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Tesis de Doctorado

Tolerancia y alteraciones de la comunidad de macroinvertebrados dulceacuícolas debida a la presencia de residuos de plaguicidas en cuerpos de agua superficial de Costa Rica

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Yo Silvia Echeverría Sáenz, estudiante del Doctorado en Ciencias Naturales para el Desarrollo, declaro que la Tesis Doctoral que presento para su exposición y defensa titulada "*Tolerancia y alteraciones de la comunidad de macroinvertebrados dulceacuícolas debida a la presencia de residuos de plaguicidas en cuerpos de agua superficial de Costa Rica*" y cuyo comité asesor de tesis son el Dr. Manuel Spínola Parallada (director de tesis), Dr. Meyer Guevara Mora (asesor) y el Dr. Pablo Gutiérrez Fonseca (asesor), es original y que todas las fuentes utilizadas para su realización han sido debidamente citadas en el mismo. Este material no lo he presentado, en forma parcial o total, como una tesis en esta u otra institución.

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Resumen

Costa Rica es un país con gran biodiversidad, sin embargo, el uso excesivo de plaguicidas ha generado contaminación de las aguas superficiales, cuyos efectos sobre la biota acuática aún no se comprenden a cabalidad. El objetivo de este estudio fue analizar los efectos de los plaguicidas sobre la distribución, la estructura y el rol ecológico de las comunidades de macroinvertebrados acuáticos, como herramienta para mejorar la conservación de los ecosistemas acuáticos tropicales. Para esto, se digitalizó y se unificó la información de las concentraciones de plaguicidas de 1036 muestras de agua superficial, así como la abundancia de las familias de macroinvertebrados en 441 muestras recolectadas entre los años 2009-2019. Los plaguicidas más frecuentemente detectados en Costa Rica durante ese período de tiempo fueron diuron, ametrina, pirimetanil, flutolanil, diazinon, azoxystrobina, buprofezin y epoxiconazol, con presencia en >20% de las muestras. Se observó que 32 plaguicidas se detectaron en concentraciones que superaron normas internacionales y se calculó un modelo de riesgo ecológico (msPAF) que reveló un riesgo de toxicidad aguda entre moderada y alta para organismos acuáticos (especialmente productores primarios y artrópodos) en el 13% de las muestras analizadas. Además, mediante análisis de umbrales ecológicos, se estimó la tolerancia/sensibilidad de las familias de macroinvertebrados respecto a 13 plaguicidas seleccionados. Los organismos que mostraron respuestas tolerantes fueron los moluscos y las familias Hyalellidae y Corophiidae (Amphipoda), así como los ácaros acuáticos (Trombidiformes), las familias Ceratopogonidae, Simuliidae, Empididae y Psychodidae (Diptera), Leptoceridae (Trichoptera), Baetidae (Ephemeroptera), Collembola, Corydalidae (Megaloptera), Elmidae (Coleoptera) y Libellulidae (Odonata). Por el contrario, las familias Blaberidae (Blattodea), Dixidae (Diptera), Ecnomidae, Lepidostomatidae, Odontoceridae (Trichoptera), Euthyplociidae, Isonychiidae (Ephemeroptera), Polythoridae (Odonata) y Atyidae (Decapoda) estuvieron completamente ausentes en sitios con plaguicidas, mientras Philopotamidae, Helicopsychidae (Trichoptera), Gyrinidae, Scirtidae, Hydrophilidae, Limnichidae (Coleoptera), Gomphidae (Odonata) y Perlidae (Plecoptera), entre otras, mostraron una respuesta de sensibilidad para ≥5 plaguicidas, indicando una muy baja tolerancia de la comunidad de macroinvertebrados dulceacuícolas a estas sustancias. La respuesta de sensibilidad o tolerancia de los organismos se discutió también mediante el análisis de caracteres anatómicos o fisiológicos de los organismos, que pudieran conferir mayor vulnerabilidad (por ej. aquellos que podrían aumentar la tasa de absorción de contaminantes o que limitan la capacidad de escape). Se observó que, si bien los caracteres tienen un gran potencial para uso predictivo en la sensibilidad de los organismos, aún falta trabajo en la definición de los caracteres más útiles para dicho fin. A través de los análisis de umbrales ecológicos se estimó los “community change points” (CCP, por sus siglas en inglés) que representan la concentración de cada plaguicida en la que se observa un cambio abrupto y sincrónico en las abundancias de muchos taxa de la comunidad. En 10 de los 13 plaguicidas analizados, el CCP fue muy inferior a los criterios de calidad ambiental para protección de la biota acuática, por lo que se revela que se producen efectos abruptos y sincrónicos a

concentraciones mucho más bajas de las que están establecidas como seguras de acuerdo con datos de toxicidad crónica para organismos acuáticos. En este estudio se ha demostrado también que otros factores de estrés asociados a zonas agrícolas como la degradación de la vegetación ribereña, el aumento de la temperatura y la presencia de nutrientes en exceso generan efectos de disminución de la biodiversidad, en conjunto con la presencia de residuos de plaguicidas y pueden incluso generar zonas deshabitadas de organismos ya sea por toxicidad o mecanismos de evasión de los contaminantes (pasivos o activos), produciendo fragmentación de la conectividad de los ecosistemas acuáticos a concentraciones considerablemente más bajas que aquellas que se consideran seguras en la normativa internacional. Por lo tanto, los resultados de este tipo de estudios podrían complementar el desarrollo de criterios numéricos de calidad de agua para la protección de ecosistemas acuáticos y también ser útiles en las evaluaciones de riesgo retrospectivas.

Palabras claves

ecosistemas lóticos; ecotoxicología de comunidades; estresores múltiples; macroinvertebrados; Neotrópico; plaguicidas.

Abstract

Costa Rica is a country with great biodiversity; however, the excessive use of pesticides has generated contamination of surface waters, with effects on aquatic biota which are still not fully understood. The objective of this study was to analyze the effects of pesticides on the distribution, structure and ecosystemic function of aquatic macroinvertebrate communities as a tool to improve the conservation of tropical aquatic ecosystems. For this, information on pesticide concentrations of 1036 surface water samples was digitized and unified, as well as the abundance of macroinvertebrate families in 441 samples collected between the years 2009-2019. The most frequently detected pesticides in Costa Rica during that time period were diuron, ametryn, pyrimethanil, flutolanil, diazinon, azoxystrobin, buprofezin and epoxiconazole, with presence in >20% of the samples. Thirty-two pesticides were detected at concentrations exceeding international standards and an ecological risk model (msPAF) was calculated that revealed a moderate to high acute toxicity risk to aquatic organisms (especially primary producers and arthropods) in 13% of the analyzed samples. In addition, the tolerance/sensitivity of macroinvertebrate families to 13 selected pesticides was estimated by ecological threshold analysis. The organisms that showed tolerant responses were mollusks and the families Corophiidae and Hyalellidae (Amphipoda), as well as aquatic mites (Trombidiformes), the families Ceratopogonidae, Simuliidae, Empididae and Psychodidae (Diptera), Leptoceridae (Trichoptera), Baetidae (Ephemeroptera), Collembola, Corydalidae (Megaloptera), Elmidae (Coleoptera) and Libellulidae (Odonata). In contrast, the families Blaberidae (Blattodea), Dixidae (Diptera), Ecnomidae, Lepidostomatidae, Odontoceridae (Trichoptera), Euthyplociidae, Isonychiidae (Ephemeroptera), Polythoridae (Odonata) and Atyidae (Decapoda) were completely absent in sites with detection of pesticides, while Philopotamidae, Helicopsychidae (Trichoptera), Gyrinidae, Scirtidae, Hydrophilidae, Limnichidae (Coleoptera), Gomphidae (Odonata) and Perlidae (Plecoptera), among others, showed a sensitivity response for ≥5 pesticides, indicating a very low tolerance of the freshwater macroinvertebrate community to these substances. The sensitivity or tolerance response of the organisms was also discussed by analyzing anatomical or physiological traits of the organisms, which could confer increased vulnerability (e.g. those that could increase the rate of contaminant uptake or that limit escape capacity). It was observed that although the traits have great potential for predictive use in the sensitivity of organisms, there is still uncertainty in defining the most useful characters for this purpose. Through the analysis of ecological thresholds, the community change points (CCP) were estimated, which represent the concentration of each pesticide at which an abrupt and synchronic change in the abundances of many taxa of the community is observed. In 10 out of 13 pesticides analyzed, the CCP was well below the environmental quality criteria for protection of aquatic biota, thus revealing that abrupt and synchronic effects occur at concentrations much lower than those established as safe according to chronic toxicity data for aquatic organisms. This study has also shown that together with the presence of pesticide residues, other stress factors associated with agricultural areas such as the degradation of riparian vegetation, the increase in temperature and the presence of excess nutrients, generate effects that diminish biodiversity, and can even generate uninhabited areas either due to toxicity or mechanisms of evasion of contaminants (passive or active), producing fragmentation of the connectivity of aquatic ecosystems at concentrations considerably lower than those considered safe in international regulations. Therefore, the results of these studies could complement the development of numerical water quality criteria for protection of aquatic ecosystems and also be useful in retrospective risk assessments in the Tropics.

Keywords

community ecotoxicology; lotic ecosystems; macroinvertebrates; multiple stressors; Neotropics; pesticides.

1. Introducción

Objeto de estudio:

Los macroinvertebrados dulceacuícolas son una comunidad de organismos que se ha utilizado para propósitos de bioindicación desde hace más de tres décadas debido a su taxonomía relativamente bien conocida, su alta riqueza de especies, su naturaleza sedentaria, la duración de los estadíos larvales acuáticos y la existencia de metodologías de recolecta de bajo costo (Bonada et al., 2006). Además, este es un grupo muy diverso que representa más del 60% de la biodiversidad animal en cuerpos de agua continentales (Balian et al., 2008) y cumplen un rol fundamental en los flujos de energía, en las cadenas tróficas acuáticas y en el ciclaje de nutrientes, tanto a lo interno del ecosistema acuático, como en los ecosistemas terrestres adyacentes (Dijkstra et al., 2014).

Muchos estudios han reportado efectos de plaguicidas sobre macroinvertebrados. Entre los que se han observado con mayor frecuencia, ya sea en estudios de campo (que contienen mezclas de plaguicidas) o en estudios de laboratorio que usan concentraciones similares a las del campo, están las alteraciones de respuestas en mecanismos de biotransformación (biomarcadores como inhibición de colinesterasas o incremento de Glutatión-S-Transferasa (GST)), disminución de tasas de ingesta, inhibición de crecimiento o reproducción, disminución de la abundancia poblacional, cambios en la estructura de las comunidades, mortalidad e inhibición de la tasa de descomposición de materia orgánica (Schäfer et al., 2011). También ha sido posible observar algunos efectos indirectos, producidos por relaciones ecológicas de depredación, como la disminución de crecimiento y reproducción de una especie de zooplancton, por efecto de la presencia de un herbicida o aumento de productores primarios por toxicidad y efectos de un insecticida sobre los invertebrados (Van den Brink et al., 2009).

Además, puesto que muchos de los organismos considerados plagas agrícolas son insectos, los mecanismos de acción de los insecticidas tienen toxicidad también para sus pares acuáticos (por ej. macroinvertebrados y zooplancton). En estudios realizados en Costa Rica por Arias-Andrés et al. (2018), Echeverría-Sáenz et al. (2018) y Rämö et al. (2018), se observó que el riesgo de la presencia de diferentes grupos de plaguicidas (insecticidas, fungicidas y herbicidas) en la cuenca del Río Madre de Dios era más alto para las comunidades de productores primarios e invertebrados. Estos estudios tomaron en cuenta tanto la toxicidad individual de las sustancias, como el efecto potencial de las mezclas encontradas en el ecosistema. Estudios en otras regiones de Costa Rica también han evidenciado cambios en la estructura de las comunidades de macroinvertebrados y disminuciones de diversidad, al comparar sitios de referencia (sin agricultura) con sitios influenciados por diferentes cultivos (Echeverría-Sáenz et al., 2012).

También en la región tropical, Cornejo et al. (2019) estudiaron los efectos de múltiples estresores asociados con prácticas agrícolas (toxicidad de plaguicidas, enriquecimiento de nutrientes y alteraciones del hábitat) sobre las comunidades de macroinvertebrados acuáticos de una cuenca en Panamá. Ellos encontraron que los estresores tuvieron efectos sobre la puntuación final de dos índices bióticos basados en macroinvertebrados (SPEAR-Pest y BMWP-PAN) y que los tres grupos de estresores evaluados generaron cambios en la riqueza y estructura de las comunidades. También, Cornejo et al (2020; 2021a, b) identificaron que los organismos detritívoros presentaron una mayor sensibilidad a los plaguicidas y que por este efecto, funciones ecosistémicas básicas como la degradación de materia orgánica se han visto reducidas.

Esta comunidad ha sufrido pérdidas de biodiversidad asociadas con la presencia de residuos de plaguicidas en muchas regiones del mundo (Schäfer, 2019). Stehle and Schulz (2015) exponen información que indica que la riqueza de las familias de macroinvertebrados se redujo ~30% en presencia de concentraciones de plaguicidas que representan los límites aceptables de dichos plaguicidas a nivel regulatorio y que es posible observar una reducción de hasta 63% en sitios con concentraciones que exceden los límites aceptables. También, otro estudio (Beketov et al., 2013) que utilizó datos de Alemania, Francia y Australia, demostró que los niveles de plaguicidas afectaron significativamente la biodiversidad de los invertebrados dulceacuícolas.

Además de los plaguicidas, existen otros factores que pueden incidir en la disminución de la diversidad de macroinvertebrados en un ecosistema acuático, tales como la contaminación de tipo orgánico (Sandin and Hering, 2004; Agboola et al., 2019), la baja disponibilidad de microhábitats para colonización dentro de los cauces (Acosta et al., 2009) o la ausencia de material alóctono por deforestación de la vegetación de ribera (Frazer, 2005; Connolly et al., 2016; Luke et al., 2019). Todos estos factores se relacionan con el uso de la tierra en las cuencas, por lo que la integridad ecológica de los ecosistemas acuáticos y la diversidad de los macroinvertebrados dependen directamente de la gestión adecuada del territorio.

En el caso de Costa Rica, este es un país cuya economía se basa en gran medida en la agricultura (SEPSA, 2018) y la mayoría de los cultivos como el melón, banano, piña, arroz, café, hortalizas, entre otros, utilizan agroinsumos que son altamente tóxicos para organismos acuáticos (Bravo Durán et al., 2013). Esta situación crea un conflicto de intereses entre la necesidad de mantener un ambiente sano y equilibrado y el impacto negativo que la actividad agrícola ejerce sobre los ambientes naturales. Además, se ha demostrado que los cuerpos de agua superficiales en Costa Rica presentan contaminación por residuos de plaguicidas en diversas regiones geográficas del país incluyendo el Caribe (Arias-Andrés et al., 2018; Castillo et al., 2000; 2006; Echeverría-Sáenz et al., 2012; 2018), la zona norte (Fournier et al., 2018), el Pacífico Norte (Mena et al., 2014; Rizo-Patrón et al., 2013), la zona sur (Ruepert et al., 2014) y las áreas hortícolas de Pacayas y Zarcero, en la Cordillera Volcánica Central (Fournier et al., 2010).

En dichos estudios, realizados en Costa Rica, se ha observado que la biodiversidad de macroinvertebrados dulceacuícolas fue consistentemente menor en los sitios donde se detectó residuos de plaguicidas (junto con otros contaminantes de origen agrícola) que en los sitios de referencia (Castillo et al., 2006; Fournier et al., 2010; Ruepert et al., 2014; Echeverría-Sáenz et al., 2012, 2018). Por esa razón, es necesario comprender a nivel ecológico, los efectos producidos por la presencia de plaguicidas y otros factores ambientales (como condiciones del hábitat fluvial, vegetación de ribera, oxígeno disuelto, pH, temperatura, nutrientes), sobre la comunidad de los macroinvertebrados dulceacuícolas. Esta información se podría utilizar para sugerir medidas de manejo de cuencas hidrográficas, así como estrategias de recuperación de ríos o quebradas con énfasis en el incremento de la biodiversidad acuática. A nivel nacional, esta información también es muy valiosa para afinar la clasificación de la calidad del agua de los cuerpos de agua superficiales que se enmarca en el Decreto 33903-MINAE-S (2007) o incluso para generar insumos útiles en la elaboración de criterios de calidad ambiental para presencia de plaguicidas en aguas superficiales de Costa Rica.

***Preguntas de investigación*:**

Dado que existe una preocupación a nivel mundial por las fuertes declinaciones de las poblaciones de insectos, tanto en países de climas templados como tropicales (Conrad et al., 2006; Janzen and Hallwachs, 2019) y al hecho ya conocido de que la presencia de plaguicidas en ecosistemas acuáticos genera efectos de disminución de la biodiversidad de macroinvertebrados dulceacuícolas (Beketov et al., 2013; Stehle and Schulz, 2015), en el presente estudio se planteó contestar a las siguientes preguntas:

¿Cuáles taxa de macroinvertebrados presentan mayor sensibilidad a la presencia de plaguicidas en un país tropical como Costa Rica?

¿Dicha sensibilidad está asociada a sus caracteres anatómicos, fisiológicos o de funcionalidad?

¿Cómo se compara el efecto de los plaguicidas con el de otros factores de estrés?

***Marco teórico*:**

Conceptos básicos sobre plaguicidas

Los plaguicidas son sustancias destinadas a prevenir, controlar o destruir cualquier organismo que sea considerado una plaga y se aplican en terrenos agrícolas de todo el orbe. Su uso ronda los cuatro millones de toneladas anuales de ingrediente activo (i.a.) en el mundo (FAO, 2018) y está en aumento principalmente en los países del continente asiático (como la India y China) y en América, donde su uso se duplicó entre los años 1990-2017. En nuestro continente, los mayores aumentos en el uso de plaguicidas en dicho período se observan en países como Brasil, Argentina y Ecuador, que incrementaron en más de siete veces su uso. Mientras tanto, el uso en Centroamérica y el Caribe se duplicó, con aumentos importantes en Nicaragua (7,67 veces más) y en Costa Rica (8,85 veces más, pasando de 1 447 ton i.a. en 1990 a 12811 ton i.a. en 2017).

Estas sustancias pueden clasificarse según su acción biocida de acuerdo con el grupo taxonómico sobre el que actúa, por ejemplo, insecticidas, fungicidas, herbicidas, nematicidas, molusquicidas, entre otros. También se suelen dividir los plaguicidas de acuerdo con su modo de acción, que se refiere la manera en la que el plaguicida ejerce la alteración fisiológica deseada, por ejemplo, inhibidores de la colinesterasa, inhibidores de biosíntesis de esteroles, inhibidores de fotosistema II, entre otros (HRAC, FRAC, IRAC, 2023).

También suele dividirse a los plaguicidas de acuerdo con su familia química. En Costa Rica, algunos de los grupos más importantes son los carbamatos, derivados de urea, organoclorados, organofosforados, piretroides, tiocarbamatos, triazinas, conazoles, neonicotinoides, entre otros. Además de esto, cada plaguicida tiene un mecanismo de acción específico (a nivel bioquímico o molecular) que es el punto exacto del organismo donde se presenta el efecto tóxico. Por la naturaleza de este proyecto y de la comunidad de organismos a estudiar (compuesta mayoritariamente por artrópodos), se mencionarán algunos mecanismos de acción relevantes dentro del grupo de los insecticidas, a saber:

Los organofosforados y carbamatos producen neurotoxicidad a través de la inhibición de la acetilcolinesterasa (neurotransmisor), los piretroides actúan sobre la bomba de sodio, rompiendo el intercambio de iones y la producción de energía celular, los organoclorados son antagonistas de la bomba de cloruro g-aminobutírica ácida en el sistema nervioso, mientras que los neonicotinoides son antagonistas de los receptores de acetilcolina, produciendo también neurotoxicidad (Rico and Van den Brink, 2015). Todos estos sistemas son muy inespecíficos, por lo que la presencia de estas sustancias en el ambiente puede causar efectos neurotóxicos sobre múltiples organismos no blanco.

¿Cómo se trasladan los residuos de plaguicidas hacia los ecosistemas acuáticos?

Los plaguicidas son susceptibles de dispersarse y transportarse ambientalmente fuera de los terrenos agrícolas (Figura 1): por el viento, a través de mecanismos de deriva, infiltrándose por el suelo, a través de la lixiviación o corriendo por encima de él, a través de la escorrentía superficial (Lefrancq, 2014). De esta forma, los residuos de plaguicidas alcanzan múltiples compartimentos ambientales y pueden viajar largas distancias, incluso hasta zonas prístinas, alejadas de los terrenos agrícolas (Daly et al., 2007a y b).

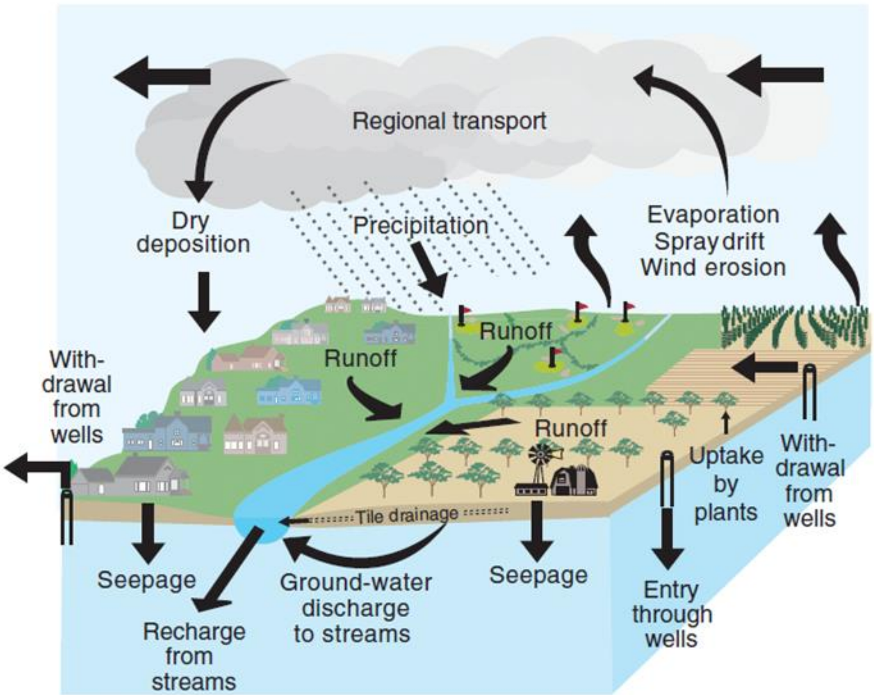


Figura 1. Movimiento de plaguicidas en el ciclo hidrológico. Fuente: Lefrancq (2014)

Las características o propiedades físicas y químicas de cada plaguicida lo harán más o menos propenso a dispersarse en cierto compartimento ambiental, es decir que determinarán su destino ambiental. Por ejemplo, la viscosidad y volatilidad de un plaguicida determinarán qué tanto puede dispersarse por deriva (atmósfera). También, la afinidad de un plaguicida por los lípidos define su probabilidad de ser absorbido por las raíces de las plantas, cuanto más lipofílico sea, mayor será la absorción en el compartimento suelo/biota. El coeficiente de partición octanol-agua (Kow) se utiliza para determinar si el plaguicida es hidrofílico o hidrofóbico, así un plaguicida muy hidrofílico tendrá mayor posibilidad de disolverse rápidamente en agua y dispersarse en el ciclo hidrológico. Sin embargo, sin importar su Kow, los eventos de precipitación que generan erosión y aumentos en la escorrentía superficial, movilizan rápidamente ambos tipos de plaguicidas hacia los cuerpos de agua (Lefrancq, 2014).

Los procesos de sorción/desorción gobiernan la lixiviación de plaguicidas hacia las capas más profundas del suelo y también el momento y la cantidad de plaguicidas movilizados por escorrentía. Cabe destacar que cuanta más materia orgánica tenga el suelo, mayor será su retención de nutrientes, ya que el humus tiene muchas cargas negativas, que le confieren gran capacidad de retener cationes. En un estudio realizado en Costa Rica, en la zona de Poás, se ha comprobado que la materia orgánica contribuye en la sorción de los plaguicidas y, por lo tanto, limita su movilidad a través del agua, disminuyendo el riesgo de que estos contaminantes alcancen las aguas subterráneas por lixiviación (García-Céspedes, 2017). Asimismo, la actividad biótica y el aumento del tiempo de retención en esta capa del suelo coadyuvan en la degradación de los plaguicidas por procesos de fotólisis, oxidación, degradación o biodegradación.

¿Cómo afectan los residuos de plaguicidas a los ecosistemas acuáticos?

Para la adecuada conservación del recurso hídrico es indispensable proteger la cobertura forestal y, donde existan actividades productivas, reforzar todas las medidas posibles de conservación de suelo y reducción de la erosión. Los sitios donde exista mayor arrastre de partículas tendrán también un mayor arrastre de plaguicidas, que se transportan con el agua de la escorrentía (los más hidrofílicos) o adheridos a las partículas de suelo (los más hidrofóbicos) (Berenzen et al., 2005; Schulz y Liess, 2001).

Cuando los plaguicidas entran a los cuerpos de agua, interactúan con los componentes bióticos y abióticos del ecosistema. Los componentes abióticos tenderán a favorecer la degradación (fotólisis, hidrólisis) o la sorción de las sustancias (en sedimentos o materia orgánica), mientras que la interacción con la biota conlleva procesos de ingreso, metabolización y/o acumulación en los organismos, que pueden producir efectos deletéreos (Schäfer et al., 2011). Por otro lado, Bopp et al. (2018) consideran que, aunque las evaluaciones de riesgo para el registro de sustancias se basan en el estudio de cada una individualmente, los marcos legales deberían incorporar la exposición multi-sustancias y empezar a considerar las evaluaciones de mezclas con una metodología uniforme. Asimismo, Altenburger et al. (2019) indican que, en la salvaguarda del ambiente, se debe comprender que: 1) actualmente, la exposición ambiental se presenta en mezclas complejas de sustancias químicas; 2) el monitoreo es importante para garantizar los objetivos de calidad ambiental que se requieren; y 3) es importante cuantificar relaciones de causa-efecto entre los contaminantes y los efectos adversos.

Existen estudios que demuestran la presencia de residuos de plaguicidas y sus efectos negativos en la biota de los cuerpos de aguas superficiales en todos los continentes (Beketov et al., 2013; Echeverría-Sáenz et al., 2018; Schäfer et al., 2016; Stehle y Schulz, 2015). Algunos de ellos han establecido relaciones claras entre el uso de la tierra y la contaminación por residuos de plaguicidas (Berenzen et al., 2005; Dabrowski et al., 2002; Glover, 2003). Stehle y Schulz (2015) se refieren a la información de más de 800 estudios que reportan concentraciones de insecticidas que exceden los límites regulatorios, por lo que se observa que esta condición es muy frecuente y que los organismos acuáticos están expuestos a concentraciones inaceptables de plaguicidas, principalmente en países tropicales, donde las medidas de protección son más laxas y el uso de plaguicidas se incrementa.

La exposición a plaguicidas puede causar efectos directos en todos los niveles de organización biológica, mientras que el modo de acción determina en gran medida cuál grupo de organismos será más probablemente afectado (productores primarios, microorganismos, invertebrados o peces) (Beketov et al., 2013; Schäfer et al., 2011; Van Den Brink y Braak, 2012). Estos efectos directos pueden desencadenar otra serie de efectos indirectos en los ecosistemas acuáticos, cuya capacidad de recuperación es incierta, pues depende de múltiples variables (tiempo de exposición, frecuencia e intensidad de la misma, presencia de ecosistemas inalterados, entre otros) (Liess y Beketov, 2011; Schäfer et al., 2011).

¿Cuáles son los principales efectos de los plaguicidas descritos en la literatura?

Los organismos acuáticos pueden ser afectados por los plaguicidas directa o indirectamente. Los efectos directos dependen de la concentración de la sustancia en el medio y son causados por la acción fisiológica del plaguicida sobre el organismo (por ej. mortalidad). Los efectos indirectos se refieren a las interacciones entre organismos de diferentes especies (como competencia, depredación, simbiosis) y no dependen de la concentración del tóxico, sino de la relación que exista entre la especie afectada directamente y otra con la cual interactúe (Schäfer et al., 2011).

Los efectos también se pueden observar en diferentes niveles de organización biológica, desde los cambios sub-organismo (como efectos bioquímicos o fisiológicos), que son los que ocurren primero, pasando por los subsiguientes efectos en los individuos (comportamiento, morfología, mortalidad) hasta llegar a observarse más tarde, los efectos que ocurren a nivel de comunidades y ecosistemas, que involucran aspectos de funcionalidad y procesos ecológicos mayores como reciclaje de nutrientes o descomposición de materia orgánica (Schäfer et al., 2011, Cornejo et al., 2020, 2021a). En eventos de contaminación severa, se espera observar disminución de la abundancia o desaparición de especies o de grupos completos de organismos que tengan mayor sensibilidad o que carezcan de mecanismos para escapar (Beketov y Liess, 2008; Jergentz et al., 2004).

El estadío del ciclo de vida, la duración de la exposición, el potencial de biomagnificación, la presencia de estresores adicionales, la densidad poblacional y las exposiciones previas a tóxicos también pueden influenciar la magnitud de los efectos (Liess y Von Der Ohe, 2005). Por lo tanto, una misma concentración de un plaguicida podría producir un efecto de mayor o menor magnitud en diferentes ecosistemas. Sin embargo, cuando los ecosistemas son similares (por ej. quebradas influenciadas por agricultura), la concentración o toxicidad de los plaguicidas en sí misma puede explicar una gran parte de la variación en la estructura de las comunidades aún en escalas regionales (Beketov et al., 2013; Stehle y Schulz, 2015; Stehle et al., 2018).

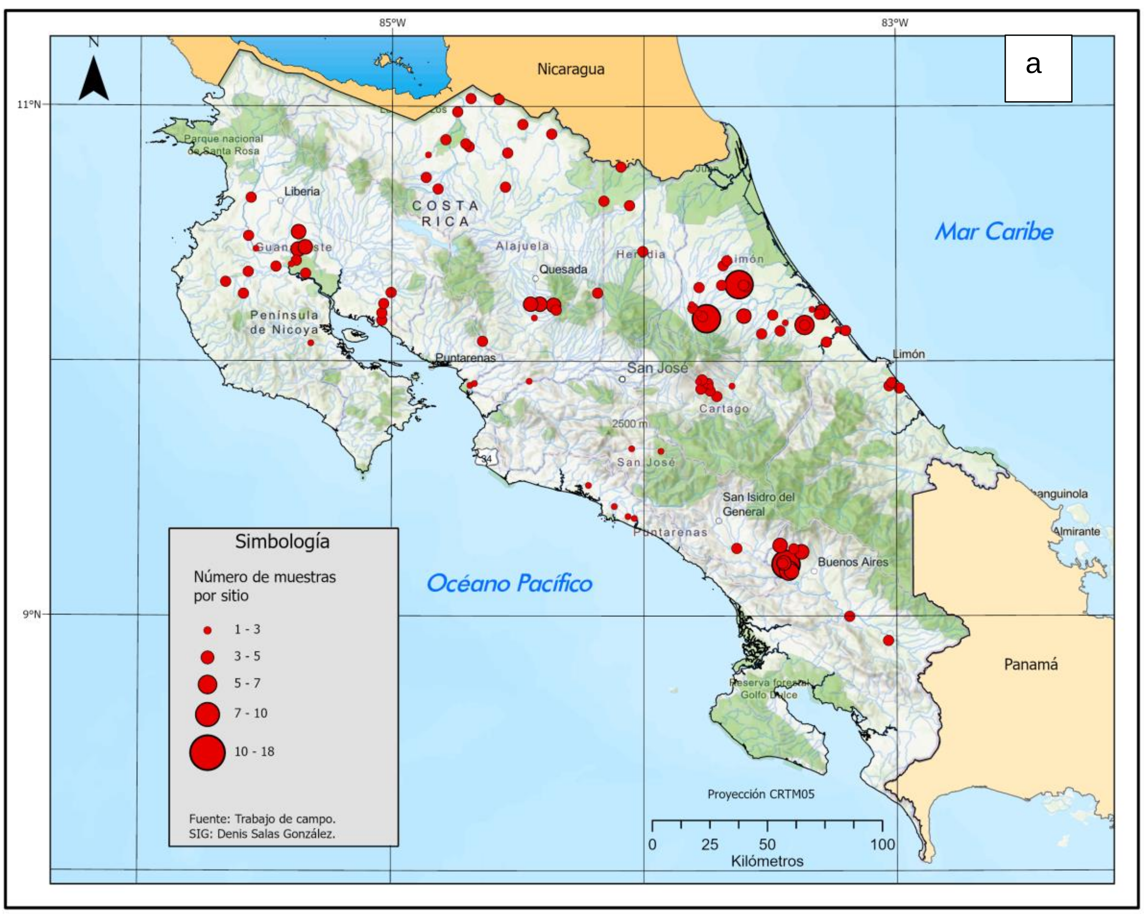
Cornejo et al. (2019) resaltaron que existe gran necesidad de realizar bioensayos de toxicidad con especies tropicales que permitan la adaptación de índices bióticos bien conocidos en otras latitudes (como el SPEAR-Pesticides; Beketov et al., 2009). Asimismo, destacan la inexistencia de estudios observacionales y manipulativos que exploren los efectos combinados de múltiples estresores en macroinvertebrados tropicales.

Es importante incorporar más componentes de investigación ecológica a la determinación de los riesgos toxicológicos de los plaguicidas y la combinación con otros estresores de los ecosistemas acuáticos, como el cambio climático. La multifactoriedad involucrada hace que las relaciones de causalidad entre la exposición y los efectos observados (tanto directos como indirectos) no sea tan clara ni tan fácil de establecer (Schäfer et al., 2011). Por lo tanto, se requiere más información sobre la biodisponibilidad de los plaguicidas (relacionada con su destino ambiental y sus propiedades físicas-químicas), los efectos de los pulsos de concentraciones "pico" de plaguicidas y sobre la interacción de otros factores ambientales en la magnitud del efecto tóxico al comparar diferentes ecosistemas. También continúa siendo limitado el conocimiento sobre el efecto tóxico de las mezclas de plaguicidas y las interacciones que se presentan en los ecosistemas naturales, sin embargo, la clave para discriminar los efectos de estas sustancias es que otros factores (por ej. crecidas o cambios de temperatura, entre otros) tienen pocas probabilidades de afectar únicamente a cierto grupo de organismos o a cierto nivel trófico, ya que sus efectos serán más inespecíficos.

Marco metodológico:

Área de estudio y fuentes de datos:

Este estudio utilizó información generada a través de recolecta de muestras de campo tomadas en 99 puntos de muestreo en cuerpos de aguas continentales lóticos de Costa Rica, en el periodo de 10 años entre 2009-2019 (Figura 2a,b).



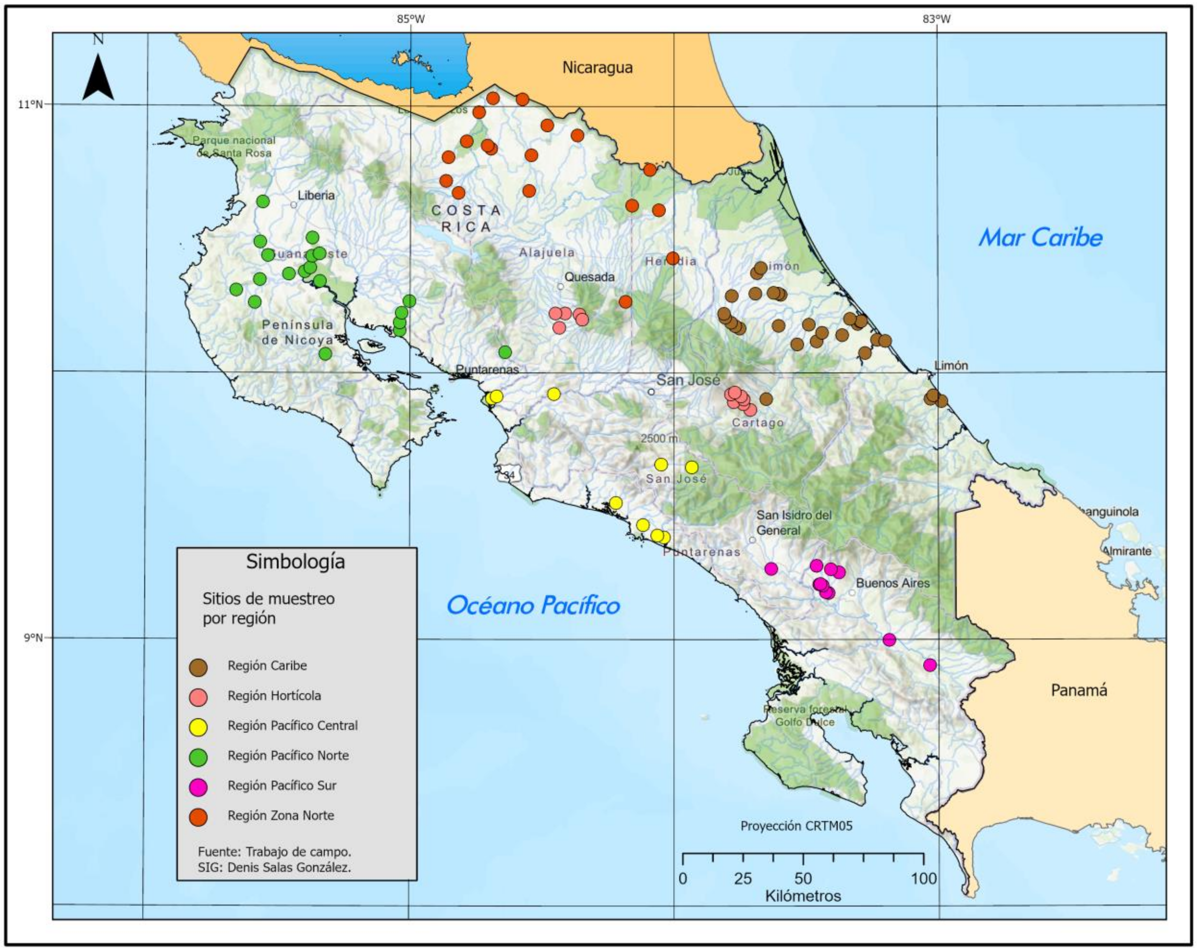


Figura 2. a. Puntos de muestreo y número de muestras de macroinvertebrados en cada sitio; **b.** Distribución de los sitios de muestreo por región. Costa Rica, 2009-2019.

Para realizar este proyecto, se unificaron todas las bases de datos que contienen información taxonómica de macroinvertebrados y variables físicas y químicas ambientales, de cada uno de los siguientes proyectos de investigación (Tabla 1):

Tabla 1. Proyectos de investigación de donde se obtuvo la información para el análisis. Costa Rica (2009-2019)

Año	**Núm. Muestras***	**Proyecto**	**Referencia**
2009	21	Diagnóstico sobre contaminación de aguas, suelos y productos hortícolas por el uso de agroquímicos en la microcuenca de las **quebradas Plantón y Pacayas en Cartago**, Costa Rica	Fournier et al., 2010

2009-2010	27	Métodos biológicos para evaluar el estado ecológico de las comunidades ribereñas, en las zonas piñeras del **Caribe** de Costa Rica	Echeverría-Sáenz et al., 2012
2009-2011	29	Impacto de los plaguicidas en el recurso hídrico de la **cuenca del río Tempisque (Palo Verde)** Costa Rica. Bases científicas para la gestión ambiental sostenible	De la Cruz, et al., 2012
2011-2012	16	Identificación de amenazas y capacitación para el uso sostenible del Refugio Nacional de Vida Silvestre **Caño Negro, Región Huetar Norte**	Fournier et al., 2018
2011-2013	45	Plan de gestión de la cuenca del **Río Volcán, Pacífico Sur** de Costa Rica	Echeverría-Sáenz et al., 2022
2011-2013	30	Evaluación del riesgo ecológico de la escorrentía de plaguicidas usados en la agricultura hacia el Río y la **Laguna Madre de Dios en la zona del Caribe**, Costa Rica "TROPICA"	Echeverría-Sáenz et al., 2018
2013-2015	24	Las buenas prácticas agrícolas en el uso y manejo de agroquímicos en la zona hortícola de **Zarcero, Alajuela**	Ramírez Muñoz et al., 2017
2016-2020	205	Plan Nacional de Monitoreo de la calidad de los cuerpos de agua superficiales (**todo el país**)	Código SIA 0093-16
2011-2019	28	Observatorio Ambiental de la Universidad Nacional; Indicador: Presencia de residuos de plaguicidas y calidad biológica del Río Jiménez, **Caribe** de Costa Rica	Echeverría-Sáenz et al., 2019
2018-2020	12	Efectos de la presencia de residuos de plaguicidas y otros factores ambientales, en el establecimiento de la comunidad de macroinvertebrados acuáticos en una quebrada con influencia piñera en el **pacífico sur** de Costa Rica	Echeverría-Sáenz et al., 2022
2018-2020	4	Procesos de Gestión Integrada del Recurso Hídrico en las subcuencas Chiz-Maravilla y Quebrada Honda, **Cartago**, Costa Rica	Código SIA 0015-17

También se buscó información sobre la toxicidad aguda (Concentración media letal - LC50 - o Concentración media de efecto - EC50 - para exposiciones de laboratorio de 24-96 horas) para diferentes grupos de organismos dulceacuícolas (plantas y algas, invertebrados y peces) en varios niveles tróficos, en las siguientes bases de datos:

- Base ECOTOX de la Agencia de Protección Ambiental de los EEUU (EPA)
- Base PPDB (Pesticide Properties DataBase) de la Universidad de Hertfordshire

Con dicha información, se generaron varias bases de datos:

1. Base de datos de plaguicidas en muestras de agua superficial lótica de Costa Rica en el periodo de los años 2009-2019 (1036 muestras totales analizadas).

2. Base de datos de características de los plaguicidas (N° CAS, grupo químico, acción biocida, modo de acción, riesgo registrado).

3. Base de datos de macroinvertebrados, con información taxonómica (clase, orden, familia, género) y de abundancia de cada uno de los taxa identificados entre los años 2009-2019 en cuerpos de agua superficiales lóticos de Costa Rica (441 muestras).

4. Base de datos de caracteres anatómicos y fisiológicos de 104 taxa de macroinvertebrados.

Objetivo 1: Tolerancia/sensibilidad de los macroinvertebrados

Se analizó la detección y frecuencia de plaguicidas a nivel nacional con la base de datos de análisis de plaguicidas en muestras recolectadas en cuerpos de agua lóticos de las regiones Pacífico Norte, Centro y Sur, Caribe Norte y Sur y zonas hortícolas de montaña en Zarcero y Cartago. Se compararon las concentraciones detectadas en Costa Rica con normativas internacionales como un indicador de la situación en el país. Además, a través de análisis msPAF (multiple substance Potentially Affected Fraction) se estimó el riesgo de las mezclas de plaguicidas para los diferentes grupos de organismos acuáticos del país (productores primarios, invertebrados y peces). Asimismo, se identificaron los plaguicidas que generan el mayor riesgo. Detalles en Artículo #1.

Paralelamente, se generó otra base de datos que incluye la información de las muestras de macroinvertebrados obtenida de las fuentes descritas anteriormente. Una vez que se unificó esta base de datos (con 441 muestras totales), se procedió a analizar la distribución de las familias de macroinvertebrados respecto a las concentraciones detectadas de diferentes grupos de residuos de plaguicidas seleccionados.

Para seleccionar los plaguicidas a incorporar en el análisis, se priorizaron las sustancias que cumplieron con los siguientes criterios: a) toxicidad aguda y crónica alta o extremadamente alta para organismos acuáticos, especialmente artrópodos; b) frecuencia de detección de la sustancia >5% en las muestras analizadas; c) riesgo ambiental estudiado en los proyectos precitados u otros a nivel nacional y considerado alto; d) representación de diferentes acciones biocidas: fungicidas, insecticidas, herbicidas).

Posteriormente, se examinó la relación entre las concentraciones de plaguicidas seleccionados obtenidas del campo y la presencia / ausencia o abundancia local de las familias de macroinvertebrados. Dicha relación se analizó mediante el paquete TITAN2 (v2.4.2, Threshold Indicator Taxa ANalysis; Baker and King, 2010), del software estadístico R (v4.2.2; R Core Team 2023), que se utilizó para identificar umbrales ecológicos, que son las zonas de cambio en las distribuciones de las familias en un gradiente ambiental (temporal o espacial). En este caso, dichos umbrales ecológicos de las familias de macroinvertebrados representan una medida indirecta de la tolerancia/sensibilidad de las familias respecto a cada uno de los plaguicidas seleccionados. Este método integró datos de presencia, abundancia y direccionalidad de las respuestas de cada familia. Detalles el Artículo #2.

Objetivo 2: Sensibilidad intrínseca y rol ecológico

Se analizó la sensibilidad intrínseca de los taxa que componen las comunidades de macroinvertebrados, mediante el análisis de caracteres anatómicos o fisiológicos. Los caracteres analizados en este proyecto son los grupos funcionales de alimentación, el tipo de respiración, la talla potencial máxima, el grado de sujeción al sustrato, movilidad, habilidad de natación, dureza o esclerotización, presencia en deriva y fuerza de vuelo del adulto, de acuerdo con la disponibilidad de información en la literatura. Un organismo cuya capacidad de migrar (o escapar) esté limitada, o cuya esclerotización sea baja, tenga respiración branquial o por integumento, entre otros, será más vulnerable ante un evento de contaminación por plaguicidas. Este tipo de análisis se ha hecho en el pasado principalmente para países de clima templado, por lo que existe poca información para los taxa originarios del neotrópico y muchos están ausentes de las bases de datos de caracteres, que han sido generadas en países europeos (por ej. SPEAR-pest; Liess and Von Der Ohe, 2005; Poff et al., 2006; Usseglio-Polatera et al., 2000). En este proyecto se recopiló la información faltante para 104 diferentes taxa de Costa Rica, que serán una primera representación del neotrópico.

Por otro lado, se desea saber si los cambios observados en la estructura de las comunidades, podrían traducirse en pérdida de roles ecológicos en los cuerpos de agua lóticos que presentan residuos de plaguicidas. Para esto, se determinó si la proporción de grupos funcionales de alimentación (GFA), estimadas para cada una de las muestras de los proyectos, era diferente en muestras con baja o alta concentración de plaguicidas detectados. Los GFA para cada muestra se identificaron principalmente con base en Ramírez y Gutiérrez-Fonseca (2014). Detalles en el Artículo #2.

Objetivo 3: Efectos de múltiples factores de estrés

Finalmente, se evaluaron los efectos de la combinación de múltiples factores ambientales en la comunidad de macroinvertebrados, como condiciones del hábitat fluvial y la calidad de la vegetación de ribera (índices IHF, QBR en Acosta et al., 2009), elevación (msnm), pH, temperatura, conductividad, nutrientes (Fósforo Total, Fósforo soluble, nitratos) y los efectos producidos por la presencia de los residuos de plaguicidas. Para esto, se realizó un análisis de Redundancia (RDA) utilizando una parte de los datos provenientes del Pacífico Sur (tres proyectos de investigación), donde existía información completa de suficientes parámetros físicos y químicos, así como de hábitat. Detalles en Artículo #3.

Adicionalmente, en la sección de Discusión Global, se incorporó un análisis de correlaciones de Pearson entre variables hidrológicas (precipitación, escurrimiento, caudal), obtenidas de la página web del Observatorio del Agua y Cambio Global (https://oacg.fcs.ucr.ac.cr/), y la concentración de nitratos.

2. Objetivos

2.1. Objetivo general

Analizar los efectos de los plaguicidas sobre la distribución, la estructura y el rol ecológico de las comunidades de macroinvertebrados acuáticos a partir de bases de datos disponibles para cuerpos de agua lóticos de Costa Rica, como herramienta para mejorar la conservación de los ecosistemas acuáticos tropicales.

2.2. Objetivos específicos

1. Identificar la relación existente entre las concentraciones de plaguicidas seleccionados y la distribución de las familias de macroinvertebrados, mediante revisión de su presencia/ ausencia o abundancia local, como una medida indirecta de la tolerancia/ sensibilidad de los taxa respecto a dichos xenobióticos.

2. Determinar la sensibilidad intrínseca de los macroinvertebrados a la presencia de residuos de plaguicidas seleccionados, mediante el análisis de caracteres anatómicos o fisiológicos y la proporción de grupos funcionales de alimentación como una medida de alteración de sus roles ecológicos en los cuerpos de agua lóticos.

3. Analizar los efectos producidos conjuntamente por múltiples factores de estrés ambiental sobre la estructura de la comunidad de macroinvertebrados, en cuerpos de agua superficial de Costa Rica.

3. Síntesis

Esta tesis está conformada por 3 artículos científicos que, juntos, responden a las diferentes preguntas de investigación planteadas y enmarcadas en el objetivo general. El artículo #1 es la base sobre la que se construyen los 3 objetivos específicos de esta tesis. Reúne toda la información sobre la presencia de plaguicidas en 1036 muestras de agua de sistemas lóticos de Costa Rica y brinda información sobre las sustancias que se han detectado en dichas muestras de agua, en el periodo 2009-2019. También se muestra la forma en que se distribuyen a nivel regional y cuáles de ellos representan el mayor riesgo para el ecosistema acuático. Este artículo brinda una primera aproximación para contextualizar la ubicuidad de la contaminación por plaguicidas en Costa Rica y, además, se calcula el riesgo de la presencia simultánea de múltiples plaguicidas sobre los organismos acuáticos. En este artículo se observa que los productores primarios y los artrópodos son los grupos más vulnerables de acuerdo con las mezclas de sustancias detectadas en los cuerpos de agua y evidencia la importancia de investigar más profundamente sobre los posibles efectos en estos grupos de organismos.

El artículo #2 comprende aspectos relacionados con los objetivos específicos 1, 2 y 3. Primeramente, se genera una selección de los plaguicidas que cumplen con los criterios establecidos para poder ser utilizados en el análisis de umbrales ecológicos. Mediante la estimación de dichos umbrales, se genera información a nivel empírico sobre la sensibilidad o tolerancia de los macroinvertebrados a los gradientes ambientales de plaguicidas. Asimismo, en este artículo se presenta una discusión sobre los caracteres anatómicos o fisiológicos que pueden ser determinantes en la sensibilidad intrínseca de cada uno de los organismos, es decir, aquella sensibilidad que se deriva de poseer características que les confieran mayor vulnerabilidad ante la exposición a plaguicidas en su hábitat natural o en ensayos de laboratorio.

Este artículo se extiende un poco más al generar una discusión sobre el posible uso de estos umbrales ecológicos, como un insumo en el desarrollo de criterios numéricos para regulación y normativa de calidad de aguas y protección ambiental en materia de presencia de plaguicidas. Por otro lado, el uso de los umbrales ecológicos como una herramienta de ecotoxicología de comunidades para la identificación de los organismos más sensibles y para determinación de criterios de calidad es un abordaje nuevo.

Este artículo discute el papel de los caracteres en la sensibilidad, las diferentes investigaciones que se han hecho en esta temática, nuestra línea de pensamiento sobre aquellos caracteres que podrían ser de mayor utilidad para definir la sensibilidad intrínseca de los macroinvertebrados en ambientes tropicales y también se abordan las inconsistencias y limitantes que observamos y que serán las nuevas preguntas de investigación para desarrollar en el futuro cercano.

El artículo #3 se enmarca en el objetivo específico 3, donde se genera la pregunta de ¿qué otros factores tienen influencia o efecto en la estructura y función de las comunidades de macroinvertebrados? Para esto, se analizó una sección de los datos correspondiente a las muestras recolectadas del pacífico sur del país, que tenía la serie más completa de información sobre varios tipos de parámetros ambientales. En este artículo se analizan los cambios de la comunidad de macroinvertebrados producto de los residuos de plaguicidas, la estacionalidad, los parámetros fisicoquímicos (nutrientes, temperatura, pH, caudal, abundancia de perifiton como fuente de alimentación), la integridad del hábitat (índices de calidad de la ribera y de diversidad de hábitat fluvial) y el potencial de recolonización. Se muestra cómo el conjunto de los factores de estrés puede generar pérdida local de biodiversidad y que la resiliencia de las comunidades de macroinvertebrados puede limitarse en el largo plazo.

En este último artículo, así como en el artículo #2 se enfatiza la necesidad de contemplar los aspectos de evasión y emigración de especies, como mecanismos de respuesta ante tóxicos. La pérdida de biodiversidad y los cambios en la estructura de las comunidades no necesariamente se pueden explicar únicamente con los datos de sensibilidad a nivel toxicológico. Además, la pérdida o inhabilitación de un hábitat por presencia de contaminantes, puede conducir a una fragmentación de la conectividad del ecosistema acuático, impidiendo la recolonización y, por ende, aumentando la vulnerabilidad de los organismos y la pérdida local de especies.

4. Artículo #1. Pesticides Burden in Neotropical Rivers: Costa Rica as a Case Study

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Article

Pesticides Burden in Neotropical Rivers: Costa Rica as a Case Study

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Abstract: Neotropical ecosystems are highly biodiverse; however, the excessive use of pesticides has polluted freshwaters, with deleterious effects on aquatic biota. This study aims to analyze concentrations of active ingredients (a.i) of pesticides and the risks posed to freshwater Neotropical ecosystems. We compiled information from 1036 superficial water samples taken in Costa Rica between 2009 and 2019. We calculated the detection frequency for 85 a.i. and compared the concentrations with international regulations. The most frequently detected pesticides were diuron, ametryn, pyrimethanil, flutolanil, diazinon, azoxystrobin, buprofezin, and epoxiconazole, with presence in >20% of the samples. We observed 32 pesticides with concentrations that exceeded international regulations, and the ecological risk to aquatic biota (assessed using the multi-substance potentially affected fraction model (msPAF)) revealed that 5% and 13% of the samples from Costa Rica pose a high or moderate acute risk, especially to primary producers and arthropods. Other Neotropical countries are experiencing the same trend with high loads of pesticides and consequent high risk to aquatic ecosystems. This information is highly valuable for authorities dealing with prospective and retrospective risk assessments for regulatory decisions in tropical countries. At the same time, this study highlights the need for systematic pesticide residue monitoring of fresh waters in the Neotropical region.

Keywords: aquatic biodiversity; msPAF; lotic ecosystems; ERA; pesticides

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1. Introduction

Neotropical regions are recognized worldwide for their biodiversity. Antonelli and Sanmartín [1] stated this is "the most species rich region on Earth", and Costa Rica is not the exception. According to data from the State of the Environment Report [2], the country has 5% of the world's biodiversity. However, the same report and [3] consider that although the country has managed to make good decisions in conservation, one of the central oversights in terms of environmental protection has been the management of agrochemicals, their excessive use, and their contaminating effects on the different environmental compartments (air, water, soil), as well as on wildlife and human health [4,5].

As stated by FAO data [6], Costa Rica used 22.9 kg/a.i./ha in 2016 and uses more than 20 kg/a.i./ha/year since the year 2000. This figure represents the third-highest use in the world, much higher than the use of European countries (e.g., The Netherlands 10.02, Belgium 6.89, and Germany 3.92 kg/a.i./ha in 2016) and also much higher than other countries in the Neotropical region (Colombia 13.17, Ecuador 12.36, Guatemala 10.02, Belize 8, El Salvador 5.95, Brazil 4.31, and Nicaragua 2.47 kg/a.i./ha in 2016). This situation is reflected in freshwater contamination by pesticide residues. In Costa Rica, pesticide residues have been detected in various geographical regions of the country, including the Caribbean [4–11], the northern zone [12], the North Pacific [13–15], the South Pacific [16], and the horticultural areas of Pacayas and Zarcero in the Central Volcanic Mountain Range [17,18]. In the last 10 years, Cornejo et al. [19,20] also detected several pesticide residues in Panama, Barizon et al. [21] in Brazil, Hernández et al. [22] in Colombia, Deknock et al. [23] in Ecuador, Leyva Morales et al. [24] in Mexico, and Cárdenas et al. [25] in Venezuela.

Tropical climates have the advantage of allowing year-round cultivation, but this implies the year-round application of agrochemicals as well. Therefore, pesticides become "pseudo-persistent" and recurrent water pollutants [26] because, even though the half-life of many pesticides is short, and they could be degraded in a few days, the high application rates in the field, result in the detection of these substances in water bodies almost permanently. For example, [11] showed that the fungicide pyrimethanil and the herbicide diuron have a detection frequency of almost 90% in the water samples from the Madre de Dios River basin, while the insecticide ethoprophos and the fungicide epoxiconazole have frequencies of more than 70%. Very high detection frequencies (>50%) are also common in other areas of the country, with different active ingredients, varying according to the predominant crops [10,27].

It is clear that monocultures (especially genetically modified crops) have expanded greatly in Latin American countries, and with this increment, higher use of pesticides has also occurred [28]. In Central America, more than 180,000 tons of 353 a.i. were imported between the years 2005 and 2009 [29], and even though not all of the imported pesticides are used in the same area, it is clear that a considerable amount of toxic substances are being released into the environment regularly in Neotropical countries.

When these substances enter water bodies, they interact with the abiotic and biotic components of the ecosystem. The interaction with biota involves processes of entry, metabolization, and/or accumulation in organisms, which can produce direct or indirect deleterious effects [30–32]. In events of severe contamination, it is expected that species or entire groups of organisms that are more sensitive or lack escape mechanisms will disappear [33,34]. Therefore, the concentration or toxicity of pesticides themselves may explain much of the variation in aquatic species community structure even at regional scales [35,36].

Stehle and Schulz [37] present information that indicates that the richness of macroinvertebrate families was reduced ~30% in the presence of pesticide concentrations that represent acceptable limits at the regulatory level and that it is possible to observe a reduction of up to 63% in sites with concentrations that exceed acceptable limits. The same authors refer to information that reports concentrations of insecticides that exceed the regulatory limits. Therefore, it is noteworthy to indicate that this situation is widespread and that aquatic organisms are exposed to unacceptable concentrations of pesticides, mainly in tropical countries, where protection measures are laxer and the use of pesticides has increased.

For this reason, this study gathered the data from 11 research projects carried out in 5 different regions of Costa Rica, as a case study to generate information on the detection frequency, toxicity, and retrospective environmental risk of pesticides measured in field samples from more than 160 sites. We aimed to reflect the conditions of Neotropical agriculturally influenced rivers and calculate the potential effects of that pesticide burden on the biota of such aquatic ecosystems.

2. Results and Discussion

2.1. Pesticide Detection and Frequency

With the collection and digitalization of the information presented in Table S1, a unified database was generated. This database contains the results of pesticide residue analyses for 1036 water samples taken throughout Costa Rica.

The pesticide residue analysis database reveals 85 different active ingredients (a.i) or degradation products that were analyzed in the water samples. From these, 72 were detected (Table 1). Amongst the analyzed (but not detected a.i.) are bifenthrin and deltamethrin (pyrethroid insecticides), cyproconazole, and fenbuconazole (triazole fungicides), fenthion and malathion (organophosphates), as well as various metabolites of organochlorine pesticides such as PCP, PCNB, DDT, and endosulfan. The majority of these organochlorine pesticides have already been forbidden or restricted in Costa Rica since 1999 and 2005 (SFE, 2020); however, their degradation products are still detectable in other environmental matrices (dust, air, [38]). Pérez-Maldonado et al. [39] also assessed DDT levels in samples from México and Central America, detecting both DDT and DDE metabolites in soil, fish tissue, and children's blood.

Table 1. Analyzed and detected pesticides from freshwater samples collected throughout Costa Rica between the years 2009 and 2019.

Active Ingredient	Num. of Analyzed Samples	Num. of Detections	Detection Frequency	Observations	Year of Prohibition/Restriction
diuron	917	339	36.97	A	
ametryn	991	315	31.79		
pyrimethanil	549	170	30.97	A	
flutolanil	432	130	30.09	A	
pentachloroaniline (M)	216	62	28.70		
diazinon	1000	279	27.90	A	
azoxystrobin	602	158	26.25	A	
buprofezin	431	99	22.97		
epoxiconazole	822	180	21.90	A	
chlorpyrifos	1029	204	19.83	R	2007
myclobutanil	456	90	19.74		
ethoprophos	914	180	19.69	R	2007
fluopyram	296	53	17.91		
bromacil	967	149	15.41	F	2017
chlorothalonil	914	136	14.88	A	
hexazinone	979	135	13.79		
bentazone	293	39	13.31		
difenoconazole	725	91	12.55	A	
metalaxyl	919	114	12.40		
propiconazole	846	99	11.70	A	
boscalid	291	32	11.00	A	
fenpropimorph	401	40	9.98	A	
thiabendazole	637	56	8.79	A	
carbendazim	126	11	8.73	A	
terbutryn	930	77	8.28		
tebuconazole	779	54	6.93	A	
carbofuran	846	58	6.86	F	2014
quintozene (PCNB)	783	41	5.24		
terbufos sulfone (M)	746	38	5.09		
fenamiphos	999	50	5.01		
imidacloprid	173	8	4.62	A	
carbaryl	837	36	4.30		
clorotalonil 4-hidroxi (M)	125	4	3.20		
profenophos	179	5	2.79		
hexachlorobenzene	545	15	2.75	F	2005
imazalil	449	12	2.67	A	
lindane	151	4	2.65	F	1999
triadimenol	827	20	2.42	A	
oxifluorfen	688	15	2.18	A	
dimethoate	750	16	2.13	A	
terbufos	992	18	1.81	R	2007

Table 1. *Cont.*

Active Ingredient	Num. of Analyzed Samples	Num. of Detections	Detection Frequency	Observations	Year of Prohibition/Restriction
triadimefon	803	14	1.74		
linuron	787	13	1.65		
clomazone	290	4	1.38	A	
triazophos	531	7	1.32	A	
oxamyl	166	2	1.20		
phorate	917	11	1.20		
permethrin	685	7	1.02	A	
carbofuran phenol (M)	846	8	0.95		
bitertanol	768	7	0.91	A	
prothiofos	660	6	0.91		
tecnazene	146	1	0.68		
a-cypermethrin	794	5	0.63	A	
piperonyl butoxide	164	1	0.61		
cadusafos	346	2	0.58		
terbuthylazine	834	4	0.48		
butachlor	633	3	0.47	A	
spiroxamine	460	2	0.43		
prochloraz	550	2	0.36	A	
parathion-methyl	842	3	0.36	R	2007
pendimethalin	654	2	0.31	A	
tolclofos-methyl	657	2	0.30		
trifloxystrobin	393	1	0.25	A	
pencycuron	801	2	0.25		
atrazine	953	2	0.21		
propanil	543	1	0.18	A	
cyhalothrin	685	1	0.15	A	
endosulfan-a	1003	1	0.10	F *	2015
metribuzin	1	1	100		
dimetomorph	5	4	80		
benfuracarb	5	1	20		
thiametoxan	5	1	20	A	
endosulfan-b	992	0	0		
deltametryn	727	0	0	A	
malathion	670	0	0		
bifenthrin	626	0	0		
fenthion	620	0	0		
cyproconazole	582	0	0	A	
fenbuconazole	439	0	0	A	
endosulfan sulfate	418	0	0		
pentachlorobenzene (M)	154	0	0		
pentachloroanisol (M)	147	0	0		
DDE-pp (M)	142	0	0		
DDD-pp (M)	134	0	0		
pp-DDE (M)	42	0	0		

* Prohibition refers to endosulfan, not to the metabolites. F Forbidden; https://www.sfe.go.cr/DocsStatusRegistro/Listado_de_prohibidos.pdf (accessed on 9 February 2021). R Restricted https://www.sfe.go.cr/DocsStatusRegistro/Listado_de_Restringidos.pdf (accessed on 9 February 2021). A Aerial application allowed https://www.sfe.go.cr/DocsStatusRegistro/Lista_productos_aplicacion_aerea.pdf (accessed on 9 February 2021). M Metabolite or degradation product.

The 72 detected a.i are representatives of several biocide actions and chemical groups, including triazole, benzimidazole, aromatic hydrocarbon, pyridine, imidazole, and chlorinated fungicides; triazine, uracil, urea, oxazolidinone, and triazinone herbicides; organophosphate, organochlorine, pyrethroid, carbamate, thiadiazine, and neonicotinoid insecticides; as well as other acaricides, nematicides, among others They are also representative of a great diversity of toxic modes of action, which is presented in Table S2.

There are some herbicides—namely, diuron and ametryn; fungicides pyrimethanil, flutolanil, azoxystrobin, epoxiconazole, and myclobutanil; insecticides diazinon, buprofezin, chlorpyrifos, and ethoprophos for which high detection frequencies ($\geq$20%) are observed at a national scale (Table 1). Furthermore, there are four forbidden substances (lindane, hexachlorobenzene, carbofuran, and bromacil) that were detected in water samples. Lindane and hexachlorobenzene were forbidden since 1999 and 2005, respectively; therefore, the detections imply illegal use of these pesticides in the mountain horticulture regions of the Central Volcanic Range. On the other hand, carbofuran, which was forbidden in 2014, was detected mostly prior to that year; however, one detection was registered in 2016. This could be the result of the use and application of product remnants already in existence (imported before the ban), but this would be highly improbable for the present and future years and should be analyzed with more detail by authorities since a high risk for aquatic biota has been demonstrated for this a.i. [7,18,27]. Bromacil is one of the most recently forbidden a.i. (2017), and it was also detected in posterior years (up to 2020); consequently, the risks associated with the potential lixiviation of this pesticide into groundwaters is still of concern, as it has been in other countries [40,41].

Differences in detection frequencies can be observed within regions in Costa Rica (Figure 1), with a higher frequency of fungicides in the Caribbean > mountain horticulture > South Pacific > North Pacific > Northern Caribbean > Central Pacific. Herbicides were more frequently detected in the South Pacific > Caribbean > North Pacific > Northern Caribbean > horticulture > Central Pacific, while insecticides and nematicides frequencies were highest in the mountain horticulture > Caribbean > South Pacific > Northern Caribbean > North Pacific > Central Pacific. It is noteworthy that the Central Pacific region has a considerably lower sampling effort than other areas, and almost no pesticides were detected in the analyzed samples; however, Rodríguez-Rodríguez et al. [42] conducted an intensive sampling (84 water samples) from 2008 to 2011 in melon and watermelon influenced catchments and found one fungicide and seven insecticides in concentrations that pose an acute and chronic risk to *Daphnia magna*, fish, and *Chironomus riparius*. This situation highlights the importance of increasing the sampling effort in that region. Furthermore, the highest individual pesticide frequencies were registered where more sampling effort has occurred; for example, for the horticulture mountain regions, chlorpyriphos was detected in 60% of the samples; in the South Pacific, diuron was detected in 64% and bromacil in 49% of the samples, while in the Caribbean, diuron, ametryn, pyrimethanil, diazinon, and azoxystrobin were detected in >40% of the samples.

Regarding the measured environmental concentration (MEC) of the a.i., Figure 2 shows all the field concentrations of 72 a.i. The majority of the pesticides were detected in concentrations <1 μg/L; however, in some cases, they reached values higher than 10 μg/L (e.g., diazinon, diuron, ametryn, and flutolanil), and at least 18 pesticides were >1 μg/L.

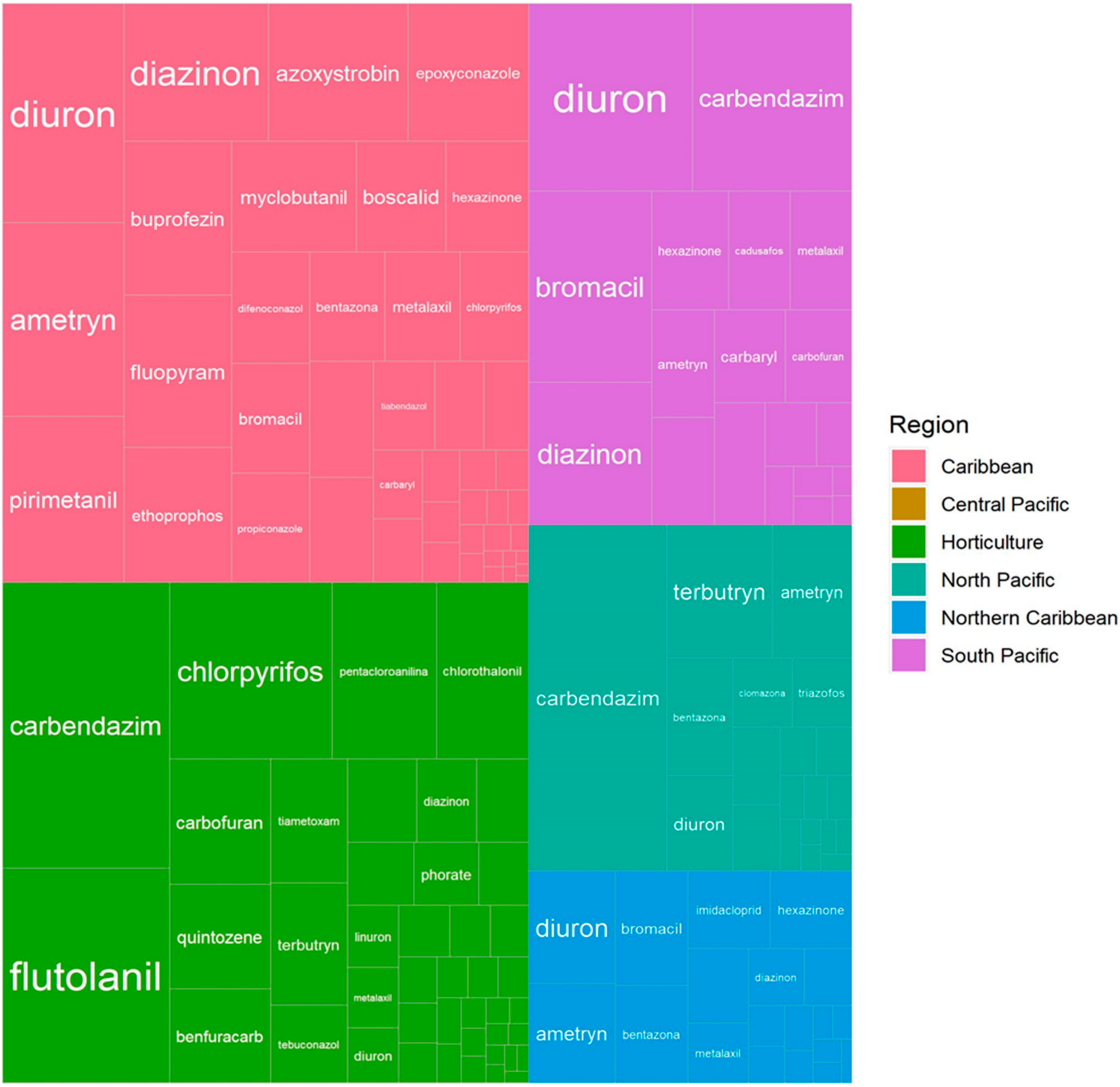

Figure 1. Detection frequency of pesticides in freshwater samples within different geographic regions of Costa Rica, between the years 2009 and 2019. Highest frequencies are located in the top left of each region box.

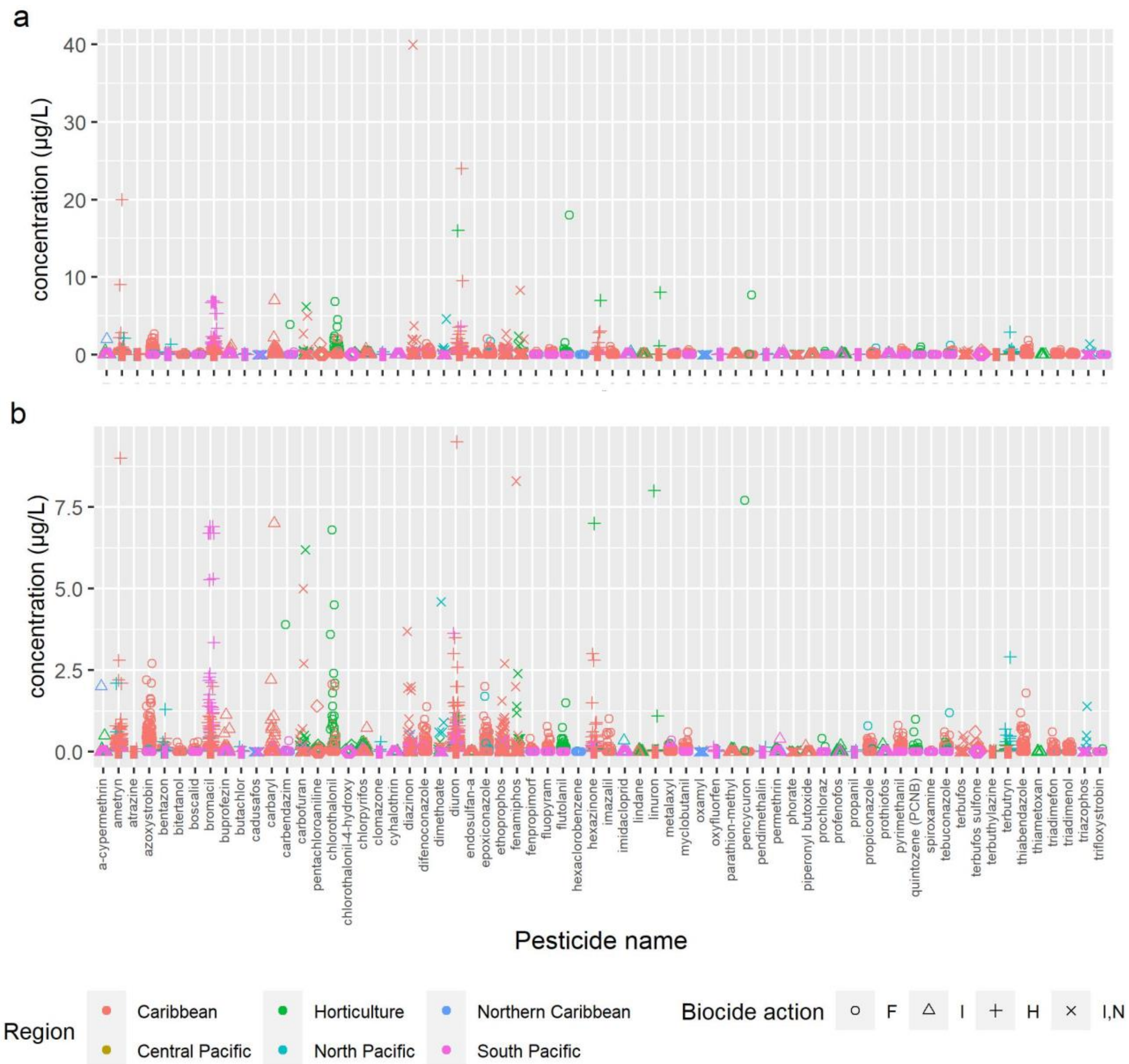

Figure 2. (**a**) Measured environmental concentration (MEC) of all pesticide active ingredients (a.i.) detected in freshwater Scheme 2009. (**b**) Zoom in of MEC <10 µg/L to increase clarity. F fungicide, I insecticide, H herbicide, I,N insecticide-nematicide.

2.2. Comparison with International Regulations

We compared the mean and maximum detected concentrations with hazardous concentration 5% (HC_5) and several international standards (EU-EQS, EPA water quality criteria, and the Australian and New Zealand Guidelines for Water Quality; Table 2). We also checked if the a.i are priority substances in the EU or US-EPA and if they were enlisted in the list of highly hazardous pesticides [43].

Table 2. The detected maximum and mean concentrations of analyzed pesticides, as compared with HC_5 and international guidelines. Marked in **bold** are a.i. with mean or maximum concentration exceeding HC_5 or international regulations.

Active Ingredient.	Biocide Action	Mean Detected Conc. (μg/L)	Max. Detected Conc. (μg/L)	HC_5 (μg/L)	AA-EQS (EU) (μg/L)	MAC-EQS (EU) (μg/L)	MTR eco (μg/L)	EPA (Chronic) (μg/L)	EPA (Acute) (μg/L)	Aust (μg/L)	HHP (PAN)	Priority (EU)	Priority (EPA)
a-cypermethrin	insecticide	**0.06**	**2**	1.77	0.00008	0.0006					YES	YES	
ametryn	herbicide	**0.27**	**20**	0.23			0.01						
atrazine	herbicide	0.09	0.09	nc	0.6	2				13		YES	
azoxystrobin	fungicide	**0.39**	2.7	43.7	0.02	4.1							
bentazone	herbicide	0.16	1.3	828	73	450							
bitertanol	fungicide	0.10	0.29	nc			0.31						
boscalid	fungicide	0.07	0.3	nc			0.55						
bromacil	herbicide	**0.66**	**6.9**	3.8			0.0068						
buprofezin	insecticide	0.06	**1.13**	nc			0.56						
butachlor	herbicide	**0.001**	**0.04**	nc			0.00023				YES		
cadusafos	insecticide	**0.03**	**0.03**	nc	0.023	0.023					YES		
carbaryl	insecticide	**0.60**	**7**	1.02			0.23	2.1	2.1		YES		
carbendazim	fungicide	0.13	0.34	11.7	0.6	0.6					YES		
carbofuran	insecticide	0.41	**6.2**	0.4			0.91			1.2	YES		
chlorothalonil	fungicide	**0.28**	**6.8**	6.2	0.06						YES		
chlorpyrifos	insecticide	**0.06**	**0.73**	0.108	0.03	0.1		0.041	0.083	0.01	YES	YES	YES
clomazone	herbicide	0.19	0.3	nc			0.56						
cyhalothrin	insecticide	**0.03**	**0.025**	nc			0.0003				YES		
diazinon	insecticide	**0.28**	**40**	0.2	0.037			0.17	0.17	0.01	YES		YES
difenoconazole	fungicide	0.15	1.38	100.9	0.76	7.8							
dimethoate	insecticide	**0.08**	**0.9**	1.25	0.07	0.7				0.15	YES		YES
diuron	herbicide	**0.43**	**24**	2.6	0.2	1.8				0.2	YES	YES	YES
endosulfan-a	insecticide	**0.03**	**0.03**	nc	0.005	0.01		0.056	0.22	0.2	YES	YES *	YES
epoxiconazole	fungicide	**0.19**	**2**	nc	0.19	1.8					YES		
ethoprophos	insecticide	**0.15**	2.7	3.1			0.063				YES		
fenamiphos	insecticide	**0.29**	**8.3**	0.8	0.012	0.027					YES		
fenpropimorf	fungicide	0.06	**0.4**	nc			0.22						
fluopyram	fungicide	0.16	0.78	nc	2.7	32							
flutolanil	fungicide	0.24	18	nc			22						

Table 2. *Cont.*

Active Ingredient.	Biocide Action	Mean Detected Conc. (µg/L)	Max. Detected Conc. (µg/L)	HC_5 (µg/L)	AA-EQS (EU) (µg/L)	MAC-EQS (EU) (µg/L)	MTR eco (µg/L)	EPA (Chronic) (µg/L)	EPA (Acute) (µg/L)	Aust (µg/L)	HHP (PAN)	Priority (EU)	Priority (EPA)
hexachlorobenzene	fungicide	0.01	0.02	nc	-	0.05				0.1	YES	YES *	YES
hexazinone	herbicide	0.22	**7**	6.1			0.56						
imazalil	fungicide	0.38	**1.01**	nc			0.87				YES		
imidacloprid	insecticide	**0.35**	**0.35**	0.52	0.0083	0.2					YES		
lindane	insecticide	**0.04**	**0.08**	nc	0.02	0.04		-	0.95	0.2	YES		
linuron	herbicide	0.025	0.025	nc	0.17	0.29					YES		
metalaxyl	fungicide	0.08	0.36	5530			46						
myclobutanil	fungicide	0.09	0.6	nc			55						
oxamyl	insecticide	0.06	0.06	nc			1.8				YES		
oxyfluorfen	herbicide	0.05	**0.15**	0.5							YES		
parathion-methyl	insecticide	0.08	0.09	nc	11						YES		
pencycuron	fungicide	1.97	**3.9**	nc			2.7						
pendimethalin	herbicide	**0.14**	**0.14**	3.26	0.018	0.024					YES		
permethrin	insecticide	**0.18**	**0.4**	nc			0.0003				YES		
phorate	insecticide	**0.03**	**0.05**	nc			0.00017				YES		YES
piperonyl butoxide	insecticide	0.17	0.17	nc									
prochloraz	fungicide	0.28	0.4	nc			1.3						
profenofos	insecticide	**0.13**	**0.2**	nc			0.00003			0.02	YES		
propanil	herbicide	0.025	0.025	12			0.07						
propiconazole	fungicide	0.10	1	386.8			10				YES		
prothiofos	insecticide	0.06	0.22	nc							YES		
pyrimethanil	fungicide	0.10	0.81	1740	7	33							
quintozene (PCNB)	fungicide	0.09	1	nc			3.1						
spiroxamine	fungicide	**0.05**	**0.05**	nc			0.002						
tebuconazole	fungicide	0.10	1.2	848.1	0.63	14					YES		
terbufos	insecticide	**0.03**	**0.5**	0.1			0.00003				YES		
terbuthylazine	herbicide	0.03	0.04	5.74	0.2	1.3							
terbutryn	herbicide	**0.10**	**2.9**	5.4	0.065	0.34						YES	
thiabendazole	fungicide	0.28	1.2	nc			3.3				YES		
thiametoxan	insecticide	0.025	0.025	nc	0.14						YES		

Table 2. *Cont.*

Active Ingredient.	Biocide Action	Mean Detected Conc. (µg/L)	Max. Detected Conc. (µg/L)	HC_5 (µg/L)	AA-EQS (EU) (µg/L)	MAC-EQS (EU) (µg/L)	MTR eco (µg/L)	EPA (Chronic) (µg/L)	EPA (Acute) (µg/L)	Aust (µg/L)	HHP (PAN)	Priority (EU)	Priority (EPA)
triadimefon	fungicide	0.28	0.6	754.3			0.91						
triadimenol	fungicide	0.17	0.31	2160			3.2				YES		
triazophos	insecticide	**0.03**	**0.5**	nc	0.001	0.02					YES		
trifloxystrobin	fungicide	0.08	0.08	nc	0.27	0.81							

* HC_5: Hazardous concentration 5%; concentration of pesticide "x" that causes a toxic effect on 5% of the species, within a species sensitivity distribution (SSD). nc = not calculated [7]; Arias-Andrés pers. com. (2021). AA-EQS Annual average environmental quality standard for long-term exposure (chronic) [44]. MAC-EQS Maximum acceptable concentration environmental quality standard for short-term exposure (acute) [44]. MTR (Maximum tolerable risk) is the concentration of a substance in the environment below which no negative effect is expected. The MTR applies to long-term (chronic) exposure [44]. EPA (Chronic and acute) water quality criteria for aquatic life [45]. Australian and New Zealand guidelines for fresh and marine water quality (protection for 95% of the species; chronic) [46]. HHP (PAN) Highly hazardous pesticides according to the criteria from the "Pesticide Action Network" [43]. Priority (EU and EPA) refers to the priority substances enlisted by the European Union [47] and the Environmental Protection Agency of the United States of America [45].

Available HC_5 calculations reflect that those concentrations detected in field samples represent a risk for the biota of the aquatic ecosystems in Costa Rica. Likewise, 50% of the detected pesticides have mean and/or maximum concentrations that do not comply with one or more international standards (Table 2). Among the non-compliant a.i. are herbicides ametryn, bromacil, butachlor, diuron, hexazinone, oxyfluorfen, pendimethalin, and terbutryn; fungicides azoxystrobin, chlorothalonil, epoxiconazole, fenpropimorph, imazalil, pencycuron, and spiroxamine; insecticides cypermethrin, buprofezin, cadusafos, carbaryl, carbofuran, chlorpyriphos, cyhalothrin, diazinon, dimethoate, ethoprophos, fenamiphos, imidacloprid, lindane, phorate, profenofos, terbufos, and triazophos. Vryzas et al. [28] state that limitations in risk assessment, coupled with the low level of implementation of pesticide regulations are partially causing the presence of pesticides above the normative, which implies that environmental protection goals might not be reached.

It is valuable to mention that several of the non-compliant pesticides are also the ones with a higher frequency of detection (Table 1) and higher toxicity for aquatic organisms (e.g., the organophosphate and carbamate insecticides), and this should raise alarm about the conservation of aquatic ecosystems throughout the country. Additionally, we are aware that some highly used pesticides in Costa Rica (e.g., mancozeb, glyphosate, 2,4-D, among others) were not evaluated in this study because of analytical and methodological limitations, but for no reason must these results be interpreted as evidence that those a.i. do not exert effects on the aquatic ecosystems of the country.

2.3. Ecological Risk Multi-Substance Potentially Affected Fraction (msPAF) Model

Of the 85 pesticides detected in this study, 21 MoA were represented. These MoAs were further subdivided when species sensitivity distribution slopes (constructed with the toxicity data) of one a.i. differed more than 10% with respect to other a.i. that shared the same MoA (Table 3).

Table 3. MoA assigned to each pesticide for the msPAF calculations. The subdivision of MoA is depicted with letters (a–d). Pesticides without an assigned TMoA for a species group were not assessed for that group in msPAF. Pesticides absent from this table did not have enough toxicity data to be incorporated in the model.

Active Ingredient	Biocide Action	MoA *	Algae	Aquatic Plants	Primary Producers	Insects	Crustaceans	Arthropods	Fish	Fish and Arthropods
metalaxyl	fungicide	FA1	1		1					1
carbendazim	fungicide	FB1								2
thiabendazole	fungicide	FB1								2a
flutolanil	fungicide	FC2							3	3
azoxystrobin	fungicide	FC3	4		4		4	4	4	4
trifloxystrobin	fungicide	FC3								4
pyrimethanil	fungicide	FD1			5				5	5
quintozene (PCNB)	fungicide	FF3								6
difenoconazole	fungicide	FG1	7		7b				7	7
imazalil	fungicide	FG1								7a
myclobutanil	fungicide	FG1							7a	7b
propiconazole	fungicide	FG1	7a		7a		7b			7a
tebuconazole	fungicide	FG1					7a	7a	7b	7a
triadimefon	fungicide	FG1						7b		7b
triadimenol	fungicide	FG1	7b		7a					7c
spiroxamine	fungicide	FG2	8		8					
chlorothalonil	fungicide	FM	9	9	9		9	9	9	9
clomazone	herbicide	H13	10		10					10
oxyfluorfen	herbicide	H14	11		11					
butachlor	herbicide	H15	12		12		12	12	12	12
pendimethalin	herbicide	H3	13	13	13				13	13
ametryn	herbicide	H5	14		14		14a	14a	14	14b
atrazine	herbicide	H5		14a		14	14a	14c	14	
bromacil	herbicide	H5	14		14a		14	14		14
diuron	herbicide	H5	14a	14b	14		14a	14a	14a	14b
hexazinone	herbicide	H5	14		14		14	14	14b	14a
linuron	herbicide	H5	14		14b				14	14a
propanil	herbicide	H5	14a		14		14a	14a	14	14

Table 3. *Cont.*

Active Ingredient	Biocide Action	MoA *	Algae	Aquatic Plants	Primary Producers	Insects	Crustaceans	Arthropods	Fish	Fish and Arthropods
terbuthylazine	herbicide	H5	14	14	14a					14d
terbutryn	herbicide	H5	14a		14		14b	14b		14c
bentazon	herbicide	H6	15		15					
buprofezin	insecticide	I16								16
carbaryl	insecticide	I1A	17		17				17a	
carbofuran	insecticide	I1A	17a		17	17	17a	17a	17	
oxamyl	insecticide	I1A	17		17a		17	17	17	17
cadusafos	insecticide	I1B							18	18
chlorpyrifos	insecticide	I1B	18b		18	18a			18b	
diazinon	insecticide	I1B	18		18b	18	18a	18a	18	
dimethoate	insecticide	I1B	18a		18a	18a	18b	18b	18b	
ethoprophos	insecticide	I1B							18	18
fenamiphos	insecticide	I1B					18	18		18
parathion-methyl	insecticide	I1B	18b		18	18			18a	
phorate	insecticide	I1B				18	18b	18b	18b	18
profenophos	insecticide	I1B				18a	18a	18b	18	18
terbufos	insecticide	I1B					18a	18a	18	18
triazophos	insecticide	I1B							18b	18
endosulfan-a	insecticide	I2A					19	19	19	19a
lindane	insecticide	I2A	19		19	19	19a	19	19a	19
a-cypermethrin	insecticide	I3A	20		20	20	20	20	20a	20
cyhalothrin	insecticide	I3A				20a	20	20	20b	
permethrin	insecticide	I3A				20	20a	20	20	20a
imidacloprid	insecticide	I4A					21			
thiametoxam	insecticide	I4A				21	21	21		

* Corresponds to codification in [48–50] and the initial of the biocide action: F= fungicide; H = herbicide; I = insecticide.

We found that 5% and 13% of the total water samples from all regions of Costa Rica (except the Central Pacific, which had the least sampling effort) pose a high (msPAF > 5%) or moderate (msPAF > 1%) acute risk, respectively, especially to primary producers (plants, algae) and arthropods (insects, crustaceans). Figure 3 shows the mean and maximum msPAF, grouped by region. In the Caribbean, several samples had an extremely high risk for arthropods (insects and crustaceans) and aquatic plants, followed by the horticulture region, South Pacific, Northern Caribbean, and North Pacific.

The msPAF model illustrates the effect of the mixture of substances with different MoA in the analyzed water samples, but it is also possible to address the specific pesticides that contribute to the higher risks in each species group (Figure 4). Top risk contributors might pose a low risk on a frequent basis, or they might pose a high risk occasionally, or both.

In our study, herbicides diuron and oxyfluorfen, and fungicides azoxystrobin, chlorothalonil, difenoconazole, and spiroxamine are the top contributors to the risk posed on primary producers. Furthermore, diuron itself contributes to 99% of the cumulative risk on aquatic plants. The study by Rämö et al. [48] found the same result with diuron, suggesting that aquatic plants are more sensitive to this a.i. than algae, given that they have the same exposure data. It is noteworthy that the fungicides that are contributing to the risks on algae, fish, and arthropods have multisite action (chlorothalonil) or are ergosterol biosynthesis inhibitors, which is vital for all eukaryotic cells and, therefore, general enough to cause effects on non-fungi organisms [49]. All other imidazole or triazole fungicides have the same MoA [50] and could also potentially affect other groups of species. Regarding fish, a-cypermethrin, cyhalothrin, and permethrin (all pyrethroid insecticides), and fungicide chlorothalonil seem to be the a.i. posing the higher risks. Lastly, cyhalothrin and permethrin, as well as other organophosphate or carbamate insecticides (carbofuran, diazinon, fenamiphos, terbufos, chlorpyrifos) and fungicide chlorothalonil, are the higher contributors to the risk for arthropods (crustaceans, insects).

However, all these estimations are based on acute toxicity (EC50, LC50), and we cannot deny the fact that many other a.i. (such as organophosphates and carbamates) might be involved in chronic toxicity in all groups of species, but especially on fish, which require higher concentration exposures to show immobility or mortality endpoints but could be affected by the neurotoxic acetylcholinesterase inhibition properties of those insecticides [51,52].

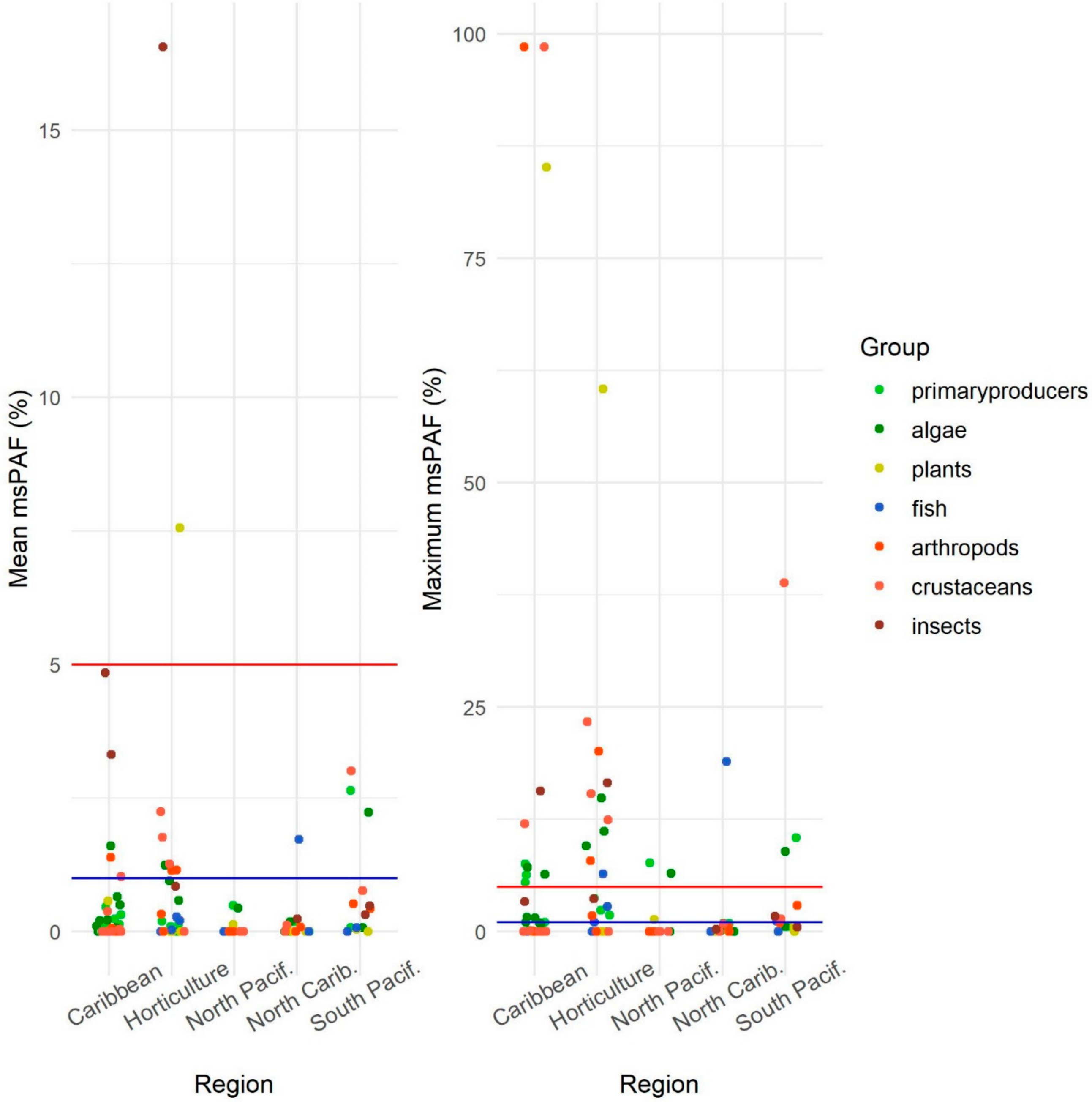

Figure 3. Mean and maximum multi-substance potentially affected fraction (msPAF) for 18 different watersheds within the studied regions in Costa Rica. Above the blue line (1% msPAF) risk is considered moderate; above red line (5% msPAF), risks are considered high.

Another relevant aspect is the presence of some high-risk pesticides identified in this study in other Neotropical countries. For example, ametryn in Ecuador [23]; azoxystrobin in Panama [19]; carbofuran in Brazil [21] and Panama; chlorpyrifos and diazinon in Ecuador, México [24], and Panama; diuron in Brazil, Colombia [22], and Ecuador; epoxiconazole in Colombia; ethoprophos in Panama; terbutryn in Ecuador. Furthermore, researchers in México and Venezuela [25] have detected very toxic pesticides such as aldrin, dieldrin, endrin, DDT, which are forbidden in many countries and are most likely posing unacceptable risks to the aquatic ecosystems.

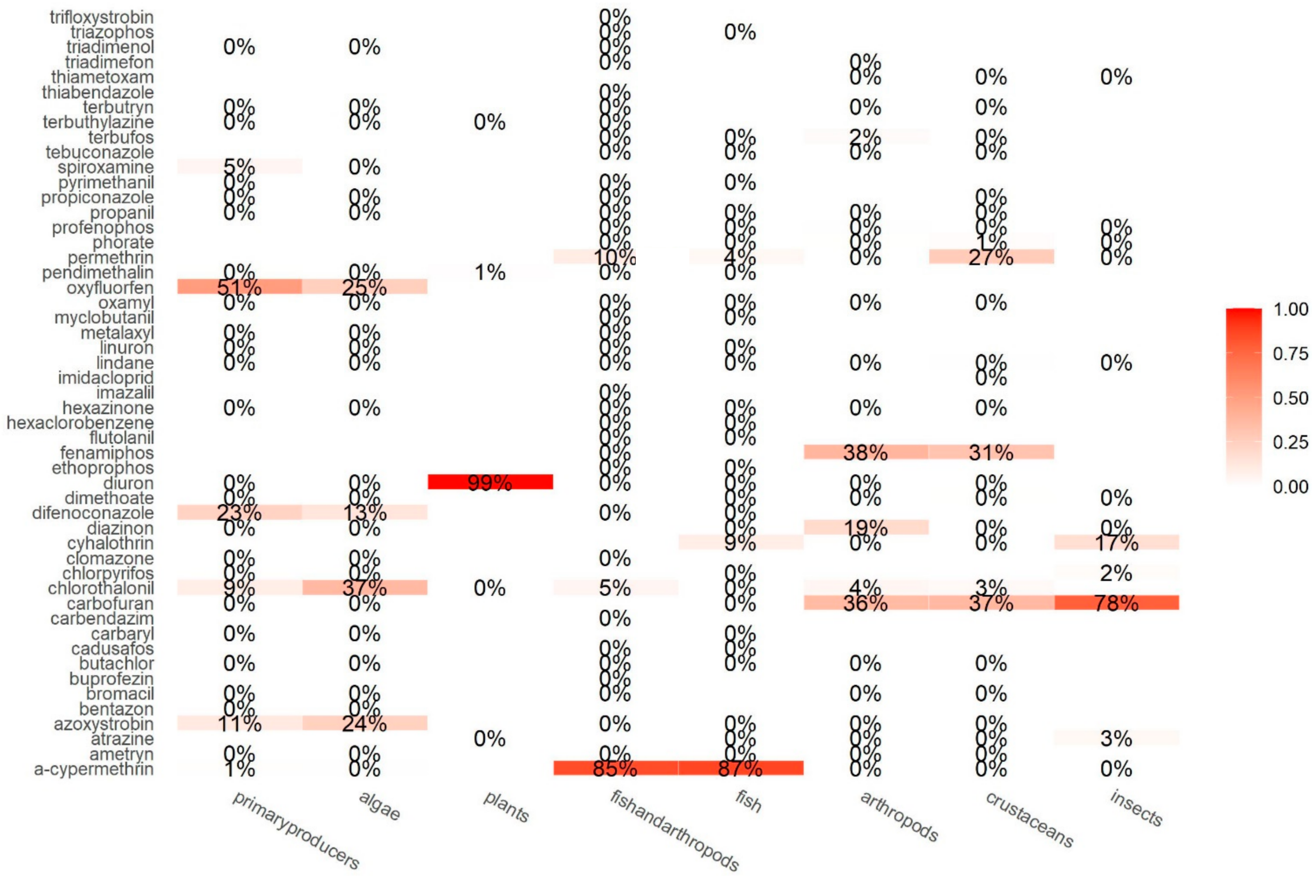

Figure 4. Fraction of the risk contributed by each pesticide in each species group.

We believe that greater efforts must be made by the government agencies and the farmers in the Neotropical region, in order to guarantee that toxic substances applied to the crops for pest control do not reach natural superficial waters in concentrations that pose unacceptable risks. The protection of the riparian vegetation is key to this purpose since it helps mitigate the effects of pesticides and excess nutrients to aquatic biota [53] and also provides habitat for refuge and later recolonization of organisms into the streams [54].

This study highlights the need for systematic pesticide residue monitoring of fresh waters in the Neotropical region, to acknowledge if the exposure to biota from specific pesticides is higher or lower than predicted by the risk analysis (toxicity tests and predictive models of exposure) executed prior to the registration [28]. Results from such a monitoring program would serve as a retrospective environmental risk assessment to address unacceptable risks.

3. Materials and Methods

3.1. Area of Study

Costa Rica is located between geographic coordinates 08°22′26′′ and 11°13′12′ North latitude and 82°33′48′′–85°57′57′′ West longitude in the Central American Isthmus. Its climate is tropical, with a mean annual temperature of 26–27.6°C and mean annual precipitation of 1300 mm in the driest regions, up to a maximum of 7467 mm in the Grande de Orosi watershed [55]. Moreover, according to [56], Costa Rica harbors 12 different life zones (dry, moist, wet, and rain forests), distributed through several altitudinal ranges (lowland, premontane, lower montane, and montane), which lead to the high variability of temperature and rainfall throughout the country. In this study, we used superficial water samples retrieved from 160 sites throughout 5 different regions of Costa Rica (Caribbean, Northern Caribbean, North Pacific, Central Pacific, and South Pacific, as well as the mountainous horticultural zones of the Central Volcanic Range).

3.2. Database

We used previously generated information. The data (region, project, date, site, watershed, and pesticide residue analysis of 1036 water samples) were derived from 11 research projects carried out by state universities in the period between 2006 and 2019 (Table S1). All samples were analyzed in the Laboratory of Pesticide Residue Analysis at the National University (LAREP, IRET, UNA) or at the Center of Investigation on Environmental Pollution, at the University of Costa Rica (CICA, UCR). This assured uniformity of data quality irrespective of the year or the research project.

3.3. Pesticide Analysis

Surface water samples were collected by inserting pre-washed 2 L brown glass bottles into the water. The collected samples were transported in cooled ice boxes to the LAREP, IRET, UNA, or to the CICA, UCR, and stored at 4 °C for a maximum of 24 h before the analyses.

LAREP-UNA. Before 2018, pesticide analysis was performed as specified in Rämö et al. [40], while after that year, samples were analyzed by gas chromatography Agilent 7890A with mass detector 5975C (GC-MS) and liquid chromatography Waters Acquity UPLC H-Class with Waters XEVO T-QS Micro mass detector (UPLC-MS/MS). In both cases, a solid-phase extraction (SPE) was made prior to the analysis. For GC, the sample was agitated and passed through a previously conditioned Isolute ENV+ (200 mg/6 mL) cartridge, which was later dried and eluted with ethyl acetate. The extract was concentrated with Nitrogen and changed into Isooctane. Final volume of the extract was 0.25 mL. For UPLC, the same extraction procedure was followed, except that the elution was made with methanol, and it was concentrated into methanol/water (10:90 *v/v* or 40:60 *v/v*). The final volume of the extract was ~0.5 mL.

CICA. The method is a solid-phase extraction (SPE) and a liquid–liquid extraction (LLE) with dichloromethane, then solvent changes to acetone (for GC analysis), or with 0.1% formic acid in deionized water (for HPLC analysis). Afterward, a high-resolution multiresidue analysis in water samples by gas chromatography and liquid chromatography was used, as detailed in [13,18].

3.4. Comparison with International Regulations

We compared the mean and maximum detected concentrations of this study with environmental quality standards (EQS) from the European Union [44,47], the United States Environmental Protection Agency water quality criteria [45], the Deutsch Institute for Health and Environment maximum tolerable risk level [44], and the Australian and New Zealand Guidelines for Water Quality [46].

3.5. Ecological Risk Multi-Substance Potentially Affected Fraction (msPAF) Model

To complement the assessments derived by single-substance ecological risk, the msPAF model calculates the toxicity risk of mixtures of pesticides with known toxic modes of action (MoA). This model uses concentration addition (CA) to calculate a unique risk value for all the substances that have the same MoA and then applies response addition (RA) to summarize the toxicity risks of all different MoA. The outcome is a msPAF value that defines the potentially affected fraction (as a percentage) of a species group, resulting from the exposure to a complex mixture of pesticides [57,58].

For this study, to calculate the msPAF, we followed the methods described in detail by Rämö et al. [48]. However, we updated the information regarding the acute toxicity of each pesticide to aquatic biota, using new studies registered in the US Environmental Protection

Agency (EPA) ECOTOX database [59]. Additionally, to assign MoA to each pesticide, we only used the classifications of the insecticide, fungicide, and herbicide resistance action committees [50,60,61]. We used the same 6 groups of organisms (algae, aquatic plants, arthropods, aquatic insects, crustaceans, and fish), we followed the same hazard unit calculation approach (geometric mean of toxicity data for each "species group-pesticide" combination), and we also set a minimum of 4 species' toxicity data (in each species group–pesticide combination) to be included within the msPAF assessment. To interpret the results, a PAF < 1% is considered low risk, 1% > PAF < 5% is considered moderate, and PAF > 5% is interpreted as a high risk. Additionally, to address the specific pesticides that contribute to the higher risks in each species group, we followed the methods described by [48].

4. Conclusions

- Pesticides are ubiquitous contaminants of fresh waters in Costa Rica and other Neotropical countries;
- Several of the highly toxic active ingredients are detected in high frequencies (>20%) throughout Costa Rica, increasing the risks for aquatic biota;
- Concentrations at which individual analyzed pesticides are found in the country exceed criteria for biodiversity protection (HC_5) and international standards, therefore representing a risk for the integrity and ecological functioning of aquatic ecosystems;
- msPAF reveals moderate and high risk derived from pesticide mixtures in water samples across Costa Rica;
- Pesticides consistently representing risk in Costa Rica (high frequency of detection, exceeding environmental standards, and identified as risk contributors within the msPAF model and literature) are a-cypermethrin, ametryn, azoxystrobin, bromacil. carbofuran, chlorothalonil, chlorpyrifos, diazinon, diuron, epoxiconazole, ethoprophos, fenamiphos, hexazinone, terbufos, and terbutryn;
- We believe these pesticides (except bromacil, which has already been forbidden) should be re-evaluated if their registration did not take into account current risk assessment tools;
- Several high-risk pesticides in Costa Rica are detected in other Neotropical countries;
- Deeper analysis of the responses of biota to the detected pesticides might be used to complement the development of numerical water-quality criteria and also for retrospective environmental risk evaluations for Neotropical countries;
- There is an urgent need for systematic pesticide residue monitoring of fresh waters in the Neotropical region.

Supplementary Materials: Table S1: Data and information sources for the analysis, Table S2: Characteristics (CAS identification number, biocide action, chemical group, and mode of action) of the detected pesticides, as well as references to studies in which they have been stated as high-risk pesticides for the aquatic environment in Costa Rica. References [62,63] are cited in the Supplementary Materials.

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Sample Availability: Samples of the compounds are not available from the authors.

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Supplementary information: Table S1. Data and information sources for the analysis.

Year	Project name (in spanish)	Reference (TR =Technical report; PA = Published article)
2009	Diagnóstico sobre contaminación de aguas, suelos y productos hortícolas por el uso de agroquímicos en la microcuenca de las quebradas Plantón y Pacayas en Cartago, Costa Rica.	[17] (TR)
2009-2010	Métodos biológicos para evaluar el estado ecológico de las comunidades ribereñas, en las zonas piñeras del Caribe de Costa Rica.	[10] (PA)
2009-2011	Impacto de los plaguicidas en el recurso hídrico de la cuenca del río Tempisque (Palo Verde) Costa Rica. Bases científicas para la gestión ambiental sostenible.	[62] (TR)
2011-2012	Identificación de amenazas y capacitación para el uso sostenible del Refugio Nacional de Vida Silvestre Caño Negro, Región Huetar Norte.	[12] (PA)
2011-2013	Plan de gestión de la cuenca del Río Volcán, Pacífico Sur de Costa Rica.	[16] (TR)
2011-2013	Evaluación del riesgo ecológico de la escorrentía de plaguicidas usados en la agricultura hacia el Río y la Laguna Madre de Dios en la zona del Caribe, Costa Rica "TROPICA".	[11] (PA)
2013-2015	Las buenas prácticas agrícolas en el uso y manejo de agroquímicos en la zona hortícola de Zarcero, Alajuela.	[27] (TR)
2016-2020	Plan Nacional de Monitoreo de la calidad de los cuerpos de agua superficiales (todo el país).	[63] (TR)
2011-2019	Observatorio Ambiental de la Universidad Nacional; Indicador: Presencia de residuos de plaguicidas y calidad biológica del Río Jiménez, Caribe de Costa Rica.	[63] (TR)
2018-2020	Efectos de la presencia de residuos de plaguicidas y otros factores ambientales, en el establecimiento de la comunidad de macroinvertebrados acuáticos en una quebrada con influencia piñera en el pacífico sur de Costa Rica.	[16] (TR)
2018-2020	Procesos de Gestión Integrada del Recurso Hídrico en las subcuencas Chiz-Maravilla y Quebrada Honda, Cartago, Costa Rica.	[63] (TR)

Supplementary information: Table S2. Characteristics (cas identification number, biocide action, chemical group and mode of action) of the detected pesticides, as well as references to studies in which they have been stated as high risk pesticides for the aquatic environment in Costa Rica.

Active ingredient	cas	High ecological risk	Biocide action	Chemical group	Entrance	FRAC/ HRAC /IRAC MoA	MoA Description
a-cypermethrin	52315-07-8	[12]; [27]; [18]; [42]	insecticide	pyrethroid	contact	I3A	Sodium channel modulator (blocks nervous stimuli)
ametryn	834-12-8	[7]; [48]; [11]	herbicide	triazine	systemic	H5	photosystem II inhibitor (D1 Serine 264 Binders)
atrazine	1912-24-9		herbicide	triazine	systemic	H5	photosystem II inhibitor (D1 Serine 264 Binders)
azoxystrobin	131860-33-8	[11]	fungicide	mathoxy-acrylate	systemic, translaminar	FC3	prevents mithocondrial respiration (cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)
benfuracarb*	82560-54-1		insecticide	carbamate	systemic, contact	I1B	Acetylcholinesterase inhibitor
bentazon	25057-89-0		herbicide	benzothiadiazole	contact	H6	photosystem II inhibitor (D1 Histidine 215 Binders)
bitertanol	55179-31-2		fungicide	triazole	systemic	FG1	demethylation in sterol biosynthesis inhibitor
boscalid	188425-85-6		fungicide	pyridine-carboxamide	foliar	FC2	Succinate dehydrogenase inhibitor
bromacil	314-40-9	[7]; [48]; [11]	herbicide	uracil, bromated	systemic	H5	photosystem II inhibitor (D1 Serine 264 Binders)
buprofezin	69327-76-0		insecticide	thiadiazine	contact	I16	chitin biosynthesis type 1 inhibitor
butachlor	23184-66-9		herbicide	chloroacetamide	systemic	H15	Very long chain fatty acids inhibitor

Active ingredient	**cas**	**High ecological risk**	**Biocide action**	**Chemical group**	**Entrance**	**FRAC/ HRAC /IRAC MoA**	**MoA Description**
cadusafos	95465-99-9		insecticide, nematicide	organophosphate	contact	I1B	Acetylcholinesterase inhibitor
carbaryl	63-25-2	[48]; [11]	insecticide	carbamate	contact	I1A	Acetylcholinesterase inhibitor
carbendazim	10605-21-7	[13]; [18]	fungicide	benzimidazole	systemic	FB1	ergosterol synthesis inhibitor
carbofuran	1563-66-2	[7]; [27]; [18]; [42]	insecticide, nematicide	carbamate	systemic, contact	I1A	Acetylcholinesterase inhibitor
carbofuran phenol (carbofuran M)	1563-38-8		unclassified	unclassified	not applicable		not applicable
chlorothalonil	1897-45-6	[11]; [27]	fungicide	chloronitrile phthalonitrile	foliar, contact	FM	multi-site activity
chlorothalonil-4-hidroxy (M)	28343-61-5		unclassified	unclassified	not applicable		not applicable
chlorpyrifos	2921-88-2	[11]; [27]; [18]	insecticide	organophosphate	contact, respiratory	I1B	Acetylcholinesterase inhibitor
clomazone	81777-89-1		herbicide	oxasolidinone, chlorinated	systemic	H13	DXP synthesis inhibitor
cyhalothrin	91465-08-6		insecticide	pyrethroid, chlorinated, flourated	contact		neurotoxic
diazinon	333-41-5	[7]; [48]; [11]; [27]	insecticide, nematicide	organophosphate	contact, respiratory	I1B	Acetylcholinesterase inhibitor
difenoconazole	119446-68-3		fungicide	triazole	systemic	FG1	demethylation in sterol biosynthesis inhibitor
dimethoate	60-51-5	[27]; [13]	insecticide, nematicide	organophosphate	systemic, contact	I1B	Acetylcholinesterase inhibitor

Active ingredient	cas	High ecological risk	Biocide action	Chemical group	Entrance	FRAC/ HRAC /IRAC MoA	MoA Description
diuron	330-54-1	[12]; [7]; [48] [11]; [13]; [18]	herbicide	urea, chlorinated	systemic	H5	photosystem II inhibitor (D1 Serine 264 Binders)
endosulfan-a	959-98-8	[13]; [42]	insecticide	organochlorine	contact		GABA-gated chloride channel blockersneurotoxic
epoxiconazole	133855-98-8	[13]	fungicide	triazole	preventive	FG1	demethylation in sterol biosynthesis inhibitor
ethoprophos	13194-48-4	[12]; [7]; [48]; [11]	insecticide, nematicide	organophosphate	contact	I1B	Acetylcholinesterase inhibitor
fenamiphos	22224-92-6	[7]; [11]	insecticide, nematicide	organophosphate	systemic, contact	I1B	Acetylcholinesterase inhibitor
fenpropimorf	67564-91-4		fungicide	morpholine	systemic	FG2	sterol biosynthesis inhibitor
fluopyram	658066-35-4		fungicide	pyridinyl-ethyl-benzamide	preventive, systemic	FC2	Succinate dehydrogenase inhibitor
flutolanil	66332-96-5		fungicide	phenyl-benzamide	systemic	FC2	Succinate dehydrogenase inhibitor
hexaclorobenzene	118-74-1		fungicide	organochlorine	contact, ingestion, respiratory		unknown for fungi
hexazinone	51235-04-2	[11]	herbicide	triazinone	systemic, contact	H5	photosystem II inhibitor (D1 Serine 264 Binders)
imazalil	35554-44-0		fungicide	imidazole	systemic	FG1	demethylation in sterol biosynthesis inhibitor
imidacloprid	138261-41-3		insecticide	neonicotinoid, chlorinated	systemic, contact	I4A	nicotinic acetylcholine receptor competitive modulator

Active ingredient	cas	High ecological risk	Biocide action	Chemical group	Entrance	FRAC/ HRAC /IRAC MoA	MoA Description
lindane	58-89-9	[27]	insecticide	organochlorine	contact, respiratory	I2A	GABA-gated chloride channel blockersneurotoxic
linuron	330-55-2		herbicide	urea, chlorinated	systemic	H5	photosystem II inhibitor (D1 Serine 264 Binders)
metalaxyl	57837-19-1		fungicide	acylalanine	systemic	FA1	protein synthesis inhibitor
myclobutanil	88671-89-0		fungicide	triazole	systemic	FG1	demethylation in sterol biosynthesis inhibitor
oxamyl	23135-22-0	[18]	insecticide, nematicide	carbamate	systemic, contact	I1A	Acetylcholinesterase inhibitor
oxyfluorfen	42874-03-3		herbicide	diphenilether, chlorinated, fluorated		H14	Protoporphyrinogen Oxidase inhibitor
parathion-methyl	298-00-0		insecticide	organophosphate	contact	I1B	Acetylcholinesterase inhibitor
pencycuron	66063-05-6		fungicide	phenylurea	protective	FB4	celular division inhibitor
pendimethalin	40487-42-1		herbicide	dinitroaniline	systemic	H3	celular division(microtubule assembly) inhibitor during germination
pentachloroaniline (quintozene M)	527-20-8		unclassified	unclassified	not applicable		not applicable
permethrin	52645-53-1	[11]; [27]	insecticide	pyrethroid, chlorinated	contact	I3A	Sodium channel modulator (blocks nervous stimuli)
phorate	298-02-2	[27]	insecticide, nematicide	organophosphate	systemic, contact	I1B	Acetylcholinesterase inhibitor

Active ingredient	cas	High ecological risk	Biocide action	Chemical group	Entrance	FRAC/ HRAC /IRAC MoA	MoA Description
piperonyl butoxide	51-03-6		insecticide		increases efficacy of other insecticides (mostly pyrethroids)		oxidase inhibitor
prochloraz	67747-09-5		fungicide	imidazole	contact	FG1	demethylation in sterol biosynthesis inhibitor
profenophos	41198-08-7	[27]	insecticide	organophosphate, chlorinated, bromated	contact	I1B	Acetylcholinesterase inhibitor and ovicidal properties
propanil	709-98-8	[13]	herbicide	amide	contact	H5	photosystem II inhibitor (D1 Serine 264 Binders)
propiconazole	60207-90-1	[11]	fungicide	triazole	systemic	FG1	demethylation in sterol biosynthesis inhibitor
prothiofos	34643-46-4	[27]	insecticide	organophosphate, chlorinated	contact	I1B	Acetylcholinesterase inhibitor
pyrimethanil	53112-28-0		fungicide	anilino-pyrimidine	protective	FD1	methionine biosynthesis inhibitor
quintozene (PCNB)	82-68-8	[27]	fungicide	aromatic hydrocarbon	contact	FF3	possible lipid peroxidation
spiroxamine	118134-30-8		fungicide	spiroketal-amine	protective and systemic	FG2	sterol biosynthesis inhibitor
tebuconazole	107534-96-3		fungicide	triazole	systemic, contact	FG1	demethylation in sterol biosynthesis inhibitor
tecnazene	117-18-0		fungicide	aromatic hydrocarbon	protective and curative	FF3	possible lipid peroxidation

Active ingredient	cas	High ecological risk	Biocide action	Chemical group	Entrance	FRAC/ HRAC /IRAC MoA	MoA Description
terbufos	13071-79-9	[7]; [48]; [11]	insecticide, nematicide	organophosphate	contact	I1B	Acetylcholinesterase inhibitor
terbufos sulfone (terbufos M)	56070-16-7		unclassified	unclassified	not applicable		not applicable
terbuthylazine	5915-41-3		herbicide	triazine, chlorinated	systemic	H5	photosystem II inhibitor (D1 Serine 264 Binders)
terbutryn	886-50-0	[13]	herbicide	triazine	systemic	H5	photosystem II inhibitor (D1 Serine 264 Binders)
thiabendazole	148-79-8		fungicide	benzimidazole	systemic	FB1	mithosis inhibitor (β-tubulin assembly)
thiametoxam	153719-23-4		insecticide, nematicide	nicotinic, chlorinated	systemic, contact	I4A	nicotinic acetylcholine receptor competitive modulator
tolclofos-methyl	57018-04-9		fungicide	aromatic hydrocarbon	contact	FF3	possible lipid peroxidation
triadimefon	43121-43-3		fungicide	triazole	systemic	FG1	demethylation in sterol biosynthesis inhibitor
triadimenol	55219-65-3		fungicide	triazole	systemic	FG1	demethylation in sterol biosynthesis inhibitor
triazophos	24017-47-8	[13]	insecticide, nematicide	organophosphate	contact	I1B	Acetylcholinesterase inhibitor
trifloxystrobin	141517-21-7		fungicide	oximino-acetate	preventive	FC3	prevents mithocondrial respiration (cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)

M = metabolite; Source for modes of action: [50, 60, 61]

5. Artículo #2. Pesticides-derived ecological thresholds: a new approach in aquatic community ecotoxicology

Manuscrito.

Revista por definir

Pesticides-derived ecological thresholds:
a new approach in aquatic community ecotoxicology

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Abstract

Tropical ecosystems are known to have high biodiversity, however, the excessive use of pesticides for agriculture has generated pollution of freshwaters, with effects on aquatic biota which are still to be thoroughly understood. The objective of this study is to generate ecological thresholds of freshwater macroinvertebrate (FWM) families, derived from an environmental gradient (pesticide concentrations from field water samples), as an indirect ecotoxicological endpoint of tolerance/sensitivity of FWM. We compiled pesticide data and FWM taxa abundances from 441 water samples obtained from 99 sites in Costa Rica between years 2009-2019. Thirteen active ingredients were selected for subsequent analysis based on toxicity, detection frequency and available information on environmental risk for aquatic ecosystems. Ecological thresholds were generated for individual pesticides, to detect changes in the distribution of FWM as a response to the field concentrations of each pesticide. Aquatic Acari (Trombidiformes), as well as Ceratopogonidae, Simuliidae, Empididae, and Psychodidae (Diptera), Leptoceridae (Trichoptera), Baetidae (Ephemeroptera), Collembola, Corydalidae (Megaloptera), Elmidae (Coleoptera) and Libellulidae (Odonata), were categorized as "*moderately tolerant*" for ≥5 pesticide gradients. Bivalve molluscs, and amphipod families Corophiidae and Hyalellidae were categorized as "*tolerant*" for at least one pesticide. Blaberidae (Blattodea), Dixidae (Diptera), Ecnomidae, Lepidostomatidae, Odontoceridae (Trichoptera), Euthyplociidae, Isonychiidae (Ephemeroptera), Polythoridae (Odonata) and Atyidae (Decapoda) were completely absent from samples with detected pesticides, while Philopotamidae, Helicopsychidae (Trichoptera), Gyrinidae, Scirtidae, Hydrophilidae, Limnichidae (Coleoptera), Gomphidae (Odonata) and Perlidae (Plecoptera), among others, were "*sensitive*" for ≥5 pesticides, indicating a very low tolerance of the FWM community to the presence of these pesticides. We discussed these results with respect to FWM anatomical and physiological traits that could confer intrinsic sensitivity. Community change points derived for these pesticides (value along the gradient where an abrupt and synchronic change in the abundance of several taxa within the community can be observed), give valuable information on the specific concentration of each pesticide that might exert pressure over the FWM communities in a neotropical setting and reveal a lack

of protection to aquatic ecosystems. Results from this study, might be used to complement the development of numerical water-quality protection criteria for neotropical countries, and for retrospective environmental risk evaluations.

Keywords: aquatic biodiversity, pesticide tolerance, superficial waters, TITAN2, tropical macroinvertebrates.

Introduction

The ecological threshold is a 1970´s theoretical concept which stated that the ecological systems are resilient and stable (or constant), but the stability of the systems might be affected by external changes or environmental drivers, producing a transition from a “stable” state into another (Holling, 1973). This transition point, at which small, continuous changes in any variable (e.g. habitat fragmentation, pollutant concentration, elevation) produce large responses in the ecosystem (including loss of species) has been defined as the ecological threshold (Groffman et al., 2006; Baker and King, 2010).

These thresholds are rapid change zones between different environmental conditions which might be important for the identification of indicator species, and also for the description of species assemblages given certain characteristics of a study site (Dufrêne and Legendre, 1997). Furthermore, the identification of such a threshold has empirical applications, it can be used as a signal for management purposes, since the crossing of the threshold might imply the transition from a functioning ecosystem to one that ceases to function (for example to harbor a specific organism).

In this line of thought, any toxicant(s) can act as a novel environmental gradient, since they are not part of the original natural environmental conditions of a given ecosystem. Therefore, communities of interacting species, which have a common preference for such original conditions, might experience synchronical and abrupt changes in their abundance or occurrence at a critical level of that novel environmental gradient (e.g. a pesticide), generating a “community threshold” that might be useful to support the creation of numerical environmental criteria for biodiversity protection (Baker and King, 2010) and also as a new endpoint to be used in community ecotoxicology.

In events of severe contamination with any toxicant, it is expected that species or entire groups of organisms that share traits like being more sensitive or lacking escape mechanisms will show a decreased abundance or will completely disappear from the affected ecosystem (Beketov and Liess, 2008; Jergentz et al., 2004; Echeverría-Sáenz et al., 2022). Therefore, the same concentration of e.g. a pesticide, could have a greater or lesser effect on different ecosystems depending on the duration of exposure, the presence of additional stressors (Liess and Von der Ohe, 2005) or the existence of refuge areas (Knillmann et al., 2018). However, when ecosystems are similar (e.g., streams influenced by agriculture), the concentration or toxicity of pesticides themselves may explain much of the variation in community structure even at regional scales (Beketov et al., 2013; Stehle et al., 2018; Stehle and Schulz, 2015). This might be partially explained by the fact that pesticides concentrations are novel anthropogenic environmental gradients, which represent un-natural chemical conditions different to those experienced by species in evolutionary time (Baker and King, 2010), and also that presenting specific traits like skin or gill respiration, longer life cycle duration or being an insect could enhance the species intrinsic sensitivity and vulnerability to toxicants (Baird and van den Brink, 2007; Rico and van den Brink, 2015).

Studies carried out in temperate regions have analyzed the effects of pesticides on freshwater macroinvertebrates (FWM), which are a very relevant component of aquatic ecosystems since they constitute more than 60% of animal biodiversity in inland water bodies (Balian et al., 2008), and play a fundamental role in energy flows, aquatic food chains and nutrient cycling, both within freshwaters and in adjacent terrestrial ecosystems (Dijkstra et al., 2014). In 2005, Liess and von der Ohe generated the SPEAR (Species at Risk) Index, which is based on the use of biological characters (traits) that enhance vulnerability to pesticides exposure. Likewise, Baird and Van den Brink (2007) related species traits to sensitivity towards stressors as a mechanistic alternative to the empirical approach of the species sensitivity distributions used for environmental risk evaluations. However, they indicated that the lack of species metadata was a limitation in the development of this approach, and more than 10 years later, Van den Berg et al. (2019) still indicated that the lack of trait data was the main obstacle in sensitivity model construction.

Buchwalter et al. (2008) demonstrated that phylogenetically linked physiological traits, like Cadmium uptake or elimination rate constants, could explain tolerance or sensitivity of certain groups of insects towards this metal. Rubach et al. (2010), Ippolito et al. (2012) and Rico and Van den Brink (2015) went further to combine the mode of action of insecticides and biological traits of species as determinants for laboratory sensitivity (e.g. EC50). Rubach et al. (2010) concluded that important traits remain to be identified in relation to the toxicokinetics and toxicodynamics, and Wiberg-Larsen et al. (2016) stated that uptake kinetics should be tightly coupled to macroinvertebrate traits such as respiration type, thickness of the cuticula, size (life-stage) or temperature and substrate preferences of macroinvertebrates. Similarly, Ippolito et al. (2012) found that behavioral complexity might also be a determinant trait for sensitivity, and Rico and Van den Brink (2015) predicted which organisms are most likely to be affected by the presence of pesticides in water, according to their anatomical and physiological traits. All of these studies aim to predict the sensitivity of species for which there are no toxicological data or which are highly under-represented in toxicological databases (e.g. insects). A good prediction of sensitivity could improve the risk assessment of pesticides since for most FWM families, no ecotoxicity data exists (Wiberg-Larsen et al., 2016).

According to Beketov et al. (2013), pesticides are responsible for global biodiversity losses and, Stehle and Schulz (2015), present information that indicates that the richness of macroinvertebrate families is reduced ~30% in the presence of pesticide concentrations that represent acceptable limits at the regulatory level and that it is possible to observe a reduction of up to 63% in sites with concentrations that exceed acceptable limits. The same authors refer to information from more than 800 studies that report concentrations of insecticides that exceed the regulatory limits. Therefore, it is noteworthy to indicate that this situation is widespread and that aquatic organisms are exposed to unacceptable concentrations of pesticides, mainly in tropical countries, where protection measures are laxer and the use of pesticides increases.

For this reason, the present study aimed to identify the relationship between a novel environmental gradient (pesticide concentrations measured in field samples) and the abundance of neotropical FWM families, as an indirect community ecotoxicology endpoint of tolerance or sensitivity to these substances. Moreover, we discussed these results with respect to anatomical and physiological traits that could confer intrinsic sensitivity of FWM families towards pesticides, and we derived community thresholds to compare them with regulatory environmental quality standards.

Materials and methods

Data origin.

The data (FWM and pesticide residue samples) comes from 11 research projects carried out by state universities between 2009-2019 in different hydrological regions of Costa Rica (Caribbean, Northern Caribbean, North, Central and South Pacific, as well as the mountainous horticultural zones of Cartago and Zarcero). The macroinvertebrate database contains the taxonomic identification (phylum, class, order, family, genus), and abundance of all macroinvertebrate organisms collected at each of 99 sites (all of them from lotic ecosystems). This database has representatives from 17 classes, 42 orders, 152 families and 284 genera of FWM (Supplementary Information Table S1). Through a unification code (identifier), called LAREP Code or UCR Code, this taxonomic information is linked to a separate database which has the results of pesticide residue analyses for the water samples taken throughout the country (Supplementary Information Table S2). Paired macroinvertebrate + pesticide residue analysis in the water exists for 441 sampling events.

Likewise, for 104 FWM families, we gathered literature information on the following physiological and anatomical traits: Functional Feeding Group (FFG), respiration type, potential body size, attachment, mobility, swimming ability, armoring, occurrence in drift and adult flying strength (Supplementary Information Table S3). It is important to mention that the only published paper that we are aware of, that has tabulated trait information for tropical FWM is Tomanova et al. (2008). Other biological information necessary to complete the traits table for this study was obtained from several sources including Damborenea et al. (2020), Guevara (2015), Hamada et al. (2014), Ramírez and Gutiérrez-Fonseca (2014), Springer et al. (2010), Domínguez and Fernández (2009), McAlpine et al. (1989), and Ruepert and Barnes (1996) for Crustacea. However, a lot of the information had to be gathered from temperate country's trait databases (e.g. Poff et al., 2006; Usseglio-Polatera et al., 2000).

Even though we understand that traits can vary amongst genus or species, this trait database was built on FWM family level because it has been demonstrated by several studies that this level is a good compromise between taxonomic refinement and the detection of differences within groups (Buchwalter et al., 2008; Rubach, 2010; Liess and von der Ohe, 2005).

Pesticide selection for environmental threshold analysis.

The pesticide residue analysis database contains 85 different active ingredients (a.i) or degradation products which have been analyzed in the water samples. From these, 72 have been detected in Costa Rica. Echeverría-Sáenz et al. (2021) presents the details and theoretical risk evaluation of all pesticides. However, to be incorporated in the threshold analysis, priority was given to 13 substances that met the following criteria: (a) high or extremely high acute toxicity to aquatic organisms, especially invertebrates; (b) detection frequency of the substance >5% in the analyzed samples; (c) evidence of high environmental risk at the national level (obtained from published research and technical reports); (d) selection of representatives of different biocide actions, fungicides, insecticides-nematicides, herbicides (Table 1). Moreover, only these 13 pesticides complied with the minimum number of samples (36) required to run the environmental threshold analysis.

Table 1. List of selected pesticides to continue with the ecological threshold analysis, with information on the specific selection criteria.

Detected pesticide (active ingredient)	CAS number	(a) acute toxicity (LC/EC50) for invertebrates (*D. magna,* mg/L)*	(b) frequency of detection (%) of the a.i. in the water samples	(c) high environmental risk for aquatic organisms **	(d) biocide action
azoxystrobin	131860-33-8	0.23	6.01	Echeverría-Sáenz et al., 2018b, 2021	fungicide
chlorothalonil	1897-45-6	0.16	5.77	Echeverría-Sáenz et al., 2018b, 2021; Ramírez-Muñoz et al., 2017	fungicide
epoxiconazole	135319-73-2	nd for invertebrates	6.01	Echeverría-Sáenz et al., 2021	fungicide

Detected pesticide (active ingredient)	**CAS number**	**(a) acute toxicity (LC/EC50) for invertebrates (*D. magna,* mg/L)***	**(b) frequency of detection (%) of the a.i. in the water samples**	**(c) high environmental risk for aquatic organisms ****	**(d) biocide action**
flutolanil	66332-96-5	0.13 (*Americamysis bahia*)	7.85		fungicide
metalaxyl	57837-19-1	117.3	8.31		fungicide
ametryn	834-12-8	28	21.01	Arias-Andrés et al., 2018; Rämo et al., 2018; Echeverría-Sáenz et al., 2018b, 2021	herbicide
bromacil	314-40-9	121	18.48	Arias-Andrés et al., 2018; Rämo et al., 2018; Echeverría-Sáenz et al., 2018b, 2021	herbicide
diuron	330-54-1	5.7	20.55	Fournier et al., 2018; Arias-Andrés et al., 2018; Rämo et al., 2018; Echeverría-Sáenz et al., 2018b, 2021	herbicide
hexazinone	51235-04-2	152	10.62	Echeverría-Sáenz et al., 2018b, 2021	herbicide
terbutryn	886-50-0	2.66		Echeverría-Sáenz et al., 2021	herbicide
chlorpyrifos	2921-88-2	0.0011	10.39	Echeverría-Sáenz et al., 2018b, 2021; Ramírez-Muñoz et al., 2017	insecticide
diazinon	333-41-5	0.0012	15.24	Arias-Andrés et al., 2018; Rämo et al., 2018; Echeverría-Sáenz et al., 2018b, 2021; Ramírez-Muñoz et al., 2017	insecticide, acaricide, nematicide
ethoprophos	13194-48-4	1.4	8.54	Fournier et al., 2018; Arias-Andrés et al., 2018; Rämo et al., 2018; Echeverría-Sáenz et al., 2018b, 2021	insecticide, nematicide

* Obtained from US Environmental Protection Agency (EPA) ECOTOX database (2021). A geometric mean was calculated when more than one toxicity value for *D. magna* was available. Also, toxicity values for other invertebrates are presented where none were available for *D. magna*.
** According to studies executed in Costa Rica.

Construction of pesticides ecological thresholds.

The relationship between pesticide concentrations detected in the field and the presence/absence or local abundance of FWM families was analyzed using the R Statistical Software (v4.2.2; R Core Team 2023) via TITAN2 R package (v2.4.2, Threshold Indicator Taxa ANalysis; Baker and King, 2010). Our goal is to notice changes in taxa distributions throughout an environmental pesticide gradient (temporal and spatial), to derive ecological thresholds of the macroinvertebrate families with respect to the field concentrations of the selected pesticides.

An analysis was conducted with each selected pesticide and FWM families at all sampled sites, including occurrence and abundance data for each taxon. To run the tests with the assigned confidence, only families that meet the minimum occurrences (>3 in the data series for each pesticide) were incorporated in the analysis. Furthermore, in order to execute the threshold analysis with each of the selected pesticides, the data must have a maximum proportion of 20% “clean” samples (pesticide not detected <LOD), and at least 80% with detected concentrations (>LOD). When the proportion of “clean” water samples was higher than 20%, a sub-sample had to be used. For this sub-sample, we selected the FWM-water pairs with the highest taxa richness. Also, we used a minimum of 36 total samples for the analysis, and set the concentration of the <LOD samples, with half the LOD.

The ecological thresholds generated for the FWM, represent an indirect measure of the *tolerance* of organisms (when they have positive directionality responses and/or multimodal distributions along the pesticide gradient), or *sensitivity* (negative directionality responses and unimodal distributions located in the lowest concentration section of the pesticide gradient). Therefore, the FWM families were classified into three categories according to the following description: A FWM family which has positive directionality (Z+), meaning its abundance increases as the environmental gradient (pesticide concentration) increases, would be categorized as “*tolerant*” to the pesticide under analysis. On the contrary, taxa that have negative directionality (Z-) and very narrow thresholds with unimodal distributions, indicating that their abundance is higher (or they are only present) in the samples that have the lowest concentrations of the pesticide (<AA-EQS or MTR; RIVM, 2021), will be classified as “*sensitive*”. Furthermore, families which have negative directionality, but very wide or multimodal abundance distributions, indicating that they are present in a wider range of pesticide concentrations, will be considered

“*moderately tolerant*”. Also, families that are present in concentrations >MAC-EQS (RIVM, 2021) are considered “tolerant”, even if they are Z-. Those organisms whose ecological thresholds and directionality do not allow them to be classified in one of these three categories will be classified as “*undefined”*.

At the end of the analysis, a community change point (CCP) or community threshold was derived for each pesticide. The CCP is the value along the gradient (pesticide concentration) where an abrupt and synchronic change in several taxa within the community can be observed, giving valuable information on the specific concentration of each pesticide that might exert pressure over the FWM communities in a neotropical setting.

Traits data analysis

In parallel to the environmental thresholds, we wanted to gain insight into intrinsic sensitivity of the taxa (explained by their specific traits). Therefore, we used two approaches. First, to understand if pesticides presence modified functionality within macroinvertebrate communities, we estimated the proportion of each FFG as a percentage in different ranges of pesticide concentrations (as a sum of all pesticides detected in the water samples, not only the ones selected for the environmental thresholds), as follows: Not detected (<LOD), 0.01-0.025 µg/L, >0.025 to <0.1 µg/L, 0.1 to <0.2, 0.2 to <0.5, 0.5 to <1, 1 to <3 and >3 µg/L.

Secondly, based on the literature (Baird and van den Brink, 2007; Rico and van den Brink, 2015; Rubach et al., 2010; Ippolito et al., 2012; Wiberg-Larsen et al., 2016; Van den Berg et al., 2019), we selected seven traits (respiration by gills or integument, being sessile/sedentary, being a crawler or epibenthic burrower, having none or weak swimming ability, having a soft body without sclerotization or cases, being rare drifters and being weak adult fliers), which have been related to sensitivity towards pesticides or that are related to mobility or escaping mechanisms. FWM families were tabulated and the traits were codified with fuzzy coding (Chevenet et al. 1994) using numbers 0-3, where 0 indicates that no member of the family has that trait, while 3 indicates that almost all of the members of the family have that trait. FWM families that had ≥4 of these traits were categorized as “trait sensitive”. Finally, we compared these “intrinsic sensitivity” outcomes with the results of the environmental threshold analysis.

Results

Analysis and construction of ecological thresholds.

Ecological threshold analyses were executed for 13 individual pesticides, 5 herbicides (ametryn, bromacil, diuron, hexazinone and terbutryn); 5 fungicides (azoxystrobin, chlorothalonil, epoxiconazole, metalaxyl and flutolanil), as well as for 3 organophosphate insecticides (chlorpyriphos, diazinon and ethoprophos) (Supplementary Information; Figures S1-S13).

With this analyses, FWM families have been categorized according to the description in the methodology section as "*tolerant*", "*moderately tolerant*" and "*sensitive*" towards each of these pesticide environmental gradients. Table 2 presents the obtained results.

Table 2. Summary of FWM family categorization (T= tolerant; MT= moderately tolerant; S= sensitive; U= undefined), with respect to each analyzed pesticide, based on environmental threshold analysis of samples taken in Costa Rica during the year period 2009-2019. ametr= ametryn; brom= bromacil; diur= diuron; hexaz= hexazinone; terb= terbutryn; azox= azoxystrobin; chlor= chlorothalonil; epox= epoxyconazole; flut= flutolanil; metal= metalaxyl; chlorp= chlorpyrifos; diaz= diazinon; ethop= ethoprophos.

Pesticide / Family	ametr	brom	diur	hexa	terb	azox	chlor	epox	flut	metal	chlorp	diaz	ethop
Insects													
Baetidae	MT	U	U	U	U	U	S	MT	MT	U	MT	U	MT
Blephariceridae	U	U	U	U	U	U	S	U	U	U	U	U	U
Caenidae	U	U	U	U	MT	U	U	U	U	U	U	U	U
Calamoceratidae	U	MT	U	U	U	U	U	U	U	U	U	U	U
Calopterygidae	MT	U	MT	U	U	U	U	MT	S	U	U	S	MT
Ceratopogonidae	MT	S	MT	S	S	MT	U	MT	S	S	U	MT	MT
Chironomidae	MT	U	U	U	U	U	U	U	U	U	U	MT	U
Coenagrionidae	MT	U	U	U	S	U	U	MT	U	MT	U	U	MT
Collembola_fam_indet	MT	U	MT	S	MT	U	U	U	U	U	U	MT	MT
Corixidae	U	MT	MT	U	U	U	U	U	U	U	U	U	U
Corydalidae	MT	U	MT	U	U	MT	S	MT	U	S	U	U	MT
Crambidae	MT	MT	MT	S	U	S	U	U	S	S	U	S	MT
Culicidae	MT	U	S	U	S	U	U	U	U	U	U	MT	MT
Dolichopodidae	S	U	S	U	U	U	U	U	U	U	U	MT	U
Dytiscidae	MT	U	U	U	U	U	U	U	U	U	U	U	U
Elmidae	MT	U	U	U	MT	MT	S	MT	S	S	U	U	MT
Empididae	MT	U	S	S	U	U	MT	MT	U	S	U	MT	MT
Ephydridae	MT	U	U	U	U	U	U	U	U	U	U	U	U
Gerridae	U	U	U	MT	MT	S	U	MT	U	U	U	S	U
Glossosomatidae	MT	MT	S	S	U	MT	U	U	S	U	S	S	MT
Gomphidae	S	MT	S	S	U	S	U	MT	U	S	U	S	MT
Gyrinidae	S	U	S	S	U	U	U	U	U	U	U	S	U

Family \ Pesticide	ametr	brom	diur	hexa	terb	azox	chlor	epox	flut	metal	chlorp	diaz	ethop
Hebridae	S	U	S	S	MT	S	U	S	S	U	U	S	S
Helicopsychidae	S	S	S	U	U	S	U	U	U	U	U	S	S
Heptageniidae	S	U	U	U	U	U	U	U	U	U	U	U	U
Hydraenidae	MT	U	S	U	U	U	U	U	U	U	U	S	U
Hydrobiosidae	U	U	U	U	U	U	S	U	U	U	U	U	U
Hydrophilidae	S	U	S	S	S	S	U	U	S	S	U	S	MT
Hydropsychidae	U	U	U	U	U	MT	S	MT	S	U	S	U	U
Hydroptilidae	MT	U	U	U	S	MT	U	MT	U	S	S	U	U
Leptoceridae	MT	U	MT	U	MT	MT	S	MT	U	U	U	S	MT
Leptohyphidae	U	U	U	U	U	U	S	MT	MT	S	S	MT	MT
Leptophlebiidae	MT	U	U	U	U	U	S	MT	U	S	S	U	U
Libellulidae	MT	U	MT	U	S	U	S	MT	U	S	U	MT	MT
Limnichidae	S	U	S	S	U	MT	U	U	U	U	U	S	S
Lutrochidae	U	U	MT	U	U	U	U	U	U	U	U	U	U
Megapodagrionidae	S	U	S	U	U	U	U	U	U	U	U	S	U
Mesoveliidae	S	U	U	U	U	S	U	U	S	U	U	S	MT
Naucoridae	MT	U	MT	S	MT	U	S	MT	U	S	U	U	S
Perlidae	S	U	MT	S	U	U	S	U	U	S	U	U	S
Philopotamidae	MT	U	S	U	S	S	U	MT	U	S	U	S	MT
Platystictidae	MT	U	U	S	U	U	U	U	U	S	U	MT	U
Polycentropodidae	S	U	MT	S	MT	U	U	U	U	S	U	MT	U
Psephenidae	MT	MT	U	U	U	S	S	MT	U	U	U	U	U
Psychodidae	U	U	MT	U	U	MT	MT	U	U	U	U	MT	MT
Ptilodactylidae	S	MT	MT	U	U	U	S	U	U	U	U	U	U
Scirtidae	S	S	S	S	U	S	U	U	U	S	U	S	S
Simuliidae	MT	U	MT	U	U	MT	U	MT	U	U	U	MT	MT
Staphylinidae	MT	S	S	S	S	MT	MT	MT	S	S	U	S	S
Stratiomyidae	S	U	S	U	U	U	U	U	U	U	U	MT	U
Tabanidae	MT	U	S	S	U	S	U	U	U	U	U	S	U
Tipulidae	MT	U	U	U	MT	MT	U	MT	U	S	U	S	S
Veliidae	U	U	MT	S	S	MT	U	MT	U	S	U	S	MT

Family \ Pesticide	ametr	brom	diur	hexa	terb	azox	chlor	epox	flut	metal	chlorp	diaz	ethop
Non-insects													
Bivalvia_fam_indet	U	S	U	U	S	U	U	T	U	U	S	MT	U
Cladocera_fam_indet	MT	U	U	U	U	U	U	U	U	U	U	U	U
Copepoda_fam_indet	MT	U	MT	U	U	U	U	U	U	U	U	U	S
Corophiidae	T	U	U	U	U	U	U	T	U	U	U	U	U
Hirudinea_fam_indet	MT	U	U	U	U	U	U	U	U	U	U	U	U
Hyalellidae	U	U	U	U	U	U	U	U	T	U	U	U	U
Hydrobiidae	U	U	U	U	S	U	U	U	U	S	S	MT	U
Isopoda_fam_indet	MT	U	U	U	U	U	U	U	U	U	U	MT	MT
Oligochaeta	MT	U	U	U	U	U	U	U	U	U	U	MT	U
Ostracoda_fam_indet	MT	U	U	U	U	U	U	U	U	S	U	MT	U
Physidae	MT	S	MT	U	U	U	U	MT	U	S	U	MT	S
Planorbidae	MT	S	U	S	S	MT	U	U	U	S	U	MT	MT
Tricladida	MT	S	MT	U	U	U	U	U	U	S	U	MT	S
Trombidiformes	MT	U	U	U	MT	MT	S	MT	MT	S	U	MT	MT

FWM such as Physidae and Planorbidae (Gastropoda) or aquatic Acari (Trombidiformes), as well as Ceratopogonidae, Simuliidae, Empididae, Psychodidae and Tipulidae (Diptera), Leptoceridae and Glossosomatidae (Trichoptera), Baetidae and Leptohyphidae (Ephemeroptera), Collembola, Corydalidae (Megaloptera), Elmidae and Staphylinidae (Coleoptera), Libellulidae, Calopterygidae and Coenagrionidae (Odonata), Naucoridae and Veliidae (Hemiptera) and Crambidae (Lepidoptera), were categorized as "*moderately tolerant*" for at least 4 of the analyzed pesticide gradients. Other FWM that had positive directionality (z+) and were therefore categorized as "*tolerant*" for at least one pesticide were bivalve molluscs, and amphipod families Corophiidae and Hyalellidae. Moreover, Chironomidae and Ephydridae (Diptera), Oligochaeta and Hirudinea (Annelida), Cladocera_fam_indet and Isopoda_fam_indet (Crustacea), Lutrochidae and Dytiscidae (Coleoptera), Caenidae (Ephemeroptera), Corixidae (Hemiptera) and Calamoceratidae (Trichoptera) were not conclusive or undefined (U) for most pesticides, but were only categorized as MT or T.

On the other hand, most FWM were categorized as "*sensitive*" in a higher proportion, which is clear from figures S1-S13, where the distribution of nearly all FWM families is positioned along the lowest concentrations of the pesticide gradients, and negative directionality (z-) is the case for almost all FWM. Moreover, Table 3 depicts FWM that were present in clean samples (<LOD) but had zero occurrences in sites were pesticides were

detected in the water. Some of these families were excluded from the analysis because they did not meet the minimum occurrences (>3 in the data series for each pesticide) required to run the tests with the assigned confidence, however, it is noteworthy to mention that they were only present in clean samples, and this might be an indication of their sensitivity. This is the case for families Blaberidae (Blattodea), Dixidae (Diptera), Ecnomidae, Lepidostomatidae, Odontoceridae (Trichoptera), Euthyplociidae, Isonychiidae (Ephemeroptera), Polythoridae (Odonata) and Atyidae (Decapoda).

Table 3. Absence ("x") of FWM families (zero occurrences) in samples with pesticide detections (>LOD), for each pesticide gradient. Costa Rica (2009-2019).

ametr= ametryn; brom= bromacil; diur= diuron; hexaz= hexazinone; terb= terbutryn; azox= azoxystrobin; chlor= chlorothalonil; epox= epoxyconazole; flut= flutolanil; metal= metalaxyl; chlorp= chlorpyrifos; diaz= diazinon; ethop= ethoprophos.

FWM family	ametr	brom	diur	hexaz	terbu	azox	chlorot	epoxi	flutol	metal	chlorp	diaz	ethop
Insects													
Belostomatidae		x	x	x		x	x		x	x	x	x	x
Blaberidae	x	x	x	x	x		x	x	x	x	x	x	x
Blephariceridae	x		x	x				x	x	x		x	x
Calamoceratidae						x	x	x	x	x	x		x
Dixidae	x	x	x	x		x	x	x	x	x	x	x	x
Ecnomidae	x		x	x	x	x	x	x	x	x	x	x	x
Euthyplociidae	x	x	x	x	x	x	x	x	x	x	x	x	x
Hebridae	x		x			x	x	x		x	x	x	x
Helicopsychidae						x	x	x		x			x
Heptageniidae							x	x	x	x	x	x	x
Hydraenidae				x		x	x		x	x	x	x	x
Hydrobiosidae	x					x		x	x	x			x
Hydroscaphidae							x	x	x		x		
Isonychiidae	x	x	x	x	x			x	x	x	x	x	x
Lampyridae	x	x			x	x		x		x		x	x
Lepidostomatidae	x	x	x	x	x	x		x		x		x	x
Limnichidae								x	x		x		x
Lutrochidae						x	x		x	x			
Megapodagrionidae	x		x	x		x	x	x		x		x	x
Mesoveliidae						x	x			x	x		
Notonectidae						x	x	x	x	x	x	x	x
Odontoceridae	x		x	x		x	x	x	x	x	x	x	x
Oligoneuriidae				x	x	x	x	x	x	x			
Platystictidae						x	x	x	x				
Pleidae		x	x		x	x	x	x	x	x	x		x

FWM family	ametr	brom	diur	hexaz	terbu	azox	chlorot	epoxi	flutol	metal	chlorp	diaz	ethop
Polymitarcyidae		x				x	x	x	x		x		x
Polythoridae	x		x	x		x	x	x	x	x	x		x
Pyralidae		x	x	x		x		x		x			x
Sciomyzidae	x	x	x	x	x	x		x		x			x
Scirtidae						x	x	x	x		x	x	x
Sisyridae		x			x	x	x		x	x	x	x	x
Stratiomyidae					x	x		x					x
Tabanidae					x	x	x	x	x				
Xiphocentronidae					x	x				x			x
TOTAL	**14**	**13**	**15**	**15**	**13**	**28**	**24**	**28**	**24**	**27**	**17**	**17**	**28**
Non-insects													
Ampullariidae						x	x		x		x		x
Atyidae			x	x	x		x	x	x	x	x	x	x
Cladocera_fam_indet					x	x		x	x		x		
Gammaridae		x				x	x	x	x		x		x
Hyalellidae		x				x		x					x
Hydridae					x	x	x	x	x		x		x
Hydrozoa_fam_indet		x		x	x				x	x	x		
Neritidae		x		x		x	x		x	x	x	x	x
Polychaeta_fam_indet		x		x					x	x			
TOTAL	**0**	**5**	**1**	**4**	**4**	**6**	**5**	**5**	**8**	**4**	**7**	**2**	**6**

Note: Marked families were present in clean samples (<LOD). Highlighted in grey are the FWM which were absent from almost all samples with pesticide detections.

Community change points (CCP).

Table 4 indicates the number of paired FWM-water samples (n) used for the analyses and depicts a comparison of the derived FWM CCP, with the respective EQS for each pesticide. As can be seen, most CCPs (except herbicides ametryn, bromacil and fungicide azoxystrobin) are lower than the EU-annual average Environmental Quality Standard (AA-EQS) or the MTR (Maximum Tolerable Risk Level), both of which are derived from chronic toxicity tests for aquatic organisms (RIVM, 2021). Especially notable are the differences between both parameters for fungicides flutolanil and metalaxyl, which have very high EQS´s.

Table 4. Number of paired FWM-water samples (n) included in the environmental threshold analyses and the derived FWM community change points (CCP) for each pesticide. Costa Rica (2009-2019).

Selected pesticide active ingredient	Number of samples (n)	LOD (µg/L)	Min-max detected concentration (µg/L)	Community change point (µg/L) fsum(z-) median[1]	AA-EQS or MTR[2] (µg/L)	MAC-EQS[2] (µg/L)
ametryn	119	0.01	0.02 - 9	0.015	0.01	-
bromacil	104	0.02	0.03 – 6.9	0.0125	0.0068	-
diuron	116	0.02	0.03 - 16	**0.01**	**0.2**	**1.8**
hexazinone	60	0.02	0.03 - 7	**0.0175**	**0.56**	-
terbutryn	47	0.02	0.03 - 0.7	**0.0175**	**0.065**	0.34
azoxystrobin	36	0.02	0.05 – 2.2	0.03	0.02	4.1
chlorothalonil	36	0.01	0.02 – 3.6	**0.015**	**0.06**	-
epoxiconazole	36	0.02	0.03 – 1.7	**0.0175**	**0.19**	1.8
flutolanil	43	0.02	0.03 – 0.38	**0.0125**	**22**	-
metalaxyl	47	0.02	0.03 – 0.36	**0.0175**	**46**	-
chlorpyrifos	54	0.01	0.02 – 0.3	**0.015**	**0.03**	0.1
diazinon	79	0.01	0.02 - 40	**0.0125**	**0.037**	-
ethoprophos	48	0.01	0.02 – 0.77	**0.0125**	**0.063**	-

1 Baker and King (2010); community-level thresholds, TITAN sum(z-) across 500 bootstrap replicates, derived in this study.

2 RIVM (2021); Environmental Quality Standards, annual average (AA; chronic) or MTR (Maximum Tolerable Risk Level; chronic) and Maximum acceptable concentration (MAC; acute toxicity data).

Community function and Intrinsic sensitivity to pesticides as analyzed by FWM traits.

Figure 1 depicts the proportion of each FFG amongst the separated pesticide ranges. All 441 paired macroinvertebrate and pesticide residue samples were used for this estimation. A clear trend of pesticide effects in the FFG proportions cannot be observed, since they are very similar within the different pesticide concentration ranges, however, it is noteworthy that shredders are completely absent from samples where the sum of all detected pesticides was >3 µg/L and an increase in the proportion of scrapers can be seen. Another worth mentioning fact is that, with the exception of collectors-gatherers (and scrapers in the >3 µg/L, range), all other FFG are represented in low proportion (<25%) in all the pesticide concentration ranges.

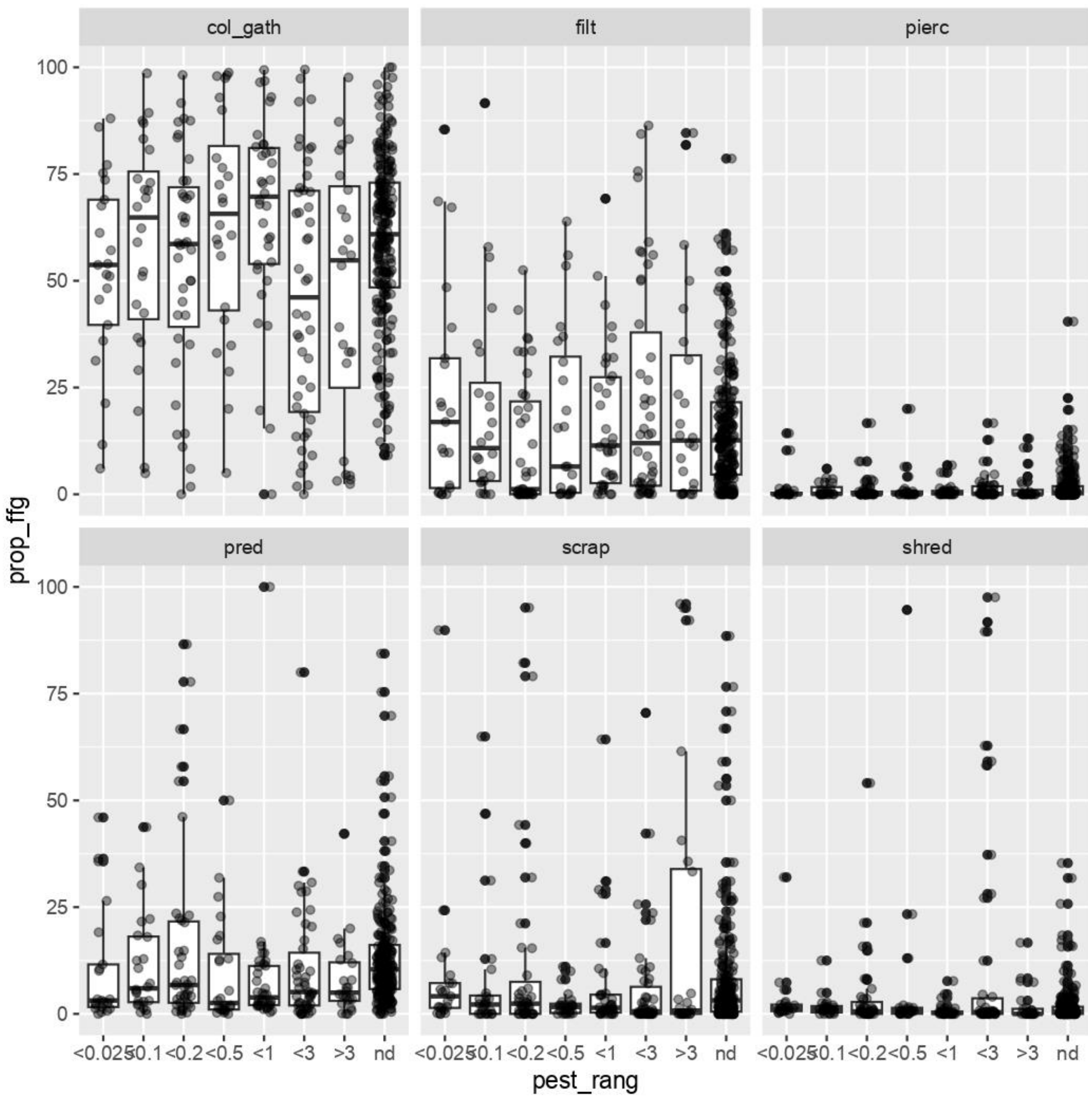

Figure 1. Proportion (%) of functional feeding groups of lotic freshwater macroinvertebrates within different pesticide concentration ranges in lotic water samples (n=441). Costa Rica (2009-2019).

col_gath: collector gatherers, *filt*: filtrators, pierc: *piercers*, *pred*: predators, *scrap*: scapers and *shred*: shredders

With respect to intrinsic sensitivity, as stated in the methodology, families with >4 intrinsic sensitivity traits, were classified as "trait sensitive". Table 5 shows the complete list of FWM families and their classification according to their respective traits. The majority of FWM which had the highest number of intrinsic sensitivity traits were families from the orders Trichoptera and Ephemeroptera. On the other hand, most of the non-insect families have fewer intrinsic sensitivity traits.

Table 5. FWM families presenting intrinsic sensitivity traits. Costa Rica (2009-2019).

Note: Traits were marked as present for a family, even if only some species of the family comply with it. Families marked with * were coherent (identified as "sensitive" in the environmental threshold analysis and also "trait sensitive", or families that were identified as "tolerant" or "moderately tolerant" in the environmental threshold analysis and showed low vulnerability according to these traits). Families marked with ** were incoherent between both approaches.

FWM family	Respiration by gills or integument	Sessile/ sedentary	Crawler or epibenthic burrower	None or weak swimming ability	Soft body without sclerotization or hard cases	Rare drifters	Weak adult fliers	# of intrinsic sensitivity traits	Trait sensitive
Calamoceratidae	1	1	1	1	1	1	1	7	Yes
Hydropsychidae	1	1	1	1	1	1	1	7	Yes
Philopotamidae	1	1	1	1	1	1	1	7	Yes*
Polycentropodidae	1	1	1	1	1	1	1	7	Yes
Sisyridae	1	1	1	1	1	1	1	7	Yes
Baetidae	1	0	1	1	1	1	1	6	Yes**
Blephariceridae	1	1	1	1	0	1	1	6	Yes*
Caenidae	1	0	1	1	1	1	1	6	Yes
Crambidae	1	1	1	1	0	1	1	6	Yes*
Ecnomidae	1	1	1	1	1	0	1	6	Yes*
Empididae	1	0	1	1	1	1	1	6	Yes**
Glossosomatidae	1	1	1	1	0	1	1	6	Yes*
Helicopsychidae	1	1	1	1	0	1	1	6	Yes*
Heptageniidae	1	0	1	1	1	1	1	6	Yes*
Hydridae	1	1	1	1	1	1	0	6	Yes
Lepidostomatidae	1	1	1	1	0	1	1	6	Yes*
Leptoceridae	1	1	1	1	0	1	1	6	Yes**
Odontoceridae	1	1	1	1	0	1	1	6	Yes*

FWM family	Respiration by gills or integument	Sessile/ sedentary	Crawler or epibenthic burrower	None or weak swimming ability	Soft body without sclerotization or hard cases	Rare drifters	Weak adult fliers	# of intrinsic sensitivity traits	Trait sensitive
Polymitarcyidae	1	0	1	1	1	1	1	6	Yes
Psephenidae	1	1	1	1	0	1	1	6	Yes
Pyralidae	1	1	1	1	0	1	1	6	Yes
Tipulidae	1	0	1	1	1	1	1	6	Yes
Calopterygidae	1	0	1	1	1	1	0	5	Yes
Ceratopogonidae	1	0	1	1	0	1	1	5	Yes**
Chironomidae	1	0	1	1	1	0	1	5	Yes
Coenagrionidae	1	0	1	1	1	0	1	5	Yes
Corydalidae	1	0	1	1	0	1	1	5	Yes**
Euthyplociidae	1	0	1	1	1	0	1	5	Yes*
Hydrobiosidae	1	0	1	1	1	0	1	5	Yes
Hydroptilidae	1	0	1	1	0	1	1	5	Yes
Hydroscaphidae	1	1	1	1	0	0	1	5	Yes
Leptohyphidae	1	0	1	1	1	0	1	5	Yes
Leptophlebiidae	1	0	1	1	1	0	1	5	Yes
Neritidae	1	0	1	1	1	1	0	5	Yes
Oligoneuriidae	1	0	1	0	1	1	1	5	Yes
Perlidae	1	0	1	1	0	1	1	5	Yes*
Platystictidae	1	0	1	1	1	0	1	5	Yes
Polythoridae	1	0	1	1	1	0	1	5	Yes*
Protoneuridae	1	0	1	1	1	0	1	5	Yes
Psychodidae	0	1	1	1	0	1	1	5	Yes**
Simuliidae	1	1	1	1	1	0	0	5	Yes**
Xiphocentronidae	0	1	1	1	1	0	1	5	Yes
Ampullariidae	1	1	1	1	0	0	0	4	Yes
Bivalvia_fam_indet	1	1	1	1	0	0	0	4	Yes**
Dixidae	0	0	1	1	1	0	1	4	Yes*
Dolichopodidae	0	0	1	1	1	0	1	4	Yes
Dryopidae	0	0	1	1	0	1	1	4	Yes
Elmidae	1	0	1	1	0	0	1	4	Yes**
Gomphidae	1	0	1	1	0	1	0	4	Yes*
Hebridae	0	0	1	1	1	0	1	4	Yes*
Hirudinea_fam_indet	1	0	1	1	1	0	0	4	Yes
Hydrobiidae	1	1	1	1	0	0	0	4	Yes
Hydrophilidae	1	0	1	1	1	0	0	4	Yes*
Isonychiidae	1	0	1	0	1	0	1	4	Yes*
Lampyridae	1	0	1	1	0	0	1	4	Yes

FWM family	Respiration by gills or integument	Sessile/ sedentary	Crawler or epibenthic burrower	None or weak swimming ability	Soft body without sclerotization or hard cases	Rare drifters	Weak adult fliers	# of intrinsic sensitivity traits	Trait sensitive
Libellulidae	1	0	1	1	0	1	0	4	Yes**
Lutrochidae	1	0	1	1	0	0	1	4	Yes
Lymnaeidae	1	1	1	1	0	0	0	4	Yes
Megapodagrionidae[1]	1	0	1	1	1	0	0	4	Yes*
Mesoveliidae	0	0	1	1	1	0	1	4	Yes
Muscidae	0	1	1	1	1	0	0	4	Yes
Nepidae	0	0	1	1	0	1	1	4	Yes
Physidae	1	1	1	1	0	0	0	4	Yes
Planorbidae	1	1	1	1	0	0	0	4	Yes
Polychaeta_fam_indet	1	0	1	1	1	0	0	4	Yes
Thiaridae	1	1	1	1	0	0	0	4	Yes
Tricladida_fam_indet	1	0	1	1	1	0	0	4	Yes
Blaberidae	0	0	1	1	0	0	1	3	No**
Copepoda_fam_indet	1	0	1	1	0	0	0	3	No
Corophiidae	1	0	1	1	0	0	0	3	No*
Curculionidae	0	0	1	1	0	1	0	3	No
Ephydridae	0	0	1	1	1	0	0	3	No
Gammaridae	1	0	1	1	0	0	0	3	No
Gerridae	0	0	0	0	1	1	1	3	No
Hyalellidae	1	0	1	1	0	0	0	3	No*
Hydrozoa_fam_indet	0	1	1	1	0	0	0	3	No
Isopoda_fam_indet	1	0	1	1	0	0	0	3	No
Limnichidae	0	0	1	1	0	0	1	3	No**
Noteridae	0	0	1	1	0	0	1	3	No
Oligochaeta_fam_indet	1	0	1	1	0	0	0	3	No
Ostracoda_fam_indet	1	0	1	1	0	0	0	3	No
Pleidae	0	0	1	1	0	0	1	3	No
Ptilodactylidae	0	0	1	1	0	0	1	3	No
Staphylinidae	1	0	1	1	0	0	0	3	No**
Stratiomyidae	0	0	1	1	1	0	0	3	No
Tabanidae	0	0	1	1	1	0	0	3	No
Trombidiformes_fam_indet	1	0	1	1	0	0	0	3	No*
Veliidae	0	0	0	0	1	1	1	3	No
Atyidae	1	0	1	0	0	0	0	2	No**

FWM family	Respiration by gills or integument	Sessile/ sedentary	Crawler or epibenthic burrower	None or weak swimming ability	Soft body without sclerotization or hard cases	Rare drifters	Weak adult fliers	# of intrinsic sensitivity traits	Trait sensitive
Belostomatidae	0	0	1	0	0	1	0	2	No
Cladocera_fam_indet	1	0	0	1	0	0	0	2	No
Collembola_fam_indet	0	0	0	1	1	0	0	2	No*
Corixidae	0	0	1	0	0	1	0	2	No
Culicidae	0	0	0	1	1	0	0	2	No
Gyrinidae	1	0	0	0	0	0	1	2	No**
Hydraenidae	0	0	0	1	0	0	1	2	No
Naucoridae	0	0	1	0	0	0	1	2	No
Palaemonidae	1	0	1	0	0	0	0	2	No
Pseudothelphusidae	1	0	1	0	0	0	0	2	No
Arachnida_fam_indet	0	0	0	1	0	0	0	1	No
Dytiscidae	0	0	1	0	0	0	0	1	No
Notonectidae	0	0	0	0	0	0	1	1	No
Sciomyzidae	0	0	0	0	1	0	0	1	No
Scirtidae	0	0	0	0	0	0	0	0	No**

[1] Megapodagrionidae is now divided into Heteragrionidae, Thaumatoneuridae and Lestidae, but we maintained this name because it is the one that appears on the original databases.

Discussion

The generated ecological thresholds can be used as an indirect community ecotoxicology endpoint of tolerance or sensitivity towards pesticides. We observed that, since pesticides are an entirely novel environmental gradient (not a natural condition), most FWM families present a highly sensitive response even to very low concentrations of these substances. Secondly, moderately tolerant (MT) or tolerant (T) organisms are representatives of FWM families that also show tolerance towards other types of pollution or general anthropogenic stress, thirdly, although traits have a great potential to be used as predictors of toxic sensitivity, there is still uncertainty over which specific traits have better validation. Finally, our CCP results indicate that concentrations of the selected pesticides, lower than the ones which can cause chronic toxicity effects, are already exerting pressure over field aquatic communities of FWM.

Retrospective analysis of macroinvertebrate community response to the presence of 13 pesticides.

In a comprehensive study comparing tolerance values (TV) in score systems worldwide, Chang et al. (2013) compiled information from 29 regions from five continents, and they showed that, similar to our results and irrespective of regional variability, non-insects were more tolerant than insects, also Dipteran families had higher TV than other insect families, EPT (Ephemeroptera, Plecoptera, Trichoptera) had lower TV than the ones for OCH (Odonata, Coleoptera, Heteroptera), and Baetidae also had higher TV than all other Ephemeroptera.

Also cohesive with our results, the non-insect families Bivalvia_fam_indet, Physidae, Planorbidae, Oligochaeta, Hirudinea, Isopoda, Cladocera and Hyallelidae have high tolerance values (TV >8 in a 1-10 scale) in the Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al., 1999). Insect families Chironomidae, Simuliidae, Empididae, Psychodidae, Tipulidae (Diptera), Baetidae, Caenidae (Ephemeroptera), Corixidae (Hemiptera), Staphylinidae, Dytiscidae (Coleoptera), Libellulidae, Calopterygidae (Odonata), and Collembola also had such high TV. Furthermore, most of the FWM families identified as tolerant or moderately tolerant in this study, are described in Costa Rican legislation as less sensitive towards other types of contamination, e.g., organic pollution or general habitat alterations (DE-33903-MINAE-S). This is the case for gastropod and bivalve molluscs, annelids, and for Chironomidae, Psychodidae, Simuliidae, Empididae, Ephydridae, Ceratopogonidae, Tipulidae (Diptera), Calopterygidae, Coenagrionidae (Odonata), Staphylinidae (Coleoptera), Naucoridae, Corixidae (Hemiptera) and Trombidifomes; while crustaceans, Baetidae, Leptohyphidae (Ephemereoptera), and Elmidae (Coleoptera) have moderate TV.

Some families, however, showed tolerance in the present study, but are not usually identified with high TV, this is the case for Trichoptera families like Leptoceridae, Glossosomatidae and Calamoceratidae, or Corydalidae (Megaloptera). The family Veliidae (Hemiptera), which was moderately tolerant for some pesticides in the present study, might not be as sensitive, also because they do not live inside the water column, but only in the surface (Merritt and Cummins, 2008). However, other families like Hebridae, also hemipterans with the same habitat type (Merritt and Cummins, 2008), were sensitive towards most evaluated pesticides.

It is predictable that FWM will not respond as strongly towards herbicides or fungicides, because their toxic mechanisms are designed for target entryways in primary producers or fungi (HRAC, 2023, FRAC, 2023). However, many FWM families evaluated in this study were sensitive to herbicides and fungicides, not only insecticides which are the most toxic to the specific community of aquatic organisms we evaluated. Furthermore, even organisms categorized as MT or T within herbicide (diuron, hexazinone) or fungicide (chlorothalonil, epoxiconazole) gradients, were distributed in concentrations <AA-EQS (indicating a relatively sensitive response). On the other hand, organisms categorized as MT or T for herbicides ametryn and bromacil, as well as fungicide azoxystrobin were distributed in concentrations >AA-EQS, more consistent with a tolerant response. We believe that the criteria of unimodal (for sensitive) vs bi-multimodal distributions (for MT) may have caused this phenomenon, because several FWM had bimodal distributions in these gradients (therefore were classified as MT), but they were distributed along the lowest concentrations of the gradient, showing a preference for cleaner sites.

Other investigations have already shown effects of herbicides over FWM communities, which might be also related to cascading indirect effects on the trophic chains derived from changes in the abundances or diversity of primary producers (Fleeger et al., 2003; Echeverría-Sáenz et al., 2022). Regarding fungicides, many of them have multi-site action (chlorothalonil) or are sterol biosynthesis inhibitors (epoxiconazole), protein synthesis inhibitors (metalaxyl) or prevent mitochondrial respiration (azoxystrobin), all of which are vital processes for eukaryotic cells and, therefore, general enough to cause effects on non-fungi organisms (Burden et al., 1989, FRAC, 2023). In a retrospective risk evaluation for mixtures of pesticides in Costa Rica, Echeverría-Sáenz et al. (2021) demonstrated that chlorothalonil, was one of the higher contributors to the risk for arthropods (crustaceans, insects), and Zubrod et al. (2019) demonstrated that fungicides can be highly toxic to a broad range of organisms.

With these results, we conclude that the vast majority of FWM in Costa Rican rivers are responding negatively to pesticides presence (not only insecticides) and, that FWM which show tolerance towards other stress factors, are more likely to also have tolerance towards very low concentrations of pesticides. An interesting approach is to understand if anatomical and physiological traits could be important to assess tolerance or vulnerability for tropical FWM.

Community function and Intrinsic sensitivity to pesticides as analyzed by FWM traits.

In temperate countries, a widely used index that relates macroinvertebrate community composition with pesticide presence and effects in field samples is the SPEcies At Risk ($SPEAR_{pesticides}$; Liess and Von der Ohe, 2005). This index distinguishes FWM in two categories of sensitivity, those that are at risk and those that are Not at Risk (based on traits like their generation time, migration ability, and presence of sensitive aquatic stages). Several organisms reported as MT or T in this study, are also categorized as species Not at Risk (Bivalvia, Physidae, Planorbidae, Oligochaeta, Hirudinea, Cladocera, Isopoda, Corophiidae, Ceratopogonidae, Simuliidae, Psychodidae, Baetidae, Elmidae, Dytiscidae, Calopterygidae, Libellulidae, Naucoridae, Corixidae and Veliidae). Furthermore, in correspondence with our results, some of the families that were present only in sites with clean water samples (<LOD; Dixidae, Ecnomidae, Lepidostomatidae, Odontoceridae and Atyidae), are reported as species at risk. Yet again, an inconsistency is present with families like Leptoceridae, Glossosomatidae, Caenidae and Coenagrionidae, which appear to have some degree of tolerance in this study but are categorized as species at risk within the $SPEAR_{pesticides}$ index (Liess and Von der Ohe, 2005).

Furthermore, according to Rico and van den Brink (2015), who evaluated the intrinsic sensitivity of macroinvertebrates to different types of insecticides, organisms from Cladocera, Ostracoda, Trichoptera and Ephemeroptera were the most sensitive orders to organophosphate and carbamate insecticides, while Bivalves and Annelids were the least sensitive in their study. These last organisms also showed tolerance towards organophosphates in our research, however the sensitive families were distributed among several orders and even a few Trichoptera or Ephemeroptera families showed some degree of tolerance. This might mean that there are differences in the factors that condition the sensitivity towards pesticides in tropical FWM. For example, given that most tropical species have short generation times and are multivoltine (Jackson and Sweeney, 1995), and that pesticides are applied all year round, the traits “voltinism/ generation time” and “presence of sensitive stages” might not be useful for selection of species at risk in the tropics. Therefore, in the present study we gave focus to traits that could relate to enhanced uptake of the toxics into the body (breathing through gills or integument, having a soft body) or that could limit the migration ability of the organisms (swimming and flying strength). Buchwalter et al. (2008) focused on toxins uptake and elimination rates and found very interesting and coherent results with both lab and field data, where families with high uptake rates or poor toxin elimination rates were more sensitive. However, this type of physiological information is not available for most of the taxa.

Regarding FWM intrinsic sensitivity, several families coincided being “trait sensitive”, and also showing sensitive responses in the field (as shown by environmental thresholds), as well as being “not trait sensitive” and showing tolerant responses in the field. However, bivalve and gastropod molluscs, several Diptera families, and Baetidae, Leptoceridae, Elmidae, Libellulidae, which are known to be more tolerant (high TVs) and were T or MT in our field pesticide gradients, were classified as “trait sensitive” with our trait selection. A possible explanation as stated by Ippolito et al. (2012), is that when considering a diverse group of organisms, instead of only arthropods, respiration type loses strength as a predictor of sensitivity, and, furthermore, in the case of molluscs, their lack of mobility and swimming ability also made them classify as “trait sensitive” in this study, even when they are consistently reported as more tolerant organisms.

Therefore, more refinement needs to be done for trait selection in order to validate or reflect more accurately the tolerance/ sensitivity of FWM observed in the field. Furthermore, even when organisms are ranked as “trait sensitive” or “at Risk” according to their lab sensitivity and traits, they can be seen as tolerant in the field, if they have a high recovery potential or are able to recolonize from surrounding refuge areas (Rubach et al., 2010; Knillman et al., 2018). Behavior of FWM to environmental stress may vary according to the ability to detect the toxicant and the specific defense mechanism of each taxa (retreat or burrow, drift or active escape responses) (Wiberg-Larsen et al., 2016).

Regarding functionality of the macroinvertebrate’s community, the proportions of FFG in this study did not reflect a noticeable change in the lower pesticide concentration ranges. Consistent with these results, Buchwalter et al. (2008), Ippolito et al. (2012) and Reiber et al. (2020) stated that no clear patterns were found between sensitivity and functional feeding habits or traits. However, when pesticides sum was >3 µg/L in our study, scrapers greatly increased their proportion (see Fig. 1). Such scrapers are represented almost exclusively by molluscs, evidencing their empirical tolerance in the field. On the other hand, shredders completely disappeared on that pesticide concentration range, which is also in agreement with Cornejo et al. (2021b), who already demonstrated with acute toxicity tests that tropical detritivores were among the most sensitive species to chlorpyrifos and chlorothalonil. Reiber et al. (2020), also identified “decreasing species” (mainly insects) with increasing pesticide pressure, as well as “increasing species” (mainly Gastropoda, Oligochaeta and Diptera), which showed an increase of frequency between reference and the sites with the highest pesticide pressure.

An insight into risk assessment and EQS compared to environmental thresholds (CCP)

Regarding the CCP´s derived from this study, in 10 out of 13 pesticide gradients (except herbicides ametryn, bromacil and fungicide azoxystrobin) the resulting community change points are much lower than the regulatory quality standards (RIVM, 2021). The CCP in this study represents the concentration of a pesticide "x" at which an abrupt change in the composition of the FWM community takes place. Especially notable are the differences between both parameters (CCP vs. AA-EQS) for fungicides flutolanil (0.0125 vs. 22 µ/L) and metalaxyl (0.0175 vs. 46 µ/L). In both cases, the CCP reveals a lack of protection for FWM if the AA-EQS is used as a safe concentration for the aquatic ecosystems. Therefore, it is clear from this study, that the FWM community is responding to much lower pesticide concentrations than the ones available from the literature regarding toxicity effects for aquatic organisms. In the case of fungicides, information of toxicity bioassays is very scarce and there are no aquatic fungi included as test organisms for risk assessment evaluations or registry regulations (Ittner et al., 2018; Zubrod et al., 2019).

Most of the times, when retrospective ERAs are estimated, insecticides and herbicides appear as the highest risk molecules (Rämo et al., 2018), while fungicides appear as low risk substances that almost never exceed their regulatory threshold levels (Brühl et al., 2023). This is not surprising since the acute toxicity of fungicides for standard test species tends to be low, and consequently estimated effect concentrations tend to be high (Zubrod et al., 2019). However, Echeverría-Sáenz et al. (2018) and Cornejo et al. (2021a) have, respectively, demonstrated effects of fungicides in FWM community composition, as well as aquatic litter microbial decomposition, which is a highly relevant ecosystem-level endpoint. More information on toxicity of fungicides to aquatic fungi is necessary to assess the risk with higher accuracy. Moreover, for all types of pesticides, ERAs have not included additional protection for mixtures of substances (which is the case in most water samples), indirect effects on food webs or evaluation of effects on biodiversity in the field.

Deeper analysis on the responses of FWM communities to pesticides might be used to complement the development of numerical water-quality protection criteria for neotropical countries, and for retrospective environmental risk evaluations. The present study is a possible input to move in that direction because it indicates how in-field biodiversity effects can be seen at concentrations much lower than those predicted by the risk evaluations. Furthermore, through this approach, we can contribute toward a new predictive ecotoxicology endpoint, decreasing the need for lab toxicity data.

Finally, it is not clear whether this synchronic shift in the abundances of several families could be a toxicity effect *per se*, however, these FWM families do show decreased numbers that could relate to toxicity or emigration from the polluted sites (evasion; Fleeger et al., 2003; Araújo et al., 2016; Wiberg-Larsen et al., 2016). At an ecosystem level, the effect of the biodiversity loss produced by the absence or lowered numbers of several taxa, disregard of the cause, might be the same, there is a rupture in the interaction of those species with the ecosystem and within species, which might be ecologically relevant, depending on the ecological function of the shifting taxa (Moe et al., 2013, De Lange et al., 2010).

We believe that greater efforts must be made by the government agencies and the researchers in the Neotropical region, to further understand population and community level responses to pesticides, and most importantly, producers and public should acknowledge the information that has been generated so far indicating unacceptable risks to ecosystems. Several studies have remarked that even with strict risk evaluations executed with complex models, there is still a lack of protection to biodiversity (Stehle et al., 2018, Knäbel et al., 2012, 2014). Therefore, it is central to consider the reduction of pesticide use as a plausible alternative in the risk reduction strategies for these substances (Brühl et al., 2023).

Recommendations

- It is relevant to further investigate the anatomical and physiological characteristics which are shared by FWM families in the different sensitivity / tolerance categories. Also, a refinement of the specific traits that confer vulnerability to pesticides and the evaluation of effects of refuge areas in species recolonization, might bring valuable insight into resilience of FWM communities.
- We recommend local governments and environmental authorities to use the information generated in this study to complement the development of numerical water-quality criteria and consider these results in retrospective environmental risk assessments.

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Supplementary Information

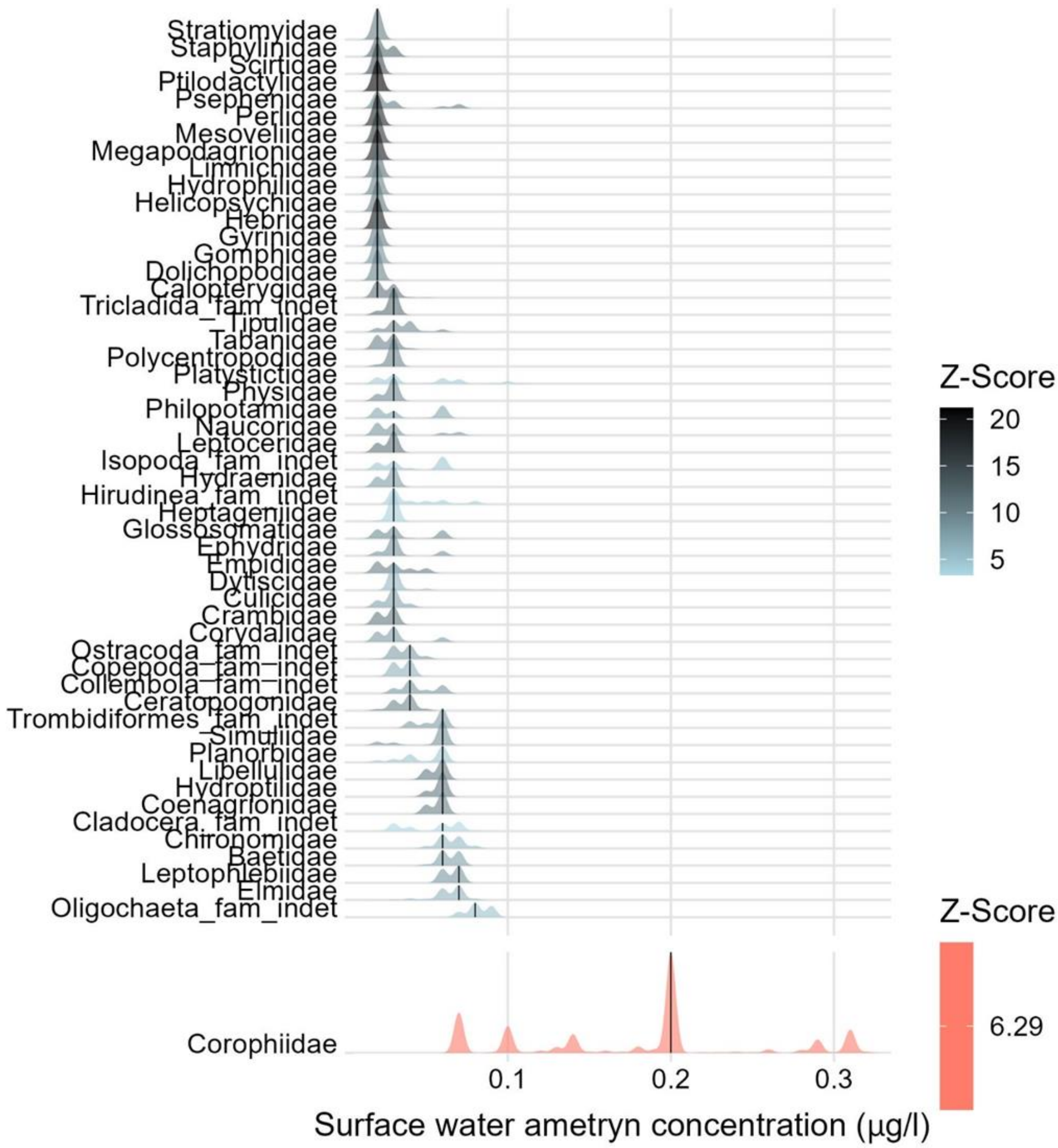

Figure S1. Ecological thresholds for ametryn (herbicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

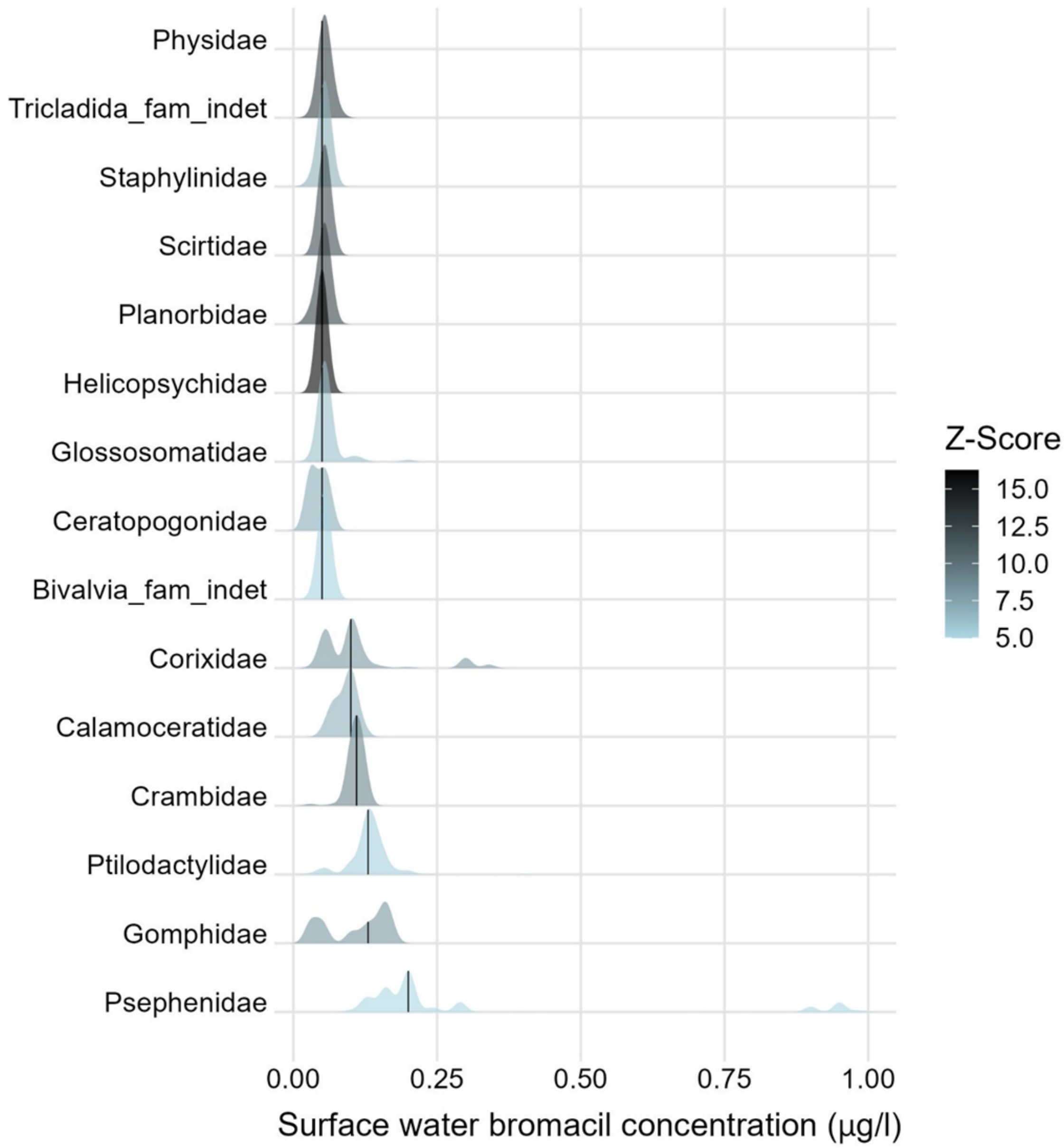

Figure S2. Ecological thresholds for bromacil (herbicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

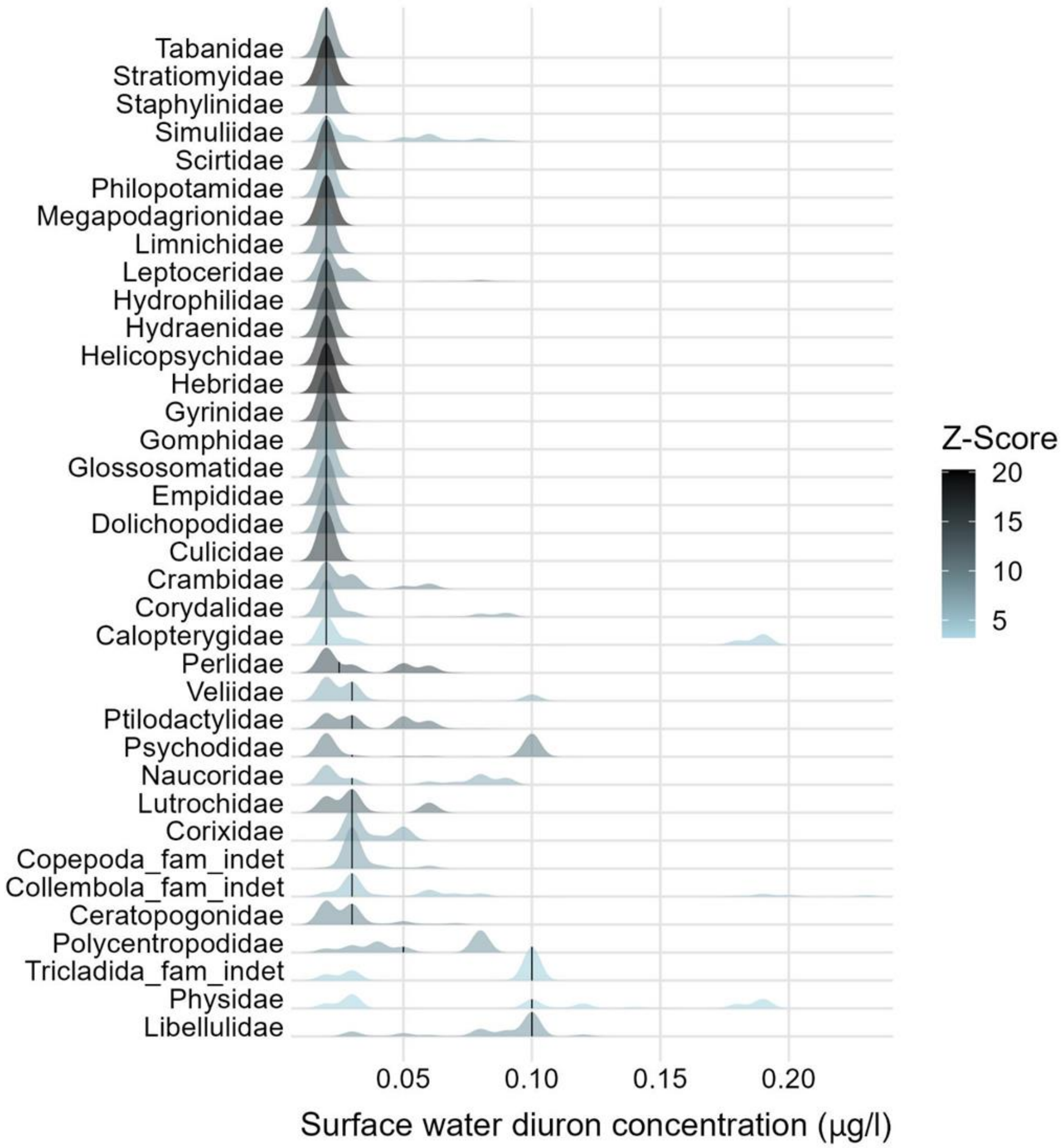

Figure S3. Ecological thresholds for diuron (herbicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

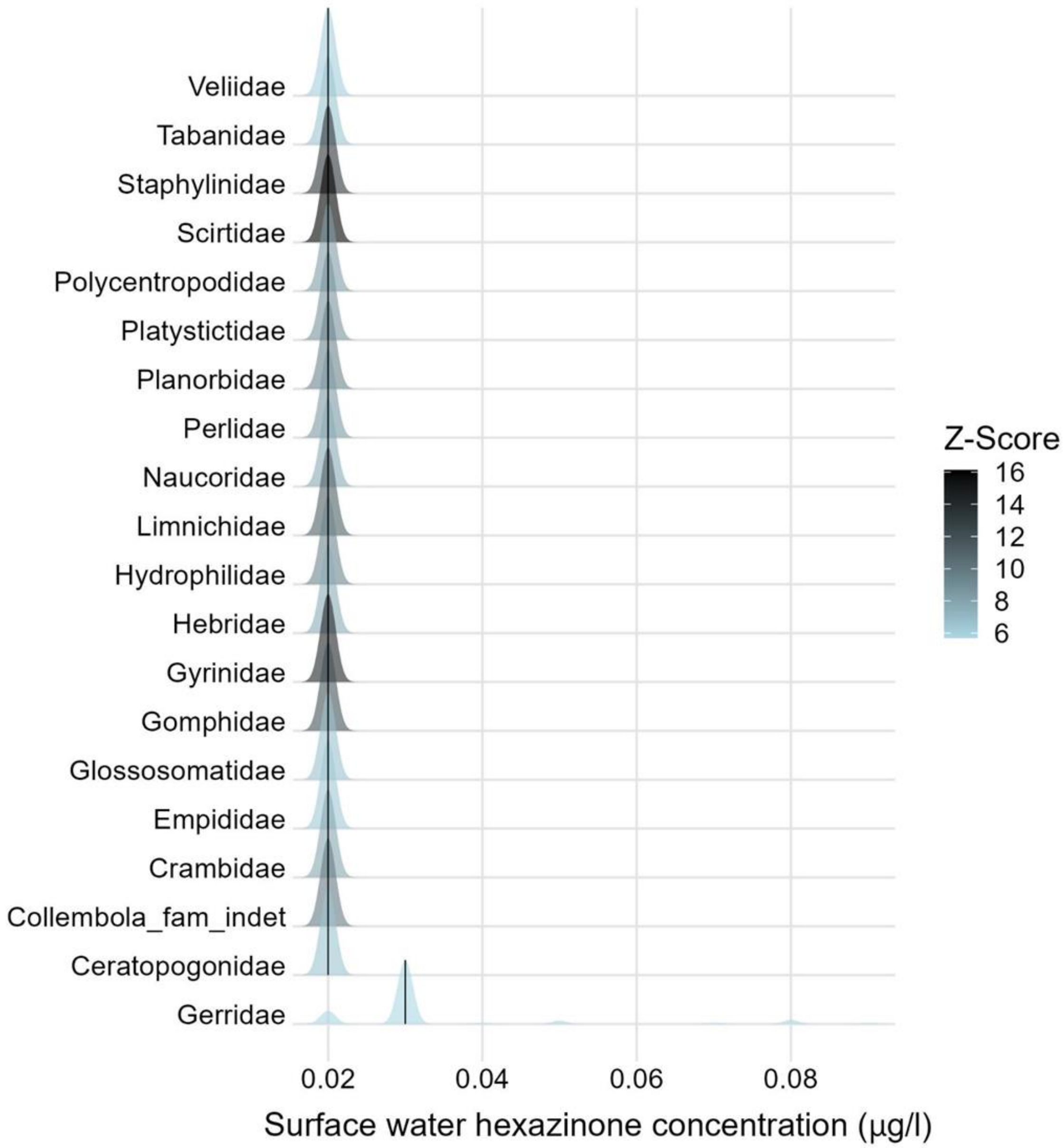

Figure S4. Ecological thresholds for hexazinone (herbicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), Grey represents negative directionality (Z-).

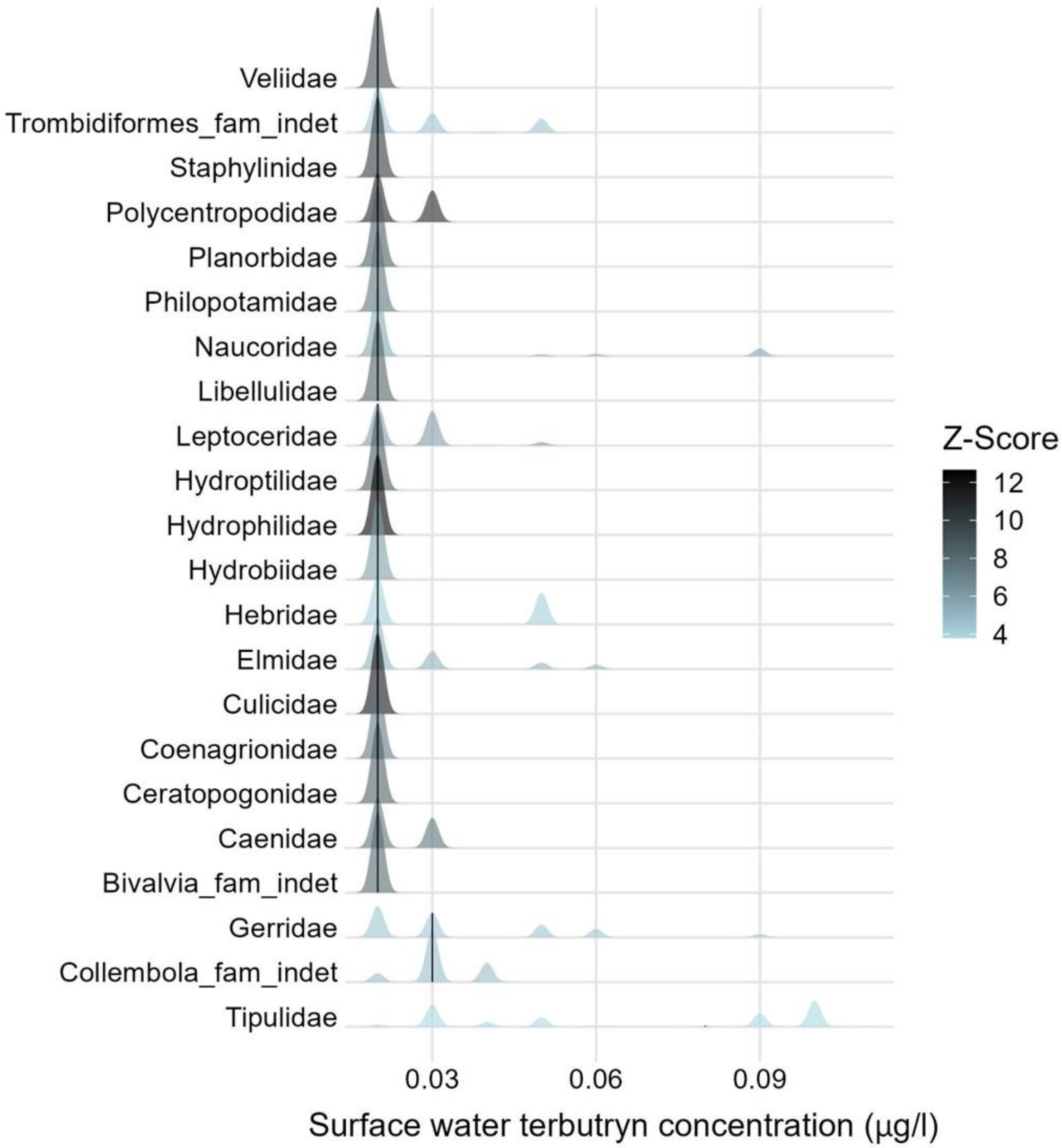

Figure S5. Ecological thresholds for terbutryn (herbicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), Grey represents negative directionality (Z-).

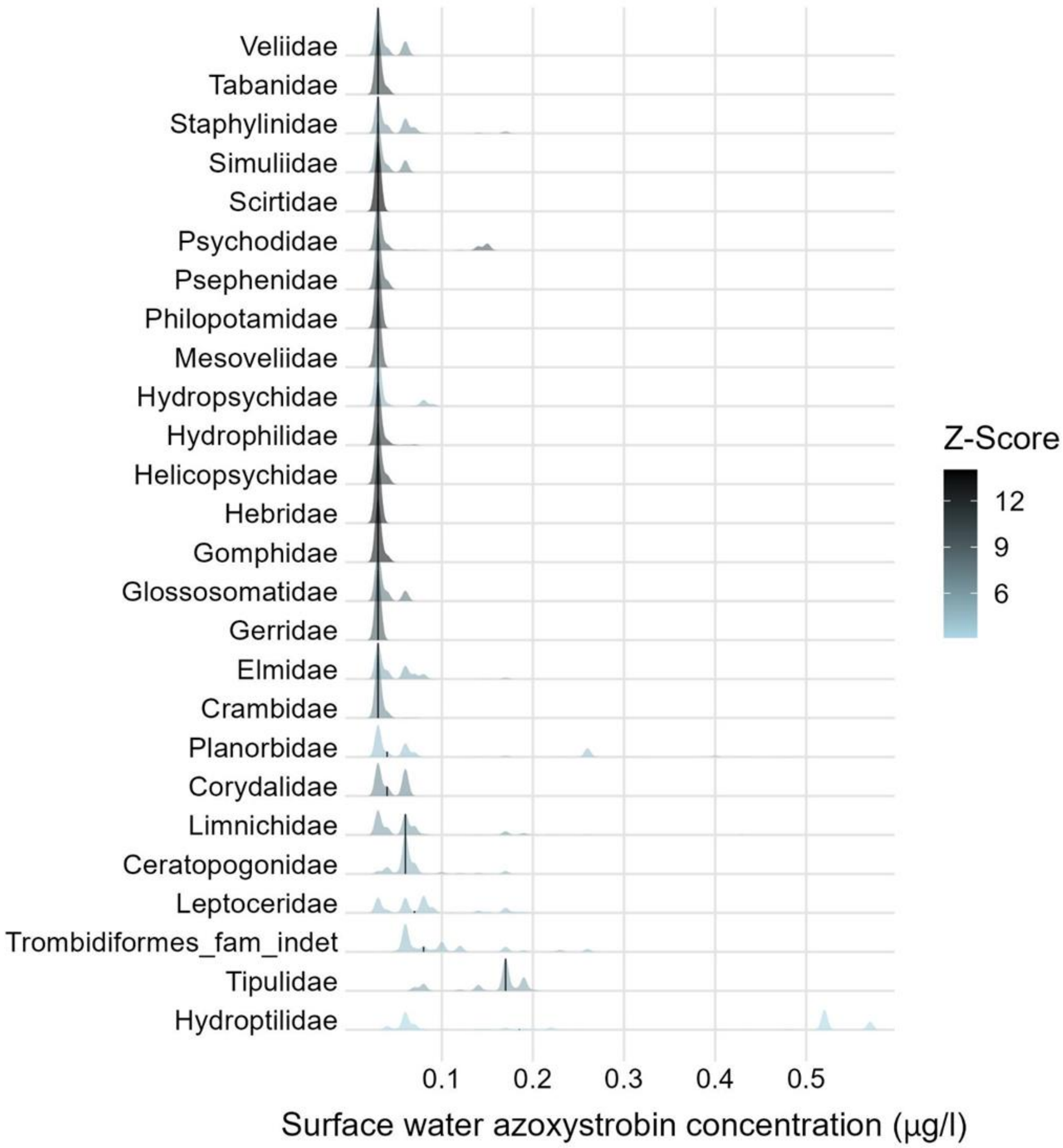

Figure S6. Ecological thresholds for azoxystrobin (fungicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

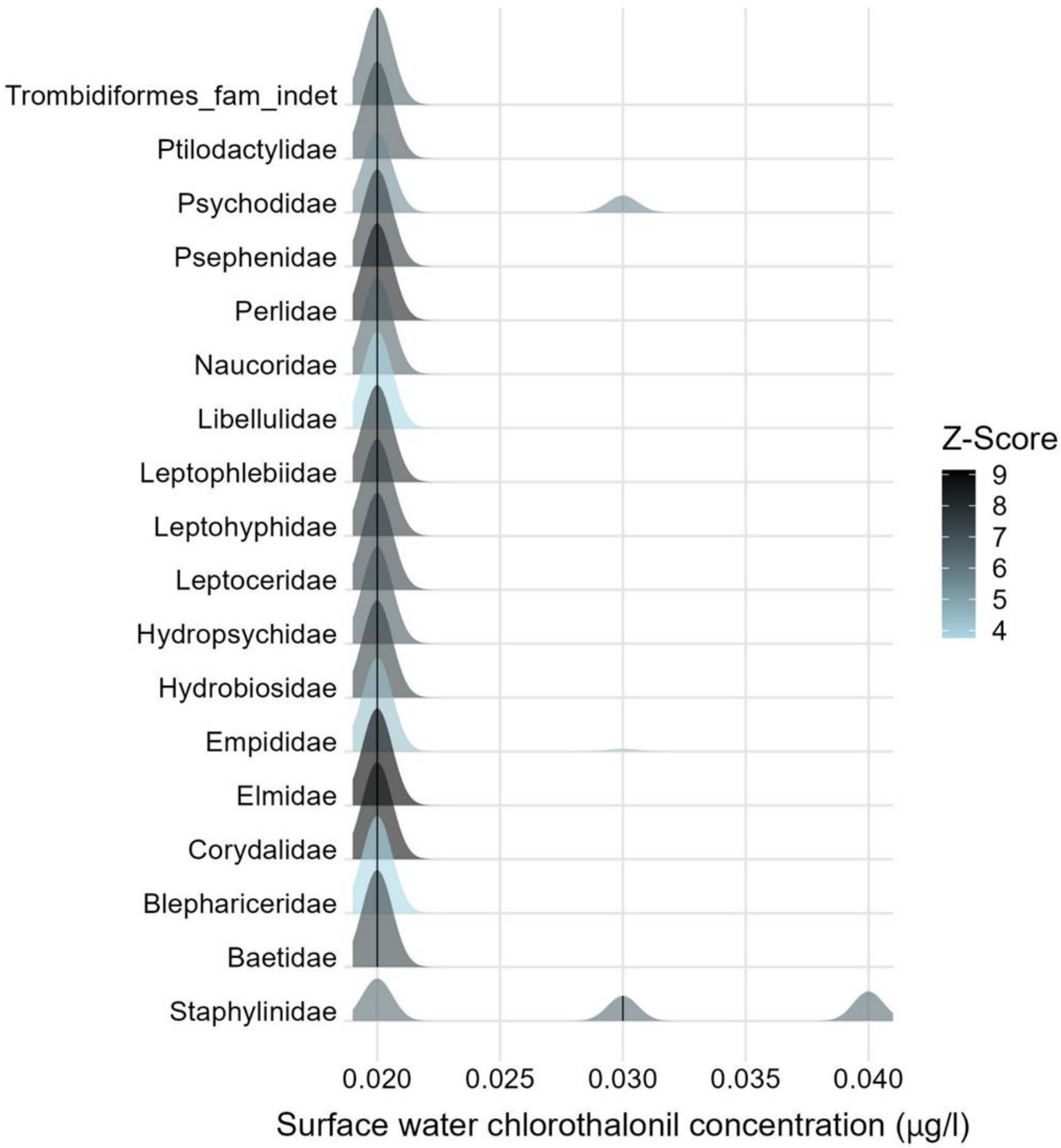

Figure S7. Ecological thresholds for chlorothalonil (fungicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

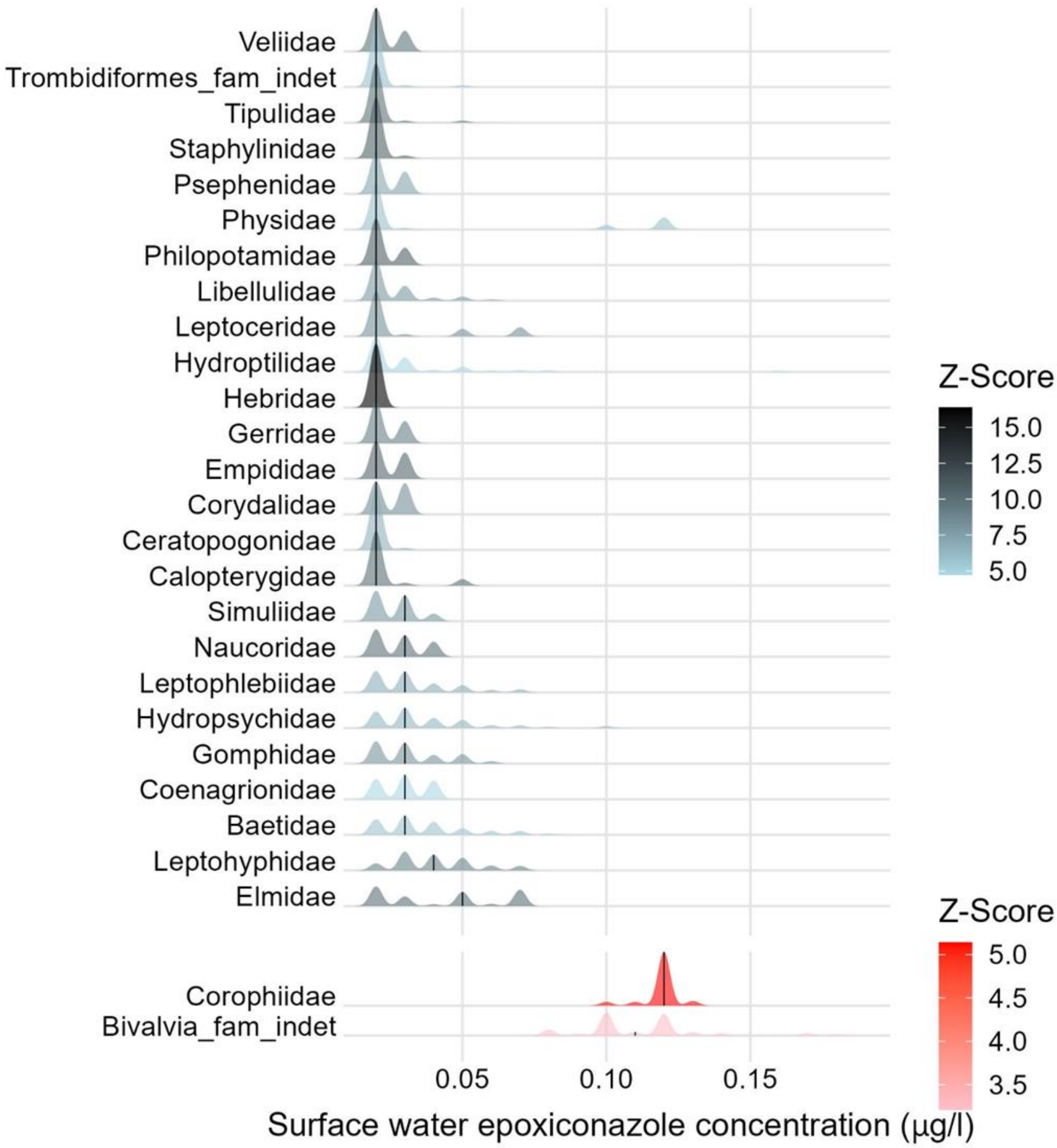

Figure S8. Ecological thresholds for epoxyconazole (fungicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), Grey represents negative directionality (Z-).

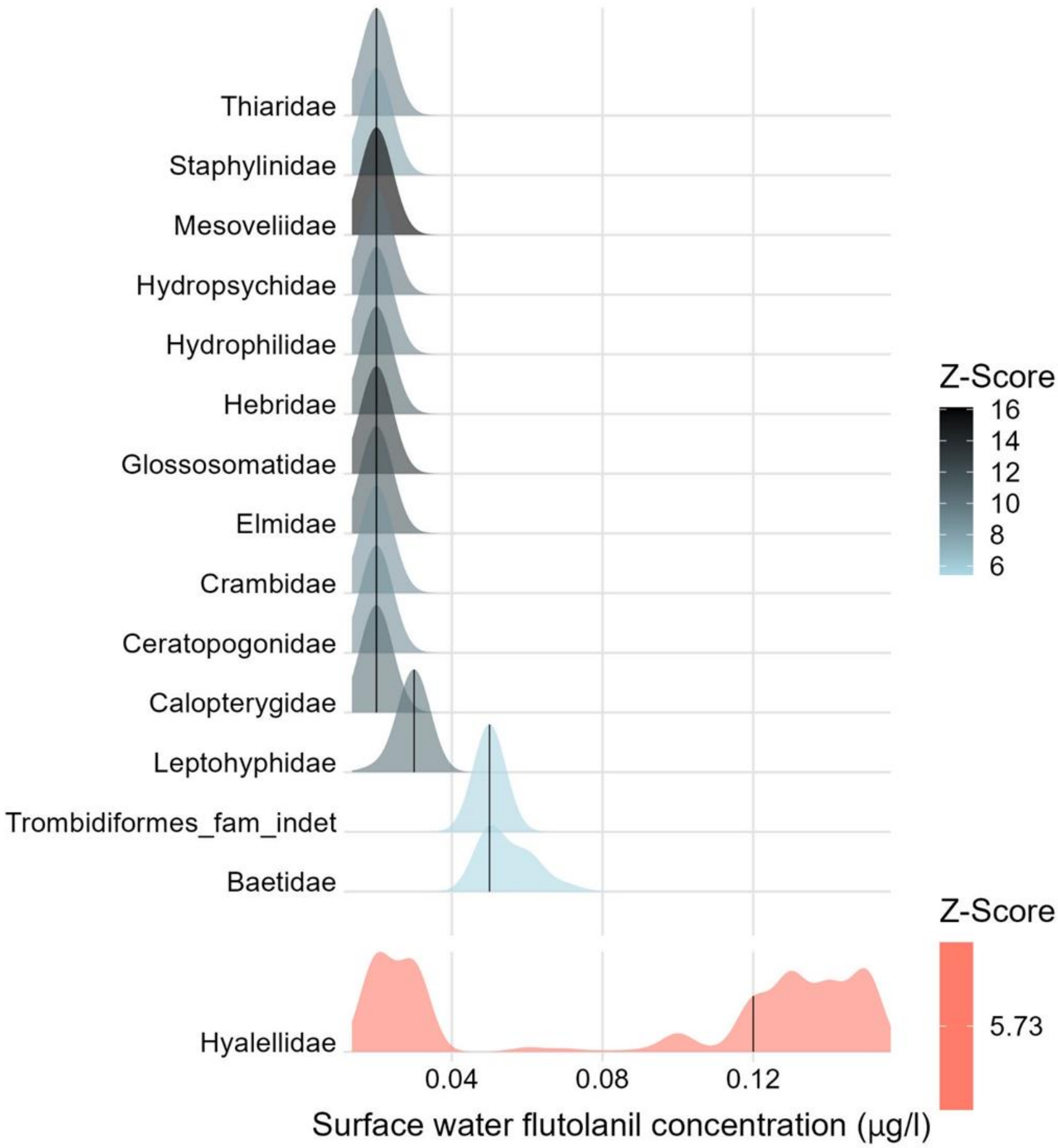

Figure S9. Ecological thresholds for flutolanil (fungicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), Grey represents negative directionality (Z-).

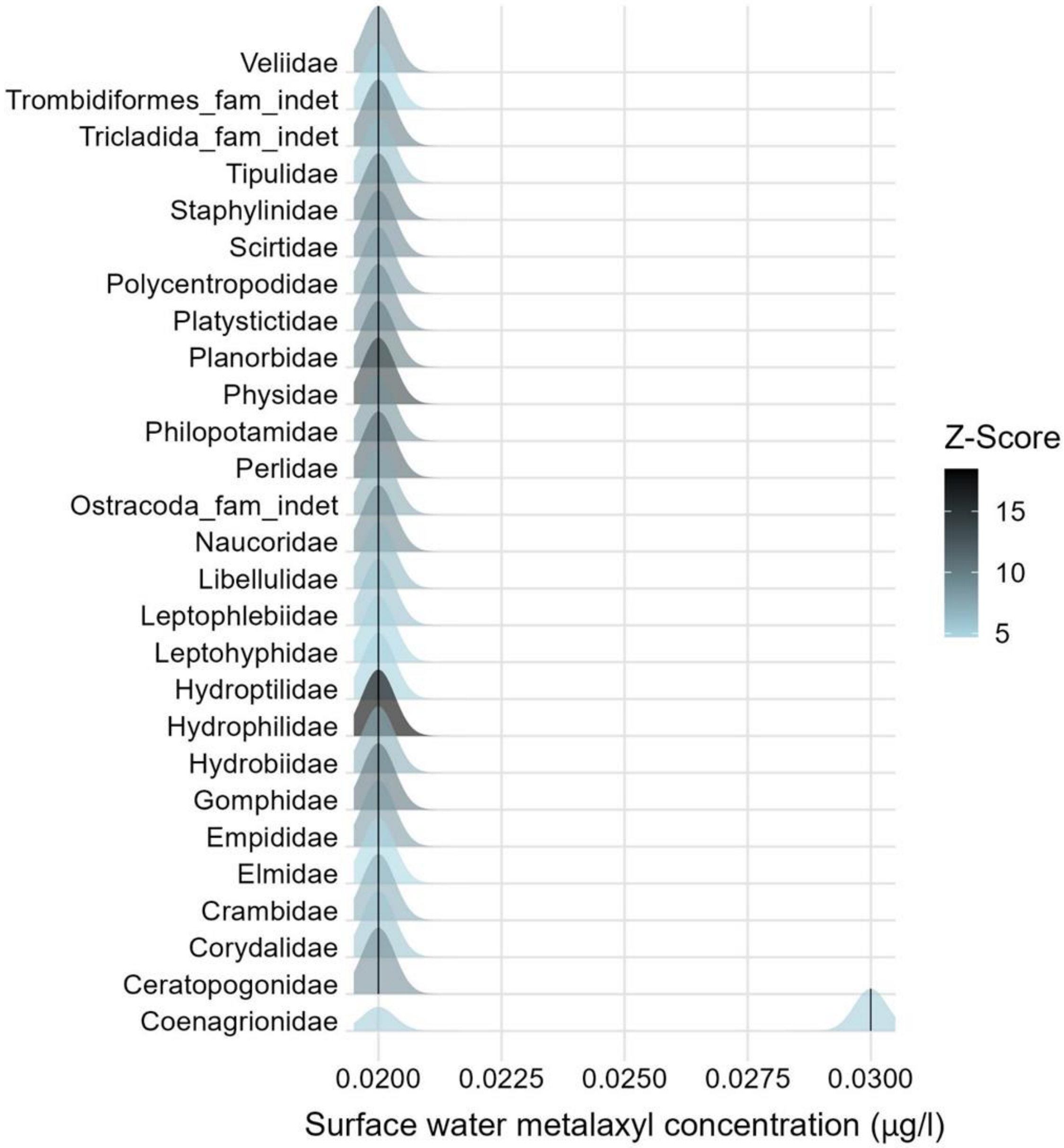

Figure S10. Ecological thresholds for metalaxyl (fungicide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), Grey represents negative directionality (Z-).

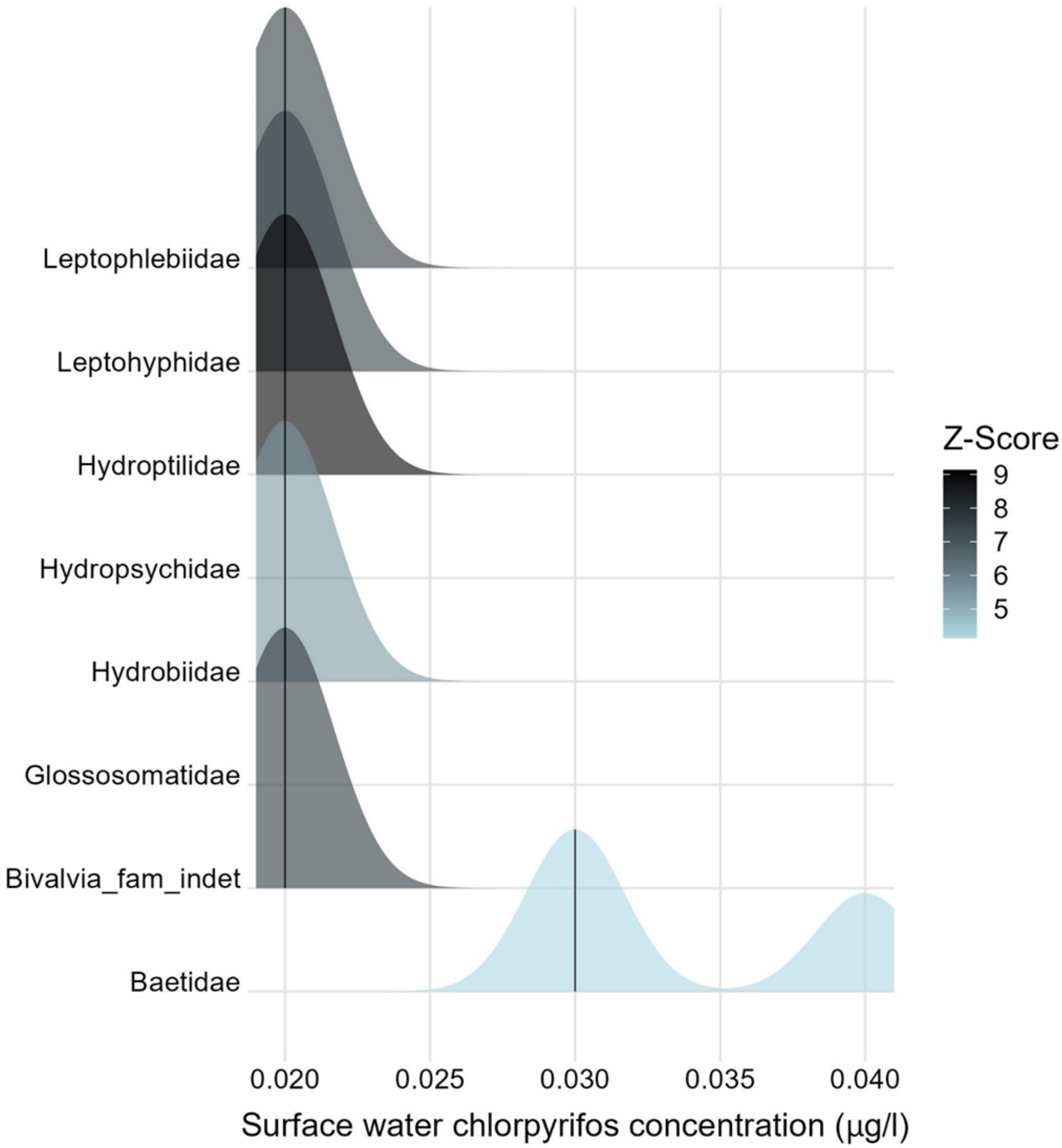

Figure S11. Ecological thresholds for chlorpyriphos (insecticide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

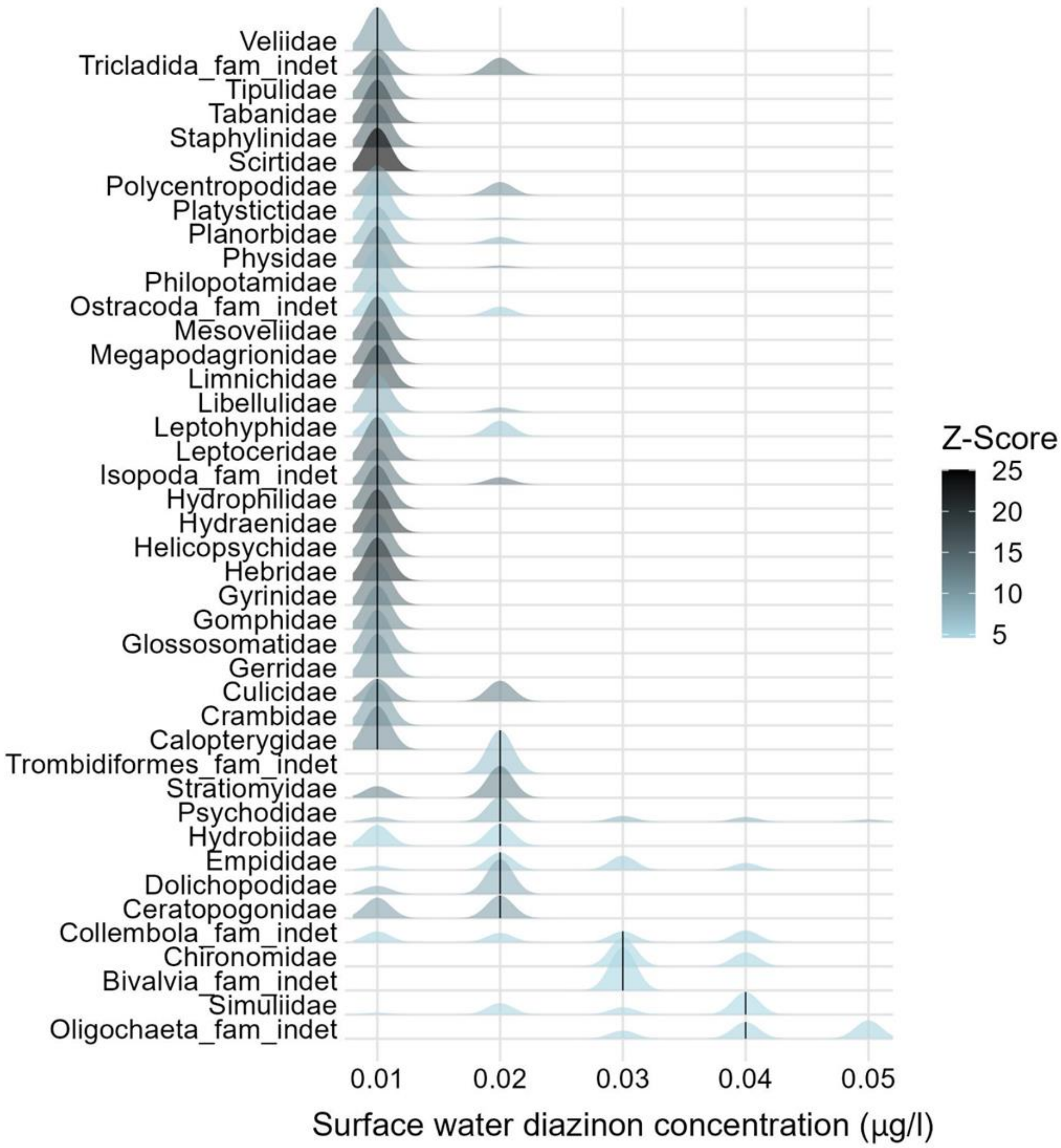

Figure S12. Ecological thresholds for diazinon (insecticide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

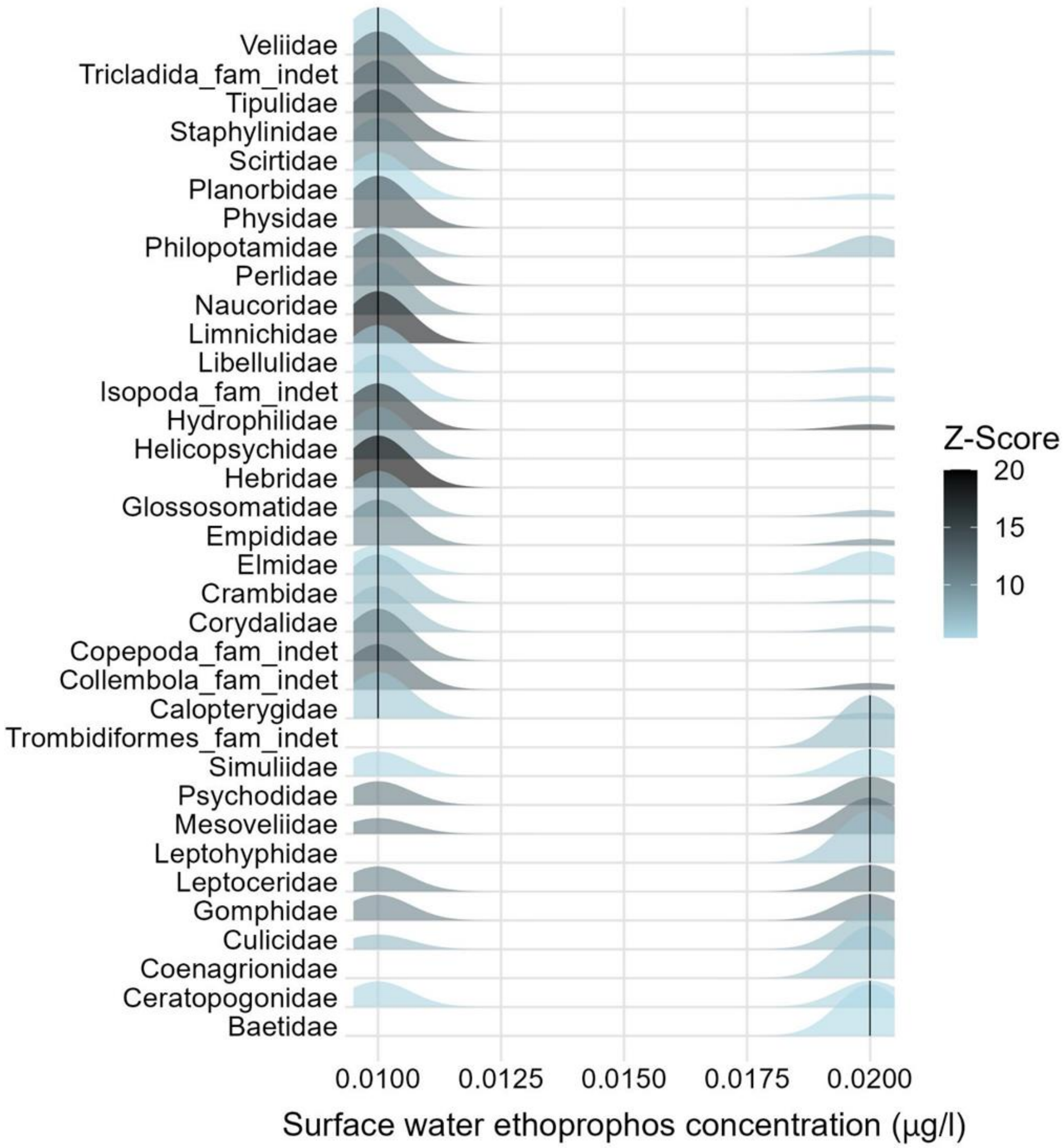

Figure S13. Ecological thresholds for ethoprophos (insecticide), derived from FWM and water samples from Costa Rica (2009-2019). Only families that complied with purity and reliability criteria of the response to the gradient are displayed. Red represents families with positive directionality (Z+), grey represents negative directionality (Z-).

6. Artículo #3. Ecological integrity impairment and habitat fragmentation for neotropical macroinvertebrate communities in an agricultural stream.

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Editor´s Choice Article

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Article

Ecological Integrity Impairment and Habitat Fragmentation for Neotropical Macroinvertebrate Communities in an Agricultural Stream

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Abstract: The Volcán River watershed in the south Pacific of Costa Rica comprises forests, small urban settlements, cattle fields, and intensive agriculture (mostly pineapple and sugarcane). The ecological integrity and quality of its waters was assessed from 2011–2013 and 2018–2019 by means of physical–chemical parameters (pH, conductivity, temperature, DO, DBO, nitrate, total phosphorus, and pesticide residues) and benthic macroinvertebrate (MI) sampling in eight sites (Volcán, Cañas, and Ángel Rivers, and Peje and Maura streams), resulting in high ecological integrity in all sites except the Peje stream, which is polluted with nitrates and pesticides. Only in this stream was there a marked seasonal variation in the abundance of 16 MI families including Leptohyphidae, Leptophlebiidae, Philopotamidae, Glossossomatidae, and Corydalidae, among others, whose presence was limited exclusively to the dry season (December to April), disappearing from the stream in the rainy season, with corresponding peaks in nitrate (max 20.3 mg/L) and pesticides (mainly herbicides and organophosphate insecticides). The characteristics of the watershed, with large areas of forest and excellent water quality, allow for the re-colonization of organisms into the Peje stream; however, those organisms are incapable of development and growth, providing evidence of a contaminant-driven habitat fragmentation in this stream during the rainy season.

Keywords: pesticides; Volcán River; Costa Rica; nitrates; community ecotoxicology; river habitat fragmentation

1. Introduction

The ecological integrity of a river or stream, meaning its suitability to offer optimal conditions for the establishment of biotic communities, is determined by a series of environmental factors [1]. Amongst the most relevant are those related to hydrology (e.g., water flow, current velocity, seasonality, and frequency of floods); habitat characteristics (quality and quantity of the riparian vegetation, substrate, river channel width, depth, and morphology); chemical and physical variables (alkalinity, temperature, dissolved oxygen, pH, turbidity, and xenobiotic presence); energy sources (nutrients, solar radiation, primary production, and organic matter); and also biotic factors related to food availability, intra and inter-species competition, reproduction rates, or predation [2].

In case of anthropogenic or naturally originated alterations of any of these factors, the availability of necessary resources for aquatic life, or the fulfillment of the ecological roles of each species, can be limited [2]. Therefore, any modification within a watershed can potentially reflect temporal and spatial variations in the ecological integrity of a river. Agricultural watersheds, for example, pose several challenges related to land use changes [3], deforestation of riparian corridors [4,5], erosion, sedimentation, changes in channel morphology [6,7], and use of fertilizers and pesticides which exert pressure on the receiving superficial waters and their biota [8,9]. Freshwater macroinvertebrate (MI) communities in continental waters worldwide have been severely affected by those pressures, with consequent threats on taxa richness and biodiversity [10–12].

The Neotropical region has the particularity of maintaining optimal temperature conditions for crops throughout the year [13]; therefore, agriculturally related stress factors are permanent, with no resting or recovery periods for the streams. Multiple authors have provided evidence of high risk for invertebrates and primary producers derived from pesticides detected in tropical agricultural watersheds [14–20]. Moreover, these stressors coexist with other Neotropical conditions such as high variation in rainfall due to climate change (especially in Central America), which might affect seasonal patterns of biota or even produce mortality in heavy drought or flood events [21].

The capability of aquatic biota to colonize or migrate through a specific river stretch can be inhibited by many different factors such as high suspended sediment loads, frequent floods or extreme drought events, high xenobiotic concentrations, and low input of allochthonous material because of riparian forest absence, among others [22–24]. Therefore, the longitudinal connectivity of a watershed (from the lower to the upper parts of the basin) can be compromised or interrupted where agriculturally related stress factors take place, creating a fragmentation of the aquatic habitat, similar to what can be found in a dammed site, but produced by a pollution barrier [25].

Studies around the globe have evidenced a profound effect of river networks habitat fragmentation (especially produced by dams) on the loss of freshwater biodiversity. In Australia [26], Japan [27], and the USA [28], researchers evidenced how in-stream physical barriers contribute to fish population declines or elimination. Regarding contaminant-caused fragmentation, [29] provided evidence that poor water quality in the watershed of River Scheldt in West Europe was acting as a barrier for the upstream migration of an anadromous fish. Moreover, [30] conducted laboratory avoidance tests and found that field-relevant concentrations of the herbicide atrazine might influence the spatial distribution and isolation of up and downstream fish populations. The same is true for many types of contaminants, from metals to PAH, pesticides, and even pulp mill effluents, which function as environmental stressors, causing organisms including fish and also invertebrates to prevent the exposure by mechanisms of active and passive avoidance, such as drift [31]. For example, pulses of neurotoxic insecticides have been proven to increase invertebrate downstream drift in stream mesocosm and microcosm experiments [32,33]. Drift initiated as fast as 2 h after the contamination at field-relevant concentrations, far lower than the LC50.

Therefore, the presence of a pollutant in the field might exert both a toxic effect and an avoidance-triggering effect. In this study, we hypothesized that several agriculturally related stress factors might be promoting fragmentation of the river network by posing a multi-factor pollution barrier which limits longitudinal habitat connectivity. Therefore, we aimed to identify the main factors influencing in-field ecological integrity, the fragmentation of the aquatic habitat, and the loss of MI biodiversity within a Neotropical watershed.

2. Materials and Methods

Study area: The Volcán River watershed is located in the south Pacific (Puntarenas province) of Costa Rica, Central America, between geographic coordinates (WGS84) −83°20.3409′ to −83°29.1345′ W; and 9°07.8204 to 9°22.3043′ N (Figure S1). It comprises a wide altitudinal range, from 221 to 3126 m.a.s.l. Consequently, this is a key watershed for connecting both terrestrial and aquatic flora and fauna between the International La Amistad Park (natural protected area), in the upper section of the basin, with coastal ecosystems in the lowlands. This watershed has highly conserved areas, mixed with pastures and coffee plantations in the upper basin and extensive pineapple and sugarcane agriculture in the middle-lower section, where the alluvial fans are formed. It extends for 22,600 ha and forms part of the Grande de Térraba River basin [34]. Mean annual precipitation in this area ranges from 3100 to 3700 mm [35]. This watershed comprises forests, small urban settlements, cattle pastures, and agriculture (mostly pineapple, sugarcane, and coffee).

Study design: This study was divided into two time periods: 1. from 2011–2013 and 2. from 2018–2019.

2.1. Ecological Integrity and Water Quality in the Volcán River Watershed from 2011–2013

We evaluated the ecological integrity and water quality from 2011–2013 through trimestral sampling in eight sites distributed in the Volcán (3 sites), Cañas (2), and Ángel (1) Rivers, and Peje (1) and Maura (1) streams (Figure S1). The ecological integrity was assessed by a combination of (a) habitat structure indexes (both in-stream and in the river bank), (b) biodiversity of aquatic biota (MI community sampling), and (c) anthropogenic stress (determined with basic physical and chemical parameters and pesticide residue analysis). Meanwhile, the water quality was assessed with a MI-based Biotic Index and also with the results from the physical and chemical parameters.

(a) Habitat structure: following Acosta et al. [36], two habitat indexes were used, the IHF: Fluvial Habitat Index, and the QBR-And: Riverbank Vegetation Quality Index. The IHF was estimated as a measure of in-stream habitat diversity and serves the purpose of differentiating between the effects of pollution and those of low availability of microhabitats in the rivers. The QBR-And also was estimated to state the quality of the riparian forest in the study sites.

(b) Aquatic MI community sampling and analysis: organisms were collected for 10 min using a D net (300 μm) and stored in 80% ethanol. At the Laboratory for Ecotoxicological Studies (ECOTOX) at the Universidad Nacional (UNA, Heredia, Costa Rica), the organisms were separated and identified to the lowest possible taxonomic level using a stereoscope and pertinent identification keys [37–39]. The abundance and richness of taxa was estimated, and the BMWP-CR biotic index [40] was calculated to determine water quality. Richness of taxa, abundance, and the BMWP-CR index were calculated also as measures of MI diversity.

(c) Physical and chemical parameters: pH, conductivity (μS/cm), temperature (°C), and dissolved oxygen (DO, mg/L) were determined in situ using a YSI 6600 portable multi-probe equipment to evaluate the basic conditions of the rivers and streams. Meanwhile, water samples were taken in 0.5 L plastic bottles and transported on ice for biological oxygen demand (BOD, mg/L), nitrate (mg/L), and total phosphorus (mg/L) analysis, to have insight into the presence of organic matter and nutrients in the water, as well as measuring anthropogenic stress. These parameters were determined at the Laboratory for Chemical Analysis and Services (LASEQ-UNA), following the Standard Methods for the Examination of Water and Wastewater [41]. For the pesticide residue analysis, surface water samples were collected by inserting pre-washed 2 L glass bottles into the water. The collected samples were transported in cooled ice boxes to the Laboratory of Pesticide Residue Analysis (LAREP-UNA) and stored at 4–6 °C for a maximum of 24 h before the analyses. In this time period (2011–2013), pesticide analyses were performed as specified in Rämö et al. [19].

2.2. Ecological Integrity and Water Quality in the Peje Stream from 2018–2019

We made a second sampling effort (12 monthly samples) only in one site: the Peje stream, between February 2018 to February 2019. In this opportunity, we determined the same parameters as before (IHF and QBR as habitat structure metrics; pH, conductivity, temperature, and DO as basic physical and chemical parameters; nitrates and total phosphorus as nutrient, energy, and food sources; and pesticide residue analysis to assess xenobiotic presence). However, we added new parameters such as phytoperiphyton abundance as additional energy and food sources for MI, and channel width, current velocity and flow as hydrology variables. The purpose of including these variables was to acknowledge other ecological processes as factors influencing MI community structure.

Furthermore, pesticide residue analyses were modified as follows: samples were analyzed by gas chromatography with mass detector Agilent 7890A-5975C GC-MS (Agilent Technologies, Palo Alto, CA, USA) using selective ion monitoring (SIM) and by liquid chromatography Waters Acquity UPLC H-Class with mass detector XEVO T-QS Micro, LC-MS/MS (Waters, Milford, MA, USA), using multiple reaction monitoring (MRM). The water samples, after adding internal standards, were extracted by solid-phase extraction (SPE) using previously conditioned Isolute ENV+ (200 mg/6 mL) (Biotage, Uppsala, Sweden) cartridges. For GC, the cartridge was eluted with ethyl acetate and the extract was concentrated with nitrogen and changed into isooctane, with a final volume of 0.25 mL. For LC, the same extraction procedure was followed, except that the elution was performed with methanol, and it was concentrated into methanol/water (10:90 v/v or 40:60 v/v), with a final volume of 0.5 mL. Target analytes were identified by retention times and confirmed with SIM or MRM ratios. Quantification was performed with internal and external calibration curves of the target analytes (quantification and detection limits can be found in Table S1).

Primary producer's community sampling and analysis: phytoperiphyton was collected following the Ebro Hydrographic Confederation protocol (2005), and five rocks submerged and exposed to sunlight were collected. Using a toothbrush, a total area of 100 cm^2 was scraped. With each scraping, the brush was placed in a bottle with 50 mL of sterile distilled water. The sample was fixed with concentrated lugol and transferred to the ECOTOX lab on ice and in darkness. With the help of a microscope, a triplicate drop of the sample was observed. Counting of cells was performed using a Neubauer counting chamber, and total abundance of phytoperiphyton was estimated.

Water flow was determined using a FH950.0 HACH digital flow meter, and the channel width, current speed (m/s), and the depth (m) of the water column were recorded in a transverse section of the channel. The distance between each measurement was 1 m. The data obtained were placed in the formula: $Q = A \times V$, where Q represents the flow (m^3/s), A is the area of the section of the course (mean depth times width), and V is the mean current velocity of the stream [42].

Data analysis: All measurements, determinations, and samples were collected and analyzed by Universidad Nacional laboratories with qualified personnel and methodologies. This assured uniformity of data quality irrespective of the time period of the research project.

For the pesticide residue data, measurements above the detection limits (LOD) and below the quantification limits (LOQ) were substituted with half of the LOQ, while data below the LOD (not detected) were substituted with an extremely low arbitrary value of 0.0001 μg/L.

Ordination exploratory analyses were carried out in R (R Core Team 2019) programming environment and vegan library [43,44]. Based on the biological community data matrix, we generated a detrended correspondence analysis (DCA), from which we obtained a length gradient of 3.24. Therefore, a redundancy analysis (RDA) was applied to clarify the relationships between environmental and MI community data. BOD was not used for this analysis because there was missing data in some of the sampling events from the Peje stream and RDA requires a complete dataset. The incorporation of this parameter would have implied omitting several sampling events; therefore, we decided to keep the totality of sites and sampling events, acknowledging that the exclusion of BOD might somehow affect the conclusions drawn from the RDA.

Previous to the execution of the RDA, individual pesticide concentrations were grouped and summarized according to their biocide action and their mode of action, following information from the Insecticides, Herbicides and Fungicides Resistance Action Committees (FRAC, IRAC, and HRAC) [45–47]. A codification was created with the initial of the biocide action: F = fungicide; H = herbicide; I = insecticide, followed by the mode of action. For example, Sum_H5 represents the addition of all concentrations of detected herbicides in a water sample, with mode of action 5 (photosystem II inhibitor; D1 Serine 264 Binders), according to HRAC [47]. Table S2 shows the represented modes of action for each pesticide active ingredient.

Before the RDA, physical and chemical variables were standardized [48] and biological data were transformed using a Hellinger transformation [49]. Variation inflation factors (VIFs) were employed to identify and eliminate variables with high collinearity [50]. To improve the model, we performed a forward selection using the adjusted R^2 as the criteria to select the best subset of physical and chemical variables that influenced the MI data with the adespatial library [51].

For visualization purposes, the biplot cannot show all the identified taxa within the watershed; therefore, we conducted a SIMPER analysis (R^2, $p < 0.05$) [52] to extract only the taxa that contribute >70% of the difference in the communities between dry and rainy seasons. These taxa are shown in the RDA biplot.

3. Results

3.1. Ecological Integrity and Water Quality in the Volcán River Watershed from 2011–2013

Between 2011 and 2013, 45 total samples were taken from the eight study sites in six trimestral field campaigns. The ecological quality indexes QBR-And and IHF showed their highest values in the upper basin sites, decreasing toward the lowlands. BMWP-CR index showed the highest values (good to excellent water quality) in the upper basin sites, with slightly lower values (good to regular water quality) in the Maura stream and the lower section of the Volcán River. The lowest values were calculated for the Peje stream sampling site during the rainy season (bad and very bad water quality) (Table 1).

Table 1. Ecological quality index values (QBR-And, IHF (min–max), and BMWP-CR) calculated in 8 sites from the Volcán River watershed, period 2011–2013.

Basin Position	QBR-And	IHF	Site	Dec-11	Mar-12	Jul-12	Sep-12	Dec-12	Mar-13
Upper	95	59–67	Volcán 1	174	158	118	129	139	108
Upper	75	53–66	Angel	-	125	122	158	140	106
Upper	95	58–74	Cañas 1	153	164	160	195	164	132
Middle	75	47–56	Volcán 2	141	111	131	142	157	-
Middle	90	36–48	Maura	106	113	102	117	83	87
Middle	45	41–47	Peje	30	102	47	19	-	91
Lower	85	48–56	Volcán 3	136	140	140	112	120	89
Lower	70	51–73	Cañas 2	140	187	123	126	122	122

QBR color interpretation: green = good vegetation quality; yellow = intermediate vegetation quality; orange = bad vegetation quality [36]. IHF < 40 = inadequate to support a diverse MI community [36]. BMWP-CR color interpretation: dark blue: excellent water quality; light blue: good water quality; green: regular water quality; yellow: bad water quality; orange: very bad water quality [40].

As can be seen from Table 1, the Volcán River watershed had (in general) high ecological integrity in all sites except for the Peje stream.

Total MI identified from the Volcán River watershed accounted for n = 26,243 individuals, distributed in 20 orders, 75 families, and 128 genera. Number of identified families was highest in the upper basin sites of the Cañas, Ángel, and Volcán Rivers, while the lowest numbers were recorded for the Peje stream (Table 2). Only in this stream we found a marked seasonal variation in the abundance of 16 MI families (Ephemeroptera: Caenidae, Leptohyphidae, and Leptophlebiidae; Trichoptera: Glossosomatidae and Philopotamidae; Plecoptera: Perlidae; Odonata: Calopterygidae, Coenagrionidae, and Libellulidae; Coleoptera: Hydrophilidae and Staphylinidae; Megaloptera: Corydalidae; Diptera: Ceratopogonidae and Tipulidae; Lepidoptera: Crambidae; and Gastropoda: Planorbidae), whose presence was limited exclusively to the dry season (December to April), disappearing completely from the stream in the rainy season (May to November).

Table 2. Summary of benthic macroinvertebrate families and seasonality pattern in the sampling sites, period 2011–2013. Number of total identified macroinvertebrate families per site and seasonality pattern of registered families.

Basin Position	Site	Total Identified Families	Present in >50% of Samples	Present Only in the Dry Season	% of Families Showing Seasonality
Upper	Volcán 1	46	25	2	4
Upper	Angel	49	22	7	14
Upper	Cañas 1	50	31	3	6
Middle	Volcán 2	35	20	0	0
Middle	Maura	40	18	5	13
Middle	Peje	32	9	16	53
Lower	Volcán 3	48	23	5	10
Lower	Cañas 2	51	26	6	12

Overall, Cañas River sites had slightly higher pH (≈8), while Cañas and Volcán 1 sites had higher DO (≈9 mg/L), Volcán 2 and 3 had higher BOD (>5 mg/L), and the Peje stream had the highest temperature (25.4 °C), conductivity (57.83 µS/cm), and concentrations of nitrates (max 20.3 mg/L), in comparison with all the other sites within the Volcán River watershed (Table 3).

Table 3. Mean and standard deviation (mean ± standard deviation) of physical, chemical, and nutrient parameters in the Volcán River watershed, period 2011–2013.

Site	Temp (°C)	pH	Cond (µS/cm)	DO (mg/L)	BOD (mg/L)	Nitrates (NO_3; mg/L)	Total P (mg/L)
Volcán 1	20.1 ± 1.3	7.3 ± 0.3	46.0 ± 5.1	8.4 ± 0.4	3.93 ± 2.15	0.38 ± 0.31	3.79 ± 8.39
Angel	22.0 ± 1.6	7.0 ± 0.3	26.8 ± 5.0	8.2 ± 0.4	3.33 ± 1.27	0.38 ± 0.31	0.03 ± 0.03
Cañas 1	19.6 ± 1.2	7.5 ± 0.4	35.8 ± 3.1	8. 5 ± 0.3	3.2 ± 2.36	0.38 ± 0.31	1.59 ± 3.47
Volcán 2	24.3 ± 2.3	7.3 ± 0.3	37.6 ± 4.6	8.2 ± 0.2	5.5 ± 4.86	0.47 ± 0.33	1.39 ± 2.74
Maura	24.5 ± 0.7	6.7 ± 0.4	22.1 ± 6.3	7.7 ± 0.3	3.4 ± 2.77	1.64 ± 0.93	0.44 ± 0.87
Peje	25.4 ± 1.2	7.0 ± 0.8	57.8 ± 7.3	8.0 ± 0.3	4.2 ± 2.28	13.78 ± 6.08	0.1 ± 0.2
Volcán 3	24. 6 ± 2.0	7.3 ± 0.5	38.8 ± 4.7	8.2 ± 0.3	5.73 ± 5.1	3.71 ± 5.26	0.77 ± 1.64
Cañas 2	22.7 ± 1.5	7.4 ± 0.3	36.1 ± 2.7	8.4 ± 0.3	3.13 ± 2.01	1.07 ± 1.66	0.04 ± 0.04

The Peje stream also had the highest concentrations of pesticide residues (mainly herbicide bromacil and organophosphate insecticide diazinon) in the first study period and throughout the complete study (Tables 4 and S3).

Table 4. Pesticide residues detected in the Volcán River watershed (2011–2013). Concentrations (µg/L) are presented as min–max (no. detections). Where no interval and parenthesis are presented, only one detection was made.

Site	Diazinon	Terbutryn	Bromacil	Oxyfluorfen	Hexazinone	Permethrin
Volcán 1	nd	nd	nd	nd	nd	nd
Angel	nd	T	nd	nd	nd	0.4
Cañas 1	nd	T	nd	nd	0.3	nd
Volcán 2	T	nd	0.1–0.14 (3)	nd	nd	T
Maura	T	T	0.21–1.2 (4)	nd	nd	nd
Peje	0.05–0.2 (4)	nd	5.3–6.9 (6)	T	0.2	nd
Volcan 3	T–0.02 (4)	T	0.6–1.3 (5)	nd	nd	nd
Cañas 2	T	nd	nd	nd	nd	nd

nd = below detection limit. T = between LOD and LOQ.

Therefore, in order to better understand the ecological processes and environmental pressures taking place at the Peje stream, we made a second sampling effort with additional ecological factors determined only in this stream to complement the existing information and aid in the understanding of the seasonal absence of MI families in the rainy season.

3.2. Ecological Integrity and Water Quality in the Peje Stream from 2018–2019

For the period 2018–2019, the QBR-And index remained the same (45; bad riparian vegetation quality), with no detectable differences in the studied stream section. The IHF index varied from 46–64, denoting a slightly better microhabitat availability for MI in this period (Table 5). However, the BMWP-CR index was lower in 2018–2019, with a minimum score of 12 in the rainy season (extremely bad water quality) and a maximum of 76 in the dry season (regular water quality), in contrast with the maximum score of 102 (good water quality) obtained during the dry season of 2012 (see Tables 1 and 5).

Table 5. Minimum, maximum, mean, and standard deviation of the variables measured or estimated in the 2018–2019 sampling period in the Peje stream. Information is presented separately for the dry and rainy seasons.

Parameter	Dry Season				Rainy Season			
	MIN	MAX	MEAN	SD	MIN	MAX	MEAN	SD
IHF score	50	64	58.40	5.13	46	62	52.57	6.35
BMWP-CR	21	76	51.8	26.44	12	62	24.71	17.38
Taxa richness MI	4	24	15.4	9.6	3	25	8.4	7.6
Temperature (°C)	23.1	26.3	24.88	1.25	24.2	26.5	25.19	0.81
pH	6.68	8.84	7.32	0.89	5.7	7.22	6.58	0.58
Conductivity (µS/cm)	55.5	73.4	63.98	7.37	47.3	58.7	52.09	3.52
DO (mg/L)	7.72	8.66	7.99	0.39	7.54	8.5	7.99	0.31
Nitrates (mg/L)	4.85	19.92 *	12.68	6.56	12.08	20.26	17.33	2.72
Total P (mg/L)	0.0201	0.075	0.05	0.039	0.052	0.115	0.074	0.036
Channel width (m)	8.2	11.5	10.06	1.47	7.1	11	8.86	1.28
Velocity (m/s)	0.03	0.43	0.24	0.18	0.39	0.78	0.55	0.15
Flow (m^3/s)	0.04	2.66	0.88	1.06	0.81	3.65	2.11	1.12
Periphyton abundance	114×10^3	1144×10^3	608×10^3	487×10^3	45.5×10^3	333×10^3	126×10^3	103×10^3

* Concentration of the first month of the transition between rainy and dry seasons.

Regarding physical and chemical parameters such as temperature, pH, conductivity, and DO, we determined very similar values as in 2011–2013, as well as similar values between the dry and the rainy seasons (Table 5). However, some parameters did show variation with respect to seasonality; they were the current velocity, the flow, the nitrate concentration, and the abundance of periphyton (Figure 1). A clear increment in the nitrate concentration could be seen in the rainy season, which followed the same pattern as the flow. Furthermore, phytoperiphyton abundance increased in two specific moments (July and December), when two factors happen at the same time: 1. flow starts to decrease as precipitation diminishes; and 2. there is a high concentration of nutrients (nitrates) available in the water column. The precipitation decrease in July obeys a climatic pattern called the "veranillo de San Juan", which is a hot and dry period (usually 5–15 days long) at some point between July and August, in the middle of the rainy season.

With respect to pesticide residues, fifteen pesticide active ingredients were detected in the study area, most of which are known to be applied to the major crops in this watershed (pineapple and sugarcane). From these pesticides, cadusafos and carbofuran were only analyzed in the 2018–2019 period, when a change in the methodology allowed the determination of more substances and at lower concentrations. Therefore, we cannot discuss or compare their detection between both study periods. However, similar to the 2011–2013 sampling period, herbicides had the highest concentrations, followed by insecticides. The major difference between these periods was the decrease in the concentration of bromacil and the increment in the concentrations of insecticides highly toxic for aquatic organisms (carbaryl, ethoprophos, and diazinon). Additionally noteworthy is that several new substances (including fungicides) were detected only in the second study period (Figure 2 and Table S3). Nevertheless, we did not observe a clear trend of pesticide concentrations increasing or decreasing according to the precipitation regimes or seasonality. Pesticides in the Peje stream were present throughout the year in similar concentrations in both study periods (Figure 2).

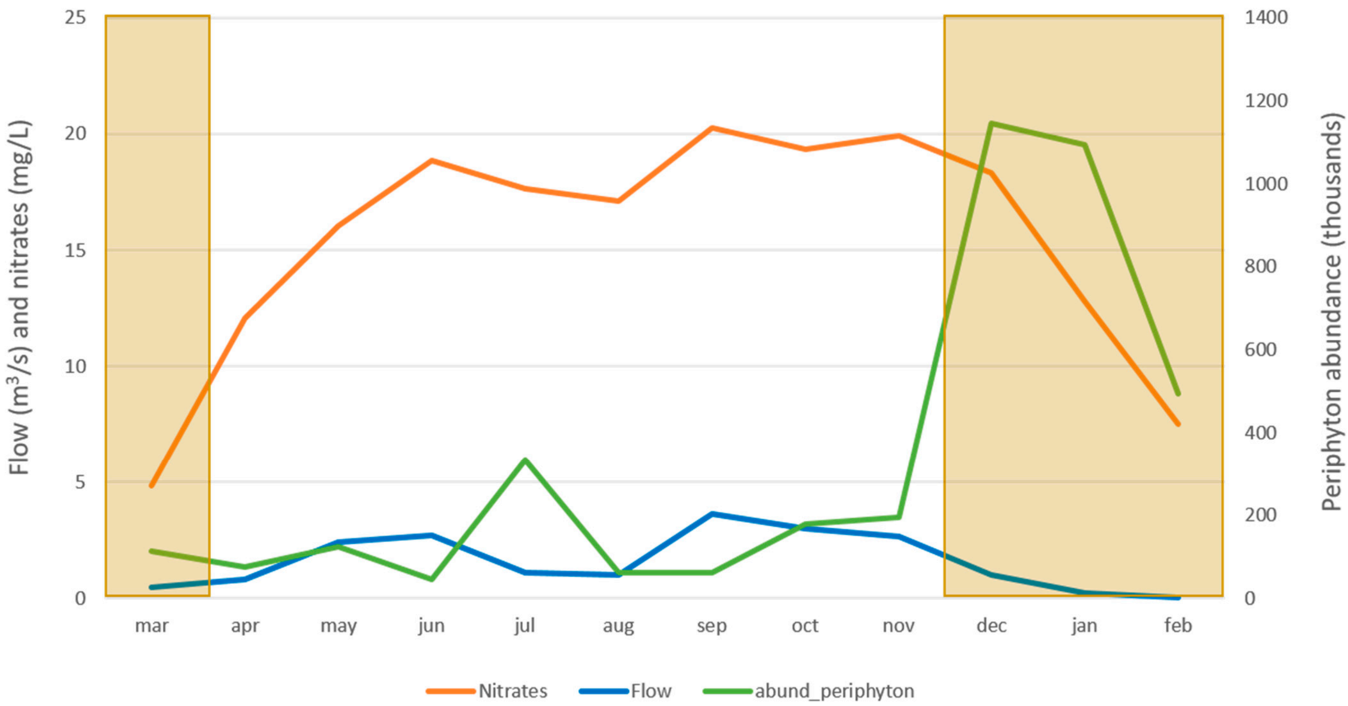
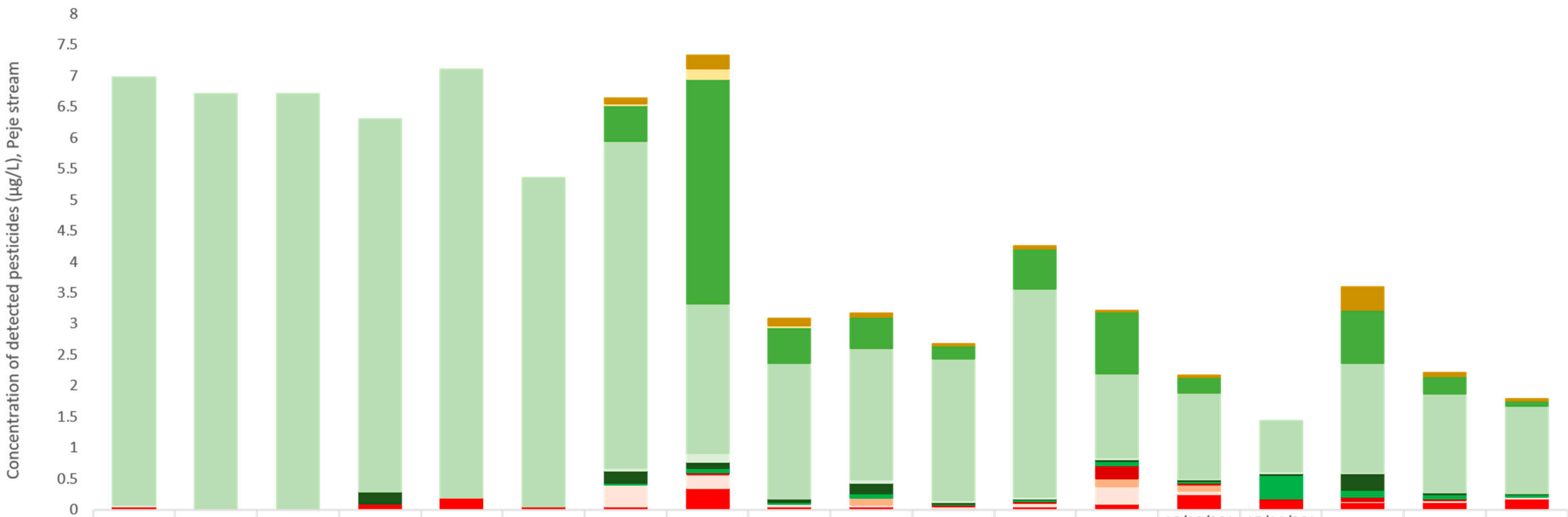


Figure 1. Temporal variations of the flow (m^3/s), nitrate (mg/L), and phytoperiphyton abundance (thousands), measured in the 2018–2019 sampling period in the Peje stream. Darker boxes represent dry season periods.

3.3. Relationships between Environmental Variables and Macroinvertebrate Community Data

We aimed to better analyze the complete dataset (study period 1 and 2) with the help of an RDA, as detailed in the methodology. This RDA model (F = 3.17, gl. = 13, 43; $p = 0.001$) and both axes (RDA 1: F 20.89; $p = 0.01$; RDA 2: F = 5.03, $p = 0.01$) explained 33% of the variation in the MI communities (adjusted $R^2 = 0.33$). According to the forward selection method, we selected the best subset of physical and chemical variables that influenced the composition of the MI community in the Volcán River watershed, and they were: 1. nitrates, 2. Sum_H5 (herbicides ametryn, bromacil, diuron, hexazinone, and terbutryn), and 3. Sum_I3A (permethrin) (Figure 3). As can be seen in the biplot, the Peje stream MI community was separated from all the other sites in the watershed. At the same time, the differences between the dry and rainy season are also reflected in the RDA biplot. MI taxa in Figure 3 are the ones which contributed to 70% of the difference between dry and rainy seasons. Only a few taxa such as *Leptonema* (Trichoptera: Hydropsychidae) or Chironomidae (Diptera) were found all year round in the Peje stream during the sampling periods (Table S4). This biplot also highlights the increased number of stressors affecting the MI of the Peje stream in comparison with all the other sites in the watershed.



	6/12/2011	12/3/2012	25/7/2012	6/9/2012	4/12/2012	18/3/2013	20/3/2018	18/4/2018	24/5/2018	25/6/2018	19/7/2018	13/8/2018	13/9/2018	10/10/2018	15/11/2018	3/12/2018	15/1/2019	22/2/2019
metalaxil							0.09	0.205	0.11	0.06	0.025	0.05	0.025	0.025		0.36	0.05	0.025
propiconazol							0.025	0.165	0.025									
diuron							0.58	3.63	0.58	0.51	0.21	0.64	0.99	0.25		0.87	0.28	0.09
bromacil	6.9	6.7	6.7	6	6.9	5.3	5.27	2.4	2.19	2.11	2.29	3.35	1.36	1.38	0.82	1.75	1.6	1.4
oxifluorfen	0.03						0.05	0.15		0.06	0.025	0.03	0.025	0.025	0.025	0.025		
hexazinona				0.2			0.19	0.09	0.05	0.17	0.025	0.025	0.025	0.025	0.025	0.26	0.025	0.025
ametrina							0.025	0.07	0.025	0.07	0.025	0.025	0.08	0.025	0.39	0.12	0.07	0.04
carbofuran								0.04			0.025	0.025	0.2	0.025	0.025	0.06	0.025	
carbaril										0.11			0.13	0.1				
cadusafos								0.025	0.025									
etoprofos							0.36	0.195	0.025	0.03	0.025	0.06	0.28	0.06		0.025	0.025	0.025
diazinon	0.05			0.1	0.2	0.05	0.05	0.35	0.05	0.05	0.025	0.05	0.1	0.25	0.15	0.12	0.13	0.18

Figure 2. Pesticide concentrations (µg/L) detected in the Peje stream and Volcán River basin for both study periods (2011–2013 and 2018–2019). Traces (>LOD; >LOQ) were replaced by half the LOQ for each substance.

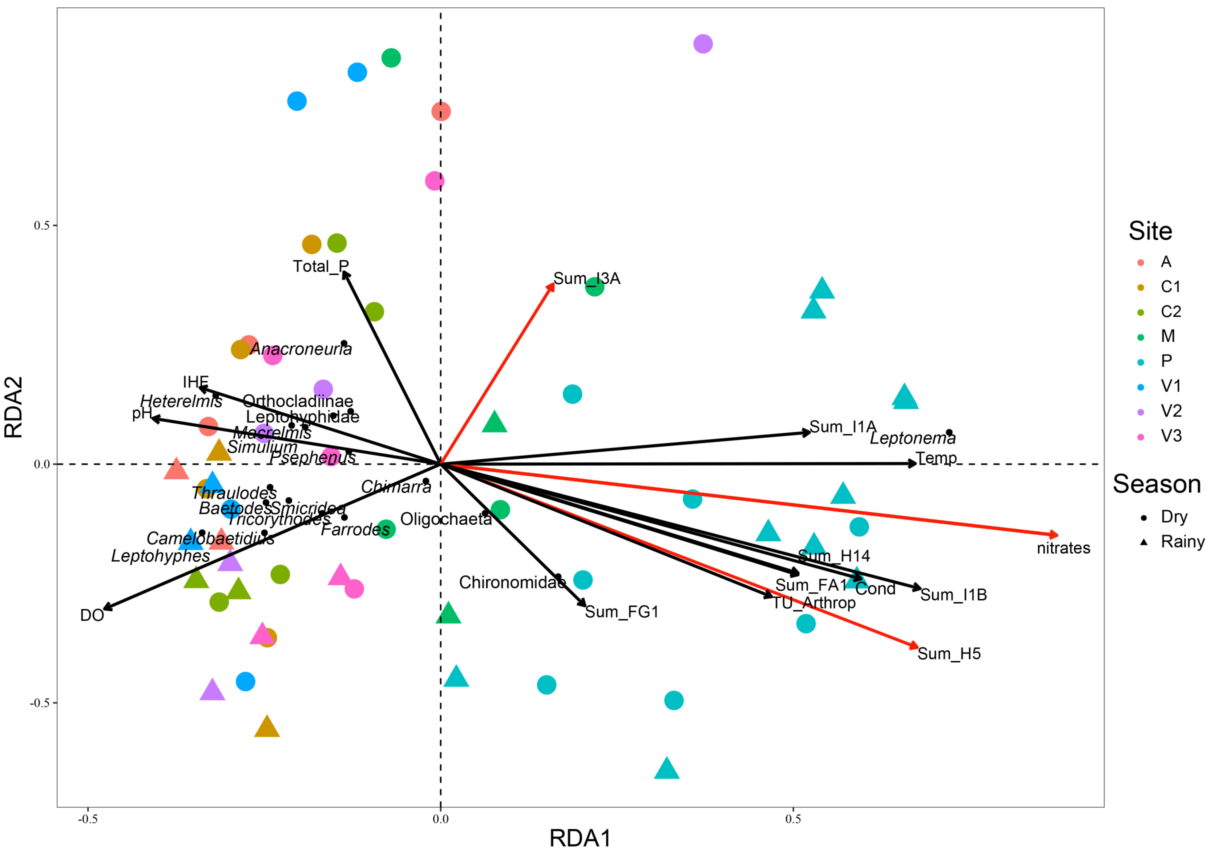


Figure 3. RDA biplot showing the relationships between environmental and macroinvertebrate community data in the Volcán River watershed. The ordination of sites with respect to the explanatory variables and selected taxa are shown. Labels: A = Angel, C = Cañas, M = Maura, P = Peje, and V = Volcán. Marked in red are the most relevant explanatory variables.

4. Discussion

As we saw from this study, agricultural pollutants (mainly nitrates, herbicides, and insecticides) have produced a fragmentation of the continuum (capacity of maintaining lateral and longitudinal connectivity for biota) of the Peje stream river network, and this has negatively affected its biodiversity.

In river networks, barriers exist of different types (natural and anthropogenic) and degrees of permeability (how much they block movement of organisms), and have divided the habitat into very small patches [53] which are less resilient, as they interact with other stressors. In our study, the Volcán River watershed had large areas of forest, excellent water quality, and riparian vegetation in most of its rivers, which could function as refuge areas [54] that allow for the re-colonization of organisms into the Peje stream. However, those organisms were incapable of continuous development and growth, providing evidence that the movement of organisms upstream from the main Volcán River into Peje stream affluent is impeded by a chemical habitat barrier that prevents life of the most sensitive organisms, even when the structural habitat conditions might be good and diverse. As stated by Araújo et al. [31], contaminants act as habitat disturbers or fragmentors, by promoting active and passive avoidance responses that end up generating uninhabited zones due to local population extinctions.

According to our results, the nitrate concentration in the Peje stream was the major disruptor for connectivity during the rainy seasons of both study periods. Nitrates followed the same pattern as the flow, which is an indication that the main source of this nitrogen is runoff from the crop fields, due to the extensive use of fertilizers and their high solubility in water, a problem well-documented worldwide since decades ago [55–58] and still relevant [59].

With increasing precipitation and runoff, an increment in the flow, the load of suspended solids, and the turbidity is also expected in the water courses [58]. Such a situation decreases the penetration of light through the water column, altering photosynthesis and lowering primary production [60], which is what we see happening in the Peje stream during the rainy season, when the lowest abundance of phytoperiphyton is registered in accordance with the higher peaks in flow.

On the contrary, the highest abundances of phytoperiphyton are registered when precipitation diminishes, with the consequent decrease in flow, and when sunlight can penetrate further into a nutrient-filled water column (highest concentrations of nitrates). Such nutrients cannot be used by the primary producers when the light penetration is low, but are rapidly consumed as soon as the flow and turbidity decrease in the stream and the higher photosynthesis rates accelerate the reproduction of periphyton [61]. These primary producers might be helping to increase MI taxa richness and abundance in two ways: 1. by uptake of the excess nitrogen from the water column, and 2. serving as food source for any re-colonizing organisms.

It is noteworthy to mention that the herbicide bromacil, which was the most detected pesticide in the first study period and was normally used in pineapple crops in the past, was forbidden in Costa Rica in 2017 [62], and this circumstance explains both the decreased detections in the 2018–2019 period and the increased appearance of other herbicides with the same mode of action, such as diuron, ametryn, and oxyfluorfen. The effect exerted by the constant presence of those herbicides in high concentrations on the diversity and abundance of the primary producers was not clear in this research. However, some studies [63] have indicated the possibility that toxic effects of herbicides on primary producers are obscured by the over-abundance of otherwise limiting nutrients (such as nitrates or phosphorus); or by the bioavailability, uptake, and toxicity of herbicides and their metabolites, which depend on factors such as temperature, pH, and DO concentrations; or due to pollution-induced community tolerance [64]. Therefore, it remains a challenge to understand the dynamics between energy sources and herbicide presence in the aquatic ecosystems overall, their direct effects on primary producers, and the indirect effects in upper trophic levels, particularly in the tropical areas.

Even though nitrates were evidenced in this study as the major pollutant affecting ecological integrity and biodiversity in the Peje stream during the rainy season, the RDA reflects that pesticide presence is certainly an aspect to continue evaluating. Although nitrate concentration ranges did not change between both sampling periods, the MI community of the Peje stream was even less diverse in 2018–2019 than in 2011–2013, as mirrored by lower BMWP-CR index scores. Moreover, MI families such as Perlidae (Plecoptera), Psychodidae (Diptera), Ptilodactylidae (Coleoptera), Gomphidae (Odonata), Leptoceridae, and Glossosomatidae (Trichoptera), which were collected in the first study period, were no longer present in the second. Some of these orders have been identified as sensitive to pesticides [65–67] or have been negatively correlated with pesticide exposure in the Caribbean region of Costa Rica [16], and their absence might be related to the presence of higher concentrations of toxic organophosphate and carbamate insecticides. On the contrary, the families inhabiting the Peje stream all year round (mainly Hydropsychidae and Chironomidae, but also Elmidae, Gerridae, Hydroptilidae, and Simuliidae) can be considered tolerant to the prevailing conditions (elevated nitrate and herbicide or insecticide concentrations). The Species at Risk (SPEARpesticide) index [65,66] identifies taxa that are at a higher risk of being affected by pesticide pollution. This approach classifies Hydropsychidae, Chironomidae, and Elmidae as species not at risk, in accordance with the present study, while Hydroptilidae is identified as a taxon at risk, contrary to our findings.

There is a gap in knowledge on the sensitivity of tropical MI toward pesticides, which needs to be filled in order to better understand the risks of these substances in conjunction with accompanying stressors. Another study by Alexander et al. [68] also found a MI community level response driven by the combined effect of nutrients and the insecticide imidacloprid in experimental outdoor artificial streams.

In the Volcán watershed, the maximum concentration of several of the detected pesticides (diazinon, ethoprophos, cadusafos, chlorpyrifos, permethrin, ametryn, bromacil, and diuron) surpassed international environmental quality standards (EQS; see Table S3) [69,70] and represents a risk for the aquatic ecosystem. Moreover, the concentrations necessary to produce an avoidance effect are far lower than the ones needed to produce toxicity [30–33]. It would be important to further understand the most relevant pathways of the used pesticides from the crop fields into the watercourses, and how this process can be reduced as a mitigation strategy [71]. For example, Bereswill et al. [7] evidenced that drainage systems rapidly transport nutrients and xenobiotics to surface waters, lowering the natural retention capacity of catchments and the efficiency of riparian forests as buffer strips.

In a recent review, Carstensen et al. [57] reported very positive evidence that diffusive nutrient losses from agricultural systems can be mitigated by >40% with different denitrification treatment measures (free water surface constructed wetlands, controlled drainage, and buffer zones). Such denitrification is highly controlled by temperature, with higher rates in high temperature conditions, which can be an advantage if a treatment is put into place in tropical ecosystems. They also stated that these measures can provide other ecosystem services such as storage of water or even biomass production.

Alternative to the construction of denitrification systems, the reduction in applied fertilizers in the crop fields, as well as restoration of previously existing lagoons and the riparian habitats alongside the watercourses, may serve the purpose of buffer zones, temperature control, sediment and nutrient retention, and food source and habitat diversification for the biota [4,56,72,73]. Therefore, the protection of the riparian vegetation may sensibly improve the habitat conditions for all aquatic organisms, and at the same time diminish the effects of agricultural activities, as has been confirmed by [74] for Brazilian and Paraguayan streams. This measure also favors connectivity by means of riverine biological corridors.

Another relevant aspect to this area is that Central America has been identified as one of the regions with the largest climate change impact, with either precipitation reductions or increments of up to 20%, depending upon the specific geographic area [75]. The south Pacific of Costa Rica (where the Volcán River watershed is located) is predicted to have high variability and increased precipitation [76]; therefore, mitigation strategies are particularly relevant given that stream impairment and habitat fragmentation due to high concentrations of nitrates (and pesticide residues) in surface waters are related to increased runoff and flow during the rainy season.

This investigation can be used as a baseline of information for follow-up monitoring and evaluation of restoration goals. We also encourage the implementation of passive alternatives and wonder: is it possible to see the recovery of the Peje stream ecosystem after only agricultural abandonment of key zones within this sub-watershed? This type of follow-up study is considered a major gap in our current understanding of stream management [21].

5. Conclusions

In this study, we provided evidence that agriculturally related contaminants might drive fragmentation of the habitat and can produce MI biodiversity loss in the field. However, fragmentation has been known and studied almost exclusively for terrestrial ecosystems, contributing to an underestimation of the threats posed to aquatic biota [25]. Up until this date, the vast majority of the research on river network fragmentation worldwide has been focused on the effects of barriers on fish populations; however, we believe that research should advance toward the understanding of the effects on other types of organisms, as well as making the evaluations at the watershed level, rather than studying only individual barriers.

Agricultural contaminants (in this case, the concentration of nitrates and pesticides) are causing an abrupt rupture of the ecological integrity of a stream and a seasonal loss of MI biodiversity. The large effect observed for nitrates might even obscure the effects produced by other highly relevant stressors in the aquatic ecosystems, such as pesticides or even modifications in the river channel morphology. Therefore, we encourage more researchers to incorporate the evaluation of the effects of fertilizer runoff on higher trophic levels and not only in primary producers, and also to provide that information to improve regulatory guidelines. The EU Nitrates Directive [59] states that this is a major water pollution problem in Europe and represents an obstacle to reach "good status" for all surface waters. In our study, the maximum nitrate concentration was 20.3 mg/L, less than half of the 50 mg/L needed to be considered a Nitrate Vulnerable Zone within this directive [59].

Chemical habitat fragmentation might be relevant as a biodiversity loss factor in many watersheds in the world, which may only have been analyzed from a water quality or ecotoxicological point of view, with disregard of the effects that the fragmentation *per se* can pose on the aquatic populations in the long term. It is necessary to conduct research leading to design and validation of stream restoration strategies based on field data (social and ecological inputs) with a river ecology focus, directed toward barrier removal to re-establishing aquatic species diversity and ecosystem functioning.

Finally, as attested by Fuller et al. [25], watershed management with biodiversity conservation goals requires the acknowledgement of the aquatic habitat fragmentation concept in order to avoid underestimation of its effects and to take specific fragmentation management actions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxics10070346/s1, Figure S1: Map of the Volcán River watershed and sampling sites, Table S1: Pesticide residue limits of detection (LOD) and of quantification (LOQ) in both study periods (2011–2013 and 2018–2019) in µg/L. Active ingredients are ordered alphabetically, Table S2: Pesticide active ingredient mode of action according to Fungicides, Herbicides and Insecticides Resistance Action Committees FRAC/HRAC/IRAC, Table S3: Maximum detected concentration (µg/L) of pesticide active ingredients in the Volcán River watershed, 2011–2013 (all sites) and 2018–2019 (only Peje stream). nd = below detection limit. na = not analyzed, Table S4: Macroinvertebrate families identified in each sampling campaign in the Peje stream (2011–2013 and 2018–2019). Yellow = dry season; green = transition; blue = rainy season.

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Supplementary Information.

"Ecological integrity impairment and habitat fragmentation for Neotropical macroinvertebrate communities in an agricultural stream"

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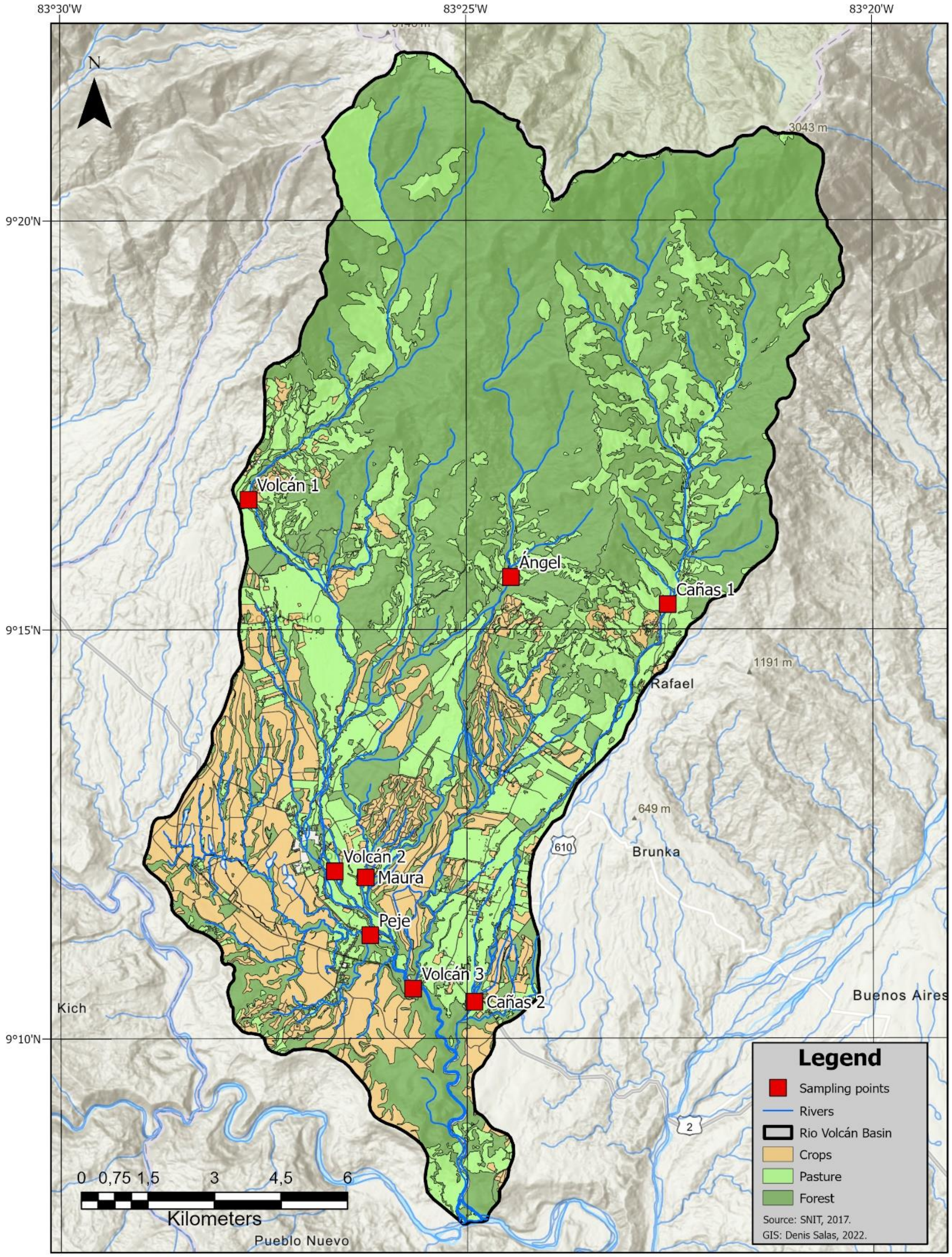

Figure S1. Map of the Volcán River watershed (South Pacific, Costa Rica) and sampling sites.

Table S1. Pesticide residue limits of detection (LOD) and of quantification (LOQ) in both study periods (2011-2013 and 2018-2019) in µg/L. Active ingredients are ordered alphabetically.

Active ingredient	Volcán (2011-2013)		Q_Peje (2018-2019)	
	LOD	LOQ	LOD	LOQ
a-cypermethrin	0.03	0.1	0.02	0.05
ametryn	0.02	0.05	0.01	0.03
atrazine	0.02	0.05	0.02	0.05
azoxystrobin			0.02	0.05
bentazone			0.02	0.05
bifenthrin	0.1	0.5	0.02	0.05
bitertanol	0.06	0.2	0.02	0.05
boscalid			0.02	0.05
bromacil	0.03	0.1	0.02	0.05
buprofezin			0.02	0.05
butachlor	0.2	0.6	0.02	0.05
cadusafos			0.02	0.05
carbaryl	0.06	0.2	0.02	0.05
carbendazim			0.02	0.05
carbofuran			0.02	0.05
cyhalothrin	0.04	0.1	0.02	0.05
cyproconazole			0.02	0.05
clomazone			0.02	0.05
chlorothalonil	0.03	0.1	0.01	0.02
chlorpyrifos	0.02	0.05	0.01	0.02
DDD-pp(M)			0.02	0.05
DDE-pp(M)			0.02	0.05
deltamethrin	0.06	0.2	0.02	0.05
diazinon	0.01	0.02	0.01	0.03
difenoconazole			0.02	0.05
dimethoate	0.04	0.1	0.02	0.05
diuron	0.03	0.1	0.02	0.05
endosulfan-a	0.04	0.1	0.02	0.05
endosulfan-b	0.04	0.1	0.02	0.05
endosulfan sulfate (M)			0.02	0.05
epoxiconazole	0.06	0.2	0.02	0.05
ethoprophos	0.04	0.1	0.01	0.04
fenamiphos	0.3	1	0.02	0.05
fenbuconazole			0.02	0.05
fenpropimorph			0.02	0.05
fenthion	0.04	0.1	0.02	0.05
fluopyram			0.02	0.05
flutolanil			0.02	0.05

Active ingredient	Volcán (2011-2013)		Q_Peje (2018-2019)	
	LOD	LOQ	LOD	LOQ
phorate	0.04	0.1	0.02	0.05
hexachlorobenzene			0.02	0.05
hexazinone	0.05	0.1	0.02	0.05
imidacloprid			0.02	0.05
lindane			0.02	0.05
linuron			0.02	0.05
malathion	0.03	0.1	0.02	0.05
metalaxyl	0.1	0.2	0.02	0.05
myclobutanil			0.02	0.05
oxifluorfen	0.06	0.2	0.02	0.05
parathion-methyl	0.06	0.2	0.02	0.05
pendimethalin	0.03	0.1	0.02	0.05
pentachloroaniline (M)			0.02	0.05
pentachloroanisol (M)			0.02	0.05
entachlorobenzene (M)			0.02	0.05
permethrin	0.03	0.1	0.02	0.05
pyrimethanil			0.02	0.05
prochloraz	0.3	1		
propanil	0.2	0.6	0.03	0.1
propiconazole	0.05	0.2	0.02	0.05
quintozene (PCNB)			0.02	0.05
spiroxamine			0.02	0.05
tebuconazole	0.05	0.2	0.02	0.05
terbufos	0.03	0.1	0.02	0.05
terbuthylazine	0.03	0.05	0.02	0.05
terbutryn	0.03	0.1	0.02	0.05
thiabendazole			0.02	0.05
triadimefon	0.3	1	0.02	0.05
triadimenol	0.3	1	0.02	0.05
triazophos			0.02	0.05
trifloxystrobin			0.02	0.05

Table S2. Pesticide active ingredient Mode of Action according to Fungicides, Herbicides and Insecticides Resistance Action Committees FRAC/ IRAC/ HRAC [45,46,47].

Active ingredient	CAS number	Biocide action	Chemical group	FRAC/HRAC/ IRAC MoA code	MoA Description
metalaxyl	57837-19-1	fungicide	acylalanine	FA1	protein synthesis inhibitor
propiconazole	60207-90-1	fungicide	triazole	FG1	demethylation in sterol biosynthesis inhibitor
oxyfluorfen	42874-03-3	herbicide	diphenilether, chlorinated, fluorated	H14	Protoporphyrinogen Oxidase inhibitor
ametryn	834-12-8	herbicide	triazine	H5	photosystem II inhibitor (D1 Serine 264 Binders)
bromacil	314-40-9	herbicide	uracil, bromated	H5	photosystem II inhibitor (D1 Serine 264 Binders)
diuron	330-54-1	herbicide	urea, chlorinated	H5	photosystem II inhibitor (D1 Serine 264 Binders)
hexazinone	51235-04-2	herbicide	triazinone	H5	photosystem II inhibitor (D1 Serine 264 Binders)
terbutryn	886-50-0	herbicide	triazine	H5	photosystem II inhibitor (D1 Serine 264 Binders)
carbaryl	63-25-2	insecticide	carbamate	I1A	Acetylcholinesterase inhibitor
permethrin	52645-53-1	insecticide	pyrethroid, chlorinated	I3A	Sodium channel modulator (blocks nervous stimuli)
carbofuran	1563-66-2	insecticide, nematicide	carbamate	I1A	Acetylcholinesterase inhibitor
cadusafos	95465-99-9	insecticide, nematicide	organophosphate	I1B	Acetylcholinesterase inhibitor
diazinon	333-41-5	insecticide, nematicide	organophosphate	I1B	Acetylcholinesterase inhibitor
ethoprophos	13194-48-4	insecticide, nematicide	organophosphate	I1B	Acetylcholinesterase inhibitor

Table S3. Maximum detected concentration and EQS* (µg/L) of pesticide active ingredients in the Volcán River watershed, 2011-2013 (all sites) and 2018-2019 (only Peje stream). nd = below detection limit. na = not analyzed.

Active ingredient	Volcán 1	Volcán 2	Volcan 3	Angel	Cañas 1	Cañas 2	Maura	Peje	EQS*
diazinon	nd	0.01	0.15	nd	nd	0.01	0.01	0.35	0.037
ethoprophos	nd	nd	nd	nd	nd	nd	nd	0.36	0.063
cadusafos	na	nd	nd	na	na	na	na	0.025	0.023
carbaryl	na	nd	0.025	na	na	na	na	0.13	0.23
carbofuran	nd	nd	0.025	nd	nd	nd	nd	0.2	0.91
permethrin	nd	0.02	nd	0.4	nd	nd	nd	nd	0.0003
chlorpyrifos	nd	nd	0.08	nd	nd	nd	nd	nd	0.03
ametryn	nd	nd	nd	nd	nd	nd	nd	0.39	0.01
hexazinone	nd	nd	0.025	nd	0.3	nd	nd	0.26	0.56
oxifluorfen	nd	nd	nd	nd	nd	nd	nd	0.15	-
bromacil	nd	0.14	1.3	nd	nd	nd	1.2	6.9	0.0068
diuron	nd	nd	0.35	nd	nd	nd	nd	3.63	0.2
terbutryn	nd	nd	0.05	0.05	0.05	nd	0.05	nd	0.065
propiconazole	nd	nd	nd	nd	nd	nd	nd	0.165	10
metalaxil	nd	nd	0.025	nd	nd	nd	nd	0.36	46

* EQS refers to AA or MAC Environmental Quality Standards of the European Union (or the MTR eco, when the EQS is not available) [69].

Table S4. Macroinvertebrate families identified in each sampling campaign in the Peje stream (2011-2013 & 2018-2019). Yellow =dry season; Green= Transition; Blue = rainy season.

2011-2013	dec-2011	mar-12	jul-12	sep-12	mar-13
Ampullaridae					X
Baetidae		X	X		X
Bulinidae		X			
Caenidae		X			
Calopterygidae					X
Ceratopogonidae		X			X
Chironomidae	X	X	X	X	X
Coenagrionidae		X			X
Corydalidae	X				X
Crambidae		X			
Elmidae	X	X	X	X	X
Empididae	X	X	X		X
Glossossomatidae		X			
Gomphidae			X		
Hydropsychidae	X	X	X	X	X
Hydroptilidae		X		X	X
Leptoceridae			X		
Leptohyphidae		X			X
Leptophlebiidae		X			X
Libellulidae		X			
Oligochaeta	X	X		X	
Perlidae					X
Philopotamidae		X			X
Psychodidae		X	X		X
Ptilodactylidae	X				
Simuliidae		X	X		X

2011-2013	dec-2011	mar-12	jul-12	sep-12	mar-13
Staphylinidae		X			
Tipulidae		X			
Trombidiformes			X		X
Turbellaria		X			
Veliidae		X			X

Note: No sample is available for December 2012.

2018-2019	19_mar_ 2018	18_apr_ 2018	22_may_ 2018	26_jun_ 2018	17_jul_ 2018	13_aug_2 018	12_sep_2 018	10_oct_ 2018	14_nov_2 018	03_dec_2 018	14_jan_ 2019	21_feb_ 2019
adult_Diptera	X											
Baetidae	X			X	X						X	X
Caenidae	X	X									X	
Calopterygidae		X									X	X
Ceratopogonidae											X	
Chironomidae	X	X	X		X	X	X	X		X	X	X
Coenagrionidae	X								X			X
Corydalidae	X			X								
Crambidae					X		X			X		
Culicidae											X	
Curculionidae										X		
Dryopidae											X	
Elmidae	X	X	X		X	X					X	
Empididae										X	X	
Gerridae	X	X	X		X	X					X	X
Hydropsychidae	X	X	X	X	X	X	X	X	X	X	X	X
Hydroptilidae	X	X				X		X	X	X		X
Hydroscaphidae												X
Leptohyphidae	X	X									X	X
Leptophlebiidae	X	X									X	X
Libellulidae	X	X		X					X		X	X

2018-2019	**19_mar_2018**	**18_apr_2018**	**22_may_2018**	**26_jun_2018**	**17_jul_2018**	**13_aug_2018**	**12_sep_2018**	**10_oct_2018**	**14_nov_2018**	**03_dec_2018**	**14_jan_2019**	**21_feb_2019**
Ochteridae	X											
Oligochaeta	X	X										X
Ostracoda	X	X										
Philopotamidae		X									X	X
Platystictidae												X
pupae_Diptera	X	X	X			X					X	X
Simuliidae		X	X	X	X						X	X
Staphylinidae												X
Tabanidae											X	X
Tipulidae												X
adult_Trichoptera						X						
Veliidae	X	X									X	X

Note: Lower precipitations occur in July, because of a weather condition called the "veranillo"

7. Discusión Global

Con la digitalización y reorganización de la información proveniente de diversos proyectos de investigación y monitoreo durante un período de 10 años (2009-2019), se demostró que existe una amplia lista de plaguicidas presentes en las aguas superficiales de Costa Rica. Dicha lista tiene representantes de varias acciones biocidas y grupos químicos, entre los que se incluyen triazoles, benzimidazoles, hidrocarburos aromáticos, piridinas, imidazoles y fungicidas clorados; herbicidas como triazinas, uracilos, ureas, oxazolidinonas y triazinonas; insecticidas organofosforados, organoclorados, piretroides, carbamatos, tiadiazinas y neonicotinoides; así como otros acaricidas y nematicidas (Echeverría-Sáenz et al., 2021).

La mayoría de los plaguicidas se detectaron en concentraciones <1 µg/L; sin embargo, al menos 18 plaguicidas fueron >1 µg/L y, en algunos casos, alcanzaron valores superiores a 10 µg/L (por ejemplo, diazinon, diuron, ametrina y flutolanil; estructuras químicas en el Anexo 2). El 50% de los plaguicidas detectados tienen concentraciones medias y/o máximas que sobrepasan una o más normas internacionales. Además, varios de los plaguicidas que no cumplen la normativa son también los que tienen una mayor frecuencia de detección y una mayor toxicidad para los organismos acuáticos (por ejemplo, los insecticidas organofosforados y carbamatos). Se puede observar que muchos de esos plaguicidas que presentan alta frecuencia son los mismos que, además, son los mayores contribuyentes al riesgo para los ecosistemas acuáticos (Echeverría-Sáenz et al., 2021), lo que debería generar alertas sobre la conservación de los ecosistemas acuáticos en todo el país.

Vryzas et al. (2020) afirman que las limitaciones en la evaluación de riesgos, junto con el bajo nivel de aplicación de la normativa sobre plaguicidas están causando en parte la presencia de plaguicidas por encima de la normativa. Además, en este estudio, se resalta la detección en agua de cuatro sustancias prohibidas: lindano, hexaclorobenceno, carbofuran y bromacil. El lindano y el hexaclorobenceno están prohibidos desde 1999 y 2005, respectivamente; por tanto, las detecciones implican el uso ilegal de estos plaguicidas en las regiones hortícolas de montaña de la Cordillera Volcánica Central. Por otro lado, el carbofuran, que se prohibió en 2014, se detectó principalmente antes de ese año; sin embargo, se registró también en 2016. Esto podría ser el resultado del uso y aplicación de remanentes del producto (importados antes de la prohibición), pero esto sería improbable para el presente y futuros años y debería ser analizado con mayor detalle por las autoridades, ya que se ha demostrado un alto riesgo de este i.a. para la biota acuática (Arias-Andrés et al., 2018; Ramírez-Morales et al., 2021; Ramírez Muñoz et al., 2017). El bromacil es uno de los i.a. prohibidos más recientemente (2017) y también fue detectado en años posteriores (hasta 2020); en consecuencia, el riesgo asociado a la potencial lixiviación de este plaguicida en las aguas subterráneas sigue siendo motivo de preocupación, como lo ha sido en otros países (De Paz y Rubio, 2006; ENSR 2005).

En este estudio, se observaron diferencias en las frecuencias de detección entre las regiones de Costa Rica. La región “menos contaminada" fue el Pacífico Central, sin embargo, esta región ha tenido históricamente un esfuerzo de muestreo considerablemente menor que otras regiones y, por lo tanto, hubo muy pocas detecciones de plaguicidas en las muestras analizadas. Por otro lado, Rodríguez-Rodríguez et al. (2021) realizaron un muestreo intensivo (84 muestras de agua) del 2008 al 2011 en cuencas con influencia de melón y sandía y encontraron un fungicida y siete insecticidas en concentraciones que suponen un riesgo agudo y crónico para los organismos acuáticos. Esta situación pone de manifiesto la importancia de aumentar el esfuerzo de muestreo en esa región.

A través del modelo de msPAF (multi-substance Potentially Affected Fraction), encontramos (Echeverría-Sáenz et al., 2021) que la mezcla de plaguicidas detectados en el 5% y 13% del total de muestras de agua de Costa Rica generan un riesgo agudo alto o moderado, respectivamente para organismos acuáticos. Dicho riesgo fue particularmente alto para productores primarios (plantas, algas) y artrópodos (insectos, crustáceos). Los plaguicidas que más contribuyen al riesgo para artrópodos fueron la cihalotrina y la permetrina, así como otros insecticidas organofosforados o carbamatos (carbofuran, diazinon, fenamifos, terbufos, clorpirifos) y el fungicida clorotalonil (crustáceos, insectos). Es importante destacar que todas estas estimaciones se basan en la toxicidad aguda (EC50, LC50), y no podemos menospreciar el hecho de que muchos otros i.a. (como los organofosforados y los carbamatos) podrían estar implicados en la toxicidad crónica en todos los grupos de especies.

Por esta razón, en este estudio se estimó la sensibilidad o tolerancia de las diferentes familias de macroinvertebrados (MI) acuáticos a través de análisis de umbrales ecológicos usando gradientes de concentración de 13 plaguicidas en muestras ambientales. Esta sensibilidad empírica, basada en las muestras de campo, se comparó con datos de valores de tolerancia (TV) en la literatura y también con una estimación de la sensibilidad intrínseca de las familias de MI, debida a la presencia de ciertos caracteres anatómicos o fisiológicos.

Nuestros resultados de sensibilidad y tolerancia de las familias de MI con respecto a los plaguicidas fueron coherentes con los de Chang et al. (2013) y Barbour et al. (1999) quienes mostraron que los organismos no insectos (crustáceos, moluscos, anélidos) eran más tolerantes que los insectos, también las familias de Dípteros tenían mayor TV que otras familias de insectos, EPT (Ephemeroptera, Plecoptera, Trichoptera) tenían menor TV que los de OCH (Odonata, Coleoptera, Heteroptera), y Baetidae también tenía mayor TV que todos los demás Ephemeroptera. Además, la mayoría de las familias de MI identificadas como tolerantes o moderadamente tolerantes en este estudio, están descritas en la legislación costarricense como menos sensibles a otros tipos de contaminación, por ejemplo, contaminación orgánica o alteraciones generales del hábitat (DE-33903-MINAE-S).

Los insecticidas son los plaguicidas más tóxicos para la comunidad de MI, sin embargo, en este estudio muchas familias de MI fueron sensibles a herbicidas y fungicidas. Otras investigaciones ya han mostrado efectos de los herbicidas sobre las comunidades de MI, que podrían estar relacionados con efectos indirectos en cascada sobre las cadenas tróficas, derivados de cambios en las abundancias o diversidad de los productores primarios (Fleeger et al., 2003; Echeverría-Sáenz et al., 2022). En cuanto a los fungicidas, muchos de ellos tienen acción multisitio (clorotalonil) o son inhibidores de la biosíntesis de esteroles (epoxiconazol), inhibidores de la síntesis de proteínas (metalaxil) o impiden la respiración mitocondrial (azoxistrobina), todos estos procesos vitales para las células eucariotas y, por tanto, lo suficientemente generales como para causar efectos sobre organismos no fúngicos (Burden et al., 1989, FRAC, 2023).

Hasta este punto, observamos que la gran mayoría de las familias de MI en los ríos costarricenses están respondiendo negativamente a la presencia de plaguicidas y que los MI que muestran tolerancia hacia otros factores de estrés, también parecen presentar tolerancia hacia concentraciones muy bajas de plaguicidas. Sin embargo, es importante notar que casi todos los TV se han desarrollado con base en datos de campo y guardan relación con aspectos de degradación del hábitat y contaminación orgánica, por lo que la coherencia no necesariamente es total. Por ejemplo, algunas familias mostraron tolerancia en el presente estudio, pero no suelen identificarse con altos TV, este es el caso de familias de Trichoptera como Leptoceridae, Glossosomatidae y Calamoceratidae, o Corydalidae (Megaloptera).

Un enfoque interesante es entender si los caracteres anatómicos y fisiológicos podrían ser importantes para evaluar la tolerancia o vulnerabilidad de los MI tropicales. En los países templados, un índice ampliamente utilizado para relacionar los MI con la presencia de plaguicidas y sus efectos en muestras de campo es el SPEcies At Risk ($SPEAR_{pesticides}$; Liess y Von der Ohe, 2005). Este índice distingue los MI en dos categorías de sensibilidad, los que están en riesgo y los que no lo están (basándose en rasgos como su voltinismo, capacidad de migración y presencia de estadios acuáticos sensibles). Varios organismos clasificados como moderadamente tolerantes o tolerantes en este estudio también se consideran especies sin riesgo en dicho índice (not at risk). En correspondencia con nuestros resultados, algunas de las familias que sólo estaban presentes en sitios sin detección de plaguicidas (Dixidae, Ecnomidae, Lepidostomatidae, Odontoceridae y Atyidae), se reportan como especies en riesgo. Una vez más, se da una incoherencia con familias como Leptoceridae, Glossosomatidae, Caenidae y Coenagrionidae, que parecen tener cierto grado de tolerancia en este estudio, pero que se clasifican como especies en riesgo en el índice $SPEAR_{pesticides}$ (Liess y Von der Ohe, 2005). Esto podría significar que existen diferencias en los factores que condicionan la sensibilidad a los plaguicidas en los MI tropicales. Por ejemplo, dado que la mayoría de las especies tropicales tienen tiempos de generación cortos y son multivoltinas (Jackson y Sweeney, 1995) y que los plaguicidas se aplican durante todo el año, los caracteres "voltinismo" y "presencia de estadios sensibles" podrían no ser útiles para la selección de especies de riesgo en los trópicos. Por lo tanto, en este estudio, se seleccionó como caracteres de sensibilidad intrínseca, aquellos que podrían aumentar el ingreso de tóxicos en el organismo (respiración por agallas o por integumento, cuerpo poco esclerotizado) o que pudiesen limitar la capacidad de migrar o evadir los contaminantes (habilidades natatorias y fuerza de vuelo en adultos). Las familias de MI de este estudio se clasificaron en “sensibles por caracteres” o “no sensibles por caracteres”.

Con respecto a la sensibilidad intrínseca de los MI, varias familias de este estudio coincidieron en ser "sensibles por caracteres" y también mostrar respuestas sensibles en el campo, así como ser "no sensibles por caracteres" y mostrar respuestas tolerantes en el campo. Sin embargo, los moluscos bivalvos y gasterópodos, varias familias de Diptera, así como Baetidae, Leptoceridae, Elmidae y Libellulidae, que tienen TV altos y fueron tolerantes o moderadamente tolerantes en nuestros gradientes de plaguicidas en campo, se clasificaron como "sensibles por caracteres". Una posible explicación, como afirman Ippolito et al. (2012), es que cuando se considera un grupo diverso de organismos, en lugar de sólo artrópodos, el tipo de respiración pierde fuerza como predictor de la sensibilidad y, además, en el caso de los moluscos, su falta de movilidad y capacidad de nado también hizo que se clasificaran como "sensibles por caracteres" en este estudio, incluso cuando son reportados consistentemente como organismos más tolerantes. Por lo tanto, es necesario afinar más la selección de rasgos para validar o reflejar con mayor precisión la tolerancia/sensibilidad de los MI observada en el campo.

El hecho de que algunas familias de MI presentaron tolerancia moderada en este estudio, pero tienen caracteres que les confieren sensibilidad intrínseca y además se les considera sensibles en la literatura, es un indicativo claro de que hay otros factores que condicionan la sensibilidad a los plaguicidas en estas comunidades. Algunas familias podrían seguir presentándose en un sitio contaminado, si tienen un alto potencial de recuperación o si pueden recolonizar desde zonas más conservadas que funcionan como un refugio (Rubach et al., 2010; Knillman et al., 2018). En este sentido, el comportamiento de evasión de los MI frente al estresor ambiental puede variar en función de su capacidad de detección del tóxico y del mecanismo de defensa específico de cada taxón (respuestas de retirada o madriguera, deriva o escape activo) (Wiberg-Larsen et al., 2016). Esta variación en las respuestas etológicas puede ser de gran relevancia en la tolerancia reflejada en muestras ambientales.

En cuanto a la funcionalidad de la comunidad de macroinvertebrados, las proporciones de GFA en este estudio no reflejaron un cambio notable en los muestreos con concentraciones relativamente bajas de plaguicidas. En consonancia con estos resultados, Buchwalter et al. (2008), Ippolito et al. (2012) y Reiber et al. (2020) afirmaron que no se encontraron patrones claros entre la sensibilidad y los hábitos o rasgos funcionales de alimentación. Sin embargo, cuando la suma de plaguicidas fue >3 µg/L en nuestro estudio, los raspadores (representados casi exclusivamente por moluscos) aumentaron mucho su proporción, evidenciándose su tolerancia en el campo. Por otro lado, los detritívoros desaparecieron completamente en ese intervalo de concentración de plaguicida, lo que concuerda con Cornejo et al. (2021b), quienes ya demostraron con ensayos de toxicidad aguda que los detritívoros tropicales estaban entre las especies más sensibles a clorpirifos y clorotalonil. Reiber et al. (2020), identificaron "especies decrecientes" (principalmente insectos) con el aumento de la presión de plaguicidas, así como "especies crecientes" (principalmente Gastropoda, Oligochaeta y Diptera), que mostraron un aumento de la frecuencia entre los sitios de referencia y los de mayor presión de plaguicidas.

En 10 de los 13 gradientes de plaguicidas analizados en este estudio, el umbral ecológico de la comunidad (CCP), fue inferior a las normas internacionales de calidad ambiental (RIVM, 2021), pero la diferencia entre los CCP y la concentración considerada “segura” en la normativa es especialmente grande en el caso de los fungicidas. Cabe destacar que estas normativas se calculan a partir de datos de toxicidad crónica de ensayos de laboratorio para esos mismos plaguicidas, por lo que la mayoría de las veces, cuando se estiman las ERA retrospectivas, los fungicidas aparecen como sustancias de bajo riesgo que casi nunca superan sus niveles umbrales reglamentarios (Brühl et al., 2023). Esto no es sorprendente, ya que la toxicidad aguda de los fungicidas para las especies estándar de ensayo tiende a ser baja y, en consecuencia, las concentraciones de efecto estimadas tienden a ser altas (Zubrod et al., 2019).

Dichos umbrales representan el punto de un gradiente ambiental, en donde se observa un cambio rápido y abrupto de la comunidad. Por lo tanto, el CCP no necesariamente es un efecto de toxicidad en sí mismo, sino que podría representar respuestas etológicas de evasión y emigración de los sitios contaminados (Fleeger et al., 2003; Araújo et al., 2016; Wiberg-Larsen et al., 2016). Dichas respuestas pueden ocurrir a concentraciones mucho más bajas que las que ocasionan efectos de toxicidad (Beketov y Liess, 2008; Berghahn et al. 2012; Araújo et al. 2016, 2018) y, en parte, esta puede ser la explicación de por qué los CCP en 10 de los 13 plaguicidas utilizados para el análisis en campo, fueron mucho menores que los criterios de calidad ambiental internacional, basados en ensayos de toxicidad crónicos a nivel de laboratorio.

Queda claro a partir de este estudio, que la comunidad MI está respondiendo a concentraciones de plaguicidas mucho más bajas que las disponibles en la literatura con respecto a los efectos de toxicidad para los organismos acuáticos. Además, para todos los tipos de plaguicidas, las ERAs no han incluido una protección adicional para las mezclas de sustancias (que es el caso en la mayoría de las muestras de agua), ni para los efectos indirectos sobre las redes tróficas o la evaluación de los efectos sobre la biodiversidad en el campo. Esta complejidad en la evaluación y predicción de los efectos producidos por los plaguicidas, aumenta con la presencia de múltiples factores de estrés en condiciones ambientales.

En Echeverría-Sáenz et al. (2022) se observó una serie de efectos indirectos en cadena debidos a la acción conjunta de diversos factores, tanto naturales como antrópicos, que afectaron la biodiversidad de MI de un ecosistema acuático. En este estudio, se observó que variables hidrológicas como la precipitación y el escurrimiento, se relacionaron directamente con el caudal ($r= 0.81$, $p= 0.009$) y que, a la vez, dicho caudal se relacionó directamente con la concentración de nitratos en el agua ($r= 0.76$, $p= 0.02$) (Figura 3). Por lo tanto, se evidencia que el aumento en la concentración de nitratos en la Quebrada Peje durante la época lluviosa proviene de la escorrentía de compuestos nitrogenados desde las laderas de la cuenca hidrográfica, posiblemente por causa de la aplicación de fertilizantes y su alta solubilidad en agua (Echeverría-Sáenz et al. 2022).

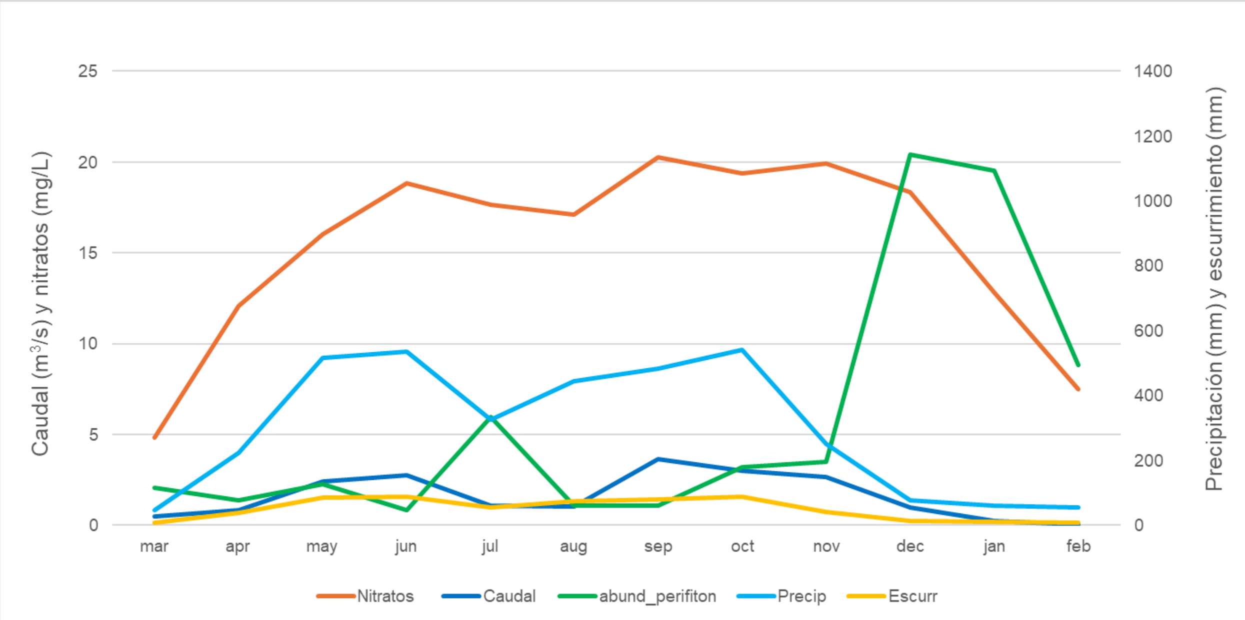

Figura 3. Comportamiento de algunas variables hidrológicas y la concentración de nitratos en la Quebrada Peje, Pacífico Sur de Costa Rica.

Las mayores abundancias de fitoperifiton se registraron en la transición de la época lluviosa a la seca, ya que la luz solar puede penetrar más y la columna de agua presentaba nitratos en las concentraciones más altas. Estos nutrientes no pueden ser utilizados por los productores primarios cuando la penetración de la luz es baja, pero se consumen rápidamente en cuanto el caudal y la turbidez disminuyen y las mayores tasas de fotosíntesis aceleran la reproducción del perifiton (Bilota y Brazier, 2008; Eassa et al. 2015). Estos productores primarios podrían estar ayudando a aumentar la riqueza y abundancia de taxones de MI de dos maneras: 1. absorbiendo el exceso de nitrógeno de la columna de agua, y 2. sirviendo como fuente de alimento para cualquier organismo recolonizador.

El efecto ejercido por la presencia constante de herbicidas en altas concentraciones sobre la diversidad y abundancia de los productores primarios, no está claro en esta investigación. Sin embargo, la Agencia de Protección Ambiental de los EEUU (EPA, 2017) ha indicado la posibilidad de que los efectos tóxicos de los herbicidas sobre los productores primarios queden ocultos por la sobreabundancia de otros nutrientes (como los nitratos o el fósforo); o por la biodisponibilidad, absorción y toxicidad de los herbicidas y sus metabolitos, que dependen de factores como la temperatura, el pH y las concentraciones de oxígeno disuelto; o debido a la tolerancia de la comunidad inducida por la contaminación (Knauer et al. 2010).

En este estudio se observó que los contaminantes agrícolas (principalmente nitratos, herbicidas e insecticidas) han producido una fragmentación del continuo (capacidad de mantener la conectividad lateral y longitudinal para la biota) de la red fluvial. Este fue un concepto clave de la presente investigación, pues aunque se pretendía comprender los efectos tóxicos de los plaguicidas en muestras ambientales, se observó que las barreras a la continuidad del ecosistema acuático, pueden ser tan importantes como la toxicidad de una sustancia. Como afirman Araújo et al. (2016), los contaminantes actúan como perturbadores o fragmentadores del hábitat, promoviendo respuestas de evitación activas y pasivas que acaban generando zonas deshabitadas debido a la extinción de poblaciones locales.

Creemos que deben hacerse mayores esfuerzos por parte de las agencias gubernamentales y de los agricultores de la región Neotropical, para garantizar que las sustancias tóxicas aplicadas a los cultivos para el control de plagas no lleguen a las aguas superficiales naturales en concentraciones que representen riesgos inaceptables. La protección de la vegetación ribereña es clave, ya que podría mejorar las condiciones del hábitat para los organismos acuáticos, ayuda a mitigar los efectos de los plaguicidas y el exceso de nutrientes en la biota acuática, además de proporcionar hábitat para el refugio y posterior recolonización de organismos en los cauces (Knillmann et al., 2018). Al mismo tiempo, disminuye los efectos de las actividades agrícolas, como ha sido confirmado por Hunt et al. (2017) para los arroyos brasileños y paraguayos. Esta medida también favorece la conectividad, mediante corredores biológicos fluviales.

8. Conclusiones y recomendaciones

Los plaguicidas son contaminantes ubicuos en las aguas continentales lóticas de Costa Rica. Estas sustancias se detectan con alta frecuencia, algunas de ellas incluso alcanzan porcentajes de detección de hasta 37% (por ej. el herbicida diuron), mientras que el herbicida ametrina, así como los fungicidas azoxistrobina, epoxiconazol flutolanil, miclobutanil y pirimetanil, y los insecticidas buprofezin, clorpirifos, diazinon, y etoprofos tienen frecuencias de detección ≥20%, aun cuando las muestras de agua analizadas en esta investigación representan muestreos puntuales y no obedecen a monitoreos permanentes.

Con esta investigación, se determinó que las concentraciones reportadas de plaguicidas individuales exceden los criterios de calidad ambiental para protección de la biodiversidad de Europa, Estados Unidos y Australia y, por lo tanto, representan un riesgo para la integridad y el funcionamiento ecológico de los ecosistemas acuáticos. Asimismo, las mezclas de plaguicidas que se observan comúnmente en las aguas de nuestro país representan un riesgo principalmente para organismos que son la base de las cadenas tróficas acuáticas.

A través del modelo msPAF se identificó que los plaguicidas que más contribuyen a generar riesgo para los ecosistemas acuáticos en Costa Rica son los herbicidas ametrina, bromacil. diuron, hexazinona y terbutrina; los fungicidas azoxistrobina, clorotalonil, epoxiconazol y los insecticidas α-cypermetrina, carbofuran, clorpirifos, diazinon, etoprofos, fenamifos y terbufos. Cabe destacar que, si bien es cierto que la mayoría de los plaguicidas más tóxicos son insecticidas, podemos ver que los herbicidas y fungicidas también deben tomarse en cuenta al estimar efectos sobre comunidades de artrópodos.

Con base en esta información, se seleccionaron 13 plaguicidas con los que se estimó los umbrales ecológicos para la comunidad de macroinvertebrados. Estos umbrales ecológicos pueden usarse como un indicador indirecto de la tolerancia o sensibilidad de los diferentes taxa a cada uno de los plaguicidas. En nuestro estudio se observó que la mayoría de las familias presentaron una respuesta de sensibilidad incluso a muy bajas concentraciones de plaguicidas y que, aquellas que presentaron respuestas que indican tolerancia, son familias que también suelen tolerar otros tipos de contaminación, estrés antropogénico o degradación ambiental.

Se utilizaron caracteres anatómicos y fisiológicos para determinar la sensibilidad intrínseca de los macroinvertebrados y comparar con los resultados de su abundancia en muestras de campo. Como resultado de esta comparación observamos que varias familias fueron consistentes en cuanto a presentar una respuesta sensible en campo y también tener caracteres de sensibilidad intrínseca. Sin embargo, hubo varias familias que mostraron respuestas tolerantes en campo, aun cuando presentan caracteres de sensibilidad intrínseca o viceversa, por lo que se concluye que esta es una línea por desarrollar.

Al igual que con los resultados de riesgo a través del modelo msPAF, se observó que muchas familias de macroinvertebrados fueron sensibles a herbicidas y fungicidas, aun cuando sus mecanismos de acción están diseñados para atacar sitios de entrada en productores primarios u hongos. Esto refuerza la necesidad de realizar más investigación sobre los efectos de estas sustancias, pues hasta el momento, el mayor énfasis se ha dado para insecticidas.

También del análisis de umbrales ecológicos se obtuvo el CCP (community change point), se observa que habría una falta de protección para las poblaciones de macroinvertebrados, si los criterios de calidad en las normativas internacionales se usan como concentraciones seguras. Además, las evaluaciones de riesgo se hacen sólo para sustancias individuales, por lo que el efecto de las mezclas que se detectan en las aguas continúa estando subestimado.

Queda claro que, en condiciones de campo, las comunidades de macroinvertebrados responden a concentraciones más bajas de plaguicidas de lo que se reporta a nivel de estudios ecotoxicológicos experimentales de laboratorio. Se mencionó que una explicación puede encontrarse en el comportamiento de evasión de los organismos y su velocidad de respuesta, pero otra razón es la presencia de múltiples factores de estrés. Por esta razón, se analizó un caso de estudio en el Pacífico Sur de Costa Rica, en donde se observó que los contaminantes agrícolas pueden generar fragmentación a nivel del hábitat acuático, así como una ruptura de la integridad ecológica y pérdida tangible de la biodiversidad, al crear una “barrera tóxica” que impide el establecimiento de la comunidad, ya sea por efectos de su propia toxicidad o porque el hábitat queda inutilizado cuando los organismos evaden o emigran de esos sitios.

La fragmentación del hábitat por barreras químicas (o contaminantes) puede ser, por sí misma, un factor relevante en la pérdida de biodiversidad en muchas cuencas hidrográficas. Sin embargo, hasta el momento es muy poco lo que se ha estudiado sobre los efectos de dicha fragmentación sobre las poblaciones en el largo plazo. Debe destacarse que, a nivel del ecosistema, sin importar cuál sea la causa de la pérdida de biodiversidad, el efecto es el mismo: hay una ruptura en las interacciones de las especies con el ecosistema y entre ellas mismas, lo que finalmente conlleva a una pérdida en la integridad y el funcionamiento ecológico.

Recomendaciones y proyección futura:

Dada la situación de contaminación por plaguicidas en Costa Rica, se recomienda continuar y mejorar el monitoreo sistemático de estas sustancias en los cuerpos de agua superficial del país, así como re-evaluar a nivel regulatorio y de registro aquellos que contribuyen mayormente al riesgo, tomando en cuenta el riesgo retrospectivo que se ha calculado en este y otros estudios. Asimismo, es importante complementar el desarrollo de criterios de calidad ambiental con los análisis de umbrales ecológicos realizados con datos de campo. Este y otros posibles usos de la información contenida en este documento para tomadores de decisiones, puede encontrarse en el Anexo 1.

Adicionalmente, el desarrollo de investigaciones relacionadas con los caracteres anatómicos, fisiológicos, ecológicos y de comportamiento en organismos tropicales, ayudarán a enlazar los datos provenientes de ensayos de toxicidad en laboratorio con las respuestas medidas en condiciones de campo, favoreciendo las evaluaciones de riesgo ambiental y la protección de los ecosistemas acuáticos. Por lo tanto, aunque los caracteres tienen un gran potencial para ser usados como predictores de sensibilidad, aún falta mucho trabajo en la validación de cuáles de ellos serían los más apropiados en ambientes tropicales y, asimismo, se requiere evaluar los efectos de la presencia de áreas de refugio y potencial de recuperación y recolonización de hábitats, como indicadores de la resiliencia de las comunidades de macroinvertebrados.

No basta con estimar el riesgo únicamente basado en toxicidad o criterios de calidad, pues tal como se observó en este estudio, la pérdida de biodiversidad ocurre en concentraciones más bajas que las que se encuentran en las normativas internacionales y los efectos de la fragmentación en sí misma, han sido muy poco estudiados en ecosistemas acuáticos.

Con este y muchos otros estudios se ha demostrado que los riesgos de los plaguicidas en los ecosistemas acuáticos son muy altos y que las medidas que se han implementado hasta ahora, no han logrado cumplir con el propósito de protección para el que fueron diseñadas. Por esto, es indispensable instar a los gobiernos a considerar la aplicación de medidas de reducción del uso de agroquímicos en la producción.

Un análisis más profundo de las respuestas de las comunidades de MI a los plaguicidas podría servir para complementar la elaboración de criterios numéricos de protección de la calidad del agua en los países neotropicales, así como para realizar evaluaciones retrospectivas de los riesgos ambientales. El presente estudio es un posible aporte para avanzar en esa dirección porque indica cómo pueden observarse efectos sobre la biodiversidad en el campo a concentraciones muy inferiores a las predichas por las evaluaciones de riesgo. Además, mediante este enfoque, podemos contribuir a un nuevo indicador de ecotoxicología predictiva, disminuyendo la necesidad de datos de toxicidad de laboratorio.

Es necesario también diseñar y validar estrategias de restauración de ecosistemas lóticos, con enfoque en ecología y dirigidas a la remoción de barreras que causan fragmentación, al restablecimiento de la diversidad y del funcionamiento del ecosistema.

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9. Anexos

Anexo 1. ¿Cómo puede utilizarse esta información por tomadores de decisiones?

La información generada en este proyecto de investigación será útil principalmente en los ámbitos académico y gubernamental, pero puede utilizarse también por personas de cualquier índole que tengan interés en la salud de los ecosistemas acuáticos continentales.

En este documento se evidencian los siguientes puntos clave con respecto a la presencia de plaguicidas en los cuerpos de agua lóticos (ríos y quebradas) de Costa Rica:

- Los plaguicidas son contaminantes ubicuos en las aguas continentales lóticas de Costa Rica.
- Algunos de los plaguicidas detectados con mayor frecuencia (herbicidas diuron, ametrina; fungicidas azoxistrobina, epoxiconazol; insecticidas clorpirifos, diazinon, y etoprofos) son también algunos de los mayores contribuyentes al riesgo por toxicidad aguda (mortalidad) para los organismos de los ecosistemas acuáticos.
- Más de 30 plaguicidas se detectaron en concentraciones que sobrepasan una o más normas internacionales para protección de la biodiversidad de Europa, Estados Unidos y Australia y, por lo tanto, representan un riesgo para la integridad ecológica de los ecosistemas acuáticos en Costa Rica.
- Las mezclas de plaguicidas detectados en las aguas generan un riesgo alto para productores primarios (plantas, algas) y artrópodos (insectos, crustáceos) que son la base de las cadenas tróficas y energéticas en los ecosistemas acuáticos.
- Se detectó en las aguas de ríos y quebradas cuatro sustancias prohibidas: lindano (desde 1999), hexaclorobenceno (desde 2005), carbofuran (2014) y bromacil (2017).

Estos puntos son de especial importancia para el Sistema Nacional de Áreas de Conservación, pues la detección de plaguicidas en los ríos y quebradas del país, en concentraciones suficientemente altas para generar toxicidad a nivel agudo (mortalidad), implica que deben generarse alertas sobre la conservación de los ecosistemas acuáticos en todo el país. Debe darse atención prioritaria a aquellas áreas protegidas cuyo objetivo de conservación es un ecosistema acuático (humedal, canales, lagunas costeras) y que reciben aguas de tributarios con altos niveles de contaminación por plaguicidas (Por ej. Parque Nacional Tortuguero, Parque Nacional Palo Verde, Humedal Ramsar Térraba-Sierpe, entre otros).

Asimismo, la Dirección de Gestión de Calidad Ambiental (DIGECA) del MINAE podría hacer uso de esta información para determinar si las condiciones de uso aprobado en los panfletos de los plaguicidas detectados, podrían ser objeto de modificaciones tendientes a disminuir el riesgo observado sobre los ecosistemas en el futuro. Para esto, se exhorta a dicha Dirección a analizar el riesgo retrospectivo (como complemento del prospectivo) de estas sustancias en los procesos de renovación de registro.

En cuanto a la detección de plaguicidas prohibidos, es importante que el Ministerio de Agricultura y el Ministerio de Salud realicen un seguimiento pues, en el caso del lindano y el hexaclorobenceno, las detecciones implican el uso ilegal de estos plaguicidas altamente tóxicos para el ambiente y la salud. Asimismo, el bromacil continúa detectándose en muestras ambientales, por lo que el riesgo de la contaminación de aguas subterráneas sigue siendo motivo de preocupación para la población en general y especialmente a las Asociaciones Administradoras de Sistemas de Acueductos y Alcantarillados Sanitarios (ASADAS) y el Instituto Costarricense de Acueductos y Alcantarillados (A y A).

El Poder Legislativo también puede utilizar la información sobre las sustancias que representan mayor riesgo, para respaldar proyectos de Ley sobre regulación, control y uso de dichos plaguicidas en el corto, mediano y largo plazo.

También, en este documento se analizan los efectos de la presencia de estos contaminantes antropogénicos sobre organismos bioindicadores acuáticos, con los siguientes puntos clave:

- Las familias de macroinvertebrados acuáticos (MIA) estudiadas presentaron sensibilidad a muy bajas concentraciones de plaguicidas y, aquellas que presentaron tolerancia, son familias que también suelen tolerar otros tipos de contaminación, estrés antropogénico o degradación ambiental.
- En 10 de los 13 gradientes de plaguicidas analizados en este estudio, el umbral ecológico (CCP; concentración en la que se observa un cambio rápido y abrupto de la comunidad de MIA), fue inferior a las concentraciones consideradas "seguras" según las normas internacionales de calidad ambiental, lo que implica que aún podría haber una falta de protección para las poblaciones de MIA, si los criterios de calidad en las normativas

internacionales se usan como concentraciones seguras en Costa Rica, especialmente en el caso de los fungicidas.

• Se observó que los contaminantes pueden producir fragmentación del ecosistema acuático (rompimiento de la conectividad entre secciones de la cuenca), al crear una “barrera tóxica” que impide el establecimiento de los organismos, ya sea por efectos de toxicidad o porque los organismos evaden o emigran de los sitios contaminados, generando zonas deshabitadas. Este comportamiento de huida puede ocurrir a concentraciones mucho más bajas que las que ocasionan efectos de toxicidad.

• Algunas familias de MIA podrían seguir presentes en un sitio contaminado, si tienen un alto potencial de recuperación o si pueden recolonizar desde zonas más conservadas que funcionan como refugio. La protección de la vegetación ribereña es clave, ya que podría mejorar las condiciones del hábitat, ayuda a mitigar los efectos de los plaguicidas y el exceso de nutrientes para los organismos acuáticos.

Esta información es de utilidad para la Dirección de Agua, pues brinda datos para la actualización de índices y reglamentación para la clasificación de la calidad de las aguas superficiales basada en monitoreo biológico con MIA. También, los CCP presentados son información muy valiosa para DIGECA, puesto que se demuestra que la biodiversidad acuática disminuye en concentraciones mucho menores que las consideradas seguras a través de evaluaciones de riesgo clásicas. Se insta a las autoridades que evalúan los riesgos ambientales de los plaguicidas a tomar en cuenta que existen efectos de disminución de diversidad biológica que se derivan de comportamientos de evasión y no necesariamente por toxicidad del contaminante.

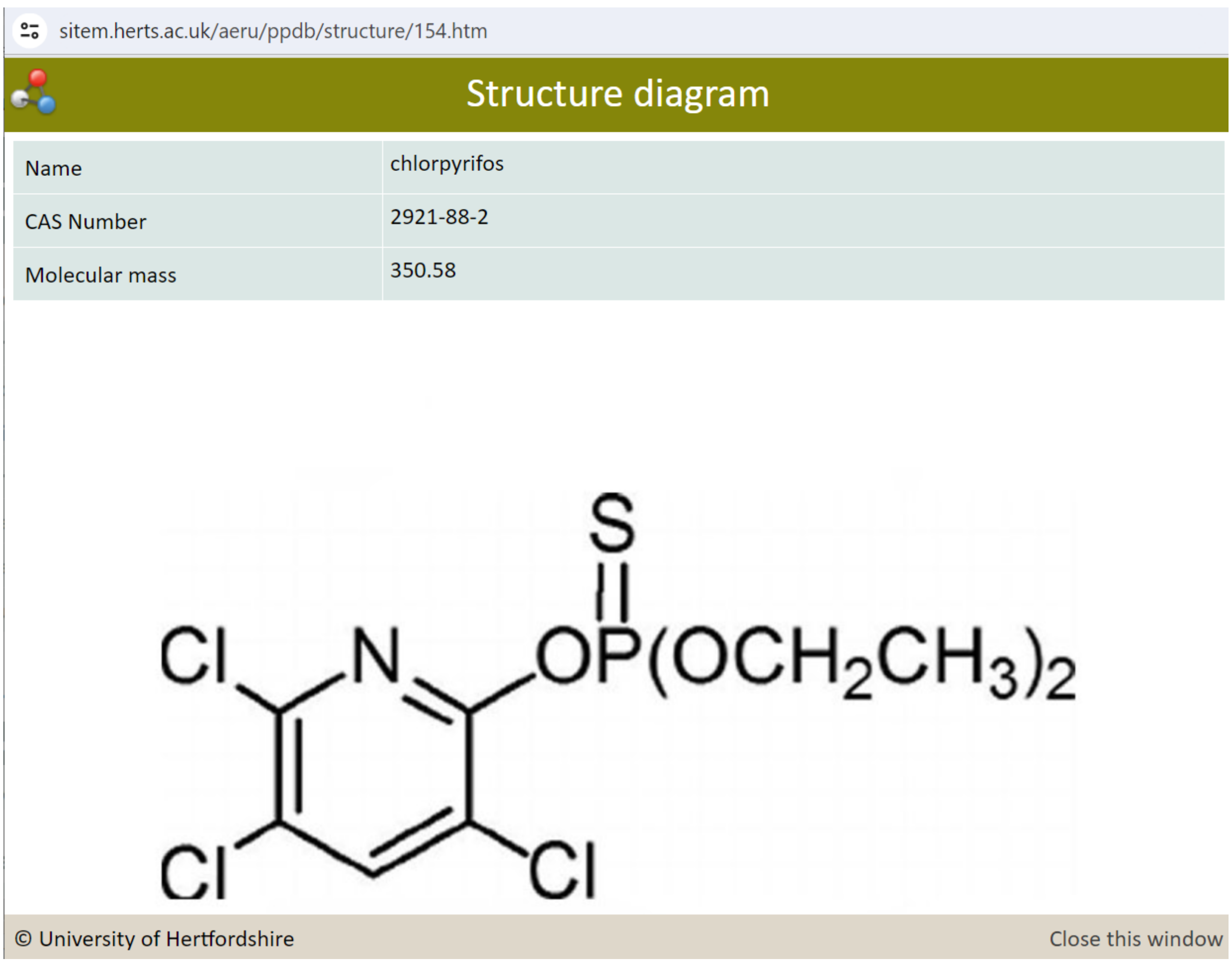
Por otro lado, es importante que la comunidad científica y las autoridades de promoción de la conservación de la biodiversidad presten mayor atención a los efectos fragmentadores de hábitat que tienen los contaminantes, ya que no sólo las barreras físicas (como represas), sino también las “barreras tóxicas” pueden ocasionar estos rompimientos en la conectividad del ecosistema acuático. El MAG y el MINAE pueden actuar conjuntamente en el restablecimiento de vegetación de ribera como una práctica ambiental y agrícola que genera zonas de refugio para los organismos acuáticos, corredores biológicos entre cuenca alta, media y baja y también actúa como filtro, disminuyendo la presencia de contaminantes y sus efectos en los ecosistemas acuáticos.

Anexo 2. Estructura molecular de los 13 plaguicidas seleccionados para el análisis de umbrales ecológicos y 3 plaguicidas prohibidos que fueron detectados en muestras de agua del periodo de estudio.

Todas las siguientes estructuras moleculares 2D fueron obtenidas de la base de datos de propiedades de plaguicidas (PPDB, por sus siglas en inglés), de la Universidad de Hertfordshire, Reino Unido (https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm).

Insecticidas

Clorpirifos

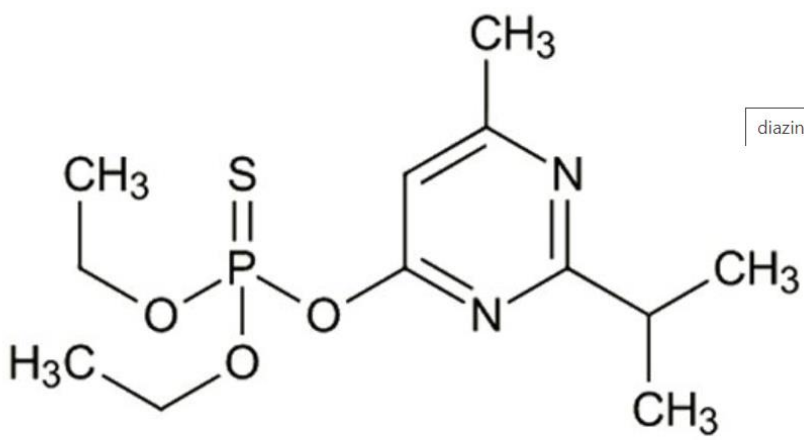


Diazinon

sitem.herts.ac.uk/aeru/ppdb/structure/212.htm

Structure diagram

Name	diazinon
CAS Number	333-41-5
Molecular mass	304.35



diazinon

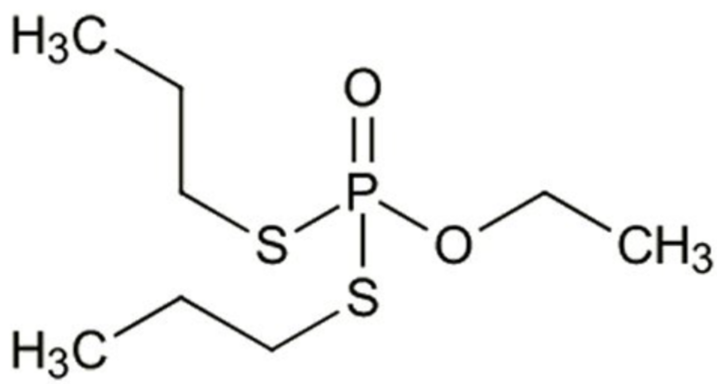
Close this window

Etoprofos

sitem.herts.ac.uk/aeru/ppdb/structure/279.htm

Structure diagram

Name	ethoprophos
CAS Number	13194-48-4
Molecular mass	242.3



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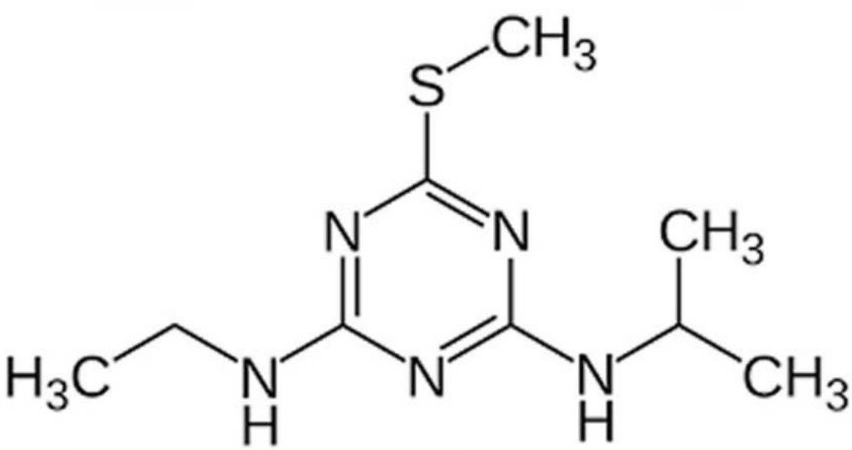
Herbicidas

Ametrina

sitem.herts.ac.uk/aeru/ppdb/structure/27.htm

Structure diagram

Name	ametryn
CAS Number	834-12-8
Molecular mass	227.12



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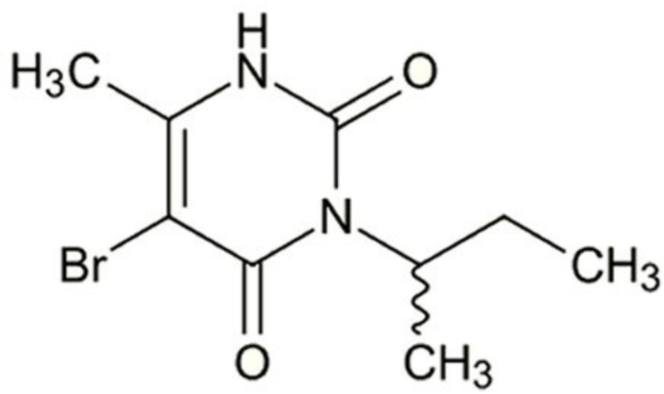
Bromacil (prohibido)

sitem.herts.ac.uk/aeru/ppdb/structure/88.htm

Structure diagram

Name	bromacil
CAS Number	314-40-9
Molecular mass	261.12



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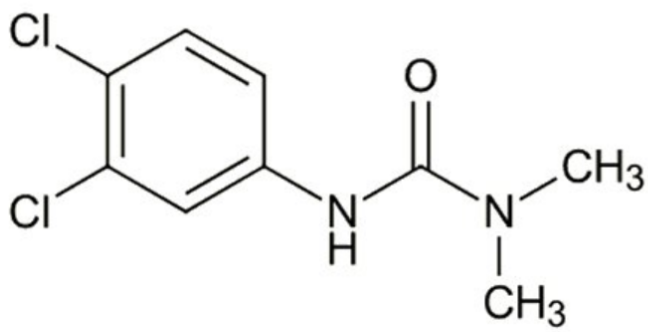
Diuron

sitem.herts.ac.uk/aeru/ppdb/structure/260.htm

Structure diagram

Name	diuron
CAS Number	330-54-1
Molecular mass	233.09



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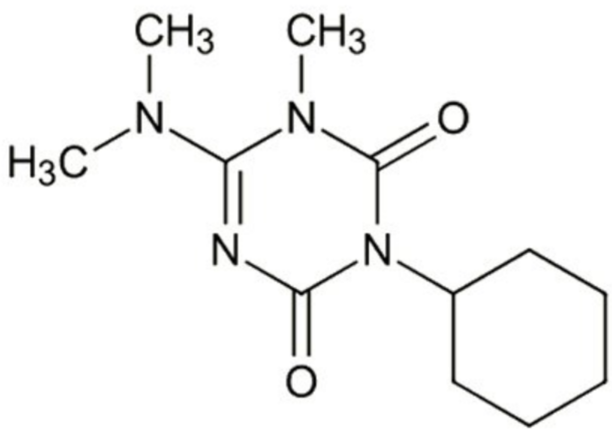
Hexazinona

sitem.herts.ac.uk/aeru/ppdb/structure/384.htm

Structure diagram

Name	hexazinone
CAS Number	51235-04-2
Molecular mass	252.31



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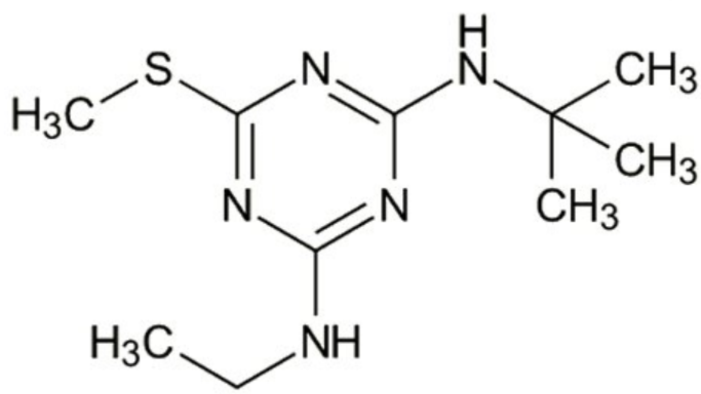
Terbutrina

sitem.herts.ac.uk/aeru/ppdb/structure/624.htm

Structure diagram

Name	terbutryn
CAS Number	886-50-0
Molecular mass	241.36



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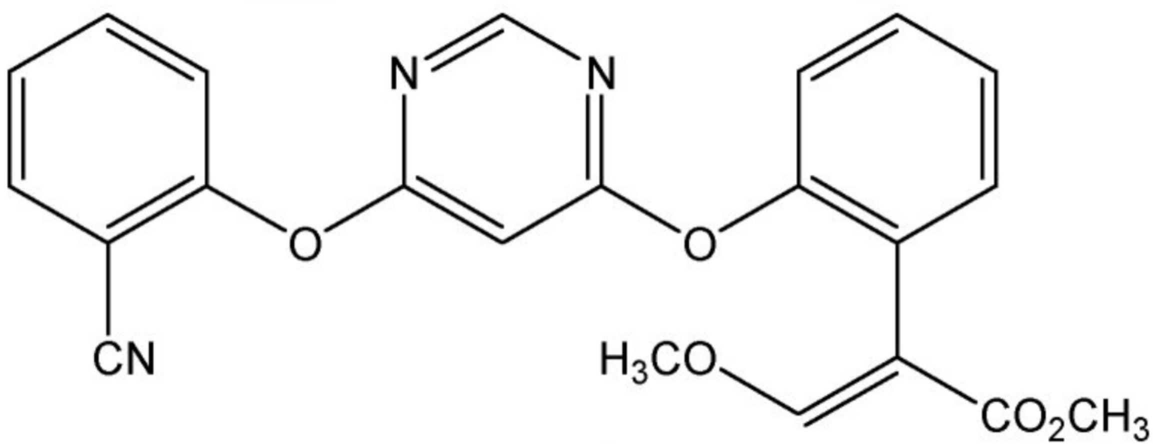
Fungicidas

Azoxistrobina

sitem.herts.ac.uk/aeru/ppdb/structure/54.htm

Structure diagram

Name	azoxystrobin
CAS Number	131860-33-8
Molecular mass	403.4



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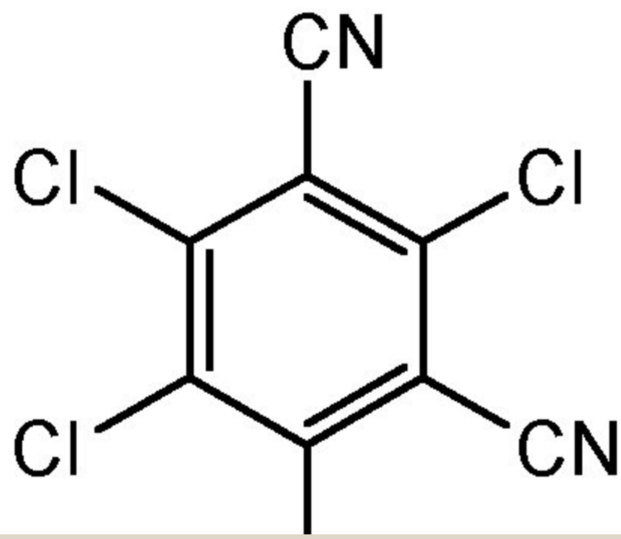
Clorotalonil

sitem.herts.ac.uk/aeru/ppdb/structure/150.htm

Structure diagram

Name	chlorothalonil
CAS Number	1897-45-6
Molecular mass	265.91



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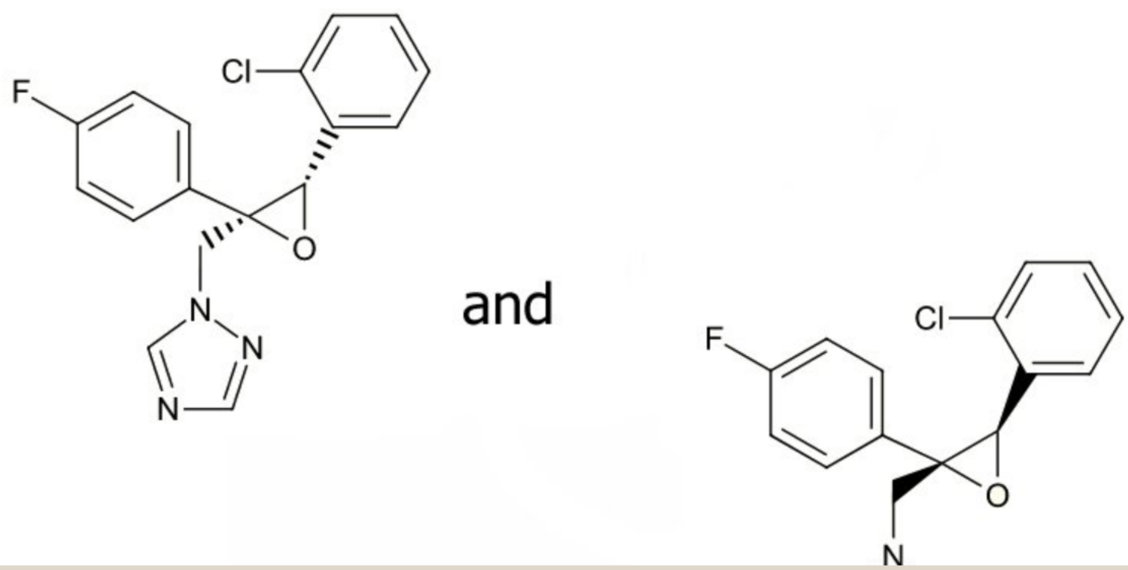
Epoxiconazol

sitem.herts.ac.uk/aeru/ppdb/structure/267.htm

Structure diagram

Name	epoxiconazole
CAS Number	135319-73-2
Molecular mass	329.76



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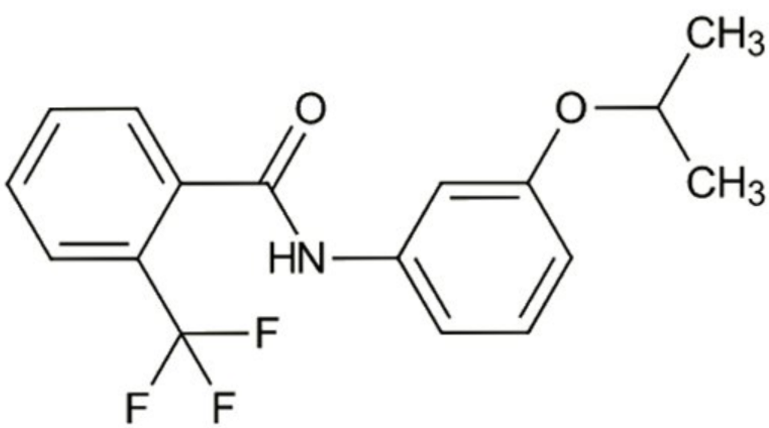
Flutolanil

sitem.herts.ac.uk/aeru/ppdb/structure/352.htm

Structure diagram

Name	flutolanil
CAS Number	66332-96-5
Molecular mass	323.31



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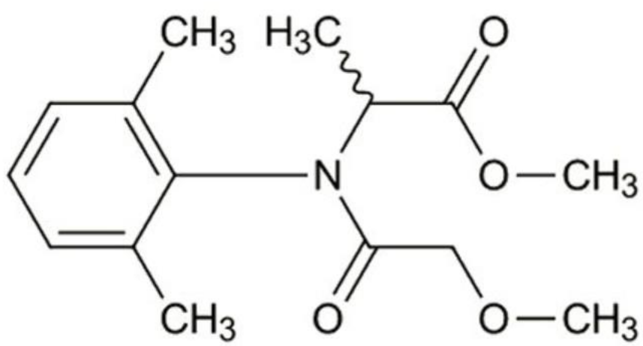
Metalaxyl

sitem.herts.ac.uk/aeru/ppdb/structure/444.htm

Structure diagram

Name	metalaxyl
CAS Number	57837-19-1
Molecular mass	279.33



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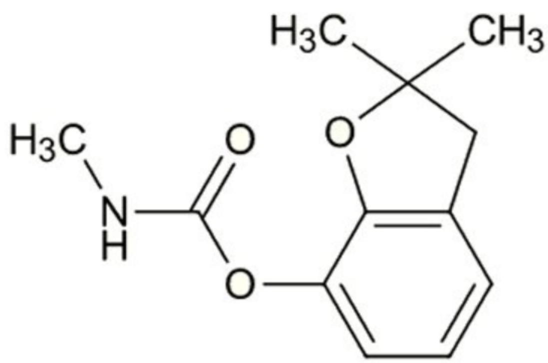
Insecticidas prohibidos y detectados

Carbofuran

sitem.herts.ac.uk/aeru/ppdb/structure/118.htm

Structure diagram

Name	carbofuran
CAS Number	1563-66-2
Molecular mass	221.26



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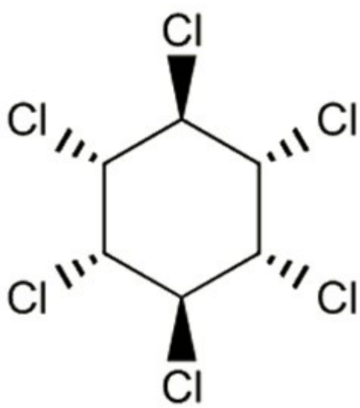
Lindano

sitem.herts.ac.uk/aeru/ppdb/structure/370.htm

Structure diagram

Name	lindane
CAS Number	58-89-9
Molecular mass	290.82



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