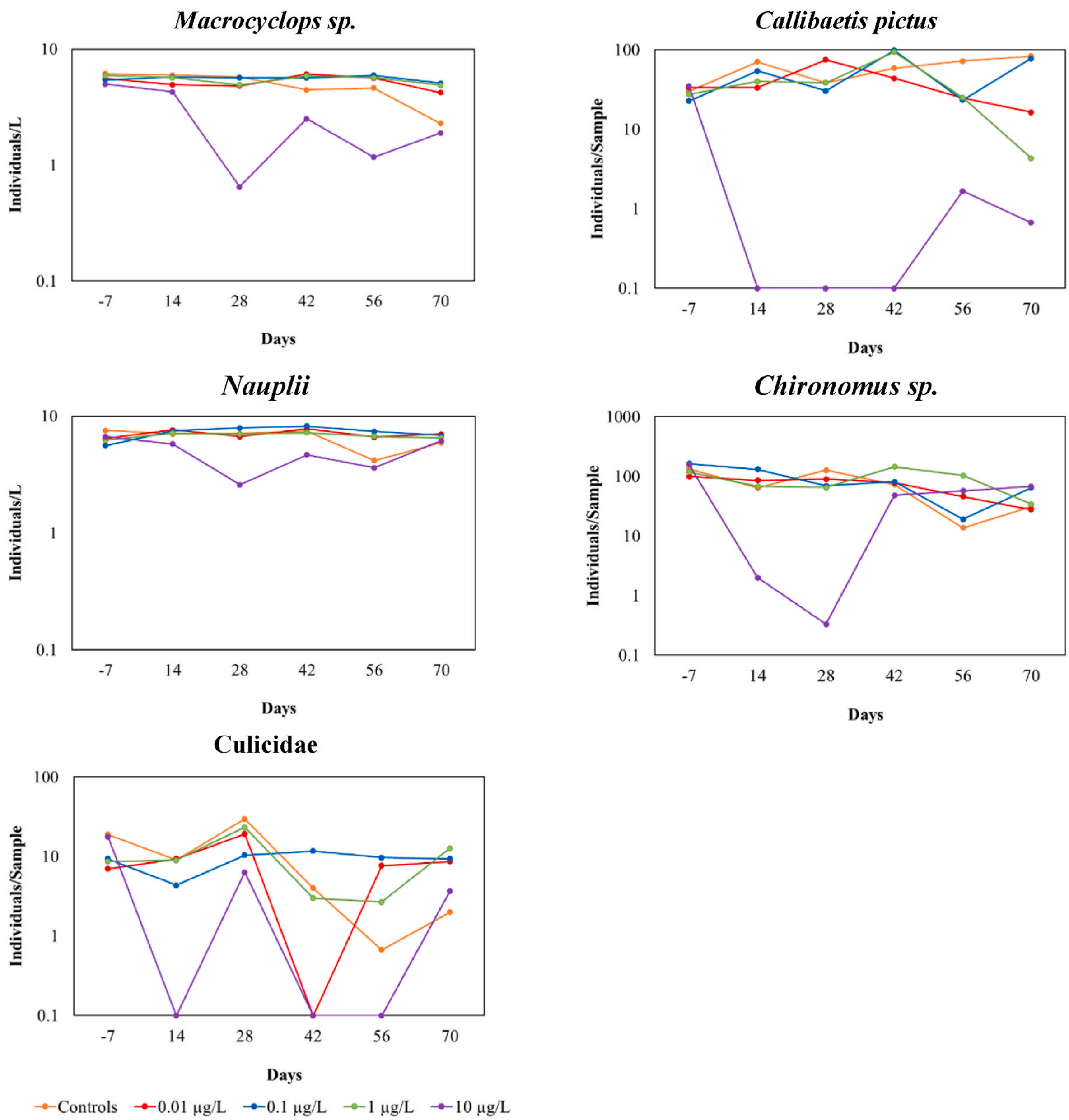

Fig. 3. Abundance of selected zooplankton (*Macrocyclops* sp and Nauplii) and macroinvertebrate (*C. pictus, Chironomus* and Culicidae) taxa in the test mesocosms over the course of the experiment. The x-axis (day) indicates the day relative to the first imidacloprid application.

as has been reported in other mesocosm experiments (e.g. Rico et al., 2018). Overall, the direct and indirect effects on the zooplankton community in our study were found to be less severe and consistent over time than those reported in previous investigations (Merga & van den Brink, 2021; Sumon et al., 2018; Rico et al., 2018). This yielded to a zooplankton community NOEC of 1 μg/L (21-d TWACs of 0.46 μg/L), which is about an order of magnitude higher than that reported by previous mesocosm studies in (sub-)tropical regions (Table 2).

3.4. Effects of imidacloprid on macroinvertebrates

The macroinvertebrate community comprised twenty-two taxa including Ephemeroptera (*Callibaetis pictus*, *Terpides* sp., *Thraulodes* sp., *Caenis* sp), Plecoptera (*Anacroneuria*), Odonata (*Pantala flavescens*, *Sympetrum* sp., *Anax longipes*, Gomphidae), Coleoptera (*Pseudodisersus* sp., *Heterelmis* sp, *Eubrianax* sp.), Diptera (*Chironomus* sp., *Podomus* sp, Culicidae), Trichoptera (*Diplectrona* sp.), Hemiptera (Notonectidae, Naucoridae, Corixidae), Megaloptera (*Corydalus* sp.), Gastropoda (*Pomacea* sp.*)*, and Annelida (Hirudinea). The PRC analysis indicated significant effects of imidacloprid on the macroinvertebrate community (Monte Carlo p-value = 0.002; Fig. 4). The most sensitive species to the imidacloprid treatment were *C. pictus, Chironomus* sp., and Culicidae, with b_k values > 1.5. The results of the RDA on each sampling day showed significant treatment-related effects on the macroinvertebrate community in all sampling days after the first application (p-values <0.05). The Williams test performed with the PCA sample scores of the macroinvertebrate dataset resulted in a community NOEC of 1 μg/L since the first application of the test compound until the last sampling day, which was 0.1 μg/L (nominal concentrations).

The results of the Williams test and the taxa classification based on their MDD are shown in Table 1. According to the results of the Williams test, the ephemeropteran *C. pictus* showed a NOEC of 1 μg/L (21-d TWACs of 0.46 μg/L) during and after the imidacloprid application (Effect class 4 A) and 0.1 μg/L (21-d TWACs of 0.05 μg/L) on the last sampling day (Effect class 2–4 A; Table S6). The magnitude of the effect was severe in the highest test concentration, leading to extinction of this

Table 2
Summary of micro- and mesocosm experiments performed with imidacloprid worldwide. The table indicates the experimental conditions, and the response of the most sensitive/relevant zooplankton and macroinvertebrate taxa together with their nominal and 21-d TWA NOECs. ND: no data.

Reference	Cosm type	Country	Climate	Experiment conditions			Most sensitive taxon	Nominal NOEC	21-d TWA NOEC	Most sensitive community	Nominal NOEC	21-d TWA NOEC
				Duration (d)	Applications (number and interval)	Mean temperature (°C)						
Ratte & Memmert (2003)	Pond mesocosm	Germany	Temperate	182	ND	ND	Macroinvertebrates: Chironomidae/Baetidae	0.6 μg/L	0.23 μg/L	ND	ND	ND
Colombo et al. (2013)	Pond microcosm	Germany	Temperate	49	3 pulses (1 week)	19	Macroinvertebrate: *Caenis* sp	1.4 μg/L	0.4 μg/L	ND	ND	ND
Hayasaka et al. (2012)	Pond mesocosm	Japan	Temperate	119	1 pulse	ND	Macroinvertebrates: *Chironomidae* sp.	49 μg/L	1.4 μg/L	ND	ND	ND
Mohr et al. (2012)	Stream mesocosm	Germany	Temperate	70	3 pulses (1 week)	17	Macroinvertebrates: *Tanypodinae/Baetis* sp	<12 μg/L	<0.85 μg/L	ND	ND	ND
Rico et al. (2018)	Pond mesocosm	Spain	Mediterranean	56	1 pulse	21	Macroinvertebrates: Chironomini/*C. dipterum*	<0.2 μg/L	<0.1 μg/L	Macroinvertebrates	1 μg/L	0.52 μg/L
Sumon et al. (2018)	Pond mesocosm	Bangladesh	Subtropical	28	4 pulses (1 week)	28	Zooplankton: *Keratella* sp./*Polyarthra* sp. Macroinvertebrate: *Cloeon* sp.	<0.03 μg/L	<0.02 μg/L	Zooplankton Macroinvertebrates	0.03 μg/L	0.02 μg/L
Dimitri et al. (2021)	Pond mesocosm	China	Subtropical	56	4 pulses (1 week)	29	Zooplankton: Copepoda/ Keratella tropica	<0.03 μg/L	<0.05 μg/L	Zooplankton Macroinvertebrates	0.03 μg/L	0.05 μg/L
Merga & Van den Brink (2021)	Pond mesocosm	Ethiopia	Tropical	168	4 pulses (1 week)	19	Zooplankton: *Trichocerca* sp/*Polyarthra* sp./ *Afrocycops* sp/*Nauplius.* Macroinvertebrates: *C. dipterum/C. horaria*	<0.01 μg/L	<0.06 μg/L	Zooplankton	<0.01 μg/L	<0.06 μg/L
This study	Pond mesocosm	Ecuador	Tropical	70	5 pulses (1 week)	25	Macroinvertebrates: *C. pictus*	0.1 μg/L	0.05 μg/L	Macroinvertebrates	0.1 μg/L	0.05 μg/L

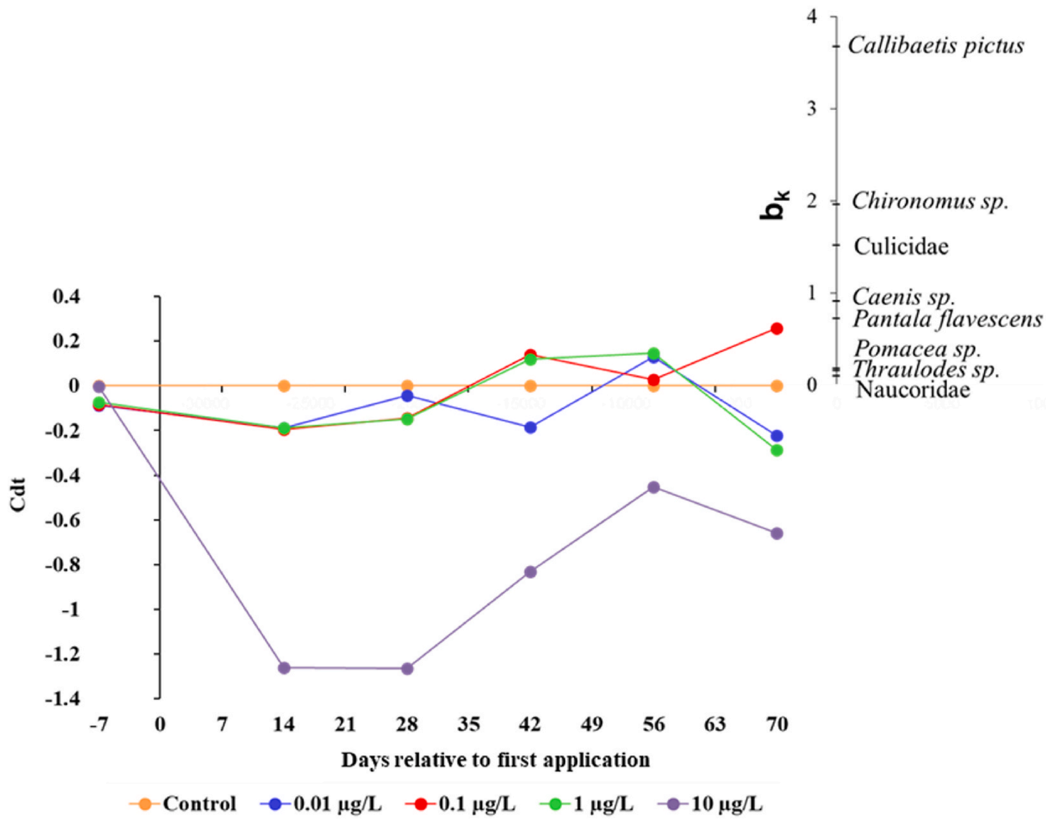

Fig. 4. Principal Response Curve (PRC) showing the effects of imidacloprid on the macroinvertebrate community throughout the experimental period. Differences between the treated macroinvertebrate community and the control group at various sampling dates are indicated by the sample weights (Cdt). The species weight (bk) reflects the affinity of each taxon with the PRC. Taxa with bk values between 0.1 and −0.1 are not shown. Of all variance 22 % could be attributed to sampling day and 44 % to the imidacloprid treatment, out of which 61 % is displayed in the first PRC (Monte Carlo p-value = 0.002).

species in the mesocosms, and was milder in the 1 µg/L treatment (Fig. 3). Other taxa that showed a significant population decline were *Caenis* sp., *P. flavescens, Chironomus* sp., Culicidae, Naucoridae, and *Pomacea* sp. All showed a NOEC of 1 µg/L (21-d TWACs of 0.46 µg/L) in an isolated sampling day (Effect class 2) except for *Chironomus* sp., which showed consistent treatment-related effects during the weeks of the imidacloprid application (Effect class 3: Fig. 3), and Naucoridae, which showed short-term effects occurring only in one sampling in all test concentrations (21-d TWACs <0.007 µg/L). Significant abundance increases were generally not found, except for *Chironomus* sp., which showed a treatment-related increase in the last two sampling days (Table 1).

This is the first study that evaluates the effects of imidacloprid on aquatic insect taxa of the Amazonian region. The high sensitivity of the ephemeropteran *C. pictus* (NOEC: 21-d TWACs of 0.05 µg/L) is in line with previous experiments testing the long-term effects of imidacloprid on other Ephemeroptera taxa such as *Caenis horaria* and *Cloeon dipterum* (<0.06 µg/L; Merga & Van den Brink, 2021), *C. dipterum* (<0.1 µg/L; Rico et al., 2018) or *Cloeon* sp. (<0.02 µg/L; Sumon et al., 2018) in freshwater mesocosms of the Mediterranean and (sub-)tropical region (Table 2). Imidacloprid generally influences the development of Ephemeroptera larvae, reducing head and thorax length, and reducing emergence capacity (Alexander et al., 2008; Raby et al., 2018a). In our experiment, the highest effect occurred on the last sampling day, suggesting that there was a delayed effect on the development of the eggs and juveniles that hatched during the exposure period.

The threshold concentration for *Chironomus* sp. and Culicidae derived from this study is very similar to that provided by Ratte and Memmert (2003) for Chironomidae in the temperate region, and Sumon et al. (2018) in sub-tropical Bangladesh but is slightly higher than the long-term NOECs provided by Merga & van den Brink (2021) and Rico et al. (2018) for both taxa in Ethiopia and in Spain, respectively. The Diptera order contains a large number of species with different morphological and physiological characteristics, which influence the recorded response for this taxonomic group, besides differences in environmental conditions among experiments. Montaño-Campaz et al. (2023) demonstrated that sublethal exposures to imidacloprid (1.38 µg/L) can modify adaptive responses, such as emergence, wing shape, and reproduction, in males and females of *Chironomus columbiensis*, being slightly more toxic for males. On the other hand, the study by Raby et al. (2018b) revealed that acute sublethal (0.24 µg/L) exposures affected both genders of *Chironomus dilutus* similarly, leading to additional effects like altered wing asymmetries, lighter adult females, and reduced fecundity rates. Our experiment shows that despite their high sensitivity, Diptera can recover relatively fast once imidacloprid has dissipated from the exposure medium (within 35 days from the last imidacloprid application). Most aquatic Diptera have a multivoltine or bivoltine life cycle, which contributes to external colonization from uncontaminated sources and fast recovery (Gergs et al., 2016). In our experiment, *Chironomus* sp. showed a treatment-related increase in the last sampling day, potentially due to the release of intra-specific competition.

The Caenidae family has been found to be very sensitive to imidacloprid and other neonicotinoid insecticides (Raby et al., 2018a; Roessink et al., 2013). In this experiment a clear dose-response was identified during the imidacloprid application period. However, the population abundance in the mesocosm was relatively variable, so that the effects could not be clearly determined in the following weeks. Clear dose-response effects were also observed on day 28 for the Odonata *P. flavescens*, which are similar to those reported by Rico et al. (2018) for other Odonata larvae. However, these effects could also be partly related to the decrease of the aquatic larvae of *Chironomidae* and Culicidae, which constitute the base diet for many predatory invertebrates (Jinguji et al., 2013).

3.5. Sensitivity differences across biogeographic regions

Imidacloprid is one of the most used, but also most researched insecticides worldwide. Including this study, imidacloprid has been tested in freshwater mesocosms of 4 different continents, and on aquatic organisms representative of three different climatic zones (i.e., temperate, Mediterranean, and sub-tropical), which are summarized in Table 2. Some important conclusions can be drawn from these experiments. First, the experiments show the most sensitive taxa in all these continents belongs to the same zooplankton (Cyclopoida, some Rotifers) and macroinvertebrate (Ephemeroptera, Diptera) groups, indicating that the relative sensitivity of aquatic organisms to a common toxicant is very similar across continents and biogeographic regions. Second, it confirms that aquatic organisms from tropical regions show a high sensitivity to imidacloprid. Sensitivity differences, based on the abundance decline of the most sensitive population, for mesocosm experiments performed in the temperate and tropical regions, vary up to one order of magnitude (<0.03–0.23 μg/L, Table 2). As pointed out earlier, one of the main factors that influences the sensitivity of aquatic organisms to imidacloprid under different latitudes may be water temperature. Laboratory and semi-field studies performed in temperate regions (Colombo et al., 2013; Hayasaka et al., 2012; Ratte & Memmert, 2003; Roessink et al., 2013) had mean water temperatures that were between 5 and 10 °C lower than those in subtropical countries of Asia (Dimitri et al., 2021; Sumon et al., 2018), Africa (Merga & Van den Brink, 2021) and in tropical Ecuador (current study; Table 2). Few studies performed with crustaceans and aquatic insects, including Ephemeroptera larvae, show that temperature enhances toxicity of imidacloprid by enhancing its uptake and biotransformation (Camp & Buchwalter, 2016; Macaulay et al., 2020), potentially resulting in a higher production of the more toxic metabolite (Huang et al., 2023). In this sense, the studies performed in sub-tropical regions of Africa and Asia and the current one have provided similar chronic toxicity thresholds for sensitive aquatic populations and communities (21-d TWACs <0.02–0.05 μg/L), although in the current study the effects on the most sensitive population was found to be less severe than those reported by Sumon et al. (2018) and Merga & Van den Brink (2021). It should be noted that the exposure profile was different among studies (with faster dissipation in the current study), and that the most sensitive Ephemeroptera taxa in these experiments belong to different genera, which may have slightly different sensitivities to the test compound.

3.6. Risks for tropical freshwater ecosystems of Latin America

Imidacloprid has been widely used in Latin America and has generated significant controversy due to its potential environmental side-effects. Several studies conducted in countries such as Argentina, Chile, Brazil, Ecuador, Mexico, and Costa Rica have reported maximum imidacloprid concentrations in surface waters that range from 0.05 μg/L to 3 μg/L (Table S7). Based on the results of this study, and the outcomes of other studies performed in other sub-tropical regions, a long-term quality standard of 0.02 μg/L may be recommended for assessing the effects of this compound in the Amazonian region and in other tropical regions of Latin America. This corresponds to the lowest population and community NOEC (21-d TWAC of 0.05 μg/L) calculated in this study divided by an assessment factor of 3, which accounts for possible differences in species composition between those tested in the current experiment and the wide range of species inhabiting freshwater ecosystems of Latin America. According to the existing information we may conclude that the current emission of imidacloprid to freshwater ecosystems (e.g. due to several spray-drift or runoff events) poses long-term risks for some aquatic insects and zooplankton taxa in many locations of Latin America. Therefore, it is imperative that Ecuador and other Latin American consider the implementation of measures to restrict or ban the use of imidacloprid for agricultural purposes due to its potential risk for freshwater ecosystems.

4. Conclusions

This is the first study that assessed the effects of imidacloprid on aquatic invertebrate communities of tropical regions of Latin America. The study shows that the sensitivity of aquatic populations is similar to that reported in other sub-tropical regions of the world and higher than that reported in temperate regions. We identified Ephemeroptera as highly sensitive to this compound, and we have proposed a long-term water quality standard of 0.02 μg/L. Furthermore, we show that exposure levels of imidacloprid in the Ecuadorian Amazon and in other tropical ecosystems of Latin America are expected to produce long-term risks for aquatic organisms, suggesting that measures to restrict or ban the use of this compound for agricultural purposes should be urgently implemented.

CRediT authorship contribution statement

Marcela Cabrera: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Mariana V. Capparelli:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **H. Mauricio Ortega-Andrade:** Writing – review & editing, Investigation, Funding acquisition. **Evencio Joel Medina-Villamizar:** Writing – review & editing, Methodology, Investigation. **Andreu Rico:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124459.

References

Alexander, A.C., Heard, K.S., Culp, J.M., 2008. Emergent body size of mayfly survivors. Freshw. Biol. 53 (1), 171–180. https://doi.org/10.1111/j.1365-2427.2007.01880.x.

Andrade-Rivas, F., Paul, N., Spiegel, J., Henderson, S.B., Parrott, L., Delgado-Ron, J.A., Echeverri, A., van den Bosch, M., 2023. Mapping potential population-level pesticide exposures in Ecuador using a Modular and Scalable geospatial strategy. GeoHealth 7 (7), e2022GH000775. https://doi.org/10.1029/2022GH000775.

Andrée, D.C., Marie Anne Eurie, F., Niels, D.T., Isabel, G.A., Arne, D., Wout, V.E., Lenin, R.F., Jasmine, D.R., Liesbeth, J., Pieter, S., Luis, D.G., Peter, L.M.G., 2021. From field to plate: agricultural pesticide presence in the guayas estuary (Ecuador) and commercial mangrove crabs. Environ. Pollut. 289, 117955 https://doi.org/10.1016/j.envpol.2021.117955.

APHA, 2012. In: Rice, E.W, Baird, R.B., Eaton, A.D., Clesceri, L.S. (Eds.), Standard methods for the examination of water and wastewater, 22nd edition. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, D.C., USA.

Braak, Ter, ter, C.J.F., Smilauer, P., 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. https://research.wur.nl/en/publications/canoco-reference-manual-and-users-guide-software-for-ordination-v.

Brock, T.C.M., Hammers-Wirtz, M., Hommen, U., Preuss, T.G., Ratte, H.-T., Roessink, I., Strauss, T., Van Den Brink, P.J., 2015. The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. Environ. Sci. Pollut. Control Ser. 22 (2), 1160–1174. https://doi.org/10.1007/s11356-014-3398-2.

Cabrera, M., Capparelli, M.V., Ñacato-Ch, C., Moulatlet, G.M., López-Heras, I., Díaz González, M., Alvear-S, D., Rico, A., 2023. Effects of intensive agriculture and urbanization on water quality and pesticide risks in freshwater ecosystems of the Ecuadorian Amazon. Chemosphere 337, 139286. https://doi.org/10.1016/j.chemosphere.2023.139286.

Camp, A.A., Buchwalter, D.B., 2016. Can t take the heat: temperature-enhanced toxicity in the mayfly Isonychia bicolor exposed to the neonicotinoid insecticide imidacloprid. Aquat. Toxicol. 178, 49–57. https://doi.org/10.1016/j.aquatox.2016.07.011.

Carriquiriborde, P., Mirabella, P., Waichman, A., Solomon, K., Van den Brink, P.J., Maund, S., 2014. Aquatic risk assessment of pesticides in Latin America. Integrated Environ. Assess. Manag. 10 (4) https://doi.org/10.1002/ieam.1561. Article 4.

Chen, C., Wang, Y., Zhao, X., Wang, Q., Qian, Y., 2014. Comparative and combined acute toxicity of butachlor, imidacloprid and chlorpyrifos on earthworm, Eisenia fetida. Chemosphere 100, 111–115. https://doi.org/10.1016/j.chemosphere.2013.12.023.

Colombo, V., Mohr, S., Berghahn, R., Pettigrove, V.J., 2013. Structural changes in a macrozoobenthos assemblage after imidacloprid pulses in aquatic field-based microcosms. Arch. Environ. Contam. Toxicol. 65 (4), 683–692. https://doi.org/10.1007/s00244-013-9940-2.

Deknock, A., De Troyer, N., Houbraken, M., Dominguez-Granda, L., Nolivos, I., Van Echelpoel, W., Forio, M.A.E., Spanoghe, P., Goethals, P., 2019. Distribution of agricultural pesticides in the freshwater environment of the Guayas river basin (Ecuador). Sci. Total Environ. 646, 996–1008. https://doi.org/10.1016/j.scitotenv.2018.07.185.

Dimitri, V., de, P., Yao, K.-S., Li, D., Lei, H.-J., Van den Brink, P.J., Ying, G.-G., 2021. Imidacloprid treatments induces cyanobacteria blooms in freshwater communities under sub-tropical conditions. Aquat. Toxicol. 240, 105992 https://doi.org/10.1016/j.aquatox.2021.105992.

Duchet, C., Hou, F., Sinclair, C.A., Tian, Z., Kraft, A., Kolar, V., Kolodziej, E.P., McIntyre, J.K., Stark, J.D., 2023. Neonicotinoid mixture alters trophic interactions in a freshwater aquatic invertebrate community. Sci. Total Environ. 897, 165419 https://doi.org/10.1016/j.scitotenv.2023.165419.

Echeverría-Sáenz, S., Spínola-Parallada, M., Soto, A.C., 2021. Pesticides burden in neotropical rivers: Costa Rica as a case study. Molecules 26 (23). https://doi.org/10.3390/molecules26237235. Article 23.

FAO, 2023. Pesticides Use. https://www.fao.org/faostat/en/#data/RP/visualize.

Gergs, A., Classen, S., Strauss, T., Ottermanns, R., Brock, T.C.M., Ratte, H.T., Hommen, U., Preuss, T.G., 2016. Ecological recovery potential of freshwater organisms: consequences for environmental risk assessment of chemicals. In: de Voogt, En P. (Ed.), Reviews of Environmental Contamination and Toxicology, 236. Springer International Publishing, pp. 259–294. https://doi.org/10.1007/978-3-319-20013-2_5.

Hayasaka, D., Korenaga, T., Sánchez-Bayo, F., Goka, K., 2012. Differences in ecological impacts of systemic insecticides with different physicochemical properties on biocenosis of experimental paddy fields. Ecotoxicology 21 (1), 191–201. https://doi.org/10.1007/s10646-011-0778-y.

Hayasaka, D., Kobashi, K., Hashimoto, K., 2019. Community responses of aquatic insects in paddy mesocosms to repeated exposures of the neonicotinoids imidacloprid and dinotefuran. Ecotoxicol. Environ. Saf. 175, 272–281. https://doi.org/10.1016/j.ecoenv.2019.03.051.

Huang, A., van den Brink, N.W., Buijse, L., Roessink, I., van den Brink, P.J., 2021. The toxicity and toxicokinetics of imidacloprid and a bioactive metabolite to two aquatic arthropod species. Aquat. Toxicol. 235, 105837 https://doi.org/10.1016/j.aquatox.2021.105837.

Hommen, U., Veith, D., Dülmer, U., 1994. A computer program to evaluate plankton data from freshwater field tests. En freshwater field tests for hazard assessment of chemicals. CRC Press.

Huang, A., Mangold-Döring, A., Guan, H., Boerwinkel, M.-C., Belgers, D., Focks, A., Van den Brink, P.J., 2023. The effect of temperature on toxicokinetics and the chronic toxicity of insecticides towards Gammarus pulex. Sci. Total Environ. 856, 158886 https://doi.org/10.1016/j.scitotenv.2022.158886.

Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem. 59 (7) https://doi.org/10.1021/jf101303g. Article 7.

Jinguji, H., Thuyet, D.Q., Uéda, T., Watanabe, H., 2013. Effect of imidacloprid and fipronil pesticide application on Sympetrum infuscatum (Libellulidae: Odonata) larvae and adults. Paddy Water Environ. 11 (1–4), 277–284. https://doi.org/10.1007/s10333-012-0317-3.

Kreutzweiser, D., Good, K., Chartrand, D., Scarr, T., Thompson, D., 2007. Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. Ecotoxicol. Environ. Saf. 68 (3), 315–325. https://doi.org/10.1016/j.ecoenv.2007.04.011.

Lewis, S.E., Silburn, D.M., Kookana, R.S., Shaw, M., 2016. Pesticide behavior, fate, and effects in the tropics: an overview of the current state of knowledge. J. Agric. Food Chem. 64 (20), 3917–3924. https://doi.org/10.1021/acs.jafc.6b01320.

Liess, M., Schäfer, R.B., Schriever, C.A., 2008. The footprint of pesticide stress in communities—species traits reveal community effects of toxicants. Sci. Total Environ. 406 (3), 484–490. https://doi.org/10.1016/j.scitotenv.2008.05.054.

Lu, Z., Challis, J.K., Wong, C.S., 2015. Quantum yields for direct photolysis of neonicotinoid insecticides in water: implications for exposure to nontarget aquatic organisms. Environ. Sci. Technol. Lett. 2 (7), 188–192. https://doi.org/10.1021/acs.estlett.5b00136.

Macaulay, S.J., Buchwalter, D.B., Matthaei, C.D., 2020. Water temperature interacts with the insecticide imidacloprid to alter acute lethal and sublethal toxicity to mayfly larvae. N. Z. J. Mar. Freshw. Res. 54 (1), 115–130. https://doi.org/10.1080/00288330.2019.1614961.

MAGAP, 2023. Dirección de Registro de Insumos Agrícolas—AGROCALIDAD. https://www.agrocalidad.gob.ec/366-2/.

Mangold-Döring, A., Huang, A., van Nes, E.H., Focks, A., van den Brink, P.J., 2022. Explicit consideration of temperature improves predictions of toxicokinetic–toxicodynamic models for flupyradifurone and imidacloprid in Gammarus pulex. Environ. Sci. Technol. 56 (22), 15920–15929. https://doi.org/10.1021/acs.est.2c04085.

Merga, L.B., Van den Brink, P.J., 2021. Ecological effects of imidacloprid on a tropical freshwater ecosystem and subsequent recovery dynamics. Sci. Total Environ. 784, 147167 https://doi.org/10.1016/j.scitotenv.2021.147167.

Mohr, S., Berghahn, R., Schmiediche, R., Hübner, V., Loth, S., Feibicke, M., Mailahn, W., Wogram, J., 2012. Macroinvertebrate community response to repeated short-term pulses of the insecticide imidacloprid. Aquat. Toxicol. 110–111, 25–36. https://doi.org/10.1016/j.aquatox.2011.11.016.

Montaño-Campaz, M.L., Oliveira, E.E., Bacca, T., Toro-Restrepo, B., Dias, L.G., 2023. Sex-specific alterations in adaptive responses of Chironomus columbiensis triggered by imidacloprid chronic and acute sublethal exposures. Environ. Sci. Pollut. Control Ser. 30 (31), 77328–77342. https://doi.org/10.1007/s11356-023-27601-1.

Pfaff, J.D., 1996. DETERMINATION of INORGANIC ANIONS by ION CHROMATOGRAPHY. En Methods for the Determination of Metals in Environmental Samples. Elsevier, pp. 388–417. https://doi.org/10.1016/B978-0-8155-1398-8.50022-7.

Raby, M., Nowierski, M., Perlov, D., Zhao, X., Hao, C., Poirier, D.G., Sibley, P.K., 2018a. Acute toxicity of 6 neonicotinoid insecticides to freshwater invertebrates: aquatic toxicity of neonicotinoid insecticides. Environ. Toxicol. Chem. 37 (5) https://doi.org/10.1002/etc.4088. Article 5.

Raby, M., Zhao, X., Hao, C., Poirier, D.G., Sibley, P.K., 2018b. Chronic toxicity of 6 neonicotinoid insecticides to Chironomus dilutus and Neocloeon triangulifer. Environ. Toxicol. Chem. 37 (10), 2727–2739. https://doi.org/10.1002/etc.4234.

Ramírez-Morales, D., Pérez-Villanueva, M.E., Chin-Pampillo, J.S., Aguilar-Mora, P., Arias-Mora, V., Masís-Mora, M., 2021. Pesticide occurrence and water quality assessment from an agriculturally influenced Latin-American tropical region. Chemosphere 262, 127851. https://doi.org/10.1016/j.chemosphere.2020.127851.

Ratte, H., Memmert, A., 2003. Biological Effects and Fate of Imidacloprid SL 200 in Outdoor Microcosm Ponds.

Rico, A., Dimitrov, M.R., Van Wijngaarden, Satapornvanit, K., Smidt, H., Van den Brink, P.J., 2014. Effects of the antibiotic enrofloxacin on the ecology of tropical eutrophic freshwater microcosms. Aquatic Toxicology 147, 92–104. https://doi.org/10.1016/j.aquatox.2013.12.008.

Rico, A., Van Den Brink, P.J., 2015. Evaluating aquatic invertebrate vulnerability to insecticides based on intrinsic sensitivity, biological traits, and toxic mode of action. Environ. Toxicol. Chem. 34 (8), 1907–1917. https://doi.org/10.1002/etc.3008.

Rico, A., Arenas-Sánchez, A., Pasqualini, J., García-Astillero, A., Cherta, L., Nozal, L., Vighi, M., 2018. Effects of imidacloprid and a neonicotinoid mixture on aquatic invertebrate communities under Mediterranean conditions. Aquat. Toxicol. 204, 130–143. https://doi.org/10.1016/j.aquatox.2018.09.004.

Roessink, I., Merga, L.B., Zweers, H.J., Van den Brink, P.J., 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. Environ. Toxicol. Chem. 32 (5), 1096–1100. https://doi.org/10.1002/etc.2201.

Silva, L.H.S., Huszar, V.L.M., Marinho, M.M., Rangel, L.M., Brasil, J., Domingues, C.D., Branco, C.C., Roland, F., 2014. Drivers of phytoplankton, bacterioplankton, and zooplankton carbon biomass in tropical hydroelectric reservoirs. Limnologica 48, 1–10. https://doi.org/10.1016/j.limno.2014.04.004.

Sumon, K.A., Ritika, A.K., Peeters, E.T.H.M., Rashid, H., Bosma, R.H., Rahman, Md S., Fatema, Mst K., Van den Brink, P.J., 2018. Effects of imidacloprid on the ecology of sub-tropical freshwater microcosms. Environ. Pollut. 236, 432–441. https://doi.org/10.1016/j.envpol.2018.01.102.

Thomas, D.H., Rey, M., Jackson, P.E., 2002. Determination of inorganic cations and ammonium in environmental waters by ion chromatography with a high-capacity cation-exchange column. J. Chromatogr. A 956 (1–2). https://doi.org/10.1016/s0021-9673(02)00141-3. Article 1-2.

Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45 (1), 247–268. https://doi.org/10.1146/annurev.pharmtox.45.120403.095930.

US EPA, 2020. Imidacloprid Proposed Interim Registration Review Decision Case Number 7605.

Van den Brink, P.J., Braak, C.J.F.T., 1999. Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environ. Toxicol. Chem. 18 (2), 138–148. https://doi.org/10.1002/etc.5620180207.

Van Den Brink, P.J., Van Wijngaarden, R.P.A., Lucassen, W.G.H., Brock, T.C.M., Leeuwangh, P., 1996. Effects of the insecticide dursban® 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. Environ. Toxicol. Chem. 15 (7), 1143–1153. https://doi.org/10.1002/etc.5620150719.

Van den Brink, P.J., Hattink, J., Bransen, F., Van Donk, E., Brock, T.C.M., 2000. Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. Aquat. Toxicol. 48 (2), 251–264. https://doi.org/10.1016/S0166-445X(99)00037-5.

Villegas, L., Cabrera, M., Capparelli, M.V., 2021. Assessment of microplastic and organophosphate pesticides contamination in fiddler crabs from a ramsar site in the estuary of guayas river, Ecuador. Bull. Environ. Contam. Toxicol. 107 (1) https://doi.org/10.1007/s00128-021-03238-z. Article 1.