



**Universidad del
Rosario**

UNIVERSIDAD DEL ROSARIO

Escuela de Medicina y Ciencias de la Salud

Doctorado en Ciencias Biomédicas y Biológicas

Análisis de alteraciones funcionales en abejas *Apis mellifera* por exposición a imidacloprid y rutina mediante un enfoque físico

Juan Pablo Hernández Benavides

Tesis doctoral presentada como requisito parcial para optar al título de Doctor en Ciencias Biomédicas y Biológicas

Director: Dr. André J. Riveros

Codirector: Dr. Ramón Fayad

Bogotá, Colombia

2025

Tabla de contenido

Resumen	4
Conclusión	5
Fundamentos teóricos y estado del arte	6
Introducción	6
Fisiología general de la abeja <i>Apis mellifera</i>	7
Comportamientos de la abeja <i>Apis mellifera</i>	8
Agroquímicos y sus usos en agricultura	10
Los flavonoides y la defensa vegetal	12
Fundamentos de la física aplicada para evaluar efectos fisiológicos en <i>Apis mellifera</i>	13
Actividad motora	14
Variación de la masa corporal y dinámica hídrica	15
Extracción y propiedades físicas del líquido extracelular	15
Conclusión	20
Referencias	20
Objetivo general	25
Objetivos de investigación	25
Problema de investigación	25
Hipótesis de investigación	25
Introducción general	27
Referencias	28
Capítulo 1. Extracción y análisis óptico del líquido extracelular de los segmentos corporales de abejas <i>Apis mellifera</i>	31

Capítulo 2. Impacto del imidacloprid en el comportamiento apetitivo, defensivo y motor de abejas <i>Apis mellifera</i>	44
Capítulo 3. Homeostasis del agua y el fluido extracelular revelan disrupciones del imidacloprid y la mitigación por la rutina en abejas	65
Capítulo 4. Características eléctricas del líquido extracelular en los segmentos corporales de abejas <i>Apis mellifera</i>	77
Capítulo 5. El flavonoide rutina protege contra las alteraciones osmóticas y eléctricas inducidas por imidacloprid en abejas africanizadas	97
Discusión general	116
Integración de resultados	116
Contraste entre los diferentes experimentos	116
Interpretación de hallazgos bajo el marco teórico general	117
Limitaciones del estudio	117
Conclusiones y perspectivas	118
Resumen de hallazgos clave	118
Respuesta a la hipótesis general	118
Nuevas preguntas	118
Proyecciones de investigación futura	119
Anexos	120
Publicaciones	120
Artículos sometidos	120
Presentaciones en congresos	120
Pasantías	120

Análisis de alteraciones funcionales en abejas *Apis mellifera* por exposición a imidacloprid y rutina mediante un enfoque físico

Resumen

La investigación abordó aspectos relacionados con los cambios comportamentales y fisiológicos en las abejas *Apis mellifera*. Las evaluaciones se basaron en métodos experimentales desde un enfoque físico. Para ello, muestras de abejas fueron expuestas a un insecticida y un flavonoide, las sustancias fueron evaluadas a diferentes concentraciones. Los efectos inducidos por las sustancias permitieron evidenciar variaciones sutiles producidas por bajas concentraciones. Los experimentos empleados fueron diseñados en mecánica, espectroscopía óptica y electromagnetismo.

La mecánica clásica empleada permitió diseñar y construir sistemas para evaluar la respuesta motora ante distintos estímulos: un inmovilizador individual y un caminador. Adicionalmente, la mecánica se empleó para la extracción del líquido extracelular mediante centrifugación; para ello, se realizaron variaciones en las siguientes variables: rapidez, tiempo y masa. La espectroscopía óptica se empleó para caracterizar el líquido extracelular de distintas partes del cuerpo del insecto. Por último, el electromagnetismo se empleó para caracterizar el líquido extracelular mediante pruebas de impedancia y capacitancia eléctrica. Los experimentos realizados permitieron identificar alteraciones funcionales relacionadas con el equilibrio osmótico, las propiedades eléctricas y el comportamiento motor.

Metodología experimental

los sistemas experimentales se diseñaron y evaluaron para:

- Medir las variaciones de masa y el contenido de agua tras la exposición a las sustancias.
- Extraer y caracterizar el líquido extracelular.
- Evaluar propiedades eléctricas (resistencia, capacitancia, impedancia e I–V) en muestras y en abejas vivas.
- Analizar alteraciones en los reflejos (PER y SER) y en los patrones de locomoción, tanto individuales como grupales.

Resultados de la investigación

- El consumo de imidacloprid redujo la ingesta de alimentos, los cambios de masa corporal y la hidratación. Esto fue evidenciado en la alteración de la evaporación y propiedades eléctricas del líquido extracelular. Lo que permite suponer la disrupción de la homeostasis osmótica e iónica en las abejas.

- El consumo de rutina promovió incrementos en su ingesta, lo que se tradujo en una mayor retención de agua y en una recuperación parcial o completa de los efectos inducidos por imidacloprid. Lo que permite suponer un tipo de acción como agente protector.
- Las propiedades eléctricas del líquido extracelular variaron según el segmento corporal evaluado. La cabeza mostró mayor resistencia y el abdomen, mayor capacitancia, mientras que el tórax presentó valores intermedios de resistencia en comparación con los segmentos anteriores.
- La exposición a imidacloprid afectó reflejos alimentarios y defensivos, y redujo la movilidad tanto individual como grupal, evidenciando un deterioro sensorimotor.

Conclusión

La investigación permitió aplicar modelos físicos para evaluar el consumo de imidacloprid y la rutina de las abejas *Apis mellifera*; los efectos se observaron en el comportamiento y la fisiología. Las alteraciones funcionales, debidas al consumo de imidacloprid, se reflejaron en la masa corporal, especialmente en el contenido de agua, lo que conlleva cambios en la dinámica del líquido extracelular. También, el consumo del neurotóxico alteró los movimientos corporales, incluso a dosis reducidas. Las propiedades eléctricas también mostraron cambios, lo que refleja disfunciones en los mecanismos de osmorregulación. Estos hechos permiten evidenciar nuevos efectos subletales y detectar con mayor sensibilidad el consumo de agroquímicos. Además, la rutina se perfila como un modulador capaz de mitigar los efectos negativos del imidacloprid. Finalmente, los resultados obtenidos nos permiten proponer este tipo de evaluaciones como parámetros fisiológicos y comportamentales, susceptibles de emplearse como biomarcadores útiles en estudios ecotoxicológicos y en estrategias de conservación de polinizadores.

Fundamentos teóricos y estado del arte

Introducción

El estudio de la abeja *Apis mellifera* mediante herramientas de la física ofrece oportunidades para comprender cómo los factores ambientales influyen en su fisiología, lo cual se refleja en su comportamiento y estado funcional. Este polinizador contribuye a un equilibrio ecológico [1,2], pero se enfrenta a amenazas crecientes debido a la exposición a insecticidas [3,4]. Aunque los plaguicidas son eficaces en el control de plagas [5,6], también pueden inducir alteraciones comportamentales y fisiológicas en las abejas [7,8]. Por ejemplo, la exposición a pesticidas deteriora la motricidad y provoca desorientación espacial [9,10], efectos que suelen estar relacionados con la acción de los insecticidas sobre las células del sistema nervioso. Estas alteraciones en el sistema nervioso han suscitado evaluaciones de comportamientos alimenticios, como la extensión de la probóscide, y de habilidades cognitivas, como las de aprendizaje y memoria.

Las alteraciones comportamentales y fisiológicas se deben a la distribución de las sustancias nocivas a través del cuerpo de las abejas. Las dinámicas de carga y descarga de los insecticidas en los tejidos se deben al flujo sanguíneo, lo que los contamina [11,12]. En este sentido, los fluidos corporales desempeñan un papel relevante en las dinámicas de intoxicación. Así, las características de estos fluidos pueden actuar como indicadores del estado funcional general de las abejas. En particular, las propiedades del líquido extracelular pueden variar debido a la exposición a sustancias externas, como en el caso del consumo de insecticidas.

Los efectos de las sustancias externas sobre los organismos rara vez se limitan a un solo nivel de organización. Por el contrario, las alteraciones suelen extenderse entre las dimensiones estructural y funcional, expresándose simultáneamente en distintos sistemas [13,14]. Esta complejidad exige una visión integradora, capaz de interpretar los cambios como parte de un proceso dinámico que afecta el equilibrio general del organismo. Reconocer esta interdependencia permite construir marcos conceptuales más amplios para estudiar el impacto de los factores ambientales sobre los seres vivos.

Considerar al organismo como una unidad funcional interdependiente permite identificar conexiones entre fenómenos aparentemente aislados y establecer relaciones causales más completas. En este marco, el presente capítulo reúne los fundamentos fisiológicos y biofísicos que sustentan el diseño experimental de esta tesis, así como el contexto científico en el que se enmarcan los análisis realizados. El desarrollo de la investigación parte de la integración entre la fisiología segmentada, las propiedades eléctricas y los posibles efectos moduladores de sustancias externas. La base teórica presentada a continuación permite abordar de manera integral el estado funcional de *Apis mellifera* en condiciones ambientales adversas.

Fisiología general de la abeja *Apis mellifera*

La fisiología de la abeja *Apis mellifera* está especializada para desempeñar funciones individuales y colectivas que garantizan la supervivencia de la colonia [15,16]. Esta organización biológica se manifiesta en la división del cuerpo en tres regiones principales: cabeza, tórax y abdomen [17,18]. Cada una alberga estructuras con funciones específicas que actúan de manera coordinada. La cabeza alberga los órganos sensoriales y los centros de procesamiento neural; el tórax alberga los músculos responsables del vuelo y la locomoción; y el abdomen participa en procesos como la digestión, la excreción y la reproducción (Fig. 1). Esta segmentación anatómica refleja una compartimentalización funcional que permite distribuir eficientemente los recursos fisiológicos y responder de manera especializada a los estímulos del entorno [19].

A nivel sistémico, estos órganos operan bajo un control fino de señales eléctricas, gradientes iónicos y procesos metabólicos. El sistema nervioso central, compuesto por ganglios distribuidos regionalmente [20], coordina las respuestas motoras y sensoriales mediante los potenciales de membrana. Por su parte, el sistema traqueal suministra oxígeno directamente a los tejidos, lo que compensa la ausencia de un sistema circulatorio cerrado [21]. Esta arquitectura fisiológica, sustentada por el equilibrio osmótico y el metabolismo energético, permite que las abejas realicen tareas complejas como la navegación, la comunicación y la regulación térmica de la colmena. Comprender esta fisiología segmentada es esencial para identificar con precisión cómo los compuestos externos inducen disfunciones localizadas o sistémicas que afectan de manera diferenciada cada región del cuerpo del insecto (Fig. 1).

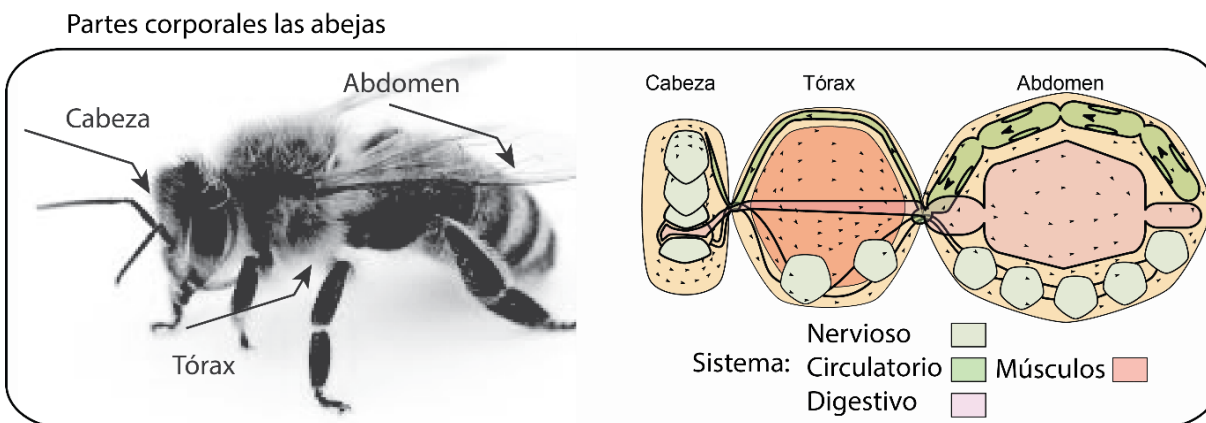


Fig. 1 Partes corporales de las abejas y distribución de sistemas en el cuerpo.

Más allá de la organización anatómica y funcional de cada región corporal, el mantenimiento de la fisiología del insecto depende de un entorno interno que conecta y regula de manera integrada las actividades celulares. Este ambiente interno está constituido por el líquido extracelular [22,23], que es el entorno inmediato de las células. Este medio acuoso actúa como matriz de intercambio, permitiendo el transporte de nutrientes y la redistribución de compuestos generados por la actividad metabólica celular. En los insectos, el líquido extracelular corresponde principalmente a la hemolinfa, que baña directamente los tejidos y garantiza el acceso a los recursos necesarios para

su funcionamiento. Esta red fluida sostiene procesos fundamentales, como la regulación del contenido de agua y de electrolitos [24,25], lo que favorece la estabilización de los gradientes electroquímicos. En el caso de *Apis mellifera*, el mantenimiento de la composición de este medio asegura funciones como la excitabilidad de las membranas, la contracción muscular y la actividad enzimática. Por ello, los cambios en sus propiedades pueden interpretarse como señales tempranas de disfunción sistémica y utilizarse como indicadores biofísicos del estado funcional del insecto.

El movimiento del líquido extracelular se produce mediante un sistema circulatorio abierto que permite el desplazamiento por las cavidades corporales. Este fluido es impulsado por un vaso dorsal contráctil que actúa como un corazón tubular, generando un flujo desde el abdomen hacia la cabeza y el tórax (Fig. 2). Las características funcionales de cada segmento corporal modifican de manera diferencial la composición del líquido extracelular, en función del tipo de tejido que alberga. Así, las demandas de nutrientes y la producción de compuestos metabólicos varían entre regiones con alta densidad muscular, como el tórax, y zonas con elevada actividad neuronal, como el cerebro. Estas diferencias locales aportan información sobre los procesos fisiológicos dominantes en cada región y permiten interpretar el líquido extracelular como un reflejo dinámico del estado funcional del insecto.

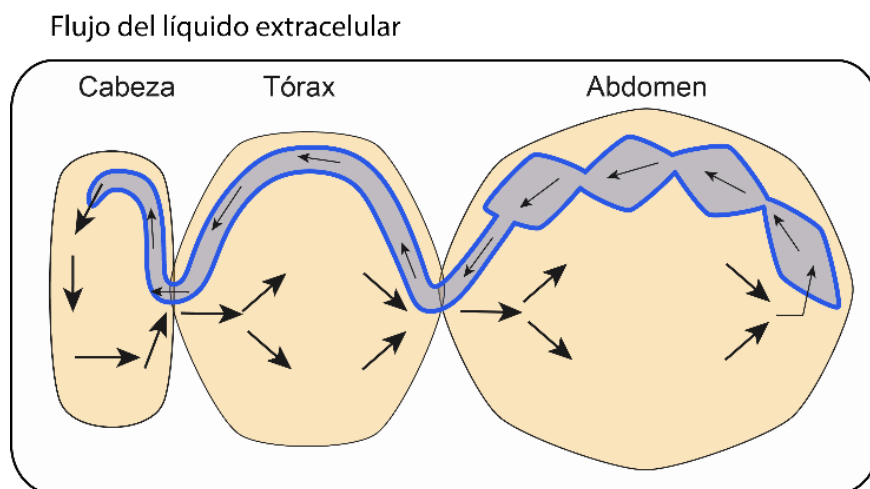


Fig. 2 Flujo del líquido extracelular en los segmentos corporales de las abejas.

La fisiología segmentada de las abejas permite interpretar las funciones del insecto desde una perspectiva integrada pero diferenciada. El estudio del líquido extracelular en cada región corporal ofrece una vía para identificar cómo varían las demandas fisiológicas locales y cómo estas variaciones se reflejan en las propiedades del líquido. Esta aproximación proporciona una base útil para analizar con precisión los efectos de los compuestos externos sobre el estado funcional del organismo, manteniendo la coherencia entre la estructura, la función y la respuesta fisiológica.

Comportamientos de la abeja *Apis mellifera*

El comportamiento de alimentación representa una de las expresiones más fundamentales en las abejas, las cuales, ante una recompensa rica en azúcares, extienden una lengua llamada probóscide.

Esta respuesta al alimento depende de la motivación interna del insecto y de la integridad del sistema sensoriomotor encargado de ejecutar la acción. La extensión de la probóscide ocurre como reacción a un estímulo sensorial específico a la respuesta a soluciones azucaradas; principalmente se da al contacto con las antenas, los tarsos o las piezas bucales. Este estímulo desencadena una señalización neuromotora rápida que coordina la apertura del aparato bucal y la proyección de la probóscide, lo que permite iniciar el proceso de alimentación. Esta conducta, conocida como reflejo de extensión de la probóscide (PER, por sus siglas en inglés) [26,27] está altamente conservada entre individuos y se ha consolidado como una herramienta experimental clave en estudios de comportamiento alimenticio, motivación fisiológica y toxicología de insectos (Figura 3).

Además del reflejo de alimentación, las abejas *Apis mellifera* manifiestan su estado funcional mediante una variedad de comportamientos motores, que también dependen de la integridad de los sistemas sensoriales, neurológicos y musculares. Estos movimientos incluyen la caminata dentro de la colmena, el vuelo durante el forrajeo y el desplazamiento exploratorio en el entorno, todos coordinados por complejos circuitos neuromusculares. La locomoción terrestre permite a las abejas desplazarse entre las celdas de la colmena con sus patas, lo que facilita el intercambio de alimento y la interacción social. En contraste, el vuelo representa un comportamiento altamente demandante en el que se emplean las alas para la búsqueda y recolección de recursos [28]. La capacidad de ejecutar estos movimientos depende directamente del estado fisiológico del insecto, por lo que su alteración constituye un signo temprano de disfunción motora.

Una tercera manifestación funcional regulada por el estado fisiológico de *Apis mellifera* es el comportamiento defensivo de extensión del aguijón. Esta respuesta rápida y estereotipada se activa ante estímulos potencialmente nocivos y consiste en proyectar el aparato punzante ubicado en el extremo del abdomen. La ejecución de esta conducta requiere la integración de señales sensoriales, el procesamiento en los centros nerviosos y la activación coordinada de la musculatura abdominal que rodea el complejo del aguijón (SER, por sus siglas en inglés) [29]. A diferencia de los comportamientos de alimentación y locomoción, la extensión del aguijón refleja una disposición de defensa y está estrechamente relacionada con la percepción de amenaza y el estado de alerta del insecto. Este tipo de comportamiento defensivo sirve para proteger la colmena y puede verse modulado por factores internos, como el nivel de energía y la integridad del sistema nervioso. Por tanto, la aparición o ausencia de esta respuesta constituye una expresión conductual valiosa para comprender el equilibrio funcional de la abeja ante condiciones adversas (Fig. 3).

Comportamientos de las abejas

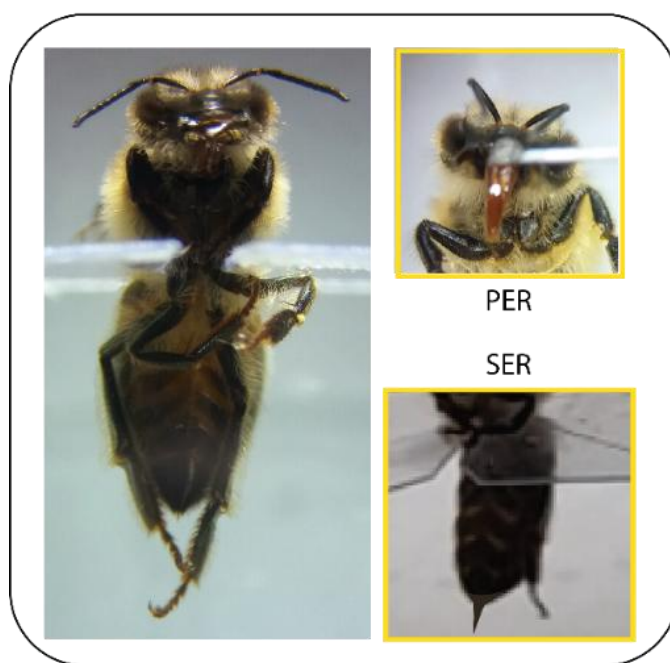


Fig. 3 Figura 3. Los comportamientos de las abejas pueden ser alimenticios, de defensa y de locomoción.

En conjunto, los comportamientos de alimentación, locomoción y defensa constituyen manifestaciones observables del estado fisiológico de *Apis mellifera*. Dichas conductas permiten inferir, de forma no invasiva, el equilibrio funcional del insecto en diversos contextos ambientales o químicos. Cada una de estas respuestas se sustenta en la integración de señales sensoriales, la transmisión nerviosa y la ejecución motora de segmentos corporales específicos, lo que las hace particularmente sensibles a alteraciones del sistema biológico. El estudio simultáneo de estos comportamientos permite construir un marco funcional integrado que vincula la fisiología segmentada con el comportamiento y sienta las bases para su aplicación como herramientas diagnósticas ante la exposición a sustancias exógenas.

Agroquímicos y sus usos en agricultura

El cuidado de los cultivos se ha realizado mediante una amplia variedad de agroquímicos diseñados para controlar organismos que afectan la productividad vegetal [30]. Entre estos, los insecticidas desempeñan un papel central en el manejo de plagas [31,32], especialmente en sistemas de monocultivo, donde las condiciones favorecen la proliferación de especies fitófagas. Con el tiempo, las formulaciones de estos compuestos se han ido modificando para volverse más especializadas, como ocurre con los insecticidas sistémicos. Estos productos son absorbidos por las raíces o las hojas de la planta, lo que permite una distribución interna que protege contra depredadores desde el interior. Los neonicotinoides son un ejemplo representativo de este tipo de insecticidas [33,34]: están diseñados para actuar sobre el sistema nervioso central de los insectos plaga, provocando su parálisis y su muerte [35,36]. Las propiedades de los neonicotinoides, como su eficacia, persistencia y capacidad de distribución sistémica, los han popularizado a nivel

mundial. Sin embargo, estas mismas características han suscitado preocupación por los posibles efectos sobre los organismos benéficos que interactúan con las plantas tratadas.

El imidacloprid es uno de los insecticidas neonicotinoides más utilizados a nivel global [37,38] debido a su alta eficacia para el control de insectos como los áfidos, las moscas blancas y los escarabajos [39]. El mecanismo de acción de esta sustancia se basa en la activación sostenida de los receptores nicotínicos de acetilcolina [40,41], lo que provoca la parálisis y la muerte de los insectos. Esta acción específica, combinada con su facilidad de aplicación mediante tratamientos foliares, del suelo o de semillas, ha consolidado su uso en cultivos extensivos. Sin embargo, la naturaleza sistémica y la persistencia del imidacloprid han generado preocupación por sus efectos colaterales sobre insectos no objetivo (Fig. 4), en particular sobre los polinizadores [42]. Diversos estudios han demostrado que, incluso a dosis subletales, el imidacloprid puede alterar el comportamiento y comprometer la supervivencia de especies benéficas, como *Apis mellifera*. Estas evidencias han motivado medidas regulatorias en varios países. La Unión Europea, por ejemplo, restringió progresivamente su uso hasta prohibirlo por completo en campo abierto en 2018 [34,43]. Estas decisiones reflejan una creciente conciencia de la necesidad de equilibrar la protección de los cultivos con la conservación de servicios ecosistémicos esenciales, como la polinización.

La exposición de *Apis mellifera* al imidacloprid ha sido estudiada debido a la alta sensibilidad de esta especie a compuestos neurotóxicos. Las abejas, en su labor de polinización, entran en contacto con residuos de imidacloprid presentes en el néctar, el polen y otras estructuras florales de plantas tratadas [13,33]. Aunque en muchos casos las concentraciones son subletales, las dosis pueden alterar funciones fisiológicas y comportamentales fundamentales del insecto. Entre los efectos más relevantes se encuentran la reducción de la respuesta alimentaria, la descoordinación motora, la desorientación espacial y la disminución de la actividad defensiva. Estas alteraciones comprometen la capacidad de forrajeo, la comunicación dentro de la colonia y, en consecuencia, la eficiencia de la polinización. La relación entre la exposición al imidacloprid y las disfunciones observadas en *Apis mellifera* ha llevado a proponer a esta especie como modelo bioindicador de toxicidad subletal en insectos sociales [44,45]. El análisis detallado de sus respuestas permite detectar con alta sensibilidad los impactos fisiológicos y ecológicos derivados del uso de insecticidas sistémicos.

El uso agrícola del imidacloprid y su impacto sobre *Apis mellifera* evidencian una tensión entre la eficacia agronómica de los insecticidas sistémicos y sus efectos sobre la fauna benéfica. A pesar de no provocar mortalidad inmediata en muchas situaciones de campo, la exposición crónica a concentraciones subletales puede inducir alteraciones funcionales que afectan directamente el comportamiento, la fisiología y la organización social de las abejas. Estas disfunciones, difíciles de detectar mediante evaluaciones convencionales de toxicidad, resaltan la necesidad de repensar los criterios de seguridad ambiental y los métodos de evaluación de impacto en insectos polinizadores.

Uso de imidacloprid para combatir plagas

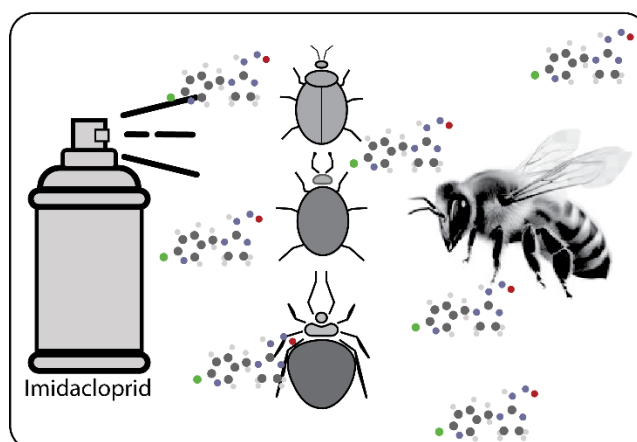


Fig. 4 El imidacloprid se emplea para combatir plagas, pero puede afectar a las abejas.

Los flavonoides y la defensa vegetal

Las plantas producen una amplia gama de compuestos secundarios como parte de sus estrategias defensivas frente a condiciones ambientales adversas, ataques de herbívoros o infecciones microbianas. Entre estos compuestos se encuentran los flavonoides, una familia de metabolitos muy extendida en el reino vegetal. Los flavonoides cumplen funciones diversas, desde la protección frente a la radiación ultravioleta hasta la señalización en las interacciones planta-microorganismo [46,47]. Además, estos compuestos poseen propiedades antioxidantes y moduladoras de procesos enzimáticos, lo que ha despertado interés en múltiples campos, entre ellos la medicina, la nutrición y la agricultura. Estas moléculas se encuentran distribuidas en hojas, flores, frutos y semillas. La presencia de los flavonoides en las plantas varía según la especie, el tejido y las condiciones de crecimiento. En el contexto agrícola, los flavonoides han sido explorados como agentes naturales con potencial para modular la fisiología de insectos [11], ofreciendo alternativas más sostenibles frente al uso de compuestos sintéticos.

La rutina, también conocida como quercetina-3-O-rutinósido, es un flavonoide presente en diversas especies vegetales, como el trigo, los cítricos, las manzanas y el eucalipto. La estructura de esta molécula combina un núcleo de quercetina con azúcares, lo que le otorga estabilidad y una baja solubilidad en agua (aproximadamente 0.13 g/L a 20°C). En las plantas, la rutina cumple funciones antioxidantes y defensivas frente a estrés biótico y abiótico. Estas propiedades han motivado su estudio en ámbitos biomédicos, donde se le atribuyen efectos antiinflamatorios, vasoprotectores y neuroactivos [48,49]. En el contexto agrícola, ha despertado interés como agente bioactivo capaz de modular la fisiología de microorganismos e insectos. El origen natural y la baja toxicidad reportada en diversos modelos biológicos han posicionado a la rutina como un compuesto con potencial para el desarrollo de estrategias sostenibles de manejo de cultivos.

A pesar de que la rutina se ha catalogado como un compuesto de origen natural con baja toxicidad en modelos animales, su efecto sobre insectos benéficos, como *Apis mellifera*, aún se está

caracterizando. Algunos estudios recientes han sugerido posibles beneficios neuroprotectores en himenópteros sociales. Por ejemplo, en el modelo de *Bombus impatiens* se ha reportado una mejora en tareas cognitivas tras la exposición a la rutina [50]. También en *Apis mellifera* se han observado efectos positivos en la actividad de las enzimas antioxidantes, lo que podría asociarse con una mayor resistencia al estrés oxidativo. Sin embargo, un aspecto relevante a tener en cuenta es que la rutina también ha sido evaluada por su capacidad insecticida frente a otras especies. Este efecto negativo plantea interrogantes sobre su impacto diferencial según el organismo, la dosis y el contexto fisiológico. Por esta razón, resulta necesario avanzar con estudios controlados que evalúen sus efectos funcionales en abejas, prestando especial atención a variables comportamentales, metabólicas y fisiológicas que puedan servir como indicadores tempranos de disfunción o protección.

El interés por la rutina como compuesto bioactivo ha crecido en el contexto de las estrategias agrícolas sostenibles, debido a su origen vegetal y a su perfil antioxidante (Fig. 5). No obstante, la evidencia sobre sus efectos en insectos benéficos, como *Apis mellifera*, sigue siendo limitada y, en algunos casos, contradictoria. Si bien se han observado respuestas potencialmente protectoras en parámetros cognitivos y antioxidantes, también se ha documentado su capacidad insecticida en otros modelos, lo que exige una evaluación cuidadosa de sus efectos según la especie, la dosis y el contexto experimental. Estas consideraciones refuerzan la necesidad de abordar el estudio de la rutina con un enfoque experimental riguroso, que permita distinguir entre respuestas adaptativas, efectos adversos y posibles aplicaciones seguras en el manejo ecológico de cultivos.

Uso de la rutina

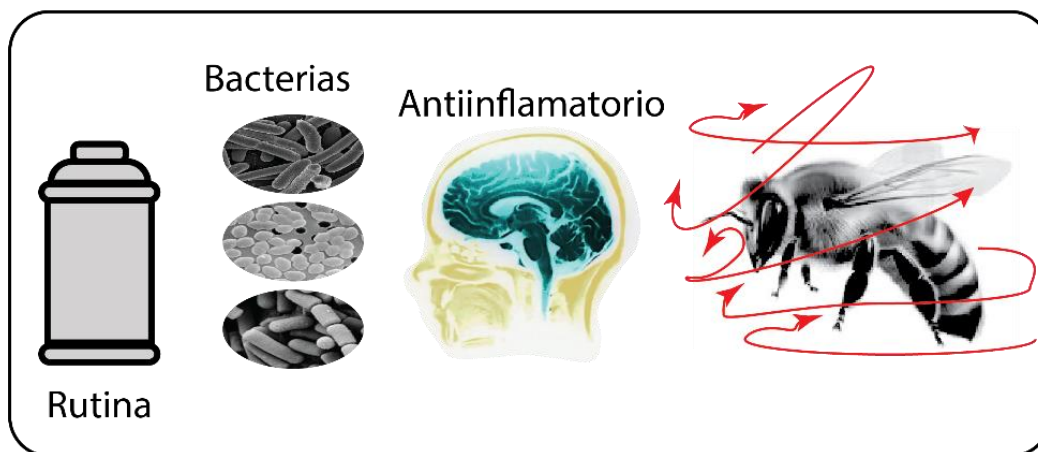


Fig. 5 La rutina tiene propiedades antibacteriales y antiinflamatorias, últimamente se ha empleado para tratar algunos problemas en las abejas.

Fundamentos de la física aplicada para evaluar efectos fisiológicos en *Apis mellifera*

El estudio del comportamiento en *Apis mellifera* ofrece una ventana al estado funcional del insecto, permitiendo detectar alteraciones motoras, sensoriales o cognitivas sin necesidad de

procedimientos invasivos. Este tipo de observación permite identificar anomalías mediante variables como el movimiento y su rapidez [51,52], que pueden reflejar el estado interno del organismo. Sin embargo, algunas disfunciones se caracterizan por su baja intensidad y resultan casi imperceptibles a simple vista, por lo que es necesario recurrir a mecanismos que permitan detectar cambios sutiles en el sistema. El enfoque físico permite establecer vínculos cuantificables entre el comportamiento y los procesos fisiológicos subyacentes mediante herramientas de medición, modelado y comparación. Esta integración entre niveles de análisis puede ampliar la comprensión del impacto de sustancias como el imidacloprid y de la rutina. Adicionalmente, la integración establece una base metodológica para el desarrollo de indicadores funcionales sensibles, reproducibles y aplicables en diversos contextos, tanto experimentales como ambientales.

Actividad motora

La actividad motora de *Apis mellifera* puede evaluarse mediante respuestas reflejas o movimientos corporales, variables que varían según el estado fisiológico del insecto [9,10]. La detección y el análisis de la respuesta pueden abordarse mediante sistemas experimentales que transforman el comportamiento en variables físicas medibles, como desplazamientos angulares, frecuencias de activación o la direccionalidad del movimiento. Este enfoque permite registrar respuestas rápidas, como la extensión de estructuras sensoriales o defensivas ante estímulos definidos, así como trayectorias más prolongadas derivadas del movimiento con grados de restricción (Fig. 6).

A nivel individual, el estudio de la locomoción puede enfocarse en patrones de caminata, cambios en la orientación o pérdida de coordinación. En contextos colectivos, el movimiento del grupo puede amplificarse mediante soportes móviles que permiten cuantificar el desplazamiento generado por la acción conjunta (Fig. 6B). Estas mediciones proporcionan información continua sobre la integridad funcional del sistema motor. La sensibilidad del sistema de medición es suficiente para detectar efectos subletales que alteren la dinámica del movimiento.

Las exposiciones a sustancias neuroactivas pueden reducir la frecuencia y la coordinación de las respuestas motoras, mientras que otros compuestos podrían modular estas alteraciones según el orden y tipo de administración. Así, la cuantificación sistemática del movimiento se convierte en una herramienta biofísica útil para identificar disfunciones motoras inducidas por agentes químicos, al articular las medidas mecánicas con los procesos fisiológicos subyacentes.

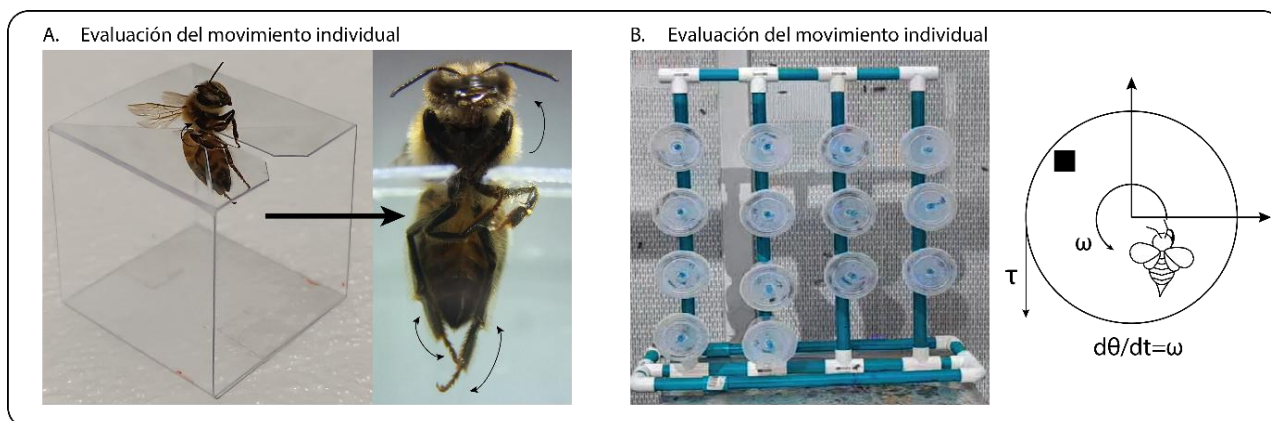


Fig. 6 La evaluación del comportamiento se logra restringiendo los grados de libertad del movimiento. A) las abejas son inmovilizadas para permitir el movimiento de las patas y la cabeza, sin permitir desplazamientos del individuo. B) las abejas son confinadas en recipientes que permiten rotaciones mientras ellas se mueven en su interior.

Variación de la masa corporal y dinámica hídrica

El mantenimiento del equilibrio hídrico es esencial para la fisiología de *Apis mellifera*, especialmente en un sistema circulatorio abierto donde los líquidos corporales se distribuyen libremente por las cavidades internas [21]. La masa corporal, compuesta por componentes sólidos y líquidos, varía en función del consumo de alimentos, de la pérdida de agua por evaporación y de los procesos de intercambio osmótico [53,54]. Estas variaciones pueden utilizarse como indicadores indirectos del estado funcional del insecto, particularmente en condiciones de exposición a compuestos que alteren la regulación hídrica o el metabolismo celular.

La evaluación de estos cambios puede abordarse mediante protocolos experimentales basados en la medición de la masa húmeda y seca del cuerpo tras distintos periodos de exposición. La comparación del contenido de agua corporal entre individuos tratados y controles permite inferir alteraciones en la capacidad del insecto para retener líquido, regular su volumen interno y mantener su fisiología básica. Una disminución de la masa húmeda puede reflejar un aumento de la pérdida por evaporación o una disfunción de los mecanismos de retención hídrica, mientras que un aumento anómalo podría sugerir una acumulación extracelular o alteraciones osmóticas. Adicionalmente, el seguimiento de la masa en función del tiempo tras exposiciones breves permite detectar dinámicas de ganancia o pérdida líquida con alta resolución temporal. Estas aproximaciones, combinadas con análisis de composición corporal, permiten construir un perfil fisiológico detallado del insecto y revelar efectos subletales de sustancias como el imidacloprid o la rutina. Así, la masa corporal no se interpreta como una medida estática, sino como un parámetro dinámico sensible a los flujos internos y a la interacción del insecto con su entorno.

Extracción y propiedades físicas del líquido extracelular

El líquido extracelular de *Apis mellifera* constituye un medio dinámico que participa en procesos clave como la osmorregulación, la distribución de metabolitos y el intercambio de señales químicas. La caracterización de este líquido permite acceder a indicadores indirectos del estado

funcional del insecto, especialmente en condiciones de exposición a sustancias. Sin embargo, la obtención de este fluido sigue representando un reto en estudios experimentales con abejas, ya que la extracción depende en gran medida de la destreza manual del experimentador y requiere procedimientos individualizados [23,55]. Este enfoque limita el número de individuos que pueden evaluarse, lo que genera una discrepancia metodológica importante si se considera que las colonias de *Apis mellifera* están compuestas por miles de abejas. Esta diferencia de escala entre la muestra accesible y la población real plantea restricciones logísticas y estadísticas para los estudios poblacionales.

Extracción mediante centrifugación

Una posible solución a estas limitaciones radica en los principios físicos de la centrifugación, una técnica que permite separar componentes biológicos en función de su densidad mediante la aplicación de una fuerza centrífuga. Adaptada al contexto de *Apis mellifera*, esta estrategia puede emplearse para recuperar el líquido extracelular contenido en los segmentos corporales sin requerir microinstrumentos especializados. Al utilizar la centrifugación, es posible procesar múltiples individuos de forma paralela, incrementando la capacidad de muestreo y reduciendo la variabilidad asociada a manipulaciones individuales. Además, esta aproximación conserva la diferenciación anatómica, lo que permite explorar variaciones fisiológicas específicas del tórax, la cabeza o el abdomen (Fig. 7). En conjunto, la centrifugación facilita el acceso a este fluido poco explorado y sienta las bases para su caracterización funcional como indicador segmentado del estado fisiológico del insecto.

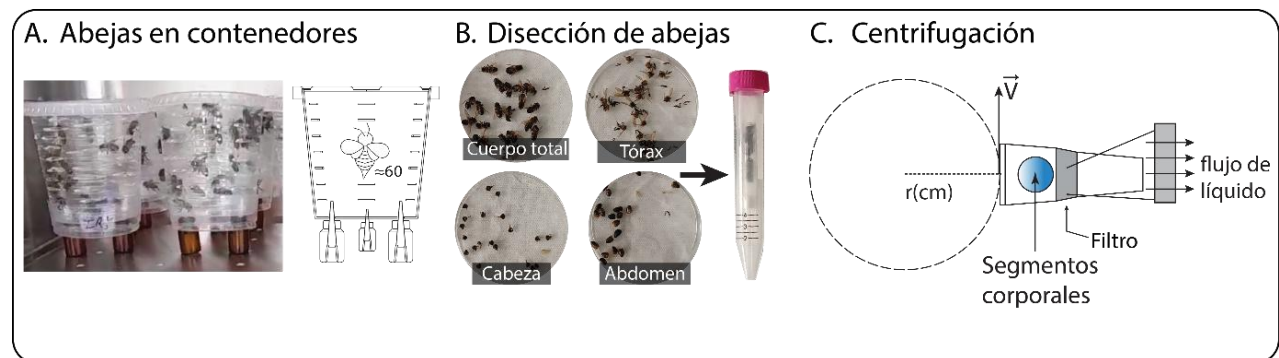


Fig. 7 La extracción del líquido extracelular. A) abejas en contenedores para la manipulación en condiciones de laboratorio. B) abejas disectadas y envasadas en tubos para centrifugado. C) modelo de centrifugación para obtención del líquido extracelular de grupos de abejas.

Caracterización óptica del líquido extracelular

La espectroscopía de absorción en el rango ultravioleta-visible (UV-Vis) constituye una herramienta valiosa para explorar la composición química del líquido extracelular de insectos. Esta técnica se basa en la interacción entre la luz y las moléculas presentes en la muestra, lo que permite identificar patrones de absorción asociados a compuestos orgánicos como pigmentos, nucleótidos, aminoácidos aromáticos o productos de degradación. En *Apis mellifera*, el análisis espectral podría revelar diferencias entre segmentos corporales o entre condiciones experimentales, relacionadas

con procesos como el estrés oxidativo, la acumulación metabólica o el daño tisular. La espectroscopía UV-Vis se distingue por su alta sensibilidad y su compatibilidad con volúmenes reducidos, lo que la hace especialmente adecuada para estudios fisiológicos en insectos. Además, la obtención de espectros de absorbancia en función de la longitud de onda permite construir perfiles comparativos que pueden integrarse con otras variables biofísicas, lo que amplía la capacidad diagnóstica de este tipo de análisis.

La detección de azúcares reductores en el líquido extracelular constituye una vía accesible para evaluar el estado energético del insecto y posibles alteraciones metabólicas. Ensayos colorimétricos, como el de Benedict, permiten identificar la presencia de monosacáridos y de algunos disacáridos que actúan como agentes reductores, generando un cambio de color en función de su concentración. Aunque de naturaleza cualitativa, esta prueba proporciona una estimación rápida del contenido de carbohidratos simples en la muestra, lo cual puede asociarse con procesos como el transporte de azúcares, la actividad enzimática o el equilibrio osmótico. La detección de azúcares en muestras obtenidas por centrifugación también constituye un criterio de validación, al confirmar que el fluido extraído mantiene componentes típicos del medio extracelular y no corresponde a una fracción puramente acuosa ni contaminada.

La aplicación segmentada de esta prueba permite explorar variaciones regionales en el contenido de azúcares, lo que podría reflejar diferencias funcionales entre la cabeza, el tórax y el abdomen. La simplicidad del procedimiento, su bajo costo y su compatibilidad con volúmenes reducidos la convierten en una herramienta útil para la evaluación inicial de alteraciones fisiológicas en estudios con insectos. La recuperación y caracterización del líquido extracelular constituyen un avance metodológico relevante en el enfoque biofísico propuesto, al permitir el acceso a una fracción sensible del sistema interno mediante procedimientos controlados y reproducibles.

La centrifugación segmentada facilita la estandarización del muestreo, mientras que la detección de componentes como azúcares reducidos contribuye a validar la naturaleza funcional del fluido extraído. Estas mediciones, junto con las propiedades ópticas obtenidas mediante espectroscopía UV-Vis, permiten construir perfiles regionales que integran información sobre la composición, el metabolismo y el estado hídrico del insecto (Fig. 8). En conjunto, el líquido extracelular se consolida como un recurso experimental valioso para identificar alteraciones fisiológicas subletales inducidas por sustancias exógenas. Lo que articula niveles de análisis que van desde la escala segmental hasta parámetros cuantificables y comparables.

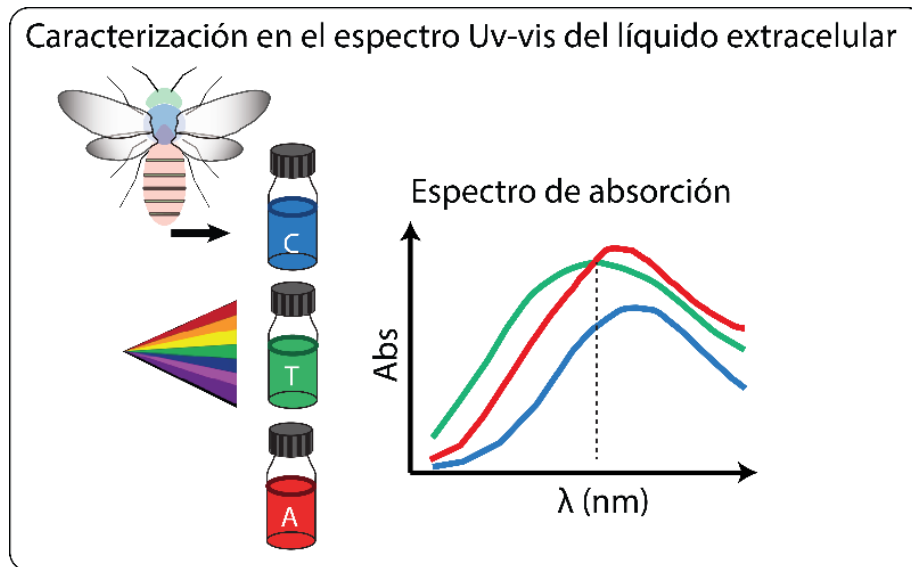


Fig. 8 La caracterización óptica permite obtener los espectros de absorción de muestras líquidas en el rango UV-Vis.

Propiedades eléctricas del líquido extracelular de *Apis mellifera*

En organismos con sistema circulatorio abierto, como *Apis mellifera*, el estudio de las propiedades eléctricas de los fluidos corporales y de los tejidos puede aportar información valiosa sobre su estado fisiológico. A diferencia de los modelos animales clásicos, donde las mediciones eléctricas suelen centrarse en potenciales de membrana o en la actividad neuronal, en insectos es posible explorar parámetros eléctricos macroscópicos como vía indirecta para evaluar la composición iónica, la organización tisular o la integridad del medio extracelular. Estas propiedades se ven influidas por factores como la concentración de solutos, la presencia de proteínas cargadas y el grado de hidratación, todos los cuales pueden alterarse por la exposición a sustancias químicas. La medición eléctrica permite detectar variaciones funcionales poco evidentes a simple vista y ofrece una vía cuantificable, no invasiva y sensible para caracterizar disfunciones fisiológicas inducidas experimentalmente.

El comportamiento eléctrico de un sistema biológico puede describirse mediante parámetros como la resistencia, la conductancia, la capacitancia y la impedancia [56–58]. Estos conceptos permiten representar la respuesta del sistema ante el paso de corriente continua o alterna. Además, estas variables reflejan propiedades estructurales y composicionales del medio evaluado. En el caso del líquido extracelular, la resistencia está determinada por la movilidad iónica y la concentración de solutos, mientras que la capacitancia puede asociarse a la capacidad del sistema para almacenar carga, especialmente si existen interfaces o membranas entre regiones [59]. La impedancia, por su parte, integra componentes resistivos y reactivos que varían con la frecuencia, lo que permite explorar la dinámica dieléctrica del medio. El análisis de estos parámetros mediante sistemas eléctricos experimentales brinda una herramienta para detectar cambios sutiles en la composición o la estructura interna, incluso cuando no se observan alteraciones morfológicas evidentes. La

caracterización eléctrica actúa como una extensión funcional del estudio fisiológico, al traducir variaciones bioquímicas en respuestas físicas medibles.

La evaluación eléctrica del cuerpo o del líquido extracelular en *Apis mellifera* adquiere especial relevancia en contextos de exposición a compuestos neuroactivos, como el imidacloprid, o a moduladores fisiológicos, como la rutina. Estas sustancias pueden alterar la distribución iónica, la permeabilidad de las membranas o la organización molecular del medio extracelular, lo que se traduce en modificaciones detectables en los parámetros eléctricos. La medición de la resistencia o de la impedancia, por ejemplo, permite identificar variaciones en la conductividad del fluido, mientras que el análisis de la capacitancia puede reflejar reorganizaciones estructurales o cambios en la polarización del medio. Estas alteraciones, difíciles de evidenciar mediante observación directa o ensayos morfológicos, pueden cuantificarse con precisión mediante circuitos eléctricos controlados, lo que constituye un recurso sensible para el diagnóstico temprano de disfunción fisiológica. Además, al tratarse de variables continuas y reproducibles, estas mediciones permiten comparar condiciones experimentales, establecer curvas de respuesta a dosis y explorar posibles efectos protectores o compensatorios. Así, el análisis eléctrico se presenta como una herramienta complementaria y poderosa para integrar la dimensión biofísica en la evaluación del impacto de las sustancias sobre la fisiología de los insectos.

La incorporación de parámetros eléctricos al estudio fisiológico de *Apis mellifera* permite ampliar el repertorio de variables funcionales empleadas para detectar disfunciones inducidas por sustancias exógenas. A diferencia de las observaciones comportamentales o las mediciones morfológicas, los datos eléctricos ofrecen una vía cuantificable, reproducible y altamente sensible para explorar alteraciones internas que afectan la dinámica iónica, la composición del líquido extracelular o la organización estructural del sistema. El cuerpo del insecto y sus fluidos se conciben como sistemas con propiedades eléctricas medibles (Fig. 9), en los que los cambios bioquímicos pueden traducirse en diferencias medibles de conductancia, capacitancia o impedancia. Esta perspectiva fortalece el enfoque biofísico del trabajo al establecer conexiones explícitas entre propiedades materiales, procesos fisiológicos y condiciones experimentales. Lo que posiciona a las mediciones eléctricas como herramientas diagnósticas de alto valor en entornos de evaluación ecológica o toxicológica.

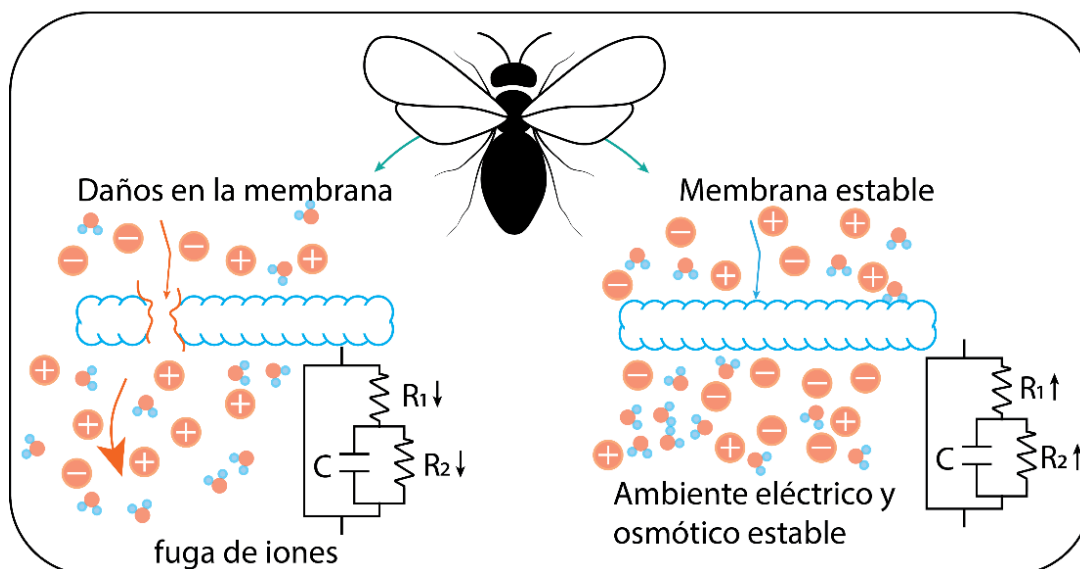


Fig. 9 Las variaciones fisiológicas pueden caracterizarse eléctricamente mediante modelos basados en circuitos conocidos.

Conclusión

El enfoque biofísico desarrollado en esta sección propone una integración metodológica que articula el comportamiento, la masa corporal, el contenido de agua, las propiedades fisicoquímicas del líquido extracelular y las características eléctricas del sistema como dimensiones complementarias del estado funcional en *Apis mellifera*. Cada una de estas variables aporta una ventana específica de observación, y su análisis conjunto permite detectar alteraciones sutiles inducidas por sustancias exógenas, incluso en ausencia de signos morfológicos evidentes o de mortalidad. Esta aproximación permite interpretar al insecto como un sistema biológico complejo y como una entidad físicamente medible, cuyas respuestas pueden cuantificarse, modelarse y compararse bajo condiciones experimentales controladas. Así, el enfoque biofísico amplía las herramientas disponibles para el estudio de insectos y ofrece una base metodológica sólida para desarrollar indicadores funcionales aplicables al monitoreo ambiental, a la ecotoxicología y a la conservación de polinizadores.

Referencias

- [1] Khalifa SAM, Elshafiey EH, Shetaia AA, El-Wahed AAA, Algethami AF, Musharraf SG, et al. Overview of bee pollination and its economic value for crop production. *Insects* 2021;12. <https://doi.org/10.3390/insects12080688>.
- [2] Saha S, Munshi SA, Laskar N. Bee pollination: From an ecological and economical perspective. 2023.
- [3] Barbosa WF, Smagghe G, Guedes RNC. Pesticides and reduced-risk insecticides, native bees and pantropical stingless bees: Pitfalls and perspectives. *Pest Manag Sci* 2015;71:1049–53. <https://doi.org/10.1002/ps.4025>.
- [4] Fairbrother A, Purdy J, Anderson T, Fell R. Risks of neonicotinoid insecticides to honeybees. *Environ Toxicol Chem* 2014;33:719–31. <https://doi.org/10.1002/etc.2527>.

- [5] Zhang Z, Li Y, Li X, Zhu X, Zhang Y. Efficacy of Imidacloprid Seed Treatments against Four Wheat Aphids under Laboratory and Field Conditions. *Plants* 2023;12. <https://doi.org/10.3390/plants12020238>.
- [6] Su Y, Ren X, Hu H, Song X, Ma X, Wang D, et al. Sublethal effects of imidacloprid and clothianidin on the biological traits of predatory lacewing *Chrysopa pallens* (Rambur) (Neuroptera: Chrysopidae). *Crop Protection* 2023;163. <https://doi.org/10.1016/j.cropro.2022.106117>.
- [7] Sánchez-Bayo F, Belzunces L, Bonmatin JM. Lethal and sublethal effects, and incomplete clearance of ingested imidacloprid in honey bees (*Apis mellifera*). *Ecotoxicology* 2017;26:1199–206. <https://doi.org/10.1007/s10646-017-1845-9>.
- [8] Parkinson RH, Fecher C, Gray JR. Chronic exposure to insecticides impairs honeybee optomotor behaviour. *Frontiers in Insect Science* 2022;2. <https://doi.org/10.3389/finsc.2022.936826>.
- [9] Williamson SM, Willis SJ, Wright GA. Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology* 2014;23:1409–18. <https://doi.org/10.1007/s10646-014-1283-x>.
- [10] Tosi S, Nieh JC. A common neonicotinoid pesticide, thiamethoxam, alters honey bee activity, motor functions, and movement to light. *Sci Rep* 2017;7. <https://doi.org/10.1038/s41598-017-15308-6>.
- [11] García LM, Caicedo-Garzón V, Riveros AJ. Oral administration of phytochemicals protects honey bees against cognitive and motor impairments induced by the insecticide fipronil. *PLoS One* 2024;19:1–17. <https://doi.org/10.1371/journal.pone.0300899>.
- [12] Alkassab AT, Kirchner WH. Assessment of acute sublethal effects of clothianidin on motor function of honeybee workers using video-tracking analysis. *Ecotoxicol Environ Saf* 2018;147:200–5. <https://doi.org/10.1016/j.ecoenv.2017.08.047>.
- [13] Xu X, Wang X, Yang Y, Ares I, Martínez M, Lopez-Torres B, et al. Neonicotinoids: mechanisms of systemic toxicity based on oxidative stress-mitochondrial damage. *Arch Toxicol* 2022;96:1493–520. <https://doi.org/10.1007/s00204-022-03267-5>.
- [14] Dirilgen T, Herbertsson L, O'Reilly AD, Mahon N, Stanley DA. Moving past neonicotinoids and honeybees: A systematic review of existing research on other insecticides and bees. *Environ Res* 2023;235. <https://doi.org/10.1016/j.envres.2023.116612>.
- [15] Chen YR, Tzeng DTW, Ting C, Hsu PS, Wu TH, Zhong S, et al. Missing Nurse Bees—Early Transcriptomic Switch From Nurse Bee to Forager Induced by Sublethal Imidacloprid. *Front Genet* 2021;12. <https://doi.org/10.3389/fgene.2021.665927>.
- [16] Wang Z, Chen X, Becker F, Greggers U, Walter S, Werner M, et al. Honey bees infer source location from the dances of returning foragers. *Proc Natl Acad Sci U S A* 2023;120. <https://doi.org/10.1073/pnas.2213068120>.
- [17] Hartfelder K, Bitondi MMG, Brent CS, Guidugli-Lazzarini KR, Simões ZLP, Stabentheiner A, et al. Standard methods for physiology and biochemistry research in *Apis mellifera*. *J Apic Res* 2013;52. <https://doi.org/10.3896/IBRA.1.52.1.06>.
- [18] Li Z, Li M, He J, Zhao X, Chaimanee V, Huang WF, et al. Differential physiological effects of neonicotinoid insecticides on honey bees: A comparison between *Apis mellifera* and *Apis cerana*. *Pestic Biochem Physiol* 2017;140:1–8. <https://doi.org/10.1016/j.pestbp.2017.06.010>.
- [19] Klowden MJ. *PHYSIOLOGICAL SYSTEMS IN INSECTS THIRD EDITION*. 2013.
- [20] Vosshall LB, Carandini M. Sensory systems. vol. 19. 2009. <https://doi.org/10.1016/j.conb.2009.08.002>.

- [21] Hillyer JF, Pass G. The Insect Circulatory System: Structure, Function, and Evolution 2019. <https://doi.org/10.1146/annurev-ento-011019>.
- [22] Blow F, Douglas AE. The hemolymph microbiome of insects 1 2. 2019.
- [23] Chan QWT, Howes CG, Foster LJ. Quantitative comparison of caste differences in honeybee hemolymph. *Molecular and Cellular Proteomics* 2006;5:2252–62. <https://doi.org/10.1074/mcp.M600197-MCP200>.
- [24] Liu L, Yan F, Zhang L, Wu Z feng, Duan D yong, Cheng T yin. Protein profiling of hemolymph in *Haemaphysalis flava* ticks. *Parasit Vectors* 2022;15. <https://doi.org/10.1186/s13071-022-05287-7>.
- [25] Cao X, Wang Y, Rogers J, Hartson S, Kanost MR, Jiang H. Changes in composition and levels of hemolymph proteins during metamorphosis of *Manduca sexta*. *Insect Biochem Mol Biol* 2020;127. <https://doi.org/10.1016/j.ibmb.2020.103489>.
- [26] Frost EH, Shutler D, Hillier NK. Effects of cold immobilization and recovery period on honeybee learning, memory, and responsiveness to sucrose. *J Insect Physiol* 2011;57:1385–90. <https://doi.org/10.1016/j.jinsphys.2011.07.001>.
- [27] Farina WM, Arenas A, Estravis-Barcala MC, Palottini F. Targeted crop pollination by training honey bees: advances and perspectives. *Frontiers in Bee Science* 2023;1. <https://doi.org/10.3389/frbee.2023.1253157>.
- [28] Parkinson RH, Gray JR. Neural conduction, visual motion detection, and insect flight behaviour are disrupted by low doses of imidacloprid and its metabolites. *Neurotoxicology* 2019;72:107–13. <https://doi.org/10.1016/j.neuro.2019.02.012>.
- [29] Mota T, Roussel E, Sandoz J-C, Giurfa M. Visual conditioning of the sting extension reflex in harnessed honeybees. *Journal of Experimental Biology* 2011;214:3577–87. <https://doi.org/10.1242/jeb.062026>.
- [30] Utono IM, Adamu RS. Effect of belt expert (Flubendiamide + Thiacloprid), Imidacloprid, Thiamethoxam seed treatment and economic impact on fall armyworm (*Spodoptera frugiperda*) infestation on Maize in Nigeria. *Cogent Food Agric* 2023;9. <https://doi.org/10.1080/23311932.2022.2164117>.
- [31] Zhang Y, Huang L, Liu L, Cao X, Sun C, Lin X. Metabolic disturbance in lettuce (*Lactuca sativa*) plants triggered by imidacloprid and fenvalerate. *Science of the Total Environment* 2022;802. <https://doi.org/10.1016/j.scitotenv.2021.149764>.
- [32] Erguven GO, Demirci U. Using *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* for bioremediation of Imidacloprid. *Environ Technol Innov* 2021;21. <https://doi.org/10.1016/j.eti.2020.101236>.
- [33] Kurwadkar S, Evans A. Neonicotinoids: Systemic Insecticides and Systematic Failure. *Bull Environ Contam Toxicol* 2016;97:745–8. <https://doi.org/10.1007/s00128-016-1968-3>.
- [34] Fuentes E, Gaffard A, Rodrigues A, Millet M, Bretagnolle V, Moreau J, et al. Neonicotinoids: Still present in farmland birds despite their ban. 2023.
- [35] Tosi S, Nieh JC, Sgolastra F, Cabbri R, Medrzycki P. Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proceedings of the Royal Society B: Biological Sciences* 2017;284. <https://doi.org/10.1098/rspb.2017.1711>.
- [36] Gauthier M, Aras P, Paquin J, Boily M. Chronic exposure to imidacloprid or thiamethoxam neonicotinoid causes oxidative damages and alters carotenoid-retinoid levels in caged honey bees (*Apis mellifera*). *Sci Rep* 2018;8. <https://doi.org/10.1038/s41598-018-34625-y>.

- [37] Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, et al. Worldwide pesticide usage and its impacts on ecosystem. *SN Appl Sci* 2019;1. <https://doi.org/10.1007/s42452-019-1485-1>.
- [38] Fouad MR, Abdel-Raheem SAA. An overview on the fate and behavior of imidacloprid in agricultural environments. *Environmental Science and Pollution Research* 2024. <https://doi.org/10.1007/s11356-024-35178-6>.
- [39] Asif MU, Muhammad R, Sohail M. Comparative efficacy of Neem derivatives and imidacloprid against some cotton pests. 2018.
- [40] Ozoe Y, Matsubara Y, Tanaka Y, Yoshioka Y, Ozoe F, Shiotsuki T, et al. Controlled expression of nicotinic acetylcholine receptor-encoding genes in insects uncovers distinct mechanisms of action of the neonicotinoid insecticide dinotefuran. *Pestic Biochem Physiol* 2023;191. <https://doi.org/10.1016/j.pestbp.2023.105378>.
- [41] Cartereau A, Taillebois E, Le Questel JY, Thany SH. Mode of action of neonicotinoid insecticides imidacloprid and thiacloprid to the cockroach *pamea*7 nicotinic acetylcholine receptor. *Int J Mol Sci* 2021;22. <https://doi.org/10.3390/ijms22189880>.
- [42] Aguiar JMRBV, Nocelli RCF, Giurfa M, Nascimento FS. Neonicotinoid effects on tropical bees: Imidacloprid impairs innate appetitive responsiveness, learning and memory in the stingless bee *Melipona quadrifasciata*. *Science of the Total Environment* 2023;877. <https://doi.org/10.1016/j.scitotenv.2023.162859>.
- [43] Marchand PA. Regulatory Evolution of Neonicotinoid Insecticides as Plant Protection Active Substances in Europe. *Agrochemicals* 2023;2:446–57. <https://doi.org/10.3390/agrochemicals2030025>.
- [44] Paloschi CL, Tavares MHF, Berte EA, Model K, Rosa KM, Conceição FG da, et al. Imidacloprid: Impact on Africanized *Apis mellifera* L. (Hymenoptera: Apidae) workers and honey contamination. *Chemosphere* 2023;338:139591. <https://doi.org/10.1016/j.chemosphere.2023.139591>.
- [45] Suchail S, Debrauwer L, Belzunces LP. Metabolism of imidacloprid in *Apis mellifera*. *Pest Manag Sci* 2004;60:291–6. <https://doi.org/10.1002/ps.772>.
- [46] Suzuki T, Kurokoh R, Murakami S, Takahashi N, Matsuura A, Katsu K, et al. Rutin Concentration and Characterization of Rutinosidase in Perennial Buckwheat (*Fagopyrum cymosum*) and Its Application in Foods. *Foods* 2023;12. <https://doi.org/10.3390/foods12071417>.
- [47] Kianersi F, Abdollahi MR, Mirzaie-asl A, Dastan D, Rasheed F. Identification and tissue-specific expression of rutin biosynthetic pathway genes in *Capparis spinosa* elicited with salicylic acid and methyl jasmonate. *Sci Rep* 2020;10. <https://doi.org/10.1038/s41598-020-65815-2>.
- [48] Cristiano MC, Barone A, Mancuso A, Torella D, Paolino D. Rutin-loaded nanovesicles for improved stability and enhanced topical efficacy of natural compound. *J Funct Biomater* 2021;12. <https://doi.org/10.3390/jfb12040074>.
- [49] Lee LC, Hou YC, Hsieh YY, Chen YH, Shen YC, Lee IJ, et al. Dietary supplementation of rutin and rutin-rich buckwheat elevates endogenous glucagon-like peptide 1 levels to facilitate glycemic control in type 2 diabetic mice. *J Funct Foods* 2021;85. <https://doi.org/10.1016/j.jff.2021.104653>.
- [50] Riveros AJ, Gronenberg W. The flavonoid rutin protects the bumble bee *Bombus impatiens* against cognitive impairment by imidacloprid and fipronil. *Journal of Experimental Biology* 2022;225. <https://doi.org/10.1242/jeb.244526>.

- [51] Karashchuk L, Li JS, Chou GM, Walling-Bell S, Brunton SL, Tuthill JC, et al. Sensorimotor delays constrain robust locomotion in a 3D kinematic model of fly walking. *Elife* 2025;13. <https://doi.org/10.7554/eLife.99005>.
- [52] Zimmer M. Insect locomotion: Flies show you how to stay on course. *Current Biology* 2021;31:R1395–7. <https://doi.org/10.1016/j.cub.2021.09.015>.
- [53] Scholarship@western S, Macmillan HA, Sinclair BJ. Ionic and Osmotic Mechanisms Of Insect Chill-Coma And Chilling Injury. 2013.
- [54] Ali H, Alqarni AS, Owayss AA, Hassan AM, Smith BH. Osmotic concentration in three races of honey bee, *Apis mellifera* L. under environmental conditions of arid zone. *Saudi J Biol Sci* 2017;24:1081–5. <https://doi.org/10.1016/j.sjbs.2016.12.006>.
- [55] Method for collecting hemolymph of insects n.d.
- [56] Jaffrin MY, Morel H. Body fluid volumes measurements by impedance: A review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods. *Med Eng Phys* 2008;30:1257–69. <https://doi.org/10.1016/j.medengphy.2008.06.009>.
- [57] Soares C, Tenreiro Machado JA, Lopes AM, Vieira E, Delerue-Matos C. Electrochemical impedance spectroscopy characterization of beverages. *Food Chem* 2020;302. <https://doi.org/10.1016/j.foodchem.2019.125345>.
- [58] Leitzke JP, Zangl H. A review on electrical impedance tomography spectroscopy. *Sensors (Switzerland)* 2020;20:1–19. <https://doi.org/10.3390/s20185160>.
- [59] Zhu H, Hu B. Dielectric Properties of Aqueous Electrolyte Solutions Confined in Silica Nanopore: Molecular Simulation vs. Continuum-Based Models. *Membranes (Basel)* 2022;12. <https://doi.org/10.3390/membranes12020220>.

Objetivo general

Establecer un marco de evaluación funcional que permita identificar alteraciones comportamentales, hídricas y eléctricas en abejas *Apis mellifera* expuestas a imidacloprid y a la rutina, fortaleciendo las herramientas diagnósticas para monitorizar abejas bajo condiciones subletales de estrés por intoxicación.

Objetivos de investigación

- Analizar las respuestas motoras de abejas *Apis mellifera* expuestas a imidacloprid y a la rutina, identificando alteraciones funcionales observables en la locomoción, la extensión de la probóscide y del aguijón.
- Evaluar la masa corporal y el contenido hídrico como indicadores de equilibrio fisiológico, relacionando sus fluctuaciones con el consumo de sustancias y con la capacidad de controlar el agua corporal.
- Establecer un procedimiento estandarizado para la extracción del líquido extracelular, considerando las diferencias regionales entre los distintos segmentos corporales, con el fin de caracterizar su composición.
- Caracterizar las propiedades eléctricas del líquido extracelular y del tórax en abejas vivas expuestas a los tratamientos, identificando variaciones en la impedancia, la resistencia y la capacitancia como indicadores del estado funcional interno.
- Comparar los efectos del imidacloprid y de la rutina, así como su interacción, sobre las variables fisiológicas y comportamentales, para identificar patrones diferenciales que permitan inferir el impacto funcional del consumo.

Problema de investigación

Aunque los efectos letales del imidacloprid han sido ampliamente documentados, persisten vacíos importantes en la comprensión de sus efectos subletales sobre la fisiología y el comportamiento de las abejas. Los efectos nocivos del consumo de sustancias comprometen funciones esenciales, como la locomoción, la hidratación o la actividad eléctrica de los tejidos. No obstante, los métodos convencionales de evaluación resultan poco sensibles o carecen de integración entre distintos niveles funcionales. Sumado a esto, se requiere profundizar en el potencial modulador de los compuestos naturales frente al daño inducido por factores estresantes. En consecuencia, el desarrollo y la implementación de enfoques funcionales son necesarios para detectar y cuantificar estas alteraciones de manera sensible, reproducible y aplicable en contextos de laboratorio o campo.

Hipótesis de investigación

El consumo de imidacloprid altera de manera significativa el comportamiento, el estado hídrico y las propiedades eléctricas del cuerpo en abejas *Apis mellifera*, mientras que la administración conjunta de rutina atenúa parcialmente dichos efectos, actuando como modulador funcional del estado fisiológico del insecto.

Introducción general

Las abejas son insectos esenciales para la fecundación y la formación de semillas en las plantas con flores, lo que garantiza la diversidad y la supervivencia de numerosas especies vegetales y animales [1]. La polinización favorece la producción de frutos, que constituyen una fuente fundamental de alimento para los humanos y otras especies [2], lo cual contribuye a la seguridad alimentaria global y al equilibrio ecológico. Por ejemplo, alrededor del 75 % de las plantas, tanto agrícolas como silvestres, dependen de este servicio de polinización [3–5]. La interacción entre las plantas y estos polinizadores garantiza la disponibilidad de hábitats y recursos alimenticios [1,6–8], además de contribuir a la estabilidad ecológica a nivel global. Sin embargo, en los últimos años, las poblaciones de abejas han disminuido drásticamente debido a múltiples factores [9,10], lo que ha generado una creciente preocupación en las comunidades científicas, agrícolas y ambientales.

El descenso de las poblaciones de abejas se debe a la pérdida de hábitat [11], al uso de pesticidas [12] y al cambio climático [13], factores que afectan su comportamiento y fisiología. Las alteraciones de estos aspectos básicos, pero esenciales, ponen en riesgo la supervivencia de estos insectos, ya que comprometen la capacidad de buscar alimento y el funcionamiento del sistema nervioso. Por ejemplo, la exposición a insecticidas provoca desorientación, movimientos involuntarios [14] y pérdida de memoria [15], fenómenos asociados al deterioro de las funciones neuronales. El consumo de insecticidas debilita a los individuos, incrementando su susceptibilidad a enfermedades [16,17] y a cambios ambientales adversos, como la variación de la temperatura y la contaminación. Así, la interacción entre estas amenazas compromete la integridad del polinizador debido a alteraciones en su salud.

El neonicotinoide imidacloprid, insecticida ampliamente utilizado en el sistema agrícola, actúa como agonista de los receptores nicotínicos de acetilcolina (nAChR) [18] en el sistema nervioso central de los insectos, interfiriendo con la neurotransmisión. El mecanismo de acción de esta sustancia consiste en la unión irreversible a estos receptores [18], lo que provoca una activación sostenida y descontrolada de las neuronas, lo que puede conducir a la excitotoxicidad y a la muerte. Particularmente, las abejas expuestas a concentraciones subletales de imidacloprid han exhibido alteraciones en la memoria, el aprendizaje y la coordinación motora [19]; comprometiendo su capacidad de navegación y búsqueda de alimento. Además, el imidacloprid induce cambios en la fisiología de las abejas, como la reducción de la actividad enzimática antioxidante [20] y alteraciones de la homeostasis iónica, lo que interfiere con su fisiología.

Siendo expuestas las vulnerabilidades de las abejas a diversos factores ambientales y químicos, los mecanismos de cuidado resultan esenciales para preservar a este polinizador. En este contexto, las estrategias terapéuticas con sustancias naturales han ganado atención, este es el caso de los flavonoides debido a sus propiedades antioxidantes [21,22]. En particular, la rutina ha demostrado efectos beneficiosos en la función cognitiva de las abejas [23,24], atenuando el deterioro de la memoria y del aprendizaje inducido por ciertos agroquímicos. Estudios han señalado que este

compuesto podría contribuir a la recuperación de la actividad neuronal en insectos expuestos a neurotóxicos, mejorando su capacidad de orientación y de recolección de alimento. Sin embargo, aunque la rutina ha mostrado potencial para mitigar el daño cognitivo, aún se requieren evaluaciones fisiológicas detalladas para identificar posibles efectos secundarios asociados a su consumo.

En este escenario de creciente vulnerabilidad y necesidad de conservación, resulta fundamental contar con estrategias de monitoreo que permitan la detección temprana y precisa de las alteraciones funcionales inducidas por agentes ambientales. Así se propone abordar algunas de estas problemáticas mediante la física, una disciplina que cuenta con herramientas procedimentales y teóricas que facilitan la observación y el análisis. Por ejemplo, mediante el diseño y la evaluación de modelos experimentales es posible acceder a indicadores sensibles del estado funcional del insecto. En particular, mediante la evaluación del comportamiento y de las propiedades eléctricas de las abejas, se podría obtener información relevante sobre alteraciones de la homeostasis del sistema biológico. Así, un enfoque interdisciplinar entre biología y física permitiría construir una lectura integrada de la salud del insecto a partir de elementos teóricos y procedimentales. Mediante el enfoque físico, se podrán establecer modelos que garanticen la precisión y la exactitud de los datos experimentales.

El presente estudio se articula en torno a la necesidad de diseñar y evaluar sistemas de monitorización de cambios comportamentales y fisiológicos en abejas. Lo que se llevará a cabo mediante un conjunto de experimentos para explorar los efectos del consumo de sustancias nocivas y benéficas en las abejas *Apis mellifera*. Las exploraciones tendrán un componente comportamental y otro fisiológico; ambos servirán para detectar alteraciones motoras y cambios osmóticos en las abejas. A través de las estrategias de observación y análisis funcional, se espera que puedan aplicarse en contextos tanto de laboratorio como de campo. Esta aproximación propone integrar los desafíos biológicos con herramientas físicas para desarrollar diagnósticos sensibles, reproducibles y aplicables a la realidad ecológica de las abejas. Estas observaciones plantean un interrogante central, sobre la detección integrada y sensible de las alteraciones funcionales subletales inducidas por agroquímicos en abejas *Apis mellifera*.

Estructura del documento y alternancia idiomática

La presente tesis doctoral se ha desarrollado en la modalidad de compendio de publicaciones, de acuerdo con la normativa institucional de la Universidad del Rosario para optar al título de doctor. En consecuencia, el documento presenta una alternancia idiomática planificada y justificada: los componentes de articulación macro, correspondientes a la Introducción General, los Fundamentos Teóricos, los Objetivos, la Discusión General y las Conclusiones y Perspectivas, se exponen en español para salvaguardar la unidad conceptual y el hilo conductor de la investigación.

Por su parte, el núcleo de la tesis (Capítulos 1 al 5) está constituido por los manuscritos científicos derivados de la investigación, que han sido publicados, aceptados en revistas indexadas de alta visibilidad internacional (como Scientific Reports, PLOS ONE e Integrative Organismal Biology). Debido a los derechos y exigencias editoriales de dichas revistas, estos capítulos se presentan íntegramente en su idioma original de publicación (inglés). Con el fin de garantizar el rigor académico y guiar la lectura, cada uno de estos apartados está precedido por un preámbulo explicativo y un resumen en castellano que contextualiza el alcance del estudio físico-biológico dentro de la hipótesis general del trabajo.

Referencias

- [1] Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, et al. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 2007;274:303–13. <https://doi.org/10.1098/rspb.2006.3721>.
- [2] Decourtye A, Alaux C, Le Conte Y, Henry M. Toward the protection of bees and pollination under global change: present and future perspectives in a challenging applied science. *Curr Opin Insect Sci* 2019;35:123–31. <https://doi.org/10.1016/j.cois.2019.07.008>.
- [3] Rocha FH, Peraza DN, Medina S, Quezada-Euán JJG. Pollination service provided by honey bees to buzz-pollinated crops in the Neotropics. *PLoS One* 2023;18. <https://doi.org/10.1371/journal.pone.0280875>.
- [4] Siviter H, Fisher A, Baer B, Brown MJF, Camargo IF, Cole J, et al. Protecting pollinators and our food supply: understanding and managing threats to pollinator health. *Insectes Soc* 2023;70:5–16. <https://doi.org/10.1007/s00040-022-00897-x>.
- [5] Saha S, Munshi SA, Laskar N. Bee pollination: From an ecological and economical perspective. 2023.
- [6] Baylis K, Lichtenberg EM, Lichtenberg E. *Economics of Pollination* 2021. <https://doi.org/10.1146/annurev-resource-101420>.
- [7] Hanberry BB, DeBano SJ, Kaye TN, Rowland MM, Hartway CR, Shorrock D. Pollinators of the Great Plains: Disturbances, Stressors, Management, and Research Needs. *Rangel Ecol Manag* 2021;78:220–34. <https://doi.org/10.1016/j.rama.2020.08.006>.
- [8] van der Sluijs JP, Vaage NS. Pollinators and Global Food Security: the Need for Holistic Global Stewardship. *Food Ethics* 2016;1:75–91. <https://doi.org/10.1007/s41055-016-0003-z>.
- [9] Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: Trends, impacts and drivers. *Trends Ecol Evol* 2010;25:345–53. <https://doi.org/10.1016/j.tree.2010.01.007>.
- [10] Nath R, Singh H, Mukherjee S. Insect pollinators decline: an emerging concern of Anthropocene epoch. *J Apic Res* 2023;62:23–38. <https://doi.org/10.1080/00218839.2022.2088931>.
- [11] Mendes SB, Timóteo S, Loureiro J, Castro S. The impact of habitat loss on pollination services for a threatened dune endemic plant. *Oecologia* 2022;198:279–93. <https://doi.org/10.1007/s00442-021-05070-y>.
- [12] Abati R, Sampaio AR, Mendes R, Maciel A, Colombo FC, Libardoni G, et al. Bees and pesticides: the research impact and scientometrics relations 2021. <https://doi.org/10.1007/s11356-021-14224-7>/Published.

- [13] Switanek M, Crailsheim K, Truhetz H, Brodschneider R. Modelling seasonal effects of temperature and precipitation on honey bee winter mortality in a temperate climate. *Science of the Total Environment* 2017;579:1581–7. <https://doi.org/10.1016/j.scitotenv.2016.11.178>.
- [14] Rigosi E, O'Carroll DC. Acute Application of Imidacloprid Alters the Sensitivity of Direction Selective Motion Detecting Neurons in an Insect Pollinator. *Front Physiol* 2021;12. <https://doi.org/10.3389/fphys.2021.682489>.
- [15] Delkash Roudsari S, Hossein Goldansaz S, Talebi-Jahromi K, Abramson CI. Learning, memory, and sensory perception are impaired by exposure to the organophosphate, ethion, and the insect growth regulator, hexaflumuron, in honey bees (*Apis mellifera* L.). *J Asia Pac Entomol* 2024;27. <https://doi.org/10.1016/j.aspen.2024.102202>.
- [16] Bartling MT, Brandt A, Hollert H, Vilcinskas A. Current Insights into Sublethal Effects of Pesticides on Insects. *Int J Mol Sci* 2024;25. <https://doi.org/10.3390/ijms25116007>.
- [17] Pham-Delègue MH, Decourtye A, Kaiser L, Devillers J. Behavioural methods to assess the effects of pesticides on honey bees. *Apidologie* 2002;33:425–32. <https://doi.org/10.1051/apido:2002033>.
- [18] Ozoe Y, Matsubara Y, Tanaka Y, Yoshioka Y, Ozoe F, Shiotsuki T, et al. Controlled expression of nicotinic acetylcholine receptor-encoding genes in insects uncovers distinct mechanisms of action of the neonicotinoid insecticide dinotefuran. *Pestic Biochem Physiol* 2023;191. <https://doi.org/10.1016/j.pestbp.2023.105378>.
- [19] Paus-Knudsen JS, Sveinsson HA, Grung M, Borgå K, Nielsen A. The Neonicotinoid Imidacloprid Impairs Learning, Locomotor Activity Levels, and Sucrose Solution Consumption in Bumblebees (*Bombus terrestris*). *Environ Toxicol Chem* 2023;42:1337–45. <https://doi.org/10.1002/etc.5611>.
- [20] Balieira KVB, Mazzo M, Bizerra PFV, Guimarães AR de JS, Nicodemo D, Mingatto FE. Imidacloprid-induced oxidative stress in honey bees and the antioxidant action of caffeine. *Apidologie* 2018;49:562–72. <https://doi.org/10.1007/s13592-018-0583-1>.
- [21] Küçükler S, Kandemir FM, Özdemir S, Çomaklı S, Caglayan C. Protective Effects of Rutin Against Deltamethrin-Induced Hepatotoxicity and Nephrotoxicity in Rats via Regulation of Oxidative Stress, Inflammation and Apoptosis 2021. <https://doi.org/10.21203/rs.3.rs-392087/v1>.
- [22] Rahmani S, Naraki K, Roohbakhsh A, Hayes AW, Karimi G. The protective effects of rutin on the liver, kidneys, and heart by counteracting organ toxicity caused by synthetic and natural compounds. *Food Sci Nutr* 2023;11:39–56. <https://doi.org/10.1002/fsn3.3041>.
- [23] Riveros AJ, Gronenberg W. The flavonoid rutin protects the bumble bee *Bombus impatiens* against cognitive impairment by imidacloprid and fipronil. *Journal of Experimental Biology* 2022;225. <https://doi.org/10.1242/jeb.244526>.
- [24] Koçancı FG, Aslim B. Neuroprotective Effects of Rutin and Quercetin Flavonoids in *Glaucium corniculatum* Methanol and Water Extracts. *International Journal of Secondary Metabolite* 2017;85–93. <https://doi.org/10.21448/ijsm.363347>.

Capítulo 1. Extracción y análisis óptico del líquido extracelular de los segmentos corporales de abejas *Apis mellifera*

J Hernández, F Mesa, A Riveros, R Fayad, J Nisperuza

Publicado en: Integrative Organismal Biology

Published: May 09 2025

<https://doi.org/10.1093/iob/obaf018>

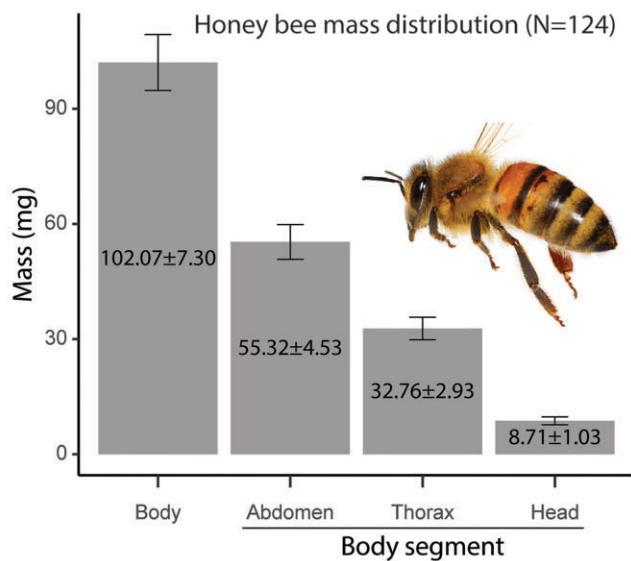
Resumen

Este estudio desarrolló y optimizó una metodología basada en la centrifugación controlada para la extracción segmentada del líquido extracelular en abejas *Apis mellifera*. El estudio se realizó con tres variables: la fuerza centrífuga relativa (RCF), el tiempo de centrifugación y el número de individuos procesados, lo que garantizó la reproducibilidad y la eficiencia del procedimiento. Los resultados mostraron diferencias significativas en el volumen de fluido recuperado entre los distintos segmentos corporales, siendo el abdomen el que presentó los mayores volúmenes, seguido del tórax y de la cabeza. La caracterización espectroscópica UV-Vis reveló propiedades ópticas distintivas en las muestras, lo que permitió identificar picos de absorbancia específicos de cada segmento. Además, el análisis bioquímico con el reactivo de Benedict confirmó la presencia de azúcares reducidos, y las muestras de la cabeza mostraron una coloración más intensa. Estos hallazgos subrayan la importancia del análisis segmentado para comprender con mayor profundidad la fisiología y el metabolismo de las abejas. La extracción propuesta constituye una herramienta novedosa y robusta para estudios fisiológicos, metabólicos y ecotoxicológicos, lo que facilita la evaluación de los impactos ambientales y de los contaminantes sobre la salud de los polinizadores.

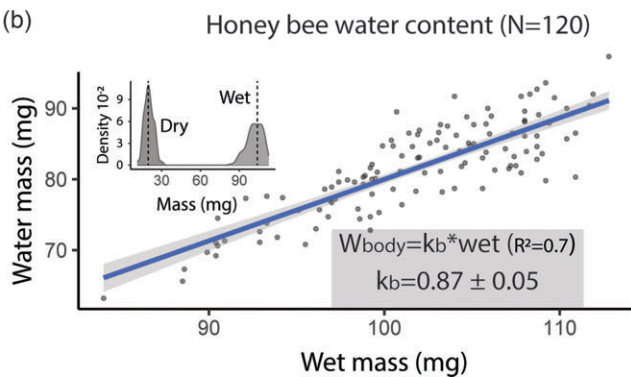
Notas metodológicas aclaratorias:

Para asegurar la pureza de las fracciones aisladas del líquido extracelular (LEC), el filtrado se realizó mediante membranas de microfiltración de acetato de celulosa con un diámetro de poro de 0.2µm. La reproducibilidad del método de centrifugación segmentada se contrastó mediante el coeficiente de variación técnica entre ensayos para los volúmenes recuperados en la cabeza, el tórax y el abdomen. Como control negativo del proceso de extracción, se emplearon capilares vacíos procesados bajo idénticas condiciones de fuerza centrífuga relativa (RCF) y tiempo, mientras que soluciones acuosas estándar de calibración refractométrica sirvieron como control positivo operacional. Adicionalmente, se consigna como recomendación prospectiva de control de calidad para futuras investigaciones que se basen en esta técnica para la cuantificación de organelos resultantes de la ruptura celular, la cual constituye una limitación analítica no controlada en el presente ensayo, ya que opera por debajo del umbral de filtración.

(a)

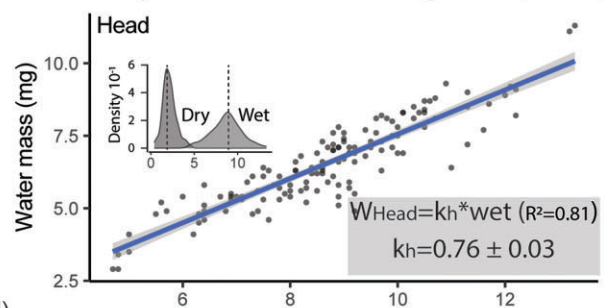


(b)

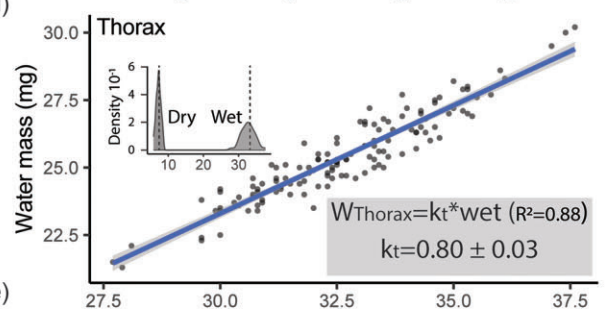


(c)

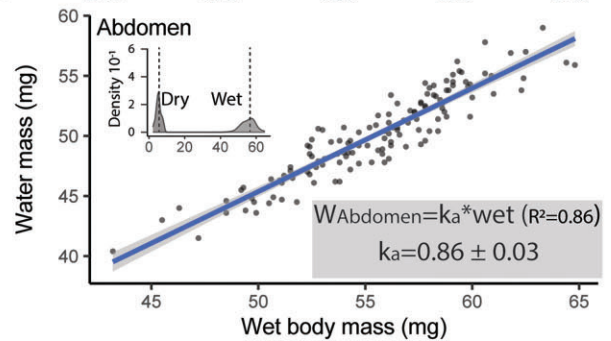
Honey bee water content in segments (N=131)



(d)



(e)



Integrative Organismal Biology

A Journal of the Society
for Integrative and
Comparative Biology

academic.oup.com/iob



OXFORD
UNIVERSITY PRESS

ARTICLE

Extraction and Optical Analysis of the Extracellular Fluid from the Body Segments of *Apis mellifera* Bees

J. Hernández*,¹ F. Mesa^{†,2}, A. Riveros*, R. Fayad* and J. Nisperuza[†]

*Departamento de Biología, Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia; [†]NanoTech Group, Facultad de Ingeniería y Ciencias Básicas, Fundación Universitaria Los Libertadores, Cra 16 No. 63a-68, Bogotá 111221, Colombia

¹E-mail: juanp.hernandez@urosario.edu.co

²E-mail: fredy.mesa@libertadores.edu.co

Synopsis This study developed and optimized a methodology based on controlled centrifugation for the segmented extraction of extracellular fluid in *Apis mellifera* bees. Three critical variables were analyzed: relative centrifugal force, centrifugation time, and the number of individuals processed, ensuring the reproducibility and efficiency of the procedure. The results demonstrated significant differences in the volume of fluid recovered from different body segments, with the abdomen yielding the highest volumes, followed by the thorax and the head. UV-Vis spectroscopic characterization revealed distinct optical features for the samples, identifying specific absorbance peaks unique to each segment. Furthermore, biochemical analysis using Benedict's reagent confirmed the presence of reducing sugars, with head samples displaying the most intense coloration. These findings underscore the importance of segment-specific analysis to gain deeper insights into the physiology and metabolism of bees. The proposed methodology offers a novel and robust tool for physiological, metabolic, and ecotoxicological studies, facilitating the assessment of environmental and contaminant impacts on pollinator health.

Introduction

The extracellular fluid in insects plays essential roles in the transport of nutrients, hormones, and metabolites (Chen et al. 2021; Strachecka et al. 2022; Elfar et al. 2023a; Tafi et al. 2024; Wang et al. 2021), as well as in osmotic regulation (Nakamatsu and Tanaka 2004; Cao et al. 2020; Holdbrook et al. 2024) and the response to stress agents (Tauchman et al. 2007; Lubawy and Słocińska 2020). In *Apis mellifera* bees, studying this fluid is key to assessing their physiological and metabolic state, especially under environmental stress conditions and contaminant exposure (Brandt et al. 2016; Kim et al. 2022). However, existing methodologies for its extraction have significant limitations regarding volume control, reproducibility, and regional resolution.

Traditional methods for fluid extraction in insects, such as using hypodermic needles or glass capillaries, focus on globally and uniformly extracting hemolymph (Borsuk et al. 2017; Butolo et al. 2020). These approaches assume that the extracellular fluid is homoge-

neously distributed throughout the insect's body, overlooking physiological and metabolic differences among body segments, such as the head, thorax, and abdomen. Previous studies have demonstrated that these regions have specialized energy demands and functions. For instance, the abdomen stores large quantities of liquid and energy reserves, whereas the thorax, dominated by muscle tissue, exhibits highly active metabolism to support flight (Blow and Douglas 2019; Chang et al. 2023).

Recently, centrifugation-based methods have been used as an alternative for efficient extracellular fluid extraction in insects (Tabunoki et al. 2019; Niu et al. 2023). However, these approaches have not considered the segmented extraction of body parts or systematically evaluated physical parameters such as angular velocity, centrifugation time, and the number of individuals processed. The segmented extraction of extracellular fluid by body regions is essential to obtain detailed information on regional metabolic processes, enabling more precise comparative analyses.

This study aimed to develop and optimize an efficient method for the segmented extraction of extracellular fluid in *Apis mellifera* bees using controlled centrifugation. Additionally, the extracted samples were optically characterized using UV-Vis spectroscopy to detect optical absorbances (Schenk et al. 2015; Park et al. 2017; Ushakova et al. 2019). Extractions were also conducted to identify the presence of reducing sugars using Benedict's reagent, as previously performed in studies with insects and food samples (Park et al. 2017; Ratanawimarnwong et al. 2022). The centrifugation method provides control over the volume and localization of the extracted fluid, offering a robust tool for physiological (Bogaerts et al. 2009; Elfar et al. 2023a; Kannan et al. 2024; Li et al. 2024), metabolic (Cournoyer et al. 2022; Chang et al. 2023; Wang et al. 2021), ecotoxicological (Paleolog et al. 2020; Migdał et al. 2021), and comparative studies in insects (Chan et al. 2006; Elfar et al. 2023b).

Traditional hemolymph extraction techniques, such as puncturing the cuticle with glass capillaries or hypodermic needles, generally produce pooled fluid from various tissues, limiting the resolution of region-specific physiological information. The method proposed here enables segment-specific sampling through centrifugation, offering the possibility of detecting localized metabolic and physiological variations that remain masked by conventional approaches (Borsuk et al. 2017; Butolo et al. 2020). While previous centrifugation-based strategies have demonstrated high efficiency in extracting hemolymph in other insect species, they have excluded both segmental separation and systematic evaluation of physical variables such as angular velocity, extraction time, and number of individuals processed (Tabunoki et al. 2019; Niu et al. 2023).

The current methodology incorporates moderate centrifugal forces and low temperatures to preserve tissue integrity and promote the recovery of extracellular fluid. The design avoids the use of sonication, chemical lysis, or mechanical disruption, which helps maintain cell structure throughout the extraction process. The analytical scope focused on optical and chromatic characterization, and the segment-dependent consistency of the results supports the interpretation that the recovered fluid predominantly reflects extracellular composition. Additional considerations regarding fluid purity and possible intracellular contributions are addressed in the discussion.

Materials and methods

Experimental animals

The Africanized *Apis mellifera* bees used in this study were collected from the apiary at the Universidad del

Rosario (Bogotá, Colombia). Collection was conducted during the early morning hours to minimize thermal stress and ensure that the insects were in optimal physiological condition (Abou-Shaara et al. 2012; Butolo et al. 2021; Stupski and Schilder 2021). Bees were manually captured using plastic containers (disposable cups) with ventilation slots to allow respiration, as recommended in various protocols (Köhler et al. 2013; Williams et al. 2013; Huang et al. 2014). Immediately after collection, they were transported to the laboratory, where they were kept under controlled temperature conditions ($35 \pm 3^\circ\text{C}$), constant relative humidity ($60 \pm 5\%$), and specific light wavelengths (730 nm) to avoid stress caused by the presence of the experimenter (Dexheimer et al. 2022; Somanathan 2024; Wang et al. 2022). The bees were fed ad libitum with a 1 M sucrose solution (31.1% w/w) provided through feeders (Fig. 1A).

In all experiments, the bees were euthanized by exposure to a temperature of -30°C in a cooling unit (Thermo Scientific Revco Freezer UGL3020A), where they remained for 24 h. The bees were then prepared for the experiments, (1) body mass measurement, (2) extracellular fluid extraction, (3) UV-Vis spectroscopic characterization, and (4) sugar detection. All activities involving the use and handling of bees were carried out in compliance with ethical standards for working with bees ensuring conditions that minimized stress to the individuals.

Experimental setup for extracellular fluid extraction

The experimental setup for extracting extracellular fluid was designed using common laboratory materials, such as pipette tips and conical centrifuge tubes. The pipette tips used were 1000 μL tips with a $0.2 \mu\text{m}$ filter (pore size), and the tubes had a capacity of 15 mL. The narrow ends of the tips were cut, resulting in lengths of $L1 = 42.0 \pm 0.1 \text{ mm}$ and $L2 = 62.0 \pm 0.1 \text{ mm}$. The filter from L1 was removed and inserted into L2, and the final tip (L) was obtained by nesting L1 into L2 (Fig. 1B). The bees were dissected into 3 segments (head, thorax, and abdomen), which were placed into the final tip (L), and this system was then inserted into the centrifuge tubes. Each segment was supplemented with 500 μL of distilled water to preserve the extracted extracellular fluid and facilitate sample flow (Fig. 1C). Finally, the setup was placed in a centrifuge (Thermo Scientific ST 16R; rot: 75003181) programmed with a constant temperature (3°C), angular velocity (relative centrifugal force [RCF]), and time (min).

The extraction of extracellular fluid was based on the centrifugal force applied to the body segments:

$$F_c = MR\omega^2, \quad (1)$$

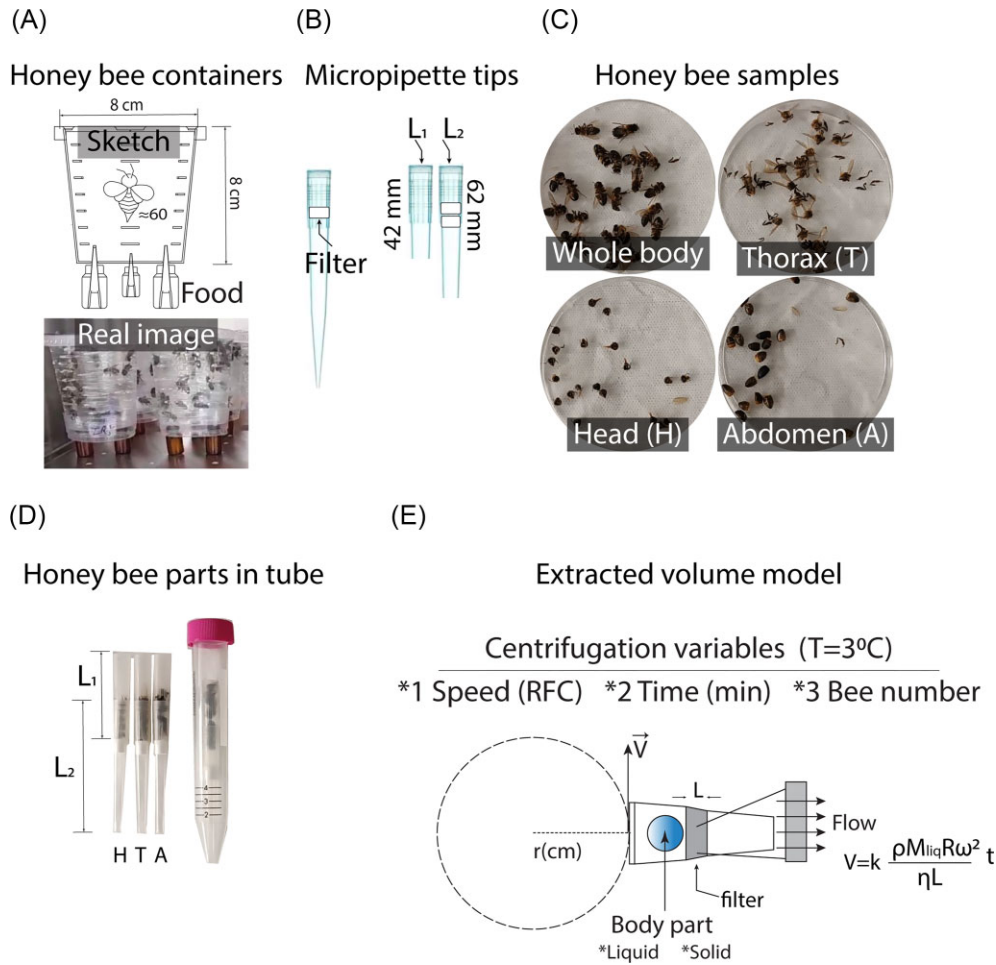


Fig. 1 Experimental setup for bee maintenance and extracellular fluid extraction. (A) Bees in containers with feeders. (B) Modified pipette tips. (C) Dissection of insects and final assembly in conical tubes. (D) Model of the extraction process.

where M is the mass of the body segment, R represents the effective centrifugation radius, and ω is the angular velocity. The volume of extracellular fluid extracted (V_{ext}) was estimated considering the pressure difference generated during centrifugation, following the equation:

$$V_{ext} = \frac{k\rho M_{liq}R\omega^2}{\eta L}t, \quad (2)$$

where k is the system's proportionality constant, ρ is the fluid density, corresponding to the mass of the extracted fluid, η is the fluid's viscosity, L is the effective length of the filter, and t is the centrifugation time (Fig. 1D).

These equations provide the theoretical foundation for the extraction system. The centrifugal force formula illustrates how differences in segment mass and angular velocity influence the mechanical stress applied during centrifugation. The second expression outlines how fluid extraction depends on pressure gradients, viscosity, and filter geometry. Although no direct calculations were performed using these equations, they serve to

conceptually support the design of the extraction setup and the interpretation of experimental trends across segments and conditions.

At the end of the centrifugation process, the tips were carefully removed from the conical tubes, and the recovered extracellular fluid was transferred to Eppendorf tubes for storage at -20°C . The samples obtained were subsequently used for optical analysis through UV-Vis spectroscopy and Benedict's reagent tests.

Experiment 1: Distribution of body mass and water content in *Apis mellifera*

The first experiment aimed to characterize the distribution of body mass and water content in *Apis mellifera*, both at the whole-body level and within its main body segments (head, thorax, and abdomen). This experiment included 3 main evaluations, (1) body mass distribution; (2) water content in the whole body; and (3) water content in the body segments. These evaluations sought to address questions related to the

distribution of body mass, water content, and its allocation among body segments.

Body mass distribution

To determine the distribution of body mass, the total mass of individual bees was measured by using an analytical balance with a precision of 0.1 mg. Subsequently, each bee was dissected into its main body segments: head, thorax, and abdomen. The mass of each segment was recorded individually immediately after dissection to prevent fluid loss due to evaporation. This data allowed for the calculation of mass distribution within the bee's body, providing a detailed characterization of the proportion of mass attributed to each segment.

Water content in the body of bees

The water content in the body was determined by measuring the wet and dry mass. Initially, the mass of each bee was measured immediately after capture, referred to as the wet mass (Wet). Subsequently, the insects were dried in an oven at 70°C for 3 days to remove all body water. At the end of this process, the dry mass (Dry) was measured. The body water content (Water) was calculated as the difference between the wet and dry mass ($\text{Water} = \text{Wet} - \text{Dry}$). Additionally, a linear regression analysis was performed between Water and Wet to develop a model describing the relationship between water content and total body mass. The slope of the model was used as the body water mass factor.

Water content in body segments

The bees were dissected into their main body segments (head, thorax, and abdomen) to evaluate the water content in each segment. The previously described protocol for measuring wet and dry mass was applied to each segment. The data obtained provided a precise reference for the distribution of water among the body segments, offering essential information for interpreting the results of extracellular fluid extraction.

Experiment 2: Extracellular fluid extraction from body segments using centrifugation

The extraction of extracellular fluid in *Apis mellifera* was conducted using centrifugation methods for each body segment. The experiment was designed to evaluate the variables of angular velocity, centrifugation time, and the number of insects. These evaluations aimed to address questions regarding the amount of extracellular fluid that could be obtained with this method and how centrifugation variables influence the extraction process.

The extraction process was evaluated using 3 variables: angular speed (Speed), centrifugation time (Time), and the number of bees (#Bees). The in-

dependent variables were tested at 5 levels: Speed (RCF) = 400, 1000, 1600, 2200, 2900; Time (min) = 2, 4, 6, 8, 10; #Bees = 2, 4, 6, 8, 10. The extraction process was designed to vary one of the 3 variables at a time while keeping the other two constant. The final evaluation of all variable changes was designed to converge at the following values: Speed = 2900 RCF; Time = 10 min; #Bees = 10 bees. The quantification of the extracted extracellular fluid was performed by measuring the mass of the samples using a precision balance (Radwag AS 220.R2) before and after centrifugation. The extracted mass was determined by the difference between the two measurements, with 5 repetitions conducted for each evaluation.

For each condition, segments from multiple bees (2, 4, 6, 8, or 10) were pooled and centrifuged together as a single sample. The mass of extracted fluid was calculated as the difference between the total mass of the container system before and after centrifugation, allowing quantification of the fluid derived exclusively from the insect tissue. Although 500 μL of distilled water was added before centrifugation to preserve fluidity and minimize sample loss, this added water was not included in the extracted mass, as it remained in the upper portion of the container and did not interfere with the measurement of the recovered fluid.

Experiment 3: UV-Vis spectroscopic characterization of extracellular fluid from body segments

The characterization was performed on the extracellular fluid extracted from the body segments of bees, which were processed through centrifugation at 2000 RCF for 4 min; this process was repeated 10 times. The fluid samples were analyzed using UV-Vis spectroscopy to explore differences among the samples.

The experiment was designed to evaluate the absorbance of the segments (head, thorax, and abdomen) across a wavelength interval of 200–590 nm, utilizing a small-sample spectrophotometer (Thermo Scientific NanoDrop 2000). This experiment aimed to address questions related to the optical absorbance of extracellular fluid based on body segments, as well as to investigate differences among the segment-derived samples.

Experiment 4: Sugar test for extracellular fluid using Benedict's reagent

The reduced sugar test was conducted on extracellular fluid samples extracted using the centrifugation method (Bees = 5; ω = 2000 RCF; t = 4 min); the extractions were repeated 16 times. In test tubes, extracellular fluid samples (0.5 mL) were mixed with Benedict's reagent (30 mL). The tubes were placed in a

beaker containing 300 mL of boiling water for 10 min. After removing the boiling water, the samples were allowed to cool to room temperature for 20 min, after which photographs of the samples were taken. Six samples of Benedict's reagent served as the control.

All photographs were taken under standardized conditions to ensure consistency in red, green, and blue (RGB) analysis. Samples were placed on a white matte background and illuminated with uniform white LED lighting (6000 K). Images were captured using a Xiaomi 12 Pro smartphone, mounted on a fixed support 20 cm above the samples. The same shooting mode, focus, and exposure settings were used for all photographs. No filters or digital enhancements were applied. RGB values were extracted using Fiji (ImageJ) under identical image-processing conditions for all samples.

The evaluation of the color changes in the mixtures was performed using Fiji (ImageJ) software, which quantified the RGB color components in the photographs. The experiment aimed to address questions regarding the presence of reducing sugars in the samples and potential variations in coloration depending on the body segment from which the fluid was extracted.

Results

Experiment 1: Distribution of body mass and water content in *Apis mellifera*

Body mass distribution

A total of 124 bees were analyzed to determine the distribution of body mass across the whole body and its main segments (head, thorax, and abdomen). The average total body mass was 102.07 ± 7.30 mg. Among the body segments, the abdomen contributed the most to the total body mass, with an average of 55.23 ± 4.53 mg, followed by the thorax (32.76 ± 2.93 mg) and the head (8.71 ± 1.03 mg). These results indicate that the abdomen accounts for more than half of the total body mass of *Apis mellifera*, while the head contributes less than 10%. The body mass distribution provides a clear reference for analyses related to extracellular fluid extraction (Fig. 2A).

Water content in the body of bees

The estimation of body water content was performed on 120 bees by measuring their wet mass and dry mass. The average wet mass was 101.92 ± 6.07 mg, while the average dry mass was 20.26 ± 3.54 mg. Based on these measurements, the body water content (Water) was calculated as the difference between wet and dry mass, resulting in an average of 81.66 ± 6.30 mg. Furthermore, a linear regression analysis between Water and Wet revealed a significant slope ($k_b = 0.87 \pm 0.05$; $R^2 = 0.7$;

$P < 0.001$), indicating that 87% of the wet mass corresponds to water content (Fig. 2B). These findings emphasize that most of the body mass in bees is composed of water, a critical factor for extracellular fluid extraction analyses.

Water content in body segments

The analysis of water content in body segments (head, thorax, and abdomen) was conducted on a total of 131 bees. The results indicated that the abdomen contains the highest water content, with an average of 50.1 ± 3.6 mg, followed by the thorax (25.4 ± 1.6 mg) and the head (6.6 ± 1.5 mg). The wet and dry masses of each segment were also measured, providing a detailed reference for relative proportions.

Regression analysis revealed a significant relationship between Water and Wet for all segments, with slopes of $k_H = 0.76 \pm 0.03$ ($R^2=0.81$) for the head, $k_T = 0.80 \pm 0.03$ ($R^2=0.88$) for the thorax, and $k_A = 0.86 \pm 0.03$ ($R^2=0.86$) for the abdomen. These results demonstrate that water content relative to wet mass varies slightly between segments, with the abdomen showing the highest percentage of water (Fig. 2C, D, and E); this distribution is crucial for interpreting extracellular fluid extraction patterns in each body segment.

Experiment 2: Extracellular fluid extraction from body segments using centrifugation

Fluid mass extracted from the head segment

To calculate the percentage of extracted fluid, we measured the total mass of the collection system (segment plus container) before and after centrifugation, subtracting the prerecorded mass of the empty container. The percentage of fluid recovered was then determined as $(\text{final mass}/\text{initial mass}) \times 100$. This approach provided a relative measure of extraction efficiency for each condition tested.

Segmental reference masses and water content values from Experiment 1 were used to interpret these percentages and contextualize extraction dynamics. Although we referred to "proportional extraction" to indicate that fluid recovery increased with centrifugation time, force, or number of bees, the trends were not strictly linear. Instead, extraction curves showed positive but saturating behaviors, consistent with physical limitations on fluid displacement under centrifugation.

Fluid mass extracted from the head segment

The independent variables (#Bees, Speed, and Time) significantly influenced the extraction of extracellular fluid from the head segment ($F_{2,72}=7.31$, $P < 0.001$; $\eta^2=0.169$). However, under the maximum variable

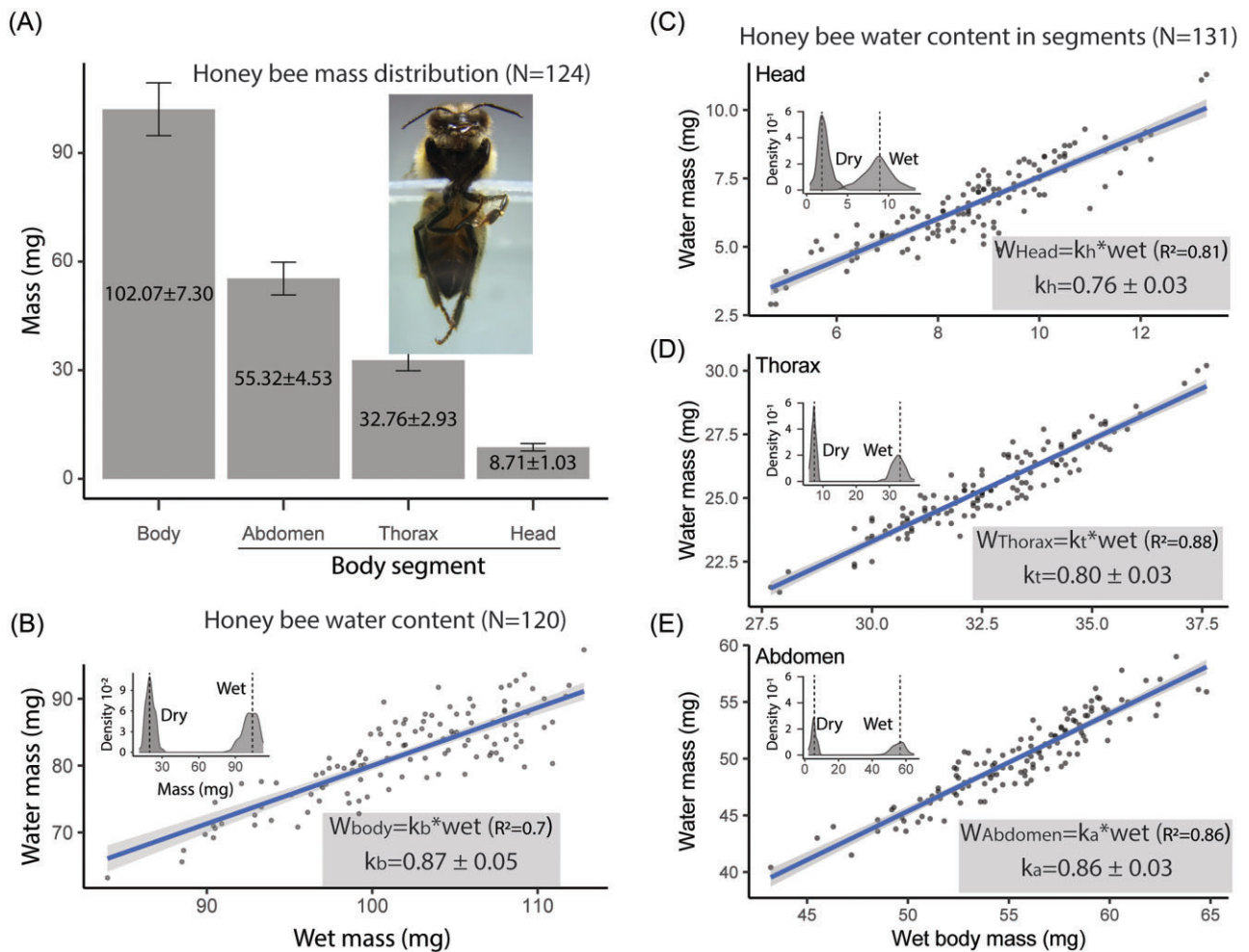


Fig. 2 Distribution of body mass and water content in *Apis mellifera*. (A) Body mass distribution. (B) Whole-body water content, showing the relationship between wet mass and dry mass. (C, D, E) Water content distribution across body segments.

conditions, the differences were not significant ($F_{2,12}=1.58$, $P = 0.25$). Increases in the number of bees (Speed = 2900 RCF; Time = 10 min) led to progressive increases in fluid recovery, with the percentage of initial to final measurements exceeding 80% (median, IQR [Low; High] %): 89.9, IQR [84.1; 90.6]. Variations in Speed (#Bees = 10; Time = 10 min) showed a trend of increasing extraction that stabilized at higher speeds, with percentages above 56% (median %, IQR [Low; High] %): 59.9, IQR [56.8; 64.0]. Finally, changes in Time (Bees = 10; Speed = 2900 RCF) resulted in increased recovery during the first intervals, followed by a plateau, with percentages exceeding 51% (median %, IQR [Low; High] %): 52.7, IQR [51.7; 53.5] (Fig. 3A). These results were interpreted in light of the segmental mass and water content data obtained in Experiment 1, which provided a reference for estimating the expected amount of fluid per segment.

Fluid mass extracted from the thorax segment

The independent variables (#Bees, Speed, and Time) significantly influenced the extraction of extracellular fluid from the thorax segment ($F_{2,72}=5.61$; $P < 0.001$; $\eta^2=0.135$). However, under the maximum variable conditions, the differences were not significant ($F_{2,12}=0.81$; $P = 0.47$). Increases in the number of bees (Speed = 2900 RCF; Time = 10 min) produced progressively higher extraction values with a trend toward saturation, with the percentage of initial to final measurements exceeding 84% (median, IQR [Low; High] %): 87.7, IQR [84.1; 91.3]. Variations in Speed (#Bees = 10; Time = 10 min) yielded consistently elevated values, with percentages above 63% (median %, IQR [Low; High] %): 64.0, IQR [63.6; 64.2]. Finally, changes in Time (#Bees = 10; Speed = 2900 RCF) showed increased recovery during the first intervals, followed by a plateau, with percentages exceeding 44% (median %, IQR [Low; High] %): 46.9, IQR [44.8; 47.6] (Fig. 3B).

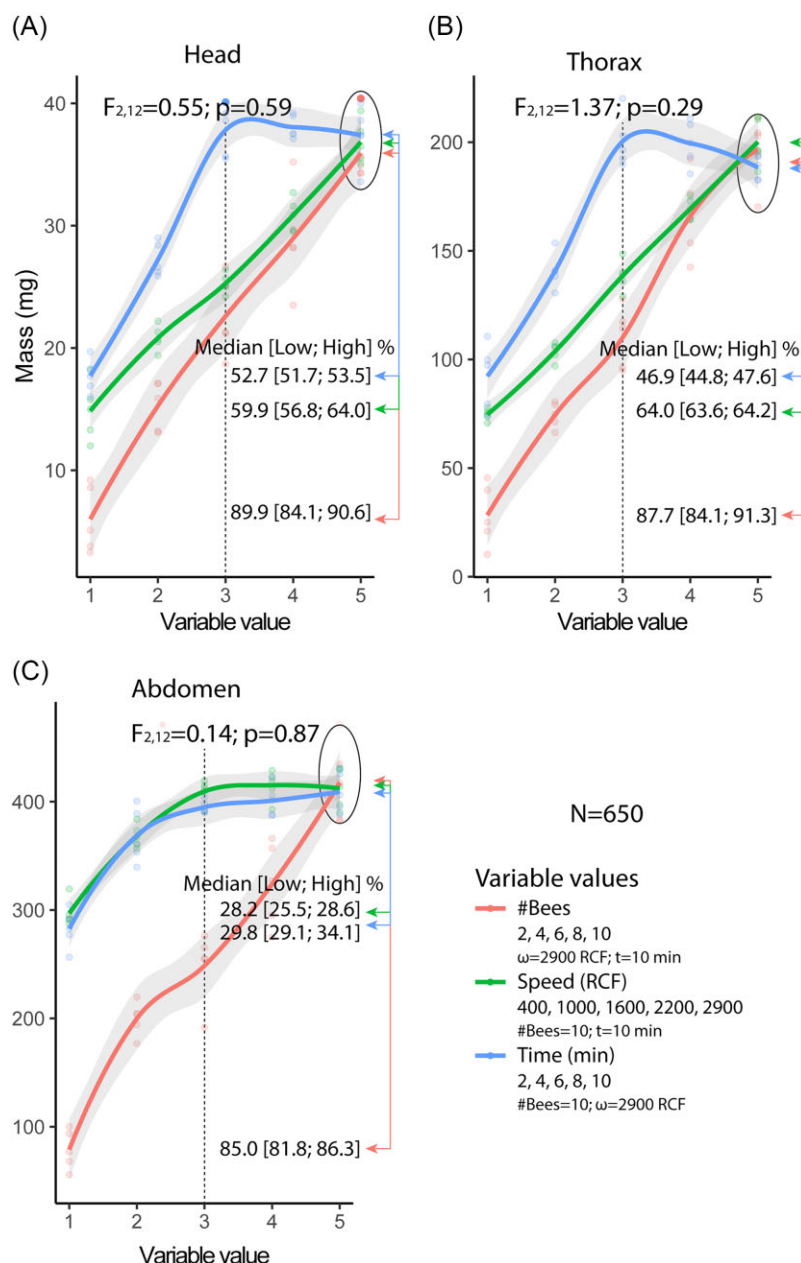


Fig. 3 Effect of independent variables on the extraction of extracellular fluid in *Apis mellifera*. (A) Volume of extracellular fluid extracted from the head segment. (B) Volume extracted from the thorax segment. (C) Volume extracted from the abdomen segment.

Fluid mass extracted from the abdomen segment

The independent variables (#Bees, Speed, and Time) significantly influenced the extraction of extracellular fluid from the abdomen segment ($F_{2;72}=19.550$; $P < 0.0001$; $\eta^2=0.352$). However, under the maximum variable conditions, the differences were not significant ($F_{2;12}=1.45$; $P = 0.27$). Increases in the number of bees (Speed = 2900 RCF; Time = 10 min) led to a strong increase in fluid recovery, with the percentage of initial to final measurements exceeding 81% (median, IQR [Low; High] %): 85.0, IQR [81.8; 86.3].

Variations in Speed (#Bees = 10; Time = 10 min) resulted in nonlinear recovery trends, with the highest yields at lower speeds, and the percentage of initial to final measurements exceeding 25% (median %, IQR [Low; High] %): 28.2, IQR [25.5; 28.6]. Finally, changes in Time (#Bees = 10; Speed = 2900 RCF) mirrored the speed condition trend, with percentages exceeding 29% (median %, IQR [Low; High] %): 29.8, IQR [29.1; 34.1] (Fig. 3C). As with the other segments, interpretation was supported by segmental mass data obtained from Experiment 1.

Under optimized conditions (2900 RCF, 10 min, 10 bees), the average fluid mass extracted per segment was approximately 1.3 mg for the head, 3.2 mg for the thorax, and 7.5 mg for the abdomen. Assuming a fluid density close to that of water (1 g/mL), these values correspond to volumes of approximately 1.3 μ L, 3.2 μ L, and 7.5 μ L, respectively, for each 10-bee sample. On a per-bee basis, this represents an average recovery of ~ 0.13 μ L for the head, ~ 0.32 μ L for the thorax, and ~ 0.75 μ L for the abdomen.

Experiment 3: UV-Vis spectroscopic characterization of extracellular fluid from body segments

The absorbance of the extracellular fluid showed maximum values between 220 and 260 nm, which were highest for the abdomen, intermediate for the thorax, and lowest for the head (Abs = mean $\pm$ SD): 0.189 ± 0.002 (Head); 0.540 ± 0.042 (Thorax); 1.671 ± 0.032 (Abdomen). These results indicate that absorbance values depend on the body segment. Relative to the abdomen, the absorbance percentage for the thorax and head was below 32% (mean $\pm$ SD %): 32.3 ± 2.6 (Thorax/Abdomen); 11.3 ± 0.3 (Head/Abdomen); this suggests that absorbance varied according to the amount of extracellular fluid extracted, with the abdomen having the highest values, the thorax intermediate, and the head the lowest (Fig. 4A).

Normalized absorbance trends showed significant differences ($F_{2;114n} = 28.71$, $P < 0.0001$) across the 210–590 nm wavelength range. Significant differences ($P < 0.001$) were also observed between pairs of segments (Diff; CI [low; high]): -0.092 ; CI $[-0.144, -0.041]$ (Abdomen-Head); -0.167 ; CI $[-0.219, -0.115]$ (Abdomen-Thorax); -0.075 ; CI $[-0.126, -0.023]$ (Head-Thorax) (Fig. 4B). The wavelengths corresponding to the maximum normalized absorbance values occurred in different regions of the spectrum depending on the body segment: 224 nm (Head); 248 nm (Abdomen); 255 nm (Thorax) (Fig. 4C). Additionally, despite significant differences between the segments, the 348 nm wavelength defined 3 local maxima within the 340–370 nm range (Fig. 4D). These findings demonstrate that the extracellular fluid varies according to the body segment it originates from; however, some regions of the spectrum exhibit similarities.

Experiment 4: Sugar test for extracellular fluid using Benedict's reagent

The RGB colors exhibited by the samples demonstrated the presence of traces of reducing sugars, as the coloration deviated from the blue of the reagent. Samples from the segments reacted with the reagent, displaying

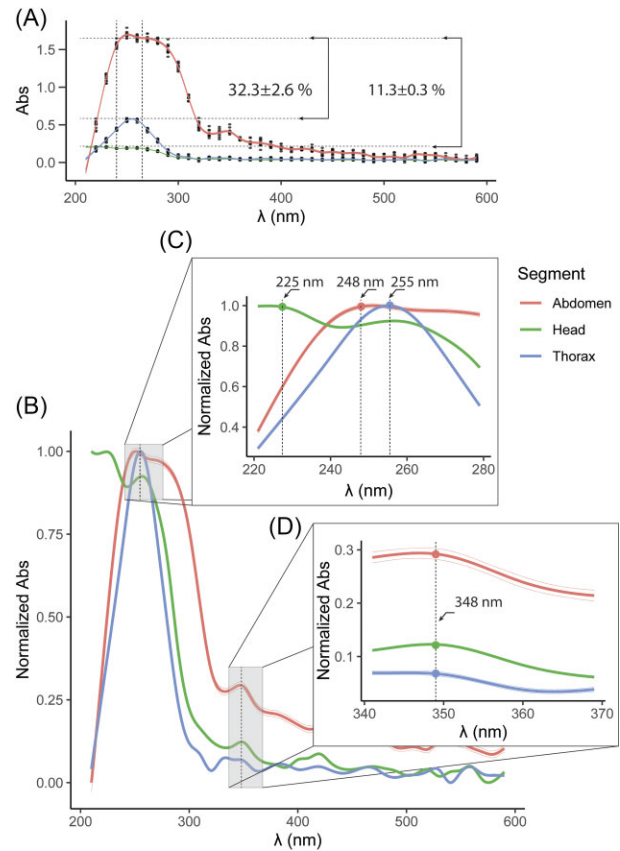


Fig. 4 Optical spectroscopy curves for extracellular fluid samples by body segment. (A) Absorbance curves for the head, thorax, and abdomen segments. The curve maxima are in the 220–260 nm range. The absorbance values for the head and thorax are below the curve of the abdomen, with percentages of 11.3% and 32.3%, respectively. (B) Normalized absorbance curves for the body segments. (C) Wavelengths defining the maximum of normalized absorbance. (D) Wavelength defining local maxima of normalized absorbance in the 340–370 nm range.

reddish coloration (mean $\pm$ SD) [mode]: 193.8 ± 18.2 [180] (Head); 156.9 ± 20.4 [162] (Thorax); 157.0 ± 23.5 [149] (Abdomen). Samples from the thorax and abdomen were characterized by low levels of green coloration compared to the head (mean $\pm$ SD) [mode]: 75.0 ± 37.3 [50] (Thorax); 75.1 ± 43.8 [44] (Abdomen); 158.0 ± 23.1 [154] (Head). The blue levels for all segments were the lowest (mean $\pm$ SD) [mode]: 65.0 ± 45.5 [45] (Head); 13.7 ± 34.6 [0] (Thorax); 17.9 ± 50.5 [0] (Abdomen) (Fig. 5A).

The coloration of the samples showed significant differences ($P < 0.0001$), indicating that the characteristics of the extracellular fluid depend on the body segment ($F_{3;13774} = 472.5$). Each RGB component exhibited significant differences ($P < 0.0001$) across all samples: $F_{3;5n6n}=788.3$ (Blue); $F_{3;5n38}=707.6$ (Green); $F_{3;3668}=724.4$ (Red). However, the green coloration for head samples showed no significant differences

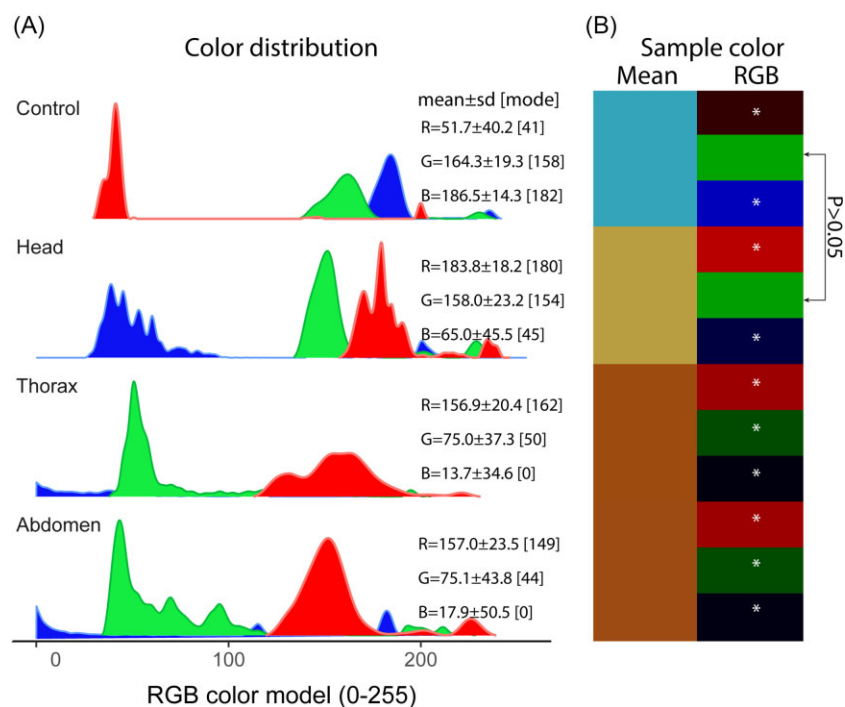


Fig. 5 Coloration of body segment samples mixed with Benedict's reagent for sugar detection. (A) Distribution of sample coloration in RGB (0–255). (B) Average combination of RGB colors to produce the coloration of the samples with the reagent.

compared to Benedict's reagent (Diff; CI [Low; High]; P): -1.38 ; CI $[-7.01; 4.31]$; 0.925 . On the other hand, although the thorax and abdomen samples appeared visually similar, their defining RGB components showed significant differences (Fig. 5B). These results demonstrate that the coloration of the samples depended on the body segment from which they were extracted.

Discussion

This study developed and validated a methodology based on segmented centrifugation for the extraction of extracellular fluid in *Apis mellifera* bees. The results demonstrated that the technique allows for the recovery of significant and reproducible volumes of extracellular fluid, highlighting important differences among body segments (head, thorax, and abdomen). The proposed methodology constitutes a robust and innovative tool for physiological, metabolic, and ecotoxicological studies in insects, addressing the limitations of traditional hemolymph extraction methods.

Importance and relevance of the methodology

The implementation of the dual-filter system and the optimization of centrifugation variables allowed for the standardization of extracellular fluid extraction. Unlike conventional methods that often rely on hypodermic needles or glass capillaries (Borsuk et al. 2017; Butolo et al. 2020), this technique ensures cleaner sample han-

dling, reduces the risk of tissue contamination, and facilitates the simultaneous processing of multiple individuals. Moreover, the use of modified pipette tips ensures efficient retention of body segments, enabling rapid and controlled separation of extracellular fluid.

The results showed that the abdomen releases larger volumes of extracellular fluid, consistent with its role as the main region for hemolymph storage (Blow and Douglas 2019). In contrast, the head and thorax exhibited lower volumes, likely due to dense tissue composition and a relatively smaller amount of extracellular fluid; these findings underscore the necessity of analyzing each segment separately, as global methods may obscure critical physiological differences.

These segmental fluid volumes are comparable to or greater than those obtained through traditional hemolymph collection methods, which typically yield 1–5 μL per bee using glass capillaries or hypodermic needles (Borsuk et al. 2017; Butolo et al. 2020). In contrast, our centrifugation-based approach enables simultaneous extraction from multiple individuals while providing segment-specific resolution and minimizing tissue damage or contamination.

Another consideration relates to the possible contamination of abdominal fluid with gastrointestinal contents. Although the dissection was performed without direct manipulation of the digestive tract, the anatomical proximity of digestive tissues in the

abdomen may lead to the presence of nonhemolymph components in the extracted fluid. In this study, we use the term “extracellular fluid” in an operational sense, referring to the liquid recovered by passive centrifugation from intact body segments, without asserting biochemical exclusivity. Future versions of this method may include improved segmental dissection, targeted exclusion of digestive tissues, or biochemical markers to verify fluid origin.

Practical applications

The methodology presented has direct applications in ecotoxicology, enabling the evaluation of contaminants, such as neonicotinoids (Brandt et al. 2016; Williamson et al. 2014; Tosi et al. 2017; Odemer et al. 2023) differentially affect the body regions of bees. Previous studies have shown that the accumulation of pesticides and their metabolic impact are not homogeneous in insects (Gonzalez et al. 2022). The segmented extraction of extracellular fluid proposed here provides a more precise approach to identifying patterns of accumulation and regional physiological effects.

Additionally, the optical characterization via UV-Vis spectroscopy demonstrates its utility as a noninvasive method for detecting biochemical differences in extracellular fluid. The presence of specific absorbance maxima in each segment suggests variability in the concentration of proteins and other metabolites (Chen et al. 2021; Chang et al. 2023; Wang et al. 2021), which could serve as biomarkers of physiological stress in response to environmental factors (Chang et al. 2023).

The absorbance spectra obtained from each segment suggest qualitative and quantitative differences in the composition of the extracellular fluid. The higher absorbance values observed in the abdominal samples may reflect a greater concentration of solutes, such as proteins, metabolic byproducts, or nitrogenous waste, which are more likely to accumulate in the storage and excretory regions of the insect. In contrast, the thoracic and head segments—dominated by muscle and neural tissue, respectively—may contain lower solute concentrations and different biochemical profiles. The segment-specific spectral peaks and differences in overall absorbance intensities support the presence of localized biochemical variation, which could be explored in future studies using targeted molecular or proteomic analyses.

This study also introduces a quantitative methodology for analyzing color changes in Benedict’s reagent tests, a technique traditionally limited to qualitative assessments. The use of Fiji (ImageJ) software (Schindelin et al. 2012) enabled the extraction of RGB values from digital images, allowing for precise quantification of color differences among samples. This approach re-

vealed that while thorax and abdomen samples appeared visually similar, significant differences were observed in their chromatic component values; this methodology not only enhances the sensitivity of the test, but also provides a reproducible and objective analysis, which is valuable for biochemical studies requiring the evaluation of subtle variations in sugar concentrations or other metabolic compounds.

The differences in coloration observed in the Benedict’s reagent test likely reflect variations in the presence and concentration of reducing sugars among body segments. The more intense reddish hues in the head samples suggest a higher content of glucose or other reactive sugars, possibly related to neural tissue metabolism or localized storage. Thorax and abdomen samples displayed lower intensities, which may correspond to reduced sugar content or the presence of less reactive compounds. While this experiment did not determine the exact identity of the sugars involved, the RGB quantification supports segment-specific biochemical variation. Future work could integrate chromatographic or enzymatic techniques to more precisely characterize the carbohydrate profile of each segment.

The quantification of colors using digital tools represents a methodological advancement that can be applied to other traditional colorimetric tests, enabling the detection of subtle differences that might be overlooked in purely visual analyses. This precision is particularly relevant in comparative physiology studies and ecotoxicological research, where small changes in metabolites could be linked to environmental stress factors.

Study limitations

Despite its advantages, the technique has certain limitations. The use of distilled water as a flow-facilitating agent may dilute specific metabolites present in the extracellular fluid, which should be considered when interpreting biochemical results. Additionally, the small size of samples obtained from smaller segments, such as the head, can pose challenges for studies requiring larger sample volumes.

Another consideration relates to the use of a fixed number of bees rather than a standardized segment mass during centrifugation. To address this, Experiment 1 provided a detailed characterization of body segment mass and water content, establishing a solid reference framework. This dataset enabled estimation of the mass applied in each extraction procedure, allowing for the inference of centrifugal forces applied to each segment type (see Fig. 2). By incorporating this information into the design and interpretation of Experiments 2–4, we ensured that differences in mechanical forces across segments were taken into account, thereby reinforcing the validity of comparative analyses.

In Experiments 3 and 4, the segment-specific samples were obtained using a fixed number of bees, as in previous steps. Although this approach introduced variations in total segment mass and, consequently, in the absolute centrifugal force, Experiment 1 provided reference values for segment masses and water content, which allowed contextual interpretation of the results.

In the case of UV-Vis spectroscopy, the absorbance values are indeed influenced by the concentration of solutes, which in turn can be affected by the volume of fluid extracted. We acknowledge that differences in segment mass and fluid yield may have contributed to the intensity of absorbance peaks. However, the segment-specific absorbance patterns, such as the position of maxima and the relative spectral profiles, reflect compositional differences that are independent of total fluid volume.

Similarly, RGB analysis from Benedict's reagent reactions focused on relative chromatic patterns rather than absolute color intensity. The consistency of these patterns across replicates supports their physiological relevance. Overall, the use of moderate centrifugation (2000 RCF for 4 min) and standardized imaging conditions helped minimize technical variability, allowing us to compare segmental biochemical signatures with confidence.

Future perspectives

The findings of this study contribute to future research focusing on the molecular composition of segmented extracellular fluid using advanced techniques such as mass spectrometry or liquid chromatography. Additionally, the relationship between the composition of extracellular fluid and the physiological performance of bees under stress conditions, such as exposure to contaminants or environmental changes, represents a priority area of interest.

Finally, the proposed approach could be adapted to other animal models, enabling comparisons across different insect species and their responses to specific environmental factors; this regional analysis capability provides a powerful tool for studies in comparative physiology and pollinator conservation.

Conclusion

This study developed and validated an efficient and reproducible methodology for the segmented extraction of extracellular fluid in *Apis mellifera* bees by using controlled centrifugation. The results demonstrated significant differences in the volume of fluid recovered, its optical properties, and the presence of reducing sugars among body segments, with the abdomen being the region with the highest extraction capacity. The spectroscopic and biochemical characterization of the

samples highlighted the importance of analyzing each segment independently to obtain more precise information about the physiology and metabolism of bees.

This methodology represents a robust tool for physiological, metabolic, and ecotoxicological studies, with applications in assessing environmental impacts and pollinator health. In the future, optimizing the method and integrating it with advanced analytical techniques will allow for a deeper exploration of the molecular composition of extracellular fluid and its relationship with environmental and stress-related factors.

Acknowledgments

The authors thank members of the Riveros' Lab (CANNON Research Group) and Fundacion Universitaria Los Libertadores.

Funding

This project was funded by the Big Grant IV-FGD003 of the Direccion de Investigación e Innovación (DIEI; Universidad del Rosario, Bogotá, Colombia) to A.J.R.

Conflict of interest

The authors do not have any competing interests to declare.

Author contributions

J.P.H. conceptualized the project, designed the experiments, collected the data, conducted the statistical analyses, elaborated figures, contributed with discussion and wrote a first draft of the manuscript. A.J.R. conceptualized the project, designed the experiments, contributed with discussion and writing of the final version of the manuscript. F.M. and J.N. contributed with discussion and writing of the final version of the manuscript.

References

- Abou-Shaara HF, Al-Ghamdi AA, Mohamed AA 2012. Tolerance of two honey bee races to various temperature and relative humidity gradients. In *Environ Exp Biol* 10: 133–8.
- Blow F, Douglas AE. 2019. The hemolymph microbiome of insects. *Journal of Insect Physiology* 115: 33–39. <https://doi.org/10.1016/j.jinsphys.2019.04.002>
- Bogaerts A, Baggerman G, Vierstraete E, Schoofs L, Verleyen P 2009. The hemolymph proteome of the honeybee: gel-based or gel-free? *Proteomics* 9: 3201–8. <https://doi.org/10.1002/pmic.200800604>
- Borsuk G, Ptasińska AA, Olszewski K, Domaciuk M, Krutmuang P, Paleolog J 2017. A new method for quick and easy hemolymph collection from apidae adults. *PLoS One* 12: e0170487. <https://doi.org/10.1371/journal.pone.0170487>
- Brandt A, Gorenflo A, Siede R, Meixner M, Büchler R 2016. The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.).

- J Insect Physiol 86, 40–47. <https://doi.org/10.1016/j.jinsphys.2016.01.001>
- Butolo NP, Azevedo P, Alencar LD, Malaspina O, Nocelli RCF 2021. Impact of low temperatures on the immune system of honeybees. J Therm Biol 101: 103082. <https://doi.org/10.1016/j.jtherbio.2021.103082>
- Butolo NP, Azevedo P, de Alencar LD, Domingues CEC, Miotelo L, Malaspina O, Nocelli RCF 2020. A high quality method for hemolymph collection from honeybee larvae. PLoS One 15: e0234637. <https://doi.org/10.1371/journal.pone.0234637>
- Cao X, Wang Y, Rogers J, Hartson S, Kanost MR, Jiang H 2020. Changes in composition and levels of hemolymph proteins during metamorphosis of *Manduca sexta*. Insect Biochem Mol Biol 127: 103489. <https://doi.org/10.1016/j.ibmb.2020.103489>
- Chan QWT, Howes CG, Foster LJ 2006. Quantitative comparison of caste differences in honeybee hemolymph. Mol Cell Proteom 5: 2252–62. <https://doi.org/10.1074/mcp.M600197-MCP200>
- Chang H, Ding G, Jia G, Feng M, Huang J 2023. Hemolymph metabolism analysis of honey bee (*Apis mellifera* L.) response to different bee pollens. Insects 14: 37. <https://doi.org/10.3390/insects14010037>
- Chen H, Wang K, Ji W, Xu H, Liu Y, Wang S, Wang Z, Gao F, Lin Z, Ji T 2021. Metabolomic analysis of honey bees (*Apis mellifera*) response to carbendazim based on UPLC-MS. Pestic Biochem Physiol 179: 104975. <https://doi.org/10.1016/j.pestbp.2021.104975>
- Cournoyer A, Plamondon L, Bau-Gaudreault L, Deschamps A, Dubreuil P, Benoit-Biancamano MO 2022. Effects of varroa destructor on hemolymph sugars and secondary infections in honeybees (*Apis mellifera*). Applied Sciences 12: 11630. <https://doi.org/10.3390/app122211630>
- Dexheimer AF, Outomuro D, Dunlap AS, Morehouse NI 2022. Spectral sensitivities of the orchid bee *Euglossa dilemma*. J Exp Biol 225: jeb244031.
- Elfar SA, Bahgat IM, Shebl MA, Lihoreau M, Tawfik MM 2023a. Intraspecific variability in proteomic profiles and biological activities of the honey bee hemolymph. Insects 14: 365. <https://doi.org/10.3390/insects14040365>
- Elfar SA, Bahgat IM, Shebl MA, Lihoreau M, Tawfik MM 2023b. Variation in hemolymph content and properties among three Mediterranean bee species. bioRxiv preprint. <https://doi.org/10.1101/2023.12.22.573070> [accessed 2025 May 19].
- Gonzalez VH, Hranitz JM, McGonigle MB, Manweiler RE, Smith DR, Barthell JF 2022. Acute exposure to sublethal doses of neonicotinoid insecticides increases heat tolerance in honey bees. PLoS One 17: e0240950 (2 February). <https://doi.org/10.1371/journal.pone.0240950>
- Holdbrook R, Andongma AA, Randall JL, Reavey CE, Tummala Y, Wright GA, Simpson SJ, Smith JA, Wilson K, Cotter SC 2024. The transition from diet to blood: exploring homeostasis in the insect haemolymph nutrient pool. Physiol Entomol 49: 227–43. <https://doi.org/10.1111/phen.12440>
- Huang SK, Csaki T, Doublet V, Dussaubat C, Evans JD, Gajda AM, Gregor A, Hamilton MC, Kamler M, Lecocq A et al. 2014. Evaluation of cage designs and feeding regimes for honey bee (Hymenoptera: Apidae) laboratory experiments. Jnl Econ Entomol 107: 54–62. <https://doi.org/10.1603/EC13213>
- Kannan M, Vitenberg T, Schweitzer R, Opatovsky I 2024. Hemolymph metabolism of black soldier fly (Diptera: Stratiomyidae), response to different supplemental fungi. J Insect Sci 24: ieae050. <https://doi.org/10.1093/jisesa/ieae050>
- Kim S, Kim JH, Cho S, Lee DE, Clark JM, Lee SH 2022. Chronic exposure to field-realistic doses of imidacloprid resulted in biphasic negative effects on honey bee physiology. Insect Biochem Mol Biol 144: 103759. <https://doi.org/10.1016/j.ibmb.2022.103759>
- Köhler A, Nicolson SW, Pirk CWW 2013. A new design for honey bee hoarding cages for laboratory experiments. In J Apicult Res (Vol. 52, pp. 12–4). <https://doi.org/10.3896/IBRA.1.52.2.03>
- Li D, Liu L, Liu ZL, Tian Y, Gao X, Cheng TY 2024. Main proteins in the hemolymph of *Haemaphysalis flava* ticks. Front Vet Sci 11: 1387719. <https://doi.org/10.3389/fvets.2024.1387719>
- Lubawy J, Słocińska M 2020. Characterization of *Gromphadorhina coquereliana* hemolymph under cold stress. Sci Rep 10: 11822. <https://doi.org/10.1038/s41598-020-68941-z>
- Migdał P, Murawska A, Bieńkowski P, Strachecka A, Roman A 2021. Effect of the electric field at 50 Hz and variable intensities on biochemical markers in the honey bee's hemolymph. PLoS One 16: e0252858 (6 June). <https://doi.org/10.1371/journal.pone.0252858>
- Nakamatsu Y, Tanaka T 2004. Correlation between concentration of hemolymph nutrients and amount of fat body consumed in lightly and heavily parasitized hosts (*Pseudaletia separata*). J Insect Physiol 50: 135–41. <https://doi.org/10.1016/j.jinsphys.2003.10.005>
- Niu Y, Zhao Y, Shi F, Li M, Zhang S, Yang J, Zong S, Tao J 2023. An efficient and simple method for collecting haemolymph of cerambycidae (Insecta: Coleoptera) adults. Insects 14: 29. <https://doi.org/10.3390/insects14010029>
- Odemer R, Friedrich E, Illies I, Berg S, Pistorius J, Bischoff G 2023. Potential risk of residues from neonicotinoid-treated sugar beet flowering weeds to honey bees (*Apis mellifera* L.). Environ Toxicol Chem 42: 1167–77. <https://doi.org/10.1002/etc.5602>
- Paleolog J, Wilde J, Siuda M, Bąk B, Wójcik Ł, Strachecka A 2020. Imidacloprid markedly affects hemolymph proteolysis, biomarkers, DNA global methylation, and the cuticle proteolytic layer in western honeybees. Apidologie 51: 620–30. <https://doi.org/10.1007/s13592-020-00747-4>
- Park JH, Lee JE, Choi SS, Park TH 2017. Protective effects of silkworm hemolymph extract and its fractions on UV-induced photoaging. Biotechnol Bioproc E 22: 37–44. <https://doi.org/10.1007/s12257-016-0588-4>
- Ratanawimarnwong N, Suksomphot V, Sornpipatpong K, Lengwan S, Donpudsa S, Choengchan N, Mantim T 2022. Microfluidic paper-based analytical device for determination of sucrose in sugarcane juice using Benedict's reagent. Anal Bioanal Chem 414: 7783–91. <https://doi.org/10.1007/s00216-022-04312-z>
- Schenk S, Schmidt J, Hoeger U, Decker H 2015. Lipoprotein-induced phenoloxidase-activity in tarantula hemocyanin. Biochim Biophys Acta (BBA) Proteins Proteom 1854: 939–49. <https://doi.org/10.1016/j.bbapap.2015.03.006>
- Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B et al. 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9: 676–82. <https://doi.org/10.1038/nmeth.2019>
- Somanathan H 2024. Why diversity matters for understanding the visual ecology and behaviour of bees. Current Opinion

- in Insect Science 101224, In Current Opinion in Insect Science (Vol. 64). Springer, Berlin: Elsevier Inc. <https://doi.org/10.1016/j.cois.2024.101224>
- Strachecka A, Kuszewska K, Olszewski K, Skowronek P, Grzybek M, Grabowski M, Paleolog J, Woyciechowski M 2022. Activities of antioxidant and proteolytic systems and biomarkers in the fat body and hemolymph of young *Apis mellifera* females. *Animals* 12: 1121. <https://doi.org/10.3390/ani12091121>
- Stupski BH, Schilder RJ 2021. Thermoregulatory responses of honey bees to cold stress. *J Therm Biol* 95: 102782.
- Tabunoki H, Hiramatsu S, Fujii H 2019. Proteomic analysis of *Bombyx mori* hemolymph. *Insect Biochem Mol Biol* 107: 57–66.
- Tafi E, Salmela H, Terenius O 2024. Molecular changes in bee hemolymph during viral infection. *Insect Mol Biol* 33: 97–110.
- Tauchman SJ, Lorch JM, Orth AP, Goodman WG 2007. Effects of stress on the hemolymph juvenile hormone binding protein titers of *Manduca sexta*. *Insect Biochem Mol Biol* 37 (8 SPEC. ISS), 847–54. <https://doi.org/10.1016/j.ibmb.2007.05.015>
- Tosi S, Nieh JC, Sgolastra F, Cabbri R, Medrzycki P 2017. Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proc R Soc B* 284: 20171711. <https://doi.org/10.1098/rspb.2017.1711>
- Ushakova N, Dontsov A, Sakina N, Bastrakov A, Ostrovsky M 2019. Antioxidative properties of melanins and ommochromes from black soldier fly *Hermetia illucens*. *Biomolecules* 9: 408. <https://doi.org/10.3390/biom9090408>
- Wang L, Van Meulebroek L, Vanhaecke L, Smagghe G, Meeus I 2021. The bee hemolymph metabolome: a window into the impact of viruses on bumble bees. *Viruses* 13: 600. <https://doi.org/10.3390/v13040600>
- Wang Z, Wang S, Ji T, Chen H, Zhang Y, Liu X, Li J, Gao F, Huang C, Wang Y et al. 2022. Hemolymph regulation in honeybees under stress. *J Insect Physiol* 136: 104340.
- Williams GR, Alaux C, Costa C, Csáki T, Doublet V, Eisenhardt D, Fries I, Kuhn R, McMahon DP, Medrzycki P et al. 2013. Standard methods for maintaining adult *Apis mellifera* in cages under in vitro laboratory conditions. *J Apic Res* 1–36, In *J Apicult Res* (Vol. 52). <https://doi.org/10.3896/IBRA.1.52.1.04>
- Williamson SM, Willis SJ, Wright GA 2014. Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicol* 23: 1409–18. <https://doi.org/10.1007/s10646-014-1283-x>

Capítulo 2. Impacto del imidacloprid en el comportamiento apetitivo, defensivo y motor de abejas *Apis mellifera*

Juan P. Hernández, Natalia Buitrago, Fredy. Mesa, Andre J. Riveros

Publicado en: Scientific Reports 30 June 2026

DOI: <https://doi.org/10.1038/s41598-026-54241-5>

Resumen

Las abejas melíferas (*Apis mellifera*) son esenciales para la polinización de cultivos y ecosistemas naturales, pero su exposición a insecticidas neonicotinoides, como el imidacloprid, representa una amenaza significativa. Este estudio evaluó los efectos subletales de dicho compuesto sobre el comportamiento apetitivo, defensivo y motor de las abejas mediante tres aproximaciones experimentales: el reflejo de extensión de la probóscide (PER), el reflejo de extensión del aguijón (SER) y el análisis del movimiento individual y grupal. Se encontró que la exposición al imidacloprid genera una disminución dosis-dependiente de las respuestas apetitivas y defensivas, con reducciones significativas del PER y del SER a concentraciones intermedias y altas. Además, se observaron alteraciones en el movimiento de los segmentos corporales, especialmente en la cabeza y el abdomen, así como una reducción de la rapidez angular y del ángulo de desplazamiento durante el comportamiento colectivo. Estos resultados indican un compromiso funcional del sistema sensoriomotor, lo que afecta la capacidad de las abejas para recolectar alimento, defender la colonia y desplazarse de manera eficiente. En conjunto, las evidencias apuntan a una afectación integral del comportamiento, en la que las deficiencias en las respuestas sensoriales, defensivas y locomotoras se interrelacionan, lo que reduce la eficiencia ecológica del insecto. Este estudio refuerza la preocupación por los efectos subletales del imidacloprid y subraya la necesidad de revisar críticamente su uso en contextos agrícolas, considerando su impacto acumulativo en la salud y la funcionalidad de los polinizadores.

Notas metodológicas aclaratorias:

Las evaluaciones de este capítulo se llevaron a cabo utilizando la formulación comercial de imidacloprid en lugar de su principio activo aislado. Esta decisión se fundamenta en el realismo ecológico: en entornos agrícolas, las abejas se exponen a mezclas que incluyen coadyuvantes y surfactantes propios del producto final, los cuales pueden alterar la toxicocinética y potenciar los efectos subletales. Evaluar la formulación comercial refleja escenarios reales de intoxicación en campo.

Se aclara al lector que las etiquetas de los gráficos a lo largo del manuscrito emplean un sistema de codificación que asocia el valor de concentración con la abreviatura de la sustancia (por ejemplo, 0.3Im hace referencia a un tratamiento de 0.3 ppm de Imidacloprid), lo que mantiene una consistencia estricta en las dosis administradas.

Sublethal effects of a commercial imidacloprid formulation on appetitive, defensive, and motor behaviors in Africanized honey bees (*Apis mellifera*)

Received: 11 September 2025

Accepted: 18 May 2026

Published online: 30 June 2026

Cite this article as: Hernández J., Buitrago-Ricaurte N., Mesa F. *et al.* Sublethal effects of a commercial imidacloprid formulation on appetitive, defensive, and motor behaviors in Africanized honey bees (*Apis mellifera*). *Sci Rep* (2026). <https://doi.org/10.1038/s41598-026-54241-5>

JP. Hernández, Natalia Buitrago-Ricaurte, F. Mesa & Andre J. Riveros

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

Sublethal effects of a commercial imidacloprid formulation on appetitive, defensive, and motor behaviors in Africanized honey bees (*Apis mellifera*)

JP. Hernández^a, Natalia Buitrago^b, F. Mesa^{c,*}, Andre J. Riveros^{a,d}

^aDepartamento de Biología. Escuela de Ciencias e Ingeniería, Universidad del Rosario, Bogotá, Colombia

^bEscuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, Colombia

^cFundación Universitaria Los Libertadores, Faculty of Engineering and Basic Sciences, Bogotá, Colombia

^dDepartment of Neuroscience, College of Science, University of Arizona, Tucson, AZ, USA

* Corresponding author: fredy.mesa@libertadores.edu.co

ABSTRACT

Honey bees (*Apis mellifera*) are essential pollinators in both agricultural and natural ecosystems, yet exposure to neonicotinoid insecticides such as imidacloprid poses a recognized risk to their behavior and physiology. We evaluated the sublethal effects of a commercial formulation of imidacloprid (Confidor 350 CS) on appetitive, defensive, and motor behaviors in Africanized honey bees using three experimental protocols: the proboscis extension reflex (PER), the sting extension reflex (SER), and analyses of individual and group locomotion. Imidacloprid exposure produced dose- and time-dependent reductions in both PER and SER, with significant impairments at intermediate and high concentrations. At the motor level, exposed bees exhibited reduced activity and altered movement dynamics across body segments, with heterogeneous reductions in head and abdominal motion, as well as decreased angular displacement and angular speed during group movement. Together, these results indicate that sublethal imidacloprid exposure disrupts multiple sensorimotor behavioral domains, resulting in broad functional impairment under controlled conditions. These findings add to growing evidence that sublethal neonicotinoid exposure can substantially alter honey bee behavior and highlight the need for careful evaluation of their use in agroecosystems.

Keywords: *Apis mellifera*, imidacloprid, sublethal effects, proboscis extension reflex (PER), sting extension reflex (SER), motor coordination.

INTRODUCTION

Honey bees (*Apis mellifera*) play a central role in pollination and biodiversity maintenance, yet their populations have been negatively impacted in intricate ways that remain to be fully explained. A combination of factors appears to be responsible for the decline in bee populations¹, reflecting

a progressive deterioration of honey bee health. One significant concern arises from the widespread use of agrochemicals ^{2,3}, which are widely used to protect crops and enhance yields. However, even at non-lethal concentrations, these compounds can disrupt fundamental behaviors that allow bees to feed, defend, and move effectively ⁴⁻⁷. Among the different classes of pesticides, neonicotinoid insecticides have received particular attention because of their high toxicity, which can cause physiological and behavioral impairments at field-relevant sublethal doses ⁸⁻¹⁰.

Neonicotinoids, a class of synthetic insecticides derived from nicotine, are widely used agrochemicals that control harmful insects in agriculture ¹¹, gardens, and even pets ¹². One relevant problem derives from the inconspicuous, subtle effects produced by low-level doses ^{10,13}, which rarely produces death immediately. At small doses, ranging from 0.4 to 40 nanograms per bee, daily foraging and navigation are impaired, suggesting that bees struggle with everyday tasks that commonly sustain the colony ¹⁴⁻¹⁶. Unfortunately, sublethal effects like these have been studied for decades ¹⁰; yet many aspects of their broader impact remain poorly understood.

Imidacloprid is a first-generation neonicotinoid used in agriculture as a commercial formulation that combines the active ingredient with various coadjuvants ¹⁷. One of the most common formulations is 350 SC, a concentrate suspension containing 350 g/L of imidacloprid ¹⁸, which is extensively applied to control aphids and whiteflies in horticultural, fruit, and field crops ^{19,20}. A major concern arises from its persistence as a contaminant in plant tissues, nectar, and pollen ^{21,22}, which affects pollinators' health. Imidacloprid consumption alters many bee systems due to its metabolites, 5-hydroxyimidacloprid and olefin. These metabolites, distributed in the head and thorax, have a half-life of approximately 4 hours ²³. The toxic effects are dose-dependent and impair brain-regulated behaviors through binding to nicotinic acetylcholine receptors (nAChRs). As a result, most research has focused on imidacloprid's effects on learning and memory ^{24,25}, sensory processing ^{26,27}, navigation ^{28,29}, communication ³⁰, and motor control ³¹ under restricted conditions.

A long-standing history of neurobiological and behavioral research on honey bees has facilitated in-depth investigations into impairments of major cognitive traits. The proboscis extension reflex (PER) is a well-established method for evaluating appetitive behaviors such as search, detection, and consumption ^{32,33}. This reflex has become a reference tool for studying learning and memory ^{34,35}, sucrose sensitivity ^{36,37}, and decision-making ^{38,39}, because it can be applied to restrained individuals while still resembling natural responses. Similarly, the sting extension reflex (SER) has most recently been employed to investigate aversive responses elicited under restrained conditions ^{40,41}. Together, these paradigms provide a useful framework for linking pesticide exposure to the health of individual bees, in which impairments of neuronal mechanisms underlie altered behaviors. However, their reliance on restrained individuals under highly controlled conditions limits their ability to capture the full complexity of sublethal effects, particularly those that emerge at the level of honey bee body segments that integrate motor control. Restraint can also alter the bee's motivational state ^{42,43}, reducing responsiveness compared to freely moving individuals.

Although significant progress has been made in understanding how imidacloprid affects bee behavior at sublethal levels, there remains a need for experimental protocols capable of capturing the finer details of motor function. Movement in bees is a complex trait that depends on the precise coordination of spatial patterns across different body segments and on the integration of neuronal and muscular activity within narrow temporal windows. The brain is generally considered the primary regulator of leg and wing coordination, but local thoracic circuits also play a substantial role in controlling posture movements. For example, a simple feed routine may include several behaviors on the flower, such as antennal scanning, proboscis extension to take nectar, and leg positioning to secure a grip^{44,45}. If any step in this sequence is disrupted, efficiency in nectar collection and pollination can decline sharply. The extent to which pesticides interfere with these fine-motor patterns remains unclear. This uncertainty raises the question of whether exposure may affect behaviors differently across neural centers within body segments, and how those individual-level impairments could scale up to alter collective motion.

Here, we aimed to investigate whether sublethal exposure to a commercial formulation of imidacloprid (Confidor 350 CS) differentially affects motor behaviors governed by distinct neural centers across body segments. Therefore, each segment is expected to respond differently to imidacloprid exposure due to differences in their neural organization and physiological function. We examined the effects of orally administered imidacloprid on individual motor responses associated with the head, thorax, and abdomen. Specifically, we evaluated three segment-specific behavioral responses: i. PER response (head), ii. walking activity and leg control (thorax), and iii. Sting extension response (SER; abdomen). The group-level movement was assessed via a novelty treadmill to capture angular displacement and angular speed. By integrating fine-scale behavioral analyses within the same individuals, our study provides a comprehensive view of how imidacloprid disrupts sensorimotor integration in honey bees and how individual deficits correlate with alterations in collective movement. These findings have significant implications for developing relevant assessment frameworks toward more refined evaluations of pesticides.

MATERIALS AND METHODS

Collection and maintenance of experimental animals

We collected Africanized honey bees (*Apis mellifera*) from a hive at the Universidad del Rosario (Bogotá, Colombia) during the morning hours to capture active foragers. The collection was performed directly at the hive exit to obtain outbound foragers rather than returning individuals. Bees were captured only while flying outward, allowing collection before contact with external food sources. Depending on the behavioral evaluation, the bees were either individually or in groups and then transferred to the laboratory. Both groups were maintained at 35 °C in the dark for 24 hours without experimental intervention to acclimate, with access to 1 M sucrose solution. This temperature range was selected to reproduce the typical thermal conditions inside an active hive, ensuring that foragers experienced a physiological environment and minimizing cold stress.

once removed from colony^{46,47}. All bees were obtained from the same hive to ensure uniform origin and minimize inter-colony behavior variability.

For individual maintenance, we employed a custom-built restraining device designed to avoid the need for anesthesia and provide an alternative to conventional tube restraints^{48,49}. The device was assembled from an acetate sheet (0.3 mm thick) to form a cube measuring 2.5 cm per side. Although cube-shaped, only the central platform physically confined the bee, keeping all the bee's segments (head, thorax, abdomen) visible for observation. The bee was introduced through a 3 mm circular opening on the top face and held at the thorax-abdomen junction, with the wings gently fixed to the cube surface using tape. This configuration allowed the bee to move its head, legs, and abdomen freely while maintaining a stable position (Figure 1 A). The mechanical modeling of the cube system includes a free-body diagram, provided in the Supplementary information.

For group experiments, bees were housed in plastic containers (volume: 250 mL). These containers were adapted from internationally validated setups for bee toxicology^{50,51}, providing both ventilation and access to food. Device feeders consisted of glass vials (40 mL) connected to 1000 μ L micropipette tips filled with tissue paper (Figure 1 B). The narrow end of each micropipette tip was inserted through the bottom of the container, allowing bees to feed. This system ensured ad libitum delivery of test substances while enabling precise monitoring of consumption and preventing losses from spillage or excessive evaporation (Figure 1 C). These containers were positioned on PVC pipes to allow free rotation by the bee movements (Figure 1 D), this system was denominated as a treadmill. The collective movements of the bees generated sufficient torque to rotate the container about its central axis, enabling quantification of activity via angular displacement and angular velocity measurements. The mechanical modeling of the treadmill is provided in the Supplementary information.

Imidacloprid concentrations

We tested the effects of oral administration of Confidor 350 CS¹⁸, a commercial formulation of imidacloprid containing 350g/L of the active ingredient. The use of a commercial product was intentional, as it represents the realistic exposure scenarios experienced by pollinators in agricultural landscapes, where the active ingredients coexist with formulation coadjutants. The insecticide was diluted in a 1 M sucrose solution (31.1% w/w) to obtain final concentrations of 0.3 ppm (0.3Im), 0.6 ppm (0.6Im), and 3.0 ppm (3.0Im). These values correspond to the range of imidacloprid residues commonly detected in nectar and pollen of treated crops, which typically vary between 0.1 and 4.0 ppm^{52,53}. The oral LD₅₀ for *Apis mellifera* is approximately 3.7 ng per bee⁵⁴, confirming that the selected concentrations fall well within the sublethal range. The 1 M sucrose solution ensured physiological relevance, providing an attractive and standardized medium for ingestion.

Evaluations 1 and 2: effects of oral administration of imidacloprid on the proboscis extension response (PER) and the sting extension response (SER)

We collected and maintained 164 forager bees in individual cubes to evaluate the proboscis extension response (PER) and the sting extension response (SER). One day after collection, each bee was randomly assigned to one of four treatments: i. Control (25 μ L of 1 M sucrose solution); ii. 0.3Im (25 μ L of 0.3 ppm solution); iii. 0.6Im (25 μ L of 0.6 ppm solution); iv. 3.0Im (25 μ L of 3.0 ppm solution).

Behavior testing began 15 minutes after the oral administration of the treatments. This timing was selected based on pharmacokinetic⁵⁵ studies showing that imidacloprid and its metabolites reach the nervous system within this interval, producing early synaptic effects. Behavioral assays conducted under controlled laboratory conditions also demonstrate that sublethal effects on learning and motor responses can appear within minutes after exposure^{56–58}. Each bee was subsequently stimulated to elicit both the proboscis extension reflex and the sting extension reflex, allowing direct comparison of appetitive and defensive responses within the same individual. PER was always tested first, followed by SER after an interval to avoid cross-stimulation between responses. The appetitive response (PER) was induced by gently touching the antennae with 1 M sucrose solution, whereas the defensive response (SER) was elicited by gently touching the posterior region of the thorax with a soft brush. Both responses were recorded every 15 minutes over a total period of 3.5 hours (Supplementary information: Video S2).

The time window for evaluation was selected to capture the initial and progressive phase of the neurotoxic response without inducing fatigue or habituation, as has been shown in previous studies^{56,59,60}. Interval-time sampling over 3.5 hours allowed detection of transient and cumulative behavioral alterations. Exhaustion or loss of responsiveness was assessed by comparing control groups that maintained stable reflex responses throughout the experiment.

Evaluation 3: effects of oral administration of imidacloprid on group movement

We collected and maintained over 700 forager bees in plastic containers (Figure 1 E). Each container housed approximately 40 bees, maintaining a constant density across treatments and ensuring comparable per-capita consumption. One day after collection, containers were randomly assigned to one of three treatments: i. Control (1 M sucrose solution); ii. 0.3Im; iii. 0.6Im. The substances were administered in a 1 M sucrose solution, which bees consumed freely over a 24-hour. Group responses were assessed by video recording the containers' rotational movement, generated by the bees' collective activity. The rotational displacement reflects the net torque produced by their movements inside the container and serves as a measure of collective motor activity. Videos were processed in Premiere Pro⁶¹ and exported as MP4s for motion-tracking analysis using dedicated software⁶². For each treatment, angular displacement and angular speed were extracted from the recordings. Since movement occurred randomly in both clockwise and counterclockwise directions, absolute values were calculated for subsequent analysis (Supplementary information: Video S4). Angular displacement and speed were extracted from 10-

minute video segments per replicate, a duration that provided stable estimates for collective motion. This evaluation was animated considering the free and restrained tests, used to track sublethal effects caused by pesticides ^{63–65}.

Evaluation 4: effects of oral administration of imidacloprid on individual movement

We collected and maintained 50 forager bees, keeping them individually in cubes. One day after collection, each bee was randomly assigned to one of two treatments: i. Control (25 μ L of 1 M sucrose solution); ii. 0.3Im (25 μ L of 0.3 ppm solution). The 0.3 ppm was selected to evaluate whether low doses of imidacloprid affect bee motor coordination. Thirty minutes after administration, individual body movements were recorded for approximately 17 seconds (17.2 ± 3.9 s) using a camera mounted on a stereoscope (Leica EZ4) (supplementary material: Video S3). The videos were processed in Adobe Premiere Pro ⁶¹ to generate a single 4-minute clip per treatment; bee images were arranged into a single frame, and external sounds were removed. The movement of the body segments was analyzed based on changes in pixel intensity, with each segment treated as a region of interest. Quantitative analysis of movement was performed using Fiji ImageJ software ⁶⁶. For each region, we computed the mean and standard deviation of pixel intensity across frames, which were then used to calculate the coefficient of variation (CoV) of pixel values. To characterize motion dynamics, CoV time series were further smoothed to extract overall trends, and the standard error of the smoothed model was calculated as a proxy for the variability of segmental movements, providing a measure of motion alteration induced by imidacloprid exposure. To ensure that changes in pixel intensity reflected bee movement rather than variations in illumination, all recordings were acquired under standardized lighting conditions with a homogeneous background. Control videos without bees confirmed that background signal variation remained below the analysis's detection threshold. This evaluation was motivated by the antenna and other tests, used to track sublethal effects caused by pesticides ^{67–69}.

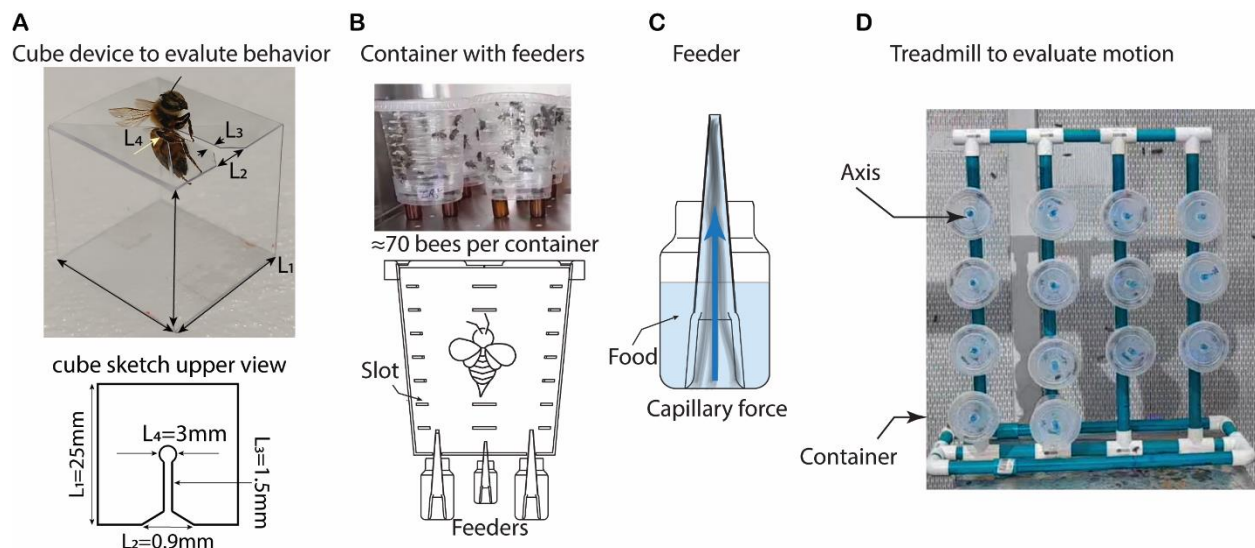


Figure 1 Experimental setups for behavioral assays in honey bees. A) Cube-shaped restraint device used for proboscis extension reflex (PER) and sting extension reflex (SER) evaluations, showing a bee inside and schematic dimensions. B) Group container with three feeding slots designed to house ~70 bees. Upper panel: photograph in use; lower panel: schematic of feeding slots. C) Feeder system consisting of a glass vial and micropipette tip, ensuring ad libitum sucrose or test substance delivery. D) Treadmill apparatus for collective locomotion, with containers attached to a central axis rotating with bee movement.

Statistical analysis

Proboscis extension response (PER; Evaluation 1) and sting extension response (SER; Evaluation 2), both binary outcomes, were analyzed using generalized linear mixed models (GLMMs) with a binomial error distribution and logit link function, fitted using the lme4 package in R. Treatment, time, and their interaction were included as fixed effects, while individual bee identity was included as a random effect to account for repeated measurements. Post hoc pairwise comparisons among treatment levels were conducted using estimated marginal means with Tukey adjustment for multiple comparisons (emmeans package). Group locomotor variables, including angular displacement and angular speed (Evaluation 3), were analyzed using mixed-effects models with treatment as a fixed effect and container identity as a random effect. For individual movement analyses (Evaluation 4), body-region activity was quantified from video recordings as changes in pixel intensity over time. These data were analyzed using mixed-effects models with treatment as a fixed effect. In addition, non-parametric regressions were applied to pixel-intensity time series, and the resulting squared errors were used as an index of movement variability; these values were compared across treatments using mixed-effects models. Model assumptions were evaluated by inspection of residuals. All statistical analyses were performed in R (version 4.5.2) using RStudio.⁷⁰

RESULTS

Evaluations 1 and 2: oral administration of imidacloprid alters the proboscis extension reflex (PER) and the sting extension reflex (SER)

We prepared 120 bees assigned to one of four treatments (none were excluded): Control (N = 30), 0.3Im (N = 30), 0.6Im (N = 30), and 3Im (N = 30). Oral administration of imidacloprid reduced the likelihood of eliciting the proboscis extension response (PER) in a concentration-dependent manner (GLMM, likelihood ratio test: $\chi^2 = 152.84$, $p < 0.0001$). Bees exposed to 0.3Im showed PER probabilities comparable to controls, suggesting that appetitive responsiveness remained preserved mainly at the lowest concentration (odds ratio Control/Low = 1.55, $p = 0.14$). In contrast, exposure to 0.6Im significantly reduced PER relative to both controls and the low-dose group (odds ratio Control/Medium = 3.20, $p < 0.0001$), and this impairment was markedly more substantial at 3Im, where the probability of PER was more than an order of magnitude lower than in controls (odds ratio Control/High = 12.49, $p < 0.0001$; Figure 2).

To further examine temporal dynamics, PER was evaluated across three intervals: I₁ (0–70 min), I₂ (70–140 min), and I₃ (140–210 min). Control and 0.3Im bees showed relatively stable appetitive responses across intervals, despite a modest overall time effect. Bees exposed to 0.6Im exhibited a tendency toward declining PER over time, although pairwise contrasts between intervals did not reach statistical significance after correction. In contrast, the highest concentration (3Im) produced a pronounced and progressive decline, with PER decreasing significantly from I₁ to I₂ and further to I₃ (Tukey-adjusted post hoc contrasts, $p < 0.0001$). For this group, PER at I₃ was considerably lower than at I₁, indicating an apparent deterioration of appetitive responsiveness as exposure time increased (Supplementary Table S1).

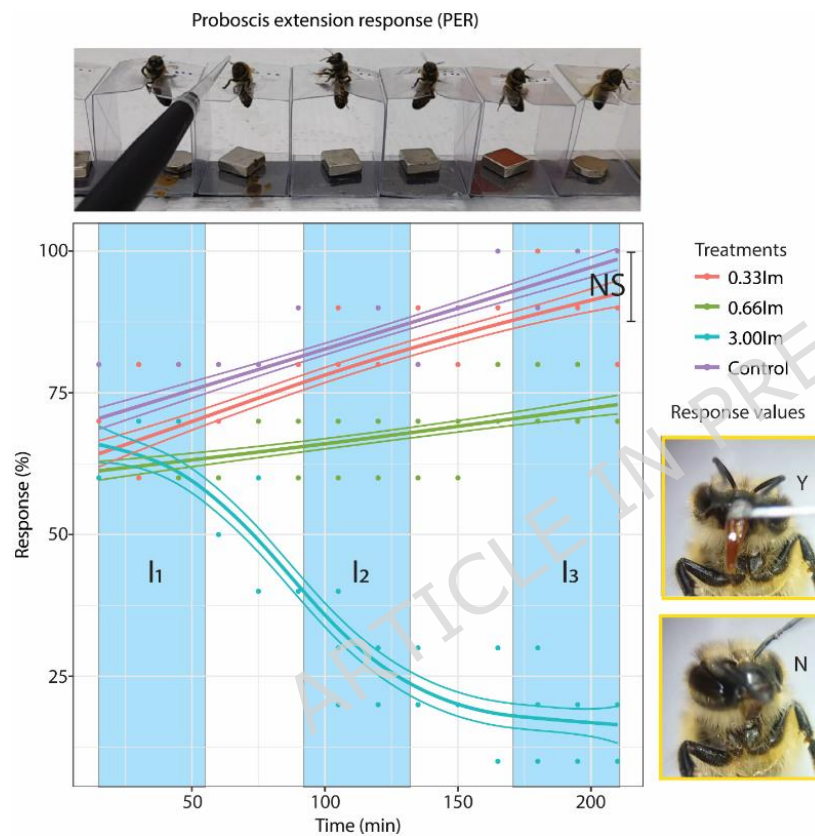


Figure 2 Dose-dependent impairment of the proboscis extension reflex (PER) following imidacloprid administration. Upper panel: schematic of PER setup. Middle panel: temporal variation in PER percentages across treatments (Control, 0.3Im, 0.6Im, 3.0Im). Shaded regions indicate evaluation intervals (I₁: 0–70 min, I₂: 70–140 min, I₃: 140–210 min). Solid lines represent regression fits; dots show individual responses. Lower images: examples of scoring criteria (1 = proboscis extension, 0 = no response). Imidacloprid significantly reduced PER in a dose- and time-dependent manner.

Oral administration of imidacloprid also altered the probability of eliciting the sting extension response (SER), with effects strongly dependent on concentration (GLMM, likelihood ratio test: $\chi^2 = 189.57$, $p < 0.0001$). Bees exposed to 0.3Im displayed SER probabilities similar to controls (odds ratio Control/Low = 1.12, $p = 0.98$), indicating that defensive responsiveness was largely

unaffected at the lowest dose. In contrast, both 0.6Im and 3Im produced substantial reductions in SER probability relative to controls (odds ratio Control/Medium = 5.99, $p < 0.0001$; Control/High = 11.08, $p < 0.0001$), with clear separation among all dose levels except between control and low dose (Figure 3).

When SER responses were examined across time intervals, a different pattern emerged compared to PER. Control, 0.3Im, and 0.6Im bees maintained relatively stable defensive responses across I_1 , I_2 , and I_3 , with no significant temporal contrasts detected after correction. Only the highest concentration (3Im) showed a significant time-dependent change, characterized by a gradual decline in SER probability between I_1 and later intervals (Tukey-adjusted post hoc contrasts, $p = 0.014$). This pattern suggests that, while SER is primarily affected by imidacloprid concentration, prolonged exposure at the highest dose is required before temporal deterioration of defensive responsiveness becomes evident (Supplementary Table S2).

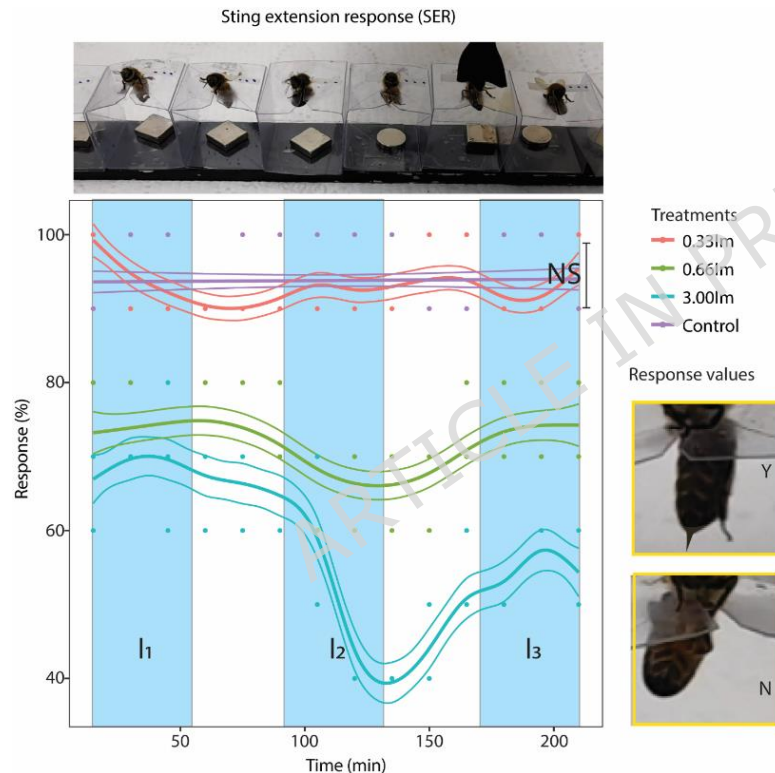


Figure 3. Sting extension reflex (SER) declines with exposure to imidacloprid. Upper panel: schematic of SER setup. Central panel: temporal variation in SER percentages across treatments (Control, 0.3Im, 0.6Im, 3.0Im). Shaded regions indicate evaluation intervals (I_1 – I_3). Solid lines represent regression fits; dots show individual responses. Right panels: examples of positive (Y) and negative (N) responses. Imidacloprid significantly reduced SER at intermediate and high doses.

Evaluation 3: oral administration of imidacloprid decreases the motion of the bee group

Similar to the PER and SER essays, oral administration of imidacloprid significantly impaired group movement in a dose-dependent manner. Analyses were conducted using the absolute values of angular displacement ($|\text{angle}|$) and absolute angular speed ($|\text{angular speed}|$) of the containers. Angular displacement differed significantly among treatments (ANOVA: $F_{2,18782} = 942.36$, $p < 0.0001$). Bees exposed to 0.6Im exhibited the lowest angular displacement (Control vs. 0.6Im: post hoc Tukey test: 95% CI of reduction: -1.38, -1.22 rad, $p < 0.0001$). A reduced dose of 0.3Im produced a slight increase in displacement relative to 0.6Im. Still, values remained significantly lower than those of controls (Control vs. 0.3Im: post hoc Tukey test: 95% CI of reduction: -1.22, -1.06 rad, $p < 0.0001$). Moreover, displacement values differed significantly between the two imidacloprid concentrations (0.3Im vs. 0.6Im: post hoc Tukey test: 95% CI of reduction: 0.08, 0.24 rad, $p < 0.0001$) (Figure 4 A).

Angular speed showed a similar pattern (ANOVA: $F_{2,18096} = 1192.7$, $p < 0.0001$). Bees exposed to 0.6Im exhibited the lowest angular speed (Control vs. 0.6Im: post hoc Tukey test: 95% CI of reduction: -3.27, -2.94 rad/s, $p < 0.0001$). At a lower dose, 0.3Im led to a moderate increase compared to 0.6Im, but values remained significantly reduced relative to controls (Control vs. 0.3Im: post hoc Tukey test: 95% CI for the reduction: -2.98, -2.65 rad/s, $p < 0.0001$). Significant differences were also observed between the two imidacloprid concentrations (0.3Im vs. 0.6Im: post hoc Tukey test: 95% CI of reduction: 0.12, 0.45 rad/s, $p < 0.0001$) (Figure 4B, supplementary table S5)

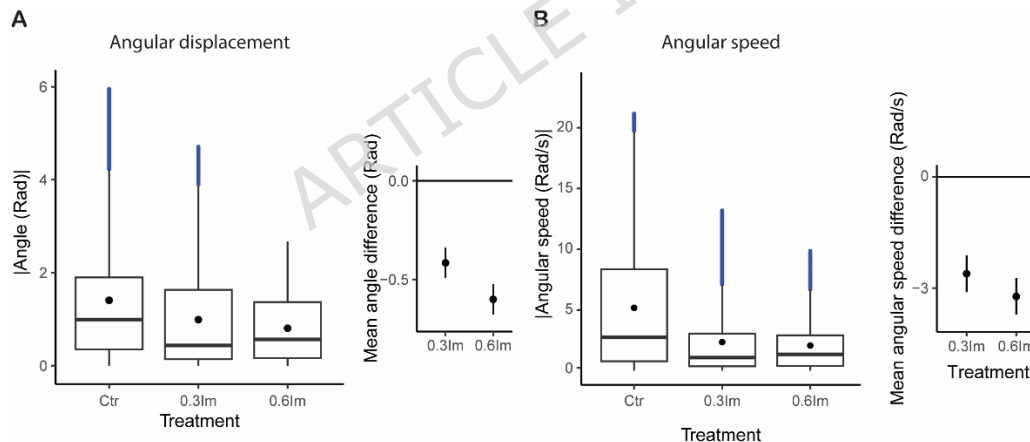


Figure 4 Collective locomotion is reduced by oral imidacloprid. A) Absolute angular displacement ($|\text{angle}|$, rad) and B) absolute angular speed ($|\text{angular speed}|$, rad/s) across treatments (Control, 0.3Im, 0.6Im). Boxplots show medians, interquartile ranges, and variability; black circles indicate means. Right panels: mean differences ($\pm 95\%$ CI) relative to controls. Both displacement and speed declined as imidacloprid concentration increased, indicating impaired group locomotion.

Evaluation 4: oral administration of imidacloprid impairs individual movement

Analysis of body segment activity revealed that oral administration of imidacloprid produced significant differences in pixel-intensity variability across all regions examined (Figure 5 A),

reflecting a treatment-induced alteration in movement dynamics. Bees exposed to 0.3 ppm imidacloprid exhibited significantly higher pixel variability in the head ($F_{1,10396} = 934.52$, $p < 0.0001$), thorax ($F_{1,10396} = 1218.0$, $p < 0.0001$), and abdomen ($F_{1,10396} = 2865.9$, $p < 0.0001$), indicating that exposure consistently increased variability across body segments. These results confirm that the treatment induced measurable changes in movement patterns, although they do not by themselves specify the qualitative nature of the motor alteration (Figure 5 B). Although all segments showed altered movement patterns, the head and abdomen exhibited the most considerable changes, indicating that the magnitude of these effects varied among segments.

Movement variability across all segments was markedly altered by imidacloprid consumption, with exposed bees exhibiting increased stillness relative to controls. These differences between treatments were quantified by the mean squared errors (SE) of the regression models fitted to each body segment, which served as a proxy for overall video activity. The analysis confirmed significant reductions in activity across all regions (ANOVA: Head $F_{1,152}=197.3$, $p < 0.0001$; Thorax $F_{1,152}=111.9$, $p < 0.0001$; Abdomen $F_{1,152}=112.9$, $p < 0.0001$), with the most significant effect observed in the head. These results show that low-dose imidacloprid not only increases pixel variability but also reduces the dynamic range of movements, consistent with impaired motor coordination (Figure 5C, supplementary table S2). The increased variability in pixel intensity could also represent small, rapid oscillations consistent with tremor-like movements rather than purposeful motion.

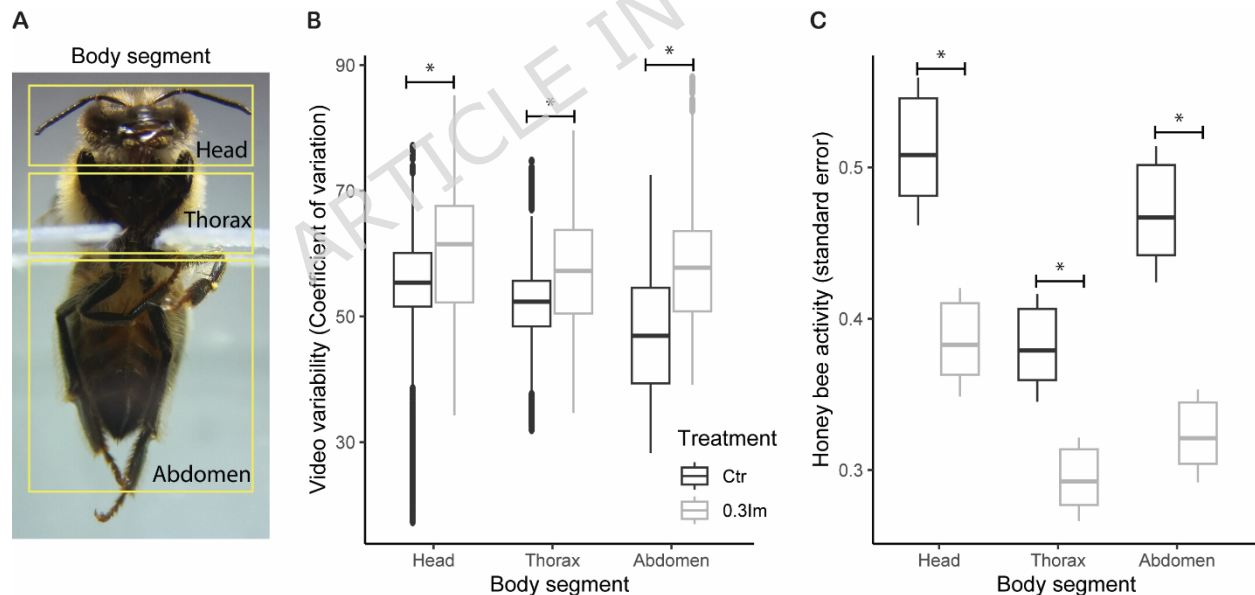


Figure 5 Individual motor coordination is disrupted by low-dose imidacloprid. A) Regions of interest (head, thorax, abdomen) used for motion analysis. B) Pixel variability expressed as the coefficient of variation (CoV) of intensity values, significantly higher in imidacloprid-treated bees. (C) Motion activity estimated from the standard error of smoothed CoV curves, showing reduced dynamic range of movements in treated bees, with the most substantial effect in the head. Together, the results demonstrate impaired segmental coordination.

DISCUSSION

The findings of this study provide evidence that sublethal exposure to imidacloprid alters multiple dimensions of honey bee behavior, affecting responses measured at both the individual and group scales. At the reflexive level, exposure was associated with a reduced probability of proboscis extension (PER) and sting extension (SER), indicating diminished appetitive and defensive responsiveness. At the motor level, treadmill-based assays revealed decreased overall activity and increased stillness, with movement variability declining more strongly in the head than in other body regions, reflecting differences in the magnitude of behavioral effects across segments rather than evidence of selective impairment. These results align with previous studies describing cognitive, sensory, and locomotor impairments in bees exposed to neonicotinoids^{29,58,71}, while extending earlier work by incorporating the defensive reflex and a group-level activity assay. These behavioral dimensions have received comparatively less attention. By evaluating appetitive, defensive, and motor behaviors within the same experimental framework, our approach integrates multiple functional domains of behavior, allowing detection of subtle or transient changes that may not be evident when a single response is assessed in isolation.

Regarding the appetitive response, the proboscis extension reflex (PER) exhibited a dose-dependent reduction, as illustrated in Figure 2. The percentage of bees responding decreased progressively with increasing concentrations of imidacloprid, with the most pronounced effect observed in the group exposed to 3.0Im. Intermediate reductions were evident at 0.3Im and 0.6Im, indicating that even relatively low concentrations were associated with reduced appetitive responsiveness⁷². This pattern is consistent with previous studies reporting that neonicotinoids interfere with conditioned feeding reflexes and olfactory learning^{73,74}. The progressive decline in PER suggests a reduced capacity to express feeding-related reflexes under exposure conditions, which may affect behaviors required during foraging. While the present study does not resolve the underlying physiological mechanisms, these results demonstrate a functional impairment of appetitive responses following sublethal exposure to imidacloprid.

In contrast to the appetitive response, an additional contribution of this study is the evaluation of the sting extension reflex (SER) as a behavioral indicator of sublethal imidacloprid exposure. The defensive response declined significantly with increasing imidacloprid concentrations, with SER responses lower than Control at the two highest doses tested (0.6Im and 3.0Im). This pattern indicates that defensive responsiveness is particularly sensitive to exposure under the experimental conditions used. Nevertheless, these effects may also reflect an overall reduction in activity or arousal rather than a disruption restricted to a specific neural circuit. From a functional perspective, changes in SER expression may influence the capacity of individual bees to express defensive behaviors when challenged, although the present study does not assess colony-level outcomes. The observed reduction in SER therefore reflects a measurable behavioral impairment rather than a direct inference about underlying sensory or motor pathways. Because PER and SER are expressed by different body regions, comparing appetitive and aversive responses provides a segment-based

behavioral framework to examine how sublethal exposure alters sensorimotor performance across functional domains.

Regarding group-level activity, an additional contribution of this study was the quantification of container rotation using angular displacement and angular speed as integrative measures of collective movement (Figure 4). Both metrics showed that imidacloprid exposure was associated with a marked reduction in overall group activity, as reflected by decreased container rotation. Containers housing bees exposed to 0.6Im exhibited significantly shorter angular displacements than controls, indicating a dose-dependent decline in aggregate movement. Similarly, angular speed decreased with increasing imidacloprid concentration, reflecting reduced activity levels and shorter angular displacements, and thus a smaller effective area covered by the rotating container. These results indicate that reductions in individual motor activity, when expressed simultaneously by multiple bees, translate into measurable changes at the group level. While this assay does not resolve coordinated or socially structured behaviors, it provides an integrated measure of collective activity that captures the cumulative effects of sublethal exposure across individuals ^{75,76}. Regarding individual locomotion, body-region activity quantified via pixel-intensity analysis showed that imidacloprid exposure significantly altered movement patterns across all three segments. Rather than indicating selective impairment, these results suggest a general suppression of movement, with differences in the magnitude of the reduction across body regions. Movement variability, quantified as mean squared error (Figure 5B), decreased following imidacloprid exposure, consistent with reduced motor output and diminished behavioral flexibility ^{77,78}.

The more substantial decline observed in head and abdominal movement relative to the thorax reflects uneven expression of behavioral impairment across segments, without implying selective targeting of specific motor systems, as shown in other behavioral evaluation paradigms ^{71,78,79}. While the present study does not resolve the physiological mechanisms underlying these patterns, the segment-based analysis highlights how global reductions in activity may manifest heterogeneously across the body. These findings are consistent with previous reports of impaired navigation, orientation, and homing in bees exposed to imidacloprid ^{28,76,80}, and provide a complementary behavioral framework for interpreting how widespread motor suppression may contribute to complex behavioral deficits.

These results show that the commercial imidacloprid is not uniform across the PER and SER behaviors. Although impairments are generally scaled with concentration, the degree of disruption differed markedly between appetitive and defensive reflexes. The PER behavior showed greater resilience at low doses but was eventually suppressed at the highest concentration, reflecting strong modulation by feeding motivation and starvation state (Figure 2). In contrast, the SER behavior was more sensitive to intermediate concentrations and exhibited a transient depression followed by partial recovery (Figure 3), indicating that its underlying mechanism responds differently to prolonged exposure. These distinct temporal profiles indicate that both behaviors respond differently to imidacloprid concentration, allowing other forms to evaluate the effects of the pesticide.

Altogether, these results are consistent with previous evidence indicating that *Apis mellifera* is highly sensitive to sublethal exposure to imidacloprid^{53,81}. By integrating appetitive, defensive, and locomotor measurements within a single experimental framework, this study broadens the behavioral contexts in which these effects can be detected. Although the present work does not directly address the underlying neurophysiological or metabolic mechanisms, the observed behavioral alterations highlight the need for future studies linking functional impairments to changes at the neural or muscular level. From an applied perspective, the defensive reflex assay may represent a complementary behavioral endpoint for detecting subtle sublethal effects under controlled conditions.

An additional aspect of this study is the use of basic physical descriptors, such as displacement and angular velocity, to aid in the interpretation of the observed behavioral changes following imidacloprid exposure. Rather than serving as a mechanistic model, this framework provides an intuitive way to relate reductions in collective motion to constraints on force generation and coordinated movement. In this sense, physical principles offer a complementary perspective for interpreting how individual-level motor impairments may scale to group-level behavior. While this approach remains descriptive, it suggests that integrating biophysical metrics with behavioral toxicology could help structure future investigations of collective impairment under sublethal stress.

CONCLUSIONS

This study indicates that sublethal exposure to imidacloprid can alter essential behaviors in *Apis mellifera*. Appetitive and defensive reflexes were reduced in a dose-dependent manner, with sting extension (SER) responding earlier and more strongly than proboscis extension (PER) under comparable exposure conditions. Segmental analyses showed reduced head and abdominal movements and greater motor pattern variability, suggesting possible coordination impairments. At the group level, collective locomotion tended to be lower, consistent with reduced individual activity. By integrating reflex, motor, and collective outcomes within a single framework, this work outlines how behavioral alterations may propagate across organizational levels. The combined use of behavioral assays and physical modelling provides a practical and adaptable approach for investigating sublethal pesticide effects in pollinators.

Acknowledgements

This work was supported by the Universidad del Rosario through its Graduate Assistantship Program and by the Fundación Universitaria Los Libertadores. We thank both institutions for their financial and academic support during the development of this research. We also thank Daniel Hernández Vargas for his assistance with data management and analysis, which helped strengthen the rigor of the study.

Author contributions

J.P.H. conceived and designed the study, performed the experiments, analyzed the data, prepared the figures, and wrote the manuscript. N.B. assisted with experimental procedures and wrote the manuscript. F.M. contributed to statistical analysis and wrote the manuscript. A.J.R. supervised the project, provided conceptual guidance, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Additional Information

Competing interests: The authors declare no competing interests.

Funding: This project was supported by the Big Grant IV-FGD003 of the Dirección de Investigación e Innovación (Diel; Universidad del Rosario, Bogotá, Colombia) to A.J.R.

REFERENCES

1. Nath, R., Singh, H. & Mukherjee, S. Insect pollinators decline: an emerging concern of Anthropocene epoch. *Journal of Apicultural Research* vol. 62 23–38 Preprint at <https://doi.org/10.1080/00218839.2022.2088931> (2023).
2. Siviter, H. *et al.* Agrochemicals interact synergistically to increase bee mortality. *Nature* 596, 389–392 (2021).
3. Mokkapati, J. S., Bednarska, A. J. & Laskowski, R. Physiological and biochemical response of the solitary bee *Osmia bicornis* exposed to three insecticide-based agrochemicals. *Ecotoxicol Environ Saf* 230, (2022).
4. Parkinson, R. H., Fecher, C. & Gray, J. R. Chronic exposure to insecticides impairs honeybee optomotor behaviour. *Frontiers in Insect Science* 2, (2022).
5. Paoli, M. & Giurfa, M. Pesticides and pollinator brain: How do neonicotinoids affect the central nervous system of bees? *European Journal of Neuroscience* vol. 60 5927–5948 Preprint at <https://doi.org/10.1111/ejn.16536> (2024).
6. Liao, L. H., Wu, W. Y. & Berenbaum, M. R. Behavioral responses of honey bees (*Apis mellifera*) to natural and synthetic xenobiotics in food. *Sci Rep* 7, (2017).
7. Murawska, A., Migdał, P., Latarowski, K., Berbeć, E. & Zarębski, K. The condition of the bee colony (*Apis mellifera*) after exposure to pesticide and their mixtures. *Apidologie* 56, (2025).

8. Goñalons, C. M. & Farina, W. M. Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. *PLoS One* 10, (2015).
9. Gonzalez, V. H. *et al.* Acute exposure to sublethal doses of neonicotinoid insecticides increases heat tolerance in honey bees. *PLoS One* 17, (2022).
10. Bartling, M. T., Brandt, A., Hollert, H. & Vilcinskas, A. Current Insights into Sublethal Effects of Pesticides on Insects. *International Journal of Molecular Sciences* vol. 25 Preprint at <https://doi.org/10.3390/ijms25116007> (2024).
11. Su, Y. *et al.* Sublethal effects of imidacloprid and clothianidin on the biological traits of predatory lacewing *Chrysopa pallens* (Rambur) (Neuroptera: Chrysopidae). *Crop Protection* 163, (2023).
12. Stehle, S. *et al.* Neonicotinoid insecticides in global agricultural surface waters – Exposure, risks and regulatory challenges. *Science of the Total Environment* 867, (2023).
13. Tosi, S., Sfeir, C., Carnesecchi, E., vanEngelsdorp, D. & Chauzat, M. P. Lethal, sublethal, and combined effects of pesticides on bees: A meta-analysis and new risk assessment tools. *Science of the Total Environment* vol. 844 Preprint at <https://doi.org/10.1016/j.scitotenv.2022.156857> (2022).
14. Girolami, V. *et al.* Effect of repeated intakes of a neonicotinoid insecticide on the foraging behaviours of *Apis mellifera* in field trials. *Environmental Science and Pollution Research* 30, 12257–12268 (2023).
15. Carroll, M. J., Brown, N. J. & Reitz, D. Sublethal effects of imidacloprid-contaminated honey stores on colony performance, queens, and worker activities in fall and early winter colonies. *PLoS One* 19, (2024).
16. Negi, B. R., Harish, &, Sharma, K., Thakur, M. & Prashad, H. Lethal and sublethal effects of thiamethoxam, a neonicotinoid molecule, on colony performance of *A. mellifera*. <https://doi.org/10.1007/s11356-021-16426-5>/Published doi:10.1007/s11356-021-16426-5/Published.
17. Zhang, J. *et al.* Comprehensive analysis of neonicotinoids in Chinese commercial honey and pollen: A corresponding health risk assessment for non-targeted organisms. *Science of the Total Environment* 919, (2024).
18. Bayer. CONFIDOR SC350 | Protección de Cultivos | Agro Bayer Colombia. https://www.agro.bayer.co/es-co/productos/product-details.html/insecticide/confidor_sc350.html (2024).
19. Fouad, M. R. & Abdel-Raheem, S. A. A. An overview on the fate and behavior of imidacloprid in agricultural environments. *Environmental Science and Pollution Research* Preprint at <https://doi.org/10.1007/s11356-024-35178-6> (2024).

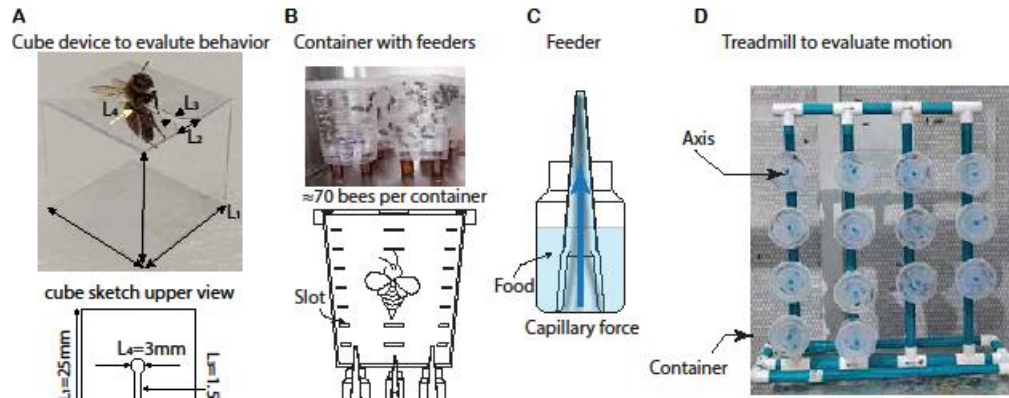
20. Al-Hawadi, J. S., Al-Sayaydeh, R. S., Al-Rawashdeh, Z. B. & Ayad, J. Y. Monitoring of imidacloprid residues in fresh fruits and vegetables from the central parts of Jordan. *Heliyon* 9, (2023).
21. Zhang, Y. *et al.* Metabolic disturbance in lettuce (*Lactuca sativa*) plants triggered by imidacloprid and fenvalerate. *Science of the Total Environment* 802, (2022).
22. Zhang, Z., Li, Y., Li, X., Zhu, X. & Zhang, Y. Efficacy of Imidacloprid Seed Treatments against Four Wheat Aphids under Laboratory and Field Conditions. *Plants* 12, (2023).
23. Suchail, S., Debrauwer, L. & Belzunces, L. P. Metabolism of imidacloprid in *Apis mellifera*. *Pest Manag Sci* 60, 291–296 (2004).
24. Paus-Knudsen, J. S., Sveinsson, H. A., Grung, M., Borgå, K. & Nielsen, A. The Neonicotinoid Imidacloprid Impairs Learning, Locomotor Activity Levels, and Sucrose Solution Consumption in Bumblebees (*Bombus terrestris*). *Environ Toxicol Chem* 42, 1337–1345 (2023).
25. Riveros, A. J. & Gronenberg, W. Learning from learning and memory in bumblebees. *Commun Integr Biol* 2, 437–440 (2009).
26. Christian, M., Kraft, M., Wilknitz, P., Nowotny, M. & Schöneich, S. Flupyradifurone, imidacloprid and clothianidin disrupt the auditory processing in the locust CNS. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* <https://doi.org/10.1007/s00359-025-01735-8> (2025) doi:10.1007/s00359-025-01735-8.
27. Jia, Q., Cai, Y., Yuan, X., Li, B. & Li, B. The Degradation Process of Typical Neonicotinoid Insecticides in Tidal Streams in Subtropical Cities: A Case Study of the Wuchong Stream, South China. *Toxics* 11, (2023).
28. Fischer, J. *et al.* Neonicotinoids interfere with specific components of navigation in honeybees. *PLoS One* 9, (2014).
29. Tan, K. *et al.* Imidacloprid alters foraging and decreases bee avoidance of predators. *PLoS One* 9, (2014).
30. Li, H. *et al.* Sublethal doses of neonicotinoid imidacloprid can interact with honey bee chemosensory protein 1 (CSP1) and inhibit its function. *Biochem Biophys Res Commun* 486, 391–397 (2017).
31. Lunardi, J. S., Zaluski, R. & Orsi, R. O. Evaluation of motor changes and toxicity of insecticides Fipronil and imidacloprid in Africanized Honey bees (Hymenoptera: Apidae). *Sociobiology* 64, 50–56 (2017).

32. Nocelli, R. C. F. *et al.* New method to Proboscis Extension Reflex to the assessment of gustatory responses for stingless bees. *Revista Cincia, Tecnologia & Ambiente* 7, 69–76 (2018).
33. Van Nest, B. N. The Olfactory Proboscis Extension Response in the Honey Bee: A Laboratory Exercise in Classical Conditioning. *J Undergrad Neurosci Educ* 16, A168–A176 (2018).
34. Giurfa, M. & Sandoz, J. C. Invertebrate learning and memory: Fifty years of olfactory conditioning of the proboscis extension response in honeybees. *Learning and Memory* vol. 19 54–66 Preprint at <https://doi.org/10.1101/lm.024711.111> (2012).
35. Smith, B. H. & Burden, C. M. A Proboscis Extension Response Protocol for Investigating Behavioral Plasticity in Insects: Application to Basic, Biomedical, and Agricultural Research. *Journal of Visualized Experiments* 1–13 (2014) doi:10.3791/51057.
36. Démares, F. J., Crous, K. L., Pirk, C. W. W., Nicolson, S. W. & Human, H. Sucrose sensitivity of honey bees is differently affected by dietary protein and a neonicotinoid pesticide. *PLoS One* 11, (2016).
37. Ali, H., Iqbal, J., Raweh, H. S. & Alqarni, A. S. Proboscis behavioral response of four honey bee *Apis* species towards different concentrations of sucrose, glucose, and fructose. *Saudi J Biol Sci* 28, 3275–3283 (2021).
38. Riveros, A. J. & Gronenberg, W. Decision-making and associative color learning in harnessed bumblebees (*Bombus impatiens*). *Anim Cogn* 15, 1183–1193 (2012).
39. Barron, A. B., Gurney, K. N., Meah, L. F. S., Vasilaki, E. & Marshall, J. A. R. Decision-making and action selection in insects: inspiration from vertebrate-based theories. *Front Behav Neurosci* 9, (2015).
40. Tedjakumala, S. R. & Giurfa, M. Rules and mechanisms of punishment learning in honey bees: The aversive conditioning of the sting extension response. *Journal of Experimental Biology* vol. 216 2985–2997 Preprint at <https://doi.org/10.1242/jeb.086629> (2013).
41. Vergoz, V., Roussel, E., Sandoz, J. C. & Giurfa, M. Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex. *PLoS One* 2, (2007).
42. Gascue, F., Marachlian, E., Azcueta, M., Locatelli, F. F. & Klappenbach, M. Antennal movements can be used as behavioral readout of odor valence in honey bees. *IBRO Neurosci Rep* 12, 323–332 (2022).
43. Wong, R. K. & Spencer, G. E. Memory formation following appetitive conditioning is variably dependent on retinoid signaling. *Neurobiol Learn Mem* 219, (2025).

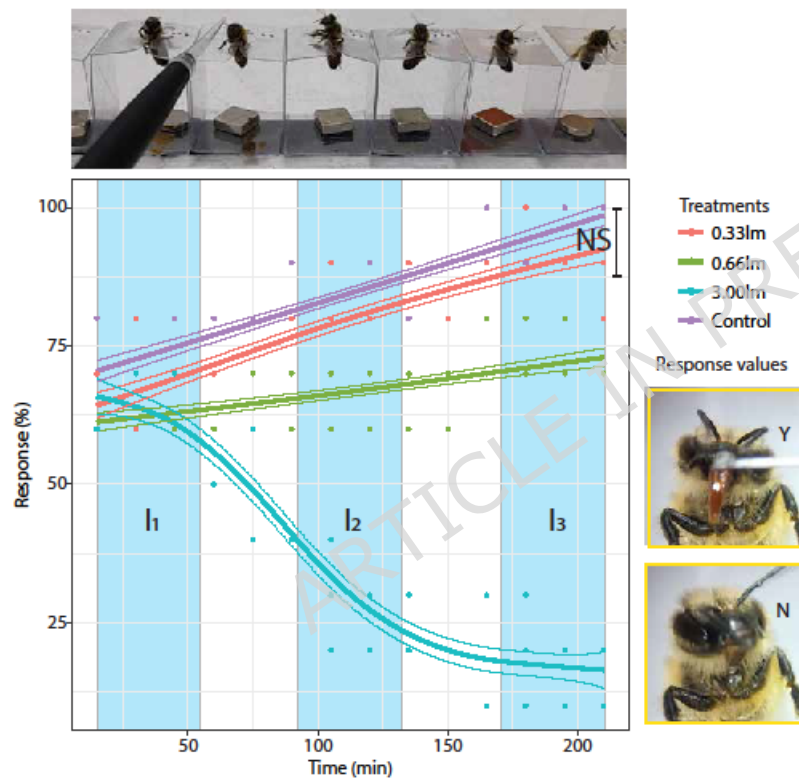
44. Tierney, S. M., Bernauer, O. M., King, L., Spooner-Hart, R. & Cook, J. M. Bee pollination services and the burden of biogeography. *Proceedings of the Royal Society B: Biological Sciences* 290, (2023).
45. Farina, W. M., Arenas, A., Estravis-Barcala, M. C. & Palottini, F. Targeted crop pollination by training honey bees: advances and perspectives. *Frontiers in Bee Science* 1, (2023).
46. Stabentheiner, A., Kovac, H., Mandl, M. & Käfer, H. Coping with the cold and fighting the heat: thermal homeostasis of a superorganism, the honeybee colony. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 207, 337–351 (2021).
47. Stabentheiner, A., Kovac, H. & Brodschneider, R. Honeybee colony thermoregulation - Regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. *PLoS One* 5, (2010).
48. Chol  , H., Junca, P. & Sandoz, J. C. Appetitive but not aversive olfactory conditioning modifies antennal movements in honeybees. *Learning and Memory* 22, 604–616 (2015).
49. Giurfa, M. & Malun, D. Associative mechanosensory conditioning of the proboscis extension reflex in honeybees. *Learning and Memory* 11, 294–302 (2004).
50. OCDE. *OCDE Guideline for the Testing of Chemicals Honey Bee (Apis Mellifera)*. <http://www.oecd.org/termsandconditions/>. (2017).
51. K  hler, A., Nicolson, S. W. & Pirk, C. W. W. A new design for honey bee hoarding cages for laboratory experiments. *Journal of Apicultural Research* vol. 52 12–14 Preprint at <https://doi.org/10.3896/IBRA.1.52.2.03> (2013).
52. Tosi, S., Nieh, J. C., Sgolastra, F., Cabbri, R. & Medrzycki, P. Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proceedings of the Royal Society B: Biological Sciences* 284, (2017).
53. Paloschi, C. L. *et al.* Imidacloprid: Impact on Africanized *Apis mellifera* L. (Hymenoptera: Apidae) workers and honey contamination. *Chemosphere* 338, (2023).
54. Verine Suchail, S., Guez, D. & Belzunces, L. P. *DISCREPANCY BETWEEN ACUTE AND CHRONIC TOXICITY INDUCED BY IMIDACLOPRID AND ITS METABOLITES IN APIS MELLIFERA*. *Environmental Toxicology and Chemistry* vol. 20 (2001).
55. Suchail, S., De Sousa, G., Rahmani, R. & Belzunces, L. P. In vivo distribution and metabolism of ¹⁴C-imidacloprid in different compartments of *Apis mellifera* L. *Pest Manag Sci* 60, 1056–1062 (2004).
56. Go  nals, C. M. & Farina, W. M. Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. *PLoS One* 10, (2015).

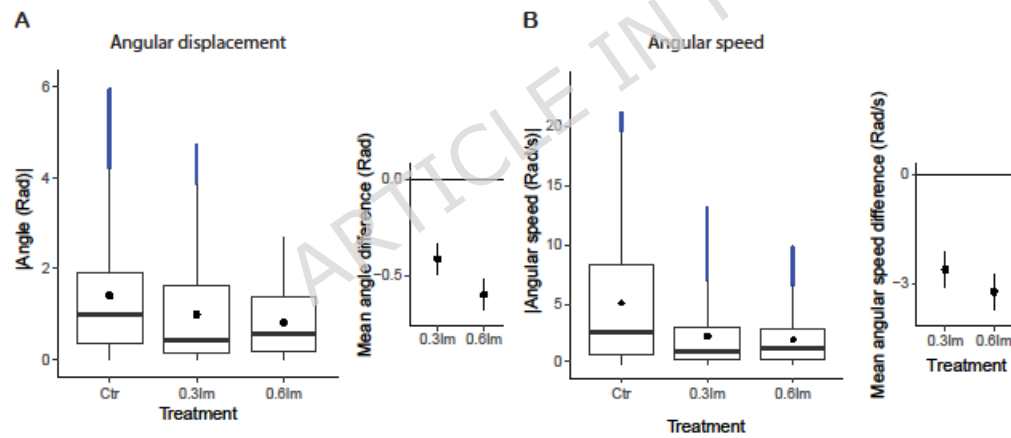
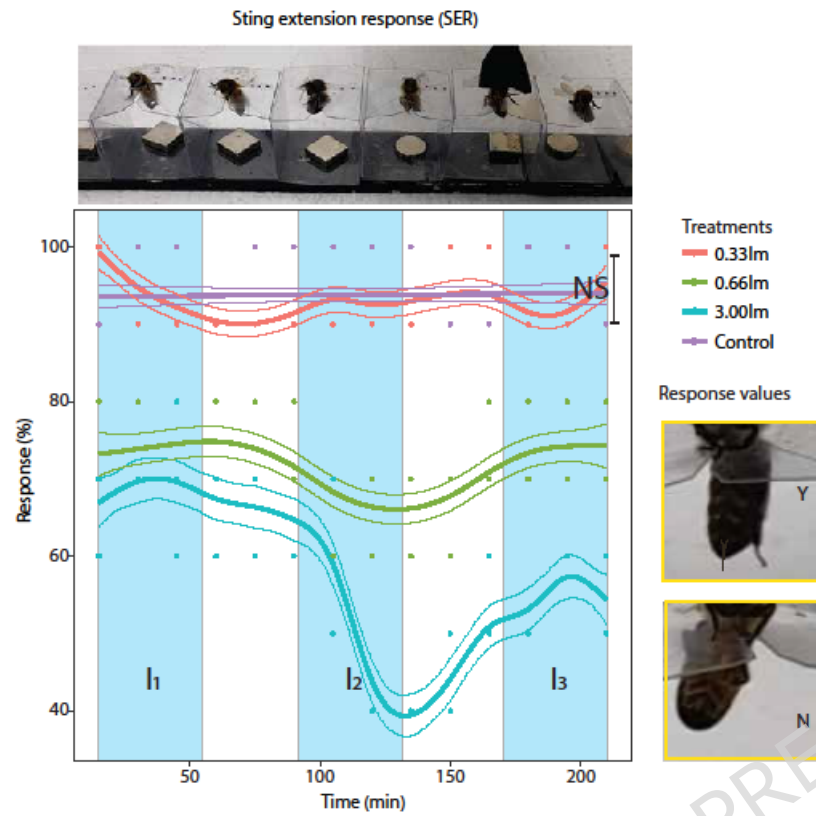
57. Williamson, S. M., Baker, D. D. & Wright, G. A. Acute exposure to a sublethal dose of imidacloprid and coumaphos enhances olfactory learning and memory in the honeybee *Apis mellifera*. *Invertebrate Neuroscience* 13, 63–70 (2013).
58. Tosi, S. & Nieh, J. C. A common neonicotinoid pesticide, thiamethoxam, alters honey bee activity, motor functions, and movement to light. *Sci Rep* 7, (2017).
59. Decourtye, A. *et al.* Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pestic Biochem Physiol* 78, 83–92 (2004).
60. Muth, F., Gaxiola, R. L. & Leonard, A. S. No evidence for neonicotinoid preferences in the bumblebee *Bombus impatiens*. *R Soc Open Sci* 7, (2020).
61. Adobe Inc. Premier Pro. Preprint at <https://www.adobe.com/premiere-pro.html> (2024).
62. Brown, D. Tracker Video Analysis and Modeling Tool. <https://physlets.org/tracker/> (2022).
63. Farder-Gomes, C. F., de Oliveira, M. A., Malaspina, O. & Nocelli, R. F. C. Exposure of the stingless bee *Melipona scutellaris* to imidacloprid, pyraclostrobin, and glyphosate, alone and in combination, impair its walking activity and fat body morphology and physiology. *Environmental Pollution* 348, (2024).
64. Arslan, O. C., Erdem, B., Somel, M., Giray, T. & Kence, M. Effects of coumaphos on locomotor activities of different honeybee (*Apis mellifera* L.) subspecies and ecotypes. *Apidologie* 54, (2023).
65. Barroso, G., Pereira, A. M., Bueno, O. C., Nocelli, R. C. F. & Malaspina, O. Behavioral impairments in Africanized *Apis mellifera* exposed to lethal and sublethal doses of acetamiprid, fipronil, and thiamethoxam. *Ecotoxicology* 34, 1169–1181 (2025).
66. Schindelin, J. *et al.* Fiji: An open-source platform for biological-image analysis. *Nature Methods* vol. 9 676–682 Preprint at <https://doi.org/10.1038/nmeth.2019> (2012).
67. Chol  , H. *et al.* Antenna movements as a function of odorants' biological value in honeybees (*Apis mellifera* L.). *Sci Rep* 12, (2022).
68. Migda  , P. *et al.* Exposure to Magnetic Fields Changes the Behavioral Pattern in Honeybees (*Apis mellifera* L.) under Laboratory Conditions. *Animals* 12, (2022).
69. Sancho, G. *et al.* A phototaxis assay to measure sublethal effects of pesticides on bees. *Sci Rep* 15, (2025).
70. Posit Software, P. Rstudio. Preprint at (2024).
71. Williamson, S. M., Willis, S. J. & Wright, G. A. Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology* 23, 1409–1418 (2014).

72. Aguiar, J. M. R. B. V., Nocelli, R. C. F., Giurfa, M. & Nascimento, F. S. Neonicotinoid effects on tropical bees: Imidacloprid impairs innate appetitive responsiveness, learning and memory in the stingless bee *Melipona quadrifasciata*. *Science of the Total Environment* 877, (2023).
73. Wright, I. R., Roberts, S. P. M. & Collins, B. E. Evidence of forage distance limitations for small bees (Hymenoptera: Apidae). *Eur J Entomol* 112, 303–310 (2015).
74. Aliouane, Y. *et al.* SUBCHRONIC EXPOSURE OF HONEYBEES TO SUBLETHAL DOSES OF PESTICIDES: EFFECTS ON BEHAVIOR. *Environmental Toxicology and Chemistry* vol. 28 <https://academic.oup.com/etc/article/28/1/113/7763727> (2009).
75. Tomizawa, M. & Casida, J. E. Neonicotinoid insecticide toxicology: Mechanisms of selective action. *Annual Review of Pharmacology and Toxicology* vol. 45 247–268 Preprint at <https://doi.org/10.1146/annurev.pharmtox.45.120403.095930> (2005).
76. Alkassab, A. T. & Kirchner, W. H. Assessment of acute sublethal effects of clothianidin on motor function of honeybee workers using video-tracking analysis. *Ecotoxicol Environ Saf* 147, 200–205 (2018).
77. Raymond-Delpech, V., Matsuda, K., Sattelle, B. M., Rauh, J. J. & Sattelle, D. B. Ion channels: Molecular targets of neuroactive insecticides. *Invertebrate Neuroscience* vol. 5 119–133 Preprint at <https://doi.org/10.1007/s10158-005-0004-9> (2005).
78. Rigosi, E. & O'Carroll, D. C. Acute Application of Imidacloprid Alters the Sensitivity of Direction Selective Motion Detecting Neurons in an Insect Pollinator. *Front Physiol* 12, (2021).
79. Belzunces, L. P., Tchamitchian, S. & Brunet, J. L. Neural effects of insecticides in the honey bee. *Apidologie* vol. 43 348–370 Preprint at <https://doi.org/10.1007/s13592-012-0134-0> (2012).
80. Schneider, C. W., Tautz, J., Grünewald, B. & Fuchs, S. RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS One* 7, (2012).
81. Hardstone, M. C. & Scott, J. G. Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest Management Science* vol. 66 1171–1180 Preprint at <https://doi.org/10.1002/ps.2001> (2010).



Proboscis extension response (PER)





Capítulo 3. Homeostasis del agua y el fluido extracelular revelan disrupciones del imidacloprid y la mitigación por la rutina en abejas

Juan P. Hernández, Fredy Mesa, Andre J. Riveros

Aceptado en: Scientific Reports

DOI10.1038/s41598-025-26833-0

Resumen

La salud de los polinizadores enfrenta amenazas crecientes debido a la exposición a agroquímicos como el imidacloprid. Los flavonoides, como la rutina, podrían tener efectos protectores. Este estudio investiga cómo el imidacloprid y la rutina influyen en la regulación de la masa corporal y el comportamiento del líquido extracelular en *Apis mellifera*. Se expusieron abejas melíferas africanizadas a imidacloprid, rutina o ambos, en concentraciones de 0.3, 0.6 y 1.0 ppm. Cuatro experimentos evaluaron cambios a corto y largo plazo en la masa corporal, el consumo de alimento, el contenido de agua y la evaporación del líquido extracelular. Los análisis estadísticos incluyeron ANOVA, pruebas post hoc de Tukey y modelos de regresión. El imidacloprid redujo el consumo de alimento y la ganancia de masa corporal en todos los experimentos, con efectos que persistieron hasta las 48 horas. La rutina aumentó la ganancia de masa y la hidratación, e incluso revirtió parcialmente algunos efectos del imidacloprid. El tratamiento combinado mostró resultados intermedios. El líquido extracelular de abejas tratadas con imidacloprid presentó tasas de evaporación más bajas, lo que sugiere alteraciones en sus propiedades fisicoquímicas. Estos hallazgos respaldan la inclusión de variables fisiológicas, como la hidratación, la variación de la masa corporal y la dinámica del fluido, en las evaluaciones de exposición subletal a pesticidas en abejas.

Fe de erratas y notas aclaratorias del manuscrito:

El uso del término “phenotypes” en los subtítulos del manuscrito original adjunto debe leerse e interpretarse estrictamente como un "perfil fisiológico intermedio" o "respuesta biofísica interactiva" del fluido extracelular, desvinculando el apartado de cualquier implicación de heredabilidad genética.

Figura 3 (Panel C) y Tabla A: Se precisa que, en la versión gráfica de la Figura 3C, el indicativo "ns" denota que no se observaron diferencias estadísticamente significativas entre los tratamientos, omitiendo el uso de letras, como se indica en la leyenda estándar. Asimismo, respecto a la Tabla A, las flechas ascendentes y descendentes indican la dirección del cambio (incremento o disminución) de las variables evaluadas en comparación con los valores del tratamiento de control.

La metodología general del grupo de control se introduce en el apartado de animales experimentales; sin embargo, se aclara que las cantidades exactas de individuos (réplicas) analizados en las curvas de evaporación se detallan directamente en la sección de Resultados y en las leyendas de la Figura 5.

Perspectivas: Comunicación química y escalabilidad

A nivel ecológico, la alteración de la homeostasis hídrica y fisiológica inducida por el imidacloprid trasciende al individuo. La disrupción del medio extracelular compromete la síntesis y la percepción de señales químicas (feromonas e hidrocarburos cuticulares), esenciales para el reconocimiento, la organización y la defensa, lo que, en última instancia, pone en riesgo la supervivencia de la colonia. Por otro lado, la metodología biofísica aquí empleada es altamente reproducible y escalable para el estudio de otras colonias o especies de polinizadores nativos, y requiere únicamente ajustes menores en las velocidades de centrifugación en función de la morfometría de la especie objetivo.



OPEN Water homeostasis and extracellular fluid dynamics reveal imidacloprid disruption and rutin mitigation in honey bees

Juan P. Hernández^{1,2✉}, Fredy Mesa² & Andre J. Riveros¹

Pollinator decline is partly driven by exposure to agrochemicals such as imidacloprid, a neonicotinoid insecticide that impairs feeding and water balance in bees. This study examines how imidacloprid and the dietary flavonoid rutin, individually and combined, affect body mass regulation and extracellular fluid in *Apis mellifera*. Africanized honey bees were exposed to 0.3, 0.6, or 1.0 ppm of each substance. We conducted four experiments to evaluate short- and long-term changes in food intake, wet and dry mass, hydration, and fluid evaporation rates. Imidacloprid consistently reduced food consumption and body mass gain, while rutin promoted weight gain, increased water retention, and partially reversed imidacloprid's effects. The combination treatment produced intermediate results. Notably, extracellular fluid from imidacloprid-treated bees showed significantly slower evaporation, suggesting altered physicochemical properties. This represents the first application of extracellular fluid evaporation as a physiological biomarker of sublethal stress in honey bees, expanding current approaches beyond conventional endpoints such as survival or behavior. These physiological changes persisted beyond the exposure period in some cases. Our results highlight the potential of mass dynamics and fluid behavior as sensitive markers of sublethal stress. Rutin appears to enhance physiological resilience, supporting its role as a candidate dietary mitigator. This work contributes to the development of new physiological endpoints for assessing the impact of agrochemicals on pollinators and offers insight into the protective role of plant-derived compounds.

Keywords Water homeostasis, Extracellular fluid evaporation, *Apis mellifera*, Imidacloprid, Rutin

Bees, particularly *Apis mellifera*, play a central role in pollination processes that sustain global agriculture and biodiversity^{1,2}. Approximately 75% of crops consumed by humans benefit from animal pollination³, with bees being the most efficient and widespread pollinators. Their ecological and economic^{4,5} importance has prompted extensive research efforts aimed at identifying and mitigating threats to their health and survival^{6,7}. However, despite their importance, bee populations have been declining due to a range of anthropogenic stressors⁸, including habitat loss⁹, climate change^{2,10,11}, pathogens^{12,13}, and especially agrochemical exposure^{14–16}.

Among the principal agrochemical threats, neonicotinoid insecticides have gained particular attention^{17–20} due to their systemic nature and persistence in the environment. Imidacloprid, one of the most widely used neonicotinoids, acts by binding irreversibly to nicotinic acetylcholine receptors in insect neurons²¹, causing sustained depolarization and leading to motor dysfunction^{22,23}, paralysis, or death²⁴. Notably, sublethal concentrations can impair foraging behavior^{25–28}, alter food consumption²⁹, induce regurgitation, and compromise water homeostasis in bees^{30,31}. Residues of imidacloprid have been detected in nectar and pollen of treated crops^{32–34}, exposing foraging bees to chronic low doses with long-term physiological consequences^{14,35}.

The regulation of water balance in insects is a tightly controlled physiological process involving ingestion, absorption³⁶, evaporation, and osmoregulation^{37–39}. Honey bees, like many terrestrial insects, rely on aquaporins and ionic pumps in the alimentary canal and excretory organs to regulate internal water content in response to environmental conditions⁴⁰. Factors such as ambient temperature⁴¹, humidity⁴², and solute concentration in food sources affect these regulatory mechanisms. Any disruption to this system, whether through environmental change or chemical exposure, can impact hemolymph composition^{43–45}, cellular function, and overall survival⁴⁶.

¹Departamento de Biología, Escuela de Ciencias E Ingeniería, Universidad del Rosario, Cra. 26 #63B-48, Bogotá, Colombia. ²Facultad de Ingeniería y Ciencias Básicas, Fundación Universitaria Los Libertadores, Bogotá, Colombia. ✉email: juanp.hernandez@urosario.edu.co

Given the high surface-area-to-volume ratio of bees and their continuous foraging activity, maintaining water balance is essential for thermoregulation, muscle performance, and neurophysiological stability^{47,48}.

Recent studies have proposed that certain phytochemicals naturally found in nectar and pollen may offer protective effects against agrochemical stress^{49–51}. Flavonoids, such as quercetin and its glycoside rutin⁵², exhibit antioxidant⁵³, anti-inflammatory⁵², and neuromodulatory properties⁵⁴. Rutin in particular has been associated with improved cognitive recovery⁵⁵, reduced oxidative stress, and enhanced detoxification in bees exposed to pesticides^{16,56,57}. In addition to modulating cellular responses to toxins, flavonoids may interact with membrane transporters^{52,58}, antioxidant systems, and metabolic enzymes, potentially enhancing physiological resilience. Moreover, rutin is part of the natural diet of bees, suggesting it may be a biologically compatible compound for supplementation⁵⁹.

Despite its promising properties, little is known about how rutin affects water balance, mass gain, or extracellular fluid dynamics in bees. Furthermore, no studies to date have assessed its potential to mitigate imidacloprid-induced physiological alterations, particularly those related to fluid regulation and mass variation. Addressing this gap is critical for understanding the full spectrum of sublethal pesticide effects and for identifying nutritional strategies that support pollinator health.

To our knowledge, extracellular fluid evaporation has received little attention as a biomarker for pesticide-induced physiological disruption in honey bees. Introducing this endpoint provides a novel approach to detect subtle alterations in water balance and homeostasis beyond conventional behavior and survival assays. The aim of this study is to evaluate the effects of imidacloprid and rutin administered individually and in combination on body mass, water content, and extracellular fluid properties in *Apis mellifera*. By focusing on physiological responses such as food intake, hydration, and fluid evaporation, this work seeks to establish quantitative markers for assessing sublethal chemical impacts on bee health and to explore the potential protective role of rutin.

Materials and methods

Experimental animals

Africanized honey bees (*Apis mellifera*) were collected from the apiary of Universidad del Rosario (Bogotá, Colombia). Foragers were randomly collected at the hive entrances from four source colonies. Since foragers represent a naturally mixed-age population of workers, our experimental groups included bees of variable age, which is standard in oral exposure assays. A total of 5035 bees were used across all experiments. Bees were housed in 250 mL disposable plastic containers, adapted for ventilation and substance administration. Prior to experiments, bees were maintained for 24 h under controlled conditions (temperature: 32 ± 6 °C; relative humidity: $51 \pm 8\%$; light wavelength: 750 nm; which minimizes disruption during feeding assays, and fed ad libitum with a 1 M sucrose solution (31.1% w/w). After this habituation period, bees were randomly assigned to experimental groups. All procedures were conducted under constant temperature and humidity to minimize environmental variation. At the end of each trial, bees were sacrificed by exposure to -30 °C for 24 h using a Thermo Scientific Revco Freezer (UGL3020A).

Feeding containers and delivery system

Feeding systems were adapted from internationally recognized protocols for assessing oral toxicity in honey bees⁶⁰. Each container was fitted with a feeding device consisting of a 40 mL glass bottle, a 1000 μ L micropipette tip, and absorbent paper to regulate flow. The narrow end of the tip was inserted through the base of the container, ensuring continuous access to the test solution while minimizing spillage and evaporation. Containers were visually monitored during exposure to confirm active feeding behavior and solution depletion.

Substances and dosages

Two substances were evaluated: the commercial insecticide imidacloprid (Confidor 350 CS, 350 g/L active ingredient) and the flavonoid rutin. Both were diluted in 1 M sucrose solutions to prepare three working concentrations: 0.3 ppm, 0.6 ppm, and 1.0 ppm. The solutions were labeled according to substance and concentration (e.g., 0.3Im, 0.6Ru). Solutions were prepared fresh on the day of each experiment. The concentration range (0.3, 0.6 and 1.0 ppm) was selected within the levels of imidacloprid residues detected in nectar and pollen under agricultural conditions. A comprehensive review reported median concentrations in nectar of 0.73 mg/kg and mean values up to ~ 2.5 mg/kg³², with observed ranges from 6.0×10^{-6} to 20 mg/kg³³. Modeling approaches further confirm that foraging bees are exposed to residuals typically within this range³⁴. Experimental studies have also employed similar sublethal concentrations ranging from 0.1 to 10 ppm³⁰. For rutin, concentrations were chosen according to its natural occurrence in nectar and pollen⁵⁶ and its experimental use as a dietary supplement in pollinators⁵⁵.

Short-term exposure assay for food intake and body mass

Bees were exposed for 4.1 h to sucrose solutions containing 1.0 ppm of imidacloprid (Im), rutin (Ru), or both (Im + Ru). Twelve groups (approximately 71 bees per group) were monitored. Mass measurements of feeders and containers were taken every 15 min using a precision balance (Radwag AS 220.R2) to calculate food intake and body mass changes. Correlations between food intake and bee mass were analyzed, and treatment-specific differences were evaluated using linear regression and ANOVA.

Dose- and time-dependent assay for hydration and body mass

Bees were exposed to ten treatments (0.3, 0.6, and 1.0 ppm of Im, Ru, and Im + Ru, plus control) across three times (1, 2, and 3 h). After exposure, bees were frozen, and groups were weighed for wet mass. Subsequently, they were dried at 70 °C for 3 days (Binder FED 720 oven) to determine dry mass and calculate water content. Each condition was replicated three times using separate containers and bee batches. Water content was estimated

by subtracting dry mass from wet mass, and values were normalized per bee. Data were analyzed with factorial ANOVA, and post hoc Tukey HSD tests were used to evaluate group differences.

Two-phase exposure assay for recovery of feeding and hydration

Bees were assigned to three groups (Im, Ru, and control) for 24 h of initial exposure (Phase 1), then subdivided and re-assigned to nine combinations for an additional 24 h (Phase 2). Each treatment combination was represented by two or three replicates, totaling 18 groups. Mass of ingested food and total bee wet and dry masses were recorded to evaluate cumulative and recovery effects on water content and satiety. This design enabled assessment of persistence and reversibility of physiological effects under chronic and sequential exposure scenarios.

Extracellular fluid extraction and evaporation assay

After 24 h of exposure to 0.3 ppm Im, Ru, or Im + Ru, extracellular fluid was extracted from centrifuged bees⁶¹ following previously described protocols. For each replicate, 15 bees were cold-anesthetized, dissected, and placed in 1.5 ml microcentrifuge tubes. Centrifugation was performed at 3000 RCF for 5 min. Four independent replicates were obtained per treatment, resulting in 12 groups in total. Fluid samples were placed at 34.5 ± 0.4 °C, and their mass was measured every 10 min over 1 h using a Radwag AS 220.R2 balance. Each replicate was analyzed independently. Evaporation curves were fitted to exponential decay models to estimate evaporation coefficients (Fig. 1).

Statistical analysis

All statistical analyses were conducted using RStudio (v4.3.1). Normality and homoscedasticity of residuals were verified via Shapiro–Wilk and Levene’s tests, respectively. When assumptions were not met, data transformations or non-parametric tests were applied. Statistical tests were selected according to the experimental design:

- Experiment 1 (short-term food intake and body mass): analyzed by regression and one-way ANOVA.
- Experiment 2 (wet and dry mass across treatments and time): analyzed by factorial ANOVA.
- Experiment 3 (two-phase recovery assays): analyzed by two-way ANOVA.
- Experiment 4 (extracellular fluid evaporation): analyzed by nonlinear regression (nls) fitted to exponential decay models; fitted coefficients were compared by ANOVA.

Tukey’s HSD was used for pairwise comparisons, and significance was established at $\alpha = 0.05$.

Results

Experiment 1: short term effects on body mass and food intake

A total of 847 bees were randomly assigned to 12 groups (mean size: 70.6 ± 4.8 bees) exposed to 1.0Im, 1.0Ru, 1.0Im + 1.0Ru, or control. Bee body mass exhibited a strong positive correlation with food consumption (slope: 0.749 ± 0.017 mg bee/mg food; $R^2 = 0.94$). However, the relationship differed significantly among treatments ($F_{1,118} = 1999.5$; $p < 0.0001$). Mass gain per milligram of consumed food varied across treatments (mean $\pm$ sd): 0.560 ± 0.051 mg bee/mg food (1.0Im), 0.642 ± 0.019 mg bee/mg food (1.0Im + 1.0Ru), 0.776 ± 0.018 mg bee/mg food (1.0Ru), and 0.752 ± 0.026 mg bee/mg food (Control) (Fig. 2A).

Consumption and body mass increased over time, but rates differed by treatment. At 2 h, food intake showed differences across treatments (mean $\pm$ sd): 25.2 ± 1.1 mg (Im + Ru), 31.5 ± 1.1 mg (Im), 34.6 ± 1.6 mg (Ru),

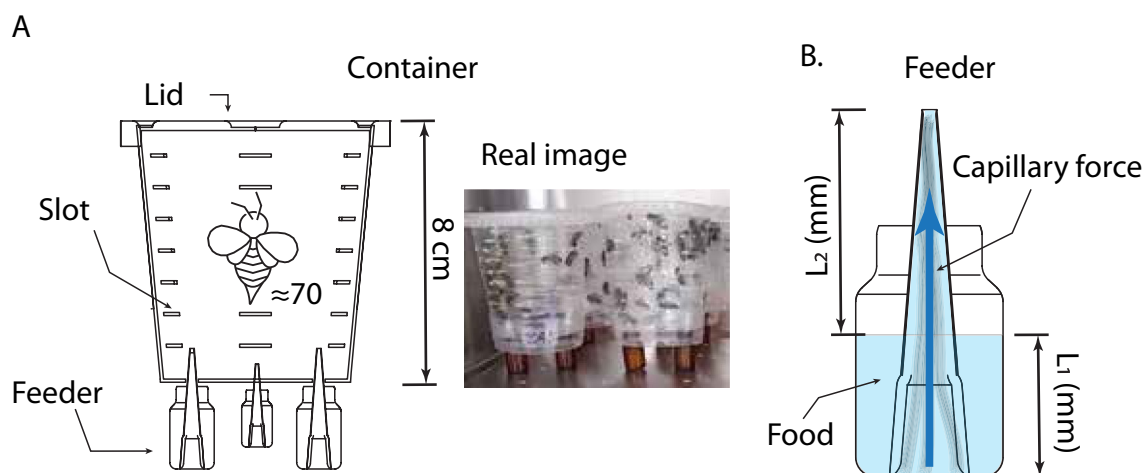


Fig. 1. Experimental setup for bee confinement and feeding. **A)** Diagram of the plastic container used to house approximately 70 bees per group. The container includes lateral ventilation slots and three feeder ports at the base. A real image of the experimental groups is shown on the right. **B)** Schematic of the feeding system consisting of a 40 mL glass bottle and a micropipette tip. Capillary forces maintain a stable fluid column. L_1 and L_2 denote fluid column heights inside the bottle and tip, respectively.

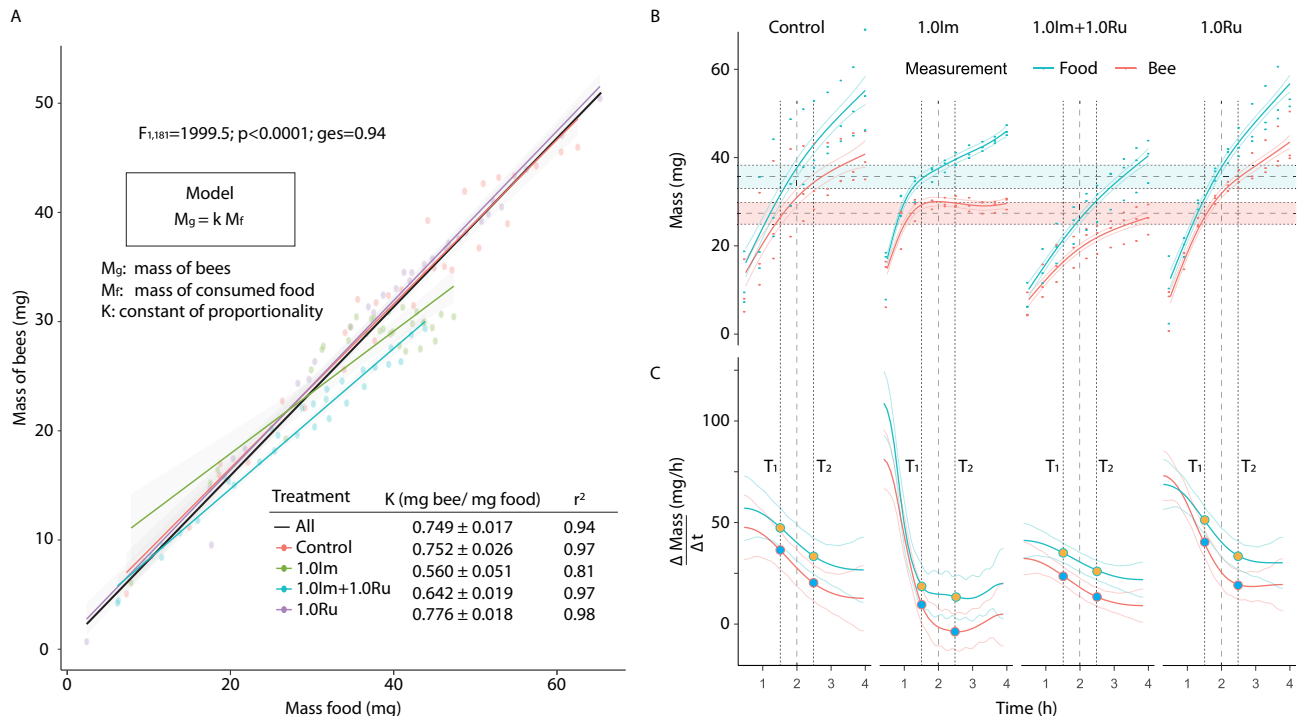


Fig. 2. Relationship between food intake and body mass during short-term exposure (Experiment 1). **A**) Linear regression between total food consumed and average bee body mass after 4.1 h of exposure to four treatments. Shaded areas indicate 95% confidence intervals. **B**) Temporal evolution of food consumption and body mass for each treatment. Dots represent group means; lines indicate model fits and trends over time.

and 35.7 ± 2.6 mg (Control). The body mass also increased initially but plateaued for 1.0Im after 2 h, despite continued feeding: 17.1 ± 1.1 mg (Im + Ru), 22.4 ± 1.1 mg (Im), 26.0 ± 1.2 mg (Ru), and 27.3 ± 2.4 mg (Control) (Fig. 2B).

Rates of change in food consumption and bee body mass were analyzed during two intervals: T₁ (1.4–1.6 h) and T₂ (2.4–2.6 h). In the interval T₁, both food consumption and body mass gain were lowest under imidacloprid exposure and highest under rutin (mean $\pm$ SD): Food_{Im} = 22.4 ± 2.2 mg/h; Bee_{Im} = 14.69 ± 3.00 mg/h; Food_{Ru} = 55.8 ± 1.3 mg/h; Bee_{Ru} = 47.4 ± 2.1 mg/h. In the interval T₂, food consumption and body mass declined in the imidacloprid group, while the rutin group maintained moderate rates (mean $\pm$ SD): Food_{Im} = 13.9 ± 0.7 mg/h; Bee_{Im} = -3.5 ± 0.5 mg/h; Food_{Ru} = 35.9 ± 0.8 mg/h; Bee_{Ru} = 21.0 ± 0.5 mg/h. In the control group, rates remained relatively stable: Food_{Control} = 48.4 ± 0.8 mg/h and Bee_{Control} = 37.3 ± 1.0 mg/h in T₁; Food_{Control} = 34.0 ± 0.7 mg/h and Bee_{Control} = 20.7 ± 0.8 mg/h in T₂. These findings indicate that imidacloprid impairs body mass gain despite continued intake, whereas rutin supports sustained weight increase (Fig. 2C).

Experiment 2: dose- and time-dependent effects on wet and dry mass

A total of 2652 bees were assigned to 36 groups (mean size: 73.7 ± 1.7 bees) exposed to ten treatments at three time points. Significant effects were observed for both wet and dry mass ($F_{\text{Wet } 3,2648} = 129, p < 0.0001$; $F_{\text{Dry } 3,2648} = 51, p < 0.0001$). Wet mass means (diff, 95% CI) were: 5.5 [4.0; 6.9] (Im), 2.5 [1.0; 3.9] (Im + Ru), $-5.1 [-6.5; -3.7]$ (Ru). Im + Ru differed from Im (3.0 [1.6; 4.4]) and Ru ($-7.6 [-9.0; -6.1]$). Concentration had no effect ($p > 0.05$) (Fig. 3 A). Time had a significant effect on the wet mass (all $p < 0.0001$). Mass followed a non-linear pattern: it increased from t₁ to t₂ but decreased at t₃ (Fig. 3 B). For Im: [94.9 $\pm$ 8.4; 102.2 $\pm$ 9.0; 100.3 $\pm$ 9.7], Im + Ru: [98.0 $\pm$ 8.8; 105.7 $\pm$ 10.5; 102.5 $\pm$ 9.4], Ru: [103.7 $\pm$ 10.0; 113.6 $\pm$ 10.5; 111.7 $\pm$ 9.3]. Water content estimates varied by treatment and time ($p < 0.0001$), with Im decreasing and Ru increasing it (Fig. 3 C).

Experiment 3: two-phase feeding and physiological recovery

A total of 1250 bees were organized into 18 groups (mean: 69.4 ± 3.9 bees). In Phase 1, bees consumed varying amounts of sucrose: lowest with Im (364–456 μ L), highest with Ru (479–570 μ L) (Fig. 4 A). During Phase 2, groups previously exposed to Im increased intake when switched to Ru. Im suppressed consumption, while Ru restored it. Wet mass differed significantly among treatments ($F_{8,1241} = 1169.5, p < 0.0001$). Lowest values were recorded for Im–Im and S–Im, with no difference between them (Diff = 0.2; 95% CI: $[-1.4, 1.8]$). Bees previously on Im but switched to sucrose recovered mass (99.6 ± 3.7 mg). Water content was lowest with Im (72.4 ± 4.3 mg) and highest with Ru (127.1 ± 4.7 mg). Switching from Im to Ru restored hydration (87.6 ± 5.3 vs. 81.6 ± 5.8 mg) (Fig. 4 B).

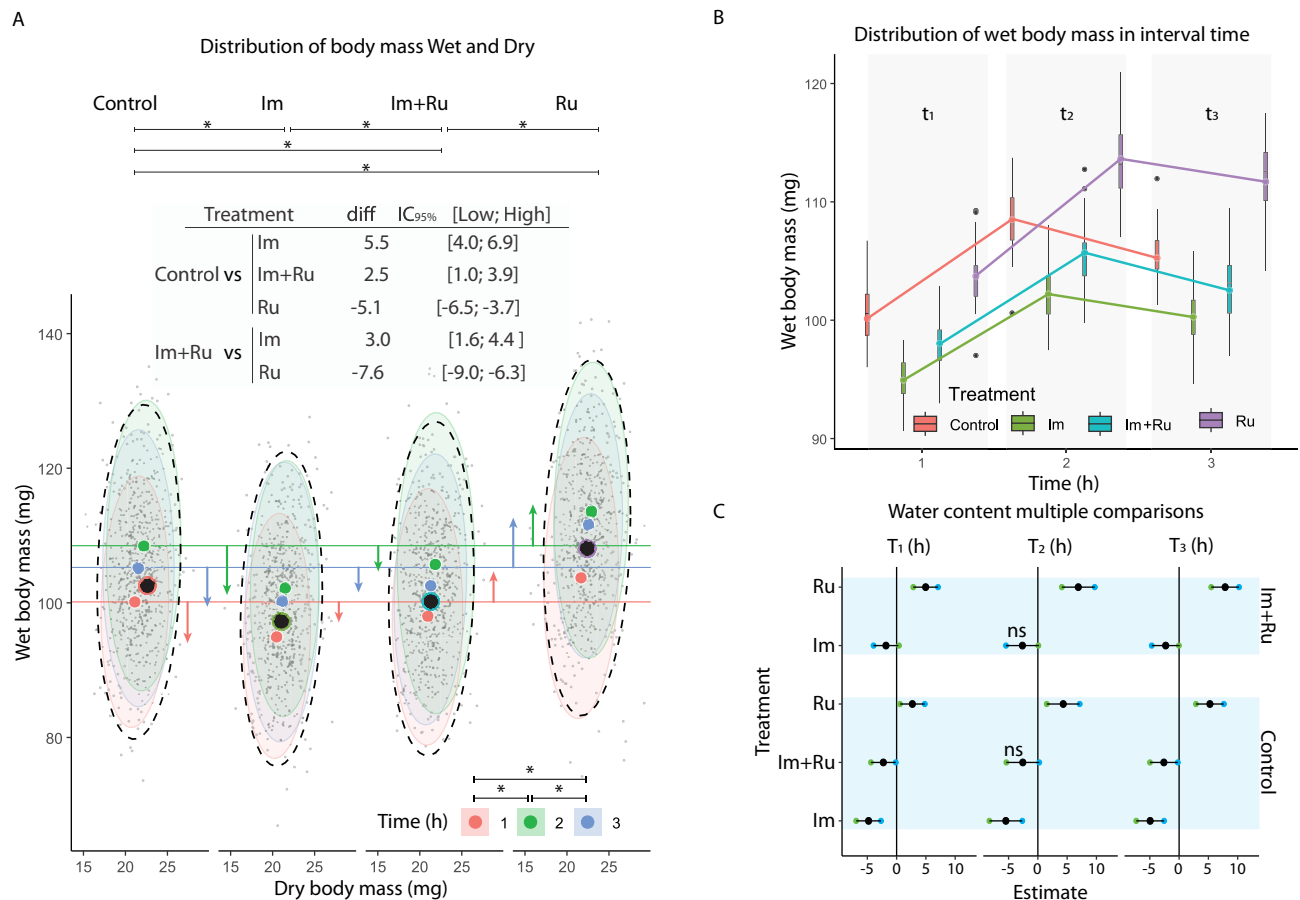


Fig. 3. Effect of imidacloprid and rutin on wet and dry body mass (Experiment 2). **A**) Bivariate plot of dry vs. wet mass after 1, 2, and 3 h of exposure. Ellipses represent 95% confidence regions. Differences between treatments are indicated. **B**) Temporal profiles of wet mass (mg) for each treatment group. **C**) Water content estimates derived from paired dry/wet mass measurements. Groups labeled with different letters are statistically different (Tukey HSD, $p < 0.05$).

Experiment 4: evaporation of extracellular fluid

A total of 286 bees were divided into 12 groups (mean: 71.5 ± 2.4 bees). During the 24 h exposure period, bees consumed different amounts of sucrose solution depending on treatment. Control bees consumed on average $40.97 \mu\text{l}$ per bee, while consumption was slightly lower in Im + Ru ($40.74 \mu\text{l}$) and higher in Ru ($42.71 \mu\text{l}$). Imidacloprid-treated bees consumed the last solution ($37.47 \mu\text{l}$). The combined mass of 15 bees per replicate showed different masses across treatments ($F_{3,16} = 13.5$, $p = 0.00367$), with lower values in Im ($1.43 \pm 0.07 \text{ g}$) and higher values in Ru ($1.89 \pm 0.04 \text{ g}$), while Control ($1.67 \pm 0.04 \text{ g}$) and Im + Ru ($1.60 \pm 0.04 \text{ g}$) showed intermediate means (Fig. 5 A). These differences indicate that exposure affected both feeding and body condition prior to fluid extraction.

Mass loss during evaporation also differed significantly among treatments ($F_{3,188} = 21.2$, $p < 0.0001$), following an exponential decay.

Evaporation coefficients ($k \times 10^{-3} \text{ s}^{-1}$) were -12.99 (Control), -9.97 (Ru), -8.02 (Im + Ru), -4.97 (Im). Compared to controls, extracellular fluid from Im-treated bees evaporated approximately 60% more slowly, while Ru-treated bees showed a $\sim 23\%$ reduction. The Im + Ru combination produced intermediate results ($\sim 38\%$ reduction) (Fig. 5 B). These quantitative differences were consistent with the temporal dynamics of evaporation. Control and Ru samples exhibited the steepest initial decline within the first 30 min, reflecting higher evaporation rates. In contrast, fluids from Im- and Im + Ru-treated bees showed slower mass loss over time, indicating reduced evaporative performance. From 30 to 60 min, these differences became more pronounced: Ru remained close to Control, while Im consistently showed the flattest curve. The Im + Ru treatment was intermediate, evaporating faster than Im alone but slower than Control or Ru. Together, these results show that imidacloprid strongly reduces the evaporative potential of the extracellular fluid, whereas rutin maintains control-like dynamics and partially mitigates the disruption when co-administered with Im (Fig. 5 C).

Discussion

This study demonstrates that the ingestion of imidacloprid and rutin, alone or in combination, produces measurable and divergent effects on body mass, water balance, and extracellular fluid properties in Africanized

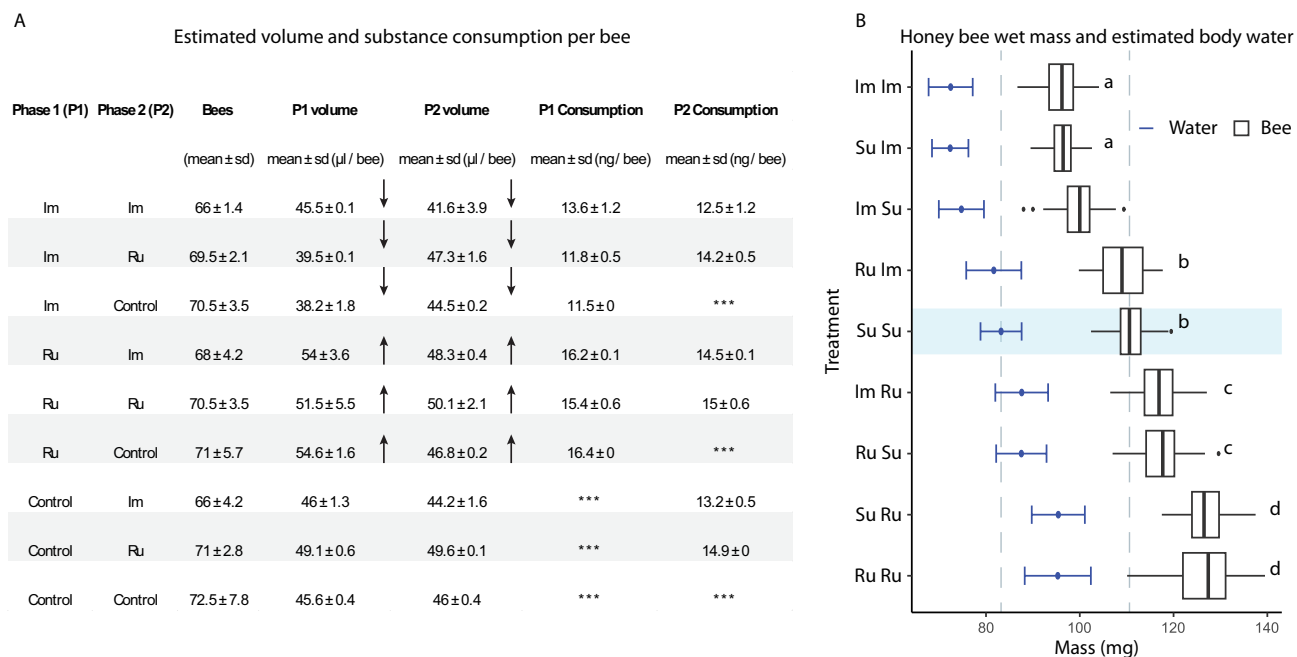


Fig. 4. Consumption and body mass recovery after two-phase exposure (Experiment 3). **A)** Table summarizing consumption volume and estimated dose (mean ± sd) per bee across both phases. **B)** Boxplots of final wet mass and estimated water content per treatment. Blue segments show estimated water; white segments show body mass. Groups with different letters differ significantly.

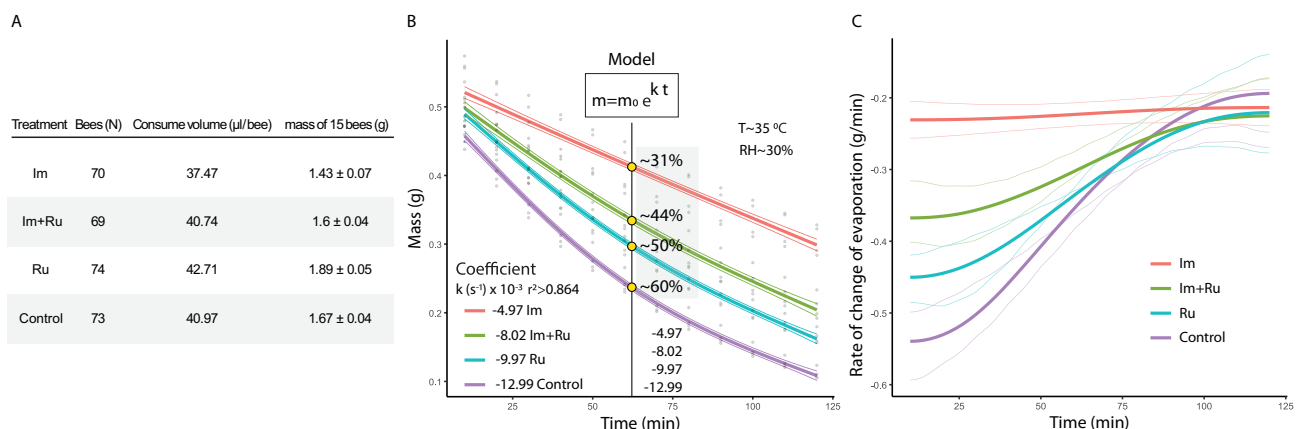


Fig. 5. Evaporation dynamics of extracellular fluid (Experiment 4). **A)** Table with the number of bees, volume of sucrose solution consumed, and the initial combined mass of 15 bees per replicate across treatments. **B)** Evaporation curves fitted to exponential decay models. Percentages indicate relative reduction in evaporation compared to controls. **C)** Temporal dynamics of extracellular fluid evaporation over 60 min, with fitted exponential curves illustrating differences in decay rates among treatments.

honey bees (*Apis mellifera*). The observed physiological alterations offer new insights into the impact of agrochemicals and dietary supplements on pollinator health, particularly in terms of water homeostasis and metabolic regulation.

Imidacloprid reduces food intake and body mass gain

Across all experiments, exposure to imidacloprid (Im) consistently resulted in lower food intake and reduced body mass. In the short-term experiment (Exp. 1), bees exposed to Im showed a lower mass-to-food ratio and a plateau in weight gain despite continued consumption. This suggests impaired nutrient absorption or increased water loss (as shown in Fig. 2B). These findings are consistent with previous reports showing that imidacloprid impairs feeding behavior and induces regurgitation, which may explain the limited weight gain observed (Wong et al., 2018; Vergara-Amado et al., 2020). Importantly, the strong correlation between food intake and mass gain

under control and Ru treatments ($R^2 > 0.95$), contrasted with the disrupted relationship under Im, supports the hypothesis that imidacloprid affects not only ingestion but also internal processing of nutrients.

In the long-term exposure assay (Exp. 3), bees under continuous Im treatment maintained low hydration levels (wet mass: 72.4 ± 4.3 mg), and even after switching to sucrose, only partial recovery was observed (Fig. 4B). These data indicate that imidacloprid exerts persistent physiological stress, potentially via disruption of osmotic gradients or inhibition of aquaporin-mediated transport⁴⁰. Furthermore, the reduced evaporation rate observed in extracellular fluid from Im-treated bees ($k = -4.97 \times 10^{-3} \text{ s}^{-1}$; Fig. 5B) suggests compositional changes in the hemolymph, possibly due to altered ionic strength or protein content, which may impair normal water exchange and evaporation dynamics.

Rutin enhances consumption, hydration, and recovery

In contrast, rutin (Ru) increased food intake and body mass in all experiments. During short-term exposure (Exp. 1), Ru-treated bees showed the highest consumption rates and consistent mass gain, supporting the idea that rutin promotes feeding behavior and efficient nutrient assimilation. In Exp. 2, Ru elevated both wet and dry body mass across all time points and concentrations, with statistical significance ($p < 0.0001$), indicating a robust physiological response. Moreover, in Exp. 3, bees previously exposed to Im and then transitioned to Ru partially recovered their hydration and food intake levels, with final wet mass values exceeding those of groups maintained on Im or switched to sucrose alone (Fig. 4B). This mitigative effect suggests that rutin enhances homeostatic recovery processes, potentially by reducing oxidative damage and restoring membrane integrity.

Interactive effects and intermediate phenotypes with Im + Ru

The combination of Im and Ru produced intermediate values across multiple endpoints, including mass-to-food ratios, hydration, and evaporation dynamics. For example, in Exp. 4, the evaporation coefficient of the Im + Ru group ($k = -8.02 \times 10^{-3} \text{ s}^{-1}$) was significantly different from both Im and Ru, suggesting a partial buffering of Im-induced changes in extracellular fluid composition. These results highlight the importance of evaluating not only isolated compounds but also their combined effects, as interactions can result in non-additive physiological outcomes.

Evaporation as a sensitive marker of physiological disruption

The use of extracellular fluid evaporation as a physiological readout provides a novel perspective on internal water dynamics. Our findings show that hemolymph extracted from imidacloprid-treated bees evaporated more slowly than that from controls, while fluid from Ru-treated bees evaporated faster, resembling control levels. The inverse correlation between evaporation rate and imidacloprid exposure suggests that this parameter reflects shifts in fluid viscosity, osmolarity, or protein content. Each of these factors is relevant for thermoregulation and metabolic function. This marker could be valuable in future sublethal toxicity studies.

Slower evaporation in fluids from imidacloprid-treated bees may indicate increased viscosity or altered composition of the hemolymph⁶². This could reduce the efficiency of evaporative cooling. Impaired thermoregulation under these conditions may amplify pesticide toxicity^{63,64}, as bees exposed to non-optimal temperatures exhibit higher mortality when challenged with imidacloprid⁶³. Moreover, osmoregulatory capacity in honey bees depends on hemolymph osmotic balance, which is disrupted under desiccation stress⁶⁵. Conversely, faster evaporation observed in rutin-treated bees suggests restoration of normal physicochemical properties of the fluid, supporting its role in maintaining homeostasis balance. Sublethal imidacloprid has also been shown to disrupt metabolic pathways in the hemolymph, reducing energy-related metabolites such as ATP and acetyl-CoA^{62,66}, which may further influence the fluid's physicochemical behavior.

Limitations and future directions

While this study provides valuable physiological insights under controlled laboratory conditions, it has some limitations. The artificial environment cannot fully replicate field conditions where bees face multiple stressors simultaneously. Moreover, molecular mechanisms underlying the observed physiological changes were not characterized, and only worker bees were tested. Future studies should extend to other castes and pollinator species, as well as integrate molecular and proteomic approaches. Importantly, the evaporation rate of extracellular fluid may serve as a practical physiological marker in field monitoring and regulatory assessments, offering a novel tool for ecotoxicological evaluation.

Implications and relevance

These findings collectively highlight that mass variation and fluid dynamics are sensitive physiological endpoints for detecting sublethal effects of agrochemicals. Unlike conventional behavioral or mortality assays, the measurement of water balance and evaporation offers a more nuanced perspective on internal physiological stress. The modulation of these parameters by dietary rutin reinforces its potential as a mitigative agent.

Given the growing concern over pollinator decline, especially in tropical environments where thermal and osmotic stresses are high, this study contributes to the broader understanding of how chemical exposures and dietary supplements interact to shape pollinator resilience. Future studies should explore the molecular basis of these effects, such as gene expression changes in water transport proteins, antioxidant enzymes, and membrane components. Additionally, field validation under natural foraging conditions would strengthen the ecological applicability of these results.

Overall, this work supports the integration of physiological biomarkers, such as mass variation and extracellular fluid behavior, into risk assessment frameworks for pollinators exposed to agrochemical mixtures.

Conclusion

This study provides compelling evidence that exposure to imidacloprid and rutin modulates physiological traits directly related to body mass, water content, and extracellular fluid behavior in *Apis mellifera*. We demonstrated that imidacloprid impairs food intake, disrupts the relationship between consumption and mass gain, suppresses hydration, and alters the physicochemical properties of extracellular fluid. These effects were evident in both short-term and long-term exposures, and persisted even after cessation of treatment in some cases.

In contrast, rutin consistently promoted higher food intake, enhanced mass gain, increased water retention, and facilitated partial recovery from imidacloprid-induced stress. The intermediate results observed in the combination group (Im + Ru) highlight the complex interactions that can occur when both substances are present, suggesting that rutin may exert protective effects without fully counteracting imidacloprid toxicity.

Importantly, the inclusion of evaporation rate as a physiological readout provided a novel and quantitative measure of internal fluid dynamics, revealing sublethal disruptions not captured by conventional endpoints. These findings support the integration of fluid-based physiological markers into ecotoxicological evaluations of pollinators.

Our results emphasize the need for more comprehensive assessments of pesticide impacts.

These assessments should consider both ingestion and internal physiological consequences. They also point to the potential role of natural dietary supplements such as rutin in mitigating agrochemical stress. Future research should expand on these findings by incorporating molecular analyses and validating physiological markers in field-relevant conditions. Ultimately, this work advances the use of physiological indicators such as mass variation, hydration status, and extracellular fluid behavior as sensitive tools for monitoring pollinator health and resilience in agricultural environments.

Data availability

Data is provided within the manuscript.

Received: 24 June 2025; Accepted: 30 October 2025

Published online: 28 November 2025

References

- Zhang, K. et al. Supplementary honey bee (*Apis mellifera* L.) pollination enhances fruit growth rate and fruit yield in *Paeonia ostii* (family: Paeoniaceae). *PLoS ONE* <https://doi.org/10.1371/journal.pone.0272921> (2022).
- Decourtye, A., Alaux, C., Le Conte, Y. & Henry, M. Toward the protection of bees and pollination under global change: present and future perspectives in a challenging applied science. *Curr. Opin. Insect Sci.* <https://doi.org/10.1016/j.cois.2019.07.008> (2019).
- Grant, K. J., DeVetter, L. & Melathopoulos, A. Honey bee (*Apis mellifera*) colony strength and its effects on pollination and yield in highbush blueberries (*Vaccinium corymbosum*). *PeerJ* <https://doi.org/10.7717/peerj.11634> (2021).
- Khalifa, S. A. M. et al. Overview of bee pollination and its economic value for crop production. *Insects* <https://doi.org/10.3390/insects12080688> (2021).
- Saha S, Munshi SA, Laskar N. Bee pollination: From an ecological and economical perspective. (2023).
- Klein, A. M., Boreux, V., Fornoff, F., Mupepele, A. C. & Pufal, G. Relevance of wild and managed bees for human well-being. *Curr. Opin. Insect Sci.* <https://doi.org/10.1016/j.cois.2018.02.011> (2018).
- Potts, S. G. et al. Global pollinator declines: Trends, impacts and drivers. *Trends Ecol. Evol.* <https://doi.org/10.1016/j.tree.2010.01.007> (2010).
- Hanberry, B. B. et al. Pollinators of the Great Plains: Disturbances, Stressors, Management, and Research Needs. *Rangel. Ecol. Manag.* **78**, 220–234. <https://doi.org/10.1016/j.rama.2020.08.006> (2021).
- Mendes, S. B., Timóteo, S., Loureiro, J. & Castro, S. The impact of habitat loss on pollination services for a threatened dune endemic plant. *Oecologia* **198**, 279–293. <https://doi.org/10.1007/s00442-021-05070-y> (2022).
- Richardson, R. T. et al. Land use changes associated with declining honey bee health across temperate North America. *Environ. Res. Lett.* <https://doi.org/10.1088/1748-9326/acd867> (2023).
- Dalmon, A., Peruzzi, M., Le Conte, Y., Alaux, C. & Pioz, M. Temperature-driven changes in viral loads in the honey bee *Apis mellifera*. *J. Invertebr. Pathol.* **160**, 87–94. <https://doi.org/10.1016/j.jip.2018.12.005> (2019).
- Parveen, N. et al. Honey bee pathogenesis posing threat to its global population: a short review. *Proc. Ind. Natl. Sci. Acad.* <https://doi.org/10.1007/s43538-022-00062-9> (2022).
- Cilia, G. et al. Occurrence of Honey Bee (*Apis mellifera* L.) Pathogens in Wild Pollinators in Northern Italy. *Front. Cell. Infect. Microbiol.* <https://doi.org/10.3389/fcimb.2022.907489> (2022).
- Mokkapat, J. S., Bednarska, A. J. & Laskowski, R. Physiological and biochemical response of the solitary bee *Osmia bicornis* exposed to three insecticide-based agrochemicals. *Ecotoxicol. Environ. Saf.* <https://doi.org/10.1016/j.ecoenv.2021.113095> (2022).
- Mokkapat, J. S., Bednarska, A. J. & Laskowski, R. The development of the solitary bee *Osmia bicornis* is affected by some insecticide agrochemicals at environmentally relevant concentrations. *Sci. Total Environ.* <https://doi.org/10.1016/j.scitotenv.2021.145588> (2021).
- Botina, L. L. et al. The impact of early-life exposure to three agrochemicals on survival, behavior, and gut microbiota of stingless bees (*Partamona helleri*). *Environ. Sci. Pollut. Res.* **30**, 70143–70158. <https://doi.org/10.1007/s11356-023-27385-4> (2023).
- Odemer, R. et al. Potential Risk of Residues From Neonicotinoid-Treated Sugar Beet Flowering Weeds to Honey Bees (*Apis mellifera* L.). *Environ. Toxicol. Chem.* **42**, 1167–1177. <https://doi.org/10.1002/etc.5602> (2023).
- Xu, X. et al. Neonicotinoids: mechanisms of systemic toxicity based on oxidative stress-mitochondrial damage. *Arch. Toxicol.* <https://doi.org/10.1007/s00204-022-03267-5> (2022).
- Huang, J. et al. Neonicotinoids: Advances in hazards of residues, screening of aptamers and design of aptasensors. *Trends Food Sci. Technol.* <https://doi.org/10.1016/j.tifs.2024.104342> (2024).
- Brandt, A., Gorenflo, A., Siede, R., Meixner, M. & Büchler, R. The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.). *J. Insect. Physiol.* **86**, 40–47. <https://doi.org/10.1016/j.jinsphys.2016.01.001> (2016).
- Cartereau, A., Taillebois, E., Le Questel, J. Y. & Thany, S. H. Mode of action of neonicotinoid insecticides imidacloprid and thiacloprid to the cockroach pamea7 nicotinic acetylcholine receptor. *Int. J. Mol. Sci.* <https://doi.org/10.3390/ijms22189880> (2021).
- Tosi, S. & Nieh, J. C. A. common neonicotinoid pesticide, thiamethoxam, alters honey bee activity, motor functions, and movement to light. *Sci. Rep.* <https://doi.org/10.1038/s41598-017-15308-6> (2017).
- Bovi, T. S., Zaluski, R. & de Orsi, R. O. Toxicity and motor changes in africanized honey bees (*Apis mellifera* L.) exposed to fipronil and imidacloprid. *An Acad. Bras. Cienc.* **90**, 239–245. <https://doi.org/10.1590/0001-3765201820150191> (2018).

24. Tomizawa, M. & Casida, J. E. Neonicotinoid insecticide toxicology: Mechanisms of selective action. *Ann. Rev. Pharmacol. Toxicol.* <https://doi.org/10.1146/annurev.pharmtox.45.120403.095930> (2005).
25. Tasman, K., Rands, S. A. & Hodge, J. J. L. The Neonicotinoid Insecticide Imidacloprid Disrupts Bumblebee Foraging Rhythms and Sleep. *iScience* <https://doi.org/10.1016/j.isci.2020.101827> (2020).
26. Zhang, G., Kersten, M., Owen, A. & Skidmore, A. Honey bee foraging and pesticide exposure in a desert urban agroecosystem. *Ecotoxicol. Environ. Saf.* <https://doi.org/10.1016/j.ecoenv.2022.114472> (2023).
27. Bänisch, S., Tschardt, T., Ratnieks, F. L. W., Härtel, S. & Westphal, C. Foraging of honey bees in agricultural landscapes with changing patterns of flower resources. *Agric. Ecosyst. Environ.* <https://doi.org/10.1016/j.agee.2019.106792> (2020).
28. Lin, Y. C., Lu, Y. H., Tang, C. K., Yang, E. C. & Wu, Y. L. Honey bee foraging ability suppressed by imidacloprid can be ameliorated by adding adenosine. *Environmental. Pollut.* <https://doi.org/10.1016/j.envpol.2023.121920> (2023).
29. Kessler, S. C. et al. Bees prefer foods containing neonicotinoid pesticides. *Nature* **521**, 74–76. <https://doi.org/10.1038/nature14414> (2015).
30. Wong, M. J., Liao, L. H. & Berenbaum, M. R. Biphasic concentration-dependent interaction between imidacloprid and dietary phytochemicals in honey bees (*Apis mellifera*). *PLoS ONE* <https://doi.org/10.1371/journal.pone.0206625> (2018).
31. Vergara-Amado, J. et al. Effects of residual doses of neonicotinoid (imidacloprid) on metabolic rate of queen honey bees *Apis mellifera* (Hymenoptera: Apidae). *Apidologie* **51**, 1091–1099. <https://doi.org/10.1007/s13592-020-00787-w> (2020).
32. Zioga, E., Kelly, R., White, B. & Stout, J. C. Plant protection product residues in plant pollen and nectar: A review of current knowledge. *Environ. Res.* <https://doi.org/10.1016/j.envres.2020.109873> (2020).
33. Zioga, E., White, B. & Stout, J. C. Pesticide mixtures detected in crop and non-target wild plant pollen and nectar. *Sci. Total Environ.* <https://doi.org/10.1016/j.scitotenv.2023.162971> (2023).
34. Li, Z. Modeling pesticide residues in nectar and pollen in support of pesticide exposure assessment for honeybees: A generic modeling approach. *Ecotoxicol. Environ. Saf.* <https://doi.org/10.1016/j.ecoenv.2022.113507> (2022).
35. Li, Z. et al. Differential physiological effects of neonicotinoid insecticides on honey bees: A comparison between *Apis mellifera* and *Apis cerana*. *Pestic Biochem. Physiol.* **140**, 1–8. <https://doi.org/10.1016/j.pestbp.2017.06.010> (2017).
36. Ostwald, M. M., Smith, M. L. & Seeley, T. D. The behavioral regulation of thirst, water collection and water storage in honey bee colonies. *J. Exp. Biol.* **219**, 2156–2165. <https://doi.org/10.1242/jeb.139824> (2016).
37. Halberg, K. V., Denholm, B. Mechanisms of Systemic Osmoregulation in Insects. Annual Review of Entomology Downloaded from www.annualreviews.org Guest. 41: 46. <https://doi.org/10.1146/annurev-ento-040323>. (2025).
38. Andersen MK, Donini A, MacMillan HA. Measuring insect osmoregulation in vitro: A reference guide. *Comp Biochem Physiol A Mol Integr Physiol.* 2025;299. <https://doi.org/10.1016/j.cbpa.2024.111751>
39. Kurio, M., Tsukasa, Y., Uemura, T., Usui, T. Refinement of a technique for collecting and evaluating the osmolality of haemolymph from *Drosophila* larvae. <https://doi.org/10.1101/2023.12.25.573325>. (2023).
40. de Souza, D. L. L., Serrão, J. E. & Hansen, I. A. Aquaporin expression in the alimentary canal of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) and functional characterization of Am_Eglp 1. *PLoS ONE* <https://doi.org/10.1371/journal.pone.0236724> (2020).
41. Stupski, S. D. & Schilder, R. J. Operative temperature analysis of the honey bee *Apis mellifera*. *J. Exp. Biol.* <https://doi.org/10.1242/jeb.231134> (2021).
42. Li, X. et al. Tolerance and response of two honeybee species *Apis cerana* and *Apis mellifera* to high temperature and relative humidity. *PLoS ONE* <https://doi.org/10.1371/journal.pone.0217921> (2019).
43. Liu, L. et al. Protein profiling of hemolymph in *Haemaphysalis flava* ticks. *Parasit. Vectors.* <https://doi.org/10.1186/s13071-022-05287-7> (2022).
44. Elfars, S. A., Bahgat, I. M., Shebl, M. A., Lihoreau, M., Tawfik, M. M. Variation in hemolymph content and properties among three Mediterranean bee species. <https://doi.org/10.1101/2023.12.22.573070>. (2023).
45. Li, D. et al. What are the main proteins in the hemolymph of *Haemaphysalis flava* ticks? *Front. Vet. Sci.* <https://doi.org/10.3389/fvets.2024.1387719> (2024).
46. Dahake, A., Persaud, S., Jones, MN., Goyret, J., Davidowitz, G., Raguso, R. A. Dying of thirst: Osmoregulation by a hawkmoth pollinator in response to variability in ambient humidity and nectar availability. <https://doi.org/10.1101/2023.06.27.546736>.
47. O'Donnell, M. J. A perspective on insect water balance. *J. Exp. Biol.* <https://doi.org/10.1242/jeb.242358> (2022).
48. McCluney, K. E. Implications of animal water balance for terrestrial food webs. *Curr. Opin. Insect Sci.* <https://doi.org/10.1016/j.cois.2017.06.007> (2017).
49. Panche, A. N., Diwan, A. D. & Chandra, S. R. Flavonoids: An overview. *J. Nutr. Sci.* <https://doi.org/10.1017/jns.2016.41> (2016).
50. Miron, A., Aprotosoaie, A. C., Trifan, A. & Xiao, J. Flavonoids as modulators of metabolic enzymes and drug transporters. *Ann. New York Acad. Sci.* <https://doi.org/10.1111/nyas.13384> (2017).
51. Dong, J. et al. Flavonoids and Naphthoflavonoids: Wider Roles in the Modulation of Cytochrome P450 Family 1 Enzymes. *ChemMedChem* <https://doi.org/10.1002/cmdc.201600316> (2016).
52. Choi, S. S., Park, H. R. & Lee, K. A. A comparative study of rutin and rutin glycoside: Antioxidant activity, anti-inflammatory effect, effect on platelet aggregation and blood coagulation. *Antioxidants* <https://doi.org/10.3390/antiox10111696> (2021).
53. Kurutas, E. B. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Nutr. J.* <https://doi.org/10.1186/s12937-016-0186-5> (2016).
54. Enogieru, A. B., Haylett, W., Hiss, D. C., Bardien, S. & Ekpo, O. E. Rutin as a potent antioxidant: Implications for neurodegenerative disorders. *Oxid. Med. Cell. Longev.* <https://doi.org/10.1155/2018/6241017> (2018).
55. Riveros, A. J. & Gronenberg, W. The flavonoid rutin protects the bumble bee *Bombus impatiens* against cognitive impairment by imidacloprid and fipronil. *J. Exp. Biol.* <https://doi.org/10.1242/jeb.244526> (2022).
56. Berenbaum, M. R. & Calla, B. Honey as a Functional Food for *Apis mellifera*. *Ann. Rev. Entomol. Annu. Rev. Entomol.* **2021**(66), 2020. <https://doi.org/10.1146/annurev-ento-040320> (2021).
57. Liao, L. H., Wu, W. Y. & Berenbaum, M. R. Behavioral responses of honey bees (*Apis mellifera*) to natural and synthetic xenobiotics in food. *Sci. Rep.* <https://doi.org/10.1038/s41598-017-15066-5> (2017).
58. Lee, L. C. et al. Dietary supplementation of rutin and rutin-rich buckwheat elevates endogenous glucagon-like peptide 1 levels to facilitate glycemic control in type 2 diabetic mice. *J. Funct. Foods* <https://doi.org/10.1016/j.jff.2021.104653> (2021).
59. Li, H., Gu, Y., Jin, R., He, Q. & Zhou, Y. Effects of Dietary Rutin Supplementation on the Intestinal Morphology, Antioxidant Capacity, Immunity, and Microbiota of Aged Laying Hens. *Antioxidants* <https://doi.org/10.3390/antiox11091843> (2022).
60. OCDE 245. Guideline for the testing of chemical honey bee (*Apis mellifera*). <http://www.oecd.org/termsandconditions/>. (2017).
61. Hernández, J., Mesa, F., Riveros, A., Fayad, R. & Nisperuza, J. Extraction and Optical Analysis of the Extracellular Fluid from the Body Segments of *Apis mellifera* Bees. *Integr. Org. Biol.* <https://doi.org/10.1093/iob/obaf018> (2025).
62. Paleolog, J., Wilde, J., Gancarz, M. & Strachecka, A. Imidacloprid decreases energy production in the hemolymph and fat body of western honeybees even though, in sublethal doses, it increased the values of six of the nine compounds in the respiratory and citric cycle. *PLoS ONE* <https://doi.org/10.1371/journal.pone.0320168> (2025).
63. Alburaki, M., Madella, S. & Cook, S. C. Non-optimal ambient temperatures aggravate insecticide toxicity and affect honey bees *Apis mellifera* L. gene regulation. *Sci. Rep.* <https://doi.org/10.1038/s41598-023-30264-0> (2023).
64. Kamboj, P., Kaur, G. & Gupta, G. Understanding the Impact of Heat Stress on Honeybee Populations: Insights into Thermoregulation, Colony Dynamics, and Environmental Interactions. *Uttar Pradesh J. Zool.* **45**, 1–13. <https://doi.org/10.56557/upjz/2024/v45i124098> (2024).

65. Atmowidjojo, A. H., Wheeler, D. E., Erickson, E. H., Cohen, A. C. Temperature Tolerance and Water Balance in Feral and Domestic Honey Bees, *Apis mellifera* L. *Comp Biochem Physiol.* Elsevier Science Inc; (1997).
66. Paleolog, J. et al. Imidacloprid Pesticide Causes Unexpectedly Severe Bioelement Deficiencies and Imbalance in Honey Bees Even at Sublethal Doses. *Animals* <https://doi.org/10.3390/ani13040615> (2023).

Acknowledgements

This work was supported by the Universidad del Rosario through its Graduate Assistantship Program and by the Fundación Universitaria Los Libertadores. The authors thank both institutions for their financial and academic support throughout the development of this research.

Author contributions

J.H. and F.M. wrote the main manuscript text and A.R. prepared all figures. All authors reviewed the manuscript.

Funding

The authors received no funding for this work.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to J.P.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2025

Capítulo 4. Características eléctricas del líquido extracelular en los segmentos corporales de abejas *Apis mellifera*

Juan Hernandez, Fredy Mesa, Anderson Dussan, Andre Riveros

Publicado en: Peer J

Published: July 17, 2025

<https://doi.org/10.7717/peerj.19691>

Resumen

Este estudio investiga las propiedades eléctricas del líquido extracelular de las abejas (*Apis mellifera*) y su relación con los distintos segmentos corporales. Mediante la caracterización de la resistencia, la capacitancia y la impedancia eléctrica, se evaluaron aspectos como la composición iónica, la polarización molecular y la respuesta diferencial de las abejas vivas ante estímulos eléctricos. Los resultados muestran que las características eléctricas varían significativamente según el segmento corporal: la cabeza presenta valores elevados de resistencia y el abdomen, valores elevados de capacitancia, lo que refleja diferencias en la composición molecular y la funcionalidad. Además, los experimentos con abejas vivas demostraron la viabilidad de medir parámetros eléctricos de manera no invasiva, lo que abre nuevas posibilidades para monitorear el estado fisiológico de estos polinizadores tanto en condiciones controladas como en entornos naturales. Este trabajo sienta las bases para el desarrollo de herramientas innovadoras para el monitoreo ecológico, la evaluación de los estresores ambientales y la gestión sostenible de las colonias de abejas.

Notas metodológicas aclaratorias:

Las abejas fueron recolectadas de diferentes colonias fuente para capturar la variabilidad biológica natural y asignadas aleatoriamente a los grupos de tratamiento. Cada secuencia de puntos reportada en las mediciones de impedancia corresponde a un grupo diferente de abejas, lo que garantiza que cada medición constituye una réplica independiente.

El sistema biológico se modeló mediante un circuito eléctrico en el que los componentes resistivos representan la conductividad iónica de los fluidos intra- y extracelulares (homeostasis osmótica), y los componentes capacitivos representan las propiedades dieléctricas las muestras de las abejas. De este modo, las alteraciones en la impedancia se interpretan físicamente como interrupciones en la distribución iónica y como pérdida de la integridad estructural de los tejidos.

Electrical characteristics of the extracellular fluid in the body segments of *Apis mellifera* bees

Juan Hernandez¹, Fredy Mesa², Anderson Dussan³ and Andre Riveros¹

¹ Faculty of Natural Sciences, Universidad del Rosario, Bogotá, Cundinamarca, Colombia

² Faculty of Engineering and Basic Sciences, Fundación Universitaria Los Libertadores, Bogotá, Cundinamarca, Colombia

³ Physics Department, Universidad Nacional de Colombia, Bogotá, Cundinamarca, Colombia

ABSTRACT

This study investigates the electrical properties of the extracellular fluid in honeybees (*Apis mellifera*) and its relationship with different body segments. By characterizing resistance, capacitance, and electrical impedance, aspects such as ionic composition, molecular polarization, and the differential response of live bees to electrical stimuli were evaluated. The results show that electrical characteristics vary significantly depending on the body segment, with the head exhibiting high resistance values and the abdomen displaying high capacitance, reflecting differences in molecular composition and functionality. Additionally, experiments with live bees demonstrated the feasibility of measuring electrical parameters non-invasively, opening new possibilities for monitoring the health of these pollinators under controlled conditions and in natural environments. This work lays the foundation for developing innovative tools in ecological monitoring, the assessment of environmental stressors, and the sustainable management of bee colonies.

Subjects Animal Behavior, Biophysics, Entomology, Zoology

Keywords Honeybees, Electrical impedance, Resistivity, Extracellular fluid, Capacitance

INTRODUCTION

The electrical properties of biological materials (Heng et al., 2023; Kuang & Nelson, 1998; Leijssen et al., 2021; Miklavčič, Pavšelj & Hart, 2006; Sasaki et al., 2022; Smolyanskaya et al., 2018) have been extensively studied due to their potential applications in biotechnology (Atkinson et al., 2023; Bedi et al., 2022; Forro et al., 2021), diagnostics (Anushree et al., 2022; Russo et al., 2022), and physiological monitoring. Biological tissues and fluids exhibit electrical behaviors (Angenent et al., 2024; Jalilnejad et al., 2023; Joshi, Mishra & Narayan, 2021), which can be explored to understand their composition, structure, and functionality. The characterization of these electrical properties provides insights into ion transport, membrane dynamics, and cellular interactions. For instance, dielectric spectroscopy has been applied in mammalian tissues to study hydration levels and cellular density, in plant tissues to assess water content and ion fluxes, and in microbial systems to monitor metabolic activity. In these systems, electrical resistance and capacitance measurements have revealed information about ion mobility, membrane integrity, and the behavior of polar molecules (Gabriel, Gabriel & Corthout, 1996; Heileman, Daoud &

Submitted 12 February 2025

Accepted 11 June 2025

Published 17 July 2025

Corresponding author

Juan Hernandez,
juanp.hernandez@urosario.edu.co

Academic editor

Lin Zhang

Additional Information and
Declarations can be found on
page 15

DOI 10.7717/peerj.19691

© Copyright

2025 Hernandez et al.

Distributed under

Creative Commons CC-BY 4.0

OPEN ACCESS

[Tabrizian, 2013](#); [Yao et al., 2020](#)). Despite this, insect extracellular fluids remain understudied, especially in ecologically relevant species such as honeybees. Exploring their electrical properties may uncover useful biomarkers of physiological condition and environmental stress.

In the case of insects, particularly honeybees (*Apis mellifera*), the electrical characteristics of their biological fluids remain largely unknown. Their role as pollinators makes their health and physiological status topics of great interest. Recent studies have emphasized the importance of monitoring their physiological and behavioral responses to environmental stressors ([Hung & Yiin, 2023](#); [Mayack et al., 2022](#); [Woodard, 2017](#)), such as pesticide exposure ([Li et al., 2022](#); [Lourenço et al., 2021](#); [Shaher & Manjy, 2020](#)) and dietary changes ([Ardalani et al., 2021](#); [Bryś, Skowronek & Strachecka, 2021](#)). However, the electrical characteristics of their extracellular fluids have received little attention, despite their potential to serve as indicators of ionic composition and metabolic activity.

The extracellular fluid of honeybees plays a fundamental role in maintaining homeostasis ([Bournonville et al., 2023](#); [Gábor et al., 2017](#); [Richardson et al., 2018](#)), transporting nutrients ([Kannan et al., 2024](#); [Li et al., 2024](#); [Tafi et al., 2024](#)), and supporting immune responses ([Gábor et al., 2017](#); [Mallon, Brockmann & Schmid-Hempel, 2003](#)). The electrical properties of this fluid, such as resistance, capacitance, and impedance, may offer new avenues for studying the physiological state of bees under various conditions. Additionally, the ability to evaluate current-voltage relationships in the thorax of live bees could provide information about ion mobility and potential interactions with the surrounding tissues.

This study aims to investigate the electrical characteristics of the extracellular fluid of honeybees under laboratory conditions. Four experiments were conducted: three focusing on the extracellular fluid and one involving live bees. These experiments determined the conductivity, capacitance, and impedance of the extracellular fluid. Finally, current-voltage measurements allowed for the characterization of the extracellular fluid in the thorax of immobilized live bees. The experiments sought to establish a foundation for evaluating the electrical properties of honeybee fluids, offering potential applications in physiological monitoring and environmental assessments. This research provides a novel perspective on bee health and their electrical interactions.

MATERIALS AND METHODS

Experimental animals

The honeybees (*Apis mellifera*) used in this study were collected from the apiary at Universidad del Rosario (Bogotá, Colombia). The insects selected were foragers captured during their morning activity (~9 a.m). The bees were housed in disposable cups modified to allow ventilation and feeding. Immediately after collection, the insects were transported to the laboratory, where temperature, relative humidity, and light type were controlled ($T = 32 \pm 0.2$ °C; $RH = 52 \pm 5\%$; $\lambda = 750$ nm). Red light (750 nm) was used to minimize behavioral alterations during handling, as bees exhibit reduced visual sensitivity at this wavelength, allowing more consistent physiological measurements. The bees were kept under these conditions for 24 h, during which they were fed with a 1 M sucrose solution

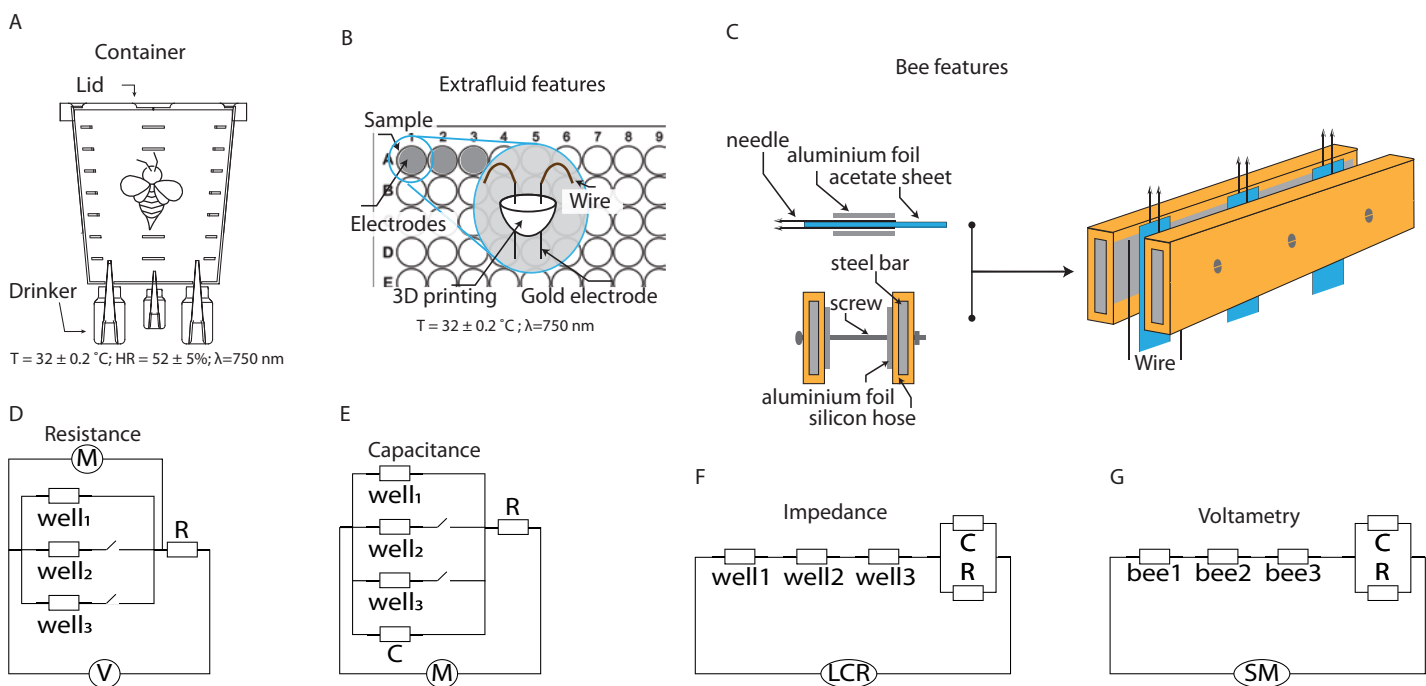


Figure 1 Schematics of the setups for characterizing extracellular fluid and live bees. (A) Container for maintaining bees under laboratory conditions. (B) System for characterizing the extracellular fluid of bees. (C) System for characterizing the extracellular fluid of live bees. (D–G) Equivalent circuits for modeling the electrical characteristics of the extracellular fluid. [Full-size DOI: 10.7717/peerj.19691/fig-1](https://doi.org/10.7717/peerj.19691/fig-1)

(33.1% w/w) (Fig. 1A). Bee maintenance protocols followed the Organisation for Economic Co-operation and Development (OECD) guidelines for insecticide exposure (OCDE 245, 2017).

Extracellular fluid extraction

The extraction of extracellular fluid was performed after euthanizing the bees using low temperatures (Kovac et al., 2014; MacMillan & Sinclair, 2011). For this, the insects were placed in a cooling chamber for 24 h. Subsequently, the body segments (head, thorax, and abdomen) were placed in filtered micropipette tips, and the fluid was extracted *via* centrifugation at 4°C . Each extraction involved seven bees subjected to 2,000 relative centrifugal force (RCF) for 3 min. To preserve the extracellular fluid, 500 μL of distilled water was added. Finally, the samples were stored at -20°C until analysis.

Devices for electrical characterization

The first device used for electrical characterization was constructed using 96-well cell culture plates, cylindrical gold electrodes (1.0 mm diameter) mounted on a 3D-printed structure. The design ensured that the electrodes were positioned 3 mm apart, and their terminals allowed for secure attachment to the plate by pressure. Wires were soldered to the electrodes to facilitate manipulation on a breadboard, enabling series or parallel configurations depending on the desired characterization (Fig. 1B).

The second device used for characterization consisted of stainless-steel electrodes and acetate sheets. The electrodes were built using 32G hypodermic needles attached to each side of acetate sheets ($2\text{ cm} \times 4\text{ cm} \times 0.02\text{ cm}$). The acetate thickness ensured the separation distance between the two needles. Aluminum foil was added to each side of the acetate to increase the conductive area of the needles. This assembly was connected to steel rods covered with silicone tubing, onto which aluminum foil was attached to ensure electrical conduction. Mechanical stabilization was achieved using screws and nuts (Fig. 1C).

For live characterizations, plastic holders were constructed to immobilize bees, facilitating electrical measurements. The holders were made using 1,000 μL micropipette tips, which were modified with longitudinal grooves to allow the bees to slide in. The insect's abdomen remained inside the tube to prevent stings and facilitate handling. The bee's thorax was secured to the tube using a small piece of paraffin paper, immobilizing the bees for characterization.

Experiment 1: electrical resistance of the extracellular fluid from bee body segments

This experiment was designed to address questions related to the resistive behavior of the extracellular fluid in honeybee body segments. Additionally, electrical resistance measurements aimed to explore the electrical properties of the samples from each body segment.

The experiment involved measuring the electrical resistance of extracellular fluid samples from each body segment of the bees. For this, 30 μL samples were placed in the wells of a cell culture plate. The experiment evaluated configurations of 1, 2, and 3 wells, which were connected in parallel. Gold electrodes were inserted into the wells, and their cables were connected in series with a 2,200 Ω electrical resistor. These configurations were connected to a digital multimeter (DMM7500 Keithley) to measure the resulting voltage drop across the samples. The instrument was set to acquire 200 data points per minute. To ensure measurement accuracy, five repetitions were performed.

Electrical model used for resistance measurements

Electrical characterization was performed using a voltage divider circuit, a configuration that distributes the voltage from a power source among two or more resistive elements connected in series. This method is widely used in electronic applications to obtain a lower voltage from a higher voltage source. A voltage divider consists of a voltage source (V_{in}) that provides the total voltage, which is then distributed among the circuit components. The resistive materials are connected in series with the source, and the potential difference (V_{out}) is measured across the components (Fig. 1D). Specifically, in the proposed configuration, the electrical resistances of the extracellular fluid samples (R_{sample}) were connected in series with a known resistor (R), following the relationship:

$$R_{sample} = -\frac{V_{out}/V_{in} R}{(V_{out}/V_{in} - 1)}. \quad (1)$$

Experiment 2: electrical capacitance of the extracellular fluid from bee body segments

This experiment was designed to address questions related to the capacitive behavior of the extracellular fluid in honeybee body segments. Additionally, the samples from each body segment were analyzed to determine their dielectric characteristics in terms of electrical capacitance.

The experiment involved measuring the electrical discharge of a capacitor connected in parallel with the extracellular fluid samples from different body segments. For this, 30 μ L samples were placed in the wells of a cell culture plate. The experiment evaluated configurations of 1, 2, and 3 wells, which were connected in parallel. Gold electrodes were inserted into the wells, and their cables were connected in parallel with a 224 nF ceramic capacitor. This configuration was then connected in series with a 5 M Ω resistor. The capacitor was charged using a voltage source set to a constant value of 0.9 V. The discharge measurements were performed by recording the voltage drop using a digital multimeter (DMM7500 Keithley), which was configured to acquire 200 data points per minute. To ensure measurement accuracy, five repetitions were performed.

Electrical model used for capacitance measurements

To analyze the electrical discharge of a capacitor, potential differences are applied to charge it, and then it is allowed to discharge through a resistor. This type of circuit is useful for studying how the energy stored in the capacitor dissipates through the resistor. In this study, the system components included the extracellular fluid samples (*C sample*), along with a capacitor (*C*) and a resistor (*R*) with known values. The resistor provided a discharge path, allowing the stored charge (*Q*) to leave the capacitor plates, resulting in a decrease in electric potential over time (Fig. 1E).

The voltage (*V*) across such a system should decrease exponentially with time (*t*), following the equation:

$$V = V_0 e^{-t/\tau} \quad (2)$$

where *V*₀ is the initial voltage and τ is the time constant (*RC*). The constant τ determines the time required for the voltage to drop to approximately 36.8% of its initial value.

Experiment 3: electrical impedance of the extracellular fluid from bee body segments

This experiment addressed questions related to the resistive and capacitive behavior of the extracellular fluid in honeybee body segments through impedance measurements. Additionally, electrical impedance measurements were incorporated to determine the resistance and reactance of extracellular fluid samples from each body segment.

The experiment involved measuring the reactance and electrical resistance of the extracellular fluid samples, which were connected to a capacitor and a resistor. For this, 30 μL samples were deposited in the wells of the culture plate, with only three wells evaluated, which were connected in series. Gold electrodes were inserted into the wells, and their cables were connected in series with a 224 nF ceramic capacitor and a 2,200 Ω resistor. Measurements were performed using an Inductance-Capacitance-Resistance (LCR) meter (E4980AL; Keysight, Santa Rosa, California, USA), configured to measure resistance and reactance five times for each frequency. The electrical excitation of the samples was selected across four frequency ranges to obtain impedance at both low and high frequencies: I1 = 20–120 Hz; I2 = 120–1,020 Hz; I3 = 1,020–10,020 Hz; I4 = 10,020–100,020 Hz. The LCR meter was programmed using the Command Expert software from Excel, and to ensure measurement accuracy, each measurement was repeated five times.

Electrical model used for impedance measurements

Electrical impedance (Z) is a generalized measure of the opposition that a circuit or sample presents to the flow of current when an alternating potential difference is applied. This measure of electrical resistance for circuits with voltages varying at different frequencies ($\omega = 2\pi f$) includes both resistance (R) and reactance (X). Impedance is a complex quantity ($j = \sqrt{-1}$), indicating that it consists of a real and imaginary component ($Z = R + Xj$). The real component represents the opposition to current flow due to energy dissipation, while the imaginary component represents the opposition to current flow due to reactive elements such as capacitors (Fig. 1F).

In systems exhibiting both capacitive and resistive behavior, as observed in the extracellular fluid samples, impedance follows the form:

$$Z = \frac{R}{1 + R^2\omega^2C^2} + \frac{R^2\omega C}{1 + R^2\omega^2C^2}j. \quad (3)$$

The limit of Z as the frequency approaches zero represents the resistive characteristic of the system; however, as the frequency approaches infinity, the impedance value becomes zero.

Experiment 4: electrical characteristics of live bees

The questions addressed in this section focused on the electrical response observed in the thorax due to electrical stimuli. The electrical responses of the bee thorax demonstrated the resistive and capacitive behavior of living tissue.

The experiment involved obtaining current vs. voltage (I–V) curves from live bees, which were immobilized and connected in parallel to a resistor-capacitor (RC) electrical circuit using hypodermic needles. Measurements were performed using a source meter (Keithley 2450), configured to produce electrical excitation and record the system's electrical current over three cycles. The excitation potential difference range was set from –900 to 900 mV, with a sweep rate of 18 mV/s. The capacitance exhibited by the thorax was calculated from the data obtained from the I–V curves.

Electrical model used for impedance measurements

Voltammetry is a set of electrical techniques used to study the characteristics of a system by measuring the electrical current generated when a potential is applied to a solution. The potential (voltage) applied to the electrodes serves to excite the mobile components of the samples, with the generated currents being proportional to the electroactive species in the solution. In linear sweep voltammetry, a potential that varies linearly with time is applied while the current is measured. The applied potential can be specified within intervals to perform forward and reverse sweeps, providing information on reversible and non-reversible processes. The relationship between current and potential is represented in a voltammogram, where the horizontal axis displays the applied potential, and the vertical axis shows the recorded current. When the samples exhibit both capacitive and resistive characteristics, the current (I) in the system will increase or decrease depending on the applied potential (Fig. 1G). The system's variations will be proportional to the applied voltage, a behavior described by a composite equation for each of the components:

$$I = C \frac{dV}{dt} + \frac{V}{R}. \quad (4)$$

The first component of Eq. (4) corresponds to the capacitive current, while the second is related to the electrical resistance of the system.

RESULTS

Experiment 1: electrical resistance of extracellular fluid by body segment

In the electrical resistance experiment, 105 bees were sacrificed to obtain extracellular fluid from each of their body segments. The highest potential differences were observed when using a single well, with the distribution range remaining constant over the evaluation period [median; quartile 1; quartile 3]: [740 mV; 736 mV; 743 mV] head; [698 mV; 694 mV; 701 mV] thorax; [678 mV; 674 mV; 682 mV] abdomen. The samples exhibited intermediate potential values when two wells were used; however, the dispersion of values increased compared to measurements with a single well [median; quartile 1; quartile 3]: [635 mV; 628 mV; 641 mV] Head; [579 mV; 571 mV; 586 mV] thorax; [554 mV; 546 mV; 561 mV] abdomen. In contrast, the values obtained with three wells were the lowest, and a growing dependence over time was also observed [median; quartile 1; quartile 3]: [564 mV; 554 mV; 573 mV] head; [503 mV; 491 mV; 511 mV] thorax; [477 mV; 465 mV; 485 mV] abdomen (Fig. 2A).

Additionally, resistance estimations using Eq. (1) indicated that the electrical characteristics changed depending on the number of wells and the body segment. In all cases, the samples from the head segment exhibited the highest electrical resistance values, in contrast to the fluid obtained from the abdomen. The observed resistances in the different segments decreased as the number of wells increased. The percentage differences observed with two and three wells, relative to the first well, remained consistent (mean $\pm$ SD): $47.77 \pm 0.21\%$ (well1–well2); $63.33 \pm 0.23\%$ (well1–well3). These results indicated that as the number of wells increased, the system behaved similarly to a parallel circuit

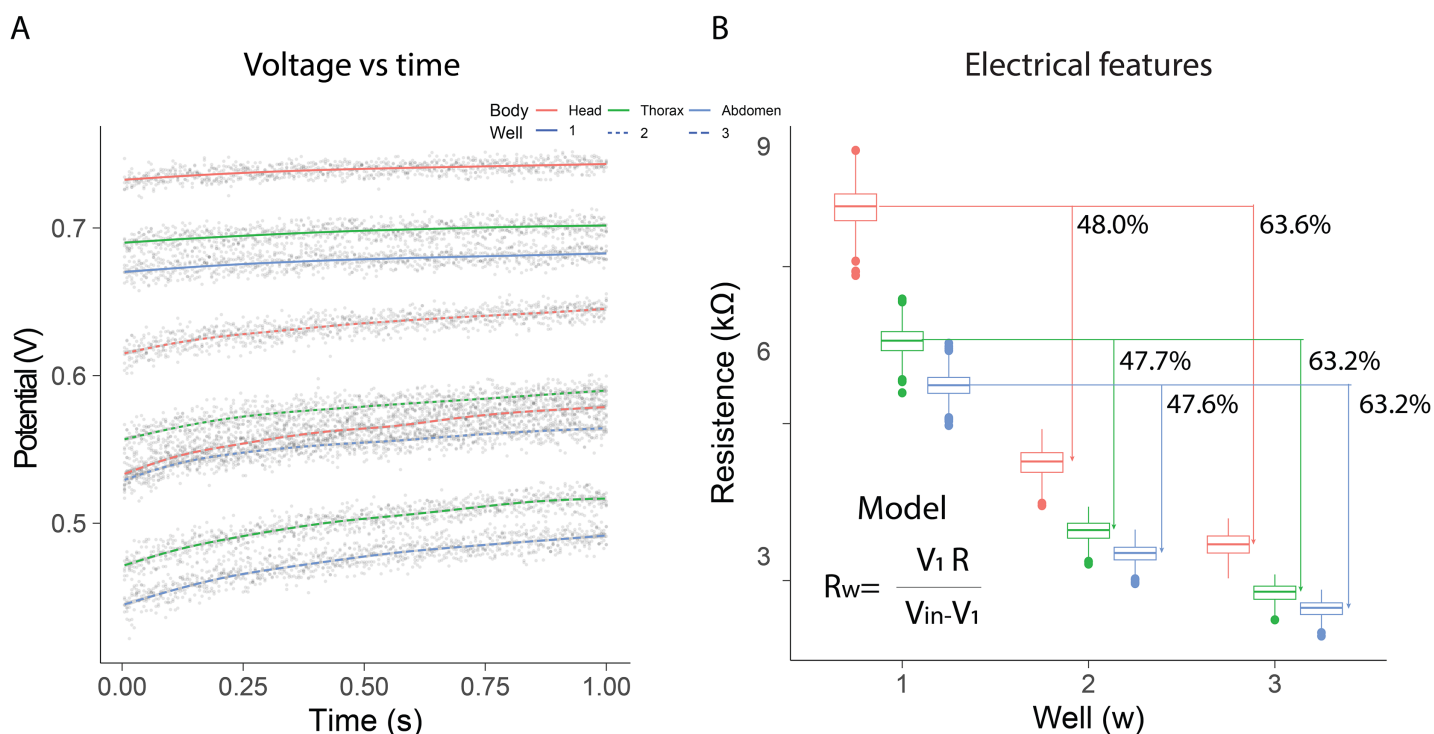


Figure 2 Electrical resistance of the extracellular fluid. (A) Potential difference observed over one minute for the fluid obtained from bee body segments. (B) Estimation of electrical resistance for each segment based on the number of wells. [Full-size DOI: 10.7717/peerj.19691/fig-2](https://doi.org/10.7717/peerj.19691/fig-2)

Table 1 Electrical resistance (kΩ) of extracellular fluid by body segment and well configuration. Values are shown as median [Q1–Q3].

Body segment	Resistance 1 well	Resistance 2 wells	Resistance 3 wells
Head	10.2 [9.9–10.4]	5.3 [5.1–5.4]	3.7 [3.5–3.9]
Thorax	7.6 [7.4–7.8]	4.0 [3.8–4.1]	2.8 [2.6–2.9]
Abdomen	6.7 [6.6–6.9]	3.5 [3.4–3.6]	2.5 [2.4–2.6]

(Fig. 2B). Consequently, the extracellular fluid samples exhibited resistive behavior to the flow of electrical current. The resistance values measured for each condition are summarized in Table 1. The median and interquartile ranges show a consistent pattern across body segments and well configurations, reinforcing the segmental differences in electrical resistance observed in this study.

Experiment 2: electrical capacitance of extracellular fluid by body segment

In the electrical capacitance experiment, 105 bees were sacrificed to obtain extracellular fluid from each of their body segments. The potential of the samples connected to the capacitor varied similarly to a discharge circuit, with an initial potential of 0.86 ± 0.02 V. The 50% discharge (0.43 ± 0.01 V) was achieved within 0.18 s, while the 36.6% discharge (0.31 ± 0.01 V) was reached in 0.27 s. In the 36.6% discharge interval, significant

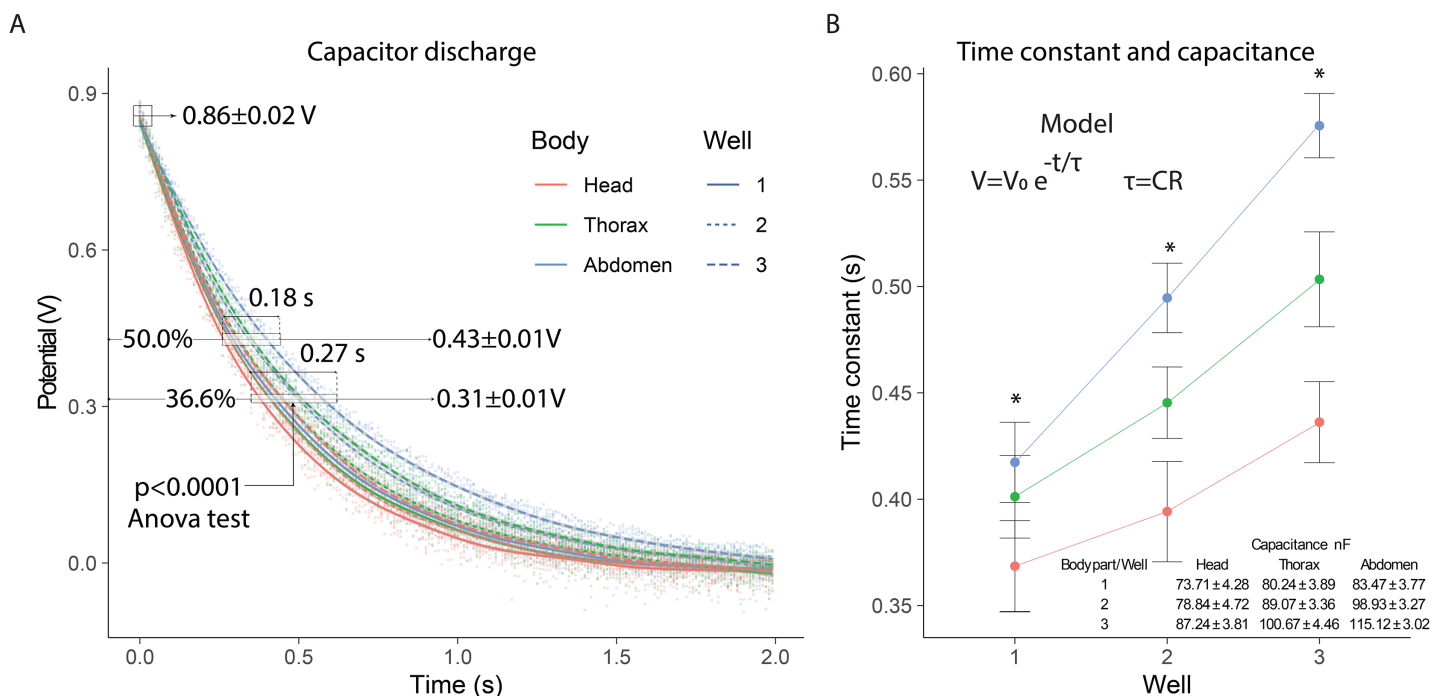


Figure 3 Electrical capacitance of the extracellular fluid. (A) Observed discharge potential difference of extracellular fluid samples from bees. (B) Estimation of the time constant and capacitance based on the number of wells. Asterisks indicate statistically significant differences between well configurations (1, 2, and 3 wells) within each body segment (one-way ANOVA, $p < 0.0001$; Tukey's test).

Full-size [DOI: 10.7717/peerj.19691/fig-3](https://doi.org/10.7717/peerj.19691/fig-3)

differences ($p < 0.0001$) were observed in the discharge potentials for each body segment depending on the number of wells used: ($F_{2,357} = 28$) head; ($F_{2,357} = 53$) thorax; ($F_{2,357} = 108$) abdomen (Fig. 3A).

In the 0.35 to 0.62 s interval, the 36.6% capacitor discharge was achieved, showing variations in the time constant for each body segment. The time constants exhibited significant differences ($p < 0.0001$) depending on the number of wells evaluated: ($F_{2,357} = 16.5$) well 1; ($F_{2,357} = 47.0$) well 2; ($F_{2,357} = 72.1$) well 3. The time constants were highest for the abdomen, intermediate for the thorax, and lowest for the head, with greater distinction as the number of wells increased. This consecutive increase in the time constant indicated that the system's capacitance also increased with the number of wells, with values in the nanofarad range (Fig. 3B).

The capacitance measurements showed consistent differences between body segments. The abdominal segment presented the highest capacitance values, followed by the thorax and the head. This trend was maintained across all well configurations. The values obtained indicate that the extracellular fluid can store electrical charge, and its capacitance is affected by anatomical and physiological differences between segments. The capacitance values recorded in the abdominal segment were consistently higher than those from the thorax and head. This may be due to the higher content of polar molecules and metabolites in the abdomen, increasing its ability to store charge. These values are comparable, in order of magnitude, to those observed in tissues with high dielectric properties such as skeletal

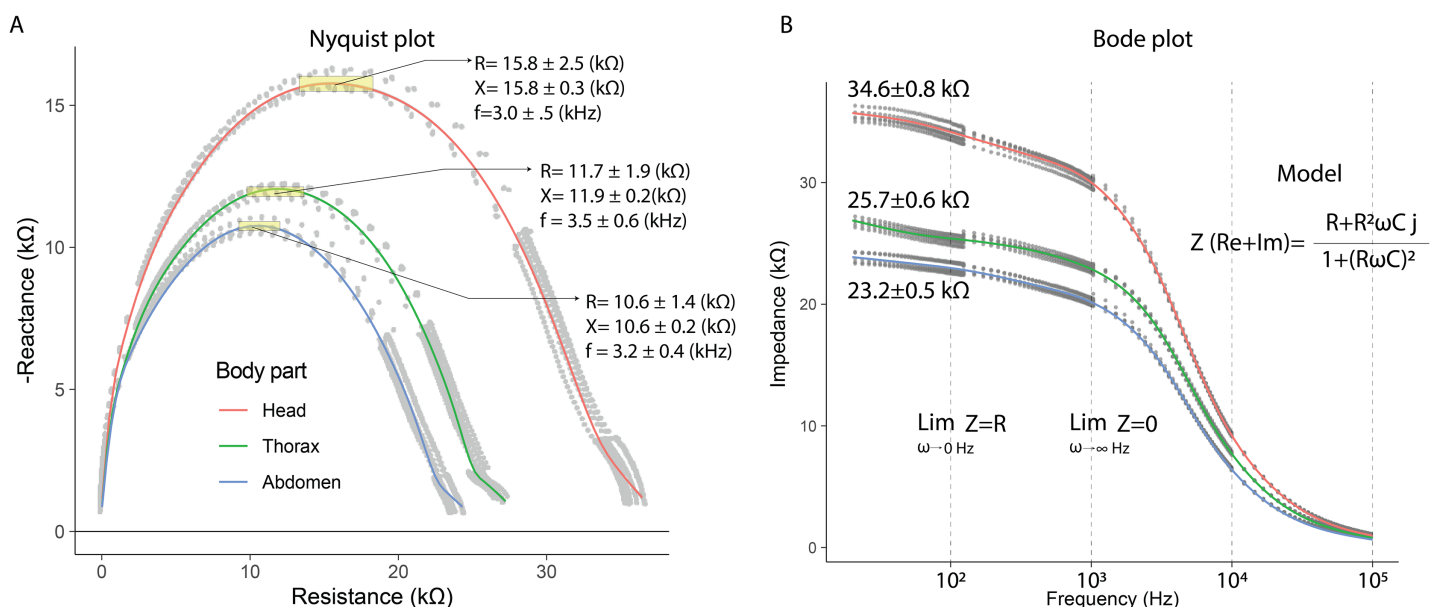


Figure 4 Capacitive and resistive behavior of extracellular fluid samples. (A) Nyquist plot showing the relationship between reactance and resistance in the electrical system. (B) Bode plot showing the relationship between electrical impedance and the excitation frequency of the sample.

Full-size [DOI: 10.7717/peerj.19691/fig-4](https://doi.org/10.7717/peerj.19691/fig-4)

muscle or lipid-rich membranes (Zimmermann & Van Rienen, 2021; Gabriel, Gabriel & Corthout, 1996).

Experiment 3: electrical impedance of extracellular fluid by body segment

In the electrical capacitance experiment, 35 bees were sacrificed to obtain extracellular fluid from each of their body segments. The electrical impedance measurements of the extracellular fluid exhibited frequency-dependent electrical characteristics. This was evidenced in the Nyquist plots, where the relationship between reactance (imaginary component) and electrical resistance (real component) displayed curved segments, indicating both capacitive and resistive behavior. The Nyquist plot showed that the curves corresponding to each body segment had a circular geometry, with the largest radius observed for the head, followed by the thorax, and lastly, the abdomen.

The Nyquist plot also revealed that reactance and resistance values varied depending on the body segment, with the highest values found in the head, followed by the thorax, and the lowest in the abdomen. The head segment was characterized by the highest reactance and resistance values at frequencies of $3.0 \pm 0.1 \text{ kHz}$ (mean $\pm$ SD): $15.8 \pm 0.3 \text{ k}\Omega$ (X); $15.8 \pm 2.5 \text{ k}\Omega$ (R). In contrast, at $3.5 \pm 0.6 \text{ kHz}$, thorax samples exhibited lower reactance and resistance values compared to the head (mean $\pm$ SD): $11.9 \pm 0.2 \text{ k}\Omega$ (X); $11.7 \pm 1.9 \text{ k}\Omega$ (R). Finally, the electrical characteristics of the abdomen samples showed further reductions at $3.2 \pm 0.4 \text{ kHz}$ (mean $\pm$ SD): $10.6 \pm 0.2 \text{ k}\Omega$ (X); $10.6 \pm 1.4 \text{ k}\Omega$ (R) (Fig. 4A). These results indicate that the components of the extracellular fluid are susceptible to interactions with electric fields.

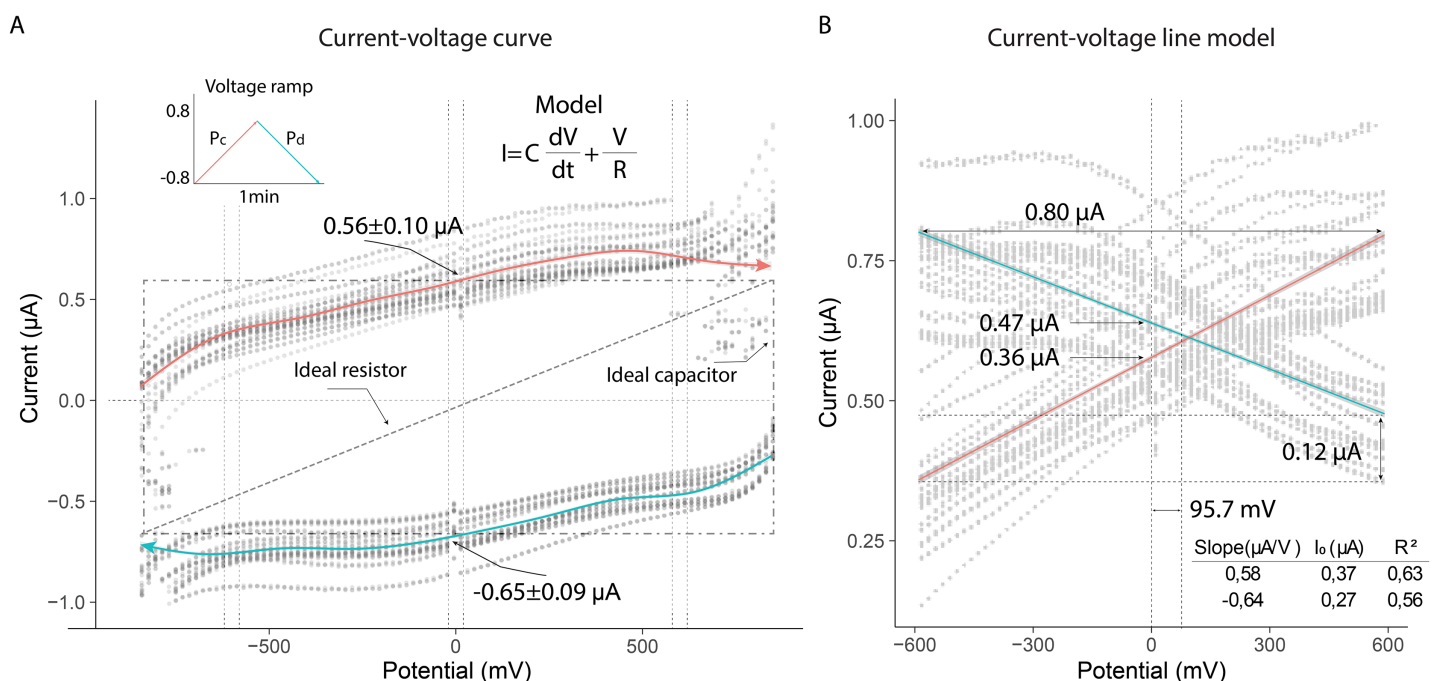


Figure 5 Current-voltage curves of the thorax of live bees connected to a resistor and capacitor under uniform potential differences. (A) Variation of current due to linear changes in electrical potential. (B) Linear model of the absolute values of electrical currents.

Full-size [DOI: 10.7717/peerj.19691/fig-5](https://doi.org/10.7717/peerj.19691/fig-5)

The Bode plot demonstrated that impedance decreased with increasing frequency, a characteristic behavior of systems that combine resistive and capacitive elements. In all cases, the electrical impedance of the extracellular fluid samples exhibited an inverse relationship with frequency (20 to 100,020 Hz), with this trend becoming more pronounced at higher frequencies. Impedance values were distinct for each body segment, with the highest values observed in the head, followed by the thorax, and the lowest in the abdomen. Specifically, at low frequencies (20 to 120 Hz), the resistive characteristics of the samples were predominant (*mean* ± *SD*): 34.6 ± 0.8 kΩ (Head); 25.7 ± 0.6 kΩ (Thorax); 23.2 ± 0.5 kΩ (Abdomen).

Experiment 4: voltammetry of live bees

The current-voltage (*I*-*V*) curves showed that the thorax of the bees exhibited electrical characteristics under the experimental conditions. The linear changes in potential difference produced variable currents, which followed opposite trends depending on whether the potential was increasing (*Pa*) or decreasing (*Pb*). The behavior of both current curves reflected a dependence typical of systems that combine resistive and capacitive properties. These characteristics were evident when the electrical potential approached zero, generating nonzero currents in the system (*mean* ± *SD*): 0.56 ± 0.10 μA (*Pa*); -0.65 ± 0.09 μA (*Pb*) (Fig. 5A).

The electrical current in the thorax exhibited nearly linear behavior in the potential range from -600 mV to 600 mV (slope (μA/V); initial current (μA)): 0.56; 0.37 (*Pa*);

−0.64; 0.27 (Pb). The current across the thorax reached values close to 0.80 μA at the extreme potential values (−600 V; 600 V). Additionally, the currents generated at zero volts differed, indicating that the extracellular fluid in the thorax responded differently to the applied potentials: 0.47 μA (Pb); 0.36 μA (Pa). This result demonstrated that the composition of the fluid in the thorax induced a higher electrical resistance under decreasing potential conditions, leading to a lower current intensity (0.12 μA) (Fig. 5B).

DISCUSSION

The results obtained in this study provide new insights into the electrical characteristics of extracellular fluids in honeybees (*Apis mellifera*) and their associated physiological implications. This approach allowed for the exploration of previously unstudied aspects, contributing to the development of precise tools for assessing pollinator health under different environmental conditions.

The experiments demonstrated that the electrical characteristics of extracellular fluid vary significantly depending on the body segment, as observed in other types of studies (Beyenbach, 2016; Crailsheim, 1985; Strachecka et al., 2022). In terms of electrical resistance, the samples from the head segment exhibited the highest values, while those from the abdomen showed the lowest. The differential resistance values of each segment may be due to the volume of fluid they contain and the electrical properties of their components. First, the amount of hemolymph in the head is lower than in the other two segments, as shown in previous studies. Additionally, the resistance gradient found suggests differences in ionic composition (sodium, chloride, and potassium) and in the dynamics of the tissues surrounding the extracellular fluid.

Regarding capacitance, the samples exhibited behavior consistent with capacitive systems during electrical discharge phases. This suggests that extracellular fluid samples exhibited electrical polarization effects when exposed to potential differences, indicating the presence of dielectric material within them (Di Meo et al., 2022; Gabriel, Gabriel & Corthout, 1996; Zimmermann & Van Rienen, 2021). This polarization-susceptible material likely corresponds to molecules with dipolar properties, which align with the field direction in the presence of an electric field. Notably, the fluid extracted from the abdomen exhibited the highest electrical capacitance, suggesting a higher presence of molecules that underwent polarization. The increased capacitance in the abdomen is consistent with the diverse elements present in this segment, such as water, pollen, sugars, and fats.

Compared to previously reported values in other biological fluids, the electrical resistance and capacitance measured in honeybee extracellular fluid fall within the expected range. For instance, hemolymph in lepidopteran insects such as *Spodoptera littoralis* and *Lymantria dispar* has demonstrated osmolarities and ionic concentrations that correspond to electrical conductivities between 4 and 6 mS/cm (Pannabecker, Andrews & Beyenbach, 1992; Smagghe & Van Leeuwen, 2004). These values are compatible with resistances in the 5–12 k Ω range, depending on developmental stage and solute content, and are consistent with the segmental values observed here for *Apis mellifera*. Likewise, capacitance values in the nanofarad range have been reported in tissues rich in

water and dipolar molecules, such as muscle fibers and hemolymph, due to their dielectric behavior (Gabriel, Gabriel & Corthout, 1996; Zimmermann & Van Rienen, 2021). The segmental pattern observed in honeybees—with higher resistance in the head and higher capacitance in the abdomen—reflects known physiological differences in fluid volume, metabolite composition, and compartmentalized function across body regions. These results support the idea that electrical measurements can serve as reliable indicators of physiological specialization in insect compartments.

An interesting observation in both resistance and capacitance measurements was the signal amplification with the addition of wells for measurements, behaving as passive electrical elements. In the case of electrical resistance, the addition of wells increased electrical effects, resembling a parallel resistor connection, where the total resistance is always lower than its components. For electrical discharge connections, the parallel configuration behaved as the sum of capacitances, exhibiting a greater ability to store electrical charge, similar to that observed in other tissues (Schwan & Kay, n.d.; Sohn et al., 2000; Tsai et al., 2020; Yao et al., 2020). These findings indicate that the evaluated biological samples can be accurately characterized using electrical resistance and capacitance models.

Electrical impedance also exhibited frequency-dependent behavior, with resistive characteristics predominating at low frequencies and capacitive behavior emerging at higher frequencies, similar to what has been observed in other biological systems (Abasi et al., 2022; Grossi & Riccò, 2017; Leitzke & Zangl, 2020; Mesa et al., 2021; Wu et al., 2021; Yao et al., 2020). This phenomenon aligns with systems containing mobile components (ions) and polarizable molecules (dipoles) (Gabriel, Peyman & Grant, 2009; Gun, Ning & Liang, 2017; Heileman, Daoud & Tabrizian, 2013; Jaffrin & Morel, 2008), as shown in the resistance and capacitance experiments. A novel finding from the impedance measurements was the dependence of reactance and resistance on excitation frequencies, indicating that frequencies between 100 and 10,000 Hz can effectively characterize extracellular fluid. Additionally, electrical impedance measurements allowed for the simultaneous characterization of both mobile and dielectric components of the extracellular fluid samples, similar to other biological systems (Bedard et al., 2022; Jönsson et al., 2022; Veil et al., 2023). Such measurements are highly relevant for assessing bee health, as they clearly differentiate each body segment. This approach could be particularly valuable for studies on particle segregation or retention due to xenobiotic exposure.

Finally, the experiments with live bees demonstrated that the thorax exhibited a differential electrical response when applying increasing and decreasing potentials. This result highlights the sensitivity of the thoracic extracellular fluid, potentially linked to the high metabolic and muscular activity in this region. The clear electrical current signal obtained from live bees is one of the strongest points of this discussion due to its potential real-world applications. As demonstrated, the current-voltage methodology required only a small number of bees to obtain data, and the bees were released after measurements. This suggests that electrical measurements could be standardized to characterize bees, enabling the collection of information to identify anomalies in their bodies. This could serve as a

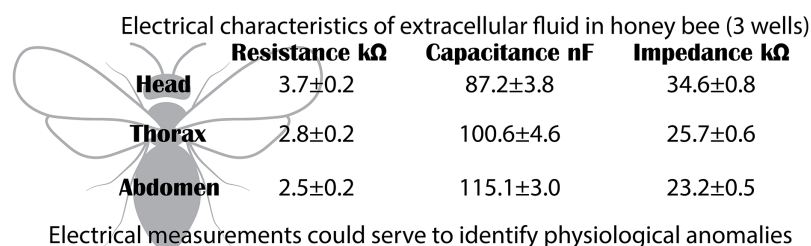


Figure 6 Electrical characteristics. Segment-specific electrical characteristics of extracellular fluid in honeybees. The head shows high resistance and impedance but low capacitance, while the abdomen shows the opposite pattern. These patterns reflect physiological specialization and may serve as biomarkers of systemic health. [Full-size !\[\]\(632f6abe01e74b8582d7456fcbe50fd0_img.jpg\) DOI: 10.7717/peerj.19691/fig-6](https://doi.org/10.7717/peerj.19691/fig-6)

valuable tool for early diagnostics, helping prevent significant bee population losses due to a lack of health status information.

Although research on the electrical characteristics of biological fluids is well documented in other organisms, this study stands out for its focus on honeybees. The results confirm previously observed patterns in biological systems, such as the dependence of capacitance and impedance on fluid density and ionic composition. The application of these methodologies to insects is novel and opens a path for non-invasive physiological monitoring in bees.

The results of our measurements have significant implications both in biological and technological contexts. The electrical differentiation among body segments may be linked to specific functions, such as nutrient transport, immune activity, or metabolism. Additionally, the development of customized experimental devices facilitates precise electrical characterization, demonstrating their adaptability to complex and small biological systems. From an applied perspective, the electrical properties of extracellular fluid could be used to assess the impact of environmental stressors, such as pesticides or dietary changes, on bee health (Fig. 6). This has considerable potential for improving ecological monitoring practices and sustainable colony management.

One limitation of this study is the reliance on measurements under controlled conditions, which may limit direct applicability in natural environments. Additionally, the relatively small sample size may leave some aspects unexplored. Future research on electrical characteristics should examine variations in extracellular fluids due to different factors, including diet, environmental conditions, and pathologies. It would also be valuable to develop more non-invasive techniques to measure these properties in live bees, enabling more frequent monitoring with minimal impact on individuals. Thus, this study represents a significant step forward in understanding the electrical characteristics of biological fluids in honeybees and lays the foundation for future applications in physiological and environmental monitoring.

CONCLUSIONS

This study not only expands the understanding of the electrical characteristics of biological fluids in honeybees (*Apis mellifera*) but also establishes a robust experimental framework for future research in physiological and environmental monitoring. The characterization of resistance, capacitance, and impedance in different body segments provides a tool to understand the biological responses of these essential pollinators to environmental challenges such as pesticide exposure and dietary changes.

Additionally, the integration of non-invasive techniques and customized devices highlights the potential of these methodologies for field applications, promoting a sustainable approach to colony management and preservation. This work represents a crucial step toward implementing strategies based on electrical parameters to ensure the health and survival of bees, which are fundamental pillars of ecosystems and global food security.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was funded by the Universidad del Rosario and Fundación Universitaria Los Libertadores. The Universidad Nacional de Colombia supported this work *via* project QUIPU 202010042199, and Min-Ciencias through Conv. 937. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Universidad del Rosario and Fundación Universitaria Los Libertadores.
The Universidad Nacional de Colombia: QUIPU 202010042199.
Min-Ciencias through Conv. 937.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Juan Hernandez conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Fredy Mesa conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Anderson Dussan performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

- Andre Riveros conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the [Supplemental File](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.19691#supplemental-information>.

REFERENCES

- Abasi S, Aggas JR, Garayar-Leyva GG, Walther BK, Guiseppi-Elie A. 2022. Bioelectrical impedance spectroscopy for monitoring mammalian cells and tissues under different frequency domains: a review. *ACS Measurement Science Au* 2(6):495–516 DOI 10.1021/acsmeasuresciau.2c00033.
- Angenent LT, Casini I, Schröder U, Harnisch F, Molitor B. 2024. Electrical-energy storage into chemical-energy carriers by combining or integrating electrochemistry and biology. *Energy and Environmental Science* 17:3682–3699 DOI 10.1039/d3ee01091k.
- Anushree U, Shetty S, Kumar R, Bharati S. 2022. Adjunctive diagnostic methods for skin cancer detection: a review of electrical impedance-based techniques. *Bioelectromagnetics* 43(3):193–210 DOI 10.1002/bem.22396.
- Ardalani H, Vidkjær NH, Kryger P, Fiehn O, Fomsgaard IS. 2021. Metabolomics unveils the influence of dietary phytochemicals on residual pesticide concentrations in honey bees. *Environment International* 152:106503 DOI 10.1016/j.envint.2021.106503.
- Atkinson JT, Chavez MS, Niman CM, El-Nagggar MY. 2023. Living electronics: a catalogue of engineered living electronic components. *Microbial Biotechnology* 16(3):507–533 DOI 10.1111/1751-7915.14171.
- Bedard C, Piette C, Venance L, Destexhe A. 2022. Extracellular and intracellular components of the impedance of neural tissue. *Biophysical Journal* 121(6):869–885 DOI 10.1016/j.bpj.2022.02.022.
- Bedi N, Srivastava D, Srivastava A, Mahapatra S, Chandra P, Srivastava A. 2022. Marine biological macromolecules as matrix material for biosensor fabrication. Available at <https://doi.org/10.22541/au.164242212.26602085/v1>.
- Beyenbach KW. 2016. The plasticity of extracellular fluid homeostasis in insects. *Journal of Experimental Biology* 219(17):2596–2607 DOI 10.1242/jeb.129650.
- Bournonville L, Askri D, Arafah K, Voisin SN, Bocquet M, Bulet P. 2023. Unraveling the bombus terrestris hemolymph, an indicator of the immune response to microbial infections, through complementary mass spectrometry approaches. *International Journal of Molecular Sciences* 24(5):4658 DOI 10.3390/ijms24054658.
- Bryś MS, Skowronek P, Strachecka A. 2021. Pollen diet—properties and impact on a bee colony. *Insects* 12(9):798 DOI 10.3390/insects12090798.
- Crailsheim K. 1985. Distribution of haemolymph in the honeybee (APIS A~ELJ~IFICA) in relation to season, age and temperature. *Journal of Insect Physiology* 31(9):707 DOI 10.1016/0022-1910(85)90051-4.

- Di Meo S, Bonello J, Farhat I, Farrugia L, Pasian M, Camilleri Podesta MT, Suleiman S, Calleja-Agius J, Sammut CV. 2022. The variability of dielectric permittivity of biological tissues with water content. *Journal of Electromagnetic Waves and Applications* 36(1):48–68 DOI 10.1080/09205071.2021.1956375.
- Forro C, Caron D, Angotzi GN, Gallo V, Berdondini L, Santoro F, Palazzolo G, Panuccio G. 2021. Electrophysiology read-out tools for brain-on-chip biotechnology. *Micromachines* 12(2):1–50 DOI 10.3390/mi12020124.
- Gábor E, Cinege G, Csordás G, Török T, Folkl-Medzihradsky K, Darula Z, Andó I, Kurucz É. 2017. Hemolymph expression reveals functional heterogeneity in honey bee (*Apis mellifera*) hemocytes. *Developmental and Comparative Immunology* 76(1):403–411 DOI 10.1016/j.dci.2017.07.013.
- Gabriel C, Gabriel S, Corthout E. 1996. The dielectric properties of biological tissues: I. Literature survey. *Physics in Medicine and Biology* 41(11):2231–2249 DOI 10.1088/0031-9155/41/11/001.
- Gabriel C, Peyman A, Grant EH. 2009. Electrical conductivity of tissue at frequencies below 1 MHz. *Physics in Medicine and Biology* 54(16):4863–4878 DOI 10.1088/0031-9155/54/16/002.
- Grossi M, Riccò B. 2017. Electrical impedance spectroscopy (EIS) for biological analysis and food characterization: a review. *Journal of Sensors and Sensor Systems* 6(2):303–325 DOI 10.5194/jsss-6-303-2017.
- Gun L, Ning D, Liang Z. 2017. Effective permittivity of biological tissue: comparison of theoretical model and experiment. *Mathematical Problems in Engineering* 2017(1):383 DOI 10.1155/2017/7249672.
- Heileman K, Daoud J, Tabrizian M. 2013. Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis. *Biosensors and Bioelectronics* 49:348–359 DOI 10.1016/j.bios.2013.04.017.
- Heng BC, Bai Y, Li X, Meng Y, Lu Y, Zhang X, Deng X. 2023. The bioelectrical properties of bone tissue. *Animal Models and Experimental Medicine* 6(2):120–130 DOI 10.1002/ame2.12300.
- Hung CC, Yiin LM. 2023. Availability of using honeybees as bioindicators of pesticide exposure in the vicinity of agricultural environments in Taiwan. *Toxics* 11(8):703 DOI 10.3390/toxics11080703.
- Jaffrin MY, Morel H. 2008. Body fluid volumes measurements by impedance: a review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods. *Medical Engineering and Physics* 30(10):1257–1269 DOI 10.1016/j.medengphy.2008.06.009.
- Jalilinejad N, Rabiee M, Baheiraei N, Ghahremanzadeh R, Salarian R, Rabiee N, Akhavan O, Zarrintaj P, Hejna A, Saeb MR, Zarrabi A, Sharifi E, Yousefiasl S, Zare EN. 2023. Electrically conductive carbon-based (bio)-nanomaterials for cardiac tissue engineering. *Bioengineering and Translational Medicine* 8(1):e10347 DOI 10.1002/btm2.10347.
- Joshi P, Mishra R, Narayan RJ. 2021. Biosensing applications of carbon-based materials. *Current Opinion in Biomedical Engineering* 18:100274 DOI 10.1016/j.cobme.2021.100274.
- Jönsson C, Johansson K, Bjurberg M, Brogårdh C. 2022. Impedance of extracellular fluid, volume, and local tissue water can be reliably measured in people with lower limb lymphedema. *Physical Therapy* 102(5):115 DOI 10.1093/ptj/pzac025.
- Kannan M, Vitenberg T, Schweitzer R, Opatovsky I. 2024. Hemolymph metabolism of black soldier fly (Diptera: Stratiomyidae), response to different supplemental fungi. *Journal of Insect Science* 24(3):591 DOI 10.1093/jisesa/ieae050.
- Kovac H, Käfer H, Stabentheiner A, Costa C. 2014. Metabolism and upper thermal limits of *Apis mellifera carnica* and *A. m. ligustica*. *Apidologie* 45(6):664–677 DOI 10.1007/s13592-014-0284-3.

- Kuang W, Nelson SO. 1998. Low-frequency dielectric properties of biological tissues: a review with some new insights. *Transactions of the American Society of Agricultural Engineers* 41(1):173–184 DOI 10.13031/2013.17142.
- Leijssen R, Brink W, Van den Berg C, Webb A, Remis R. 2021. Electrical properties tomography: a methodological review. *Diagnostics* 11(2):176 DOI 10.3390/diagnostics11020176.
- Leitzke JP, Zangl H. 2020. A review on electrical impedance tomography spectroscopy. *Sensors* 20(18):1–19 DOI 10.3390/s20185160.
- Li Z, Duan J, Chen L, Wang Y, Qin Q, Dang X, Zhou Z. 2022. Melatonin enhances the antioxidant capacity to rescue the honey bee *Apis mellifera* from the ecotoxicological effects caused by environmental imidacloprid. *Ecotoxicology and Environmental Safety* 239(5):113622 DOI 10.1016/j.ecoenv.2022.113622.
- Li D, Liu L, Liu ZL, Tian Y, Gao X, Cheng TY. 2024. What are the main proteins in the hemolymph of *Haemaphysalis flava* ticks? *Frontiers in Veterinary Science* 11:367 DOI 10.3389/fvets.2024.1387719.
- Lourenço AP, Guidugli-Lazzarini KR, de Freitas NHA, Message D, Bitondi MMG, Simões ZLP, Teixeira ÉW. 2021. Immunity and physiological changes in adult honey bees (*Apis mellifera*) infected with *Nosema ceranae*: the natural colony environment. *Journal of Insect Physiology* 131(3):104237 DOI 10.1016/j.jinsphys.2021.104237.
- MacMillan HA, Sinclair BJ. 2011. Mechanisms underlying insect chill-coma. *Journal of Insect Physiology* 57(1):12–20 DOI 10.1016/j.jinsphys.2010.10.004.
- Mallon EB, Brockmann A, Schmid-Hempel P. 2003. Immune response inhibits associative learning in insects. *Proceedings of the Royal Society B: Biological Sciences* 270(1532):2471–2473 DOI 10.1098/rspb.2003.2456.
- Mayack C, Macherone A, Zaki AG, Filiztekin E, Özkazanc B, Koperly Y, Schick SJ, Eppley EJ, Deb M, Ambiel N, Schafsnitz AM, Broadrup RL. 2022. Environmental exposures associated with honey bee health. *Chemosphere* 286:131948 DOI 10.1016/j.chemosphere.2021.131948.
- Mesa F, Paez-Sierra BA, Romero A, Botero P, Ramírez-Clavijo S. 2021. Assisted laser impedance spectroscopy to probe breast cancer cells. *Journal of Physics D: Applied Physics* 54(7):075401 DOI 10.1088/1361-6463/abc380.
- Miklavčič D, Pavšelj N, Hart FX. 2006. Electric properties of tissues. In: Akay M, ed. *Encyclopedia of Biomedical Engineering*. Hoboken, NJ: John Wiley & Sons, 1–12 DOI 10.1002/9780471740360.ebs0403.
- OCDE 245. 2017. Guideline for the testing of chemical honey bee (*Apis mellifera*). Available at <http://www.oecd.org/termsandconditions/>.
- Pannabecker TL, Andrews F, Beyenbach KW. 1992. A quantitative analysis of the osmolytes in the hemolymph of the larval gypsy moth, *Lymantria dispar*. *Journal of Insect Physiology* 38(11):823–830 DOI 10.1016/0022-1910(92)90092-R.
- Richardson RT, Ballinger MN, Qian F, Christman JW, Johnson RM. 2018. Morphological and functional characterization of honey bee, *Apis mellifera*, hemocyte cell communities. *Apidologie* 49(3):397–410 DOI 10.1007/s13592-018-0566-2.
- Russo GI, Musso N, Romano A, Caruso G, Petralia S, Lanzaò L, Broggi G, Camarda M. 2022. The role of dielectrophoresis for cancer diagnosis and prognosis. *Cancers* 14(1):198 DOI 10.3390/cancers14010198.
- Sasaki K, Porter E, Rashed EA, Farrugia L, Schmid G. 2022. Measurement and image-based estimation of dielectric properties of biological tissues-Past, present, and future-Past. *Physics in Medicine & Biology* 67(14):14TR01 DOI 10.1088/1361-6560/ac7b64.

- Schwan HP, Kay CF. n.d. Capacitive properties of body tissues. Available at <https://www.ahajournals.org/doi/10.1161/01.RES.5.4.439>.
- Shaher KW, Manjy MS. 2020. Degradation of honey bees and environmental pollution: a review. *Plant Archive* 20(1):339–344.
- Smagghe G, Van Leeuwen T. 2004. The hemolymph of caterpillars Spodoptera littoralis: physico-chemical properties and ionic composition compared to culture media. *Communications in Agricultural and Applied Biological Sciences* 69(1):15–22.
- Smolyanskaya OA, Chernomyrdin NV, Konovko AA, Zaytsev KI, Ozheredov IA, Cherkasova OP, Nazarov MM, Guillet JP, Kozlov SA, Kistenev YV, Coutaz JL, Mounaix P, Vaks VL, Son JH, Cheon H, Wallace VP, Feldman Y, Popov I, Yaroslavsky AN, Shkurinov AP, Tuchin VV. 2018. Terahertz biophotonics as a tool for studies of dielectric and spectral properties of biological tissues and liquids. *Progress in Quantum Electronics* 62:1–77 DOI 10.1016/j.pquantelec.2018.10.001.
- Sohn LL, Saleh OA, Facer GR, Beavis AJ, Allan RS, Notterman DA. 2000. Capacitance cytometry: measuring biological cells one by one. *Proceedings of the National Academy of Sciences* 97(20):10687–10690 DOI 10.1073/pnas.200361297.
- Strachecka A, Kuszewska K, Olszewski K, Skowronek P, Grzybek M, Grabowski M, Paleolog J, Woyciechowski M. 2022. Activities of antioxidant and proteolytic systems and biomarkers in the fat body and hemolymph of young Apis mellifera females. *Animals* 12(9):1121 DOI 10.3390/ani12091121.
- Tafi E, Sagona S, Meucci V, Bortolotti L, Galloni M, Bogo G, Gatta D, Casini L, Barberis M, Nepi M, Felicioli A. 2024. Effect of amino acid enriched diets on hemolymph amino acid composition in honey bees. *Archives of Insect Biochemistry and Physiology* 115(1):137 DOI 10.1002/arch.22085.
- Tsai RJ, Aldaoud A, Redoute JM, Garrett DJ, Prawer S, Grayden DB. 2020. Analysis of the capacitance of minimally insulated parallel wires implanted in biological tissue. *Biomedical Microdevices* 22(1):673 DOI 10.1007/s10544-019-0467-9.
- Veil C, Muller D, Walz S, Schule J, Somers P, Tarin C, Stenzl A, Sawodny O. 2023. Enhancing tissue impedance measurements through modeling of fluid flow during viscoelastic relaxation. *IEEE Transactions on Biomedical Engineering* 70(2):650–658 DOI 10.1109/TBME.2022.3199468.
- Woodard SH. 2017. Bumble bee ecophysiology: integrating the changing environment and the organism. *Current Opinion in Insect Science* 22:101–108 DOI 10.1016/j.cois.2017.06.001.
- Wu Y, Hanzae FF, Jiang D, Bayford RH, Demosthenous A. 2021. Electrical impedance tomography for biomedical applications: circuits and systems review. *IEEE Open Journal of Circuits and Systems* 2:380–397 DOI 10.1109/OJCAS.2021.3075302.
- Yao J, Wang L, Liu K, Wu H, Wang H, Huang J, Li J. 2020. Evaluation of electrical characteristics of biological tissue with electrical impedance spectroscopy. *Electrophoresis* 41(16–17):1425–1432 DOI 10.1002/elps.201900420.
- Zimmermann J, Van Rienen U. 2021. Ambiguity in the interpretation of the low-frequency dielectric properties of biological tissues. *Bioelectrochemistry* 140(1):107773 DOI 10.1016/j.bioelechem.2021.107773.

Capítulo 5. El flavonoide rutina protege contra las alteraciones osmóticas y eléctricas inducidas por imidacloprid en abejas africanizadas

Juan P. Hernández, Fredy Mesa, Andre J. Riveros

Publicado en: PLOS One

Published: September 9, 2025

<https://doi.org/10.1371/journal.pone.0331855>

Resumen

Las abejas melíferas (*Apis mellifera*) son polinizadoras esenciales que se encuentran amenazadas por los efectos subletales de pesticidas como el imidacloprid, un neonicotinoide que interfiere con la señalización colinérgica. Sin embargo, sus efectos sistémicos sobre la homeostasis fisiológica aún no se comprenden con claridad. En este estudio evaluamos los efectos de la administración oral de imidacloprid y del flavonol rutina sobre las propiedades del líquido extracelular (LEC) en *Apis mellifera*. Las mediciones realizadas incluyen el contenido de agua, la tasa de evaporación, la impedancia eléctrica y la movilidad iónica. El imidacloprid redujo significativamente el contenido de agua, disminuyó la evaporación y alteró la resistencia y la reactancia eléctricas en el tórax, lo que sugiere una disrupción del equilibrio osmótico y electroquímico. La rutina mitigó estos efectos de manera dosis-dependiente, parcial o totalmente, posiblemente mediante la estabilización de las membranas y la mejora de los procesos de desintoxicación. Nuestros resultados indican que el imidacloprid altera la homeostasis iónica y osmótica más allá de sus blancos neuronales. La rutina podría proteger frente a estas alteraciones mediante mecanismos fisiológicos que van más allá de la neuroprotección. Estos hallazgos abren nuevas posibilidades para proteger a los polinizadores mediante estrategias que incluyen dietas.

Notas aclaratorias:

La atenuación de las secuelas del neonicotinoide observada en los individuos suplementados con rutina se explica por un modelo fisiológico de protección sinérgica multifactorial. El daño inducido por el imidacloprid no se limita exclusivamente a sus blancos neuronales tradicionales, sino que desencadena de manera agresiva una cascada sistémica de estrés oxidativo intracelular.

En este escenario, la rutina actúa, en primera instancia, como un posible agente depurador de radicales libres, lo que mitiga el deterioro tisular. En segunda instancia, es posible que el flavonoide ejerza un rol biofísico estabilizador directo sobre las bicapas lipídicas de las membranas celulares. Dicha estabilización resulta crítica para prevenir la fuga descontrolada de iones y solutos, bloqueando de manera efectiva la disrupción electroquímica y salvaguardando la homeostasis osmótica, hídrica y eléctrica del líquido extracelular frente al desafío tóxico del insecticida.

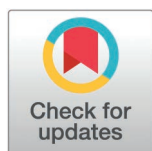
RESEARCH ARTICLE

The flavonoid rutin protects against imidacloprid-induced osmotic and electric disruptions in Africanized honey bees

Juan P. Hernández¹, Fredy Mesa², Andre J. Riveros^{1,3*}

1 Departamento de Biología, Escuela de Ciencias e Ingeniería, Universidad del Rosario, Bogotá, Colombia, **2** Facultad de Ingeniería y Ciencias Básicas, Fundación Universitaria Los Libertadores, Bogotá, Colombia, **3** Department of Neuroscience, College of Science, University of Arizona, Tucson, Arizona, United States of America

* ajosafat@arizona.edu



OPEN ACCESS

Citation: Hernández JP, Mesa F, Riveros AJ (2025) The flavonoid rutin protects against imidacloprid-induced osmotic and electric disruptions in Africanized honey bees. PLoS One 20(9): e0331855. <https://doi.org/10.1371/journal.pone.0331855>

Editor: Yahya Al Naggar, Tanta University Faculty of Science, EGYPT

Received: May 4, 2025

Accepted: August 21, 2025

Published: September 9, 2025

Copyright: © 2025 Hernández et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data availability statement: The data has been provided in a [Supporting information](#).

Funding: This project was founded by the Big Grant IV-FGD003 of the Dirección de Investigación e Innovación (DleI; Universidad del Rosario, Bogotá, Colombia) to AJR.

Abstract

Honey bees (*Apis mellifera*) are essential pollinators threatened by sublethal effects of pesticides such as imidacloprid, a widely used neonicotinoid that disrupts the central nervous system. However, many of the systemic effects are poorly understood, especially on the physiological homeostasis of the honey bee. We evaluated the effects of oral administration of imidacloprid and the flavonol rutin on the properties of extracellular fluid (ECF) in *Apis mellifera*. We measured water content, evaporation rate, electrical impedance, and ion mobility of the ECF. Our results show impacts of imidacloprid consumption, such as water content decrease, slowed evaporation, and altered electrical characteristics of the thorax segment. All these events suggest disruption of osmotic and electrochemical balance. Particularly, the rutin consumption partially mitigated the imidacloprid effects in a dose-dependent manner, enhancing detoxification. Our results point out that imidacloprid alters ionic and osmotic homeostasis beyond neural targets; and on the other hand, rutin may protect against these disruptions through physiological mechanisms beyond neuroprotection. These findings highlight new alternatives and evaluations for protecting pollinators via dietary strategies.

Introduction

Wild and managed bees play a fundamental role in ecosystems, not just as pollinators of wild flora but also as silent workers behind much of the food humans and livestock depend on [1–6]. Yet, in recent decades, their numbers have dropped at an alarming rate [7,8]. This decline does more than reduce pollination, it chips away at biodiversity [9,10] and, in a broader sense, shakes the foundation of global food security [11–13]. Among the many factors behind this crisis, pesticide exposure stands out, particularly in intense monocultures where these chemicals are applied

Competing interests: The authors have declared that no competing interests exist.

almost routinely [6,14–16]. Neonicotinoids, a class of insecticides praised for their broad-spectrum effectiveness [17,18], also cast a long shadow. Imidacloprid, a first-generation neonicotinoid patented 35 years ago [19], remains widely used. While its toxicity is well documented, many of its sublethal effects on bees continue to be a growing concern [20–22].

The sublethal effects of imidacloprid can interfere with a range of biological functions. As a partial agonist of cholinergic nicotinic receptors [23–25], the compound primarily targets neurons in the insect brain [26,27]. This interaction alters sodium channel conductance, which in turn disrupts the delicate balance of the membrane potential [28,29]. It is hardly surprising that bees exposed to the insecticide often show impaired brain functions, where learning and memory seem to suffer [30–32], and so do motor control and navigation [33–36]. For example, studies have reported that even a dose as low as 20ng per bee may trigger tremors and loss of motor coordination, signs that neural signaling is taking a serious hit [33,35,37].

An idea that has been gaining traction to counter the impact of imidacloprid, and similar insecticides, is something researchers call the precision nutrition approach [38–42]. The concept is straightforward: supply bees with specific molecules that may offer prophylactic protection and boost their resilience [43–45]. It turns out that nutrition plays a bigger role than many expect. Diets rich in antioxidants and anti-inflammatory agents appear to help bees keep their physiological systems running under stress [46,47]. Plant-derived secondary metabolites have drawn attention here, as they seem to improve tolerance against toxic exposures [48–50]. Previous studies suggest that rutin can protect cognitive functions, not only in the bumble bee *Bombus impatiens* but also in the Africanized honey bee (*Apis mellifera*) exposed to imidacloprid and even fipronil [51].

The reported protection offered by rutin and similar plant metabolites raises more questions than it answers. What are these compounds really doing at the physiological level? Their action may not be limited to neurons alone. When insecticides disrupt membrane conductance [52,53], the distribution of ions can become skewed, and this imbalance might ripple out beyond local sites to affect systemic electrical conditions. How far this disturbance may extend remains unclear. Ions like Na⁺, K⁺ and Cl[−] are more than just players in neural signaling; they also act as osmolytes, meaning any shift in their conductance can easily spill over into osmotic balance. This raises a pressing question: could these secondary metabolites help restore not only electrical properties but also the osmotic stability of bee tissues?

Thus, we explore these questions by examining how the extracellular fluid (ECF) of the Africanized honey bee (*Apis mellifera*) responds to imidacloprid, rutin, or both. The ECF is an ideal target because it surrounds tissues and internal organs, acting as a dynamic environment where nutrients, gases, and waste are constantly exchanged [54–58]. The composition and volume of this fluid contribute to internal balance by regulating water and solute levels [59–62]. Moreover, specialized organs such as Malpighian tubules and rectal glands regulate extracellular composition by adjusting water and ion excretion or reabsorption [63–67], allowing insects to maintain optimal homeostasis in response to environmental changes [68–72]. Thus,

similar to a vertebrate blood test, analyzing the extracellular fluid may provide insights into changes in the overall physiological state. We deliberately focused on the ECF of the thorax. The abdomen was excluded to avoid the variability introduced by crop contents. The thorax, with its greater rigidity, was better suited for our standardized setup. This configuration allowed us to reliably measure electrical properties while remaining minimally invasive compared to approaches involving the head.

Our goal was fourfold. First, we investigated whether *ad libitum* administration of low and high doses of imidacloprid and rutin would differentially influence osmotic balance, as reflected by changes in water content. Second, we analyzed possible alterations in the colligative properties of the extracellular fluid, focusing on evaporation dynamics. Third, we assessed modifications in resistance and reactance under different treatments with sucrose, rutin, and imidacloprid. Finally, we examined solute electrical mobility to gain insight into the electrical behavior of dissolved ions.

Based on the known toxicodynamics of imidacloprid as a partial agonist of $\text{Na}^+/\text{Ca}^{2+}$ channels and its chemical properties, we expected that the enhanced inward cation movement could lower resistance and reactance in the ECF. However, previous studies suggest that pesticide exposure may also alter ion distribution through mechanisms unrelated to receptor activation, including indirect effects on membrane integrity. While our data do not directly address such processes, they prompted us to consider that imidacloprid could modify the extracellular environment in ways that produce an opposite electrical pattern. Regarding rutin, we anticipated that its presence would mitigate these alterations, yielding values closer to those observed in control bees.

Our findings point to clear dose-dependent effects of both rutin and imidacloprid. What stood out is how their influences seemed to pull in opposite directions across nearly every measured parameter. Bees that consumed imidacloprid tended to show signs of dehydration, a faster evaporation rate, and an increase in electrical resistance. In contrast, those receiving rutin retained more water, evaporated fluid more slowly, and displayed lower resistance values. These patterns appear to support the idea that rutin's protective role may, at least partly, involve mechanisms that help maintain osmotic balance, although other factors could also be at play.

Results

Estimation of dosages

We found that the volume ingested by bees varied depending on the administered treatment (ANOVA: $F_{6,77} = 103.374$; $p < 0.0001$; [Table 1](#)). We found differences in the volume of solutions ingested, with higher values in groups exposed to rutin (0.6Ru, 0.3Ru) compared to those treated with imidacloprid (0.6Im, 0.3Im) or combinations of both compounds (0.6Im+0.6Ru, 0.3Im+0.3Ru). In particular, bees in the 0.6Ru treatment consumed the highest volume (mean $\pm$ s.e.m. = 55.2 ± 6.2 μL /bee; [Table 1](#)), while those in the 0.6Im group exhibited the lowest consumption (mean $\pm$ s.e.m. = 24.2 ± 1.9 μL /bee; [Table 1](#)). The acceptance of the solutions varied depending on the treatment

Table 1. Estimated consumption and doses across all experimental groups.

Treatment	N	Estimated volume (μL) consumed per bee (mean $\pm$ s.e.m.)	Estimated dose (ng) per bee (mean $\pm$ s.e.m.)
0.6Im	415	24.2 ± 1.9	14.5 ± 1.1
0.3Im	415	27.7 ± 2.6	8.3 ± 0.8
0.6Ru	424	55.2 ± 6.2	33.1 ± 3.7
0.3Ru	404	44.6 ± 3.6	13.4 ± 1.1
0.6Im+0.6Ru	401	31 ± 2.1	18.6 ± 1.3
0.3Im+0.3Ru	423	38 ± 4	11.4 ± 1.2
Control	418	41.3 ± 3.4	—

Data is presented as mean $\pm$ s.e.m.

<https://doi.org/10.1371/journal.pone.0331855.t001>

composition, which influenced the effective exposure to each compound. The administered dose, calculated in ng/bee, reflected consumption differences, with the highest concentrations found in groups treated with rutin alone (0.6Ru: 33.1 ± 3.7 ng/bee; Table 1), whereas the lowest values were observed in the high concentration imidacloprid treatment (0.3Im: 8.3 ± 0.8 ng/bee; Table 1).

Evaluation 1: wet and dry mass of thorax

We weighed 100 bees per treatment. We found that the administration of imidacloprid or rutin to bees led to statistically significant dose-dependent and contrasting effects relative to the administration of sucrose in the Control group (ANOVA: $F_{6,693} = 41.10$, $p < 0.0001$; Fig 1A). Bees receiving imidacloprid exhibited significantly lower wet masses (mean $\pm$ s.e.m: $m_{w0.3Im} = 33.4 \pm 2.2$ mg; $m_{w0.6Im} = 32.3 \pm 2.1$ mg) than bees in the Control group (mean $\pm$ s.e.m: $m_{wControl} = 35.16 \pm 2.23$ mg; Control vs. 0.3Im *post hoc* Tukey test: 95%CI: $-2.6, -0.8$ mg, $p < 0.0001$; Control vs. 0.6Im: *post hoc* Tukey test: 95%CI: $-3.8, -1.95$ mg, $p < 0.0001$).

Conversely, bees receiving rutin exhibited wet masses that did not significantly differ from bees in the Control group (mean $\pm$ s.e.m: $m_{w0.3Ru} = 35.1 \pm 2.2$; Control vs. 0.3Ru: *post hoc* Tukey test: 95%CI = $-1.0, 0.9$ mg, $p = 0.98$; Fig 1A) or exhibited significantly higher mass when the rutin concentration was increased (mean $\pm$ s.e.m: $m_{w0.6Ru} = 36.5 \pm 2.3$ mg; Control vs. 0.6Ru: *post hoc* Tukey test: 95%CI = $0.4, 2.3$ mg, $p < 0.0001$; Fig 1A). Interestingly, bees receiving the low dose mixture (0.3Ru + 0.3Im) did not exhibit significant differences in wet mass relative to bees in the Control group (mean $\pm$ s.e.m: $m_{w0.3Ru+0.3Im} = 35.4 \pm 2.3$ mg; Control vs. 0.3Ru + 0.3Im: *post hoc* Tukey test: 95%CI = $-0.7, 1.2$ mg, $p = 0.98$; Fig 1A). However, bees receiving the high dose mixture (0.6Ru + 0.6Im) exhibited significant differences in wet mass relative to bees in the Control group (mean $\pm$ s.e.m: $m_{w0.6Ru+0.6Im} = 33.8 \pm 2.3$ mg; Control vs. 0.6Ru + 0.6Im: *post hoc* Tukey test: 95% CI = $-2.3, -0.44$ mg, $p < 0.0001$; Fig 1A).

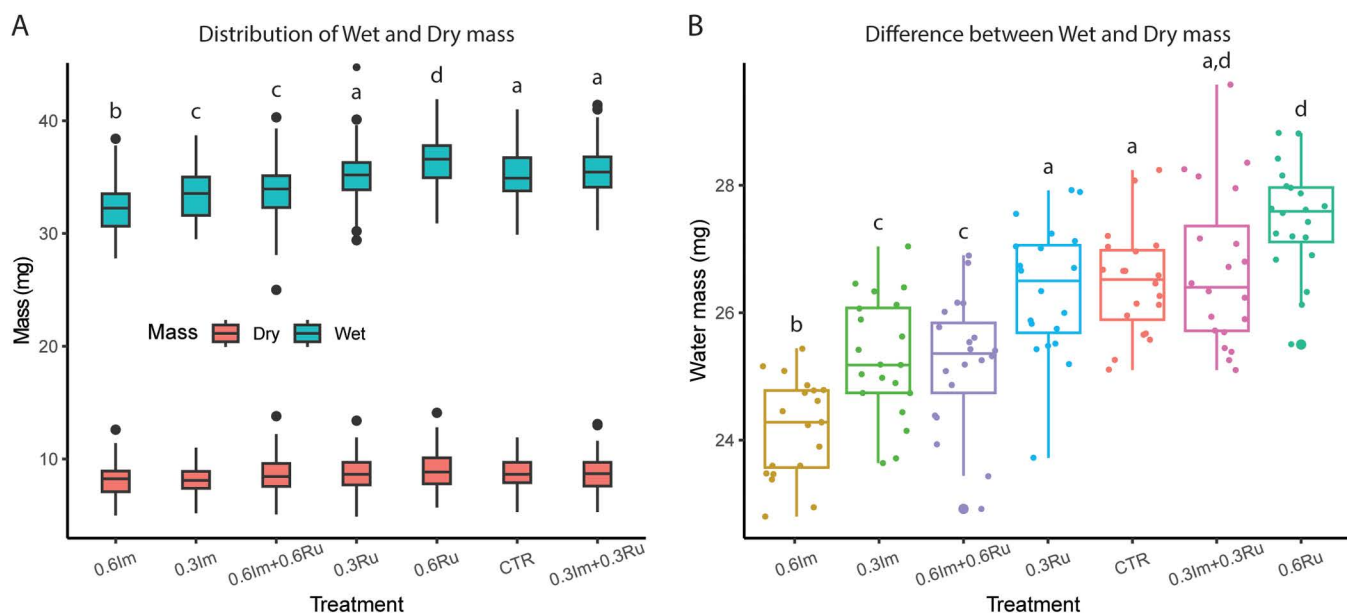


Fig 1. Effect of imidacloprid and rutin administration on wet mass, dry mass, and water content of the thorax in Africanized honey bee foragers. (A) Wet mass (blue bars) and dry mass (red bars) of thoraxes dissected after 24 h of ad libitum feeding on the assigned treatments. (B) Estimated water content calculated as the difference between wet and dry mass. Treatments include control (Ctr), imidacloprid (Im) at two concentrations (0.3 and 0.6 ppm), rutin (Ru) at two concentrations (0.3 and 0.6 ppm), and their combinations. Bars represent mean values $\pm$ SEM. Different letters above bars indicate statistically significant differences among treatments (ANOVA followed by Tukey's HSD, $p < 0.05$).

<https://doi.org/10.1371/journal.pone.0331855.g001>

When assessing water balances ($m_w - m_d$), we also found dose and molecule dependence in the significant differences between the bees exposed to different treatments ($F_{6,133} = 26$, $p < 0.0001$). Bees receiving imidacloprid exhibited decreases at low (Control vs. 0.3Im: *post hoc* Tukey test: 95%CI = -2.1, -0.3 mg, $p < 0.003$; Fig 1B) and high concentrations relative to bees in the Control group (Control vs. 0.6Im: *post hoc* Tukey test: 95%CI = -3.2, -1.4 mg, $p < 0.0001$; Fig 1B). In contrast, bees receiving rutin either maintained (Control vs. 0.3Ru: *post hoc* Tukey test = 95%CI: -1.0, 0.8 mg, $p = 0.98$; Fig 1B) or increased the water content relative to bees in the Control group (Control vs. 0.6Ru: *post hoc* Tukey test = 95%CI: 0.08, 1.9 mg, $p < 0.02$; Fig 1B). Interestingly, bees receiving the mixtures exhibited a dose-dependent response (Control vs. 0.3Ru+0.3Im: *post hoc* Tukey test: 95%CI = -0.7, 1.1 mg, $p = 0.98$; Control vs. 0.6Ru+0.6Im: *post hoc* Tukey test: 95%CI = -2.2, -0.3 mg, $p < 0.0001$; Fig 1B).

Evaluation 2: extracellular fluid evaporation

We collected and processed 100 bees per treatment. We found that the evaporation curves followed an exponential trend that varied across treatments. Bees receiving only sucrose (Control) had an ECF with an evaporation trend that closely fit an exponential model with a low evaporation constant [$k = (-16.26 \pm 0.25) \times 10^{-3}$; $R^2 = 10.97$]. Importantly, the administration of imidacloprid led to a dose-dependent decrease in the evaporation rate (0.3Im: $k = (-5.73 \pm 0.29) \times 10^{-3}$; 0.6Im: $k = (-7.03 \pm 0.15) \times 10^{-3}$). In contrast, when administered with rutin, ECF of bees exhibited evaporation rates that more closely resembled those of bees in the Control group (0.3Ru: $k = (-10.00 \pm 0.29) \times 10^{-3}$; 0.6Ru: $k = (-13.82 \pm 0.28) \times 10^{-3}$). Finally, when bees received the combination, their ECF exhibited intermediate values (0.3Ru+0.3Im: $k = (-7.88 \pm 0.31) \times 10^{-3}$; 0.6Ru+0.6Im: $k = (-9.58 \pm 0.26) \times 10^{-3}$; Fig 2A).

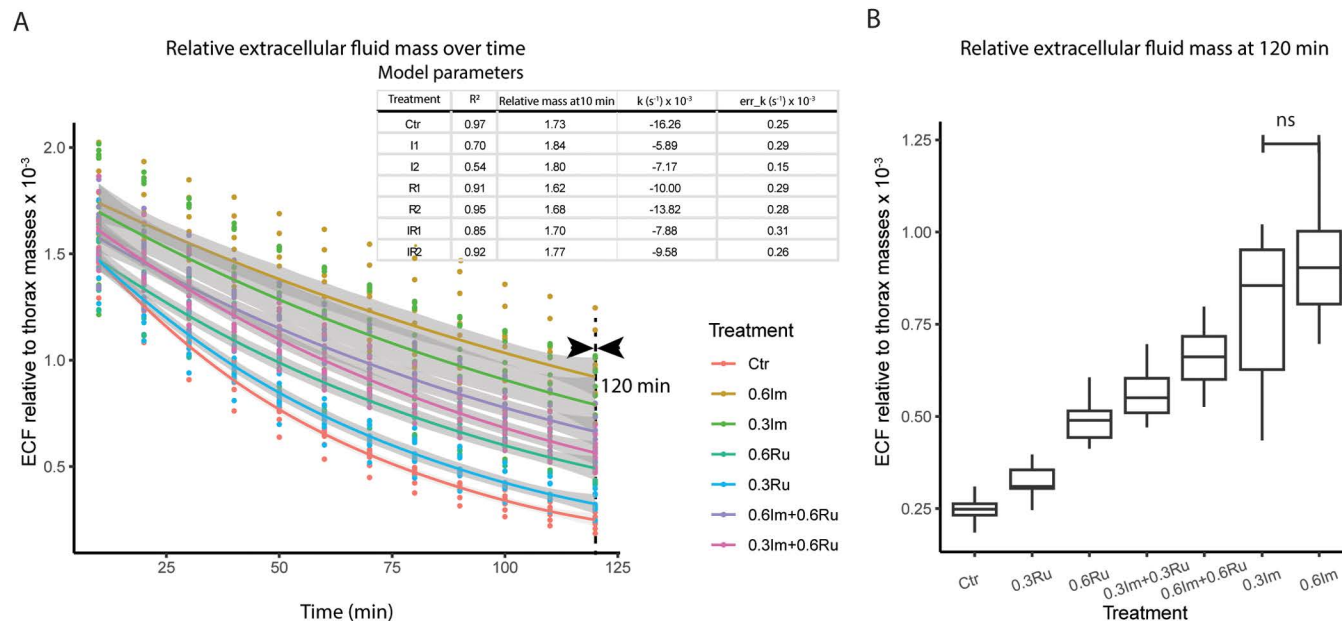


Fig 2. Effect of imidacloprid (Im) and rutin (Ru) administration on evaporation dynamics of extracellular fluid (ECF) extracted from thoraxes of Africanized honey bee foragers. (A) Evaporation curves of ECF showing mass loss (relative mass, ECF mass/ pooled thorax mass) over time (minutes) at 35°C for each treatment. The upper panel summarizes the fitted model parameters, including correlation coefficients (R^2), relative mass, and evaporation constant (k). (B) Final ECF evaporation measured at 120 minutes. Treatments include control (Ctr), imidacloprid (Im) at 0.3 and 0.6 ppm, rutin (Ru) at 0.3 and 0.6 ppm, and their combinations. Lines represent mean values, and shaded areas denote SEM. Different letters above bars in panel B indicate statistically significant differences among treatments (Kruskal–Wallis test, $p < 0.05$).

<https://doi.org/10.1371/journal.pone.0331855.g002>

Statistical comparisons between treatments at minute 120 further supported the pattern observed in the mass of ECF samples relative to the thorax mass from which they were extracted (Kruskal-Wallis: 120 min: $\chi^2_6 = 58.05$, $p < 0.0001$; Fig 2B). In all cases, the administration of imidacloprid, rutin, or their combinations resulted in a lower evaporation rate. The ECF of bees exposed to imidacloprid remained in the highest quantity at the end of the 120-minute period (mean $\pm$ SD: 0.6Im=0.92 $\pm$ 0.18; 0.3Im=0.79 $\pm$ 0.22). Similarly, rutin consumption led to fluid retention levels higher than those observed in the Control group (mean $\pm$ SD: Control=0.24 $\pm$ 0.04; 0.6Ru=0.49 $\pm$ 0.06; 0.3Ru=0.32 $\pm$ 0.04).

Evaluation 3: electrical impedance analysis

We collected, prepared, and processed 50 bees per treatment. The impedance exhibited by the bees showed both capacitive and resistive behavior, as evidenced in the Nyquist plots. The electrical properties of the thorax ECF were influenced by the presence of both charged and uncharged molecules; charged species contributed to ionic conduction and uncharged species affected polarization effects. Charged molecules exhibited mobility due to the applied potential difference, contributing to ionic conduction, whereas uncharged molecules underwent polarization due to the same potential, affecting capacitive behavior. The Nyquist plots revealed semicircular impedance behavior, where the resistive component reflects ion mobility in the extracellular fluid, and the capacitive component represents charge storage at membrane interfaces (Fig 3A).

Thorax reactance, measured at resistance values between 18k Ω and 32k Ω , exhibited significant differences across treatments ($F_{6,168} = 107.2$, $p < 0.0001$; Fig 3B). In the bees within the Control group, the mean reactance in this resistance range was 16.9 $\pm$ 0.5k Ω , whereas lower values were observed in all treated groups. Bees exposed to high-concentration of imidacloprid (0.6Im) exhibited a 2.4k Ω reduction in reactance relative to bees in the Control group (*post hoc* Tukey test=95%CI: -2.8, -2.1k Ω , $p < 0.0001$; Fig 3B), while the exposure to low-concentration (0.3Im) reduced reactance by 2.0k Ω (*post hoc* Tukey test=95%CI: -2.4, -1.7k Ω , $p < 0.0001$; Fig 3B). In contrast, bees administered with the low concentration of rutin (0.3Ru) did not exhibit significant changes in reactance relative to bees in the Control group (*post*

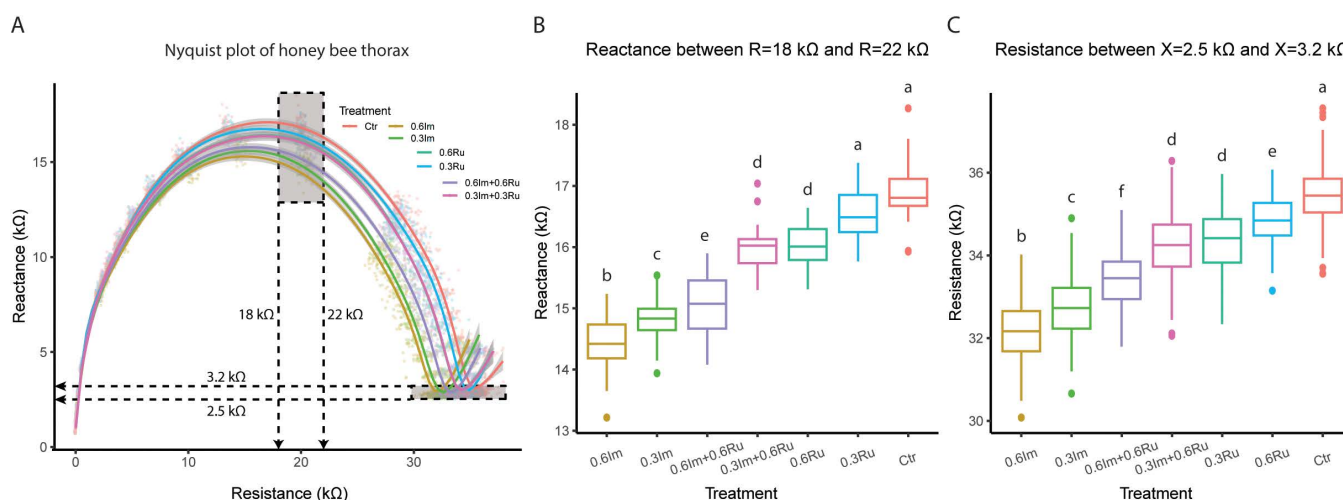


Fig 3. Effect of imidacloprid (Im) and rutin (Ru) administration on thoracic electrical impedance in Africanized honey bee foragers. (A) Nyquist plots showing reactance (k Ω , imaginary component) as a function of resistance (k Ω , real component) for the thorax under different treatments. Curves illustrate treatment-dependent variations in electrical properties, where a rightward shift reflects increased extracellular resistance and larger semicircle radii indicate higher tissue capacitance. (B) Comparison of resistance values within the 18–22 k Ω range, highlighting differences in reactance among treatments. (C) Comparison of resistance in the 2.5–3 k Ω range, showing variations in electrical resistance. Bars represent mean $\pm$ SEM, and different letters above treatments indicate statistically significant differences (ANOVA followed by Tukey's HSD, $p < 0.05$).

<https://doi.org/10.1371/journal.pone.0331855.g003>

hoc Tukey test=95%CI: -0.3, 0.7k Ω , $p=0.06$; Fig 3B). Nevertheless, bees receiving high concentration rutin (0.6Ru) decreased their reactance by 0.8k Ω (*post hoc* Tukey test=95%CI: -1.1, -0.8k Ω , $p<0.0001$; Fig 3B). The administration of the mixture of imidacloprid and rutin resulted in intermediate effects: the high-dose composition (0.6Ru + 0.6Im) reduced reactance by 1.8k Ω (95%CI: -2.1, -1.4k Ω , $p<0.0001$; Fig 3B), while the low-dose composition decreased by only 0.8k Ω (95%CI: -1.2, -0.7k Ω , $p<0.0001$; Fig 3B).

Thorax resistance, measured at reactance values between 2.5k Ω and 3.2k Ω , also exhibited significant treatment-dependent differences (ANOVA: $F_{6,1510}=627.7$, $p<0.0001$; Fig 3C). Bees in the Control group had a mean resistance of 35.5 ± 0.7 k Ω (mean $\pm$ s.e.m), while lower values were observed in all treated groups. Bees exposed to high-concentration imidacloprid (0.6Im) exhibited a 3.3k Ω decrease in resistance (*post hoc* Tukey test=95%CI: -3.5, -3.1k Ω , $p<0.0001$; Fig 3C), whereas the exposure to low-concentration (0.3Im) reduced resistance by 2.7k Ω (*post hoc* Tukey test=95%CI: -3.0, -2.6k Ω , $p<0.0001$; Fig 3C). The administration of a low-concentration of rutin (0.3Ru) led to a decreased resistance by 1.1k Ω compared to the bees in the Control group (*post hoc* Tukey test=95%CI: -1.3, -0.9 k Ω , $p<0.0001$; Fig 3C), while the administration of high-concentration rutin (0.6Ru) reduced resistance by 0.6k Ω (*post hoc* Tukey test=95%CI: -0.8, -0.4 k Ω , $p<0.0001$; Fig 3C). Similar to the effects on reactance, the administration of the compositions of imidacloprid and rutin produced intermediate effects: the high-dose combination (0.6Ru + 0.6Im) reduced the resistance by 2.0k Ω (*post hoc* Tukey test=95%CI: -2.2, -1.8k Ω , $p<0.0001$; Fig 3C), while the administration of the low-dose composition (0.3Ru + 0.3Im) resulted in a 1.2k Ω reduction (*post hoc* Tukey test=95%CI: -1.3, -1.1 k Ω , $p<0.0001$; Fig 3C).

Evaluation 4: current-voltage (I-V) curves

We collected, prepared, and processed 50 bees per treatment to evaluate the I-V characteristics of the thorax extracellular fluid. The electrical response exhibited both resistive and non-ohmic behavior, confirming the presence of charge transporting mechanisms influenced by ionic mobility and interface polarization. The applied potential generated a nonlinear I-V relationship, where charged species in the extracellular fluid contributed to current flow due to electrophoretic forces, while uncharged molecules exhibited capacitive effects due to dielectric polarization. The asymmetric shape of the I-V curves confirmed that charge redistribution and membrane-associated electrochemical interactions played a role in the electrical response (Fig 4A).

The electrical current exhibited significant differences among treatments in the thorax's electrical response under ascending and descending voltages. The recorded currents increased or decreased according to the applied potential, displaying non-ohmic behavior. The current across all treatments was different from zero at zero applied voltage, indicating additional electrochemical components in the system, such as polarization effects or capacitive contributions from the extracellular fluid. This deviation from pure ohmic behavior suggests that electrical conduction is governed both by the fluid's resistive properties and charge accumulation at electrode interfaces (Fig 4A). Within the voltage range of -30mV to 30mV, absolute current values exhibited similar trends for both ascending and descending voltage conditions, indicating that charge mobility was influenced by the applied electric field orientation. The current measured through the thorax in this voltage range showed significant treatment-dependent differences for both increasing (ANOVA: $F_{6,1001}=471.16$, $p<0.0001$) and decreasing (ANOVA: $F_{6,1001}=412.03$, $p<0.0001$) voltages.

Bees that consumed imidacloprid exhibited the highest currents, indicating a greater presence of mobile ions in the extracellular fluid (0.6Im vs. Control: *post hoc* Tukey test, 95%CI: 0.55, 0.63 μ A, $p<0.0001$; 0.3Im vs. Control: *post hoc* Tukey test, 95%CI: 0.43, 0.39 μ A, $p<0.0001$). The consumption of rutin also increased electrical currents in the bees compared to the bees in the Control group, but to a lesser extent than imidacloprid (0.6Ru vs. Control: *post hoc* Tukey test, 95%CI: 0.30, 0.26 μ A, $p<0.0001$; 0.3Ru vs. Control: *post hoc* Tukey test, 95%CI: 0.21, 0.17 μ A, $p<0.0001$). A remarkable observation was that when bees consumed 0.3Im + 0.3Ru, the values of currents were closer to those exhibited by the bees within the Control group (0.3Im + 0.3Ru vs. Control: *post hoc* Tukey test, 95%CI: 0.06, 0.02 μ A, $p<0.0001$; Fig 4B).

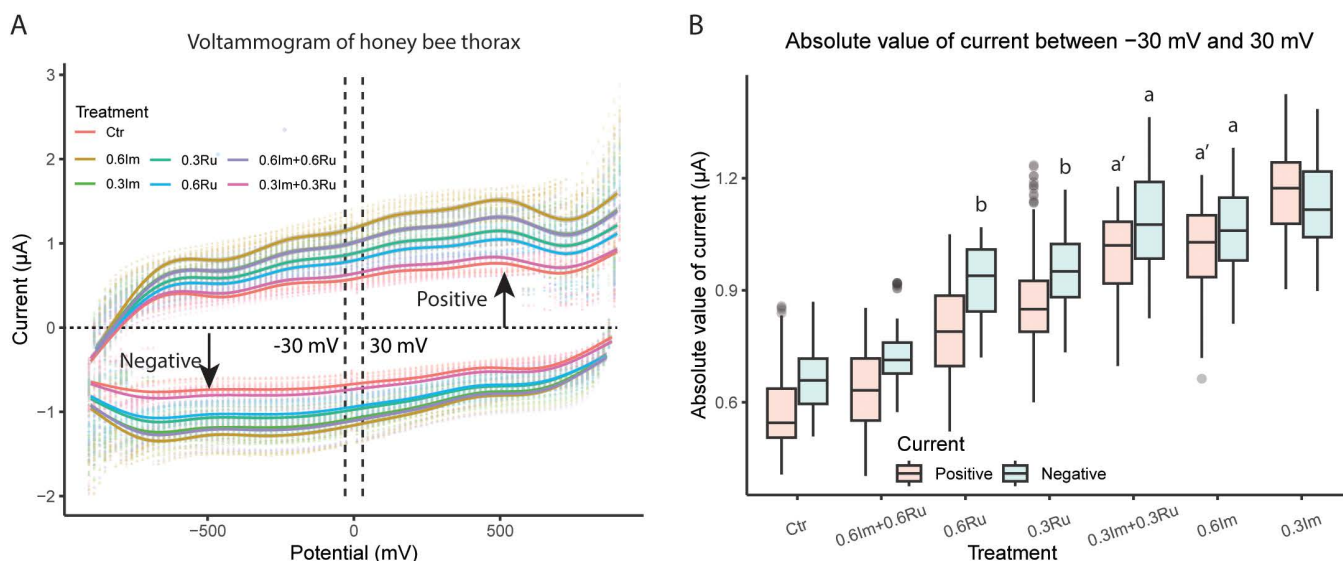


Fig 4. Effect of imidacloprid (Im) and rutin (Ru) administration on thoracic electric currents in Africanized honey bee foragers. (A) Voltammograms showing the current response as a function of applied potential (−800 to 800 mV) for each treatment. The slope of the I–V curve reflects ionic conductivity, while deviations from linearity indicate capacitive contributions of thoracic tissue. (B) Absolute values of current recorded within the −30 to 30 mV range, where both positive and negative components of current were quantified separately to compare treatments. Treatments include control (Ctr), imidacloprid (Im) at 0.3 and 0.6 ppm, rutin (Ru) at 0.3 and 0.6 ppm, and their combinations. Bars represent mean $\pm$ SEM, and different letters above treatments indicate statistically significant differences (ANOVA followed by Tukey's HSD, $p < 0.05$). Only significant comparisons are displayed to improve visualization.

<https://doi.org/10.1371/journal.pone.0331855.g004>

Discussion

The impairments caused by pesticide and pollutant exposure remain a major concern for the pollination services provided by bees. Insecticides such as neonicotinoids target neural receptors and have been associated with sublethal impairments in cognitive functions, including learning, memory, navigation, and motor control. However, other systemic effects are still not fully understood. Here, we aimed to determine whether the oral administration of imidacloprid could alter the electrochemical properties of the extracellular fluid (ECF). Additionally, we tested whether rutin, a flavonol known for its potential protective effects against insecticides, might mitigate the impact of imidacloprid. Our results indicate that, relative to control conditions, imidacloprid exposure generally caused dose-dependent decreases in water content, ECF evaporation rate, electrical reactance, and resistance, while increasing electrical currents. In contrast, rutin administration, either alone or in combination with imidacloprid, resulted in values closer to those observed in control bees. These findings suggest three main aspects: (i) a substantial alteration in the extracellular electrochemical environment caused by imidacloprid, (ii) dose-dependent changes in response to imidacloprid and/or rutin likely due to their chemical properties and associated physiological actions, and (iii) partial amelioration of imidacloprid's impact when co-administered with rutin.

First, our results indicate that imidacloprid-induced ECF alterations may extend beyond receptor activation. Like other neonicotinoids, imidacloprid is known to affect membrane ion conductance. As a partial agonist of nicotinic acetylcholine receptors (nAChR), it is expected to enhance inward cationic currents (Na^+ , Ca^{2+}) [73,74], which theoretically could increase ECF resistance. However, we observed a decrease in both resistance and reactance, along with higher electrical currents, which may suggest an increase in free charged particles within the ECF. This pattern is consistent with the possibility of membrane leakage or damage caused by the 24 h ad libitum exposure to the insecticide. Imidacloprid is known to induce oxidative stress, leading to the production of reactive oxygen and nitrogen species (ROS and RNS, respectively) [75–77]. Evidence from zebrafish [78], earthworms [79], mice [80] and rats [81,82] indicates that physiological responses

to ROS and RNS can enhance lipid peroxidation, as reflected by elevated levels of malondialdehyde and thiobarbituric acid-reactive substances, both major byproducts of lipid oxidation. It is plausible that oxidative damage destabilizes membrane lipids, increasing ion leakage and thereby contributing to the reduction in resistance and reactance, as well as to the observed increase in current. Such disruption could impair muscle and nerve function, potentially affecting motor coordination and responsiveness.

Second, we observed a non-linear dose-dependent effect caused by the administration of imidacloprid (0.3Im vs. 0.6Im), rutin (0.3Ru vs. 0.6Ru), and their combination (0.3Im + 0.3Ru vs. 0.6Im + 0.6Ru). This non-linearity could be partly attributed to variations in self-administered doses. Doubling the concentration of imidacloprid led to an approximate 9% decrease in liquid consumption, resulting in only a 0.75-fold increase in the administered dose ([Table 1](#)). In contrast, doubling the rutin concentration increased ingestion by around 21%, leading to a 2.5-fold increase in dose. Interestingly, the combined administration of imidacloprid and rutin resulted in intermediate consumption behavior, with a 1.6-fold increase in dose. These patterns may arise from non-mutually exclusive mechanisms. Imidacloprid might induce a satiation-like effect, explaining the ~42% reduction in consumption relative to control bees ([Table 1](#)), whereas rutin could have the opposite influence, consistent with the ~34% increase in ingestion observed. Alternatively, imidacloprid may impair crop emptying, mechanically restricting further intake, while rutin could enhance thirst or reduce satiety signals.

Despite these differences in consumption, the physiological effects observed were consistent with dose-dependent responses. The reduction in water content caused by imidacloprid increased from ~5% (0.3Im) to ~8% (0.6Im) relative to control bees. Conversely, rutin administration led to greater water retention, increasing from ~0% (0.3Ru) to 4% (0.6Ru) above control levels. These differences are unlikely to be solely explained by ingestion variations, as the changes in water content did not scale directly with consumption. Instead, they may reflect intrinsic properties of rutin as an osmolyte, its potential to stabilize membranes [82,83], or the upregulation of aquaporins, a mechanism previously demonstrated in rats [84]. Dose-dependent effects were also evident in ECF evaporation rates ([Fig 2](#)). Imidacloprid significantly reduced extracellular fluid evaporation compared to the control group, which may indicate compositional changes that increase viscosity or solute concentration. From an ecological perspective, disturbances in water balance could impair thermoregulation, potentially making bees more vulnerable to dehydration and adverse climatic conditions [85–87].

Third, we observed that the co-administration of imidacloprid and rutin generally mitigated several of the negative effects associated with imidacloprid. For example, bees receiving 0.6Im showed an approximate 8% reduction in water content relative to controls, while the addition of 0.6Ru (0.6Im + 0.6Ru) appeared to reduce this loss by about 50% ([Table 1](#)). Likewise, administration of 0.3Im alone resulted in a ~5% reduction in water content, whereas the combination with 0.3Ru (0.3Im + 0.3Ru) prevented this decrease almost entirely. Similar patterns of amelioration were detected in ECF evaporation rates ([Fig 2](#)). These protective effects may be linked to rutin's influence on osmoregulatory processes, possibly through modulation of Malpighian tubule activity [67,70], which is known to play a central role in insect excretion and water balance [68]. The enhanced ECF evaporation rate observed in rutin-treated groups could indicate that this flavonoid helps stabilize hemolymph composition while supporting water retention.

Additionally, the concurrent administration of rutin attenuated the imidacloprid-induced changes in resistance, reactance, and electrical current. These observations are consistent with the hypothesis that rutin contributes to membrane stabilization and improved ion regulation [87–90]. Competitive or allosteric interactions between rutin and imidacloprid at receptor level may also play a role, although further studies are needed to confirm this mechanism. Moreover, rutin, similar to other flavonoids, may influence detoxification pathways, such as the cytochrome P450 enzyme family [44,90], potentially enhancing insecticide metabolism. While these possibilities remain speculative, they provide plausible explanations for the observed protective effects. Importantly, previous studies have primarily evaluated prophylactic administration of rutin [51,91], whereas our results suggest that protection may also occur during concurrent exposure, highlighting rutin's potential as a mitigating agent under realistic exposure scenarios ([Fig 5](#)).

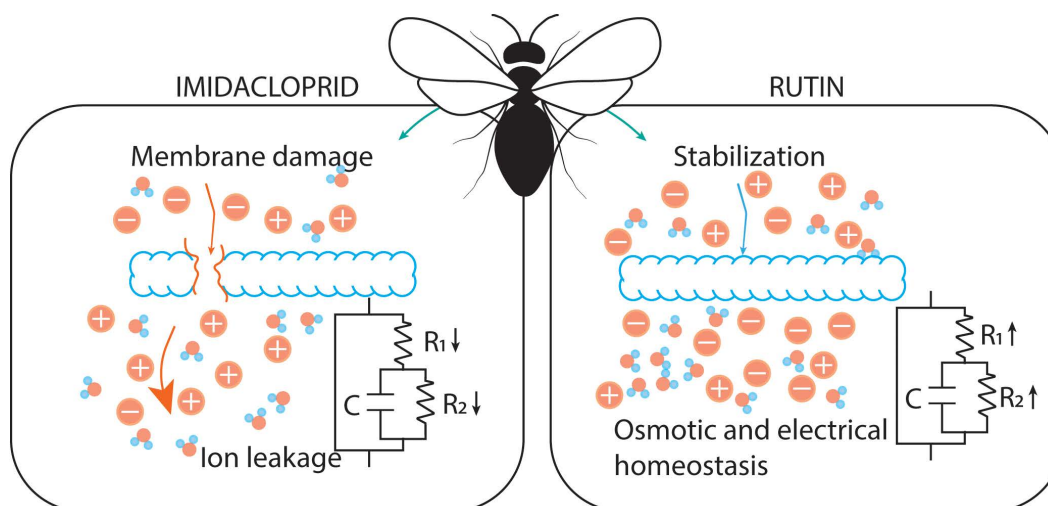


Fig 5. Diagram summarizing the physiological effects of imidacloprid and rutin on extracellular ionic and osmotic balance in Africanized honey bees (*Apis mellifera*). The diagram illustrates how imidacloprid exposure may destabilize membrane integrity, leading to ion leakage, reduced electrical resistance and reactance, and decreased water content in thoracic tissues. These alterations potentially impair motor and neural functions by disrupting osmotic and electrical homeostasis. In contrast, rutin administration is depicted as contributing to membrane stabilization, improved ion regulation, and enhanced water retention, which may counteract the negative effects of imidacloprid. Together, these pathways highlight the opposing physiological actions of the two compounds on bee extracellular fluid properties.

<https://doi.org/10.1371/journal.pone.0331855.g005>

Despite its protective effects, rutin alone also induced some dose-dependent physiological alterations relative to control bees. Higher rutin dosage (0.6Ru) was associated with increased water retention, reduced ECF evaporation rates (0.3Ru, 0.6Ru), decreased resistance (0.3Ru, 0.6Ru), decreased reactance (0.6Ru), and increased electrical currents (0.3Ru, 0.6Ru). Although no behavioral or physiological impairments were detected in bees receiving rutin in our previous or current experiments, it is important to consider that rutin is a plant-derived secondary metabolite originally evolved to deter herbivores and insects [92–94]. Therefore, some degree of physiological stress may be expected as part of its action.

We have previously proposed that rutin's protective effects could involve hormetic responses [51,91], where low levels of stress trigger adaptive mechanisms that enhance resistance to insecticides. The present results are consistent with this idea, as the physiological changes induced by rutin alone did not appear detrimental and may instead prime bees to better cope with imidacloprid exposure. However, confirming this mechanism will require future experiments explicitly designed to test hormesis and its relationship to ion regulation and membrane stability.

Even though our experiments point to clear patterns in how imidacloprid disrupts bee physiology and how rutin might mitigate those effects, there are things we need to keep in mind. For one, the work was done in a lab with bees from just a few colonies, so it probably doesn't capture the messy reality of field conditions where many stressors act at once. Also, we only looked at the thorax and its extracellular fluid, meaning other tissues and systemic effects remain a question mark. When it comes to the electrical measurements, they gave us a new perspective, but part of what we infer still needs biochemical or molecular backing to be sure. And lastly, our tests with rutin were under controlled co-exposure; how this plays out when bees forage freely in the wild is still an open question. These gaps are not deal breakers; instead, they set the stage for the next steps, where field work and deeper analyses could reveal how far these findings truly extend.

Our findings suggest that imidacloprid disrupts water homeostasis and the electrochemical balance in Africanized honey bees, likely compromising tissue function and extracellular fluid stability. Such alterations may reduce resilience to environmental stressors, impair foraging efficiency, and interfere with navigation. Co-administration of rutin appeared to mitigate several of these negative effects, pointing to its potential as a protective compound under pesticide exposure.

From an ecological perspective, these results open the possibility that dietary flavonoids could enhance pollinator resilience, although field validations are still needed to confirm this protective role under natural conditions.

These observations also emphasize the importance of developing mitigation strategies to safeguard pollinators from pesticide-induced harm, while reinforcing the need for tighter regulations to minimize sublethal stress in bees. Beyond their applied implications, the physiological principles explored here, including ion regulation, osmotic balance, and electrical conductance, are broadly conserved across insect taxa. Therefore, the experimental framework presented in this study may be adapted to other species, such as *Drosophila melanogaster* or *Bombus terrestris*, enabling comparative analyses of systemic responses to environmental toxins. Ultimately, this approach offers a promising avenue for advancing insect physiology and ecotoxicology research while highlighting the value of electrical and osmotic markers as early indicators of stress in pollinators.

Materials and methods

Experimental animals and conditions

We collected adult foragers of the Africanized honey bee *Apis mellifera* from hives located at the apiary of the Universidad del Rosario (Bogotá, Colombia). The bees were captured in disposable plastic cups (9 oz) equipped with feeders containing a 1M sucrose solution. Immediately after capture, the bees were transported to the laboratory and maintained for 24h in an incubator ($T=35^{\circ}\text{C}$; $\text{RH}=55\%$). At the end of this acclimation period, the bees were administered according to the assigned experiment with the insecticide imidacloprid, the flavonol rutin, or their combination (see below). In all cases, the compositions were dissolved in a 1M sucrose solution to encourage consumption.

This research did not require approval from an institutional animal care committee, as honey bees are invertebrates and are not subject to animal experimentation regulations in Colombia. Nevertheless, all handling and experimental procedures were conducted according to international guidelines to minimize stress and ensure the welfare of the bees.

Imidacloprid and rutin dosage

We relied on a commercial form of imidacloprid (Confidor350, Bayer), aiming to test realistic conditions experienced by bees in crops. The imidacloprid concentrations were prepared to maintain sublethal concentrations based on reported LD_{50} data (the dose at which 50% of a test population is lethally affected). This concentration exceeded the reported in pollen (0.001 ppm; Jiang et al., 2018) or nectar (0.065 ppm; Jiang et al., 2018) in the field, but served as a critical challenge to test hypotheses of protection by the administration of rutin. Thus, we used two concentrations of the insecticide: 0.3 ppm (0.3Im), 0.6 ppm (0.6Im).

Rutin concentrations were prepared in the same magnitude order as imidacloprid, ensuring proportionality and functionality. Defining realistic concentrations is challenging because of the broad variation of rutin found in nectar and pollen (Guffa et al, 2017; Gullón et al. 2017). However, we have previously shown effectiveness of cognitive protection in bumble bees and honey bees using concentrations of 0.6 ppm [44,51]. Thus, we prepared two concentrations of rutin: 0.3 ppm (0.3Ru), 0.6 ppm (0.6Ru). Following these concentrations and the mixtures, groups of bees were randomly assigned to one of seven treatments: Control (1M sucrose water), 0.3Rut, 0.6Ru, 0.3Im, 0.6Im, 0.3Ru + 0.3Im, 0.6Ru + 0.6Im. The bees were maintained in the incubator (see conditions above) and allowed to drink the assigned composition *ad libitum* for 24h. Then, the bees were allocated to one of four evaluations.

Each treatment group consisted of 100 bees for wet and dry mass measurements and 50 bees for current-voltage (I–V) characterization. These sample sizes were determined based on the need to minimize variability while ensuring reliable measurements with our experimental setup. For the electrical evaluations, preliminary tests indicated that using at least three bees per measurement was necessary to obtain a detectable signal, and replicating measurements with multiple batches increased robustness. Although no previous studies have used this exact methodology, the chosen sample sizes provided sufficient data to identify differences between treatments.

All solutions were prepared fresh daily to prevent degradation. Rutin was dissolved directly in 1 M sucrose by gentle stirring until fully dissolved. Both rutin and imidacloprid solutions were stored at 4°C during short intervals of the experimental period and protected from light to minimize chemical breakdown.

Evaluation 1: wet and dry mass of thorax

Following the exposure period, batches of 20 bees per treatment were sacrificed by freezing at −20°C for 24h. This extended freezing period minimized evaporation upon removal from freezer. Then, the bees were dissected to obtain the thorax. The wet mass of each thorax was measured using a precision scale (0.1 mg precision; Radwag AS 220. R2). The thoraxes were then placed in a drying oven at 70°C for 48h. After this period, the dry mass was recorded using the same scale. The difference between wet (m_w) and dry mass (m_d) was used to estimate body water content (water content = $m_w - m_d$).

Evaluation 2: extracellular fluid evaporation

This evaluation aimed to identify changes in colligative properties of the extracellular fluid (ECF). After the exposure period, batches of 10 bees per treatment were sacrificed by freezing at −20°C for 24h. The bees were then removed from the freezer, and their thoraxes were dissected and weighted as pools according to treatment. The thoraxes were placed in pipette tips with filters to extract extracellular fluid by centrifugation (3000RCF, 5 min, 4°C). Samples of 0.5 mL of extracellular fluid were deposited into cell culture wells, and the initial mass of the wells was recorded (m_0). The ECF samples were subjected to evaporation at 35°C for 2h, with mass (m) measurements recorded every 10 min. This process allowed characterization of evaporation properties for each treatment over time, with evaporation rates reflecting solute concentrations ($\text{mass} = m - m_0$). The centrifugation procedure typically yielded between 0.4 and 0.6 mL of extracellular fluid per 10 thoraxes, consistent with our previously published extraction protocol [95].

Evaluation 3: electrical impedance analysis

Electrical impedance (Z) describes the opposition of a biological sample to alternating current flow when a voltage difference is applied. Here, a parallel resistive-capacitive (RC) model was used, suitable for representing heterogeneous biological tissues such as the bee thorax, where extracellular fluid with high ion concentrations acts as a resistive conductor, while cell membranes function as capacitive elements due to their lipid bilayer structure. Mathematically, impedance is expressed as:

$$Z = \frac{R}{1 + R^2\omega^2C^2} - \frac{R^2\omega C}{1 + R^2\omega^2C^2}j$$

where R is the resistance of the extracellular fluid, and C is the capacitance from the cell membranes. Impedance (Z) consists of a real component (R), representing ionic conduction resistance in extracellular fluid, and an imaginary component ($-X$), representing capacitive reactance due to charge storage in cell membranes.

For our evaluation, we characterized the electrical properties of live bees connected to a capacitor and a resistor. Electrical measurements were performed using batches of three bees *per* treatment simultaneously to increase the electrical signal. Measurements were conducted using two stainless steel electrodes made of hypodermic needles (diameter = 0.18 mm) inserted into the thorax and arranged in parallel. This configuration was connected in series with a 224 nF ceramic capacitor and a 2200 Ω resistor. Measurements were performed using an LCR meter (Keysight E4980AL), configured to measure resistance and reactance with an excitation potential of 300 mV. The LCR meter was controlled using the Command Expert software from Excel to ensure measurement accuracy. The results were represented in Nyquist diagrams, plotting reactance versus resistance. A larger semicircle radius indicated higher tissue capacitance, while a

rightward shift suggested increased extracellular fluid resistance, providing insights into changes in ionic composition and tissue structure under different treatments. The experiment was repeated five times. The LCR meter was calibrated using the manufacturer's standard calibration procedure before each set of measurements to ensure accuracy and consistency.

Evaluation 4: current-voltage (I-V) curves

Current (I)-voltage (V) curves (hereafter I-V curves) characterize biological samples by analyzing the current response to an applied voltage difference. In this study, an electrical model was applied to describe the bioelectrical behavior of the bee thorax, where the measured current is the sum of two main components:

$$I = C \frac{dV}{dt} + \frac{V}{R}$$

The first term, $C \frac{dV}{dt}$, represents capacitive current due to charge accumulation on cell membranes acting as natural capacitors. The second term, V/R , corresponds to resistive current from ionic conduction through extracellular fluid, particularly hemo-lymph rich in ionic solutes. Voltage was applied within a range of -800mV to 800mV at a 0.18mV/s rate, selected to evaluate physiological electrical behavior without inducing electroporation damage. The current-voltage relationship was represented in voltammograms, with voltage on the x-axis and current on the y-axis. Variations in ionic conductivity and tissue capacitance provided insights into solute composition and mobility in the thorax of bees exposed to different experimental treatments.

Electrical conductivity of the thorax was evaluated using I-V curves in live bees to assess the presence of electrically mobile solutes. Measurements were performed on three bees with two stainless steel electrodes inserted in the thorax (see Evaluation 3 for details). The bees were connected in series with a 2200 Ω resistor. Measurements were performed using a source measurement unit (Keithley 2400 Graphical Series SMU). The applied voltage ranged from -800mV to 800mV, with a change rate of 0.18mV/s. Data were filtered within the -30mV to 30mV range to compare absolute currents obtained across treatments. The full procedure for bee immobilization, electrode insertion, and electrical connection is shown in Supplementary Videos S1–S7 in [S3 File](#), which detail each step of the preparation and measurement process. The source measurement unit (Keithley 2400) was calibrated according to the manufacturer's specifications before each experimental session to minimize instrumental drift.

Statistical analysis

The data obtained were analyzed using parametric and non-parametric tests depending on the distribution and sample size. For data that met the assumptions of normality and homogeneity of variances, an analysis of variance (ANOVA) was performed, followed by Tukey's *post-hoc* tests to identify significant differences between experimental groups. When data did not meet these criteria or when sample sizes were small ($n \leq 35$), non-parametric tests such as the Kruskal-Wallis test were applied, followed by pairwise Wilcoxon tests for *post-hoc* comparisons. A significance level of $p < 0.05$ was considered in all statistical analyses. Data processing, statistical analyses, and figure generation were performed using R statistical software (v.4.1.2). Data were imported and cleaned using the tidyverse package; statistical tests such as ANOVA, Tukey's HSD, and Kruskal–Wallis were conducted using rstatix, and all plots were generated with ggplot2 and ggh4x. A detailed and reproducible R Markdown script, including all analysis steps and figure export commands, is provided as Supplementary [S2 File](#).

Supporting information

S1 File. Supplementary File S1 contains the complete raw dataset, including individual measurements of thorax wet and dry mass, water loss, evaporation dynamics over time, impedance variables (resistance, reactance, impedance modulus), and current–voltage values for all experimental groups.
(XLSX)

S2 File. Supplementary File S2 provides the full R Markdown script used for data cleaning, statistical analyses, and figure generation related to mass, evaporation, impedance, and I–V curves, ensuring transparency and reproducibility of the analyses.

(RMD)

S3 File. Supplementary Videos (File S3) show a step-by-step protocol for preparing and connecting *Apis mellifera* workers to the electrical measurement system. The videos demonstrate bee stabilization using micropipette tips, thorax immobilization with parafilm, and the subsequent fixation, electrode insertion, and connection required to perform electrical measurements.

(M4V)

Acknowledgments

The authors thank members of the Riveros' Lab (CANNON Research Group).

Author contributions

Conceptualization: Juan P. Hernández, Fredy Mesa, Andre J. Riveros.

Data curation: Juan P. Hernández.

Formal analysis: Juan P. Hernández.

Funding acquisition: Andre J. Riveros.

Investigation: Juan P. Hernández.

Methodology: Juan P. Hernández, Fredy Mesa.

Project administration: Juan P. Hernández, Andre J. Riveros.

Resources: Andre J. Riveros.

Supervision: Andre J. Riveros.

Validation: Juan P. Hernández.

Visualization: Juan P. Hernández.

Writing – original draft: Juan P. Hernández, Andre J. Riveros.

Writing – review & editing: Juan P. Hernández, Fredy Mesa, Andre J. Riveros.

References

1. Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, et al. Importance of pollinators in changing landscapes for world crops. *Proc Biol Sci.* 2007;274(1608):303–13. <https://doi.org/10.1098/rspb.2006.3721> PMID: [17164193](https://pubmed.ncbi.nlm.nih.gov/17164193/)
2. Decourtye A, Alaux C, Le Conte Y, Henry M. Toward the protection of bees and pollination under global change: present and future perspectives in a challenging applied science. *Current Opinion in Insect Science.* 2019;35:123–31. <https://doi.org/10.1016/j.cois.2019.07.008>
3. Grant KJ, DeVetter L, Melathopoulos A. Honey bee (*Apis mellifera*) colony strength and its effects on pollination and yield in highbush blueberries (*Vaccinium corymbosum*). *PeerJ.* 2021;9:e11634. <https://doi.org/10.7717/peerj.11634> PMID: [34395063](https://pubmed.ncbi.nlm.nih.gov/34395063/)
4. Klein A-M, Boreux V, Fornoff F, Mupepele A-C, Pufal G. Relevance of wild and managed bees for human well-being. *Curr Opin Insect Sci.* 2018;26:82–8. <https://doi.org/10.1016/j.cois.2018.02.011> PMID: [29764666](https://pubmed.ncbi.nlm.nih.gov/29764666/)
5. St. Clair AL, Zhang G, Dolezal AG, O'Neal ME, Toth AL. Agroecosystem landscape diversity shapes wild bee communities independent of managed honey bee presence. *Agriculture, Ecosystems & Environment.* 2022;327:107826. <https://doi.org/10.1016/j.agee.2021.107826>
6. St. Clair AL, Zhang G, Dolezal AG, O'Neal ME, Toth AL. Diversified Farming in a Monoculture Landscape: Effects on Honey Bee Health and Wild Bee Communities. *Environ Entomol.* 2020;49(3):753–64. <https://doi.org/10.1093/ee/nvaa031> PMID: [32249293](https://pubmed.ncbi.nlm.nih.gov/32249293/)
7. Yang Y, Wu Y, Long H, Ma X, Shariati K, Webb J, et al. Global honeybee health decline factors and potential conservation techniques. *Food Sec.* 2023;15(4):855–75. <https://doi.org/10.1007/s12571-023-01346-8>

8. Nath R, Singh H, Mukherjee S. Insect pollinators decline: an emerging concern of Anthropocene epoch. *Journal of Apicultural Research*. 2022;62(1):23–38. <https://doi.org/10.1080/00218839.2022.2088931>
9. Turley NE, Biddinger DJ, Joshi NK, López-Urbe MM. Six years of wild bee monitoring shows changes in biodiversity within and across years and declines in abundance. *Ecol Evol*. 2022;12(8):e9190. <https://doi.org/10.1002/ece3.9190> PMID: [35983174](#)
10. Parreño MA, Alaux C, Brunet J-L, Buydens L, Filiak M, Henry M, et al. Critical links between biodiversity and health in wild bee conservation. *Trends Ecol Evol*. 2022;37(4):309–21. <https://doi.org/10.1016/j.tree.2021.11.013> PMID: [34955328](#)
11. van der Sluijs JP, Vaage NS. Pollinators and Global Food Security: the Need for Holistic Global Stewardship. *Food ethics*. 2016;1(1):75–91. <https://doi.org/10.1007/s41055-016-0003-z>
12. Sillman J, Uusitalo V, Tapanen T, Salonen A, Soukka R, Kahiluoto H. Contribution of honeybees towards the net environmental benefits of food. *Sci Total Environ*. 2021;756:143880. <https://doi.org/10.1016/j.scitotenv.2020.143880> PMID: [33302069](#)
13. Siviter H, Fisher A II, Baer B, Brown MJF, Camargo IF, Cole J, et al. Protecting pollinators and our food supply: understanding and managing threats to pollinator health. *Insect Soc*. 2023;70(1):5–16. <https://doi.org/10.1007/s00040-022-00897-x>
14. Tarakini G, Chemura A, Tarakini T, Mashavakure N, Musundire R. Foraging Behaviour of *Apis mellifera scutellata* and *Hypotrigona gribodoi* Bees in Monoculture and Polyculture Vegetable Gardens. *Proc Zool Soc*. 2021;74(3):294–304. <https://doi.org/10.1007/s12595-021-00373-7>
15. Quigley TP, Amdam GV, Harwood GH. Honey bees as bioindicators of changing global agricultural landscapes. *Curr Opin Insect Sci*. 2019;35:132–7. <https://doi.org/10.1016/j.cois.2019.08.012> PMID: [31541967](#)
16. Chakrabarti P, Sarkar S, Basu P. Pesticide induced visual abnormalities in Asian honey bees (*Apis cerana* L.) in intensive agricultural landscapes. *Chemosphere*. 2019;230:51–8. <https://doi.org/10.1016/j.chemosphere.2019.05.050> PMID: [31102871](#)
17. Huang J, Wang H, Dong H, Liu M, Geng L, Sun J, et al. Neonicotinoids: Advances in hazards of residues, screening of aptamers and design of aptasensors. *Trends in Food Science & Technology*. 2024;144:104342. <https://doi.org/10.1016/j.tifs.2024.104342>
18. Zhang J, Wang Y, Wurjih S, Ruan H, Huang Y, Guo M, et al. Comprehensive analysis of neonicotinoids in Chinese commercial honey and pollen: A corresponding health risk assessment for non-targeted organisms. *Sci Total Environ*. 2024;919:170937. <https://doi.org/10.1016/j.scitotenv.2024.170937> PMID: [38360305](#)
19. Kagabu S. Discovery of imidacloprid and further developments from strategic molecular designs. *J Agric Food Chem*. 2011;59(7):2887–96. <https://doi.org/10.1021/jf101824y> PMID: [20718435](#)
20. Dirilgen T, Herbertsson L, O'Reilly AD, Mahon N, Stanley DA. Moving past neonicotinoids and honeybees: A systematic review of existing research on other insecticides and bees. *Environ Res*. 2023;235:116612. <https://doi.org/10.1016/j.envres.2023.116612> PMID: [37454798](#)
21. Tosi S, Sfeir C, Carnesecchi E, vanEngelsdorp D, Chauzat M-P. Lethal, sublethal, and combined effects of pesticides on bees: A meta-analysis and new risk assessment tools. *Sci Total Environ*. 2022;844:156857. <https://doi.org/10.1016/j.scitotenv.2022.156857> PMID: [35760183](#)
22. Bartling M-T, Brandt A, Hollert H, Vilcinskis A. Current Insights into Sublethal Effects of Pesticides on Insects. *Int J Mol Sci*. 2024;25(11):6007. <https://doi.org/10.3390/ijms25116007> PMID: [38892195](#)
23. Carteau A, Taillebois E, Le Questel J-Y, Thany SH. Mode of Action of Neonicotinoid Insecticides Imidacloprid and Thiacloprid to the Cockroach *Pernambuco Nicotinic Acetylcholine Receptor*. *Int J Mol Sci*. 2021;22(18):9880. <https://doi.org/10.3390/ijms22189880> PMID: [34576043](#)
24. Shi C, Tian Y, Wang Y, Guo W, Jiang W. The interaction of nicotinic acetylcholine receptor subunits Lda3, Lda8 and Ldb1 with neonicotinoids in Colorado potato beetle, *Leptinotarsa decemlineata*. *Pestic Biochem Physiol*. 2023;195:105558. <https://doi.org/10.1016/j.pestbp.2023.105558> PMID: [37666594](#)
25. Ozoe Y, Matsubara Y, Tanaka Y, Yoshioka Y, Ozoe F, Shiotsuki T, et al. Controlled expression of nicotinic acetylcholine receptor-encoding genes in insects uncovers distinct mechanisms of action of the neonicotinoid insecticide dinotefuran. *Pestic Biochem Physiol*. 2023;191:105378. <https://doi.org/10.1016/j.pestbp.2023.105378> PMID: [36963946](#)
26. French-Constant RH, Williamson MS, Davies TGE, Bass C. Ion channels as insecticide targets. *J Neurogenet*. 2016;30(3–4):163–77. <https://doi.org/10.1080/01677063.2016.1229781> PMID: [27802784](#)
27. Ihara M. Ligand-gated ion channels as targets of neuroactive insecticides. *Bioscience, Biotechnology, and Biochemistry*. 2021;86(2):157–64. <https://doi.org/10.1093/bbb/zbab202>
28. Christian M, Kraft M, Wilknitz P, Nowotny M, Schöneich S. Flupyradifurone, imidacloprid and clothianidin disrupt the auditory processing in the locust CNS. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2025;211(3):311–25. <https://doi.org/10.1007/s00359-025-01735-8> PMID: [39939492](#)
29. Rigosi E, O'Carroll DC. Acute Application of Imidacloprid Alters the Sensitivity of Direction Selective Motion Detecting Neurons in an Insect Pollinator. *Front Physiol*. 2021;12:682489. <https://doi.org/10.3389/fphys.2021.682489> PMID: [34305640](#)
30. Mustard JA, Dobb R, Wright GA. Chronic nicotine exposure influences learning and memory in the honey bee. *J Insect Physiol*. 2023;151:104582. <https://doi.org/10.1016/j.jinsphys.2023.104582> PMID: [37918514](#)
31. Stanley DA, Smith KE, Raine NE. Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. *Sci Rep*. 2015;5:16508. <https://doi.org/10.1038/srep16508> PMID: [26568480](#)

32. Aguiar JMRBV, Nocelli RCF, Giurfa M, Nascimento FS. Neonicotinoid effects on tropical bees: Imidacloprid impairs innate appetitive responsiveness, learning and memory in the stingless bee *Melipona quadrifasciata*. *Sci Total Environ*. 2023;877:162859. <https://doi.org/10.1016/j.scitoenv.2023.162859> PMID: 36933743
33. Williamson SM, Willis SJ, Wright GA. Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology*. 2014;23(8):1409–18. <https://doi.org/10.1007/s10646-014-1283-x> PMID: 25011924
34. Tosi S, Nieh JC. A common neonicotinoid pesticide, thiamethoxam, alters honey bee activity, motor functions, and movement to light. *Sci Rep*. 2017;7(1). <https://doi.org/10.1038/s41598-017-15308-6>
35. Bovi TS, Zaluski R, Orsi RO. Toxicity and motor changes in Africanized honey bees (*Apis mellifera* L.) exposed to fipronil and imidacloprid. *An Acad Bras Ciênc*. 2018;90(1):239–45. <https://doi.org/10.1590/0001-3765201820150191>
36. Lunardi JS, Zaluski R, Orsi RDO. Evaluation of Motor Changes and Toxicity of Insecticides Fipronil and Imidacloprid in Africanized Honey Bees (Hymenoptera: Apidae). *Sociobiology*. 2017;64(1):50. <https://doi.org/10.13102/sociobiology.v64i1.1190>
37. Rigosi E, O'Carroll DC. Acute Application of Imidacloprid Alters the Sensitivity of Direction Selective Motion Detecting Neurons in an Insect Pollinator. *Front Physiol*. 2021;12:682489. <https://doi.org/10.3389/fphys.2021.682489> PMID: 34305640
38. Agarwal R, Bansal A, Saini AS, Raj A, Kumar A, Saini S. Bee nutrition and artificial food. *The Pharma Innovation Journal*. 2023;12:1635–41.
39. Brodschneider R, Crailsheim K. Nutrition and health in honey bees. *Apidologie*. 2010;41(3):278–94. <https://doi.org/10.1051/apido/2010012>
40. Negri P, Villalobos E, Szawarski N, Damiani N, Gende L, Garrido M, et al. Towards Precision Nutrition: A Novel Concept Linking Phytochemicals, Immune Response and Honey Bee Health. *Insects*. 2019;10(11):401. <https://doi.org/10.3390/insects10110401> PMID: 31726686
41. Paray BA, Kumari I, Hajam YA, Sharma B, Kumar R, Albeshr MF, et al. Honeybee nutrition and pollen substitutes: A review. *Saudi J Biol Sci*. 2021;28(1):1167–76. <https://doi.org/10.1016/j.sjbs.2020.11.053> PMID: 33424413
42. Frizzera D, Del Fabbro S, Ortis G, Zanni V, Bortolomeazzi R, Nazzi F, et al. Possible side effects of sugar supplementary nutrition on honey bee health. *Apidologie*. 2020;51(4):594–608. <https://doi.org/10.1007/s13592-020-00745-6>
43. Daisley BA, Pitek AP, Torres C, Lowery R, Adair BA, Al KF, et al. Delivery mechanism can enhance probiotic activity against honey bee pathogens. *ISME J*. 2023;17(9):1382–95. <https://doi.org/10.1038/s41396-023-01422-z> PMID: 37311937
44. García LM, Caicedo-Garzón V, Riveros AJ. Oral administration of phytochemicals protects honey bees against cognitive and motor impairments induced by the insecticide fipronil. *PLoS One*. 2024;19(3):e0300899. <https://doi.org/10.1371/journal.pone.0300899> PMID: 38527045
45. Naree S, Ellis JD, Benbow ME, Suwannapong G. The use of propolis for preventing and treating *Nosema ceranae* infection in western honey bee (*Apis mellifera* Linnaeus, 1787) workers. *Journal of Apicultural Research*. 2021;60(5):686–96. <https://doi.org/10.1080/00218839.2021.1905374>
46. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J*. 2016;15(1):71. <https://doi.org/10.1186/s12937-016-0186-5> PMID: 27456681
47. Mousavi SM, Hashemi SA, Behbudi G, Mazraeadoost S, Omidifar N, Gholami A, et al. A Review on Health Benefits of *Malva sylvestris* L. Nutritional Compounds for Metabolites, Antioxidants, and Anti-Inflammatory, Anticancer, and Antimicrobial Applications. *Evid Based Complement Alternat Med*. 2021;2021:5548404. <https://doi.org/10.1155/2021/5548404> PMID: 34434245
48. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci*. 2016;5:e47. <https://doi.org/10.1017/jns.2016.41> PMID: 28620474
49. Semwal R, Joshi SK, Semwal RB, Semwal DK. Health benefits and limitations of rutin - A natural flavonoid with high nutraceutical value. *Phytochemistry Letters*. 2021;46:119–28. <https://doi.org/10.1016/j.phytol.2021.10.006>
50. Zeng X, Du Z, Ding X, Jiang W. Protective effects of dietary flavonoids against pesticide-induced toxicity: A review. *Trends in Food Science & Technology*. 2021;109:271–9. <https://doi.org/10.1016/j.tifs.2021.01.046>
51. Riveros AJ, Gronenberg W. The flavonoid rutin protects the bumble bee *Bombus impatiens* against cognitive impairment by imidacloprid and fipronil. *J Exp Biol*. 2022;225(17):jeb.244526. <https://doi.org/10.1242/jeb.244526> PMID: 36000283
52. He X, Lu L, Huang P, Yu B, Peng L, Zou L, et al. Insect Cell-Based Models: Cell Line Establishment and Application in Insecticide Screening and Toxicology Research. *Insects*. 2023;14(2):104. <https://doi.org/10.3390/insects14020104> PMID: 36835673
53. Lin M-H, Lin J-F, Yu M-C, Wu S-N, Wu C-L, Cho H-Y. Characterization in Potent Modulation on Voltage-Gated Na⁺ Current Exerted by Deltamethrin, a Pyrethroid Insecticide. *Int J Mol Sci*. 2022;23(23):14733. <https://doi.org/10.3390/ijms232314733> PMID: 36499059
54. Kannan M, Vitenberg T, Schweitzer R, Opatovsky I. Hemolymph metabolism of black soldier fly (Diptera: Stratiomyidae), response to different supplemental fungi. *J Insect Sci*. 2024;24(3):5. <https://doi.org/10.1093/jisesa/ieae050> PMID: 38713543
55. Elfar SA, Bahgat IM, Shebl MA, Lihoreau M, Tawfik MM. Variation in hemolymph content and properties among three Mediterranean bee species. *Cold Spring Harbor Laboratory*. 2023. <https://doi.org/10.1101/2023.12.22.573070>
56. Feng M, Ramadan H, Han B, Fang Y, Li J. Hemolymph proteome changes during worker brood development match the biological divergences between western honey bees (*Apis mellifera*) and eastern honey bees (*Apis cerana*). *BMC Genomics*. 2014;15(1):563. <https://doi.org/10.1186/1471-2164-15-563> PMID: 24996860
57. Chang H, Ding G, Jia G, Feng M, Huang J. Hemolymph Metabolism Analysis of Honey Bee (*Apis mellifera* L.) Response to Different Bee Pollens. *Insects*. 2022;14(1):37. <https://doi.org/10.3390/insects14010037> PMID: 36661964

58. Cao X, Wang Y, Rogers J, Hartson S, Kanost MR, Jiang H. Changes in composition and levels of hemolymph proteins during metamorphosis of *Manduca sexta*. *Insect Biochem Mol Biol*. 2020;127:103489. <https://doi.org/10.1016/j.ibmb.2020.103489> PMID: 33096211
59. Halberg KV, Denholm B. Mechanisms of systemic osmoregulation in insects. *Annual Review of Entomology*. 2025;41:46. <https://doi.org/10.1146/annurev-ento-040323>
60. Nearman A, vanEngelsdorp D. Water provisioning increases caged worker bee lifespan and caged worker bees are living half as long as observed 50 years ago. *Sci Rep*. 2022;12. <https://doi.org/10.1038/s41598-022-21401-2>
61. Andersen MK, Donini A, MacMillan HA. Measuring insect osmoregulation in vitro: A reference guide. *Comp Biochem Physiol A Mol Integr Physiol*. 2025;299:111751. <https://doi.org/10.1016/j.cbpa.2024.111751> PMID: 39341353
62. Rady MM, Boriek SHK, Abd El-Mageed TA, Seif El-Yazal MA, Ali EF, Hassan FAS, et al. Exogenous Gibberellic Acid or Dilute Bee Honey Boosts Drought Stress Tolerance in *Vicia faba* by Rebalancing Osmoprotectants, Antioxidants, Nutrients, and Phytohormones. *Plants (Basel)*. 2021;10(4):748. <https://doi.org/10.3390/plants10040748> PMID: 33920494
63. Piermarini PM, Denton JS, Swale DR. The molecular physiology and toxicology of inward rectifier potassium channels in insects selectivity filter: a structural feature of K channels that contains the signature GYG motif and forms the K selective channel pore. *Annual Review of Entomology*. 2025;55:58. <https://doi.org/10.1146/annurev-ento-062121>
64. Silver S, Donini A. Physiological responses of freshwater insects to salinity: molecular-, cellular- and organ-level studies. *J Exp Biol*. 2021;224(20):jeb222190. <https://doi.org/10.1242/jeb.222190> PMID: 34652452
65. Missirlis F. Regulation and biological function of metal ions in *Drosophila*. *Curr Opin Insect Sci*. 2021;47:18–24. <https://doi.org/10.1016/j.cois.2021.02.002> PMID: 33581350
66. Gorman MJ. Iron homeostasis in insects. 2025;56. <https://doi.org/10.1146/annurev-ento-040622>
67. Farina P, Bedini S, Conti B. Multiple Functions of Malpighian Tubules in Insects: A Review. *Insects*. 2022;13(11):1001. <https://doi.org/10.3390/insects13111001> PMID: 36354824
68. Lee CE, Charmantier G, Lorin-Nebel C. Mechanisms of Na⁺ uptake from freshwater habitats in animals. *Front Physiol*. 2022;13:1006113. <https://doi.org/10.3389/fphys.2022.1006113> PMID: 36388090
69. Gálíková M, Klepsatel P. Ion transport peptide regulates energy intake, expenditure, and metabolic homeostasis in *Drosophila*. *Genetics*. 2022;222(4):iyac150. <https://doi.org/10.1093/genetics/iyac150> PMID: 36190340
70. Dow JAT, Krause SA, Herzyk P. Updates on ion and water transport by the Malpighian tubule. *Curr Opin Insect Sci*. 2021;47:31–7. <https://doi.org/10.1016/j.cois.2021.02.018> PMID: 33705976
71. Rey S, Ohm H, Klämbt C. Axonal ion homeostasis and glial differentiation. *FEBS J*. 2023;290(15):3737–44. <https://doi.org/10.1111/febs.16594> PMID: 35943294
72. Dates J, Kolosov D. Voltage-gated ion channels as novel regulators of epithelial ion transport in the osmoregulatory organs of insects. *Front Insect Sci*. 2024;4:1385895. <https://doi.org/10.3389/finsc.2024.1385895> PMID: 38835480
73. Liu Z, Williamson MS, Lansdell SJ, Han Z, Denholm I, Millar NS. A nicotinic acetylcholine receptor mutation (Y151S) causes reduced agonist potency to a range of neonicotinoid insecticides. *J Neurochem*. 2006;99(4):1273–81. <https://doi.org/10.1111/j.1471-4159.2006.04167.x> PMID: 16981889
74. Raymond-Delpech V, Matsuda K, Sattelle BM, Rauh JJ, Sattelle DB. Ion channels: molecular targets of neuroactive insecticides. *Invert Neurosci*. 2005;5(3–4):119–33. <https://doi.org/10.1007/s10158-005-0004-9> PMID: 16172884
75. Xu X, Wang X, Yang Y, Ares I, Martínez M, Lopez-Torres B, et al. Neonicotinoids: mechanisms of systemic toxicity based on oxidative stress-mitochondrial damage. *Arch Toxicol*. 2022;96(6):1493–520. <https://doi.org/10.1007/s00204-022-03267-5> PMID: 35344072
76. Zhang X, Fu H, Wu Q, Chen L, Lu Y, Gao S. Imidacloprid Disturbs the Nitrogen Metabolism and Triggers an Overall Stress Response in Maize Seedlings. *Agronomy*. 2022;12(12):3161. <https://doi.org/10.3390/agronomy12123161>
77. He B, Liu Z, Wang Y, Cheng L, Qing Q, Duan J, et al. Imidacloprid activates ROS and causes mortality in honey bees (*Apis mellifera*) by inducing iron overload. *Ecotoxicol Environ Saf*. 2021;:112709. <https://doi.org/10.1016/j.ecoenv.2021.112709> PMID: 34895731
78. Ge W, Yan S, Wang J, Zhu L, Chen A, Wang J. Oxidative stress and DNA damage induced by imidacloprid in zebrafish (*Danio rerio*). *J Agric Food Chem*. 2015.
79. Zhang Q, Zhang B, Wang C. Ecotoxicological effects on the earthworm *Eisenia fetida* following exposure to soil contaminated with imidacloprid. *Environ Sci Pollut Res Int*. 2014;21(21):12345–53. <https://doi.org/10.1007/s11356-014-3178-z> PMID: 24938817
80. El-Gendy KS, Aly NM, Mahmoud FH, Kenawy A, El-Sebae AKH. The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. *Food Chem Toxicol*. 2010;48(1):215–21. <https://doi.org/10.1016/j.fct.2009.10.003> PMID: 19833166
81. Kapoor, Srivastava, Bhardwaj, Prasad. Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its no observed effect level (NOEL). *J Toxicol Sci*. 2010.
82. Lonare M, Kumar M, Raut S, Badgujar P, Doltade S, Telang A. Evaluation of imidacloprid-induced neurotoxicity in male rats: a protective effect of curcumin. *Neurochem Int*. 2014;78:122–9. <https://doi.org/10.1016/j.neuint.2014.09.004> PMID: 25261201
83. Wang X, Anadón A, Anadón A, Wu Q, Qiao F, Ares I, et al. Mechanism of neonicotinoid toxicity: impact on oxidative stress and metabolism. *Annu Rev Pharmacol Toxicol*. 2018;58:471–507. <https://doi.org/10.1146/annurev-pharmtox>

84. Pandey P, Khan F, Qari HA, Oves M. Rutin (Bioflavonoid) as Cell Signaling Pathway Modulator: Prospects in Treatment and Chemoprevention. *Pharmaceuticals* (Basel). 2021;14(11):1069. <https://doi.org/10.3390/ph14111069> PMID: 34832851
85. Choi S-S, Park H-R, Lee K-A. A Comparative Study of Rutin and Rutin Glycoside: Antioxidant Activity, Anti-Inflammatory Effect, Effect on Platelet Aggregation and Blood Coagulation. *Antioxidants* (Basel). 2021;10(11):1696. <https://doi.org/10.3390/antiox10111696> PMID: 34829567
86. Caglayan C, Kandemir FM, Yildirim S, Kucukler S, Eser G. Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats. *J Trace Elem Med Biol*. 2019;54:69–78. <https://doi.org/10.1016/j.jtemb.2019.04.007> PMID: 31109623
87. Cristiano MC, Barone A, Mancuso A, Torella D, Paolino D. Rutin-Loaded Nanovesicles for Improved Stability and Enhanced Topical Efficacy of Natural Compound. *J Funct Biomater*. 2021;12(4):74. <https://doi.org/10.3390/jfb12040074> PMID: 34940553
88. Pandey P, Khan F, Qari HA, Oves M. Rutin (Bioflavonoid) as Cell Signaling Pathway Modulator: Prospects in Treatment and Chemoprevention. *Pharmaceuticals* (Basel). 2021;14(11):1069. <https://doi.org/10.3390/ph14111069> PMID: 34832851
89. Choi S-S, Park H-R, Lee K-A. A Comparative Study of Rutin and Rutin Glycoside: Antioxidant Activity, Anti-Inflammatory Effect, Effect on Platelet Aggregation and Blood Coagulation. *Antioxidants* (Basel). 2021;10(11):1696. <https://doi.org/10.3390/antiox10111696> PMID: 34829567
90. Ydyrys A, Zhaparkulova N, Aralbaeva A, Mamataeva A, Seilkhan A, Syraiyl S, et al. Systematic Analysis of Combined Antioxidant and Membrane-Stabilizing Properties of Several Lamiaceae Family Kazakhstani Plants for Potential Production of Tea Beverages. *Plants* (Basel). 2021;10(4):666. <https://doi.org/10.3390/plants10040666> PMID: 33808498
91. García LM, Caicedo-Garzón V, Riveros AJ. Oral administration of phytochemicals protects honey bees against cognitive and motor impairments induced by the insecticide fipronil. *PLoS One*. 2024;19(3):e0300899. <https://doi.org/10.1371/journal.pone.0300899> PMID: 38527045
92. Riddick EW. Evaluating the Effects of Flavonoids on Insects: Implications for Managing Pests Without Harming Beneficials. *Insects*. 2024;15(12):956. <https://doi.org/10.3390/insects15120956> PMID: 39769558
93. Al-Khayri JM, Rashmi R, Toppo V, Chole PB, Banadka A, Sudheer WN, et al. Plant Secondary Metabolites: The Weapons for Biotic Stress Management. *Metabolites*. 2023;13(6):716. <https://doi.org/10.3390/metabo13060716> PMID: 37367873
94. Mouden S, Klinkhamer PGL, Choi YH, Leiss KA. Towards eco-friendly crop protection: natural deep eutectic solvents and defensive secondary metabolites. *Phytochem Rev*. 2017;16(5):935–51. <https://doi.org/10.1007/s11101-017-9502-8> PMID: 29167631
95. Hernández J, Mesa F, Riveros A, Fayad R, Nisperuza J. Extraction and Optical Analysis of the Extracellular Fluid from the Body Segments of *Apis mellifera* Bees. *Integr Org Biol*. 2025;7(1):obaf018. <https://doi.org/10.1093/iob/obaf018> PMID: 40438523

Discusión general

Integración de resultados

Los experimentos desarrollados a lo largo de esta tesis permitieron evaluar, de forma segmentada y progresiva, los efectos del imidacloprid y de la rutina. Las evaluaciones se realizaron sobre el comportamiento, la fisiología hídrica y las propiedades eléctricas de las abejas *Apis mellifera*. Mediante un enfoque funcional se articularon respuestas comportamentales (reflejos, locomoción), variaciones en la masa corporal (contenido de agua) y características eléctricas del líquido extracelular. Los hallazgos muestran que la exposición a imidacloprid provoca alteraciones sistémicas que comprometen múltiples niveles funcionales del insecto. En contraste, la administración de la rutina, ya sea por sí sola o en combinación con el insecticida, atenuó algunas de estas alteraciones y promovió valores cercanos a los del control.

Contraste entre los diferentes experimentos

En el plano comportamental, la reducción de los reflejos de alimentación (PER) y de defensa (SER) evidenció que el imidacloprid afecta circuitos sensoriomotores esenciales para la supervivencia. Paralelamente, los análisis de movimiento individual y colectivo evidenciaron una disminución de la coordinación motora, del desplazamiento angular y de la velocidad grupal. En el plano fisiológico, se documentaron reducciones de la masa húmeda, del contenido hídrico y de la tasa de evaporación del líquido extracelular. A nivel eléctrico, se registraron disminuciones en la resistencia y la reactancia, con un aumento de la corriente, lo que sugiere una pérdida de las propiedades dieléctricas del fluido. Los experimentos abordan distintos niveles de organización (comportamiento, masa corporal, propiedades eléctricas); los cambios observados evidencian una convergencia funcional. Lo anterior, visto desde los efectos deletéreos derivados de la exposición al insecticida, que deteriora la capacidad del insecto para responder de manera coordinada y eficiente a estímulos ambientales. Lo contrario ocurre con la administración de la rutina de sustancias que mostraron un efecto modulador.

Interpretación de hallazgos bajo el marco teórico general

Los resultados obtenidos indican que el imidacloprid altera la homeostasis funcional de *Apis mellifera* en al menos tres dimensiones:

- Neurofuncional: al afectar los reflejos y la coordinación, compromete la integración sensorial y la ejecución motora.
- Hídrica: al reducir el contenido y dinámica del agua corporal, debilita procesos osmóticos y de regulación volumétrica.
- Eléctrica: al modificar la impedancia del líquido extracelular, altera las propiedades dieléctricas necesarias para el correcto funcionamiento celular y la transmisión de señales.

Estas alteraciones son consistentes con un modelo en el que el daño inducido por el insecticida se propaga a través de vías funcionales interrelacionadas. Las respuestas observadas no son fenómenos aislados, sino manifestaciones complementarias de una disfunción sistémica que puede comprometer la viabilidad ecológica del insecto.

Limitaciones del estudio

Entre las limitaciones más relevantes se encuentran:

- La dificultad para aislar por completo los efectos de la rutina e imidacloprid en escenarios de campo, debido a la presencia de múltiples estresores ambientales.
- El uso de dosis y tiempos de exposición controlados en laboratorio que, aunque necesarios para la estandarización, no siempre reflejan las condiciones reales de exposición crónica.
- La imposibilidad de medir directamente la actividad neuronal o metabólica, lo que impide vincular causalmente los cambios observados con procesos celulares específicos.
- Las diferencias morfofisiológicas entre segmentos corporales no siempre se tradujeron en respuestas diferenciadas, lo que limita la interpretación segmental en algunos casos.

Contribución al conocimiento científico y aplicaciones prácticas

Este trabajo contribuye al conocimiento científico al ofrecer:

- Un enfoque experimental integrado que permite evaluar simultáneamente múltiples niveles funcionales.
- Evidencia de que las alteraciones de los reflejos, la masa corporal y las propiedades eléctricas están funcionalmente interrelacionadas y pueden emplearse como biomarcadores no letales.
- Propuestas metodológicas estandarizadas aplicables a la evaluación de otras sustancias neuroactivas en insectos.

En cuanto a sus aplicaciones prácticas, los hallazgos permiten avanzar en el desarrollo de herramientas de monitoreo funcional para evaluar la salud de polinizadores, y ofrecen evidencia para la regulación de agroquímicos como el imidacloprid. Además, la identificación de efectos protectores de la rutina abre la puerta al diseño de estrategias de mitigación basadas en compuestos naturales.

Mecanismo propuesto de mitigación biológica:

La atenuación de los efectos del imidacloprid observada en los individuos suplementados con rutina se debe a un mecanismo protector multifactorial. Fisiológicamente, el daño neonicotinoide desencadena una cascada de estrés oxidativo intracelular agresiva. En este contexto, la rutina actúa como un potente agente depurador de radicales libres, neutralizando las especies reactivas de oxígeno (ROS). A nivel estructural, las propiedades polifenólicas de este flavonoide le permiten

interactuar con las bicapas lipídicas, estabilizando las membranas celulares y preservando la función de las uniones estrechas en los tejidos epiteliales. Esta estabilización previene la fuga descontrolada de iones y fluidos, lo que explica cómo la rutina resguarda la homeostasis hídrica y eléctrica del líquido extracelular.

Conclusiones y perspectivas

Resumen de hallazgos clave

Esta tesis muestra que la exposición subletal a imidacloprid compromete funciones fundamentales en *Apis mellifera*, afectando su capacidad para alimentarse, defenderse, desplazarse y mantener su equilibrio fisiológico. Las alteraciones observadas incluyen la disminución de las respuestas comportamentales de alimentación y defensa, la pérdida de coordinación motora, tanto individual como grupal, la reducción del contenido hídrico corporal y las modificaciones en las propiedades eléctricas del líquido extracelular. La integración de estos resultados sugiere que las alteraciones inducidas por el insecticida no se manifiestan de manera aislada, sino como un conjunto de fallas funcionales interconectadas que reducen la eficiencia ecológica del insecto. Por otro lado, el consumo de rutina permitió mitigar algunos de estos efectos, lo que evidencia un posible rol modulador frente al daño inducido por compuestos tóxicos.

Respuesta a la hipótesis general

Los datos obtenidos respaldan la hipótesis central de esta investigación. La exposición a imidacloprid provoca múltiples alteraciones funcionales que afectan el comportamiento, la hidratación corporal y la actividad eléctrica del sistema interno. Estas alteraciones pueden detectarse y cuantificarse mediante metodologías experimentales estandarizadas que permiten evaluar el estado funcional del insecto sin recurrir a procedimientos invasivos ni letales. La rutina, al modular la respuesta ante las alteraciones inducidas, resulta un candidato interesante para futuras estrategias de protección fisiológica en contextos de estrés químico.

Nuevas preguntas

A partir de los hallazgos de esta tesis surgen nuevas interrogantes sobre los mecanismos que subyacen a la disfunción funcional. Resulta necesario explorar cómo se relacionan estas respuestas comportamentales con alteraciones del sistema nervioso central o con patrones de activación muscular. También queda abierta la pregunta sobre la reversibilidad de estas alteraciones, su evolución en el tiempo y su interacción con otros factores ambientales como la temperatura, la nutrición y la exposición simultánea a diferentes contaminantes. Otra línea de investigación relevante consiste en caracterizar con mayor profundidad la composición fisicoquímica del líquido extracelular para comprender su papel como medio de transmisión y como reflejo del estado interno del organismo.

Proyecciones de investigación futura

Los resultados de esta tesis permiten proyectar un enfoque funcional aplicable a los estudios de ecotoxicología, fisiología comparada y biología de la conservación. Las metodologías desarrolladas pueden ser refinadas para su implementación en condiciones de campo, lo que permitirá el monitoreo temprano de alteraciones en insectos expuestos a agroquímicos. Asimismo,

se abre la posibilidad de incorporar herramientas complementarias, como registros electrofisiológicos, análisis metabólicos y caracterizaciones moleculares, que permitan vincular los cambios funcionales con procesos celulares específicos. Finalmente, este enfoque biofísico podría adaptarse al estudio de otras especies de polinizadores, fortaleciendo las estrategias de evaluación ambiental mediante indicadores sensibles, cuantificables y reproducibles.

En conjunto, esta tesis no solo confirma el impacto negativo del imidacloprid sobre *Apis mellifera*, sino que también propone una forma coherente de abordar dicho impacto, basada en la articulación entre el comportamiento, la fisiología corporal y las propiedades eléctricas. Esta visión integradora permite comprender al insecto como un sistema funcional cuyos desequilibrios pueden detectarse antes de que se manifiesten como mortalidad o colapso ecológico.

Anexos

Publicaciones

1. Extraction and Optical Analysis of the Extracellular Fluid from the Body Segments of *Apis mellifera* Bees. Integrative Organismal Biology, Volume 7, Issue 1, 2025
2. Electrical characteristics of the extracellular fluid in the body segments of *Apis mellifera* bees. PeerJ 13:e19691
3. The flavonoid rutin protects against imidacloprid-induced osmotic and electric disruptions in Africanized honey bees. PLOS ONE.
4. Water homeostasis and extracellular fluid dynamics reveal imidacloprid disruption and rutin mitigation in honey bees. Scientific Reports.

Artículos sometidos

1. Sublethal effects of the neonicotinoid imidacloprid on behavior across body segments in honey bees. Scientific Reports.

Presentaciones en congresos

1. VI Congreso colombiano de Zoología. Córdoba. 27-31 marzo de 2023.
2. LVIII Congreso Nacional y IX Internacional de Ciencias Biológicas. Risaralda. 10-13 octubre 2023.

Pasantías

1. Universidad Nacional de Colombia. Grupo de investigación Materiales Nanoestructurados y sus aplicaciones. Dr. Anderson Dussan.
2. Fundación Universitaria Los Libertadores. Grupo de investigación NanoTech. Dr. Jorge Nisperuza; Dr. Fredy Mesa