Efectos letales y subletales de la exposición a contaminantes emergentes en el caracol manzana *Pomacea sp.*

Programa de Desarrollo de las Ciencias Básicas – Área Ciencias Biológicas

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(*) El estudiante está en más de un acta

Nota Defensa de Doctorado

La defensa de Doctorado de la MsC. Florencia Féola "*Efectos letales y sub-letales de la exposición a contaminantes emergentes en el caracol manzana Pomacea sp*." bajo la tutoría del Dr. Angel Segura, Dra. Carolina Crisci y Dr. Julio Gómez, involucró estudios ecofisiológicos centrados en una especie nativa, el caracol Pomacea sp, con diferentes aproximaciones experimentales para analizar los efectos de contaminantes de preocupación emergente, uno orgánico el herbicida clomazone, y uno inorgánico el Sulfuro de Bismuto.
El trabajo abordó en forma genèrica los marcos teóricos que sustentaron su investigación contemplando un abordaje transversal que incluyó resultados e informaciòn sobre la autoecología de la especie utilizada, efectos toxicológicos sobre diversas variables metabólicas y comportamentales, el uso de herramientas estadísticas originales para este tipo de estudio, hasta recomendaciones en las formas de cultivo, lo que en conjunto constituyen un importante aporte a esta rama del conocimiento. Se destaca que los principales resultados fueron publicados en tres revistas arbitradas internacionales.
La presentación fue clara, e incorporó las sugerencias emitidas por el tribunal.
Por todo lo anterior, este tribunal considera que Florencia Féola aprobó su doctorado.

Javier García Alonso

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esta etapa y la transformó, haciéndola más desafiante, pero también dándole un sentido especial que vuelve aún más valioso haber llegado hasta aquí.

Indice

1. Resumen

En esta tesis doctoral se evalúan los efectos letales y subletales de productos químicos derivados de actividades antropogénicas sobre un organismo acuático nativo de agua dulce en Uruguay. Se estructura en capítulos autocontenidos, con una introducción y una discusión general.

En las últimas décadas, actividades antropogénicas como la agricultura y la industria han registrado un aumento sustancial. Parte de los productos aplicados/producidos en las mismas pueden alcanzar ambientes acuáticos cercanos, en donde causan efectos negativos sobre las comunidades biológicas asociadas. Esto puede afectar las funciones ecosistémicas, viéndose afectados, por ende, los servicios ecosistémicos incluyendo la disponibilidad de alimento y de agua potable, lo que pone el riesgo la salud humana.

Los agroquímicos y las nanopartículas se incluyen dentro de los denominados contaminantes emergentes y forman parte del conjunto de compuestos que llegan a los ecosistemas acuáticos. Diversos trabajos han demostrado que pueden generar cambios a distintos niveles de organización, desde el molecular (en los individuos) hasta el ecosistémico. En particular, los efectos letales son bien conocidos, pero los subletales se realizan con menor frecuencia.

El clomazone es un herbicida de uso extendido a nivel mundial, incluyendo Uruguay, empleado principalmente en cultivos de arroz, soja, algodón, entre otros, y que se ha encontrado en cuerpos de agua. Por otra parte, las nanopartículas de sulfuro de bismuto (Bi_2S_3) son empleadas en diversas áreas, como la medicina, la electrónica, en la remoción de contaminantes de descargas industriales; pero existe escaso conocimiento sobre su efecto en los ecosistemas acuáticos.

El caracol manzana *Pomacea* sp. es un organismo nativo que habita en cuerpos de agua dulce de todo el Uruguay, desde pequeños estanques y cañadas hasta el Río Uruguay. Varias especies de este género son utilizadas como bioindicadores en del mundo. Sin embargo, información fisiológica y sus respuestas a diversos contaminantes es reducida.

En este contexto, el objetivo de este trabajo fue evaluar, mediante ensayos de laboratorio, los efectos letales y subletales del herbicida clomazone y nanopartículas de Bi_2S_3, sobre el caracol manzana (*Pomacea* sp.).

Previo a la realización de los bioensayos, se evaluó el efecto de la temperatura sobre *Pomacea* sp., caracterizando la curva de desempeño térmico para consumo de oxígeno, tasa de excreción de amonio, tasa de ingestión, además de la incidencia sobre crecimiento y mortalidad. Para ello, los organismos fueron expuestos a un gradiente de temperatura, representativo de las condiciones en el ambiente. Esto permitió determinar la temperatura o rango de temperaturas que no implicaran estrés térmico para los organismos, dato que fue

posteriormente utilizado en las evaluaciones de exposición para evitar interferencias metodológicas.

Con el fin de evaluar los efectos de clomazone y Bi_2S_3, los caracoles fueron sometidos a distintas concentraciones de ambos compuestos, por separado. En estos ensayos se determinó la tasa de mortalidad, la concentración letal media de clomazone a las 96 h (96h-LC_{50}), y los efectos subletales de ambos compuestos sobre el consumo de oxígeno, la actividad, tasa de ingestión y de excreción de amonio, y/o el crecimiento. Los residuos generados fueron descartados según las normas de bioseguridad vigentes, siguiendo las indicaciones del fabricante y los protocolos de la empresa encargada de la gestión de residuos peligrosos.

Los resultados mostraron un claro efecto del clomazone. El 96h-LC_{50} = 14,59 mg L^{-1}; La actividad y el consumo de oxígeno se vieron afectados en concentraciones elevadas. En cuanto al Bi_2S_3, se observó un aumento en el consumo de oxígeno y la bioacumulación de este compuesto en el tejido de *Pomacea* sp., siendo este el primer reporte de la bioacumulación de dicha nanopartícula en tejidos de *Pomacea sp.*

Estos resultados muestran el efecto del herbicida Clomazone y nanopartículas de Bi_2S_3 sobre el caracol manzana *Pomacea* sp.. Esta es una especie con potencial para ser considerada como bioindicador de contaminación ambiental, al igual que otras especies de este género. Al no observase efectos a concentraciones subletales, se recomienda la evaluación a otros niveles de organización, como moleculares, que suelen ser sensibles y permiten observar respuestas en el corto plazo, para continuar generando bioindicadores de referencia que puedan luego ser considerados herramientas de evaluación de riesgo ambiental.

2. Introducción general

La agricultura basada en insumos y la industria se han intensificado considerablemente a nivel mundial desde las décadas de 1940 y 1950. Parte de los productos que se aplican o producen, alcanzan los ambientes acuáticos por diversos mecanismos impactando sobre múltiples aspectos de los ecosistemas, como su integridad, ya que afectan la fauna y flora que allí habita. Esto puede generar cambios en la biodiversidad y potencialmente en la estructura de la cadena trófica de los mismos (**Meer et al., 2020**).

La presencia de estos compuestos también afecta las funciones ecosistémicas, pudiendo alterar como consecuencia, la calidad de los servicios brindados por los ecosistemas, poniendo en riesgo la salud humana (**Martín-López & Montes, 2010; Meer et al., 2020; Zhou et al., 2025)**. Los compuestos químicos pueden bioacumularse en los organismos residentes de los sistemas acuáticos en forma pasiva y mediante interacciones tróficas, causando efectos como su propia mortalidad o la biomagnificación de los compuestos en la trama trófica. Esto puede tener consecuencias también a nivel humano, influyendo en la provisión de alimento, y también sobre los servicios de recreación y hasta en la provisión de agua potable (**Zhou et al., 2025**). Diversos estudios han demostrado que la exposición a, por ejemplo, pesticidas, puede producir cáncer, enfermedades respiratorias, pérdida de visión, problemas neurológicos (**Zhou et al., 2025**).

Algunos de estos compuestos son considerados contaminantes emergentes, definidos como sustancias químicas o materiales recientemente descubiertos, que pueden presentar un riesgo potencial para el humano y/o el ambiente, pero que aún no ha sido correctamente evaluado (**Singh et al., 2019; Li et al., 2022; El-Kalliny et al., 2023; Onotu et al., 2025**). A pesar de que en general se encuentran en concentraciones muy bajas, pueden presentar una elevada toxicidad (**Bayabil et al., 2022**). Se caracterizan por presentar estructuras químicas complejas y/o ocurrir en bajas concentraciones, lo que ha implicado el desarrollo de nuevos procedimientos analíticos de alta resolución para su detección en distintas matrices como agua y tejidos (**Sauvé & Desrosiers**, **2014**; **Radwan et al., 2023; Li et al., 2024**).

La llegada de los contaminantes a los ecosistemas acuáticos puede ser a través de fuentes puntuales, asociadas a descargas directas y localizadas, como tuberías de industrias; o por fuentes difusas, que implican el transporte de contaminantes desde área extensas del paisaje, y que están relacionadas a distintos procesos como la escorrentía, como ocurre en las zonas agrícolas (**Carpenter et al. 1998; USEPA 2003; FAO 2017**).

Entre los contaminantes emergentes se encuentran los agroquímicos y las nanopartículas, en los cuales se hará foco en este trabajo. Los primeros son compuestos empleados en la agricultura con distintos objetivos; dentro de ellos se encuentran los fertilizantes, aplicados para el mejoramiento del cultivo; y los pesticidas, utilizados como mecanismo de control de

plantas, insectos u hongos considerados perjudiciales para las actividades agrícolas. De acuerdo con el organismo blanco que se pretende controlar, se los clasifica en herbicidas, insecticidas, fungicidas, acaricidas, molusquicidas.

Las nanopartículas son materiales con un tamaño de hasta 100 nm, que pueden variar en su composición -metálicas, poliméricas, de carbono- y que se aplican en diversos ámbitos, como cosmética, electrónica, medicina y hasta en el medio ambiente. En las siguientes secciones, se abordan en detalle las características de los pesticidas y las nanopartículas.

2.1. *Pesticidas*

El aumento en la producción agrícola surge principalmente a partir de lo que se denominó la Revolución Verde en la década de 1960; en ella se desarrollaron variedades de diferentes especies de cultivo (e.g. arroz, trigo y soja) altamente resistentes a pesticidas, con el fin de mejorar su calidad y rendimiento. La contracara de estas nuevas variedades es que pueden requerir la aplicación de grandes volúmenes de agroquímicos, como fertilizantes y pesticidas, lo que potencialmente genera problemas a nivel ambiental (**Briggs, 2009; REDES, 2017**). Los “paquetes tecnológicos” asociados a estos cultivos, que incluyen el material vegetal y todos los insumos relacionados, como agroquímicos, maquinaria, sistemas de riego y conocimiento técnico para realizar un manejo adecuado de los insumos con el fin de mejorar la producción, la calidad y la rentabilidad de los productos, se aplican en grandes superficies (**REDES, 2017**).

En 1996, en Uruguay se incorporaron cultivos de cepas transgénicas de semillas como la soja. Luego de 20 años, son 15 tipos de cultivos transgénicos desarrollados; 5 de soja y 10 de maíz. Todo esto trae aparejado el aumento en el volumen de agroquímicos aplicados, principalmente herbicidas (**REDES, 2017**). Entre los años 2000 y 2023 el uso de pesticidas a nivel mundial se incrementó en un 70% (**FAO, 2024**), siendo América el mayor consumidor con un 44,6%, seguido por Asia (32,5%), Europa (15,8%), África (4,7%) y finalmente Oceanía (2,4%) (FAOStat). Además, entre 1999 y 2020, la venta de estos compuestos en América del Sur se incrementó en un 119,4%, el mayor crecimiento a nivel mundial (**RAP-AL, 2023**).

Uruguay no es ajeno a este escenario, ya que entre los años 1990 y 2000, el consumo de pesticidas se duplicó, y luego continuó en aumento hasta que en el 2014 alcanzó 25.845.000 kilos (**Comisión de Ganadería, Agricultura y Pesca, 2025**). Esto también concuerda con las contrataciones de empresas de fumigación por servicios agropecuarios, las cuales se triplicaron entre los años 2000 y 2011 de acuerdo con los censos agropecuarios (**Mondelli, 2014**).

Luego de este período y hasta el 2021 se ha observado un descenso de aproximadamente 14.135 toneladas en la importación de agroquímicos, donde los herbicidas representan el mayor volumen. Desde 2021, las importaciones de herbicidas vienen en aumento, llegando a

30.573.278 kg o L de formulado en 2023. Los insecticidas y fungicidas son utilizados en menor volumen, pero en general con efectos más potentes (Fig. 1) (**MGAP, 2023; Palladino et al., 2023**), observándose una brusca caída de los primeros entre los años 2014 y 2015, de 3.841.068 a 1.100.697 kg o L, respectivamente, que luego se mantiene hasta el 2024 (Fig. 1).

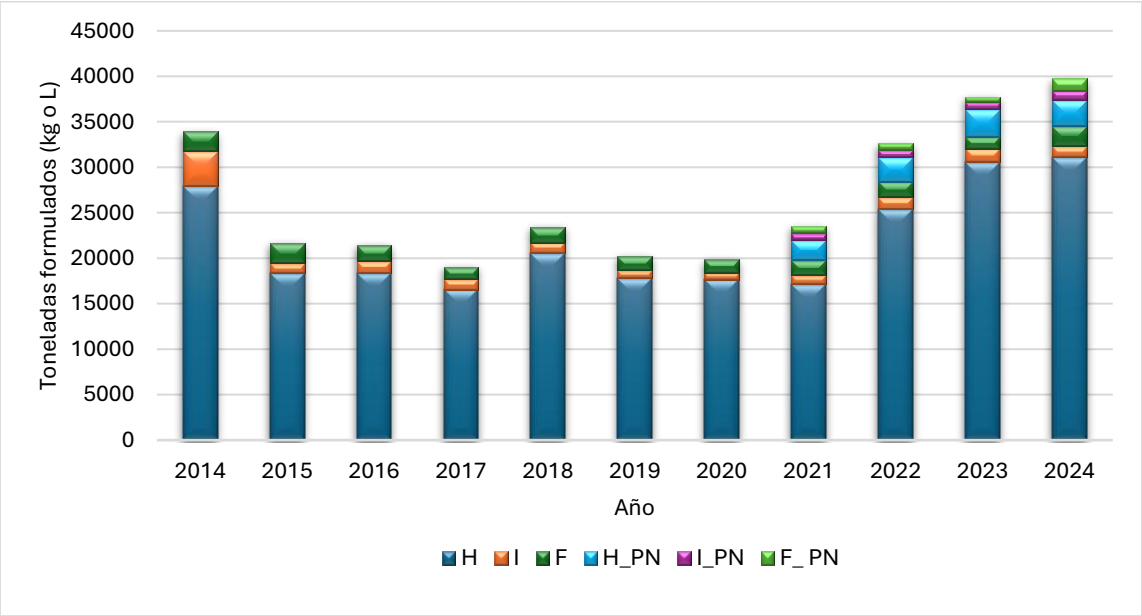

Figura 1. Toneladas (kg o L) de formulados de pesticidas importados y de formulación nacional en Uruguay entre 2014 y 2024 (MGAP). H=herbicidas, I= insecticidas, F= fungicidas, H_PN= herbicidas de producción nacional, I_PN= insecticidas de producción nacional, F_PN= fungicidas de producción nacional.

Por su parte, los fungicidas disminuyen casi a la mitad en el período entre 2014 y 2023 (de 2.165.288 a 1.293.579 kg o L, respectivamente), volviendo a aumentar en 2024 (2.216.480 kg o L). Además, se debe mencionar que desde el 2021 existen formulados de producción nacional, lo que aumenta el volumen en casi 3.000.000 kg o L (**MGAP, 2021 al 2024**).

La presencia de estos compuestos en el ambiente causa efectos en organismos no-blanco, que no son su objetivo de acción, pertenecientes a la fauna y/o flora de zonas cercanas a las áreas de aplicación. Estos efectos se ven reflejados en diversos indicadores que corresponden a distintos niveles de organización, desde moleculares hasta comunitarios (Fig. 4; **Cossi et al. 2018; Ghaffar et al., 2021; Schuhmann et al., 2022; Mishra et al., 2024; Souza et al., 2024; Michel et al. 2025).** En la sección 2.3 se desarrolla en detalle los efectos observados en organismos no blanco, además de abordar los tipos de ensayos realizados para evaluarlos.

2.1.1. Clomazone

El Clomazone ($C_{12}H_{14}ClNO_2$) (Fig. 2) es un herbicida que pertenece al grupo químico de las isoxazolidinonas, con un peso molecular de 239.70 g mol^{-1}. Es un compuesto que afecta la

actividad de enzimas asociadas a la producción de carotenoides en plantas que afectan los cultivos. Es aplicado principalmente en cultivos como soja, arroz, algodón y tabaco, principalmente en períodos de preemergencia (**Zimdahl, 2018**).

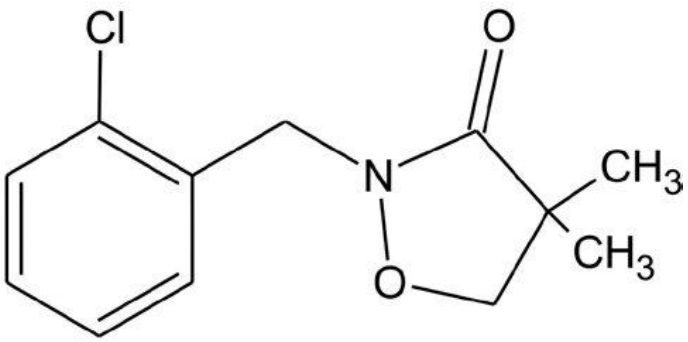

Figura 2. Estructura química de Clomazone

Este es un herbicida muy soluble en agua (1100 mg L^{-1} a 23°C), lo que vuelve relevante y preocupante su potencial impacto en organismos que habitan cuerpos de agua cercanos a las zonas de cultivo (**van Scoy & Tjeerdema, 2013**).

El clomazone es usado en América Latina, donde se ha encontrado en distintos cuerpos de agua debido a la deriva producida desde las zonas de aplicación (**van Scoy & Tjeerdema, 2013; Cabrera et al., 2025**). En Uruguay no existe un marco legal que establezca los límites permitidos para este compuesto en cuanto a agua de consumo humano ni en aguas superficiales. Aunque si existe normativa para otros herbicidas como el Glifosato, para el cual el valor máximo permitido, junto con su metabolito AMPA, en agua potable es de 700 µg L^{-1} (norma UNIT 833:2008). En cuanto al uso de clomazone en Uruguay, su importación ha sufrido una drástica caída entre 2014 y 2022 (de 262,5 a 82,1 toneladas, respectivamente); para luego comenzar a aumentar hasta alcanzar 176,5 toneladas en 2024 (Fig. 3**; MGAP, 2014 al 2024**). Los datos mostrados corresponden al período 2014-2024, según la información disponible en la página web del Ministerio de Ganadería, Agricultura y Pesca (MGAP; https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/datos-y-estadisticas/datos/importaciones-productos-fitosanitarios).

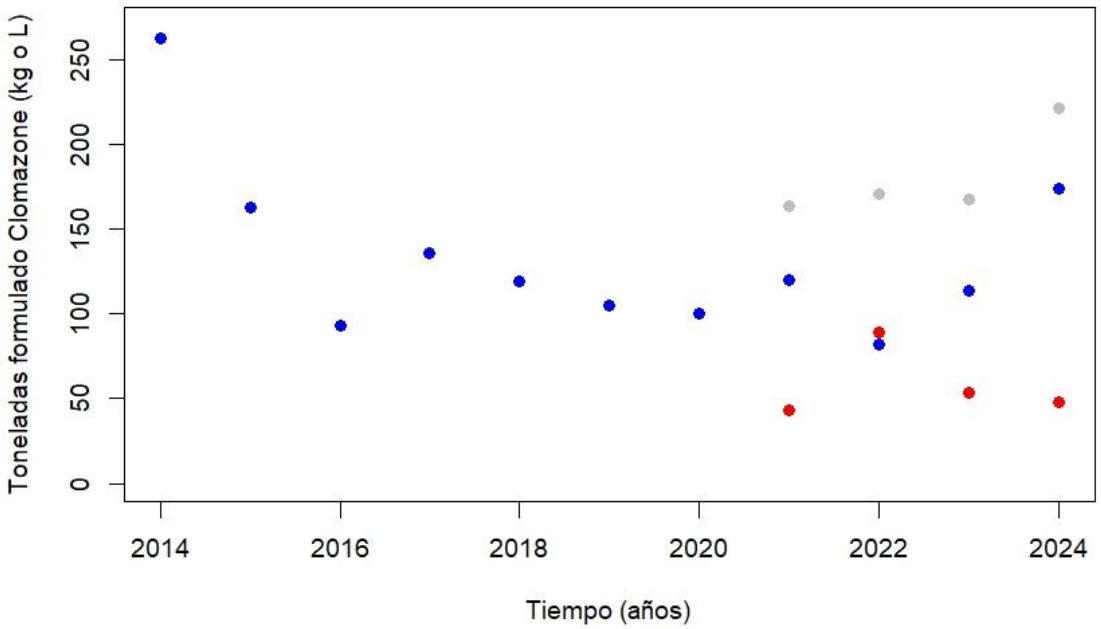

Figura 3. Toneladas (kg o L) de formulados del herbicida Clomazone; los puntos azules representan las toneladas importadas, mientras que los rojos representan las producidas en Uruguay, entre 2014 y 2024 y los grises, la suma entre los importados y los producidos a nivel nacional (MGAP).

Este compuesto puede tener efectos adversos sobre organismos que no son sus objetivos de acción, como por ejemplo, cambios en actividad enzimática y en el metabolismo/fisiología, producir estrés oxidativo, afectar la reproducción, la alimentación y el comportamiento (**Miron et al., 2004**; **Cattaneo et al., 2012**; **Singh & Garg, 2022**), entre otros; algunos de estos efectos son tratados en profundidad en el capítulo 2 de esta tesis.

2.2. Nanopartículas

Las nanopartículas son partículas de tamaño muy pequeño (menores a 100 nm), generalmente compuestas por un núcleo, que constituye la parte central y está formado por el material que le da el nombre a la nanopartícula (por ejemplo, metales, óxidos metálicos, carbono). Una capa superficial, diferente al núcleo, en la que se introducen productos químicos, como por ejemplo, estabilizantes; y una interfase de interacción con la matriz circundante (**Shin et al. 2016**). Se emplean en diversos ámbitos, como la medicina, la cosmética, el medioambiente —donde pueden actuar como reguladoras de contaminación— , y la electrónica. Su utilización se debe a propiedades como capacidad de adsorber metales, degradar compuestos orgánicos, su elevada relación superficie/volumen, así como sus características ópticas y catalíticas, entre otras (**Daniel & Astruc, 2004**; **Biswas and Wu, 2006; Singh et al., 2019; Astruc et al., 2020; Kafeel et al., 2022**).

Su elevada capacidad de transporte permite utilizarlas para transportar medicinas dentro del cuerpo o como nanomonitores, detectando posibles amenazas dentro y fuera del cuerpo humano (**Chow, 2005**). Además, por sus variaciones de color y forma pueden ser utilizadas en aplicaciones de bio-imagen (**Dreaden et al., 2012**).

En los últimos años, las inversiones en nanotecnología han aumentado considerablemente de forma global (**Alsaba et al., 2020**); en Uruguay, es una actividad principalmente de investigación en la que se vinculan actores públicos y privados, que fue reconocida como un área de estudio/trabajo importante en el Plan Nacional Estratégico de Ciencia, Tecnología e Innovación en el año 2010 (**Chiancone y Larrechea, 2012**).

Esta intensificación en la actividad ha significado el incremento en la producción de nanopartículas y en la preocupación y el cuestionamiento de los posibles impactos que pueden causar en el medio ambiente, principalmente en el medio acuático (**Ray et al., 2009**). Es por ello que se ha desarrollado la nanoecotoxicología, una rama dentro de la ecotoxicología que evalúa los efectos que estos materiales pueden tener sobre los ecosistemas (desde el nivel de organismo hasta el nivel ecosistémico) (**Asharani et al., 2008; Xue, 2013; Nagamatsu et al., 2021; Oya-Silva et al., 2021; Kafeel et al., 2022; Féola et al., 2025**).

2.2.1. Sulfuro de bismuto (Bi_2S_3)

El sulfuro de bismuto es un compuesto semiconductor a partir del cual pueden generarse nanopartículas por distintas técnicas. Presenta aplicaciones en diversos campos como la electrónica, en la formación de equipos como celdas solares, baterías, sensores (e.g. termoeléctricos). También ha sido ampliamente utilizado en medicina, en el monitoreo y tratamiento de células cancerígenas, y como vehículo para el transporte de medicinas y en imagenología (rayos X) (**Ajiboye et al., 2021**). Las nanopartículas de sulfuro de bismuto pueden presentar diversas formas, como estrellas, flores, balones, tubos, varillas, entre otras (**Ajiboye et al., 2021**).

Este compuesto ha sido utilizado en la remoción de contaminantes que han llegado al ambiente a través de descargas industriales, como plásticos, cueros, textiles, entre otros (**Sang et al., 2020**) No obstante, se tiene poco conocimiento del efecto que estas nanopartículas pueden tener sobre los ecosistemas y la salud humana (**Ergenler et al., 2023**).

2.3. Impactos de pesticidas y nanopartículas en el medio acuático

Los impactos de estos compuestos en los ecosistemas acuáticos pueden perjudicar a los organismos que habitan en ellos y que no son su objetivo (organismos no-blanco) (**Stevanovic et al. 2017; Ernst et al. 2018; Chen et al. 2019**), provocando efectos a distintos niveles de organización biológica (por ejemplo, celular, metabólico/fisiológico o poblacional), cuyas respuestas pueden medirse mediante diversos indicadores conocidos como

biomarcadores. El daño celular, el consumo de oxígeno, cambios estructurales en distintos tejidos, la tasa de ingestión, la mortalidad o el crecimiento son indicadores a distintos niveles (Fig. 4; **Montagna & Collins 2008; Montagna 2010; dos Santos & Martínez 2014; Cossi et al. 2018; Michel et al. 2025).** Cabe resaltar que todos los niveles son importantes, aunque resaltan aspectos diferentes y contribuyen de forma diferente en la comprensión de los efectos de los contaminantes. Los inferiores, como el bioquímico- en general son más sencillos de abordar y relacionar con una causa específica, en comparación a los niveles superiores (**Newman, 2015**). Además, puede existir una concatenación de efectos, o sea que un evento observado a niveles superiores, puede ser el resultado de efectos a niveles inferiores; por ejemplo, cambios en hormonas relacionadas a la reproducción debido a la exposición a un contaminante puede causar efectos en la reproducción de los organismos, afectando la población (**Kidd et al., 2007**). De acuerdo con **Newman (2015)** y **Cossi (2018)**, las respuestas en niveles inferiores son altamente sensibles y específicas frente a la exposición a contaminantes, pero no siempre resulta sencillo su interpretación desde el punto de vista de efectos ecológicos. Sin embargo, las respuestas a niveles superiores, como el poblacional y el comunitario, presentan mayor relevancia ecológica, aunque al ser menos específicos hace difícil identificar el agente causal.

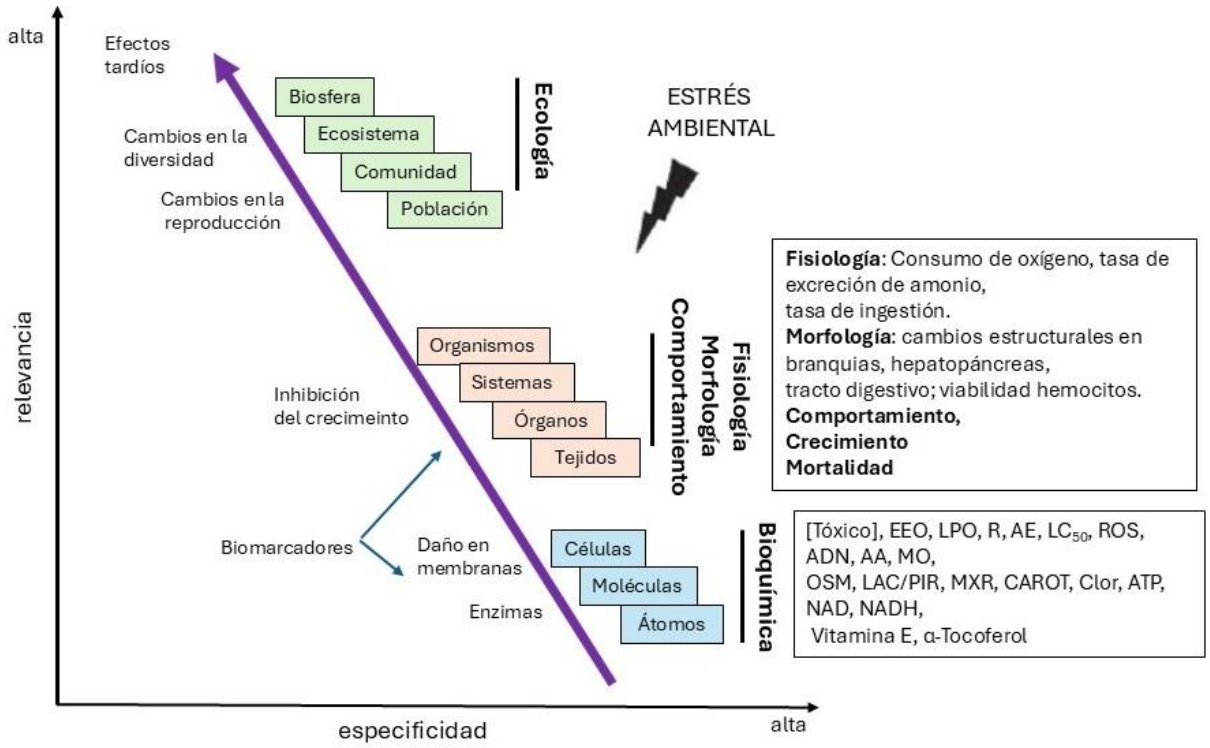

Figura 4. Biomarcadores evaluados en los diferentes niveles de organización que surgen a partir de la revisión bibliográfica de macroorganismos invertebrados y productores primarios macroscópicos nativos de la región sur de América del Sur. Tomado de Cossi (2018) y adaptado por Féola. EEO: enzimas estrés oxidativo; LPO: peroxidación lipídica; R: reservas energéticas (lípidos, carbohidratos, proteínas); AE: actividad enzimática (Na/K ATPasa, colinesterasa y acetilcolinesterasa); ROS: especies reactivas de oxígeno; AA: aminoácidos (concentración); MO: materia orgánica; MT: metalotioneínas; OSM: osmolaridad; LAC/PIR: lactato piruvato; MXR: resistencia a xenobióticos múltiple; CAROT: carotenoides; Clor: clorofilas a y b.

Para evaluar el potencial impacto que una sustancia/compuesto puede ocasionar en un organismo, se realizan bioensayos. Estos consisten en exponer a organismos, células, tejidos, a diferentes concentraciones de la sustancia/compuesto de interés y evaluar su respuesta eco-fisiológica. En una primera instancia se realizan ensayos denominados letales o de toxicidad aguda, en los que se determina la concentración letal (LC_{50}; concentración que mata al 50% de los organismos expuestos) en un período de tiempo relativamente corto (entre 24 y 96 hs) (**Lavarías & García 2015; Suvetha et al. 2015; USEPA, s.f.**). Si bien estos ensayos se enfocan principalmente en evaluar la mortalidad, también pueden incluir la evaluación de efectos subletales de respuesta rápida, como cambios en el comportamiento (**Newman, 2014**). Generalmente, el LC_{50} obtenido se utiliza para definir las concentraciones de los ensayos subletales o de toxicidad crónica, en los que los organismos son expuestos a concentraciones menores que las letales y por tiempos más prolongados (y variables). En estos ensayos se evalúan efectos sobre distintos biomarcadores, así como a procesos como el crecimiento y la reproducción (**Crupkin et al. 2013; Ma et al. 2014; Santadino et al. 2014**). Los ensayos subletales no presentan un procedimiento completamente estandarizado ya que difieren en aspectos como la duración, las metodologías empleadas y los biomarcadores a evaluar, en función de los recursos disponibles, el objetivo del estudio, los requerimientos del organismo a estudiar, entre otros factores (**USEPA et al., 2002; Crupkin et al., 2013; Santadino et al., 2014**).

En cuanto a la dimensión espacial de los experimentos, estos pueden realizarse a distintas escalas: a- macrocosmos, que son a gran escala y pueden tomar lugar en los ambientes naturales de los organismos, estando sujetos a las características de éstos (variables ambientales sin controlar); b-mesocosmos, de menor escala que el anterior y donde pueden controlarse algunas variables, lo que permite acercarse a lo que sucede en un ambiente pero con cierto grado de control; c-microcosmos, que se desarrollan a pequeña escala, empleando recipientes pequeños (e.g. peceras) y bajo condiciones de laboratorio controladas. En estos bioensayos, si bien hay alto nivel de control de las condiciones experimentales, representan el ambiente de manera muy simplificada. Dada su menor complejidad de implementación y seguimiento, son los más empleados en ensayos de toxicidad acuática, aunque no existe un diseño único, sino que aspectos como el número de réplicas, número de organismos por réplica y tiempo experimental, pueden variar (**Matheson, 2008; Lin et al., 2021**).

En estos ensayos se emplean tanto organismos que son definidos como organismos modelo como el cladócero *Daphnia* y el pez cebra *Danio rerio* que se caracterizan por presentar protocolos estandarizados, lo que permite obtener resultados reproducibles y comparables entre estudios, así como extrapolar los efectos observados a otros organismos (**Festing & Altman, 2002**); como otras especies que presentan potencial como bioindicadores. Se caracterizan por presentar pequeño porte, ciclo de vida corto, de fácil manipulación y

mantenimiento en laboratorio (**Montagna & Collins, 2005**). En general se han utilizado principalmente especies exóticas; sin embargo, en los últimos años ha aumentado el uso de especies nativas. Esto resulta ventajoso ya que se trata de organismos propios de los ecosistemas que habitan, lo que permite evaluar de forma más realista los efectos de los contaminantes (**Bertrand & Iturbutu, 2023**). Además, evita las consecuencias de las especies exóticas en los ecosistemas nativos.

En América del Sur son muchas las especies de macroinvertebrados de diversos grupos taxonómicos utilizados en ensayos de evaluación de efectos de factores bióticos/abióticos y de toxicidad. Para conocer el estado del arte de ese campo y en el marco de esta tesis, fue realizada una revisión bibliográfica con el fin de evaluar la cantidad de especies nativas acuáticas de macroinvertebrados (MI) y productores primarios macroscópicos (PPM) de la región comprendida por Argentina, Brasil, Chile y Uruguay, utilizados con diversos fines (mantenimiento, cultivo y/o experimentación). Se obtuvo que 20 especies de MI y 2 de PPM han sido utilizadas en bioensayos, en su mayoría del tipo microcosmos. Entre los MI se encuentran crustáceos decápodos y anfípodos, moluscos gasterópodos y bivalvos, entre otros **(Montagna & Collins, 2005; Jacomini et al., 2006; Sauco et al., 2010; Giusto et al., 2012; Arrighetti et al., 2018; Cossi et al., 2020**). Esta revisión permitió conocer el estado del arte en cuanto a las especies cultivadas, ya sea para comercializar o con fines experimentales, lo que facilitó la selección del organismo modelo de estudio de este trabajo, el caracol dulceacuícola *Pomacea* sp.

2.4. *Caracol manzana Pomacea sp.*

El género *Pomacea* (Phylum: Mollusca; Clase: Gastropoda; Familia: Ampullariidae) es nativo del continente americano, donde habita en zonas tropicales y subtropicales (**Horgan, 2017**). En lugares como Asia y Europa ha sido ingresado, donde se convirtió en un organismo invasor, causando importantes daños en zonas de cultivo, principalmente de arroz (**Horgan, 2017**).

Son organismos operculados, que cuentan con respiración branquial y pulmonar. Su dieta es principalmente herbívora, generando gran impacto sobre la vegetación de la zona en que habitan (**Boland et al., 2008**). Son organismos ovíparos que desovan masas de huevos que depositan en estructuras que se encuentran sobre el nivel del agua (e.g. vegetación) **(Cowie, 2002**). El mantenimiento en cautiverio ha sido bien establecido para algunas especies del género (**Vazquez-Silva et al., 2011; Rojas & Perez, 2012**). Algunas especies de *Pomacea* se utilizan como bioindicadores a nivel mundial, evaluando el efecto de diferentes factores como variables ambientales, agroquímicos, metales, medicamentos, nanopartículas y otras sustancias (**Seuffert y Martin, 2013, 2017; Xu et al., 2017; Jeyavani et al., 2023; Yang et al., 2023**).

En Uruguay han sido reportadas cuatro especies de Pomacea: *P. canaliculata, P. maculata, P. megastoma y P. scalaris*, (**Röhrdanz, 2017**). Pero **Hayes et al. (2012**) previamente habían definido una quinta especie denominada *Pomacea* sp., críptica a *P. canaliculata*, clasificada bajo el mismo nombre por ser muy similares morfológicamente, pero genéticamente distintas (**Bickford et al., 2007**). *P. canaliculata* se encuentra dispersa por cuerpos de agua dulce de todo el Uruguay (**Röhrdanz, 2017**), solapando la presencia de *Pomacea* sp. Esta última ha sido encontrada en zonas como como la Laguna de Briozzo o Escondida (Aguas Dulces, Rocha) (**Bañobre et al., 2020**). Este cuerpo de agua presenta una superficie de 0.167 km^2, sin conexión con actividades agrícolas (**Noguera et al., 2022**).

Desde el 2020 se realizaron colectas de ejemplares de *Pomacea* sp. y sus puestas en la Laguna de Briozzo que se mantienen en el Laboratorio de experimentación y cultivo de organismos acuáticos del Centro Universitario Regional del Este. Con el tiempo se ha logrado la reproducción en cautiverio, lo que nos permite contar con un pool de organismos aclimatados por más de una generación a condiciones de laboratorio para la realización de los distintos ensayos (Fig. 5). Si bien esto requiere de un trabajo de mantenimiento constante, como limpieza y alimentación, es ventajoso porque brinda una disponibilidad casi constante de organismos e independiza de realizar colectas para los ensayos.

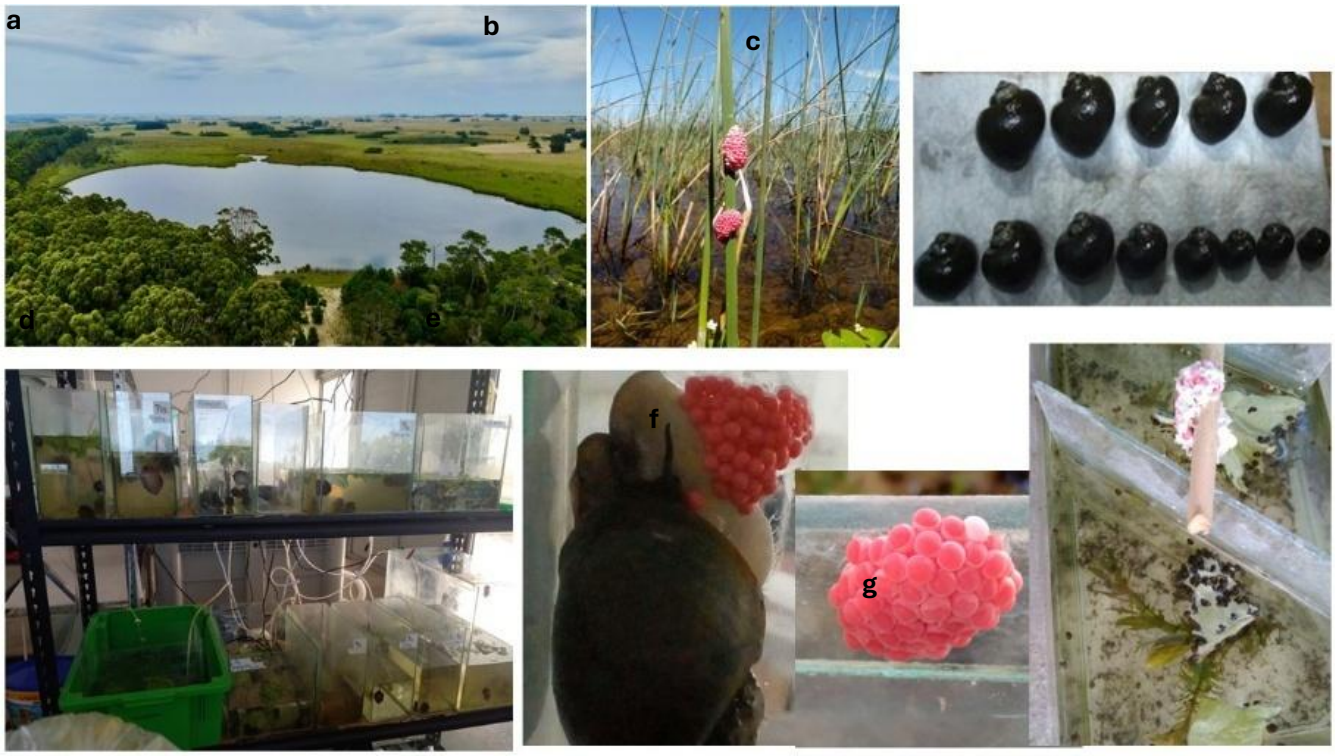

Figura 5. Colecta y mantenimiento de *Pomacea* sp. a: en la laguna de Briozzo (Rocha, Uruguay) fueron realizadas los muestreos; b y c: puestas y ejemplares de caracol manzana colectados; d: mantenimiento en laboratorio; e y f: evento de desove y puesta, respectivamente; g: caracoles recién eclosionados en el laboratorio.

Todos los residuos generados en los bioensayos realizados en este trabajo fueron descartados según las normas de bioseguridad vigentes, siguiendo las indicaciones del fabricante y los protocolos de la empresa encargada de la gestión de residuos peligrosos.

2.5. Efecto de las variables ambientales sobre organismos acuáticos

La temperatura, la salinidad, el pH, entre otras variables ambientales, tienen influencia sobre los organismos que habitan los distintos ecosistemas acuáticos. En los ambientes de agua dulce, la temperatura es considerada el factor más relevante (**Johnson et al., 2024**), ya que afecta tanto las condiciones fisicoquímicas del medio, como ocurre con el oxígeno disuelto —que disminuye con el aumento de la temperatura—, como los organismos. Estas variaciones térmicas pueden provocar cambios en las poblaciones y/o comunidades de diversos organismos, como los macroinvertebrados. Entre los efectos observados se incluyen modificaciones en los tiempos de eclosión y desarrollo, en la coloración, en riqueza de especies, en la composición de la cadena trófica, entre otros **(Bonacina et al., 2023**); y también puede generar efectos a nivel individual, en distintos biomarcadores como el consumo de oxígeno, la alimentación, el crecimiento, el comportamiento, la reproducción **(Montagna, 2011; Abram et al., 2016; Deaton et al., 2016**)

El aumento de la temperatura, una vez superado cierto umbral, afecta a los organismos (estrés térmico) provocando alteraciones en diversos biomarcadores y aumentando su susceptibilidad frente a la presencia de sustancias contaminantes, y, por lo tanto, potenciando sus efectos (**Kazmi et al., 2022; de Souza et al., 2023**). Tanto para clomazone como para las nanopartículas de sulfuro de bismuto existen muy pocos estudios en los que se tome en cuenta la interacción del contaminante y la temperatura (**Freitas, 2017**).

Comprender estos procesos requiere determinar los efectos de la temperatura y las correspondientes curvas de desempeño térmico, gráficos que registran el comportamiento de una determinada respuesta, como consumo de oxígeno, alimentación, crecimiento, comportamiento, frente a las variaciones de temperatura. Se caracterizan por comenzar con un aumento de la respuesta frente al incremento de temperatura, hasta alcanzar un valor máximo u óptimo, para luego disminuir frente a temperaturas elevadas; esto indica que luego del óptimo, los organismos se encuentran frente a estrés térmico, lo que afecta su desempeño (**Gutiérrez-Pesquera, 2015; Segura et al. 2018; Arroyo et al. 2022**). A partir de ellas se definen los límites de tolerancia térmica de las especies y se establecer los rangos óptimos en los que la performance de los organismos no se ve comprometida.

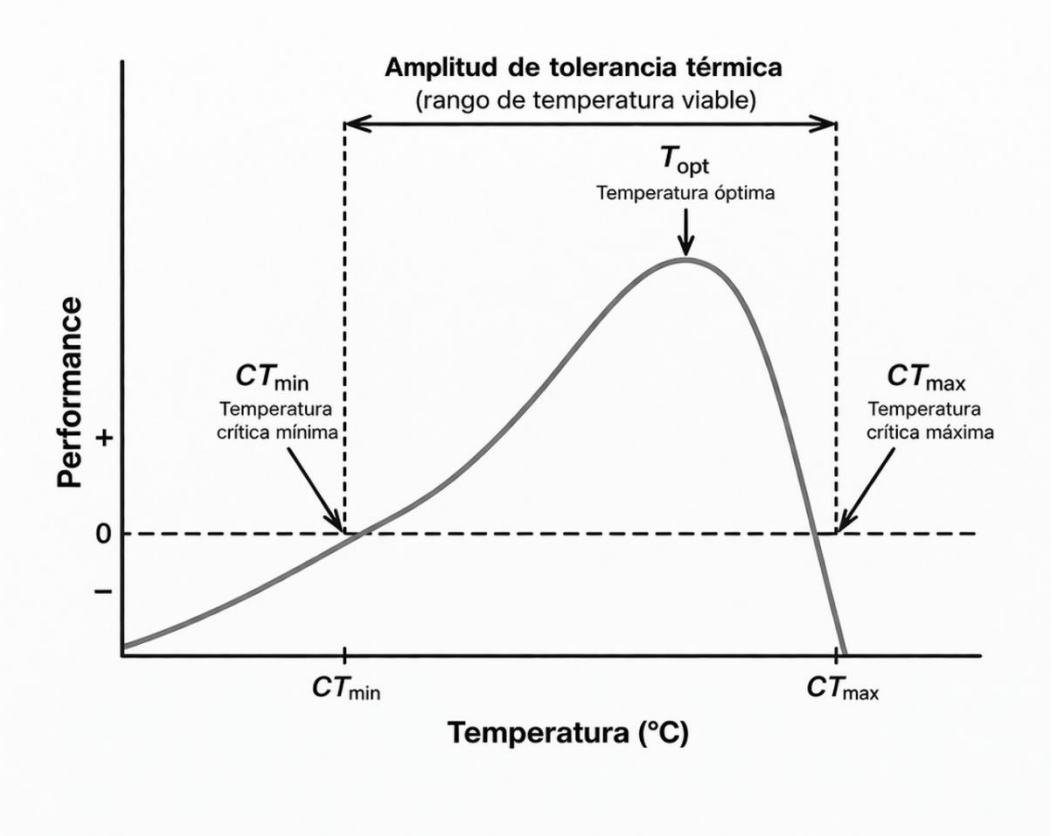

Figura 6. Curva de desempeño térmico. Indica el comportamiento de una respuesta de un organismo, como consumo de oxígeno, alimentación, crecimiento, frente a variaciones de temperatura. La temperatura mínima (CTmin) y la temperatura máxima (CTmax) representan los límites de tolerancia térmica; mientras que Topt representa la temperatura en que la respuesta evaluada se encuentra en su óptimo.

Esta información resulta esencial para evaluar con precisión los efectos de los contaminantes emergentes, porque permite discernir si las respuestas observadas en los organismos se deben exclusivamente a la exposición a dichas sustancias o si están moduladas por la temperatura. Además, el análisis y la comprensión del efecto combinado de ambos factores resulta fundamental, dado que en los ambientes naturales las variaciones térmicas pueden amplificar o atenuar los efectos inducidos por los contaminantes sobre los organismos no-blanco y, como fue mencionado, son escasos los estudios al respecto.

3. Hipótesis

El aumento de la temperatura y la exposición a sustancias contaminantes afectará el balance energético de *Pomacea* sp., reflejada en un aumento del consumo de oxígeno y la tasa de excreción de amonio. Esto aumentará el consumo de energía de los organismos, lo que llevará a un incremento en el consumo de alimento (TI) y disminuirá la energía disponible para crecimiento. A temperaturas extremas, el metabolismo disminuirá hasta alcanzar niveles de inactividad.

4. Objetivos

4.1. General

Determinar los efectos letales y subletales de Clomazone y de nanopartículas de Sulfuro de Bismuto sobre el caracol manzana (*Pomacea* sp.), con el fin de aportar información de referencia.

4.2. Objetivos específicos

a- Realizar una revisión del estado del arte sobre las especies de macroinvertebrados acuáticos nativos (de la región sur de América del Sur) que han sido utilizadas en laboratorio con diversos fines (cultivo, experimentación, mantenimiento) a nivel regional (**Material suplementario**);
b- Evaluar los efectos de la temperatura sobre el metabolismo de *Pomacea* sp y caracterizar las curvas de desempeño térmico de las distintas respuestas, con el fin de determinar las condiciones de temperatura óptimas para realizar ensayos de exposición a contaminantes emergentes;
c- Determinar el LC_{50}-96hs del herbicida Clomazone para *Pomacea* sp.;
d- Evaluar los efectos subletales de Clomazone en *Pomacea* sp.;
e- Evaluar los efectos subletales de nanopartículas de sulfuro de bismuto (Bi_2S_3) en *Pomacea* sp.

5. Estructura de la tesis

La presente tesis se presenta en la modalidad de compendio de artículos, los cuales están asociados a los objetivos específicos de la misma. Además, cada uno constituye un capítulo que incluye una breve introducción al tema central, seguida de una descripción de la metodología realizada, los resultados obtenidos y la discusión de los principales hallazgos. Finalmente se presenta una discusión general y las conclusiones.

1-Capítulo 1: Efectos de la temperatura en la fisiología, crecimiento y sobrevivencia en el caracol manzana *Pomacea* sp (artículo publicado en la revista Aquatic Ecology)

2-Capítulo 2: Efectos agudos y crónicos del herbicida clomazone sobre *Pomacea* sp (artículo publicado en la revista Ecotoxicology)

3- Capítulo 3: Impacto ecotoxicológico del material semiconductor Bi_2S_3 en la pulga de agua (*Daphnia magna*) y en el caracol manzana *Pomacea* sp (artículo publicado en Journal of Nanoparticle Research)

6. Capítulo I.

Artículo 1: Publicado en Aquatic Ecology (https://doi.org/10.1007/s10452-025-10171-4) en enero de 2025.

Temperature effects on the physiology, growth and survival of the apple snail *Pomacea* sp. (Perry, 1810)

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En este artículo y en el marco del objetivo "b", analizamos y caracterizamos las curvas de desempeño térmico para las distintas respuestas (consumo de oxígeno, OC; tasa de excreción de amonio, TEA; tasa de ingestión, TI), el crecimiento y la mortalidad de juveniles de *Pomacea* sp., sometido a un amplio rango de temperatura. Encontramos que existe una temperatura óptima para el mantenimiento de los organismos, sin que sea observado un efecto estresante de dicho parámetro. Esto es útil para conocer a qué temperatura, o en que rango de temperatura es viable mantener a los organismos en cautiverio, sin estar produciendo un efecto negativo, lo que porta al objetivo "b". Además, el conocimiento de los rangos térmicos óptimos es importante en el contexto de cambio global, ya que aumentos en la temperatura podrían desplazar a los organismos fuera de su rango de tolerancia, afectando su desempeño fisiológico, crecimiento y/o sobrevivencia. Sin embargo, otras especies podrían verse favorecidas y expandir su distribución, colonizando nuevos ambientes.

Aquat Ecol
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Temperature effects on the physiology, growth and survival of the apple snail *Pomacea* sp. (Perry, 1810)

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Abstract

The energetic balance of organisms depends on the energy assimilated from food to fulfill vital functions (e.g. maintenance and somatic growth). Temperature drives the energetic balance and the performance of organisms. Evaluating the effect of temperature on multiple components is relevant to understanding the response to climate change. Here, we evaluated the thermal performance curve (TPC) for oxygen consumption (OC), ammonia excretion (AER) and ingestion rates (IR) in temperatures from 6 to 30 °C in the freshwater apple snail *Pomacea sp*. Additionally, we evaluated the evolution of somatic growth, IR and survival for *ca.* 400 days snails exposed to fluctuating environmental temperature (OT; T range = 7–27 °C) and laboratory conditions (IT; T range = 12–19.6 °C). The TPC of OC and AER showed a unimodal pattern, with an optimum at 22 and 28 °C, respectively. IR showed a monotonic increase towards the warmest temperature (30 °C). Between ~ 15–20 °C weight increases with temperature while IR remains constant; suggesting snails invest energy mostly in growth. The final size achieved by snails in IT and OT were similar (~ 500 mg) while maximum IR was lower in IT (~ 400 mg/g.d vs ~ 800 mg/g.d of ET). Survival was similar between treatments, but growth parameters fitted by a modified Von Bertalanffy growth function with a temperature dependence on growth coefficient differed. TPC were different, which could generate mismatch between resource acquisition, assimilation and excretion affecting growth patterns. Evidence on a high capacity to deal with large thermal variability suggests adaptations of the snail to cope with climate change.

Keywords Apple snail · Climate change · Energetic metabolism · Thermal performance curve

Introduction

Understanding the energetic balance of organisms in changing environments is critical to predict the response of organisms, populations and communities to the effects of global climatic change (Abram et al. 2016). Heterotrophic organisms are able to keep their integrity by using assimilated energy obtained from food to sustain somatic maintenance, growth and reproduction as well as excreting toxic compounds (*e.g.* ammonia) (Hill and Wyse 1992; Brown et al. 2004; Berneche and Allen 2015). The energetic balance of an organism can be represented following Winberg (1956) as:

$$C - H = A = R + U + P$$

where C is the energy consumed from food, H is the energy lost through feces, A is the assimilated energy, R is the energy spent in respiration, U is the energy invested in ammonia excretion and P is the energy available for processes like growth, somatic maintenance and reproduction. This is similar to more recent formulations of the energetic balance (Hou et al. 2008; White et al. 2022) and reflects general properties of living systems. Environmental conditions affect this balance and the particular effect on each of the components will drive the eco-physiological response of the organism. Temperature is a key factor affecting metabolic rates of ectothermic organisms (Buckingham and Freed 1976; Kooijman 2010) at multiple scales, from daily fluctuations, seasonal patterns and climate change (Kingsolver et al. 2015).

Previous works evaluated temperature effects over the different components in different groups of aquatic animals (Gophen 1976; Hao et al. 2014; Deaton et al. 2016) but evaluations of multiple components at different scales are scarce. Temperature is a relevant driver of metabolic activity (Gophen 1976; Gillooly et al. 2001; Seuffert et al. 2010; Kooijman 2010) particularly for ectotherms, which mostly depends on environmental temperature to perform their activities, but with consequences for the whole food web (Gibert et al. 2022). In the face of an increasing global trend for temperature, as well as a predicted change in amplitude of the fluctuations (Salinger 2005), the effects on the different components of the energetic balance are critical. Thus, an adequate characterization of the different metabolic rates to temperature (*i.e.* thermal performance curves; TPC) is key to understanding organisms' response.

The TPC is generally unimodal and skewed with an exponential increase and a rapid decrease after the optimum (Stamou et al. 2000; Amarasekare and Savage 2012; Segura et al. 2018; Arroyo et al. 2022). The thermal response is expected to differ among organisms acclimated to stable or variable environments (Amarasekare and Savage 2012; Seo et al. 2020) within the ontogenetic lifetime (Kingsolver et al. 2015), but the evaluation of these predictions is difficult under controlled conditions.

Aquatic macroinvertebrates (> 5 mm) are useful models' study to explore these patterns under laboratory conditions. For example, experiments directed to evaluate temperature responses, showed an increase in R and U in the bivalve *Dreissena polymorpha,* the prawn *Macrobrachuim rosernbergii* and the shrimp *Farfantepanaeus paulensis* (Aldridge et al. 1995; Niu et al. 2003; Barbieri et al. 2016). But in some cases, C and A decreased with temperature, thus decreasing the energy available to invest in growth (P) (Aldridge et al. 1995). The general decrease of metabolic activity at temperatures higher than the optimum was also observed (Henry et al. 1993; Hao et al. 2014) while mortality also increases with temperature (Hao et al. 2014; Zhang et al. 2019). Freshwater snails follow the same patterns in the TPC (Santos et al. 1987; Henry et al. 1993; Pascual and Drake 2008; Wenasa Frifer 2016; Calvo 2016), while growth was observed to increase with temperature in *P. canaliculata* (Seuffert and Martin 2013). However, the characterization of multiple metabolic responses in the same group of organisms is scarce, which precludes its generalization.

Freshwater snails of the genus *Pomacea* are an important part of food webs in their native range in South America, while it is an invasive species in Asia and Europe, generating relevant damage to ecosystem and economic loss in crops (mainly rice) and as vectors for human diseases (Agudelo Patino 2017). Besides, its laboratory maintenance and culture are relatively straightforward (Vazquez-Silva et al. 2011; Rojas and Perez 2012). Experimental evidence suggests a temperature effect on activity, growth and feeding rates in *P. canaliculata, P. maculata* and *P. megastoma* (Heiler et al. 2008; Seuffert et al. 2010; Seuffert and Martin 2013; Calvo 2016). However, the specific response of multiple metabolic rates to temperature and variability remains unclear. Hayes et al. (2012) determined through morphological analysis and DNA sequencing, that there exists a species of *Pomacea* (named *Pomacea* sp 7), cryptic with respect to *P. canaliculata*, that inhabits freshwater bodies in Uruguay and was analyzed previously (as *Pomacea* sp.) by Bañobre et al. (2020). However, there is no information about the effects of temperature over its performance. In this context, the aim of this work is to evaluate the effects of temperature and/or characterize the TPC for different components of the metabolism (oxygen consumption, ammonia excretion rate and ingestion rate), growth and survival of *Pomacea* sp. juveniles.

Materials and methods

Adult snails of *Pomacea* sp. were collected in the Briozzo Lagoon (34°17′40″S 53°48′18″W; Rocha, Uruguay; Fig. 1) in December 2020. Although the individuals collected in the Briozzo lagoon are morphologically very similar to *P. canaliculata*, they belong to a cryptic species as suggested by morphological and DNA analysis (Hayes et al. 2012). We employ *Pomacea* sp. following previous publications using organisms from this lagoon (Bañobre et al 2020). Snails were maintained in environmental conditions under natural photoperiod, where they spawned; the newly hatched snails were used for experiments. Then, organisms were taken to the laboratory (except those used in the outdoor treatment in Trial II) and maintained in aquaria at a temperature range of 12 to 18 °C, and fed ad libitum with hydroponic fresh lettuce, 2 or 3 times a week following Estebenet and Cazzaniga (1992).

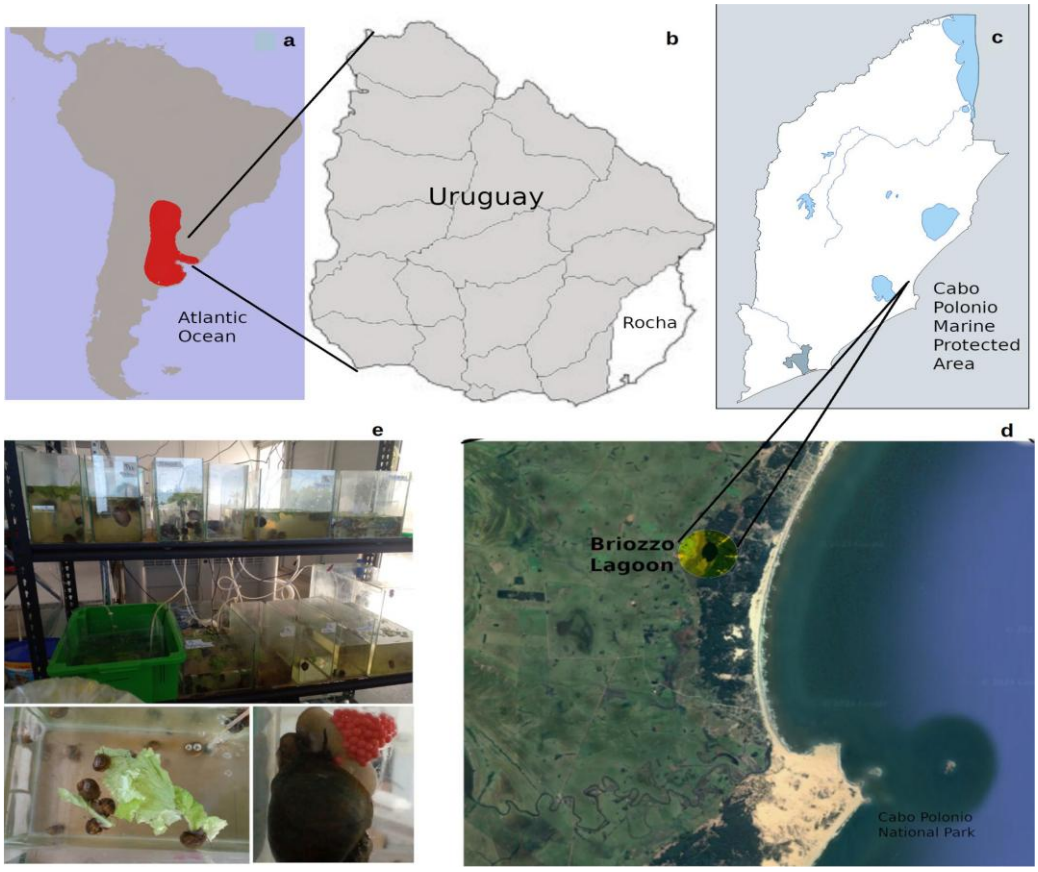

Fig. 1 Distribution and site of collection of the apple snails *Pomacea* sp used in the present study. **a** Distribution of *P. canaliculata* in South America, modified from Hayes et al. (2012). **b, c** Snails were collected in the East coast of Uruguay in (**d**) a freshwater lagoon (Briozzo Lagoon) close to the Cabo Polonio Marine Protected Area. **e** After reproduction events, snails were maintained in cultures under controlled laboratory conditions. New hatched juvenile snails were used in the present experiments

Trial I: characterization of the thermal performance curve (TPC)

In November 2021, juvenile snails (~ 10 months old) weighting on average 0.19 ± 0.04 g (mean ± SD) and measuring 4–5 mm long were exposed to different temperature treatments (6, 12, 18, 24 and 30 °C). This low size was due to the low temperature at which they were kept (12–18 °C), then we make sure that they did not reach sexual maturity (a factor that could influence the metabolic response); related species of *Pomacea* reach this stage at a size between 20–60 mm, depending on the temperature at which they were raised (Tiecher 2015; Gurovich 2021). Besides, the rearing temperature is close to the temperature suggested by Seuffert and Martin (2013) to raise snails for experimentation. Temperature range for experiments was selected based on the temperature when snails were maintained at environmental conditions (maximum temperature of 27 °C) and in Trial II (where the minimum temperature recorded was 7 °C). These temperatures are well within the range in which species of this genus can survive (from 4 to 36 °C) (Ramakrishnan 2007; Rawlings et al. 2007) and are useful to characterize the metabolic response.

In each treatment, we measured rates for six different snails (replicas). They were kept individually (Sauco et al. 2013; Velasco 2014) in plastic chambers, with 50 ml of dechlorinated tap water (DTW). First, they were placed in an incubator, where temperature was increased/decreased from laboratory conditions (18 °C at the moment of the trails) at a rate of 1 °C/day to the temperature of each treatment and then transferred to a second incubator, where the experiment took place (e.g. first, snails of the 24 and 30 °C were placed in the first incubator were temperature increased 1 °C/day to reach 24 °C; then, snails selected for this treatment were transferred to the second incubator, while temperature continued to rise 1 °C/day, until reaching 30°C) (Leung et al. 2021). We started the trail with the treatment of 18 °C. The light-to-dark cycle was 12 h:12 h; temperature and pH were measured daily, with a pH sensor (EcoSense pH100A).

After 24 h in the second incubator, snails were placed individually in sealed chambers with 10 ml of DTW to measure oxygen consumption. The volume was evaluated in previous tests in order to record the signal. Oxygen consumption was measured by the optic method using a respirometer Oxy-4 m (Portner et al. 2006). Oxygen (O2) concentration was registered continuously for ~ 40 min and the Oxygen consumption rate (OC) was calculated according to the following equation:

$$O(mg/g.h) = \frac{(O_{2f} - O_{2i}) * v}{W * t} \quad (1)$$

where O_{2i} and O_{2f} are the initial and final oxygen concentration (mg/L) respectively, *v* is the volume of the vial (L), W is the snail weight (g), and *t* is the elapsed time (hours) between initial and final records.

Then, snails were placed again in the plastic chambers with renewed DTW. Samples of 12 ml were taken from each chamber at t = 0 and t = 48 h with a syringe and filtered with glass fiber filters MGF (Munktell, Ahlstrom). Ammonia (NH_4) levels were determined using the Indophenol blue technique (Koroleff 1970) and ammonia excretion rate (AER) was calculated according to the following equation:

$$AER(\mu g/g.d) = \frac{(NH_{4f} - NH_{4i}) * v}{W * t} \quad (2)$$

where NH_{4i} and NH_{4f} are the initial and final ammonia concentration (μg/L), and t is the time (days) elapsed between the two records.

Snails were fed with the triple of their weight with hydroponic lettuce rinsed with tap water, dried in paper towel for 5 min and weighed in precision balance (0.0001 g). Ingestion rate (IR) was estimated as the difference in lettuce fresh weight (*LW* in mg) after 24 h:

$$IR(mg/g.d) = \frac{LWi - LWf}{Wt} \quad (3)$$

Trial II: temperature variability effect in growth, IR and survival

We placed 42 juvenile snails of two months old and wet weight (mean ± SD) of 0.02 ± 0.007 g in two individual plastic chambers (with 21 divisions each) in March 2021, for 400 days. One chamber was exposed to environmental temperature (outdoor; OT) protected from direct sunlight, while the other chamber was kept inside laboratory with reduced temperature fluctuations (indoor; IT). Water temperature was registered 2 times a week in both chambers with an EcoSense pH100A sensor. Water was renewed with DTW once a week. Snails weight was registered every 15–20 days with precision balance (0.0001 g) and mortality were monitored daily. Snails were fed two times a week with their own weight of hydroponic lettuce. IR was estimated following Eq. 3. Growth rate was estimated as the difference in weight between successive sampling events divided by the difference in time.

Statistical analysis

Trial I

The likelihood ratio test (LRT) was used to determine differences in the mean or variance of rates (OC, AER, IR) among temperature treatments. When differences in variances were found, we performed a weighted regression using the inverse of standard deviation of each treatment as weights (Linnet 1993).

Thermal Performance Curves (TPC) were constructed for OC, AER and IR using water temperature as the independent variable. We evaluated the fit of five models to TPC (Table 1) which included simple linear regression, a quadratic and an exponential model. We also fitted

(i) a piecewise segmented model (Muggeo 2003) which is more flexible than a quadratic model yet simple (Segura et al. 2018) and (ii) a more realistic variant of the Sharpe-Schoolfield (SH) model (named Schoolfield High model) recently proposed (Kontopoulos et al. 2018). For a description of models and parameters we refer to Table 1. The segmented model is composed of two slopes (a1, a2), a break point (*bp*) and one intercept (b1). In the Schoolfield High model, c is the specific metabolic rate, e is the Euler number, Ea is the activation energy, Eh is the deactivation energy of high temperature (where the enzymatic activity is reduced at a half), k is the Boltzmann constant (8.6 $x10^{-5}$ eV K^{-1}), Th is the temperature at which enzyme is half active and half suppressed due to high temperatures and TempK is the temperature in Kelvin (Schoolfield et al. 1981; Kontopoulos et al. 2018). Parameters were estimated using the function provided in Kontopoulos et al. (2018) and minimizing the likelihood in R. The difference in the Akaike Information Criteria (ΔAIC) was used to rank models. We also explored the root of the mean squared errors (RMSE), significance of parameters and residuals to evaluate the models.

Table 1 Models, formulas and associated coefficients selected to be fitted for the responses of different metabolic parameters (Oxygen consumption, Ammonia Excretion Rate and Ingestion Rate) of juvenile *Pomacea* sp. exposed to different temperature regimes. Ref = reference (denomination given in the text); bp = break point

Model	Formulae	Coefficients	References
Lineal	$y = a + bx$	a, b	Equation 4
Quadratic	$y = a + bx + cx^2$	a, b,c	Equation 5
Exponential	$y = d^{fx}$	d, f	Equation 6
Segmented	$y_1 = b_1 + a_1x$ if $x < bp$; $y_2 = b_2 + a_2x$ if $x > bp$	a, b, bp	Equation 7
Schoolfield High(SH)	$y = c*e^{(Ea/k(1/273+15)-1/TempK)} * 1/(1+e^{(Eh/k(1/Th-1/TempJ))})$	c, e, Ea, Eh, Th, k, TempK	Equation 8

Trial II

We analyzed and compared survival between treatments by creating a Kaplan–Meier cumulative survival curve (non-parametric method) (Kishore et al. 2010). The probability of difference between curves is based on the Chi-square statistic, that was determined using the *survdiff* function of the R {survival} package (Therneau 2024).

We fitted a modified Von Bertalanffy growth function (VBGF) including a temperature (T in kelvin) dependence of the growth coefficient ($K(T)$):

$$W_t = W_{\inf} * \left(1 - e^{-K(T)*(t-t_0)}\right) \quad (4)$$

in which Winf is the asymptotic weight, t0 the initial time, *e* is the exponential function and *K(T)* is the temperature (T) dependent growth coefficient which was defined as

$$K(T) = k_{20} e^{\frac{-EA}{k_b}\left(\frac{1}{T} - \frac{1}{293}\right)} \quad (5)$$

The *k*20 is the growth coefficient at an arbitrary chosen reference temperature (20 °C), Ea is the activation energy (eV; electron volt), and *kb* is the Boltzmann Arrhenius constant (8.62 x10^{-5} eV K^{-1}), while T is the environmental temperature (in Kelvin). We fitted this model using the *nlm* function from the {stats} package in R for the growth data under outdoor (IT) and indoor (IT) conditions. We based the function on the seasonal growth function presented in Haddon (2023), which allows the inclusion of the effects of environmental variables (such as temperature).

We also explored growth patterns in each treatment by fitting generalized additive mixed models (GAMM) using the R packages *MuMIn*, *gamm4* and *Rcmdr*. GAMM models allow to include the effects of time and temperature as co-variates, including individual, day and treatment as random factors (Therneau 2024).

All statistical analyses were performed in the R software (https:// www. rproj ect. org/).

Results

Trial I

The pH remained stable (pH = 7.30 ± 0.31; mean ± sd) in all the temperature treatments. There was a single dead snail at 18 °C.

The Thermal Performance Curves (TPC) were characterized, and the shape of the response varied among rates (Fig. 2). We found heteroscedasticity for most treatments mostly caused by reduced rates and variance when snails were exposed to 6 °C (Fig. 2). Model results did not change quantitatively when weighted regressions were employed and thus, we keep with results from models fitted without weighting.

Oxygen Consumption (OC) mean values varied between 0.00 and 0.11 mg g^{-1} h^{-1} and presented differences in average and variance (LRT = 52.48; $p < 0.0001$; N = 29). The better models for TPC of OC showed a unimodal response (ΔAIC > 4 with respect to linear model) (Table 2). Peak performance in OC was found close to 22 °C with a less pronounced subsequent drop (Fig. 2a).

Ammonia Excretion Rate (AER) mean values were between 0.00 and 7.61 μgNH_4 $g^{-1}d^{-}1$ and differed also, in variance according to the LRT (LRT = 94.94, $p < 0.0001$, N = 28). In terms of

AIC, the best models showed a linear/unimodal response (Table 3). Peak performance for AER was found at 28.3 °C according to the quadratic model (Table 3), with a small decrease afterwards (Fig. 2b). The segmented model did not converge, and it is not reported.

Ingestion rate (IR) mean values varied between 3.07 and 776.69 mg $g^{-1}d^{-1}$. Average and variance differed among temperature values as evidenced by the LRT (LRT = 66.51, p < 0.0001, N = 54). The best models for TPC of IR showed a monotonic response (Table 4; Fig. 2c); SH model did not converge.

Trial II

Outdoor and indoor temperatures (mean ± sd) were 15.94 ± 6.33 and 16.35 ± 2.3 °C, respectively. Their average was similar between treatments, but the variance larger for OT (LRT = 15.78; p = $1x10^{-04}$; N = 36). Temperature in the OT treatment showed an abrupt drop between day 1 and 75, from 24 to 10 °C and then continued to drop until reaching 7 °C at day 146. Then increased to 27°C at the end of the trial in day 390. The decrease in temperature in the IT was less pronounced, with the minimum temperature higher (12 °C), and the maximum temperature lower (19 °C) than in the OT (Fig. 3a and b). The first mortality event was registered 12 days in OT and 15 days in IT after the beginning of the trail. Eight individuals died during the first 50 days. Mortality was observed until days 243 and 253 in the IT and the OT, respectively (Fig. 3e and f). Chi-square statistics of the Kaplan–Meier survival analysis showed no significant differences between treatments (Chisq = 0.1; p = 0.72). During the entire experiment, nine snails died under IT and eight in OT.

Growth trajectories showed different patterns in the IT and the OT treatments (Fig. 3c and d). There was a delay in growth (between July and November; days ~ 100–250) in the OT treatment, coincident with lower temperatures in relation to IT. However, at the end of the experiment, average weight was not statistically different between treatments (LR = 0.17; p = 0.67; N = 24). The parameters of the von Bertalanffy fitted model for the IT growth were W_{inf} = 1155 mg, k_{20}= 0.003, t_0 = − 93 and Ea = 0.07; while for OT were W_{inf} = 521 mg, k_{20}= 0.004, t_0 = − 70 and Ea = 0.25.

GAMM models suggested that only temperature had significant effects over IR and growth (p<0.05). Random effects were not significant, indicating that time, treatment and individual (random factors) had no effect over IR and growth. The temperature effects over IR and weight showed different patterns (Fig. 4). Wet weight increments presented a linear relationship with temperature (increasing from 6 to 30 °C). IR increased between 6 and ~15 °C, remained constant until ~20 °C, and then increased again until 30 °C. The maximum IR was ~800 mg $g^{-1}d^{-1}$ for ET and ~ 400 mg $g^{-1}d^{-1}$ for LT.

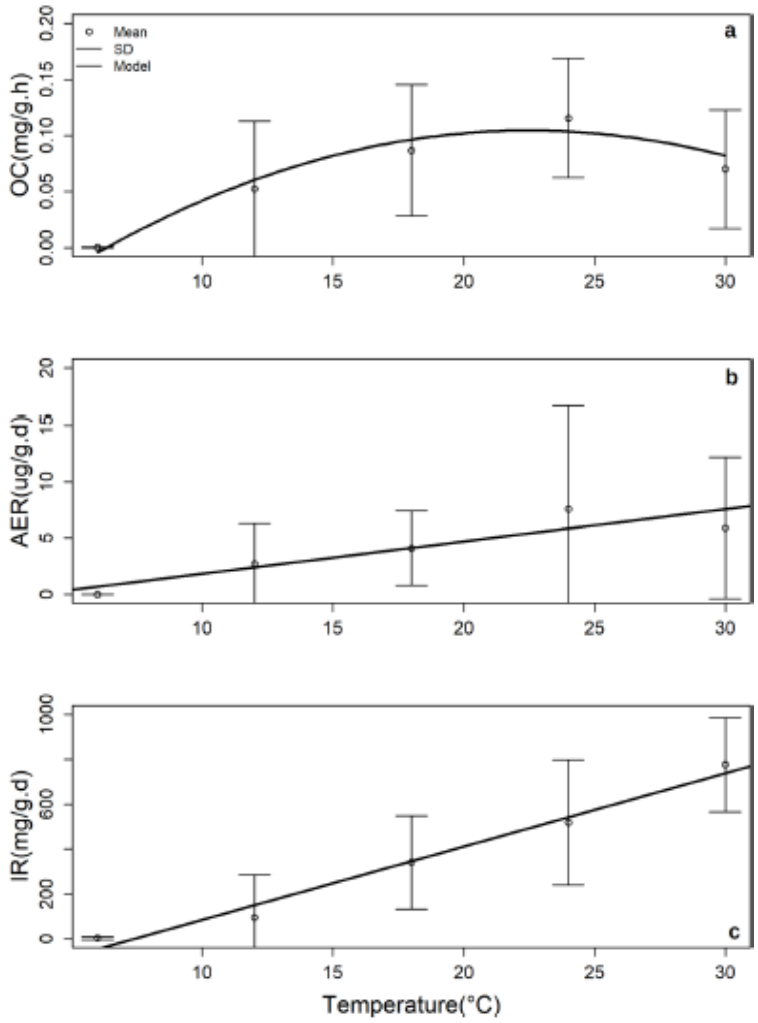

Fig. 2 Thermal performance curves (TPC) for multiple components: **a** Oxygen consumption (OC; mgO2/ g.h), **b** Ammonia excretion rate (AER; µgNH4/g.d) and **c** Ingestion rate (IR; mg/g.d), of juveniles of the freshwater snail *Pomacea* sp. exposed to 5 levels of temperature (6, 12, 18, 24 and 30 °C). Data are presented as average (dot) ± SD (segment). Thick continuous lines represent the model that best fits the data (Table 1)

Table 2 Statistical models fitted for the thermal performance curve (TPC) of Oxygen Consumption (OC) of juvenile *Pomacea* sp. snails exposed to different temperature regimes (6, 12, 18, 24 and 30 °C)

Model	a	b	c	e	f	Max	df	*p*-value	R^2	RMSE	ΔAIC
Linear	0.003*	0.0033					27	7.0e−3	0.21	0.05	4.95
Quadratic	0.018*	−0.098*	−0.0004*			22.2	26	1.0e-3	0.35	0.05	0
Exponential				0.0306	0.0391		27			3	7.11
Segmented	0.0073*	−0.04				22.8 ± 2.6	25	na	0.35	0.05	0.99
	0.0076	0.3									
	c	Ea	Eh	Th							
SH(nls)	0.07*	1.25	2.3*	21.9*			25	na		0.05	1.89

The estimated coefficients for the different models are shown: maximum temperature (for quadratic and segmented models), degrees of freedom (df), *p*-value, R2, Root Mean Square Error (RMSE) and Akaike Information Criteria (ΔAIC). Models: lineal (Eq. 4), quadratic (Eq. 5), exponential (Eq. 6), segmented (Eq. 7) and Schoolfield High (SH) (Eq. 8) Max = temperature (°C) at which OC was maximum. Asterisk represents $p < 0.05$

Table 3 Statistical models fitted for the thermal performance curve of ammonia excretion rate (AER) of juvenile *Pomacea* sp. snails exposed to different temperature regimes (6, 12, 18, 24 and 30 °C)

Model	a	b	c	e	f	Max	df	p-value	R^2	RMSE	ΔAIC
Linear	0.287*	−1.05					26	0.02	0.16	5.05	0
Quadratic	0.77	−4.38	−0.01			28.3	25	$5.3e^{-2}$	0.15	4.98	1.27
Exponential				1.358	0.057		26			26.54	1.17
	c	Ea	Eh	Th							
SH	5.3	0.6	1.2	24.9			25	na	na	198.04	208.94

The coefficients, maximum temperature (for quadratic model; Max.), degrees of freedom (df), *p*-value, R2, Root Mean Square Error (RMSE) and Akaike Information Criteria (ΔAIC) are shown. Models: lineal (Eq. 4), quadratic (Eq. 5), exponential (Eq. 6) and Schoolfield High (SH) (Eq. 8). Asterisk represents $p < 0.05$

Table 4 Statistical models fitted for the thermal performance curve of Ingestion Rate (IR) of juvenile *Pomacea* sp. snails exposed to different temperature regimes (6, 12, 18, 24 and 30 °C).

Model	a	b	c	e	f	Max	df	*p*	R^2	RMSE	ΔAIC
Linear	32.7*	−242.4*					52	0	0.66	194.97	0
Quadratic	0.52	13.94	−111.6			−13.3	51	0	0.66	192.42	0.58
Exponential				52.66*	0.09*		52			201.33	3.47

The coefficients, degrees of freedom (df), *p*-value (*p*), R^2, Root Mean Square Error (RMSE) and Akaike Information Criteria (ΔAIC) are shown. The models fitted were: lineal (Eq. 4), quadratic (Eq. 5) and exponential (Eq. 6). Asterisks represent significant differences ($p < 0.05$)

Discussion

In this work, we characterized the thermal response of *Pomacea* sp. and showed a differential behavior for the different physiological traits and mortality. While there is evidence pointing to an optimum at ~ 22 °C in oxygen consumption, the ingestion rate showed no decline in the evaluated range while the evidence on the shape of excretion rate was not conclusive.

Results showed that variability in environmental temperature promotes differential response of the ingestion and growth rates, but there are no differences in overall mortality patterns. There is scarce information in the study of responses of natural populations to global warming for the *Pomacea* genus, where multiple physiological traits are evaluated at the same time and in the same species.

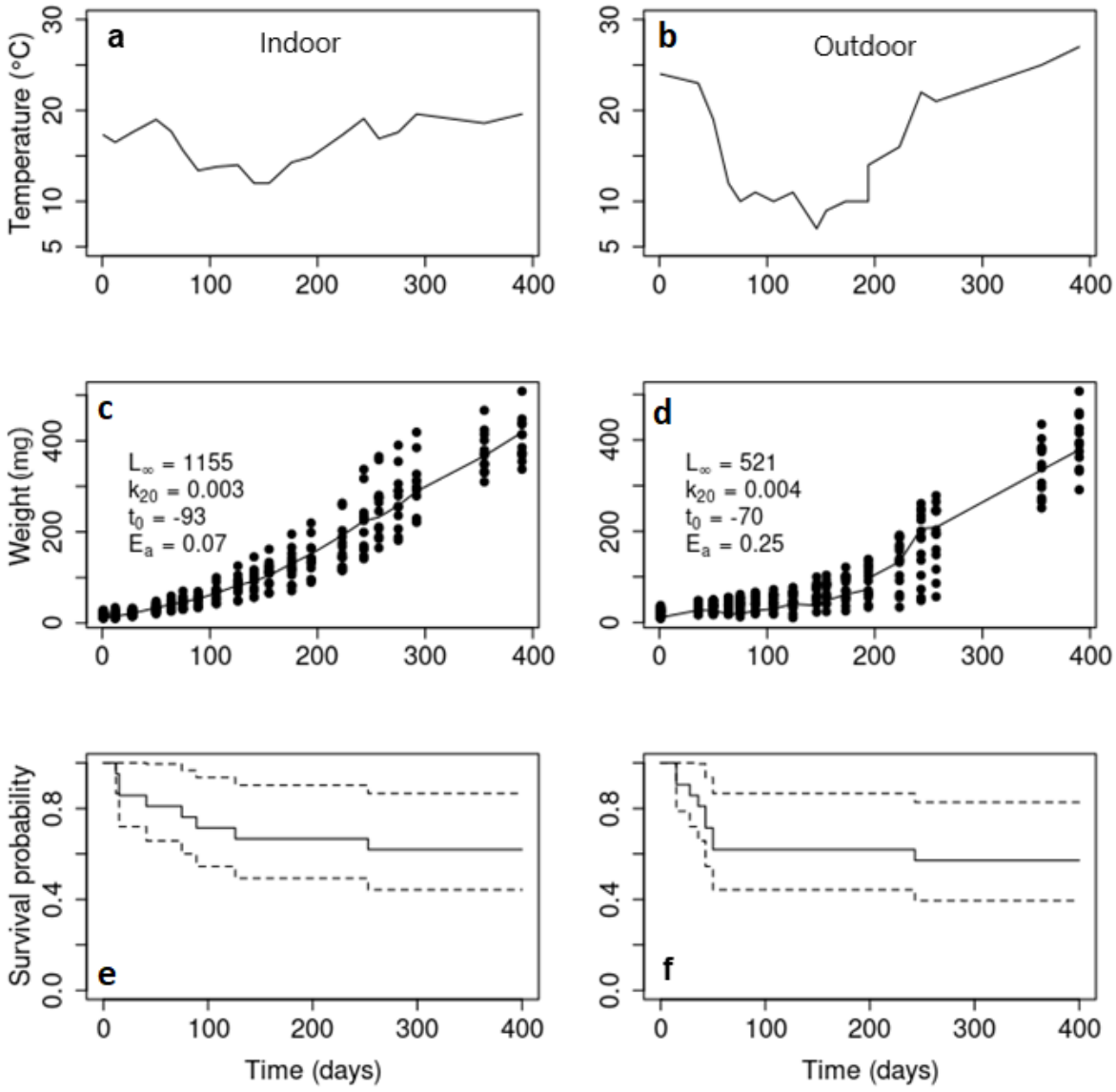

Fig. 3 Growth and survival of juvenile *Pomacea* sp. snails maintained under semi-controlled and variable temperature regimes for 400 days. **a** Water temperature in indoor (IT) and in the **(b)** outdoor treatments (OT). Growth patterns in **(c)** IT and **d** OT with the results of the fit to the modified von Bertalanffy growth function. Kaplan–Meier survival plots for **(e)** IT and **f** OT which were not significantly different (p = 0.72). Dashed lines represent the 95% confidence interval for the survival curve

Oxygen Consumption (OC) presented a unimodal thermal response which is one of the first characterizations for the *Pomacea* genus and was observed previously in other mollusks (e.g. *Mizuhopecten yessoensis, Ruditapes philippinarum* and *Paphia undulata;* Hao et al. 2014; Nie et al. 2017; Zhang et al. 2019). Oxygen consumption for *P. bridgesii* of larger size (0.32 ± 0.07 g between 0.16 and 1.86 mgO2 g^{-1} h^{-1} at 25 °C; Wenasa-Frifer 2016) were larger than present findings (between 0.06 and 0.21 mgO2 g^{-1} h^{-1} at 24 °C), differing from expectations based on reduced specific oxygen consumption with mass (Hill and Wyse 1992; Healy et al. 2013). Species specific differences could cause deviations or differences in the acclimation pattern. Also, the acclimation period setup must be taken in consideration, while in some works,

organisms are transferred to treatment temperatures abruptly and then acclimated (Yoshida et al. 2014); in others, temperature changes are gradual and then a period of acclimatization occurs (Seuffert et al. 2010). The effect of the different strategies should be analyzed more in depth, with explicit consideration of the different alternatives, as climate change could induce both a shift in average values and also in the associated variance (Van Der Wiel and Bintanja 2021) with relevant effects for the metabolisms of organisms and populations.

In the extremes, the observed decline of OC rate at 30 °C suggests that snails are close to the thermal limit (Wenasa-Frifer 2016). This implies that they live close to the thermal limit in summer in most of their distributional range, particularly in shallow water ecosystems in which they must develop further eco-ethological strategies to survive. The reduced response of OC at lower temperatures (6 °C) agrees with previous observations (Seuffert and Martin 2009) and suggest in winter they are under a low activity regime.

The Thermal Performance Curve for Ammonia Excretion Rate (AER) followed a linear and a quadratic model as equally plausible (according to AIC and the parameters p-value, R^2 and RMSE).

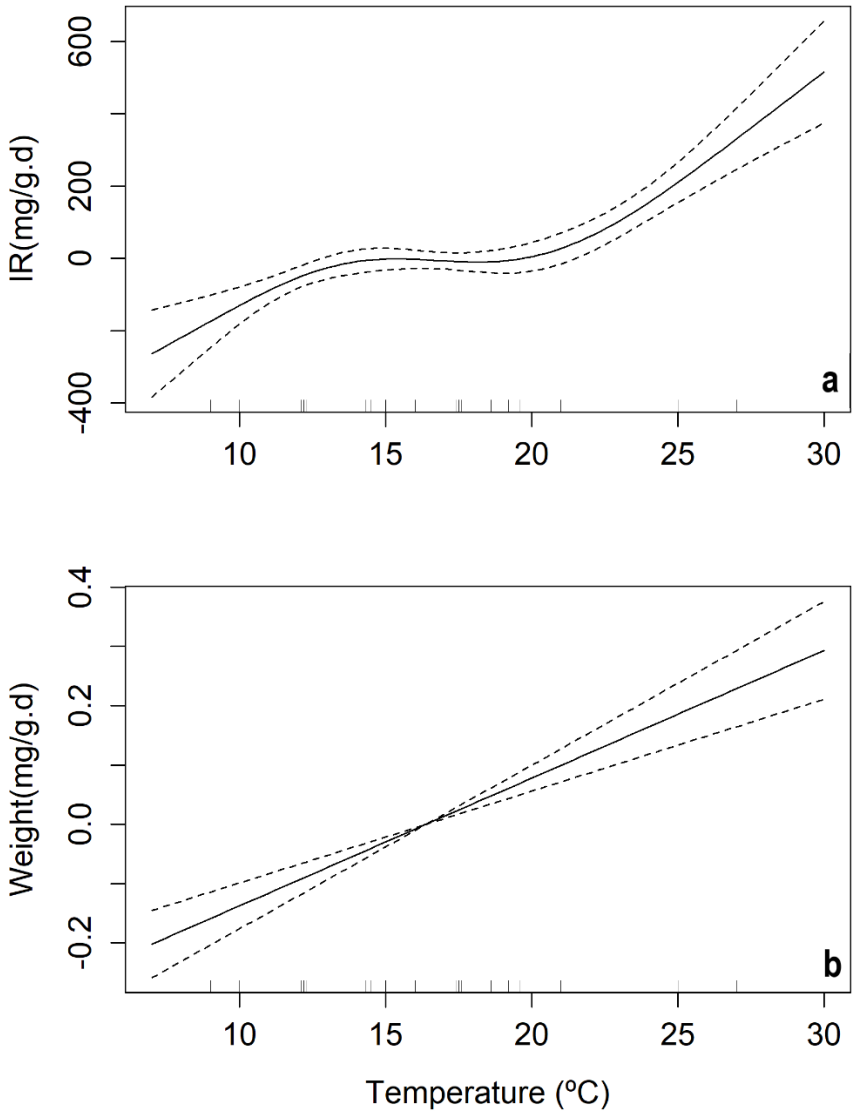

Fig. 4 GAMM plots showed the effects of Temperature (fixed factor) over **a** Ingestion Rate (IR) and **b** Weight ($p < 0.05$ for both indicators; time, treatment and individual were included as random factors) of juvenile *Pomacea* sp snails exposed to indoor (IT) and outdoor (OT) conditions. The zero in the response variable axis represents the mean value of the variable (IR and Weight) in both graphics

Absolute values of AER were lower than figures found for larger-sized *Pomacea maculata* at lower temperatures (20 °C) and consistent with the reduced activity below 10 °C (Deaton et al. 2016). According to Chaturvedi and Agarwal (1983), a direct relationship exists between AER and snails' activity. Our results are in agreement with this fact and consistent with the observed increase in snails' activity (feeding and respiration) with temperature.

Ingestion rate (IR) showed a monotonic increase with temperature; the linear and exponential curves are the most plausible models according to the AIC results (ΔAIC < 4); both presented a positive slope and provide an adequate characterization of the TPC (Fig. 2c; Table 4). It has been shown that snails of *Pomacea insularum* and *P. canaliculata* have similar ingestion rates to those recorded in this work (between 53 and 900 mg $g^{-1}d^{-1}$) (Baker et al. 2010; Calvo 2016).

However, the flat response presented by the IR (between 15 and 20 °C) and the continuous growth shown by the GAMM model, could be interpreted as that in this temperature range snails are not under thermal stress and the energy assimilated from feeding is fully invested in growth. At higher temperatures, there was an increase in locomotory activity and snails spent time in different activities (feeding, moving), increasing oxygen consumption (Bae and Park 2015). Growth and activity also increased for *P. canaliculata* exposed to temperatures between 20 and 32 °C (Heiler et al. 2008). These results are relevant for the laboratory maintenance of organisms, which ideally should minimize energy expenditure during long periods to diminish associated stress (*e.g.* oxidative stress, generation of metabolites). The monotonic increase of IR with temperature suggests that the process of food acquisition is not the rate that limit overall metabolism and should be taken into consideration for culture, maintenance and modeling eco-physiological responses. It is also consistent with observations made for multiple aquatic invertebrates (Gophen 1976; Niu et al. 2003). However, in natural conditions, it has been observed that they bury themselves in the sediment at high temperatures and do not feed (Cowie 2002) which imposes an upper limit to feeding caused by another physiological restriction (probably the metabolic capacity for oxygen consumption). It should be considered that the results obtained from these trials correspond to snails kept in confined spaces and individually, without interspecific interaction and this increase of IR in resource limited environment could favor an increase in competition for food, which will also be exacerbated by increasing trends in temperature as predicted under climate change. The reduced metabolic response observed at 6 °C is consistent with previous studies in which activity ceased below 10 °C in *P. maculata* and *P. canaliculata* (Deaton et al. 2016; Seuffert et al. 2010). In some cases, the snails remained inside the shell with no activity as observed in the present controlled experiments (Fig. 2), and also in the dependence of growth rate which was close to zero in the OT between July and November (days ~ 100–250) consistent with austral winter in which temperatures were below the thermal limit. However, after an inactive period under low temperatures, when temperatures rise in the OT, and particularly above the optimum (~ 20 °C), organism's growth rate overcompensate to reach the same size at the end of the trial to the organisms reared under moderate temperatures (IT; Fig. 3). This capacity to tolerate low temperatures without a significant effect is probably related to the evolution of these snails to thrive in shallow water bodies which are subjected to large environmental fluctuations (Cowie 2002). However, the estimated asymptotic weight differed between OT and IT suggesting a long-term effect of temperature fluctuations, at least under experimental conditions, which deserve to be explored in further trials.

The use of a modified von Bertalanffy growth function (VBGF) allows us to identify the effect of temperature in a mechanistic way as has been done previously for marine shrimps (Sampognaro and Segura 2024). According to the results of the von Bertalanffy model, the maximum size (W_{inf}) reached would be lower than maximum sizes observed in nature (Cowie

2002). The effect of experimental conditions (feeding, isolation, cleaning, reservoirs) must be revised, as for example minerals or other necessary compounds relevant for shell formation could limit growth (Chen et al. 2023). Temperature effects on growth have been observed in *P. canaliculata* and *P. patula*, where growth was favored by higher temperatures although in some cases it entailed an increase in mortality (Meyer-Willerer and Santos-Soto 2006; Seuffert and Martin 2013).

All these indicators are part of the energy balance of an organism that can be affected by the incidence of external factors, such as temperature. In this work, the drop in OC and AER at higher temperatures could indicate that beyond 22 and 28 °C (respectively) snails are under thermal stress and must realize changes in the metabolic pathways (e.g. anaerobic metabolism) as to deal with it (Nash et al. 2022). It would be necessary to evaluate other indicators (energy storages, hormones, enzymes) to determine that metabolic changes. Maybe snails obtain the energy needed to deal with thermal stress (at least in part) from food, which could explain the increase in IR even at high temperatures (at least in the range of temperature evaluated) (Zhang et al. 2019).

The among individual's variance associated with the TPC was large, as observed in *Pomacea maculata* (Deaton et al. 2016). The use of juvenile snails (born and maintained in the same conditions) from the same cohort discards the effect of genetic differences caused by maternal line and different reproductive stages but raise the point of a marked differential response at the individual phenotype level. Organisms evolving in a fluctuating environment will express more amplitude in the thermal response than organisms subjected to continuous thermal landscapes (Amarasekare and Savage 2012). *Pomacea* snails inhabiting the subtropical and temperate region, are inherently subjected to seasonal changes in temperature (− 5 to 40 °C), particularly those snails living in shallow water bodies in the littoral zone, where water temperature closely follows air temperature (Cowie 2002). There are some strategies that can be applied in future trials to minimize variance, such as the use of immature males only (Bae and Park 2015). Increasing sample size could help to better characterize the pattern and also to better describe the underlying distribution (*e.g.* in terms of skewness, multimodality, among others) and to detect individual behavior. Large variability difficult parameter estimation, as heteroscedastic data can lead to violation of model assumptions. It is also difficult the identification of the better model among the competing options and preclude the fitting of complex and more realistic models (i.e. Sharpe- Schoolfield). However, keeping the number of animals used for experimentation at a minimum is recommended by the principle of the 3Rs (Replacement, Reduction and Refinement) (Sneddon et al. 2017).

In the context of climatic change, the results obtained in this work suggest that a raise in temperature could increase snails' activity, mainly an increase in food consumption; this could imply a significant reduction in macrophyte biomass, changing the ecosystem state of the water

bodies where the snails live (Rahel and Olden 2008; Mi-Jung et al. 2012) by causing negative effects, even affecting the food web and the interactions between species (Woodward et al. 2010; Knouft and Ficklin 2017). Besides, all this could favor the dispersion of snails, expanding to new temperate areas where they could establish a population. The complex, trait-specific responses obtained in this work is in line with recent findings on the complexity of thermal responses in ectotherms (Pawar et al. 2024) and reinforce the need to characterize multiple responses at different scales to advance in the comprehension of the response of natural populations/communities to global warming (Woodward et al. 2010), including works of different disciplines that cover practice and theoretical studies (Knouft and Ficklin 2017).

Conclusion

Overall, the results of this study showed that temperature causes different effects over oxygen consumption, ammonia excretion, ingestion rate and growth of juvenile of *Pomacea* sp. The optimal temperature range for this species lies between 22 and 28 °C, when OC and AER are considered together. Additionally, 6°C could be considered near the lower limit of tolerance but trials above 30 °C are required to explore the upper limits of the thermal niche. All this information could be considered to predict the snail's distribution in the wild and to improve the culture of *Pomacea* sp. in laboratory. It has also been recorded that toxic chemical sensitivity changes with respect to optimum temperature (Wang et al. 2019), thus conjugating impacts from land use and climate change.

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Author contributions FF: Designing and conducting trials, data collection and analyses, writing. CC: Designing trials, statistical analyses, review and editing. JG: Designing trials, statistical analyses, review and editing. AS: Designing trials, supervision, statistical analyses, writing, review and editing. All authors read and approved the final version of the manuscript.

Data availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this paper.

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7. Capítulo 2

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Acute and chronic effects of the herbicide Clomazone over the apple snail *Pomacea* sp.

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En este artículo y en el marco de los objetivos "c" y "d", evaluamos los efectos del herbicida Clomazone sobre distintos bioindicadores de *Pomacea* sp. En primera instancia se realizó un experimento de toxicidad aguada (Letal) en el que se determinó la LC50-96h; y luego otro experimento, pero se toxicidad crónica (de 76 días), con concentraciones menores a la LC50, en el que se evaluaron las siguientes respuestas: actividad, consumo de oxígeno, tasa de excreción de amonio, tasa de ingestión, crecimiento y mortalidad. Fue observado que algunos de estos bioindicadores son afectados por concentraciones subletales de Clomazone.

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Acute and chronic effects of the herbicide clomazone over the apple snail *Pomacea* sp.

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Abstract

The intensification of industrial agriculture has resulted in an increased utilization of agrochemicals, a significant proportion of which enter aquatic ecosystems via surface run-off, exerting an impact on non-target species. The consequences of acute intoxication are well-documented, however sub-lethal exposure is a less well-researched topic. The present study provides an experimental evaluation of the lethal and sub-lethal effects of clomazone, a widely used herbicide, on the apple snail *Pomacea* sp. In the initial series of trials, the organisms were exposed to four nominal concentrations of clomazone (0, 4.86, 9.25 and 15.93 mg L^{-1}) for of 96 h to evaluate mortality and determine the lethal concentration (LC_{50}). In addition, sub-lethal responses such as behavioral activity, oxygen consumption (OC), ingestion rate (IR), ammonia excretion rate (AER) and growth were evaluated after being exposed for 76 days to concentrations of 15 and 150 µg L^{-1}. The LC_{50} for clomazone was 14.59 mg L^{-1} (95% CI: 13.67–15.51). A significant ($p < 0.05$) dose-dependent reduction in both behavioral activity (quantified as area under the curve, AUC) and OC was observed in the short term. In contrast, during chronic exposure at 150 µg L^{-1}, no significant changes in OC, AER, IR, or growth were registered along the whole period. These findings, based on a multi-endpoint approach, suggest that physiological and behavioral responses of *Pomacea* sp. are sensitive to clomazone exposure. This highlights the species' potential as a bioindicator, but more studies are needed to explore their responses at multiple levels and life stages to develop environmental management tools in the context of risk assessment.

Keywords: Metabolic response, Ecotoxicology, Physiological and behavioral biomarkers, Risk assessment, Clomazone

Introduction

Agricultural activity has expanded significantly worldwide, leading to increased use and release of agrochemicals (He et al. 2020). Biocides including herbicides, insecticides, and fungicides are applied to control unwanted species in crops but often reach aquatic ecosystems (Hoffman et al. 2002; Gonzalez-Piana et al. 2017), where they can affect nontarget flora and fauna at multiple levels of biological organization, ranging from individuals to entire ecosystems (Hoffman et al. 2002; Chen et al. 2019; Michel et al. 2025). Clomazone ($C_{12}H_{14}ClNO_2$), a member of the isoxazolidinone family, is an herbicide that inhibits photosynthesis (USEPA 2007). It is used to control grass and weeds in common crops such as rice, soybeans, and canola (Curran et al. 1992; Burdett et al. 2001). After application, clomazone reaches aquatic environments, where it remains present for up to 130 days. It can travel several kilometers downstream of treated areas (Zanella et al. 2002) and enter marine environments via irrigation canals (Zanella et al. 2002; Sauco et al. 2010). Depending on environmental conditions such as oxygen availability, light exposure and temperature, its half-life in water ranges from 3.1–47.3 days (Tomco et al. 2010; Tomco and Tjeerdema 2012; Zanella et al. 2008). Reported concentrations varying from below detection limits to approximately 1.7 µg L^{-1} in aquatic ecosystems (Zanella et al. 2002; Sauco et al. 2010). The effects of clomazone have primarily been studied in fish, which can bioaccumulate this herbicide in various tissues, including muscle tissue in species such as *Cyprinus carpio* and *Perca fluviatilis* (0.32 ng g^{-1}; Lazartigues et al. 2011, 2013a, 2013b), and liver tissue in *Micropogonias furnieri* (up to 1.25 mg kg^{-1}; Caldas et al. 2013). The environmental quantification and bioaccumulation in fauna make it important to advance the ecotoxicological assessment of this substance in order to understand its effects on natural populations (Vinardell 2007). Understanding the lethal and sub-lethal effects of biocides is key to developing effective environmental management tools (Ma et al. 2014; Nema and Bhargava 2018). The assays to evaluate these effects can be classified as acute or chronic. Extensive standardization of acute tests is used to determine the lethal concentration (LC_{50}), which is defined as the concentration causing 50% mortality of a population after a given exposure time (between 24 and 96 h; Akhila et al. 2007; USEPA n.d.). Chronic tests, on the other hand, generally last longer and evaluate sub-lethal effects, but are less commonly performed. Among the last, the evaluation of multiple physiological responses is especially useful for tracking sub-lethal effects over time (Paracampo et al. 2015; Kunwar et al. 2022). Examples of the former assays indicate that the LC_{50} of clomazone for the fish species *Cyprinodon variegatus*, *Rhamdia quelen*, *Menidia menidia*, *Lepomis macrochirus* and *Oncorhynchus mykiss* is between 5.2 and 34 mg L^{-1} over 96 h (Miron et al. 2004; Shaner 2014). Additionally, LC_{50} values have been reported for Daphnia sp. (5.2 mg L^{-1}), the mysid shrimp *Americamysis bahia* (0.56 mg L^{-1}) and the aquatic hemipteran *Limnocoris submontandoni* (1012.4 mg L^{-1}) (Jonsson et al. 1998; Miron et al. 2004; Shaner 2014; Souza et al. 2024). However, there are no ecotoxicological reports for molluscs. Similarly, chronic

bioassays in fish species like *Channa punctatus*, *Cyprinus carpio*, and *Danio rerio* have examined sub-lethal endpoints such as behaviour, spawning time, DNA damage, enzyme activity, oxidative stress, and hematological changes (Cattaneo et al. 2012; Stevanovic et al. 2017; Singh and Greg 2022; Bojarski et al. 2024). In contrast, evaluations of the chronic effects on aquatic invertebrates are scarce (EFSA 2025). Reports on the effects on macroinvertebrate communities and in semi-aquatic insects like *Chironomus tepperi* and *Limnocoris submontandoni* include a decrease in richness, diminishing of emergent adults and effects on the reproductive system (Burdett et al. 2001; Souza et al. 2024; Stenert et al. 2018). Furthermore, simultaneous evaluations of multiple responses within the same species are relatively scarce and can provide a more comprehensive understanding of how organisms respond to toxic substances. We are not aware of any reports evaluating the toxicity in molluscs. Therefore, evaluating multiple endpoints for acute and chronic effects in molluscs appears to be a timely topic.

Although apple snails of the genus *Pomacea* are native to South America, they have become invasive in parts of Asia and Europe, causing considerable damage to crops (Arfan et al. 2014). Their diverse habitat ranges from large rivers to small ponds close to agricultural regions, making them particularly susceptible to agrochemical exposure. Previous studies have shown that species in this genus are affected by metals and agrochemicals (Arrighetti et al. 2018; Campoy- Diaz et al. 2018; Yap et al. 2023). In some cases, these substances are bioaccumulated in their tissues (Pena 2017; Banerjee et al. 2023). The cultivation and maintenance of *Pomacea* in laboratory settings is well established and relatively straightforward (Rojas and Perez 2012; Feola et al. 2025) and it has been used as a bioindicator species. However, to date, there is no available information regarding the effects of clomazone on the biology of *Pomacea* sp.

This study aims to evaluate the acute and chronic toxicity of Clomazone in the apple snail (*Pomacea* sp.) using a multi-endpoint approach. This non-target, generalist species was selected to provide an initial assessment of the effects of clomazone across multiple biological responses.

Materials and methods

Clomazone

The herbicide Cibelcol, produced by Cibeles in Uruguay, contains the active ingredient clomazone (2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone) at a concentration of 480 g L^{-1}. The formulation presents Xilene, that acts as a solvent, and other inert ingredients (not mentioned in the formulation).

Initial clomazone concentrations were measured for the acute test after preparing the solutions, by taking a sample of solution and processing under high performance liquid

chromatography (HPLC) by using a Thermo Scientific Ultimate 3000 LC coupled to a diode array detector (DAD) by direct injection of the sample. A Thermo Scientific Hypersil Gold C18 (250 × 4.6 mm id. 5 µm) column was used. The column oven temperature was ser at 30 °C. The mobile phase consisted of (A) 5 mM Ammonium acetate buffer (pH = 9.5; pH is adjusted with diluted solution of NH_4OH) and (B) LC grade MeCN. The separation was performed at a flow rate of 1.0 mL min^{-1}. The injection volume was 10 µL. The peak was determined using a UV detector set at wavelengths of 220 and 254 nm. Chromeleon v.7.2.9 software from Thermo Scientific was used for instrument control and data processing.

Where possible, measured concentrations of clomazone are presented; otherwise, nominal concentrations are shown.

Study organisms

The wide distribution of the apple snail and the variety of environments it inhabits make it a good sentinel species for detecting toxic contamination (Banerjee et al. 2023). Furthermore, maintaining them in a laboratory is straightforward. Adult *Pomacea* sp. (Hayes et al. 2012) were collected from the shallow, freshwater coastal lagoon known as Briozzo (34°17′40“S, 53°48′18“W), located in Rocha, Uruguay. This lagoon is part of a system of lagoons that has been designated a Biosphere Reserve by the United Nations Educational, Scientific and Cultural Organization (UNESCO), and it is also situated within a Ramsar site. Snails were transported in ten-liter plastic buckets filled with lagoon water to the laboratory at the Centro Universitario Regional del Este (CURE) in Rocha, which is located approximately 100 km away from the lagoon. The snails were acclimatized to laboratory conditions and then kept in aquaria containing dechlorinated tap water (DTW) at temperatures between 12 and 18 °C and under a natural photoperiod and pH = 7.15 ± 0.29 for more than one year (Feola et al. 2025). They were fed ad libitum with hydroponic fresh lettuce 2 or 3 times a week following Estebenet and Cazzaniga (1992) and Campoy-Diaz (2018). Reproductive events occur at temperatures above 20 °C, with slight variability depending on the feeding regime (Estebenet and Cazzaniga 1992; Tanaka et al. 1999). Experiments were conducted using recently hatched snails (F1) and adults that were considered to be non-reproductive. We determined the maturity stage of the organisms used for experimentation by comparing their size to the estimated size at maturity of *Pomacea canaliculata* (22–31 mm for males and 26–38 mm for females). This species is a cryptic member of the *Pomacea* sp.

Acute toxicity test

We determined the lethal concentration (LC_{50}) of clomazone for *Pomacea* sp. and evaluated its effects on oxygen consumption and snail activity. A total of 32 adult specimens of *Pomacea* sp., weighing on average 7.40 ± 1.54 g and a range between 4 and 10 g and measuring between 24–34 mm of total length, were placed individually in 250 mL plastic flasks. Each

specimen was considered an experimental replicate (Feola et al. 2025). The effect of three clomazone concentrations (measured concentrations; T1 = 4.86 ± 0.13 mg L^{-1}, T2 = 9.25 ± 0.05 mg L^{-1} and T3 = 15.93 ± 0.25 mg L^{-1}) and a control group (C) maintained in DTW were evaluated. In each treatment group and the control group, eight snails were randomly selected and placed in their individual containers for 96 h. The clomazone concentrations were selected based on preliminary assays conducted on a different set of organisms.

Experimental solutions were prepared using the commercial clomazone formulation and DTW. After 48 h, the water and solutions were replaced to maintain the herbicide concentrations and water quality. Snails were not fed during the trial. Snail´s mortality and activity were assessed daily. The snails' behaviour was observed for five to ten minutes each day, and this information was recorded. Activity was then classified in four categories following Heiler et al. (2008) and Seuffert et al. (2010): (1) active (snails moving or attached to the container walls), (2) low activity (partially fully retracted into the shell), (3) detached (body completely out of the shell and immobile) and (4) dead.

Oxygen consumption (OC) of 3 snails per treatment was registered at the beginning of the trial, and at 48 and 96 h by the optic method using a respirometer Oxy-4 meter (Portner et al. 2006). Oxygen concentration was registered continuously for approximately 40 min, and the oxygen consumption rate (OC) was calculated according to the following equation:

$$O(mg/g.h) = \frac{(O_{2f} - O_{2i}) * v}{W * t} \quad (1)$$

where O_{2i} and O_{2f} are the initial and final oxygen concentration (mg L^{-1}) respectively, v is the volume of the container (L), W is the snail weight (g), and t is the elapsed time (hours) between initial and final records.

Chronic toxicity test

To evaluate clomazone sub-lethal effects over *Pomacea* sp., juvenile F1 snails (0.212 ± 0.022 g; mean ± SD; 7 months old, approximately) were exposed to two nominal concentrations of clomazone (15 and 150 µg L^{-1}; approximately 0.1 and 1% of the maximum concentration of the acute test) and a control group exposed to DTW for 76 days. We placed ten snails in each treatment group and ten in the control group, with each snail in its own 50 mL bohemian glass. Renewal of water and solutions was carried out two times a week to ensure clomazone concentration and adequate oxygen levels. Snail´s activity was registered twice a week, while mortality was evaluated daily.

The OC of three randomly selected snails per treatment was measured at the beginning of the test and on days 1 and 76 using the same method as described above.

The ammonia excretion rate (AER) was determined for each snail every week in the first five weeks (at days 7, 16, 22, 30, 37) and at the end of the experiment (day 76). Water samples (12 mL) were taken after the water was renewed and again after 48 h to estimate ammonia concentrations. The samples were filtered with glass fiber filters MGF (Munktell, Ahlstrom). Ammonia levels were determined using the Indophenol blue technique (Koroleff 1970) and AER was calculated according to the following equation:

$$AER(\mu g/g.d) = \frac{(NH_{4f} - NH_{4i}) * v}{W * t} \quad (2)$$

where NH_{4i} and NH_{4f} are the initial and final ammonia concentration (µg L^{-1}), v is the volume of the container (L), W is the snail weight (g), and t is the time (days) elapsed between the two records.

Snails were fed twice a week with an amount of hydroponically grown lettuce approximately equivalent to their body weight. The lettuce was rinsed with tap water, blotted dry with a paper towel for five minutes, and then weighed using a precision balance (± 0.0001 g). Lettuce consumption was measured 18 times out of a total of 21 throughout the trial. The ingestion rate (IR) was estimated at the beginning (LW_i) and end (LW_f) of the experiment as the difference in fresh lettuce weight (LW, in mg) over a 24-h feeding period:

$$IR(mg/g.d) = \frac{(LW_i - LW_f)}{W * t} \quad (3)$$

Snails were weighed at the beginning and at the end of the test. The growth rate (GR) was estimated as the difference in weight between successive sampling events divided by the difference in time (days). In both trials, temperature was registered daily whit a HOBO sensor.

Statistical analysis

All statistical analyses were performed in the R software (R Core Team 2025).

The lethal dose (LC50) at 72 and 96 h (72-LC_{50} and 96-LC_{50}, respectively) was estimated by fitting a logistic model to the measured concentration of clomazone and mortality, using the "logit" link for mortalities registered in the acute toxicity test at 72 and 96 h:

$$p = \frac{e^{(a+bCCL)}}{1 + e^{(a+bCCL)}} \quad (4)$$

where CCL is the concentration of clomazone, and a and b are the fitted intercept and slope in logit space, respectively. The LC_{50} is defined as:

$$LC_{50} = -\frac{a}{b} \quad (5)$$

The analytical 100(1–α) % confidence interval (CI) is defined as:

$$CI_{(1-\alpha)} = (1/\beta_1)(-\beta_0 \pm z_\alpha / 2v(P_{50}))$$ where $z_\alpha/2$ is the quantile of the normal distribution and v denotes the square root of the analytical variance of the logit link function (Roa 1999; Segura and Delgado 2012).

We analyzed the Oxygen consumption in both acute and chronic toxicity tests (OC) by means of the response ratio to evaluate the effect size (Lajeunesse 2011). This metric quantifies the ratio of the response variable in the treatment group relative to the control (Hedges et al. 1999). A two-way repeated measures ANOVA was conducted to assess simultaneously the effect of Treatment and Time on OC, using the {rstatix} package in R. When the assumption of normality was not met, a sqrt(log(Y + 1)) transformation was applied. Post-hoc comparisons were conducted using t-tests with Bonferroni correction, assuming 0.05 as the limit for significance ($p < 0.05$). The temporal changes in OC were visualized in plot of the original (untransformed) variable with time for ease of interpretation. The categories of snails' activity were coded into four values (0 = dead, 1 = detached, 2 = low or no activity, 3 = active) to construct a time-integrated index.

For both tests (acute and chronic) the area under the time-activity curve (AUC) was calculated using the *bayestestR* package in R (Makowski 2025). ANOVA was used to compare the AUCs of the control and treatment groups when the assumptions of normality and homogeneity of variance were met. Tukey's HSD test was employed for post-hoc analysis ($p < 0.05$) using the stats R package. When AUC did not meet the normality assumption, the non-parametric Kruskal–Wallis test was applied. We evaluated the generalized eta squared (GES), a statistic that refers to the effects size and quantifies the proportion of variance in the dependent variable that is explained by a specific factor in an ANOVA model (Olejnik and Algina, 2003; Richardson et al. 2011). Values greater than 0.14 are related to relevant effects (Cohen 1988).

The AER, IR and growth comparisons between both the treatments and the control were performed using the Friedman test, which is a non-parametric alternative to repeated measures ANOVA (Hollander and Wolfe 1973), as the distributions were not normal and some outliers were present.

Results

Acute toxicity test

Temperature was kept close to 19.15 ± 0.4 °C in the whole assay. pH values were 7.62 ± 0.10 in C and 7.43 ± 0.08 in the solutions. One snail was found dead in T2 and five in T3. The 72 and 96h-LC_{50} were estimated as 19.83 mg L^{-1} (CI95% = 18.91–20.74) and 14.59 mg L^{-1} (CI95% = 13.67–15.51), respectively. The probability of mortality increased by increasing the concentration of clomazone over 72 and 96 h (Fig. 1).

OC ranged from 0–0.123 mg g^{-1} h^{-1} and was not statistically different among groups in the initial time. However, it presented significant differences for the T2 and T3 treatments. The response ratio illustrates changes in OC overtime (Fig. 2a). At the beginning of the experiment (t = 0), OC levels across the three clomazone treatments and the Control were similar.

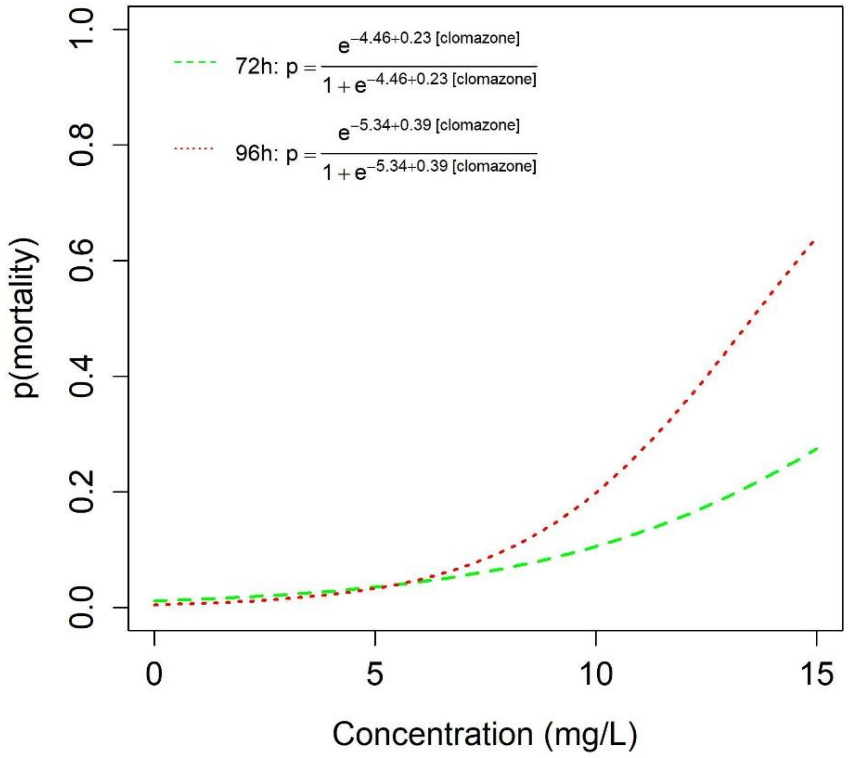

Fig. 1 Plot showing the acute mortality probability of apple snails (*Pomacea* sp.) in relation to clomazone concentration (C = 0, T1 = 4.86, T2 = 9.25 and T3 = 15.93 mg L^{-1}). Lines represent GLM models fitted for 72 h (green) and 96 h (red). The equations of the fitted models are shown in the top left corner of the plot

The two-way ANOVA revealed a significant interaction between time and treatment ($F(6, 16) = 7.14$, $p < 0.001$), indicating that OC differed significantly both among treatments and over time. Additionally, the generalized eta squared (ges = 0.63) indicated a large effect size. Pairwise comparisons, using paired t-test, show that the mean OC was significantly different between times 0 (0.035 ± 0.039 mg g^{-1} h^{-1}) and 48 h (0.037 ± 0.012 mg $g^{-1}h^{-1}$) with respect to 96 h (0.073 ± 0.01 mg g^{-1} h^{-1}) in T1; between 48 (0.055 ± 0.4 mg g^{-1} h^{-1}) and 96 (0.0016 ± 0.00038 mg g^{-1} h^{-1}) h in T2; and 0 (0.029 ± 0.0052 mg g^{-1} h^{-1}) with respect to 48 (0.0006 ± 0.00036 mg g^{-1} h^{-1}) and 96 (0.000067 ± 0.000058 mg g^{-1} h^{-1}) in T3 (Fig. 2b; Table 1).

ANOVA revealed a significant effect of the treatment factor on snail activity ($p = 7.1 \times 10^{-11}$), indicating differences in activity levels (as measured by AUC) among treatments. Activity decreased with increasing clomazone concentration (Fig. 4), with a moderately high effect size

(generalized eta squared, ges = 0.83). Post-hoc comparisons confirmed significant differences between all pairs of treatment levels. Mean activity values (± SD) were as follows: C = 254 ± 13.5; T1 = 213 ± 31.9; T2 = 178 ± 20.7; and T3 = 118 ± 26. At 48 h, there were differences between C (0.023 ± 0.004 mg g−1 h−1), T1 (0.034 ± 0.012 mg g^{-1} h^{-1}) and T2 (0.055 ± 0.04 mg g^{-1} h^{-1}) with respect to T3 (0.0006 ± 0.00036 mg g^{-1} h^{-1}). At 96 h, significant differences were observed in the CO of the C (0.053 ± 0.018 mg g^{-1} h^{-1}) and T1 (0.073 ± 0.01 mg g^{-1} h^{-1}) compared to T2 (0.0016 ± 0.00038 mg g^{-1} h^{-1}) and T3 (0.00007 ± 0.00006 mg g^{-1} h^{-1}) (Fig. 2b; Table 1).

Snail activity was negatively affected by clomazone exposure, decreasing progressively with increasing herbicide concentration. In the control group, only individuals displaying active or low activity were observed (Fig. 3C). Although low activity individuals were present across all treatments, they were most prominent in T2, particularly between 24 and 48 h, coinciding with a marked reduction in overall activity (Fig. 3, T1). Detached snails were observed in all clomazone treatments, particularly in T2 and T3. In T2, their numbers increased towards the end of the exposure period (Fig. 3, T2). In T3, detachment was observed in all snails from 24 h onwards, followed by an increase in mortality at 72 and 96 h (Fig. 3, T3).

Chronic toxicity test

The temperature was on average 19.84 ± 1.17 °C. One snail was found dead in each clomazone treatment (15 and 150 µg L^{-1}).

Oxygen consumption varied between 0 and 10.33 mgO2 g^{-1} h^{-1}. A significant effect of time ($p = 0.012$) was found in ANOVA, with an associated ges of 0.48, which indicates a large effect size (Fig. 5). In contrast, the post-hoc test showed no differences in OC. All treatments tend to increase in OC after 24 h of exposure (C = 3.63 ± 1.33 mg g^{-1} L^{-1}; T15 = 4.80 ± 1.19 mg g^{-1} L^{-1}; T150 = 3.56 ± 2.53 mg g^{-1} L^{-1}). After 76 days, only the T150 presented an increased in OC (8.56 ± 2.24 mg g^{-1} L^{-1}) with respect to C and T15 (Fig. 5a, b).

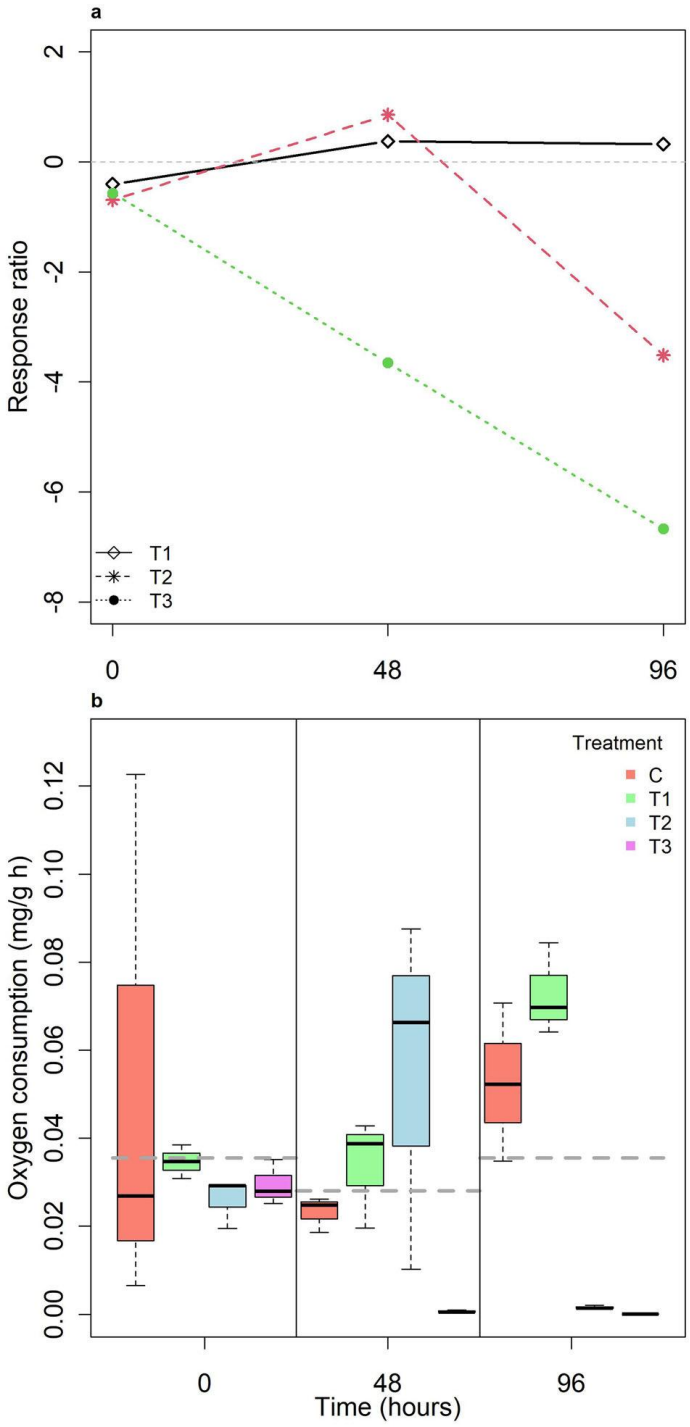

Fig. 2 Effect of different clomazone concentrations (C = 0, T1 = 4.86, T2 = 9.25 and T3 = 15.93 mg L^{-1}) on the oxygen consumption (OC) of Pomacea sp. during a 96-h acute toxicity test. **a** response ratio plot showing the effect of clomazone on OC; 0 on the Y-axis represents the control (C); **b** boxplot of the OC values (mean ± sd) for each treatment at different time points (0, 48, and 96 h). Horizontal grey dotted lines indicate the overall mean OC at each time point

Table 1 Post-hoc bonferroni results for oxygen consumption in *Pomacea* sp. exposed to four clomazone concentrations (C = 0, T1 = 4.86, T2 = 9.25 and T3 = 15.93 mg L^{-1}) over 96 h

Time (hours)								
Treatment	48				96			
	C	T1	T2	T3	C	T1	T2	T3
C	–			0.02	–		5.8×10^{-6}	16.9×10^{-7}
T1		–		0.007		–	12.9×10^{-7}	45.3×10^{-8}
T2			–	0.002	5.8×10^{-6}	12.9×10^{-7}	–	
T3	0.02	0.007	0.002	–	16.9×10^{-7}	45.3×10^{-8}		–

In the initial time there were no significant differences in any group. Empty spaces mean that there were no statistical differences

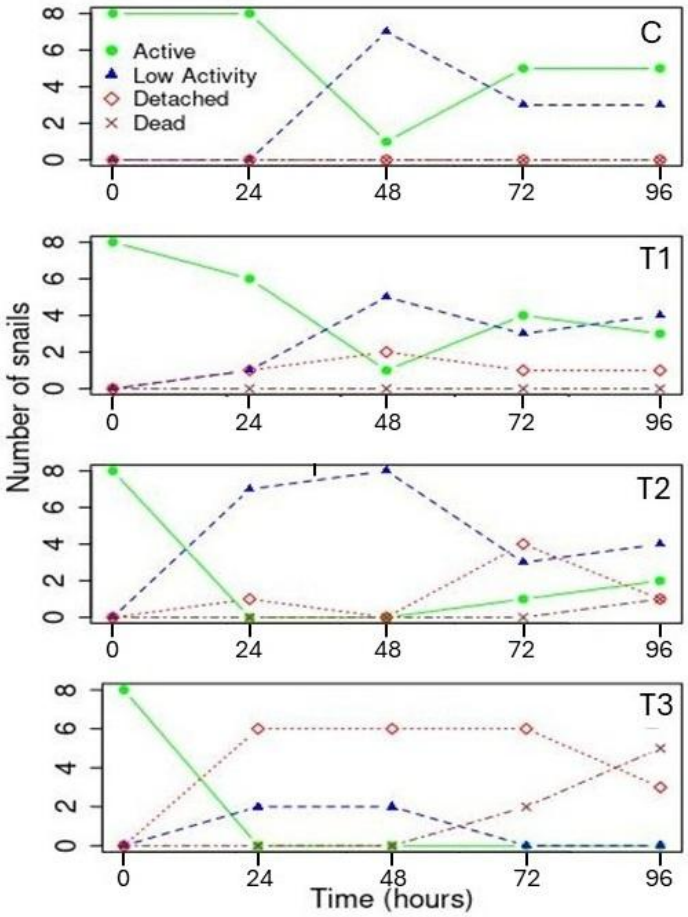

Fig. 3 Activity of adult apple snails (*Pomacea* sp) exposed to four clomazone concentrations (0, 4.86, 9.25 and 15.93 mg L^{-1}; C, T1, T2 and T3, respectively) for 96 h. Number of snails in each activity stage (active, low activity, detached or dead) recorded per treatment over the exposure time

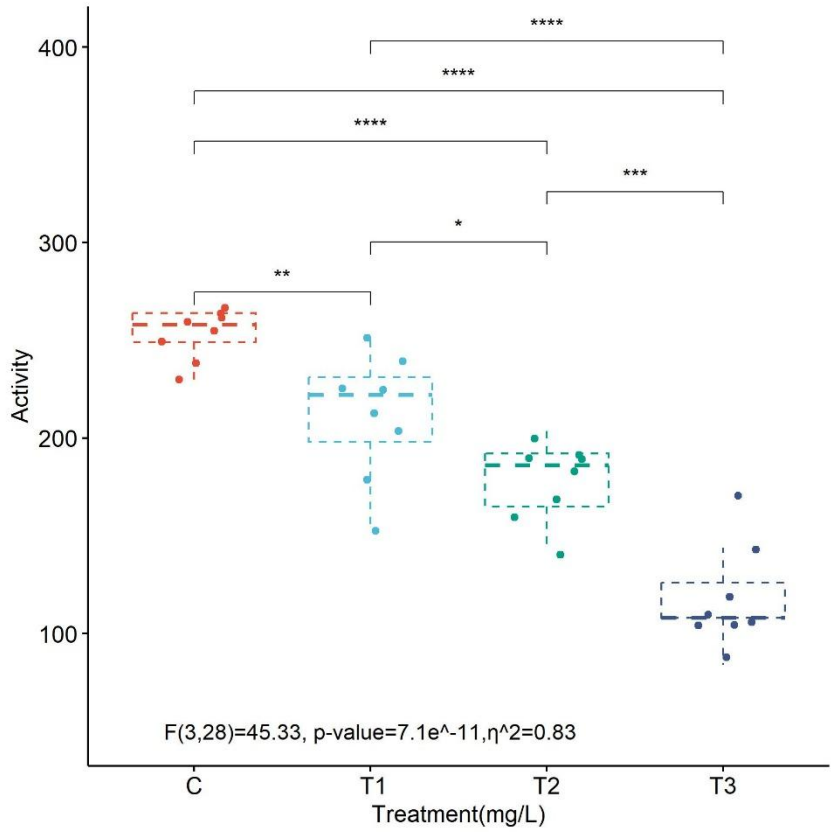

Fig. 4 Activity of adult apple snails (*Pomacea* sp) exposed to four clomazone concentrations (0, 4.86, 9.25 and 15.93 mg L^{-1}; C, T1, T2 and T3, respectively) over 96 h, calculated using the area under the curve (AUC) for each treatment. Points represent individual activity levels. Asterisks indicate significant differences ('****' < 1e–04, '***' < 0.001, '**' < 0.01, '*' < 0.05, ns not significant)

Active and low activity snails were recorded in this trial while detached and dead were not. The Kruskal-Wallis test was not significant (p = 0.61), suggesting no effect of clomazone on snail's activity.

AER values were between 0 and 158.17 µg g^{-1} d^{-1}, but the Friedman test showed no significant effect of clomazone; the overall average (±sd) was 35.95 ± 35.90 µg g^{-1} d^{-1}. The ingestion rate and growth rate did not show significant differences among groups. The overall average (± sd) for IR was 722.88 ± 310.61 mg g^{-1} d^{-1} and for GR was 0.33 ± 0.13 g.

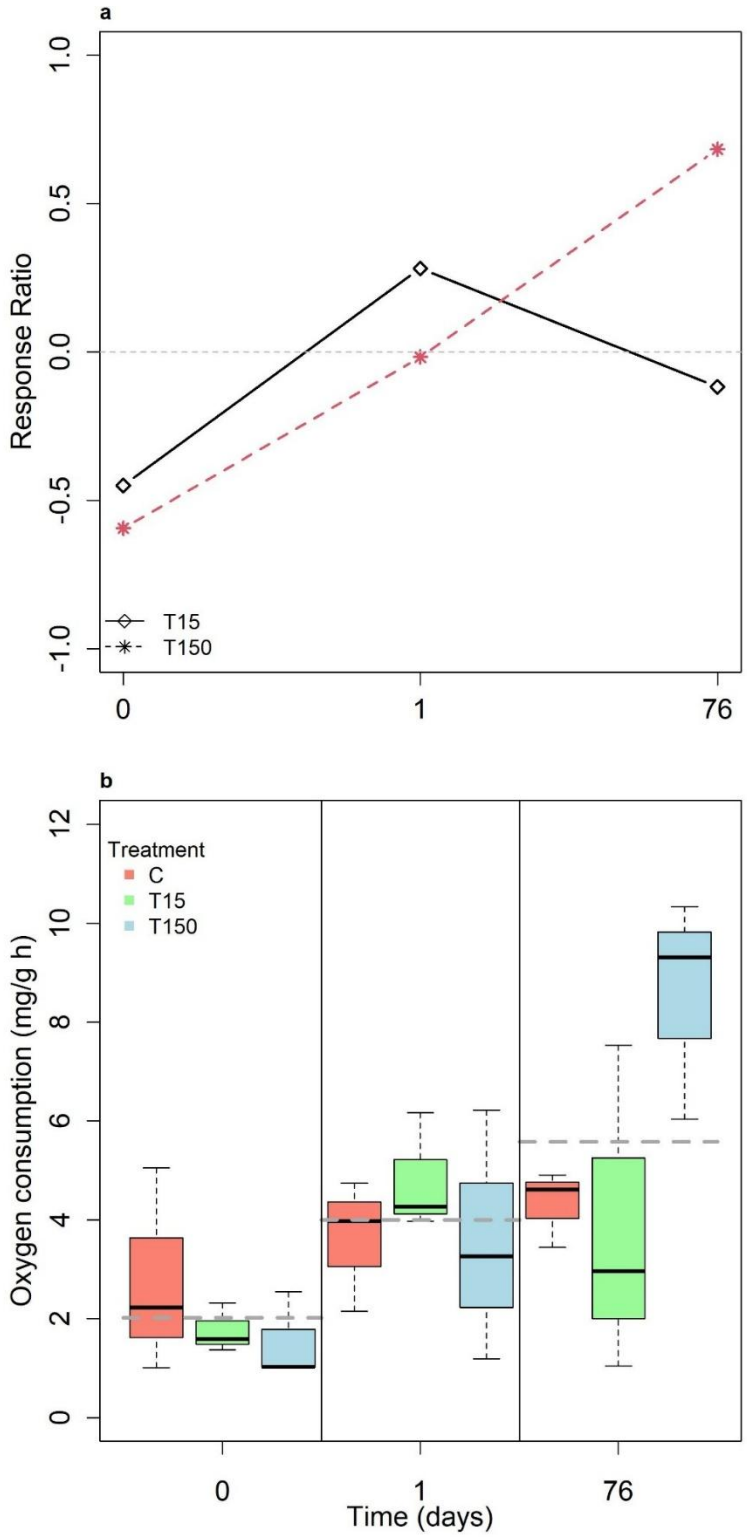

Fig. 5 Effect of clomazone concentrations (C = 0, T15 = 15 and T150 = 150 µg L^{-1}) on the oxygen consumption (OC) of *Pomacea* sp. during a 76-day chronic toxicity test. a Response ratio plot showing the effect of clomazone on OC; 0 in the Y-axis represents the Control (C). b boxplot of the OC values (mean ± sd) for each treatment at different time points (0, 48, and 96 h). Horizontal grey dotted lines indicate the overall mean OC at each time point

Discussion

In the present study, we demonstrate that the herbicide clomazone has both lethal and sub-lethal effects on the apple snail *Pomacea* sp. under laboratory conditions. This shows that this species is sensitive to clomazone, as survival, oxygen consumption and activity decrease with increasing concentration and exposure time. However, not all the bioindicators were affected at sub-lethal concentrations (chronic toxicity test). This work is one of the first approaches that evaluates the effects of clomazone on *Pomacea* (dos Santos et al. 2019), a genus used as bioindicator worldwide (Dummee et al. 2015; Campoy-Diaz et al. 2018).

Acute toxicity test

Survival of the apple snail diminished when increasing clomazone concentrations and exposure time. This is one of the first estimations of the lethal dose for molluscs and there is not much information about the lethal dose for aquatic invertebrates, which are highly variable (from 0.56-1012 mg L^{-1}, Shaner 2014; Souza et al. 2024) as this heterogeneous group encompasses organisms with different sizes and lifestyles. The 96 h lethal dose determined for clomazone was an order of magnitude higher than the concentrations measured and reported in the environment, such as rivers and canals associated with agricultural areas, where the determined levels were between 0.37 and 8.85 µg L^{-1} (Marchesan et al. 2007; Sauco et al. 2010). However, the concentrations of clomazone found in the floodwaters one week after application were substantially higher (16 and 292 µg L^{-1}) (Schreiber et al. 2017; Zanella et al. 2008) as were concentrations after 1 h of application (307 y 505 µg L^{-1}; Zanella et al. 2008). Direct exposure to the biocide at the time of application is plausible in rice fields (Arfan et al. 2014), suggesting that the concentrations to which the snails are actually exposed might be closer to the lethal dose than expected. This herbicide could threaten the survival of *Pomacea* sp. and other organisms inhabiting the same environment in the short term. *Pomacea* sp. seems to be more sensitive to insecticides than to herbicides (the LC_{50} for the insecticides Chlorpyrifos, Dichlorvos, Carbaryl and Cypermethrin were 6.1, 49, 14.6 µg mL^{-1} and 400 µg L^{-1}, respectively; and 175 mg L^{-1} for Glyphosate; Mora et al. 2000; Putkome et al. 2008; Xu et al. 2017; Arrighetti et al. 2018). The main reason is that insecticides are developed to act against a group of animals, so they include products that affect different systems (e.g. neurological, muscular, growth and development) and a wide range of metabolic pathways (Sparks and Nauen 2015). Herbicides, on the other hand, are designed to deal with weeds by mainly inhibiting photosynthetic metabolic pathways, which are toxic to animals, but generally at a higher concentration. Furthermore, the effects of adjuvants in commercial formulations should be explicitly considered when assessing ecotoxicology due to their recorded impact (Chris et al. 2022).

Most of the work involves evaluating biomarkers that require organisms to be killed (e.g. genetic damage, enzymatic activity, oxidative stress, histology; Xu et al. 2017; Bojarski et al.

2024; Souza et al. 2024). According to Melvin and Wilson (2013), the use of biomarkers that do not require invasive techniques or killing the organisms to evaluate the effects of a toxic compound, are reliable tools that can be used to estimate the consequences of a real exposure event and allow to estimate the effect of sustained exposure. Oxygen consumption (OC) and activity are types of noninvasive markers that were shown to be affected by clomazone in *Pomacea*, with both decreasing as the concentration of this herbicide increased. No studies were found that evaluated the oxygen consumption of organisms exposed to clomazone. However, the behaviour of this metabolic response in stressful environments varies between species, primarily due to their toxicokinetic and toxicodynamic traits (Nyman et al. 2014). The OC increased in *Pomacea canaliculata* exposed to Glyphosate (Xu et al. 2017), while it decreased in the freshwater snail *Bellamya bengalensis* and in the clam *Corbicula fluminea* exposed to mercury (Oliveira et al. 2018; Dhara et al. 2022). The decrease in OC at the highest clomazone concentrations could be due to some effects over respiratory organs (gills), such as mucus secretion or changes in gills structure (Das and Gupta 2012; Kamble and Kamble 2014). *Pomacea* exhibits alternative pulmonary respiration, which was limited in the experiment due to the experimentation chambers being completely filled with solutions. According to Dhara et al. (2022), the reduction in OC could have negative effects over food intake or predation risk and can lead to a decrease in activity (Montagna and Collins 2008; Vaquer-Sunyer and Duarte 2008) as was observed in this study. Active snails were observed in C and in T1 during the entire treatment. The number of active snails and the activity (quantified as AUC) decreased as herbicide concentration increases, while detached and dead organisms increased. The observation of detached organisms coincides with observation of dead snail of the species *Bellamya bengalensis* when exposed to mercury (Dhara et al. 2022). Activity is mainly used as a bioindicator in crustaceans and fish, but not in molluscs, particularly gastropods (Dhara et al. 2022). However, we demonstrated that it is an interesting response with which to evaluate the effects on snails in bioassays. Besides, the analysis of activity/behaviour is not invasive, does not imply animal handling or invest considerable time and can be further automatized.

AUC is a method of activity quantification that is generally applied in the field of medicine to know about the performance of some drugs and diseases (Scheff et al. 2011; Turner 2020). No work was found employing AUC as a method to quantify the activity of animals in any situation (effects of different foods, environmental changes, exposure to toxic compounds). This is one of the first studies to apply this method to quantify the activity of an organism in response to a stressful situation. According to Melvin and Wilson (2013), behaviour is a satisfactory trait with which to evaluate this kind of trial. Additionally, it shows a clear response compared to other endpoints, such as development and reproduction, and changes can be observed over short timeframes. Some aquatic and terrestrial organisms tend to decrease their activity in the presence of clomazone and other agrochemicals. For example, El-Gendy et al.

(2021) observed decreased activity in terrestrial snails, while Miron et al. (2004) observed erratic swimming and convulsions in *Rhamdia quelen* fingerlings exposed to clomazone concentrations over 5 µL L^{-1}. Similarly, Saglio and Trijasse (1998) observed burst swimming in goldfish exposed to atrazine and diuron. According to Bretaud et al. (2002), behavioural changes could be linked to the effects of agrochemicals on the nervous systems of exposed organisms (e.g. hormone levels and enzymatic activity). Methodological caution should be exercised in choosing the coding values to be used in classifying categories, as this choice could influence the expected area under the curve (AUC) response.

Chronic toxicity

Oxygen consumption (OC) was higher when compared to the results of the acute test (two orders of magnitude) due to the smaller size of the snails used. This agrees with the explanation that smaller organisms present a higher specific oxygen consumption (relative to body mass) than bigger ones (Hill and Wise 1992).

OC increases in the treatment of 150 µg L^{-1}, but the post-hoc test showed no significant differences; this may be due to lack of statistical power, perhaps due to a low number of samples within the factors. Given this, we suggest that for this type of statistical analysis a larger number of samples must be analyzed for each treatment and time.

The concentrations used in the sub-lethal trial did not affect the rest of the endpoints (activity, ammonia excretion rate, ingestion rate and growth), perhaps because they are very low to cause effects, at least at these levels and in the time of exposure. Effects caused at other levels of organization (e.g. oxidative stress, lipid peroxidation, genetic damage, enzyme activity, hormone levels, energy reserves, histological), some of which can be observed in short periods of time (one week, approximately) cannot be discarded (Putkome et al. 2008; Luna-Acosta et al. 2012; Griboff et al. 2014; Lavarias and Garcia 2015; Souza et al. 2024). But most of these procedures imply the killing of the organisms.

The activity measured as the area under the curve is not usually used as an endpoint in animal experimentation. Therefore, we do not have information from other studies with which to compare the present results. The AER can be affected by the presence of agrochemicals, but the response could depend on the exposure substance and/or its concentration, as was observed in the freshwater crab *Trichodactylus borellianus* (AER increases when exposed to chlorpyrifos and does not change when it was exposed to endosulfan, with respect to the control) (Montagna and Collins 2008). Besides, it decreases when juveniles of the same species were exposed to Glyphosate (Montagna and Collins 2005). This response difference can be explained by the metabolic pathways (use of proteins, carbohydrates and/ or lipids to obtain energy) that organisms execute to deal with the situation. In the case of silver catfish (*Rhamdia quelen*) exposed to clomazone, it was observed that there was a decrease in

muscular glycogen and liver protein (protein catabolism) that could indicate the use of this compound as an energy source (Crestani et al. 2006).

Ingestion rate was not affected by clomazone in the 76 days of the trial; something similar was observed for *Pomacea canaliculata* exposed to Glyphosate (from 0–120 mg L^{-1}), where IR began to decrease significantly after 75 days of exposure; the same was observed with growth rate (Xu et al. 2017). The effects over these endpoints were observed previously after 105 days of exposure. The fact that the growth remained the same in the three treatments could mean that 15 and 150 µgL^{-1} do not alter the energetic balance of juveniles of *Pomacea* sp. at least in a significant way.

These results suggest that biological responses such as oxygen consumption and activity could be sensitive (at least at concentrations over 3.75 mg L^{-1}), easy to measure and no invasive tools to evaluate in ecotoxicological assays to reveal if certain substances have effects on the energy balance of organisms.

According to Harayashiki et al. (2023), there is a lack of studies that evaluate the effects of toxic substances at a multigenerational level, mainly in molluscs; this allows information to be generated at other levels of organization, such as the population level. In this sense some experiments could be carried out, such as those made by Kong et al. (2024), where the copepod *Pseudodiaptomus annandalei* was exposed to some biocides for multiple and consecutive generations. In this kind of experiment, traits such as clutch size, population growth, expression of different genes, can be evaluated to determine the effects of toxic substances and allow us to know if there exist, for example, epigenetic modifications. Besides, abiotic factors (such as temperature, pH, oxygen, salinity) can interfere with the effects of toxic substances over living organisms; so, it is important to evaluate these interactions in the context of risk assessment, too (Thuyet et al. 2013; Silva et al. 2020).

Although chronic exposure to clomazone at concentrations up to 150 µg L^{-1} affected OC, the differences were not statistically significant. Based on previous studies involving other compounds, two complementary approaches are recommended to improve the sensitivity of future assessments: (i) extending the exposure duration and (ii) incorporating additional biomarkers capable of detecting early or subtle physiological responses, as demonstrated in *Pomacea canaliculata* by Arrighetti et al. (2018). These biomarkers could enhance environmental monitoring and be integrated into ecological risk assessment frameworks. Furthermore, given that the LC_{50} value obtained in this study (14.91 mg L^{-1}) is very close to the highest concentration tested (15.93 mg L^{-1}), we propose conducting new acute toxicity assays with higher concentrations to more accurately define the LC_{50} and to evaluate sub-lethal effects across a broader concentration range. This combined approach would contribute to a more comprehensive understanding of the ecotoxicological profile of clomazone.

Conclusions

In this study, the acute and chronic effects of the herbicide clomazone on the apple snail *Pomacea* sp. were evaluated. The results indicate that clomazone induces acute effects (mortality) at high concentrations. Overall, the findings suggest that *Pomacea* sp. may have potential as a bioindicator species; however, further research is required to evaluate its responses across different biological levels and life stages.

Data availability

Requests for data access can be made to the corresponding author.

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Author contributions FF: Designing and conducting trials, data collection and analyses, writing. CC: Designing trials, statistical analyses, review and editing. JG: Designing trials, statistical analyses, review and editing. AS: Designing trials, supervision, statistical analyses, writing, review and editing. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there are no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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8. Capítulo 3

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Ecotoxicological impact of the semiconductor nanomaterial Bi2S3 on the water flea (*Daphnia magna*) and the apple snail (*Pomacea* sp.)

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En este artículo y en el marco del objetivo "e", evaluamos los efectos de nanopartículas de Sulfuro de Bismuto (Bi_2S_3) sobre distintos bioindicadores de *Pomacea* sp, mediante un ensayo de exposición a concentraciones subletales durante 28 días. En él fueron evaluados el consumo de oxígeno, la mortalidad, el crecimiento, la tasa de ingestión y el factor de bioacumulación. Como resultados principales, fue observado que el consumo de oxígeno es el bioindicador que presenta cambios frente a la exposición ya desde el comienzo del ensayo, y que estos organismos son capaces de bioacumular las nanopartículas en sus tejidos. Esto puede significar, posteriormente, una transferencia a otros niveles de la cadena trófica, pudiendo afectar a otros organismos.

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RESEARCH

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Abstract

Nanoecotoxicology is essential for understanding the environmental impact of nanomaterials. This study evaluated the impact of Bi_2S_3- PVP nanorods on two model aquatic organisms with contrasting biology: *Daphnia magna* and *Pomacea* sp. The physicochemical properties of the nanorods were characterized. Nanorods presented a uniform hydrodynamic size (217.9 + / − 25.8 nm), Z-potential of − 5.24 + / − 0.76 mV, and thermal stability up to 500 °C. *Daphnia* incorporated Bi2S3- PVP nanorods in the digestive tract, but no evidence of immobilization/ mortality (in presence or absence of xanthan gum as stabilizer) was observed up to 100 mg L^{-1}. Snail's growth rate and ingestion rate were not affected by Bi2S3-PVP at concentrations of T1 = 1 and T10 = 10 mg L^{-1} in the evaluated timeframe. Oxygen consumption increased significantly in both treatments T1 and T10, with respect to the control after 18 and 28 days of exposure. Bismuth concentrations were detected and quantified in snail muscle in both treatments (T1 = 13.66 ± 20 mg kg^{-1} and T10 = 94.74 ± 144 mg kg^{-1}), and the bioaccumulation factor (BAF) was positive (BAF = 13.96 ± 20.99) and equal for both treatments. Bi_2S_3-PVP nanorods interact with both aquatic organisms and were ingested by both organisms, highlighting their bioavailability across different trophic levels. We report for the first time the bioaccumulation of these NPs in the tissues of a freshwater snail and in the gut content of *Daphnia*. These results contribute to the knowledge of nanomaterials' ecological risks, requiring the understanding of their dynamics and the development of regulatory frameworks for assessing emerging technologies.

Keywords Nanoecotoxicology · Bismuth sulfide ·Nanoparticles · Model organisms · Metabolic/physiological responses · Bioaccumulation · Risk assessment

Introduction

The rapid development and widespread application of semiconductor nanomaterials (NMs) have revolutionized various industries, ranging from electronics and medicine to environmental sensing and energy storage. These NMs, including quantum dots, metal oxides, and two-dimensional nanostructures, exhibit unique physicochemical properties that make them essential for modern technology. However, the increasing production and use of semiconductor NMs raise significant concerns regarding their potential environmental impact, particularly on aquatic ecosystems [1]. Given the unique physicochemical properties of semiconductor NMs, such as high surface area, reactivity, and the potential for generating reactive oxygen species, it is vital to investigate the ecotoxicology in different species [2]. Nanoecotoxicology, an emerging field within ecotoxicology, focuses on understanding the interactions between NMs and biological systems, encompassing the mechanisms of toxicity, bioaccumulation, and their ecological consequences. The effects of these materials on various biological endpoints, including survival, growth, reproduction, and behavior, are crucial. Moreover, understanding the bioaccumulation and biodistribution of semiconductor NMs in the organism's tissues is essential for assessing potential trophic transfer and long-term ecological impacts.

The effect of nanoparticles (NPs) has been evaluated in multiple organisms. In the freshwater fishes *Danio rerio* and *Rhamdia quelen*, the exposure to SiO_2, ZnO, TiO_2, and Ag NPs produces behavioral changes associated with neurotoxic effects, reproductive changes such as a change in the hatching time, effects on the immune system, malformations, increased mortality, oxidative stress, and genetic damage [3–8]. Invertebrate organisms exposed to NPs evidenced reactive oxygen species/oxidative stress, growth inhibition, mortality, lysosomal destabilization, bioaccumulation, body burden, and gene/ protein expression changes [9–12]. Specifically, the effect of exposure to TiO_2 NPs increased mortality and generated reproductive, behavioral, and physiological changes such as heartbeat, swimming speed, changes in vertical migration, and feeding rate in the filter feeding *Daphnia magna* [10]. The freshwater snail *Lymnaea luteola* exposed to Bi_2O_3 NPs evidenced changes in levels of oxidative stress enzymes, changes in behavior, and DNA damage [12]. These species are at the base of the trophic food web, and thus, NPs effects can propagate to the whole food web by bioaccumulation or biomagnification.

Generally, the studies focused on snails predominantly involve metal NMs and semiconductor oxides such as ZnO, TiO_2, CeO_2, among others [13, 14]. Some of these materials can already

be detected in the environment, while others remain undetectable. Surface water concentrations of TiO_2 NPs have been estimated by environmental fate models to range between 0.021 and 10.000 µg L^{-1} [15]. Measured concentrations in sewage and effluents vary widely, from 18 to 1233 µg L^{-1} [16, 17]. Cerium oxide nanoparticles have been detected in the Loire River at levels ranging from 1.3 x 10^4 to 3.4 x10^5 particles mL^{-1}, with the highest concentrations observed near wastewater treatment plants [18]. ZnO nanoparticles have been reported in various water sources—including wastewater effluents, tap water, and lake water—at concentrations between 0.2 and 1.8 µg L^{-1} [19]. Although systematic monitoring of nanoparticle concentrations in the environment is still lacking, assessing their toxicological potential remains crucial, particularly given the expected increase in their environmental presence as their production and application continue to increase [20].

Bismuth-based compounds represent a group of NMs gaining research attention because of their potential presence in natural environments. Bismuth, a non-toxic, abundant metal with a high atomic number, is notable for its biocompatibility. In NP form, bismuth compounds exhibit promising properties for applications in water treatment and medicine, including the diagnosis and treatment of diseases [21–24]. This has led to an increasing research interest in understanding bismuth compounds (Fig. 1), with large-scale production which will be followed by an increase in the concentration of these products in natural environments. One of the most prominent compounds is bismuth sulfide (Bi2S3), which exhibits remarkable properties such as high photoconductivity and significant thermoelectric power. These characteristics make Bi_2S_3 suitable for a wide range of applications, including photodetectors, electrochemical hydrogen storage devices, and sensors. In the medical field, it is utilized in photothermal therapy and as a contrast agent in X-ray computed tomography [25–28]. More recently, Bi_2S_3 NMs have been explored as radiosensitizers, further expanding their potential biomedical applications [29, 30]. An unavoidable consequence of the rapid development of

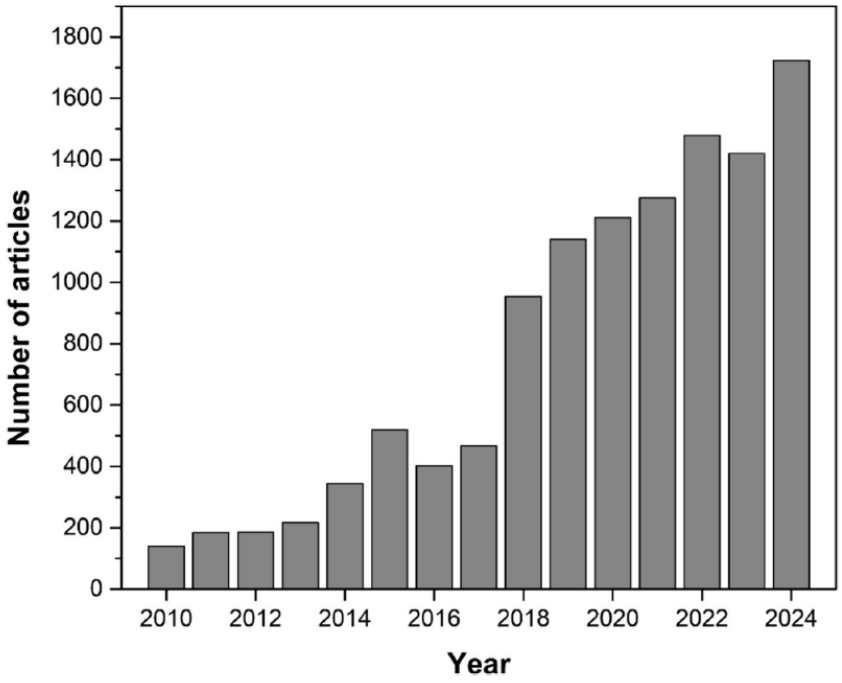

Fig. 1 The rise of published studies about bismuth semiconductor in the last 15 years. The words included in the literature review were bismuth semiconductor conducted on June 20th, 2025

bismuth sulfide NMs is their increasing concentration in the environment. Therefore, it is crucial not only to evaluate their impact on various organisms but also to identify a natural indicator of their presence.

Most of the reports on the toxicity of Bi_2S_3 NPs have been conducted on mammalian cell lines. These studies are carried out as a first step in evaluating their potential applications in nanomedicine [29, 31, 32]. To our knowledge, only one study has been published on the effects of Bi_2S_3 NPs (in the form of nanoflowers) on the carp *Cyprinus carpio*. The authors found that a concentration of 175 mg L^{-1} of nanoflowers has a genotoxic effect on this species, causing DNA damage and abnormalities in the nuclei [33]. He et al. [34] exposed early-stage zebrafish embryos to bismuth–asparagine coordination polymer spheres of 800 nm (with applications in nanomedicine) and recorded concentration-dependent developmental abnormalities [34]. The embryos displayed reduced head size (notably in the eyes), shorter body length, pericardial edemas, and delayed epiboly. These morphological defects coincided with altered expressions of developmental genes [34]. Yoon et al. [35] evaluated bismuth-doped zerovalent iron NPs in experiments that included aquatic bacteria (*Escherichia coli* and *Bacillus subtilis*) and the water flea *Daphnia magna*. They reported cell death in the bacteria and an inhibition of normal physiological states in *Daphnia magna*. The authors attributed these effects to oxidative stress, cell membrane disruption, and hypoxia [35]. In order to advance in the comprehension of the effects of bismuth-based NPs, a careful evaluation of multiple organisms with contrasting feeding strategies is required.

In this work, we assessed the nanoecotoxicology of Bi_2S_3- PVP nanorods on two aquatic model organisms with contrasting feeding strategies: the water flea *Daphnia magna* (Cladocera) and the apple snail *Pomacea sp.* (Prosobranchia). The former is a pelagic filter-feeder [36], whereas the latter feeds mainly on macrophytes, as well as periphyton and organic matter [37]**.** We examined the physicochemical properties of Bi_2S_3-PVP nanorods that influence their ecotoxicological behavior and evaluated their effects on the biological responses of these organisms at different concentrations of nanorods. By integrating perspectives from nanotechnology, ecotoxicology, and environmental science, this study aims to advance our understanding of the environmental implications of nanomaterial release in nature.

Materials and methods

Synthesis and characterization of Bi_2S_3–PVP nanoparticles

Polyvinylpyrrolidone (PVP)-coated bismuth sulfide NPs (Bi_2S_3-PVP) were synthesized using a hot injection method followed by a ligand exchange procedure, as described in a previous work [29]. Briefly, a solution of bismuth neodecanoate (10 mM, $Bi(Neo)_3$, 99% Aldrich), 5 mL of oleic acid (90%, Aldrich), and 20 mL of octadecene (ODE, 90%, Aldrich) was heated at 180 °C under an argon atmosphere. Next, a 50 mM solution of sulfur (S, reagent grade Aldrich) in oleylamine (OLA, > 98% Aldrich), along with 10 mL of ODE at room temperature, was swiftly injected into the mixture. The reaction was stopped after 40 s by adding cold toluene and using compressed air. For purification, a 1:1 ethyl acetate:ethanol (EtOAc:EtOH) was added, and the mixture was centrifuged several times.

The dried NPs were dispersed in 60 mL of EtOAc and combined with 30 mL of a 17.5 mM PVP (MW 10000 Aldrich) solution in EtOH to perform a ligand exchange. This mixture was heated under reflux for 4 h, then cooled with cold EtOAc. To collect the precipitate, the mixture was centrifuged and washed with 1:1 EtOH:EtOAc. The main characteristics of the synthesized NPs, such as crystallographic composition, size, and morphology, are available elsewhere [29]. A JEOL 2100 with 200 kV of voltage acceleration transmission electron microscopy (TEM) was used to confirm the size and morphology of NPs. Additionally, the hydrodynamic size and surface charge (Z-potential) were measured using dynamic light scattering (DLS), with a Zetasizer Nano-SZ90 from Malvern Panalytical. For these measurements, a suspension of approximately 20 μg mL^{-1} was prepared in Milli-Q water and sonicated for 30 min prior to the measurement.

The amount of ligand and the thermal events involved in its decomposition were evaluated through differential thermal analysis and thermogravimetry (TGA) using a simultaneous thermal analyzer STA/ TG-DSC, Netsztch STA F5, with an alumina crucible, a flow rate of 10 mL cm^{-3}, in a nitrogen atmosphere.

Ecotoxicity assays

Ecotoxicity of Bi_2S_3-PVP NPs was assessed in two model organisms, the water flea *Daphnia magna* and the apple snail *Pomacea* sp. The filter feeding *D. magna* inhabits the water column, potentially ingesting NPs suspended in the water column, while *Pomacea* sp. is a benthic detritivore organism that might ingest NPs from the sediment. This allows for two potentially different ways of ingesting NPs. Bioaccumulation in *D. magna* might serve as a rapid indicator of NPs in the pelagic environment due to short term accumulation, while *Pomacea* sp. could provide an integrated view including longer temporal scales and sedimentation of NPs in aquatic environments. In each species, a different set of experiments was conducted, and responses were measured.

Experiments on Daphnia magna

An acute toxicity test was conducted on the water flea *Daphnia magna* cladoceran using a Bi_2S_3-PVP NP suspension in Milli-Q water, following OECD standards [38]. Previously, the NPs were tested both with and without the addition of xanthan gum to the suspension. Xanthan gum was used at a concentration of 100 mg L^{-1}. The experiments with gum were carried out to simulate the presence of NP dispersing agents known to exist in the aquatic environment, such as organic matter or minerals [13, 14].

The cladocerans were 2 to 24 h old and originated from the third to fifth brood. They were reared at a temperature of 20 ± 2 °C, with a photoperiod of 16 h of light and 8 h of darkness. They were kept in reconstituted hard water with a pH of 7.6 ± 0.3 and hardness of 160–180 mg CaCO3 L^{-1} (according to OECD 2004 and ASTM 2001 standards), which was refreshed three times per week [38, 39]. Water fleas were fed three times per week with a concentration of 3 x 10^5 cells mL^{-1} of the algae *Raphidocelis subcapitata* [39].

Four replicates per treatment were performed, with five cladocerans in each replicate (n = 20). Cladocerans were exposed for 48 h to ten concentrations of NPs from 0.05 to 100 mg L^{-1} (0.05, 0.5, 1.0, 5.0, 6.25, 10.0, 12.5, 25.0, 50.0, 100.0 mg L^{-1}), with and without xanthan gum. A control treatment with no NPs using culture water was also evaluated. During the experiment, organisms were not fed. Immobilization/ mortality was registered for each organism at 24 h and at the end of the assays [34]. NP accumulation in the digestive tract was observed using a polarized optical microscope Nikon Eclipse Ti (Nikon Corporation, Tokyo, Japan) with a Nikon DS-Ri1 (magnification 4x).

Experiments on Pomacea sp.

Adult specimens of the apple snail *Pomacea* sp. Were collected from Briozzo Lagoon (34° 17′ 40″ S 53° 48′ 18″ W; Rocha, Uruguay) and kept under laboratory conditions with semi-controlled temperature ranging from 12 to 18 °C and natural photoperiod for more than a year [40]. They were fed ad libitum with hydroponically grown fresh lettuce two or three times a week, following the protocol of Estebenet and Cazzaniga [41]. New hatched snails from adults

grown in the laboratory were selected for the experiment (10-month-old; F1 generation; mean weight: 1.39 ± 0.32 g). Juvenile snails were acclimated for one week to the temperature used in experimental conditions (~ 20 °C).

Thirty Juvenile *Pomacea* sp. snails were placed individually (replicates) in 50 mL glass containers [40]. Ten snails were exposed for 28 days to 50 mL of dechlorinated tap water (DTW; control, C), ten to a concentration of 1 mg L^{-1} (T_1), and ten to a concentration of 10 mg L^{-1} (T_{10}) of Bi_2S_3-PVP NP suspension. The experimentation using individualized snails enables us to isolate and observe the effects caused solely by the NPs and eliminates potential intraspecific density-dependent interactions, such as competition for food or the decline in water quality caused by mortality within the flask [42, 43].

To keep the same concentration of NPs, the solutions were renewed twice a week, and the flasks were washed each time. The solutions were prepared by weighing dried Bi_2S_3-PVP NPs and suspending them in DTW by ultrasound. The same protocol but with DTW was applied to the control to avoid confounding effects of manipulation. The water temperature was kept at 20.01 ± 1.10 °C, a range that supports optimal performance of snails and prevents thermal stress [40]. The photoperiod followed natural light conditions (~ 12:12).

The concentration of bismuth in the suspensions was determined at the moment of the suspension preparation (t = 0, prior to exposing the snails) and after 72 h of snails' exposition by microwave-induced plasma optical emission spectrometry (MIP OES). Water samples (10 mL) were taken from the solution matrix (avoiding the capture of wall-adhered NPs) and were kept at − 80 °C until the analytical determination. The analytical determinations were performed in an Agilent 4210 spectrometer (Santa Clara, CA, USA) equipped with an inert One Neb nebulizer with a double-pass glass cyclonic spray chamber system and a standard torch. The spectrometer used an Agilent 4107 online nitrogen generator (Santa Clara, CA, USA). The plasma gas flow was fixed at 20 L min^{-1} and the auxiliary gas flow at 1.5 L min^{-1}. The following operational settings were applied: uptake time 15 s, plasma stabilization time with sample aspiration 15 s, read time 3 s (in triplicate), viewing position 0, nebulizer flow 0.75 L min^{-1}, and wavelength 306.772 nm. Automatic background correction was used.

Metabolic responses of Pomacea sp.

Oxygen consumption (OC) for six organisms randomly selected per treatment was recorded at the beginning of the trial (10 min after exposure to NPs) and on days 18 and 28 of the treatment. Oxygen concentration was estimated using the optical method, with an Oxy-4 m respirometer [39]. Each measurement session lasted approximately 40 min and ended before the oxygen concentration reached stressful levels (< 5 mg L^{-1}) [44]. The OC rate was calculated using the following equation [35]:

$$O(mg/g.h) = \frac{(O_{2f} - O_{2i}) * v}{W * t}$$

where O_{2i} and O_{2f} are the initial and final oxygen concentrations in mg L^{-1}, *V* is the container volume in L, *W* is the weight of the snail in *g*, and *t* is the time in hours between the initial and final readings.

The snails were fed with hydroponic lettuce twice a week, and the ingestion rate (IR) was estimated. Lettuce was weighed before feeding, and the remains were measured after 24 h. IR was calculated at the beginning and end of the experiment according to the following equation [40]:

$$IR(mg/g.d) = \frac{LWi - LWf}{Wt}$$

where LW_i and LW_f are the initial and final lettuce weights (g), while W is the snail weight and *t* is one day.

Growth rate (GR) was assessed by weighing the organisms at the start (W_i) and end (W_f) of the experiment using a precision balance (accuracy: 0.0001 g). This approach minimized handling during the trial and reduced stress, which could otherwise affect their response to NPs. The GR was obtained from Eq. 3:

$$GR\ (g\ day^{-1}) = (W_f - W_i)/\ \triangle T$$

Determination of bismuth concentration in snails' tissue

At the end of the experiment, snails were weighed and slaughtered. The body of each snail was removed and separated from the intestine, washed with distilled water, and lyophilized (Biobase freeze dryer, model BK-FD10PT) to determine bismuth bioaccumulation by MIP OES, as described before.

Prior to the determination by MIP OES, the samples were weighed and subjected to a microwave-assisted digestion using a Mars 6 digestion system (North Carolina, USA) equipped with a rotor with 8 Xpress Vessels (PFA, 20 mL). Each sample was treated with 4.0 mL of dilute HNO3 (6 mol L^{-1}) + 1.0 mL of ultrapure water using the following program: heat to 200 °C for 15 min, maintain at 200 °C for 15 min, and cool to room temperature (power 400–1800 W) [45].

The concentration of bismuth in the snails was determined and expressed as mg kg^{-1}. The bioaccumulation factor (BAF) was calculated as follows:

$$BAF\ (L\ kg^{-1}) = C_B/C_{WD}$$

where C_B is the bismuth concentration in the snails in mg kg^{-1} and C_{WD} is the concentration in the solution in mg L^{-1} [46]. The latter was determined by averaging the calculated bismuth concentrations of the prepared solutions throughout the trial, considering the PVP content.

Statistical analysis

Evaluation of changes in rates

Homogeneity of variance and normality were evaluated in the response variables obtained from the trials with *Pomacea* (OC, IR, GR, and BAF), using the Shapiro and Levene tests, respectively. The GR and BAF variables were logarithmically transformed to log(x + 1) since they did not meet assumptions. One way ANOVA was used to evaluate the effect of the treatment on GR and BAF, and two-way repeated measures ANOVA was used to simultaneously evaluate the effect of Treatment and Time in the OC and IR. The *rstatix* R package was used for ANOVA testing. The *t* test with the Bonferroni correction was used as a post-hoc test, using the nominal 0.05 level as the limit for significance.

Results and discussion

Bi_2S_3-PVP nanoparticles

A representative TEM image of the NPs used in the ecotoxicity assays is shown in Fig. 2a. Particles present rod morphology with an average width of 4.2 ± 1.4 nm and an average length of 27.4 ± 19.3 nm [29].

Among many factors, the size and surface charge of NPs are critical factors that can greatly affect their uptake and interactions in aquatic organisms [13]. For instance, it has been demonstrated that the size of NPs plays a crucial role in their cellular uptake [47, 48]. Given a similar morphology, smaller NPs generally exhibit enhanced cellular internalization as they have a high surface area and the ability to navigate through biological barriers in a more effective way [49, 50]. Xue et al. [50] found that nanorods and helical/striped surface morphologies have higher internalization efficiency compared to spherical particles and could be thus more ecotoxic at a given concentration.

The DLS profile (shown in Fig. 2b) of our NPs exhibited a single, narrow peak, centered at 217.9 ± 25.8 nm, corresponding to a uniform hydrodynamic size. Their Z-potential resulted in − 5.24 ± 0.76 mV.

The hydrodynamic size of NPs plays a crucial role in determining their behavior, bioavailability, and toxicity in aquatic environments. Unlike primary particle size (as measured by TEM), the hydrodynamic size accounts for solvent or surface coating [51]. This parameter directly influences sedimentation rate, diffusion, aggregation tendency, and the ability of NPs to cross biological membranes. Our NPs appear less likely to be internalized by filtering organisms compared to smaller ones, as some studies indicate that NPs above 100 nm tend to aggregate,

which reduces their bioavailability in the water column. Conversely, larger aggregates may settle more rapidly, being bioavailable to benthic organisms [52]. For example, in mussels, digestive glands and hemocytes readily take up smaller, non-aggregated particles, while in snails, biodynamic studies with Ag and ZnO NPs reveal that aggregation and low zeta potential limit uptake [53, 54].

The surface charge (measured as Z-potential) of NPs also plays a key role in their interaction with biological systems and cells. NPs with a positive surface charge have shown an enhanced cellular uptake compared to their counterparts. This is explained in terms of their electrostatic attraction to the cell membrane (negatively charged) [47, 55]. The Z-potential result of our NPs suggests that the cell internalization of Bi2S3PVP nanorods by the snails would likely be lower than that of particles of similar morphology bearing a positive Z-potential. Nevertheless, the bismuth content measured in snail tissues (Table 1) and the presence of NPs in the digestive tract of *Daphnia magna* indicate substantial active uptake at the organism level. This suggests that, at least in these species, accumulation may predominantly occur in the gut lumen or extracellular compartments, potentially through ingestion and retention rather than translocation across cellular membranes.

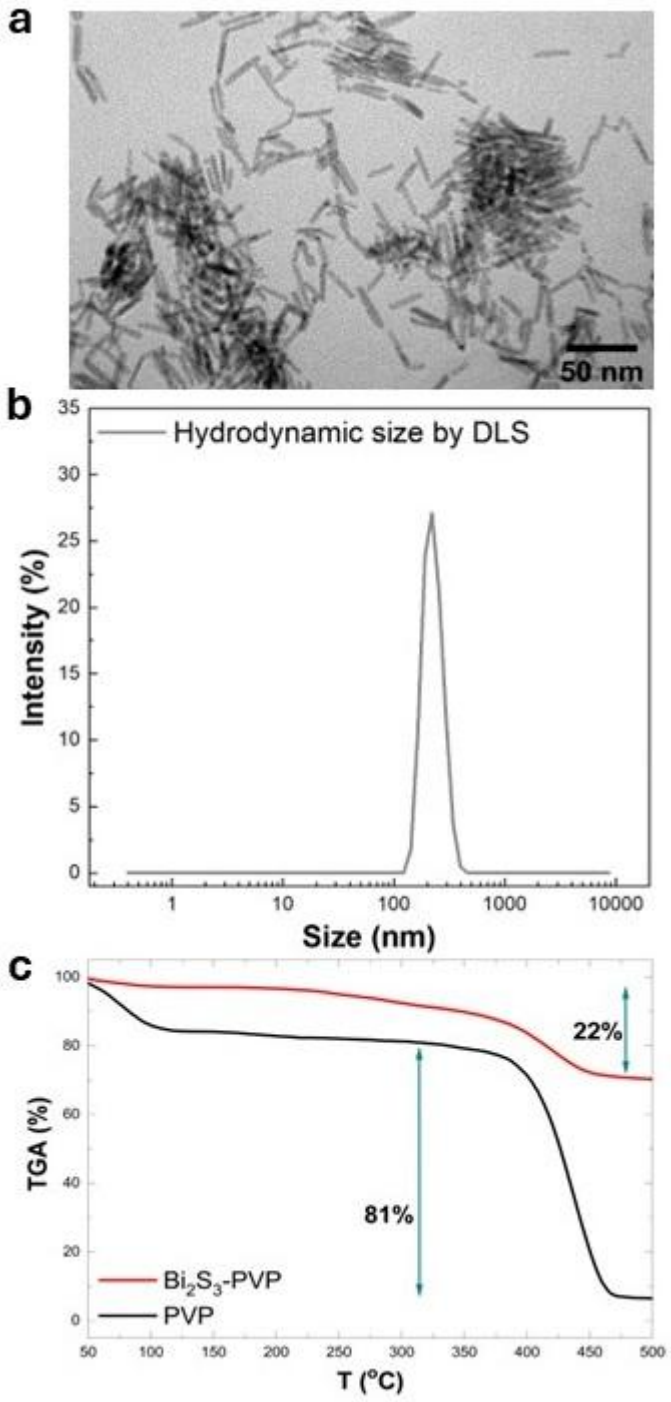

Fig. 2 A A transmission electron microscopy image of representative rod-like Bi_2S_3-PVP nanoparticles, **b** dynamic light scattering (DLS) profile of Bi_2S_3-PVP nanoparticles suspended in water, and **c** thermogravimetric analysis (TGA) profile of Bi_2S_3-PVP nanoparticles compared to the PVP one

TGA confirmed the thermal stability of the NPs up to 500 °C, showing no decomposition within this range (Fig. 2c). A single mass loss event was observed, corresponding to the presence of PVP, with a polymer content of approximately 22% by mass. The presence of PVP, a commonly used ligand and stabilizing agent, contributes to the colloidal stability of the NPs by preventing aggregation through steric hindrance. In this context, surface-bound PVP can

influence the NPs' interactions with biological systems, potentially affecting their uptake, distribution, and toxicity in aquatic organisms.

Ecotoxicity assays in *Daphnia magna* (Cladocera)

No immobilization or mortality of *D. magna* was observed at 24 or 48 h of exposure at concentrations of Bi_2S_3-PVP NPs between 0.05 and 100 mg L^{-1}, whether xanthan gum was used as a dispersant or not. This indicates that Bi_2S_3-PVP NPs do not exhibit acute toxicity in *D. magna* at these concentrations.

Table 1 Bismuth concentration measured in the suspensions (mg L^{-1}) after 72 h of *Pomacea* sp. exposure and in the snail's tissue (mg kg^{-1}). C-X, T1-X, and T10-X identify the individuals in each treatment (C = control; T1 = 1 mg L^{-1}1; T10 = 10 mg L^{-1})

C			T1			T10		
Snail	Tissue [Bi] (mg kg^{-1})	Suspension [Bi] (mg L^{-1})	Snail	Tissue [Bi] (mg kg^{-1})	Suspension [Bi] (mg L^{-1})	Snail	Tissue [Bi] (mg kg^{-1})	Suspension [Bi] (mg L^{-1})
C-1	ND	—	**T1-1**	1.17	—	**T10-1**	—	—
C-2	ND	ND	**T1-2**	6.03	ND	**T10-2**	—	—
C-3	ND	—	**T1-3**	62.22	ND	**T10-3**	58.43	ND
C-4	ND	—	**T1-4**	4.05	—	**T10-4**	39.91	—
C-5	ND	—	**T1-5**	5.22	—	**T10-5**	21.29	—
C-6	ND	—	**T1-6**	—	—	**T10-6**	13.69	—
C-7	ND	—	**T1-7**	13.79	—	**T10-7**	51.94	—
C-8	ND	—	**T1-8**	2.36	—	**T10-8**	91.76	—
C-9	ND	ND	**T1-9**	ND	—	**T10-9**	446.95	0.0554
C-10	—	—	**T1-10**	14.40	—	**T10-10**	33.88	—

C-10, T1-6, T10-1, and T10-2 dead organisms

ND, non-detectable

Error: 5%

In the absence of xanthan gum, nanoparticles rapidly settled at the bottom of the flask, indicating that the presence of the gum improved nanoparticle suspension stability. Figure S1 shows photographs of the bottom of the containers used in the analysis after 48 h of incubation of the Cladocera with the NPs. At an NP concentration of 10 mg L^{-1}, no deposition was observed at the bottom of the vessel when gum was used. However, at concentrations above 100 mg L^{-1} (Figures S1 c and f 100 mg L^{-1}), deposition was evident. This sedimentation was also observed in the trials evaluating snail's responses, highlighting the importance of measuring bismuth concentration in the water column when experiments last more than 24/48 h.

NPs incorporated into the digestive tract of *D. magna* were identified by the black coloration of the tract, which persisted throughout the experiment (Fig. 3). This pattern was observed in experiments with and without xanthan gum, but in the latter case, a more intense coloration was registered. The change in the gut coloration might increase predation in natural ecosystems, as most predators of *Dapnhia* are visual predators [56].

NP ingestion may be hazardous to these organisms as it can reduce their feeding rate, leading to an energy deficit that ultimately decreases reproduction, causing a population decline [57]. However, despite NP ingestion, optical microscopy shows that they remain in the digestive tract for at least 48 h, indicating that the NPs were not internalized into other organs, especially the brood chamber, where they could impact reproduction. Furthermore, there is no visible rupture of the digestive tract membrane [58].

It is noteworthy that neither the NPs nor the xanthan gum adhered to the Cladocera's antennae. This is important because *Daphnia magna* uses its antennae for both feeding and mobility. Feeding occurs through the filtration of food as the antennae's movement creates a constant flow to direct food toward the digestive system, while also allowing the necessary movement for the organism to continue its life cycle [59]. If these compounds were present on the antennae, they could obstruct both feeding and movement in the water, which would result in the death of the organisms.

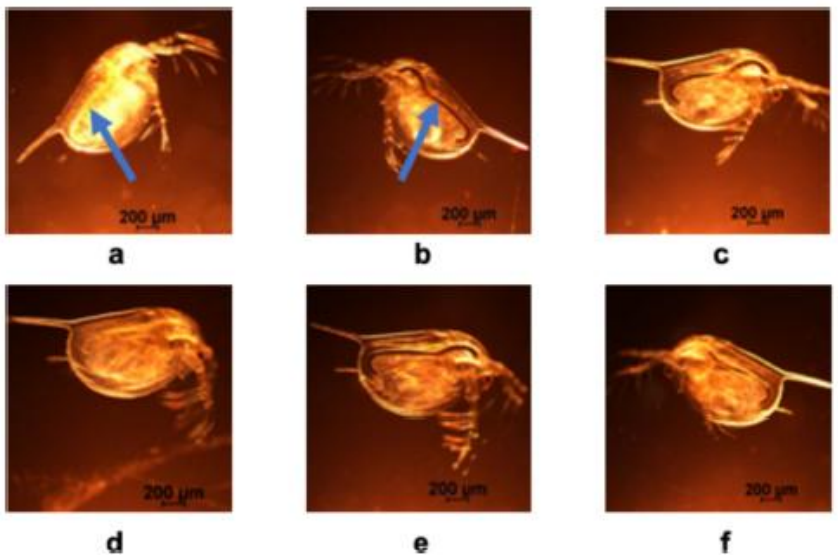

Fig. 3 Optical microscopy images of *Daphnia magna* from the Acute Test Assay for **a** and **d** controls without and with xanthan gum, respectively, **b** 6.25 mg L^{-1} of Bi_2S_3-PVP in water, **c** 100 mg L^{-1} Bi_2S_3-PVP in water, **e** 6.25 mg L^{-1} in water with xanthan gum, and **f** 100 mg L^{-1} in water with xanthan gum

Ecotoxicity assays in *Pomacea* sp.

The measured bismuth concentration in the prepared suspensions prior to contact with the organisms was below the limit of detection for the 1 mg Bi2S3-PVP L^{-1} suspension. For the 10 mg Bi_2S_3-PVP L^{-1} suspension, the measured concentration was, on average, 0.0659 mg of Bi L^{-1}. Considering that 22% of the NPs' mass consists of PVP, the calculated bismuth concentration for the two prepared suspensions is 0.52 mg Bi L^{-1} and 5.2 mg Bi L^{-1}, respectively. These results indicate a reduction in the measurable bismuth concentration by an order of magnitude, suggesting that the NPs rapidly settle at the bottom of the vessel after suspension preparation. It is worth mentioning that xanthan gum was not used in the experiments with snails because they are not filter-feeding organisms. Therefore, maintaining the NPs in suspension was not a critical factor.

We registered four dead organisms in either the control (N = 1) or treatments (NT_1 = 1, NT_{10} = 2) not following an evident pattern. The average GR and specific IR did not differ among treatments (p > 0.05), suggesting that concentrations up to 10 mg L^{-1} do not affect these responses in the evaluated timeframe. Table 2 shows the summary of IR and GR for both treatments.

Evidence suggests that aquatic snails (like many other aquatic organisms) may be vulnerable to the harmful effects of NPs exposure, which can alter their oxygen consumption patterns and, in turn, impact their overall metabolic and respiratory functions.

As shown in Fig. 4, OC in C remained stable throughout the experiment (p > 0.05). Pairwise comparisons among treatments provide evidence that snails after 10 min of exposure to 10 mg L^{-1} of NPs concentration increased OC (T10 = 0.152 ± 0.067 mg g^{-1} h^{-1}). There is a significant difference between T10 and T1 (OC = 0.052 ± 0.034 mg g^{-1} $h^{-1)}$ for time = 0 day (Fig. 4). This increase in OC for the higher NP concentration persists throughout the treatment (OC 0.216 ± 0.074 and 0.157 ± 0.023 mg g^{-1} h^{-1} for 18 and 28 days, respectively). This pattern is consistent with typical physiological responses observed in invertebrate organisms under stress, where ventilation rate increases to meet elevated energetic demands [60]. However, metabolic responses to environmental stress can vary among species due to differences in toxicokinetics and toxicodynamics, as well as specific characteristics of the contaminant [61].

Table 2 Ingestion rate and growth of snails exposed to 1 and 10 mg L^{-1} Bi_2S_3-PVP (T1 and T10, respectively) for 28 days. C represents the control, without NPs. No significant differences were observed between treatments

Treatment	Ingestion rate (mg g^{-1} day^{-1})		Growth (g)
	t = 0 day	t = 28 days	
C	209.6 ± 55.9	215.3 ± 96.9	0.12 ± 0.07
T_1	204.1 ± 49.0	189.7 ± 64.7	0.16 ± 0.14
T_{10}	219.5 ± 128.2	153.5 ± 80.8	0.11 ± 0.08

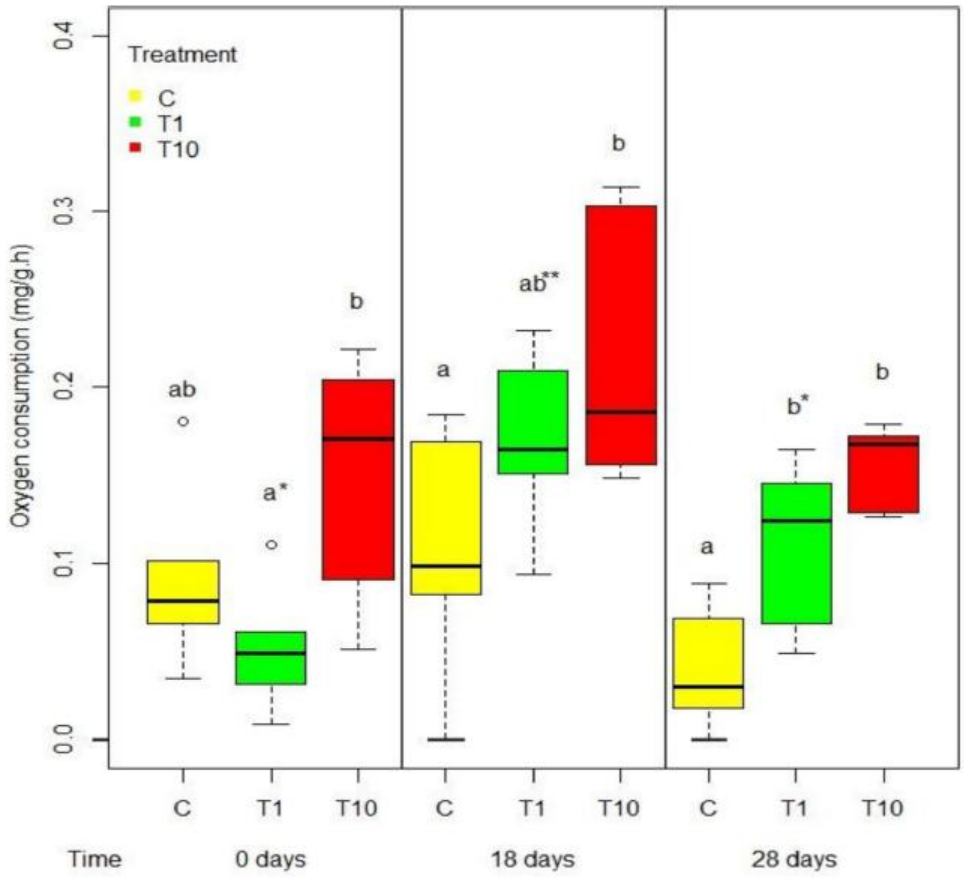

Fig. 4 Mass-specific oxygen consumption (mg g^{-1} h^{-1}) of the freshwater snail *Pomacea* sp., exposed to different concentrations of Bi_2S_3-PVP NPs (T1 = 1 mg L^{-1} and T10 = 10 mg L^{-1}) and a control set (C) with no NPs, for 28 days. Letters represent significant differences between treatments, within each time. Asterisks represent significant differences among times within the same treatment. Note: Oxygen consumption began to be recorded *ca.* 10 min after the snails were placed in the solutions with NPs

Second, comparisons within treatments (and between times) (Fig. 4) show that exposure to 1 mg L^{-1} of NPs induced a significant increase in OC compared to C, followed by a significant drop by day 28 (OC 0.052 ± 0.034, 0.169 ± 0.048, and 0.112 ± 0.047 mg g^{-1} h^{-1} for 0, 18, and 28 days, respectively; p0-18 d = 0.013, p0-28 d = 0.086, and p18- 28 d = 0.036). The T_{10} treatment showed the highest value, but no significant change with time, suggesting that this higher concentration may have exceeded the snail's physiological tolerance, suppressing their compensatory mechanisms.

The concentration of bismuth in the snail muscle in C was always under the detection limit, but it was quantifiable in most replicates of the two treatments (Fig. 5). The quantification limit was exceeded in eight out of nine replicates in T_1, and in all eight replicates in T_{10}. The quantified bismuth concentration in tissue differed significantly ($p < 0.05$) between the treatments and the control (T_1 = 13.66 ± 20 mg kg^{-1} and T_{10} = 94.74 ± 144 mg kg^{-1}; see Fig. 5).

As shown in Table 1, in both treatments (T_1 and T_{10}) one individual displayed an "abnormal" pattern (T_1-3 and T_{10}-9), ingesting a considerably higher amount of bismuth. Previous studies have reported highly variable responses among aquatic invertebrates exposed to external agents (e.g., nanoparticles, metals), although the underlying causes of such variability remain

poorly understood [62, 63]. Some authors have attributed inter-individual differences to factors such as size, sex, age, genotype, or behavior, yet no direct correlations have been established [62, 63]. In our study, all organisms were siblings, reared and maintained under identical laboratory conditions. Moreover, based on the evaluated response parameters (GR, IR, OC), these outliers did not exhibit any distinctive behavior that could explain their elevated bismuth uptake. Considering these findings, a plausible explanation for our extreme results is that these individuals engaged in increased browsing activity during the experiment, which may have resulted in greater nanoparticle ingestion.

The measured concentration in the suspensions at the end of the experiment was below the limit of detection (LOD = 0.0047 mg L^{-1}), except for one trial in the T_{10} treatment. This indicates that the NPs had sedimented and/or adhered to the surface of the borosilicate vessel and/or the snail shell, as determined before and observed during the experiments. Additionally, despite the slightly negative surface charge of NPs, they were absorbed by the snails. This was confirmed by the determinations presented in Table 1 and Fig. 5. Bismuth concentrations in the snail's tissue exceeding the initial levels in the suspensions were observed in several organisms (< LOD 0.0047 mg Bi L^{-1} and 0.0659 mg Bi L^{-1} for suspensions of 1 and 10 mg Bi_2S_3- PVP L^{-1}, respectively). Given that the suspension of treatments was renewed twice a week and the flasks were washed, the concentrations measured in the snails indicate, on the one hand, that the snails ingested NPs that adhered to the vessel and, on the other hand, that there was bioaccumulation of bismuth in the tissues, as confirmed by BAF. This has significant implications for the food web, as bioaccumulation and biomagnification at lower trophic positions can result in high concentrations of contaminants in organisms feeding higher in the trophic web. Although there is no evidence of trophic transfer in the wild for these species, mercury selenide (HgSe) has been quantified in the liver of the golden eagle, which was hypothesized to result from scavenging on marine birds and cetaceans, possibly linked to NPs of this compound [64]. Hepatic damage to birds caused by zinc oxide NPs, the mutagenic and cytotoxic effects caused to the freshwater turtle *Podocnemis expansa* [65], or the effect on reproductive activity in the turtle *Pelodiscus sinensis* [66], suggest that bioaccumulation across trophic levels can be detrimental for the whole food web, including mammals [67].

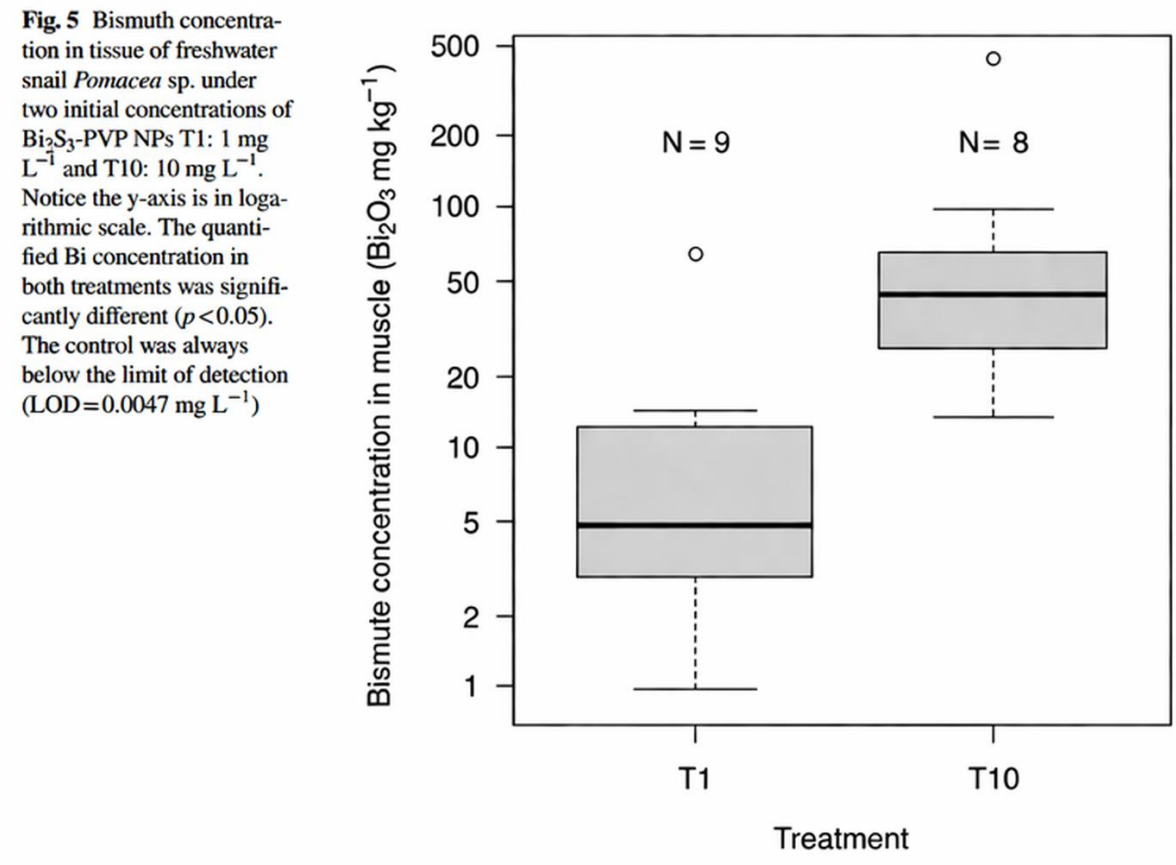

Fig. 5 Bismuth concentration in tissue of freshwater snail *Pomacea* sp. under two initial concentrations of Bi_2S_3-PVP NPs T1: 1 mg L^{-1} and T10: 10 mg L^{-1}. Notice the y-axis is in logarithmic scale. The quantified Bi concentration in both treatments was significantly different ($p < 0.05$). The control was always below the limit of detection (LOD = 0.0047 mg L^{-1})

Mean (sd) BAF values ($BAFT_1$ = 15.16 ± 24.31 L kg^{-1} and $BAFT_{10}$ = 11.84 ± 18.04 L kg^{-1}) were not statistically different between treatments (ANOVA, $p > 0.05$). An extremely high BAF value was observed in each treatment (77.73 and 55.84 L kg^{-1} in T_1 and T_{10}, respectively), which are observed in bioaccumulation measurements. We propose some hypotheses to explain these large deviations. First, it is expected that snails feeding on aggregated NPs within the flask may generate extreme BAF values, as patchy feeding could increase variance compared to the assumption of a homogeneous distribution. Further testing of this hypothesis is relevant as it could imply that some organisms can accumulate disproportionately large concentrations of bismuth NP in their tissues.

Another possible explanation for these high values is the semi-static experiment design; ideally, experiments with the aim of evaluating bioaccumulation in snails should be performed on continuous and high flow to avoid NPs sedimentation [68].The fact that in our experiment the suspensions were in a semi-static regime led to the sedimentation of the Bi_2S_3-PVP NPs and thus, an increased concentration available to concentrate in the tissues [68].

We are not aware of any reports that have determined the BAF for Bi_2S_3-PVP NPs in animals. However, it has been reported that aquatic organisms like fishes could bioaccumulate these NPs in various body tissues (e.g., intestine, skin, scales) [33]. BAF was calculated for other compounds, such as Ag NPs, and organisms; it was between 1.89 and 52.3 L g^{-1} (mean values), being higher in smaller organisms such as microalgae and freshwater crustaceans

[69], mainly because of their feeding habits. This is one of the first works that reports this index for the *Pomacea* genus exposed to NPs. There is information only for biocides, such as Tributyltin TBT [70].

The effects of other bismuth compound NPs, such as bismuth oxide, were studied on the freshwater snail *Lymnaea luteola*. These NPs have shown toxic effects including oxidative stress, DNA damage, and increased cell death, with a 96-h LC_{50} of 72.6 μg mL^{-1} [71]. However, in *Daphnia magna*, no experimental data on bismuth-based compounds or bismuth metal NPs are available.

Conclusions

The Bi_2S_3-PVP NPs with a rod-like morphology exhibited a moderate hydrodynamic size and a slightly negative surface charge. While it is not expected that a high cellular uptake occurs for both studied organisms due to the near-neutral Z-potential, the significant accumulation of bismuth in snail tissues and cladocera gut suggests important active uptake of NPs, highlighting their bioavailability across different trophic strategies. This emphasizes that factors beyond simple electrostatic interactions, such as particle morphology, aggregation behavior, or biological affinity, also influence NP assimilation, highlighting the complex dynamics governing NP-biological interactions and underscoring the need for further investigation into the mechanisms of NP uptake and their potential ecological risks in freshwater systems.

We demonstrated that Bi_2S_3-PVP NPs interact with aquatic organisms with contrasting feeding habits through multiple pathways. While the presence of semiconductor NPs in the gut of *Daphnia* has been previously reported and is not considered an uncommon phenomenon, this is, to our knowledge, the first report describing the presence of Bi_2S_3-PVP NPs in this part of the organism.

Although no acute lethal effects were observed in water fleas during short-term exposure assays, digestive tract suggest potential sublethal impacts. These alterations may increase the vulnerability of exposed organisms to visual predators in natural ecosystems, potentially leading to indirect mortality and broader ecological consequences. These findings highlight the need to further investigate the ecological and evolutionary implications of NPs exposure across food webs.

No significant differences were observed in growth or ingestion rates for snails among treatments, but OC analyses revealed clear physiological responses of *Pomacea* sp. to Bi_2S_3-PVP NPs exposure. These findings indicate that Bi_2S_3-PVP NPs can induce dose-dependent metabolic effects even in the absence of observable behavioral or mortality outcomes. The alteration of OC emphasizes the importance of incorporating sublethal physiological endpoints in nanoecotoxicological assessments, as they may reveal early signs of stress not captured by conventional toxicity parameters. Further studies are warranted to elucidate the long-term

ecological consequences of NP exposure on aquatic invertebrate populations and food web dynamics.

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Author contribution MPB and FF contributed equally to this work. FF and IG conducted the majority of the ecotoxicological experiments, focusing on freshwater snails and cladocerans, respectively. Their work included the preparation of laboratory suspensions, experimental setup, and assessments. IG was also responsible for the synthesis and physicochemical characterization of the nanoparticles. MPB, FF, AS, IA, and IG jointly contributed to the conceptualization of the study and the formal analysis of the results. MP and IM carried out the analytical determinations of bismuth. The original draft of the manuscript was written by MPB, AS, and FF. MPB and IA played leading roles in securing funding for the project.

Data availability No datasets were generated or analyzed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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9. Discusión general y conclusiones

Esta tesis aporta conocimiento sobre la biología/ecología del caracol manzana *Pomacea* sp. y sus respuestas frente a la exposición al herbicida Clomazone y a nanopartículas de Sulfuro de Bismuto, dos contaminantes emergentes. La alimentación, reproducción y respuestas a la temperatura han sido estudiadas para distintas especies del género (**Kumara et al., 1999; Ruiz-Ramírez et al., 2005; Seuffert et al., 2010; Seuffert & Martin, 2013; Tamburi et al., 2018**); sin embargo, en este trabajo se abordan aspectos que hasta ahora no han sido explorados para *Pomacea* sp., caracterizando las curvas de performance térmico para algunos bioindicadores y evaluando los efectos de la exposición al herbicida clomazone y a nanopartículas de sulfuro de bismuto.

En el marco del primer objetivo, se analizaron los efectos de las variaciones de temperatura en la fisiología a corto y largo plazo. A corto plazo, se caracterizaron las curvas de desempeño térmico (TPC, por su sigla en inglés), obteniendo como resultado una temperatura óptima de desempeño a ~22 °C, en la cual los caracoles no presentarían signos de estrés asociados a este factor. En temperaturas inferiores o superiores a dicho valor, se observó una disminución del consumo de oxígeno (OC), indicando que los organismos están lidiando con una situación de estrés térmico.

La Tasa de Ingestión (TI) aumenta con la temperatura, lo que podría indicar que la energía asimilada a partir del alimento se destina a compensar el incremento en los requerimientos fisiológicos asociados al estrés térmico. No obstante, permanece casi constante entre ~15-20 °C, mientras que el crecimiento continuó incrementándose. De acuerdo con **Pörtner et al. (2017)**, el comportamiento de esta respuesta frente a la temperatura es unimodal, como la observada para el consumo de oxígeno; comienza con un aumento hasta alcanzar un óptimo y luego disminuye (Laurel et al., 2016). Si bien no se encontraron trabajos con resultados similares a los de esta tesis, esta diferencia en el comportamiento podría indicar que, durante exposiciones prolongadas (c.a. 400 días), la energía asimilada dentro de ese rango de temperatura, entre 15 y 20 °C, es utilizada principalmente en procesos crecimiento, y no para cubrir otras funciones metabólicas, como podría ocurrir a temperaturas por debajo o por encima de este rango (Capítulo 1).

Los efectos de la exposición de *Pomacea* sp. al herbicida clomazone evidenciaron afectación del herbicida en la actividad, el consumo de oxígeno y la mortalidad, mientras que sólo el consumo de oxígeno fue levemente afectado en el ensayo subletal. Este es el primer registro del consumo de oxígeno de un organismo acuático expuesto a clomazone, mientras que las demás respuestas ya han sido evaluadas en otros organismos, principalmente peces (**Saglio & Trijasse, 1998; Miron et al., 2004**) y caracoles terrestres. En estos últimos también se observó una disminución de la actividad (**El-Gendy et al., 2021**; Capítulo 2).

Las nanopartículas de sulfuro de bismuto provocaron cambios en el consumo de oxígeno desde el comienzo del experimento. Se observó que los caracoles bioacumularon este compuesto en sus tejidos, lo que puede tener efectos en las cadenas tróficas de las que este caracol forma parte (Capítulo 3).

Los resultados obtenidos indican que *Pomacea* sp. tolera un amplio rango de temperaturas, y que, si bien presenta niveles en los que su desempeño no se ve afectado, también presenta estrategias para lidiar frente a condiciones extremas, como la disminución del metabolismo y la actividad a temperaturas bajas. Este comportamiento ya ha sido observado previamente en otras especies de este género (**Seuffert & Martin, 2010; Deaton et al., 2016**). Los resultados que surgen de este trabajo son útiles para predecir la distribución de estos caracoles en la naturaleza y mejorar su cultivo en cautiverio.

Las variaciones de temperatura también pueden tener influencia sobre la sensibilidad de los organismos a la exposición a productos químicos, haciéndolos más susceptibles cuando se encuentran en niveles de temperatura que representan una situación de estrés (**Kazmi et al., 2022**). Esto sucede porque la actividad de los organismos aumenta con el incremento de temperatura (**Seuffert et al., 2010**). Asimismo, el calor también aumenta la tasa respiratoria y puede afectar la permeabilidad de las membranas celulares, facilitando la absorción de sustancias contaminantes (**Sokolova & Lannig, 2008**). Estos factores resaltan la importancia del uso de suelo y procesos como el cambio climático y las interacciones entre ambos factores.

Se debe resaltar el uso de análisis como el Área Bajo la Curva (Area Under the Curve, AUC; Capítulo 2), método prácticamente no aplicado en esta clase de evaluaciones y que permitió asignar valores numéricos al comportamiento de los caracoles para poder comparar este indicador entre los distintos tratamientos. Este resultado demuestra la viabilidad del método al aplicarlo en este contexto y establece un precedente para su aplicación en investigaciones futuras de esta naturaleza. En estudios previos, ejemplares de este género han sido expuestos a diversos agentes generadores de disturbios en el medioambiente, como metales y agroquímicos, observándose efectos sobre biomarcadores como enzimas asociadas al estrés oxidativo, acetilcolinesterasa, proteínas, además de cambios estructurales en tejidos como branquias, glándula digestiva y pie (**Dumee et al., 2015; Martínez et al., 2017; Arrighetti et al., 2018; Campoy-Díaz et al., 2018**). Los resultados obtenidos en este trabajo muestran que *Pomacea* sp. es sensible a la presencia de compuestos como clomazone y sulfuro de bismuto, lo que fue evidenciado a través de respuestas tanto letales como subletales. Entre los primeros se destacan cambios en el consumo de oxígeno y la actividad. Estos hallazgos, sumado a los antecedentes mencionados para este género y para otras especies de gasterópodos

dulceacuícolas, como *Pomacea canaliculata* y *Biomphalaria alexandrina*, utilizados como bioindicadores de contaminación química en ecosistemas acuáticos (**Campoy-Díaz et al., 2018; Morais et al., 2022; Juarez et al., 2025**), refuerzan la idea de que *Pomacea* sp. presenta potencial como bioindicador. Además, el hecho de que sea una especie nativa aumenta su relevancia, ya que permite una evaluación más representativa de las condiciones ambientales locales.

Es importante destacar el uso de herramientas no invasivas para el registro de biomarcadores (e.g. actividad, consumo de oxígeno, tasa de excreción de amonio, tasa de ingestión, crecimiento), porque no implican el sacrificio de los organismos, al menos a lo largo de los experimentos, permitiendo realizar el seguimiento de estos. También permite disminuir el número de ejemplares utilizados, cumpliendo con el componente de Reducción del principio de las 3 R (Reemplazo, reducción y refinamiento; **Sneddon et al., 2017**).

Los resultados obtenidos a partir de los trabajos expuestos indican que *Pomacea* sp. podría ser un organismo bioindicador de los ambientes que habita, que pueden ser complementados con otros ensayos para evaluar efectos a otros niveles de organización que permitan ampliar el abanico de herramientas de evaluación de riesgo ambiental.

Por otra parte, tomando en cuenta el aumento en las actividades que generan o utilizan compuestos que potencialmente pueden alcanzar los sistemas naturales por distintas vías, los efectos que pueden ocasionar en los organismos a diversos niveles de organización y los resultados observados en esta tesis, se considera relevante continuar profundizando en esta línea de investigación y en el amplio espectro de compuestos que han sido detectados en los ecosistemas acuáticos.

En esta tesis se abordaron la temperatura, variable ambiental clave en los ecosistemas dulceacuícolas, y dos contaminantes emergentes de distinta naturaleza: el herbicida clomazone y las nanopartículas de sulfuro de bismuto (Bi_2S_3). Ambos compuestos, especialmente las nanopartículas, han sido escasamente estudiados, por lo que existen importantes vacíos de conocimiento sobre sus efectos en organismos acuáticos.

Los resultados obtenidos aportan nueva información sobre la biología y el metabolismo del caracol manzana *Pomacea* sp., al analizar su respuesta frente a distintos escenarios. A las variaciones de temperatura, lo que puede ser considerado para el cultivo y mantenimiento de estos organismos en cautiverio, y que también permite predecir su comportamiento ante escenarios de aumento térmico asociados al cambio climático, y los efectos sobre los ecosistemas que habitan y las tramas tróficas que conforman.

Tanto el clomazone como las nanopartículas de Bi_2S_3 produjeron efectos sobre algunos de los biomarcadores evaluados (consumo de oxígeno, actividad, sobrevivencia). Todo

esto, aporta sustento a que el caracol *Pomacea* sp. pueda ser utilizado como especie bioindicadora tanto en el contexto de cambio climático, como de contaminación en sistemas dulceacuícolas.

10. Perspectivas

Los métodos no invasivos permiten evaluar los efectos de estos contaminantes emergentes sin sacrificar a los organismos, pero en algunos casos no se observaron efectos significativos; por lo que, a futuro, se pueden estudiar otros biomarcadores a nivel molecular, como estrés oxidativo y/o daño genético, cuyos cambios pueden evidenciarse a corto plazo **(dos Santos & Martínez, 2014; Sánchez et al., 2017**).

Realizar ensayos que incluyan distintas temperaturas y contaminantes emergentes, para estudiar cómo varían sus efectos, en un escenario de cambio climático, aprovechando que se conoce qué sucede en situaciones en que la temperatura está en niveles adecuados para el desempeño de la especie, es una vía para explorar.

Los resultados obtenidos aportan en el contexto de la generación de biomarcadores para ser integrados como herramientas en evaluaciones de riesgo ambiental.

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12. Material suplementario

Cultivo, mantenimiento en cautiverio y experimentación con especies nativas acuáticas del Sur de Sudamérica

Cultivation, captive maintenance and experimentation with native aquatic species of Southern South America

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RESUMEN. Especies acuáticas son llevadas al laboratorio para mantenimiento, experimentación y/o cultivo. Su desempeño es evaluado experimentalmente para conocer las condiciones óptimas para su mantenimiento en cautiverio con diversos fines (e.g. cultivo) y se realizan ensayos para conocer efectos de contaminantes. Macroinvertebrados y productores primarios registran el menor volumen de información en el Sur de América del Sur. Este trabajo pretende aportar información actualizada y sistematizada sobre organismos nativos de estos grupos a nivel regional. Se evaluaron trabajos en los que fueron mantenidos en cautiverio. Se reportó la duración y las condiciones experimentales a las que fueron expuestos, tipos de compuestos, concentraciones utilizadas y biomarcadores evaluados. Veinticuatro especies presentan registro de cultivo, mantenimiento y/o experimentación: nueve crustáceos decápodos, ocho moluscos bivalvos, tres gasterópodos, un anfípodo, un cnidario y dos especies de productores primarios. Once especies son dulceacuícolas, nueve marinas y cuatro eurihalinas. Tres han sido cultivadas. De los trabajos evaluados, cincuenta y cuatro son referidos a aclimatación, cultivos y ensayos con factores abióticos/bióticos; en treinta y cuatro se evalúa el efecto de la exposición a sustancias tóxicas. Los biomarcadores estudiados corresponden a los niveles bioquímico, poblacional, morfológico y fisiológico/metabólico. Este tipo de información es importante ya que permite conocer el estado del arte sobre la biología/ecología y experimentación con especies nativas, además de ser considerada al momento de seleccionar especies para realizar ensayos y conocer el estado de los organismos en el ambiente frente a diversos cambios generados por el cambio global (aumento de sustancias tóxicas, cambio climático, entre otras).

Palabras Clave: cultivo, especies acuáticas nativas, Sudamérica.

ABSTRACT. Aquatic species are taken to the laboratory for maintenance, experimentation and/or cultivation. Its performance is evaluated experimentally to know the optimal conditions for its maintenance in captivity for various purposes (e.g. culture) and tests are carried out to

know the effects of contaminants. Macroinvertebrates and primary producers register the lowest volume of information in Southern South America. This work aims to provide updated and systematized information on native organisms of these groups at the regional level. Works in which they were kept in captivity were evaluated. The duration and experimental conditions to which they were exposed, types of compounds, concentrations used, and biomarkers evaluated were reported. Twenty-four species have recorded cultivation, maintenance and/or experimentation: nine decapod crustaceans, eight bivalve mollusks, three gastropods, one amphipod, one cnidarian and two species from primary producers. Eleven species are freshwater, nine marine and four euryhaline. Three have been cultivated. Of the works evaluated, fifty-four are related to acclimatization, cultures and tests with abiotic/biotic factors. Thirty-four evaluate the effect of exposure to toxic substances. The biomarkers studied correspond to the biochemical, population, morphological and physiological/metabolic levels. This type of information is important because it allows to know the state of the art on biology/ecology and experimentation with native species, in addition to being considered when selecting species to perform tests and know the state of organisms in the environment against various changes generated by global change (increase in toxic substances, climate change, among others).

Keywords: aquatic native species, captive, South America.

Introducción

Especies acuáticas de diversos grupos son estudiadas en laboratorio por su importancia económica, por ser potencialmente cultivables y/o potenciales bioindicadoras (Bertrand et al., 2016; Cossi et al., 2018; Nie et al., 2016). Generalmente son utilizadas especies nativas del hemisferio norte, y, por ende, están adaptadas a las condiciones del mismo. De ellas son estudiados aspectos biológicos (alimentación, osmorregulación, reproducción, crecimiento) y ecológicos (tipo y características de hábitat). Para aquellas especies que pueden ser mantenidas en cautiverio, es importante conocer las condiciones óptimas para su desarrollo considerando variables o factores abióticos (*e.g.* temperatura, salinidad, fotoperíodo) (Carvalho et al., 2015; Ituarte et al., 2010; Montagna, 2011; Seuffert & Martin, 2013;). Esto evita que los organismos sean expuestos a condiciones de estrés y así no deban invertir parte de su energía en mantener la homeostasis. Los resultados de la fisiología de base deben considerarse para ajustar las condiciones en ensayos ecotoxicológicos (bioensayos).

Diversas sustancias tóxicas empleadas en actividades antropogénicas (agricultura, ganadería, industria), llegan a los cuerpos de agua e incluso son detectados en el mar lejos de su fuente (Hunt et al., 2016; Sauco et al., 2010; Solis et al., 2017). Esto afecta la calidad del agua, causando daños a los organismos que habitan la zona, con efectos en su reproducción, fisiología y/o abundancia (Chen et al., 2019; Jergentz et al., 2004; Tsvetkov et al., 2017; Yang et al., 2018). La contaminación por sustancias tóxicas puede evaluarse analíticamente a través de muestreos en el ambiente (identificando la presencia y niveles de los mismos en el agua y en organismos) (Ernst et al 2018, Rodriguez-Bolaña et al., 2023) y desarrollando bioensayos de dos tipos: i) Letales (agudos): son aquellos en los que la exposición a una sustancia produce un desequilibrio en el balance energético de los organismos provocando la muerte de los mismos. En estos ensayos se determina la concentración letal media del compuesto (LC50), como aquella en la que el 50% de los organismos expuestos muere en un período de tiempo breve determinado (96 horas en general) (Paracampo et al., 2015; Stephenson, 1982; Suvetha et al., 2015). ii) Sub-letales (crónicos): se basan en analizar el cambio en biomarcadores cuando los organismos se exponen a niveles de sustancias inferiores al LC50 o similares a los encontrados en el ambiente. Este tipo de ensayo comenzó a ser explorado más recientemente (Abujamara et al., 2014; Cossi et al., 2018). Aquí, el desbalance energético no genera la muerte inmediata, pero tiene consecuencias importantes a nivel poblacional y sobre la composición de las comunidades (Di Fiori et al., 2012; Morrisey et al., 2015).

Los biomarcadores utilizados para medir el efecto de la exposición a estos compuestos se pueden categorizar en diferentes niveles de organización biológica. Los que presentan mayor especificidad (ocurren a nivel de individuo) pertenecen a los niveles inferiores (atómico, molecular y celular), generalmente afectados tempranamente; mientras que los superiores (poblacional, comunitario, ecosistémico y de biósfera) presentan menor especificidad, pero

mayor relevancia y son visualizados en un plazo mayor (Vera et al., 2012). A nivel regional se han realizado bioensayos evaluando biomarcadores con elevada especificidad (actividad enzimática, reservas y niveles de energía) (Griboff et al, 2014; Lavarias et al., 2011; Montagna & Collins, 2005). La concentración de los compuestos en tejidos y agua son útiles para evaluar la capacidad de bioacumulación de los organismos (Giusto et al., 2012; Giusto & Ferrari, 2014; Griboff et al., 2014; Lavarías et al., 2011). En menor medida se determinan biomarcadores fisiológicos como consumo de oxígeno, excreción de amonio, tasa de filtración y crecimiento (Al Subiai et al., 2011; Montagna & Collins, 2005; Verónica & Collins, 2003) y son menos utilizados aquellos procedimientos que evalúan daños a nivel genético (de Boissel et al., 2017).

En los bioensayos usualmente se emplean especies nativas o que puedan ser mantenidas en cautiverio durante períodos prolongados (Cossi et al., 2018; Martínez et al., 2017; de Siqueira et al., 2020). Cuando la especie presenta las condiciones para ser mantenida en cautiverio y se conocen los efectos de los contaminantes a diferentes niveles, se las conoce como especies modelo de estudio. Además, el conocer las respuestas ecotoxicológicas en un amplio rango permite evaluar el estado de los organismos en condiciones naturales y generar información de calidad útil para gestión ambiental (Peluso et al., 2011).

En Argentina, Brasil Chile y Uruguay, han sido realizados ensayos de exposición a compuestos tóxicos con especies acuáticas nativas (invertebrados y productores primarios macroscópicos) de distintos ambientes. Pero no existe una revisión que sistematice la información al respecto e identifique las fortalezas y los vacíos de información. Desde el punto de vista biogeográfico, estos países tienen ambientes que pertenecen al reino Neotropical (Olson et al., 2001) y a la ecorregión cálida-templada del Atlántico Suroeste (Spalding et al., 2007). Además, Argentina, Chile y Uruguay forman parte del denominado Cono Sur. Paraguay no fue incluido ya que no fueron encontrados trabajos relacionados a la temática de este trabajo; la única especie de macroinvertebrados que ha sido cultivada en este país es el camarón de río *Macrobrachium rosernbergii*, de origen exótico (FAO, 2005).

En esta revisión se pretende proporcionar una visión actualizada sobre el cultivo y mantenimiento de especies nativas de macroorganismos acuáticos invertebrados y productores primarios macroscópicos (plantas acuáticas y macroalgas) en el Sur de SA, utilizadas como bioindicadores. Se explorará la taxonomía, tipos de alimentación, el tipo de ambiente que habitan (marino/dulceacuícola) y las condiciones de experimentación. Se evaluará el tipo de compuesto al que fueron expuestas (agroquímicos, metales, hidrocarburos, etcétera), el tiempo de exposición, variables ambientales, biomarcadores evaluados, entre otros.

En este contexto, el objetivo de este trabajo es sistematizar la información existente sobre rasgos biológicos, ecológicos y experimentales, de las especies acuáticas nativas de macroorganismos invertebrados (MI) y productores primarios macroscópicos (PPM)

cultivadas o mantenidas en cautiverio en la región sur de Sudamérica (Argentina, Brasil Chile y Uruguay). Siendo los objetivos específicos: 1- relevar los grupos de MI y PPM (macrófitas y macroalgas) acuáticos nativos que han sido mantenidos en cautiverio y sus características filogenéticas, el ambiente que habitan (marino/dulceacuícola), el tipo de alimentación que presentan, entre otras; 2- determinar durante qué período de tiempo fueron mantenidos en cautiverio y con qué propósito (aclimatación, experimentación y/o cultivo); en el caso de la experimentación, se evaluará qué tipo de ensayos fueron realizados (microcosmos, mesocosmos o ambiente); 3- describir los valores de las condiciones ambientales (temperatura, salinidad, pH y oxígeno disuelto), en los cuales fueron mantenidos/cultivados; 4-evaluar qué especies han sido utilizadas en bioensayos de toxicidad, los compuestos empleados y los biomarcadores estudiados.

Materiales y métodos

La búsqueda bibliográfica fue realizada entre enero y diciembre de 2020, siendo el foco las especies de MI y PPM acuáticos nativos de la región, que han sido mantenidos en cautiverio con diferentes fines (aclimatación, cultivo, experimentación) o para realizar ensayos en el ambiente. Se realizó una selección primaria en base al conocimiento de la zona que posee el equipo de trabajo (considerando especies nativas, implicancia económica, si son consideradas bioindicadores, potencialmente cultivables) y búsquedas digitales. Además, se consultó a colegas especialistas de los países vinculados al trabajo, quienes además de aportar información relevante, recomendaron otros especialistas en un esquema del tipo bola de nieve. Se exploraron fuentes de acceso electrónico como portal Timbó (https://foco.timbo.org.uy/home), Science Direct, SCIELO, JSTOR (JSTOR Home), Google Académico, Boletín de la Sociedad de Biología de Concepción | Concepción, Chile (bolsocbiolconcepc.cl), Repositorio Universidad Católica de Chile (Repositorio UC | Dirección de Bibliotecas UC), Instituto de Fomento Pesquero (https://www.ifop.cl), Wiley Online Library (https://onlinelibrary.wiley.com), Repositorio Institucional Académico Universidad Andrés Bello (https://repositorio.unab.cl), ResearcheGate (Home Feed | ResearchGate) e instituciones gubernamentales como el Museo Natural de Historia Natural (https://www.mnhn.gub.uy), el Sistema Nacional de Áreas Protegidas (SNAP; https://www.snap.gub.uy/sisnap), la Dirección Nacional de Medio Ambiente (DINAMA; https://www.dinama.gub.uy/especies), Dirección Nacional de Recursos Acuáticos (DINARA; https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/direccion-nacional-recursos-acuaticos), entre otros. Para la búsqueda fueron utilizadas las siguientes palabras clave (individualmente o realizando combinaciones entre ellas): agricultura, agroquímicos, bioindicadores, cautiverio, cultivo, ecotoxicología, efectos sub-letales, efectos letales, especies acuáticas nativas, fungicidas, herbicidas, insecticidas, macroinvertebrados, macroalgas, metales, Neotropical, plantas acuáticas, Argentina, Brasil, Chile, Paraguay, Uruguay y los nombres científicos de las especies. Posteriormente, la información obtenida de los trabajos fue sistematizada y organizada en

tablas. Las especies fueron clasificadas de acuerdo con características biológicas (niveles de clasificación taxonómica, tipo de alimentación, forma de osmorregulación), ecológicas (ambiente en el que habitan y características de este), si presentan importancia comercial y/o son consumidas por el ser humano. Además, fue relevada la información de los ensayos tanto de factores bióticos/abióticos como de exposición a compuestos tóxicos, incluyendo las condiciones en que fueron realizados (qué ítems alimenticios fueron ofrecidos, valores o rangos de factores abióticos) y durante qué período de tiempo. En los bioensayos se incluyeron los compuestos tóxicos empleados y los biomarcadores relevados.

Resultados

Fueron revisados 228 trabajos científicos con información relevante, publicados entre 1973 y 2020. Ochenta y dos surgieron de estudios realizados en Argentina, cuarenta y ocho de Brasil, noventa y cuatro de Chile y cuatro de Uruguay. De todos los trabajos analizados, ciento setenta y tres corresponden a especies mantenidas en cautiverio con fines de aclimatación, cultivo y/o ensayos con factores bióticos/abióticos (alimentación, acidificación, técnicas de marcaje/recaptura, temperatura, salinidad, entre otros); mientras que setenta y nueve tuvieron como objetivo evaluar efectos de la exposición a diferentes compuestos tóxicos como agroquímicos, agua de ambientes poluídos, hidrocarburos, metales, etcétera (Figura 1).

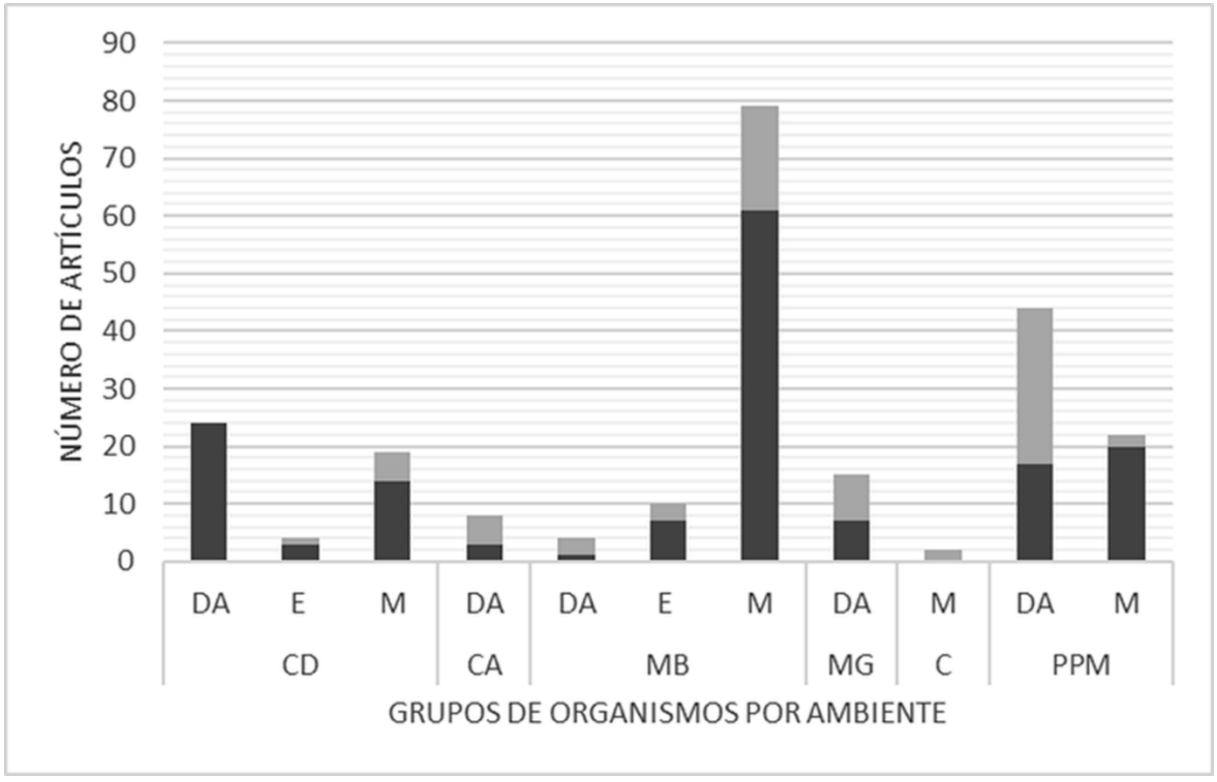

Figura 1. Número de artículos referentes a mantenimiento en cautiverio y ensayos de factores bióticos/abióticos (barra oscura) y bioensayos (barra clara) de los diferentes grupos de macroinvertebrados acuáticos nativos (crustáceos decápodos, CD; crustáceos anfípodos, CA; moluscos bivalvos, MB; moluscos gasterópodos, MG; cnidarios, C y productores primarios macroscópicos, PPM) clasificados por el ambiente que habitan (dulceacuícola, DA; eurihalino, E; marino, M).

A partir de la revisión bibliográfica y de los aportes recibidos por los colegas consultados, se registraron un total de cincuenta y dos especies, correspondientes a ocho filos/divisiones (tres animales y cinco productores primarios) y siete clases/órdenes (cinco animales y ocho productores primarios); veinticuatro corresponden a agua dulce, seis a ambientes eurihalinos y treinta son marinas. El mecanismo de osmorregulación fue osmoconformador y/u osmorregulador (en algunos casos sin información); treinta son consumidas y/o comercializadas (Tabla 1). Todas ellas fueron mantenidas en cautiverio con distintos objetivos (aclimatación, mantenimiento, experimentación y/o cultivo), bajo distintas condiciones abióticas (salinidad, temperatura, pH y oxígeno), períodos de tiempo y alimentación. La mayoría de los registros corresponden a especies dulceacuícolas (19 especies), seguido por las marinas (17 especies) y, por último, las que habitan ambientes eurihalinos (6 especies). En cuanto a los ensayos, predominan aquellos que evaluaron los efectos de alimentación, salinidad y/o temperatura (Tabla 2). Los crustáceos decápodos y los moluscos bivalvos tuvieron representantes en los tres ambientes, mientras que los productores primarios y moluscos gasterópodos lo hicieron en el dulceacuícola y el marino, los anfípodos en el dulceacuícola y los cnidarios, en el marino (Fig. 2). De esas cincuenta y dos especies, treinta y una han sido utilizadas en bioensayos: siete crustáceos decápodos (CD), ocho moluscos bivalvos (MB), dos moluscos gasterópodos (MG), un anfípodo (CA), un cnidario (C) y doce productores primarios macroscópicos (PPM) (Tabla 3). Las especies más empleadas en estos ensayos (en general del tipo microcosmos) corresponden al ambiente dulceacuícola y marino. La Figura 3 muestra la cantidad de veces que los organismos de cada grupo y ambiente fueron expuestos a los distintos compuestos tóxicos. Los PPM dulceacuícolas son los más utilizados, seguidos por los MB marinos. Los compuestos más empleados son los metales, los agroquímicos y el agua de efluentes, cuyos efectos fueron evaluados en cuarenta, veintiuno y once bioensayos, respectivamente.

Los biomarcadores más estudiados corresponden al nivel bioquímico, evaluados en setenta y cinco trabajos. Le siguen los ensayos que incluyen registro de crecimiento y/o mortalidad, evaluados en cuarenta y tres ensayos. Con menor representación siguen las mediciones de efectos a nivel fisiológico/metabólico (evaluados en diecinueve ocasiones) y morfológico (trece ensayos). Solamente en los CD y PPM dulceacuícolas y MB marinos, fueron evaluados biomarcadores en los cuatro niveles (Figura 4). A continuación, se presenta información detallada para cada grupo.

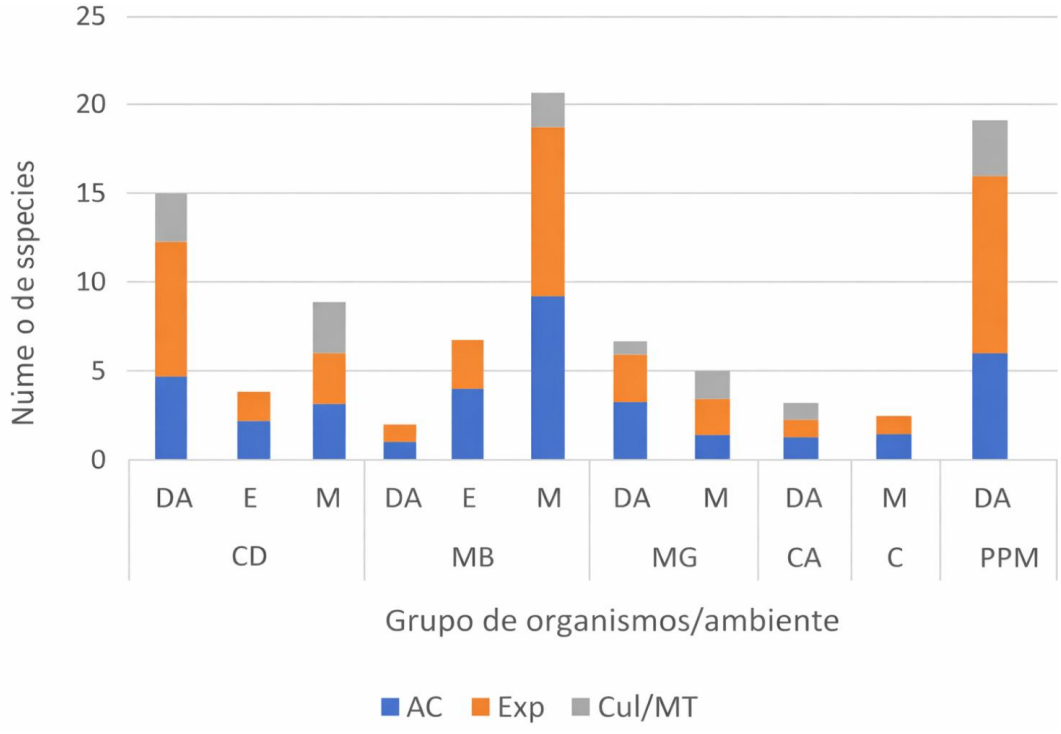

Figura 2. Número de especies de invertebrados y productores primarios macroscópicos acuáticos nativos de la región sur de América del Sur, que han sido mantenidos en cautiverio con diferentes propósitos: aclimatación (AC), experimentación (Exp) y cultivo (Cul), clasificados por grupo al que pertenecen (crustáceos decápodos, CD; moluscos bivalvos, MB; moluscos gasterópodos, MG; crustáceos anfípodos, CA; cnidarios, C y productores primarios macroscópicos, PPM) y ambiente (dulceacuícola, DA; eurihalino, E; marino, M).

Crustáceos decápodos (Filo: Arthopoda, Clase: Decápoda)

Es el grupo más representativo con trece especies, de las cuales ocho son dulceacuícolas, dos estuarinas y tres marinas. Todas presentan alimentación omnívora, excepto *E. brasiliensis*, que es suspensívoro filtrador (Lercari & Defeo, 1999). El cangrejo sirí *C. sapidus,* el camarón *A. longinaris*, *C. caementarius, L. santolla* y *S. spinifronts*, son capturados por la pesca artesanal con fines comerciales (Consultora CEA Valdivia, 2013; Melnikov, 2014) (Tabla 1). Además, todas las especies han sido mantenidas en cautiverio con fines de aclimatación y experimentales por entre 2 días y 7 meses para evaluar los efectos de la dieta, la temperatura, la salinidad, intensidad de luz y ciclo de marea (Tabla 2) (Cancino et al., 2003; Collins, 1977; Ferreira et al., 2005; Ituarte et al., 2006; Martínez et al., 2008; Navarro et al., 2002; Montagna, 2011; Neves et al., 2000; Otegui & Soares-Gomes, 2007; Pereira-Almerão, 2005; Romero et al., 2004; Silva-Castiglioni, et al., 2016).

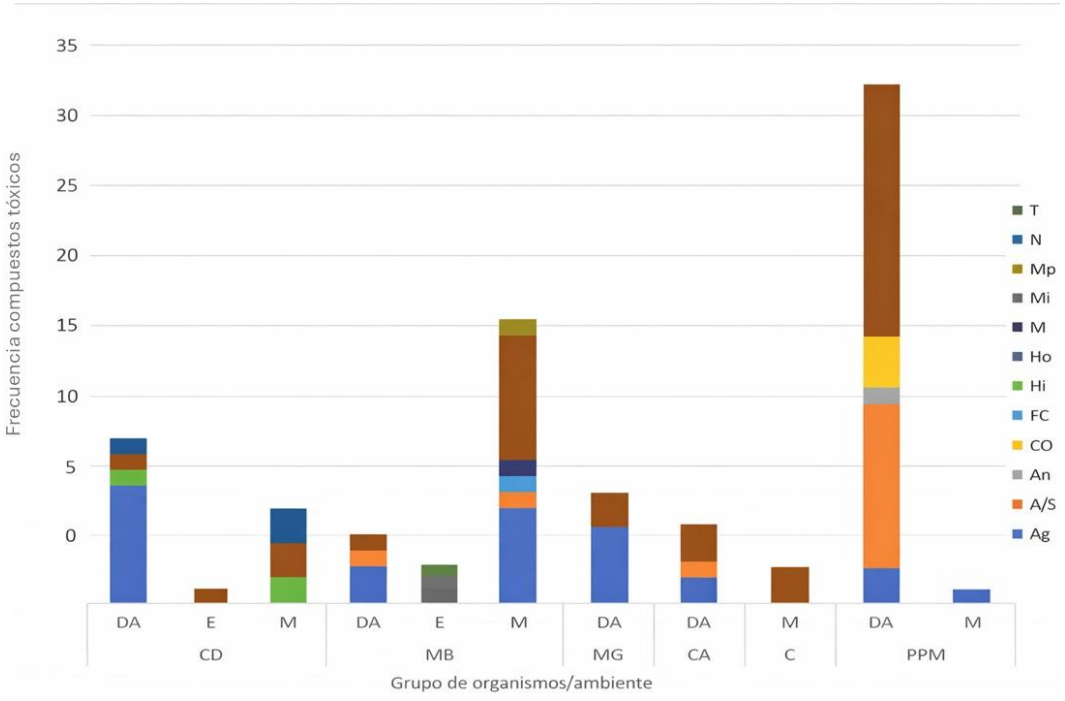

Figura 3. Frecuencia de los compuestos (metales pesados, agroquímicos, hidrocarburos, etcétera) utilizados en los bioensayos con invertebrados y productores primarios macroscópicos acuáticos nativos de la región sur de América del Sur, clasificados por grupo al que pertenecen (crustáceos decápodos, CD; moluscos bivalvos, MB; moluscos gasterópodos, MG; crustáceos anfípodos, CA; cnidarios, C y productores primarios macroscópicos, PPM) y ambiente (dulceacuícola, DA; eurihalino, E; marino, M).

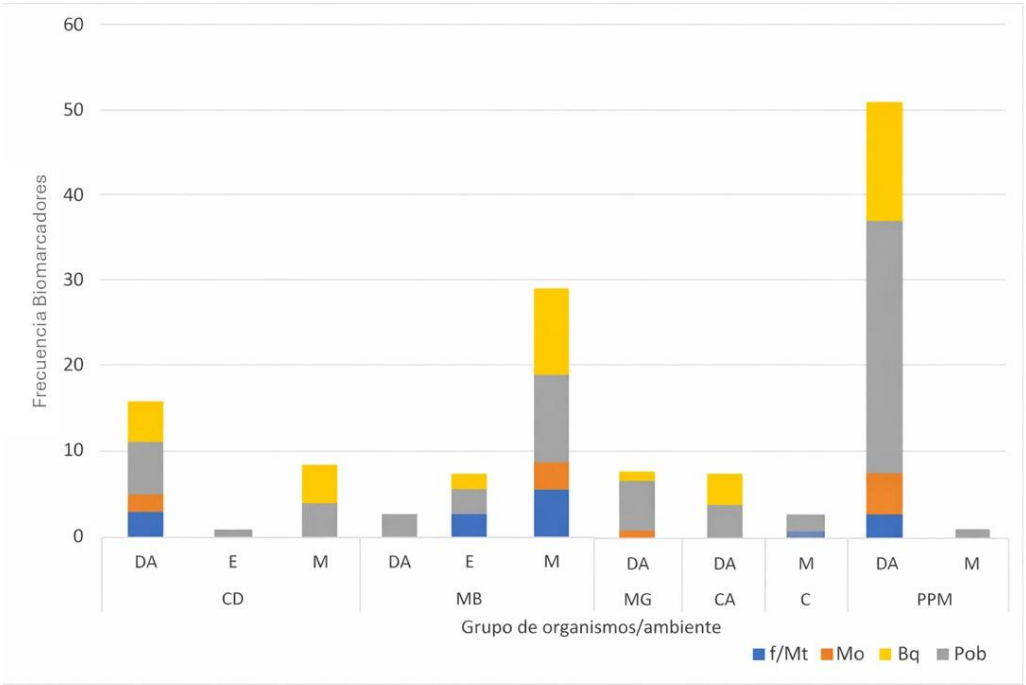

Figura 4. Frecuencia de los biomarcadores evaluados (crecimiento y/o mortalidad, C/M; bioquímicos, Bq; morfológicos, Mo y fisiológicos/metabólicos, F/Mt) en cada grupo de invertebrados y productores primarios macroscópicos acuáticos nativos de la región sur de América del Sur, clasificados por grupos de organismos expuestos (crustáceos decápodos, CD; moluscos bivalvos, MB; moluscos gasterópodos, MG; crustáceos anfípodos, CA; cnidarios, C y productores primarios macroscópicos, PPM) y ambiente acuícola (dulceacuícola, DA; eurihalino, E; marino, M).

Tabla 1. Especies de macroinvertebrados y productores primarios acuáticos nativos mantenidos en cautiverio con diversos objetivos (aclimatación, cultivo, experimentación) en Argentina, Brasil, Chile y Uruguay.

Table 1. Macroinvertebrate and native aquatic primary producer's species kept in captivity for different purposes (acclimatization, culture, experimentation) in Argentina, Brasil, Chile and Uruguay.

Grupo	Filo/División	Clase/Orden	Ambiente	Especie	Alimentación	Osmorregulación	Consumo/comercio
Crustáceos decápodos	Arthropoda	Decapoda	Dulceacuícola	*Aegla platensis*	Omnívoro	Osmorregulador	No
				Aegla septentrionalis	Na	Na	No
				Aegla uruguayana	Omnívoro	Osmorregulador	No
				Macrobrachium borelli	Omnívoro/carnívoro	Na	Na
				Palaemonetes argentinus	Omnívoro	Osmorregulador	Na
				Trichodactylus borellianus	Na	Na	Na
				Samastacus spinifronts	Omnívoro	Na	Si
				Cryphiops caementarius	Omnívoro	Osmorregulador	Si
			Eurihalino	*Callinectes sapidus*	Omnívoro	Osmoconformador/osmorregulador	Si
				Cyrtograpsus angulatus	Omnívoro	Osmoconformador/osmorregulador	Na
			Marino	*Emerita brasiliensis*	Filtrador	Osmorregulador	No
				Lithodes santolla	Omnívoro	Osmorregulador	Si
				Artemesia longinaris	Generalista	Osmorregulador	Si

Moluscos bivalvos	**Mollusca**	**Bivalvia**	**Dulceacuícola**	***Anodontites trapesialis***	**Filtrador**	**Osmorregulador**	**Na**
			Eurihalino	*Brachidontes darwinianus*	Filtrador	na	Na
				Erodona macroides	Filtrador	Osmorregulador	No
				Mulina edulis	Filtrador	na	Si
				Ostrea chilensis	Filtrador	Osmoconformador	Si
			Marino	*Argopecten purpuratus*	Filtrador	Osmoconformador	Si
				Zygochlamys patagónica	Filtrador	Osmorregulador	Si
				Mytilus chilensis	Filtrador	Osmoconformador	Si
				Aulacomya ater	Filtrador	na	Si
				Choromytilus chorus	Filtrador	Osmoconformador	Si
				Venus antiqua	Filtrador	na	Si
				Gari solida	Filtrador	na	Si
				Mesodesma donacium	Filtrador	Osmoconformador	Si
				Ensis macha	Filtrador	na	Si
				Amiantis purpurata (A. purpuratus)	Filtrador	Osmorregulador	Si
				Brachidontes rodriguezii	Filtrador	Osmoconformador	Si
				Donax hanleyanus	Filtrador	Osmorregulador	Si
				Mesodesma mactroides	Filtrador	Osmoconformador	Si
				Mytilus sp.	Filtrador	Osmoconformador	Si
Moluscos gasterópodos	Mollusca	Gasterópoda	Dulceacuícola	*Biomphalaria sp.*	Herbívoro	na	No
				Heleobia australis	Filtrador	na	No

				Pomacea sp	Herbívoro	Osmorregulador	No
			Marino	*Thais chocolata*	Carnívoro	na	Si
				Chorus giganteus	Carnívoro	Osmorregulador	Si
Crustáceos Anfípodos	Arthropoda	Amphípoda	Dulceacuícola	*Hyalella curvispina*	Omnívoro	na	No
Cnidarios	Cnidaria	Anthozoa	Marino	*Bunodosoma cangicum*	Omnívoro	Osmoconformador	No
Productores primarios macroscópicos	Angiospermae	Alismatales	Dulceacuícola	*Egeria densa*	Fotosíntesis	na	Si
	Pteridophyta	Lycopsida	Dulceacuícola	*Azolla filiculoides*	Fotosíntesis	na	No
	Magnoliophyta	Alismatales	Dulceacuícola	*Lemna minuta*	Fotosíntesis	na	si
		Alismatales	Dulceacuícola	*Lemna valdiviana*	Fotosíntesis	na	No
		Alismatales	Dulceacuícola	*Spirodela intermedia*	Fotosíntesis	na	No
		Cyperales	Dulceacuícola	*Schoenoplectus californicus*	Fotosíntesis	na	Si
		Myrtales	Dulceacuícola	*Ludwigia peploides*	Fotosíntesis	na	No
		Saxifragales	Dulceacuícola	*Myriophyllum aquaticum*	Fotosíntesis	na	Si
			Dulceacuícola	*Myriophyllum quitense*	Fotosíntesis	na	Si
	Fanerógama	Apiales	Dulceacuícola	*Hydrocotyle ranunculoides*	Fotosíntesis	Na	No
	Chlorophyta	Ulvales	Marina	*Ulva sp*	Fotosíntesis	Osmoconformador	Si
	Rhodophyta	Gracilariales	Marina	*Gracilaria chilensis*	Fotosíntesis	na	Si

En estos trabajos fueron evaluados efectos sobre crecimiento de juveniles y adultos, sobrevivencia, reservas energéticas, entre otros aspectos, con el fin de adquirir conocimiento sobre la biología/ecología de las especies.

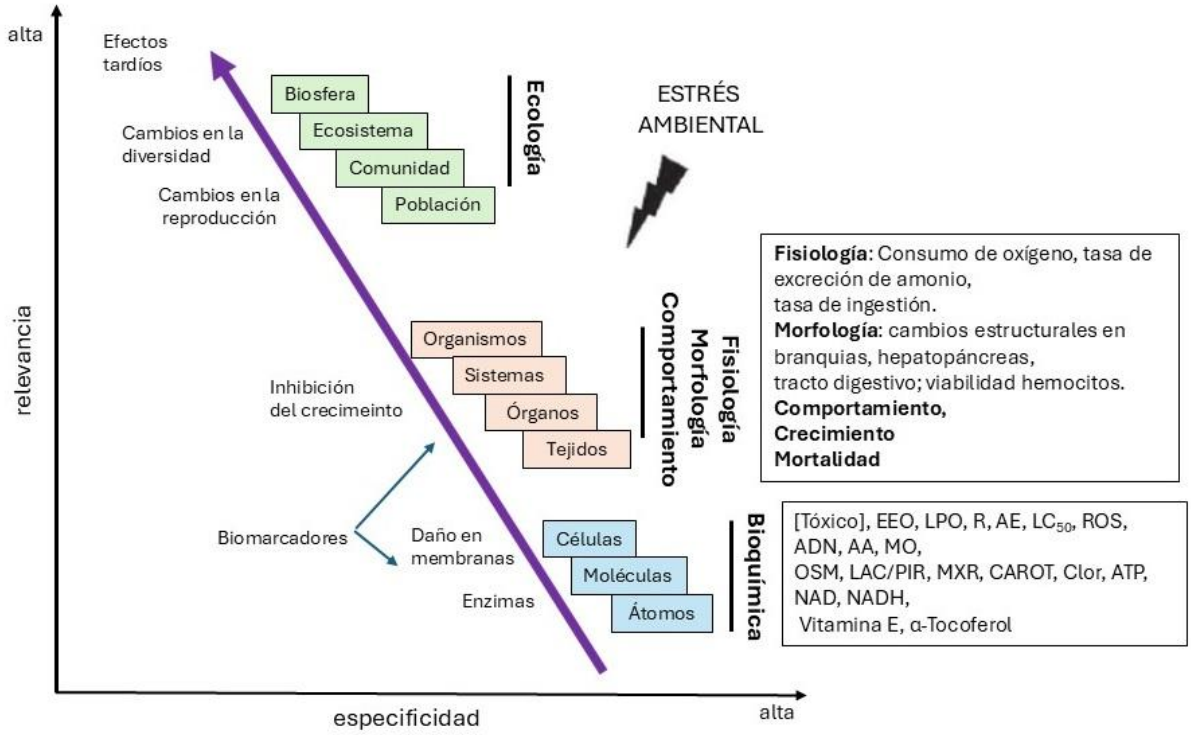

Figura 5. Biomarcadores evaluados en los diferentes niveles de organización que surgen a partir de la revisión bibliográfica de macroorganismos invertebrados y productores primarios macroscópicos nativos de la región sur de América del Sur. Tomado de Cossi (2018) y adaptado por Féola. EEO: enzimas estrés oxidativo; LPO: peroxidación lipídica; R: reservas energéticas (lípidos, carbohidratos, proteínas); AE: actividad enzimática (Na/K ATPasa, colinesterasa y acetilcolinesterasa); ROS: especies reactivas de oxígeno; AA: aminoácidos (concentración); MO: materia orgánica; MT: metalotioneínas; OSM: osmolaridad; LAC/PIR: lactato piruvato; MXR: resistencia a xenobióticos múltiple; CAROT: carotenoides; Clor: clorofilas a y b.

Siete especies de CD han sido utilizadas para estudiar el efecto de compuestos tóxicos: tres dulceacuícolas (*M. borellii*, *P. argentinus* y *T. borellianus*), una eurihalina (*C. sapidus*) y tres marinas (*E. brasiliensis*, *L. santolla* y *A. longinaris*). Con mayor frecuencia fueron evaluados los efectos de los agroquímicos (herbicidas e insecticidas), seguido por ensayos con metales pesados e hidrocarburos (Tabla 3). La especie más estudiada fue *P. argentinus* con siete ensayos, donde se evaluaron efectos sub-letales (consumo de oxígeno, actividad de enzimas relacionadas al estrés oxidativo, reservas energéticas, cambios estructurales) de la exposición a agroquímicos, metales y nitrito (Bertrand et al, 2016; Chiodi-Boudet et al., 2015; Espino et al, 2015). En la especie *M. borelli* fue evaluada la actividad de enzimas relacionadas al estrés oxidativo y los niveles de peroxidación lipídica (LPO) frente a la exposición a petróleo (Lavarías et al., 2011). *T. borellianus* fue expuesto a insecticidas para determinar respuestas a nivel fisiológico (consumo de oxígeno y excreción de amonio) (Montagna & Collins, 2008;

Verónica & Collins, 2003). El cangrejo *C. sapidus* (eurihalino) fue expuesto a diferentes concentraciones de cobre para evaluar su efecto sobre el nivel de aminoácidos en la hemolinfa y la actividad de enzimas asociadas a las branquias (Na/K-ATPasa, H-ATPasa and Catalasa) (Guerreiro-Gómes et al., 2019). *L. santolla* fue expuesto a petróleo, amonio, y cadmio y plomo, evaluando su efecto sobre biomarcadores poblacionales; también fue determinada la concentración letal media (LC50) para los dos primeros tóxicos (Amin et al., 1998; Amin & Comiglio, 2002; Diodato et al., 2019). La LC50 también fue determinada para el herbicida Propanil en el tatucito *E. brasiliensis;* la misma fue de 5,64 y 6,06 mg/l para organismos pequeños y grandes, respectivamente (Sauco et al., 2010). Los períodos de exposición fueron de entre 24 horas y 160 días (Montagna & Collins, 2005; Montagna & Collins, 2008). Las especies dulceacuícolas fueron estudiadas a temperaturas entre 15 y 30 °C, pH de 6,6 a 8,3 y entre 4,5 y 7 mg/l de oxígeno disuelto (Chiodi-Boudet et al., 2015; Montagna, 2011; Lavarías-García, 2015). Las eurihalinas fueron mantenidas a temperaturas de 20 a 22°C, salinidad entre 2 y 30, pH de 7,2 a 8,34 y de 2,24 a 3,84mg/l de oxígeno disuelto (Guerreiro-Gómes et al., 2019; Longo & Díaz, 2017; Pinoni & Mañanes, 2004). Mientras que las marinas fueron expuestas a temperaturas entre 4 y 26°C, 23 y 34 de salinidad, pH entre 8.03 y 8,5, y de 4,7 a 10,5 mg/L de oxígeno disuelto (Diodato et al., 2019; Lovrich et al., 2003; Otegui & Soares-Gomes, 2007; Scelzo et al., 1997; Schvezov et al., 2017). Los ítems alimenticios ofrecidos incluyeron ración, larvas de mosquito, tatucito, carne vacuna, pescado, mariscos, fitoplancton, zooplancton (Bond & Buckup, 1983; Collins, 1977; Longo & Díaz, 2017; Loyola, 2009; Montagna, 2011; Silva-Castiglioni et al., 2016) (Tablas 2 y 3). Solo *M. borelli*, fue cultivado exitosamente por un período de 2 años (Bond & Buckup, 1983) (Tablas 2 y 3).

Moluscos bivalvos (Filo: Mollusca, Clase: Bivalvia)

Este grupo está representado por diecinueve especies: una dulceacuícola, cuatro eurihalinas y catorce marinas. En su mayoría son osmoconformadores. Aquellos que son consumidos/comercializados corresponden al ambiente marino (obs. personales; Olivier & Penchaszadeh, 1971; Scarabino, 2021) (Tabla 1). *M. edulis* es la única especie que se ha intentado cultivar en Uruguay, sin éxito (DINARA-FAO. 2008); *A. purpuratus* y *M. chilensis* son cultivados en Chile, con fines comerciales (APE – Acuicultura de Pequeña Escala en Chile » APE (sembrandoelmar.cl)). Todas ellas han sido aclimatadas por entre 6 horas y 15 días (Espinoza et al., 2003; Torres, 2019; Torroglosa & Giménez, 2019) bajo condiciones controladas de laboratorio. En cuanto a los experimentos, fueron realizados ensayos de alimentación (Milanesi-Camejo, 2015; Proverbio, 2017), marcaje (Carvalho et al., 2015; Herrmann et al., 2009; Proverbio, 2017) y otros para estudiar los efectos de acidificación, densidad de cultivo, salinidad y/o temperatura sobre indicadores eco-fisiológicos como presión osmótica, crecimiento, mortalidad, regeneración del sifón inhalante, cambios estructurales en la glándula digestiva, desarrollo larval, función enzimática (Carvalho et al., 2015; Gilles, 1972; Martínez et al., 2000; Núñez et al., 2013; Santos et al., 2020) (Tabla 4). Los ensayos se

extendieron por entre 1 y 183 días (Milanesi-Camejo, 2015; Proverbio, 2017; Ramajo et al., 2020). Para *A. mactroides* se obtuvieron larvas en cautiverio mediante la técnica de stripping (masajeo) (Santos et al., 2020).

Diez especies de bivalvos han sido utilizadas para estudiar el efecto de compuestos tóxicos: una dulceacuícola (*A. trapesalis*), siete marinas (*A. mactroides, A. purpuratus, A. ater, C. chorus, D. hanleyanus* y *M. chilensis*) y dos eurihalinas (*M. donacuim* y *O. chilensis*). En general, fueron expuestas a metales (nueve bioensayos), agroquímicos (siete bioensayos), agua de sitios poluídos (2 bioensayos), filtros de cigarro, hormonas, microplásticos, bacterias y toxinas (un bioensayo en cada caso) (Tabla 5).

Tabla 2. Crustáceos decápodos nativos utilizados en la región sur de América del Sur con diversos fines (aclimatación, mantenimiento, cultivo y/o experimentación). Se reportan datos de períodos de tiempo, factores abióticos (temperatura, salinidad, pH y oxígeno), alimentación y las referencias correspondientes. a=año, d=días, h=horas, m=meses. Na= información no reportada.

Table 2. Native decapod crustaceans used in the south of South America for various purposes (acclimatization, maintenance, cultivation and/or experimentation). Data on time periods, abiotic factors (temperature, salinity, pH and oxygen), diet and corresponding references are reported. Na = unreported information.

Grupo	Ambiente (especies)	Aclimatación/ ensayos	Duración	T (ºC)	Salinidad	pH	O2	Alimentación	Referencias
Crustáceos decápodos	**Dulceacuícola** *A. platensis*, *A. septentrionalis*, *A. uruguayana, C. caementarius, M. borellii, P. argentinus, S. spinofronos, T. borellianus*	Aclimatación	2d-3m	16-25	Dulce	7,6-7,8	Na	Ración	Collins, 1997; Giri & Collins, 2003; Ferreira et al, 2005; Pereira-Almerao, 2005; Montagna & Collins, 2007; Castiglioni et al., 2009; Chiodi Boudet et al., 2015; Diawol et al., 2015; Espino et al., 2015; Silva-Castiglioni et al., 2016.
		Alimentación	15-60d	16-25	Dulce	7	5,2mg/L	Larvas de mosquito, ración de diferente composición	Collins, 1997; Ferreira et al., 2005; Salgado-Leu & Tacon, 2015; Silva-Castiglioni et al., 2016
		Cultivo	3m-2a	17 -26	dulce-20	Na	Na	*E. brasiliensis*, carne vacuna, microalgas, zooplancton	Bond & Buckup, 1983; Morales et al., 2006; Morales & Meruane, 2012
		Temperatura	2-4m	15-30	Dulce	7,6-8	5,5-7mg/L	Pescado fresco y ración	Montagna, 2011; Renzulli & Collins, 2000
		Salinidad	110d	21	1 -25	Na	Na	Artemia,calamar y ración	Ituarte et al., 2006
		Mantenimiento	15d	20-25	dulce-10	Na	Na	Carne molida, mariscos y pescado	Moreno et al., 2012; Neves et al., 2000
	Eurihalino *C. sapidus, C. angulatus*	Aclimatación	14 a 20 d	20-22	2-30	Na	Na	Ración, zooplacton, pescado	Pinoni & López, 2004; Longe & Díaz, 2017; Guerreiro-Gomes et al., 2019
		Salinidad	2 -30d	20-22	2-35	Na	Na	Ración	Pinoni & López, 2004; Longe & Díaz, 2017; Asaro et al., 2018
	Marino *E. brasiliensis, L. santolla*	Aclimatación	colecta hembras ovígeras-1m	6-12	Mar	Na	Na	Calamar, mejillón, microalgas, ración	Otegui & Soares-Gomes, 2007; Sauco et al., 2010; Schvezov et al., 2013; Schvezov et al., 2017; Schvezov et al., 2019; Pretterebner et al., 2019; Sacristán et al., 2019
		Alimentación/Sali-nidad/temperatura	Na	18-26	34	8,03	4,7mg/L	Microalgas	Otegui & Soares-Gomes, 2007
		Alimentación	138d	12	30	Na	Na	Ración, artemia, mejillón	Loyola, 2009
		Cultivo	Na	7-12	30-32	8,1	8 mg/L	Na	Paschke et al., 2010; Sotelano et al., 2018
		Exposición al aire y reinmersión	36-h48h	4-12	mar	Na	Na	Sin alimento	Schvezov et al., 2017; Schvezov et al., 2019
		Inanición	12d	8	30	Na	Na	Sin alimento	Comoglio et al., 2008

	Mantenimiento	1m-10s; Na	5,5-12	23-34	Na; 8-8,5	Na; 10,5mg/L	Peces, mejillones, calamar; Na	Lovrich et al., 2003; Calcagno et al., 2004; Sotelano et al., 2012; Tapella et al., 2012; Urbina et al., 2013; Schvezov, 2015; Diodato et al., 2019
	Oxígeno	10d	12	31	Na	2,1-21,1kPa	Mejillón	Paschke et al., 2010

Tabla 3. Crustáceos decápodos acuáticos nativos de la región sur de América del Sur utilizados en bioensayos, compuestos a los que son expuestos, características de los ensayos (alimentación, tiempo, temperatura, salinidad, pH, oxígeno) y bioindicadores evaluados (fisiológicos/metabólicos, morfológicos, bioquímicos, crecimiento y mortalidad) y las referencias asociadas. d=días, h=horas. na=información no reportada

Table 3. Aquatic decapod crustacean's native to the southern region of South America used in bioassays, compounds to which they are exposed, characteristics of the assays (feeding, time, temperature, salinity, pH, oxygen) and bioindicators evaluated (physiological/metabolic, morphological, biochemical, growth and mortality) and the associated references. d=days, h=hours. na=unreported information.

Grupo	Ambiente	Especie	Compuesto tóxico	Tipo	Ensayo	Tiempo	T(ºC)	Salinidad	pH	O2	Alimentación	Fisiológicos/metabolicos	Morfológicos	Bioquímicos	Crecimiento	Mortalidad	Referencias
Crustáceos decápodos	Dulce-acuícola	*Macrobrachium borellii*	Petróleo	Hidrocarburo	Microscosmos	4-7d	22	dulce	Na	Na	Na			SOD, GPx, CAT,GST, LPO			Lavarías et al.,2011
		Palaemonetes argentinus	Clorpirifos	Insecticida	Microscosmos	96h	25	dulce	Na	Na	Na			[CPF,bioacumulación), LPO, CP, GST, GR,GPx, SOD, Ch, MTs, α-tocoferol			Bertrand et al, 2016
			Glifosato	Herbicida	Microscosmos	96h-160d	Na	dulce	Na	Na	Na	Consumo oxígeno, excreción amonio			si	si	Montagna & Collins, 2005
			Cadmio	Metal	Microscosmos	3-15d(exposición) + 7-28d (depuración)	17	dulce	8,3	Na	Ración		Daño hepatopáncreas	LPO			Choidi Boudet et al., 2015

			Fenitro-tion	Insecticida	Microscosmos	96h	20 -22	dulce	6,6-6,9	4,5 -5	Na			Colineste-rasa, hemo-linfa;SOD, CAT,GST, LPO		si	Lavarías-García, 2015
			Clorpiri-fos y endosul-fán	Insecticida	Microscosmos	4 ciclos de muda	25	dulce	7,8	6	Ración				si	si	Montagna & Collins, 2007
			Atrazina	Herbicida	Microscosmos	22d	25	dulce	7,6	Na	2 dietas			Vit-E, Peróxidos, GST, GSH, GR, SOD,LPO, Lípidos, Carbohidratos, Proteínas			Griboff et al., 2014
			Nitrito	Nutriente	Microscosmos	96h	20	dulce	7,6	Na	Sin alimen-tación		Daño branquias y hepatopáncreas	SOD, CAT		si (LC50)	Espino et al, 2015
		Trichodactylus borellianus	Clorpiri-fos y endosulfán	Insecticida	Microscosmos	24h	25	dulce	7,8	6	Na	Consumo oxígeno, excreción amonio					Montagna & Collins, 2008
			Cipermet rina	Insecticida	Microscosmos	96h	25	dulce	7,2	Na	Na	Consumo oxígeno, excreción amonio				si	Verónica & Collins, 2003
	Euri-halina	*Callinectes sapidus*	Cobre	Metal	Microscosmos	96h	20	2- 30	7,38-8,34	Na	Na			[Na],[Amino-ácidos] hemolinfa; Na/K ATPasa, CA y Cu en branquias			Guerreiro-Gómes et al., 2019
	Marina	*Emerita brasiliensis*	Propanil	Herbicida	Microscosmos	72h	Na	Na	Na	Na	Na					si (LC50)	Sauco et al., 2010
		L.itodes santolla	Petróleo Diesel	Combusti-ble	Microcosmos	Na	7-8	32			Sin alimen-tación					si (LC50)	Amin & Comiglio, 2002
			Cadmio y Plomo	Metal	Microcosmos	30d	7-8	28-32	Na	Na	Na						Amin et al., 1998

		Amonio	Nutriente	Microcosmos	96h	7-8	23-24	8,15	Na	Sin alimentación			si (LC50)	Diodato et al., 2019
	A.rtemesia longinaris	Cobre	Metal	Microcosmos	72h	18-22	33-34	Na	Na	Sin alimentación			si (LC50)	Scelzo, 1997

A.trapesalis fue expuesto a diferentes concentraciones de metales y agroquímicos (ensayo microcosmos y en el ambiente),

evaluando sus efectos sobre actividad de enzimas relacionadas al estrés oxidativo en diferentes tejidos, niveles de especies reactivas de oxígeno (ROS), peroxidación lipídica (LPO), daño en el ADN (en los hemocitos), entre otros biomarcadores; además de determinarse la concentración de los diferentes compuestos en el agua y en los tejidos de los organismos (de Oliveira et al., 2018; Jacomini et al., 2006; Lopes et al., 1992) (Tabla 3). *M. donacium* y *O. chilensis* fueron expuestas a agroquímicos, bacterias y toxinas (ensayos microcosmos) para evaluar el consumo de oxígeno, la tasa de depuración del compuesto y su concentración en el organismo, los efectos sobre el sistema inmune y la sobrevivencia (Álvarez et al., 2015; Maldonado-Aguayo et al., 2013; Navarro et al., 2016). En las especies marinas expuestas a agroquímicos, agua de sitios poluídos, filtros de cigarro, metales, etc., fueron evaluados bioindicadores a nivel fisiológico (consumo de oxígeno, tasa de excreción y tasa de filtración, producción de biso), morfológico (cambios estructurales en branquias, glándula digestiva, gónadas, músculo, pie, manto y hepatopáncreas), comportamental, bioquímico (osmolaridad, reservas energéticas, ATP, actividad de la enzima Na/K ATPasa, viabilidad de hemocitos, daño en ADN, entre otros); además fue evaluada la mortalidad y la concentración de los compuestos en los tejidos de los organismos y en el agua (Giaccomin et al., 2014; Jorge et al., 2016; Sauco et al., 2013; Torres, 2019). En general los organismos no fueron alimentados durante los ensayos, pero cuando recibieron alimento, estuvo compuesto por microalgas (de Oliveira et al., 2018; Zapata et al., 2009). Los bioensayos del tipo microcosmos tuvieron una duración de entre 48 horas y 17 meses (Führer et al., 2012; Lopes et al., 1992; Romero et al., 2018; Torres, 2019) (Tabla 5).

Durante los períodos de aclimatación, mantenimiento, cultivo y experimentación, *A. trapesalis* fue mantenida a temperaturas entre 18 y 23°C, pH entre 6,5 y 7,8 y niveles de oxígeno mayores a 85% de saturación (de Oliveira et al., 2018; Loayza-Muro & Elías-Letts, 2007). Las especies eurihalinas fueron mantenidas a temperaturas de 10 a 22°C, salinidad de agua dulce a 50 y oxígeno de 6,2 a 8 mg/l (Cunha-Nalesso, 1988; Milanesi Camejo, 2015; Navarro et al., 2020); el pH fue entre 7,5 y 7,9 (reportado sólo en un trabajo) (Navarro et al., 2020). Las especies marinas fueron mantenidas en temperaturas de 4 a 30°C (Carvalho et al., 2015; Di Salvo et al., 1983; Gilles, 1972), salinidad de agua dulce (0 ppm) a 42 (Carvalho et al., 2015; Proverbio, 2017; Soria et al., 2007), pH de 7,5 a 8,2 (Führer et al., 2012; Jorge et al., 2016; Torres, 2019) y niveles de oxígeno entre 6,99 y 13,5 mg/L (Führer et al., 2012; Jorge et al., 2016). La alimentación, cuando existió, fue en base a microalgas (de Oliveira et al., 2018; Milanesi Camejo, 2015; Toro et al., 2003) (Tablas 4 y 5).

Moluscos gasterópodos (Filo: Mollusca, Clase: Gasterópoda)

Los moluscos gasterópodos nativos mantenidos en cautiverio incluyen tres especies dulceacuícolas y dos marinas. Estas presentan distintos tipos de alimentación; los hay herbívoros como *Biomphalaria* sp., *Pomacea* sp. y *Heleobia. Australis*, y carnívoros como *Thais chocolata* y *Chorus giganteus*. Este último y *Pomacea* sp. son osmorreguladores (Tabla 6). Respecto a su mantenimiento en cautiverio, este ha sido con fines de aclimatación por entre 24 horas y 4 semanas (Magalhaes et al., 2014; Seuffert & Martín, 2013), previo a los ensayos, cuya duración fue de entre 2 horas y 4 años. Fueron evaluados efectos de alimentación, ciclo de marea intensidad de luz, oxígeno y/o temperatura sobre el crecimiento, la sobrevivencia, la tasa de ingestión y la plasticidad morfológica (Cancino et al., 2003; Magalhaes et al., 2014; Seuffert & Martín, 2013; Tamburi et al., 2018). Tanto el caracol manzana *Pomacea* sp. como *Biomphalaria* sp. han sido cultivados exitosamente (con fines experimentales) (Cossi et al., 2018; de Siqueira et al., 2020; Martínez et al., 2017); mientras que *T. chocolata* es un recurso pesquero. Para *A. ater*, *C. chorus* y *T. chilensis* han sido desarrolladas tecnologías de cultivo, pero este aún no se desarrolla con fines comerciales (APE – Acuicultura de Pequeña Escala en Chile » APE (sembrandoelmar.cl)).

Dos especies dulceacuícolas han sido expuestas a metales pesados, pesticidas, bajo condiciones controladas: *Biomphalaria* sp. y *Pomacea* sp. Los tiempos de exposición variaron entre 48 horas y 6 meses. Los bioindicadores evaluados fueron principalmente bioquímicos (actividad de enzimas vinculadas al estrés oxidativo, concentración de compuestos en tejidos y agua, proteínas, glucógeno, entre otros) y en menor medida, morfológicos (cambios estructurales en branquias, glándula digestiva y pie) y poblacionales (mortalidad) (Arrighetti et al., 2018; Campoy-Díaz et al., 2018; Cossi, 2018; Cossi et al., 2018; Cossi et al., 2020; de Oliveira-Filho et al., 2004; Martínez et al, 2017) (Tabla 7). La temperatura tomó valores entre 15 y 35°C (Seuffert & Martín, 2013; Tamburi et al., 2018), el pH entre 7 y 8 (Cossi et al., 2018; Martínez et al., 2017) y el oxígeno entre 8 y 9 mg/L (de Oliveira-Filho et al., 2004). Los organismos fueron alimentados con lechuga y/o ración (Arrighetti et al., 2018; Campoy-Díaz et al., 2018; Cossi et al., 2020; Magalhaes et al., 2014) (Tablas 6 y 7).

Crustáceos anfípodos (Filo: Arthropoda, Clase: Anfípoda)

El anfípodo de agua dulce *Hyalella curvispina* presenta una alimentación omnívora (Somma et al., 2011) y la capacidad de osmorregular (Dutra et al., 2008) (Tabla 1). Ha sido cultivada para la obtención de organismos para experimentación (García et al, 2010; Sansiñena et al., 2018). Los ensayos han tenido una duración de entre 48 horas y 10 días, evaluando el efecto de la temperatura sobre la respiración (Doyle & Momo, 2009) (Tabla 6). *H. curvispina* fue expuesta a diferentes compuestos, como metales pesados (cadmio, cobre y zinc), insecticidas y sedimentos de zonas poluídas. Los períodos de exposición variaron entre 48 horas y 10 días. Durante los ensayos, los organismos fueron alimentados con ración para

Tabla 4. Moluscos bivalvos acuáticos nativos utilizados en la región sur de América del Sur con diversos fines (aclimatación, mantenimiento, cultivo y/o experimentación). Se reportan datos de períodos de tiempo, factores abióticos (temperatura, salinidad, pH y oxígeno), alimentación y las referencias correspondientes. a=año, d=días, h=horas, m=meses. Na= información no reportada.

Table 4. Native aquatic bivalve mollusks used in the south of South America for various purposes (acclimatization, maintenance, cultivation and/or experimentation). Data on time periods, abiotic factors (temperature, salinity, pH and oxygen), diet and corresponding references are reported. a=year, d=day, h=hours, m=months. Na = unreported information.

Grupo	Ambiente (especies)	Aclimatación/ ensayos	Dura-ción	T (ºC)	Salinidad	pH	O2	pCO2(µatm)	Profundidad(m)	Alimentación	Referencias
Molus-cos bivalvos	**Dulceacuícola** *A. trapesalis*	Aclimatación	10d	21	Dulce	6,5-7,5	>85%			Microalgas	de Oliveira et al., 2018
	Eurihalina *B. darwinianus*, *E. mactroides, M. edulis, O. chilensis*	Aclimatación	1-2s	12-22d	dulce-32	Na	Na			Microalgas	Chaparro & Thompson, 1998; Cunha-Nalesso, 1988; Mardones-Toledo et al., 2015; Milanesi Camejo, 2015; avarro et al., 2016; Navarro et al., 2020
		Alimentación	20d	15	Mar	Na	Na			Microalgas, ración	Vivanco et al., 2014
		Densidad de cultivo y alimentación	44d	16-18	Na	Na	Na			Microalgas	Oliva et al., 2013
		Temperatura y acidificación	60d	10-20	27,4-27,8	7,5-7,9	Na	400-1000		Microalgas	Navarro et al., 2020
	Marina *A. ater, A. purpurata, A. purpuratus, B. rodriguezii, C. chorus, D. hanleyanus, M. mactroides, Mytilus sp., M. chilensis, Z. patagonica*	Aclimatación	6h-15d	5-23	21,9-35	8-8,02	>80%; 2,5-13,5mg/L			Microalgas	Benítez et al., 2018; Brokordt et al., 2015; Caers et al., 1999; Carvalho et al, 2015; Duarte et al., 2014; Espinoza et al., 2003; Fernández-Reiriz et al., 2005; Führeret al., 2012; García et al., 2012; García & Winkler, 2012; Garrido et al., 2012; González et al., 2017; Hégaret et a., 2012; Herrmann et al., 2009; Herve-Fernández et al., 2010; Lagos et al.,2016; Lardies et al., 2017; Navaro & Winter, 1982; Navarro & González, 1998; Núñez et al., 2013; Ramajo et al., 2019; Ramajo et al., 2020 ; Romero et al., 2018; Ruiz-Velásquez et al., 2017; Proverbio, 2017; Saavedra et a., 2012; Torroglosa & Giménez, 2019; Uriarte et al., 2004; Winter et al., 1984
		Acidificación	30d	14	30	7,6-8,1	Na	500-1200		Na	Benítez et al., 2018
		Agua marina filtrada	7d	20	Na	Na	Na			Microalgas	Riquelme et al., 1996

	Alimentación	5h-60 d	15-21	21-35	Na	Na		Microalgas; microalgas+sedimento	Caers et al., 1999; Farías et al., 1998; Ibarrola et al., 2012; Martínez et al., 1995; Navarro & Winter, 1982; Nevejan et al., 2003; Proverbio, 2017; Velasco et al.,2003; Winter et al., 1984
	Alimentación y temperatura	72d-3m	16-20	Na	Na	Na		Microalgas, fécula de papa, emulsión de lípidos	Navarro et al., 2000
	Alimentación y acidificación	30d	17-19	34	7,69-8,14		400-1519	Microalgas	Ramajo et al., 2016
	Crecimiento	20d	Na	Na	Na	Na		Microalgas	Riascos et al., 2007
	Cultivo	60-90d	14-18	Na	Na	Na		Microalgas; microalgas ambiente	Abarca et al.,2012; Oliva et al., 2020
	Exposición al aire	2-36hs	18-22	Na	Na	Na		No	Zaixso et al., 1979
	Marcaje	1 a 20 d	23	33	Na	Na		Microalgas	Herrmann et al., 2009; Carvalho et al., 2015; Proverbio, 2017
	Salinidad	8h-2m	dic-22	Dulce-42	Na	4,6-5,8mg/L		Microalgas	Carvalho et al., 2015; Gilles, 2019; Navarro, 1988; Navarro & González, 1998; Soria et al., 2007
	Temperatura	6h-18d	12-24	Na	Na	Na		Microalgas; sin alimento	Brokordt et al., 2015; Núñez et al., 2013
	Desarrollo larval en laboratorio	21d	18-26	35	Na	Na		Microalgas	Santos et al., 2020
	Salinidad/desarrollo larval	27h	20-22	20-35	Na	Na		Microalgas	Santos et al., 2020
	Hipoxia	6h	18	Na	Na	1,5-2,0mg/L		Sin alimento	Brokordt et al., 2015
	Mantenimieto y/o Larvicultura	35d-93d	12-25	33,2	Na	Na		Microalgas	Contreras et al., 2014
	Profundidad	183d	12-17,2	34	Na	1,45-4,17mg/L	9-22	na	Ramajo et al., 2020
	Reproducción	4s-100d	10-30	26-28	Na	Na		Microalgas, sin alimento, agua ambiente	DiSalvo et al., 1983; Toro et al., 1996; Wilson et a., 1996; Videla et al., 1998
	Salinidad y acidificación	60d	13-16	20-30	7,64-8,17	Na	380-1200ppm	Microalgas	Duarte et al., 2018; Grenier et al., 2020

Temperatura	3-60d	1-20	30	Na	Na		Microalgas	Agüero-Velázquez, 2018; Zaixso et al., 1979
Temperatura y pH	18d	14-18	33-35,9	7,7-8,0	Na		Microalgas	Lagos et al., 2016
Temperatura y acidificación	18d- 70d	12-18	31—35	7,57-8,05	Na	380-1200 ppm	Microalgas	Duarte et al., 2014; Navarro et al., 2013; Lardies et al., 2017
Temperatura, pH y oxígeno	11d	14-18	34	7,58-8,11	45-80%		Mezcla de fitoplancton, zooplancton, vitaminas, minerales y ácidos grasos	Ramajo et al., 2019

Tabla 5. Moluscos bivalvos acuáticos nativos de la región sur de América del Sur utilizados en bioensayos, compuestos a los que son expuestos, características de los ensayos (alimentación, tiempo, temperatura, salinidad, pH, oxígeno) y bioindicadores evaluados (fisiológicos/metabólicos, morfológicos, bioquímicos, crecimiento y mortalidad) y las referencias asociadas.d=días, h=horas. na=información no reportada.

Table 5. Native aquatic bivalve mollusks to the southern region of South America used in bioassays, compounds to which they are exposed, characteristics of the assays (feeding, time, temperature, salinity, pH, oxygen) and bioindicators evaluated (physiological/metabolic, morphological, biochemical, growth and mortality) and the associated references. D=days, h=hours. na=unreported information.

Grupo	Ambiente	Especie	Compuesto	Tipo	Ensayo	Tiempo	T(ºC)	Salinidad	pH	O2	Alimentación	Fisiológicos/metabólicos	Morfológicos	Bioquímicos	Mortalidad	Referencias
Moluscos bivalvos	Dulce-acuícola	*Anodontites trapesialis*	Zinc, Manganeso y/o Hierro	Metales	Microcosmos	96hs	21-23	Na	6,5-7,5	85%	Microalgas			[Zn], [Mn], [Fe] (agua, M, B, GD, Mu y HL); ROS (B); TAC (B y Mu); LPO, PC SOD y GST (B, Mu, GD); MT (B y M); ChE y ASCh (M y Mu); daño ADN (H)		de Oliveira et al., 2018
			Atrazina	Herbicida	Microcosmos	48hs- 1 s	21,9	Na	7,7	89%	sin alimentar			[Atrazina] tejidos y agua		Jacomini et al., 2006
			Agua ambiente	Pesticidas	Ambiente	17m	Na	Na	Na	Na	Na			[pesticidas] en agua y tejidos		Lopes et al., 1992
	Eurihalina	*Mesodesma doacium*	*Vibrio anguillarum*	Bacteria	Microcosmos	32h	17	Na	Na	Na	No			Componentes de sistema inmune		Maldonado-Aguayo et al., 2013
			Ácido domoico	Toxina	Microcosmos	350h	18	Na	Na	Na	Microalgas	Tasa depuración de ácido domoico		Concentración ácido domoico		Álvarez et al., 2015
		Ostrea chilenis	Toxinas paralizantes de los mariscos	Toxina	Microcosmos	30d	14	30	Na	Na	Microalgas	Tasa de Aclaramiento, Consumo O2		Lípidos	Si	Navarro et al., 2016
	Marina	*Donax hanleyanus*	Filtros cigarro	Na	Microcosmos	48h	19,6	30	7,78	8,18	sin alimentar	Consumo oxígeno			Si	Torres, 2019

Mesodesma mactroides	Cobre	Metal	Micro-cosmos	96h	20	30	7,5	6,99	sin alimentar	Consumo oxígeno		[iones] (HL, B, GD), osmolaridad (HL), Na/K ATPasa (B, GD), AC (B, GD)		Jorge et al., 2016
	Cobre	Metal	Micro-cosmos	96h	20	30	Na	Na	sin alimentar			[Cu] agua; ATP, NAD, NADH (Mu, B, GD), proteínas, glucosa, glucógeno, lípidos (HL,Mu, B, GD); piruvato y lactato (Mu y HL)	Si	Giaccomin et al., 2014
	Agua/salini-dad	Pesticidas	Micro-cosmos	2m	na	4-12	Na	Na	Na				Si	Sauco et al., 2013
Argopecten . purpuratus	Cadmio y Plomo	Metales	Micro-cosmos	48h-96h	18-20	34,5	8,05	4,5 mg/l	Na				Si	Romero et al., 2018
	Cobre	Metal	Micro-cosmos	8d	20-22	35			Microalgas			genes (respiración, regulación síntesis de proteínas, estrés,desarrollo)		Zapata et al., 2009
Miytius. chilensis	Cadmio	Metal	Micro--cosmos	100d	12	30	Na	Na	Microalgas			Acumulación deCd		Hervé-Fernández et al., 2010
Aulacomya ater	Clorpirifos	Insecticida	Micro-cosmos	96h	14-16	32-34	8,2	2,5-13,5	No			AchE, proteinas		Führer et al., 2012
	Clorpirifos	Insecticida	Micro-cosmos	21d	14-16	32-34	8,2	25-13,5	Microalgas	Consumo O2, TEA		AchE, proteinas		Führer et al., 2012
	Estradiol	Hormona	Micro-cosmos	21d	Na	Na	Na	Na	Na		Gónadas	Vitelogenina		Saavedra et al., 2012
	Cobre y Cadmio	Metales	Micro-cosmos	168h	16-18	30-32	Na	Na	No				si (LC50)	Espinoza et al., 2003
C.horomytilus chorus	Azamethi-phos y deltametrina	Insecticida	Micro-cosmos	5d					Na			ARN	si (LC50)	Núñez-Acuña et al., 2019

	azametiphos, deltamethrina ,abamectin benzoato	Insecticidas	Microcosmos	6d	14	Na	Na	Na	sin alimentar				Sanhueza-Guevara et al., 2018
	Policloruro de Vinilo	Microplástico	Microcosmos	12s	12-18	Na	Na	Na	Microalgas	Tasa de Aclaramiento, producción de biso			Olmedo, 2017
	Agua de ambientes poluídos	Organoclorados y Hidricarbonos	Microcosmos	1,5h	12-15	30	Na	Na	Microalgas	Tasa de Aclaramiento, Consumo O2, Eficiencia de absorción, Tasa de Abosorción			Toro et al., 2003
	Tributilestaño	Metal	Microcosmos	192h	10-14	34	7,8	Na	Microalgas		Branquias y hepatopáncreas		Román et al., 1992
	Tributilestaño	Metal	Microcosmos	96h	10-14	34	7,8	Na	Microalgas			si (LC50)	Román et al., 1992

Tabla 6. Moluscos gasterópodos, crustáceos anfípodos y cnidarios acuáticos nativos utilizados en la región sur de América del Sur con diversos fines (aclimatación, mantenimiento, cultivo y/o experimentación). Se reportan datos de períodos de tiempo, factores abióticos (temperatura, salinidad, pH y oxígeno), alimentación y las referencias correspondientes. a=año, d=días, h=horas, m=meses. Na= información no reportada.

Table 6. Native aquatic gastrophod mollusks, amphipods crustaceans and cnidarians used in the south of South America for various purposes (acclimatization, maintenance, cultivation and/or experimentation). Data on time periods, abiotic factors (temperature, salinity, pH and oxygen), diet and corresponding references are reported. a=year, d=day, g=generations, h=hours, m=months. Na = unreported information.

Grupo	Ambiente (especies)	Aclimatación/ ensayos	Duración	Temperatura (ºC)	Salinidad	pH	O2	Alimentación	Referencias
Moluscos gasterópodos	**Dulceacuícola** *Biomphalaria* sp., *H. australis*, *Pomacea* sp.	Aclimatación	3-4 s	20-25	Dulce	6,6-7	Na	lechuga	Sueffert & Martín, 2013; Magalhaes et al., 2014; Arrighetti et al., 2018
		Alimentación/temperatura	2h	20-30	Dulce	Na	Na	ración	Magalhaes et al., 2014
		Cultivo	2g, >6a	21-25	Dulce	7-8	8,8mg/L	lechuga	Martínez et al., 2017; Cossi et al., 2018; de Siquieira et al., 2020
		Mantenimiento	4 m	23-26	Dulce	Na	Na	lechuga	Campoy-Díaz et al., 2018
		Temperatura	10s-4a	15-35	Dulce	Na	Na	lechuga	Sueffert & Martín, 2013; Tamburi et al., 2018
	Marino (*T. chocolata, C. giganteus)*	Aclimatación	Na	12-18,7	Mar	Na	Na	Vieiras y otros bivalvos	Romero et al., 2004
		Alimentación	33-60d	13-15	32-33	Na	Na	Mejillones	Gutiérrez & Gallardo, 1999; Gallardo et al., 2004
		Alimentación y temperatura	5m	13-18	Mar	Na	Na	Bivalvos	Navarro et al., 2002
		Intensidad de luz y ciclo de marea	1h-7d	13-16	Mar	Na	Na	Na	Gallardo et al., 2013
		Mantenimiento	4m; na	12-23	mar; 30-33	Na	Na	Microalgas, almejas, mejillón	Gutiérrez & Gallardo, 1999; Gallardo et al., 2004; Romero et al., 2004; Martínez et al., 2008; Gallardo et al., 2013
		Temperatura	Na	9-15	Mar	Na	Na	Na	Martínez et al., 2008
		Temperatura y oxígeno	60d	12-18	Mar	Na	50-100%	Na	Cancino et al., 2003
Crustáceos anfípodos	**Dulceacuí-cola** *H. curvispina*	Aclimatación	4-10d	15-26,5	Dulce	7,4-7,86	Na	Algas, ración	Doyle & Momo, 2009; Dutra et al., 2008; Giusto et al., 2012; Giusto & Ferrari, 2014
		Cultivo	varias generaciones	20	Dulce	Na	Na	Na	Sasiñena et al., 2018
		Mantenimiento	2d	19-25	Dulce	Na	8,2-9,1mg/L	Ración	García et al., 2010
		Temperatura	4-8h	11,5-30	Dulce	Na	Na	Na	Doyle & Momo, 2009

Cnidarios	**Marina** *B. cangicum*	Aclimatación	15d	20	30-33	Na	Na	Peces	Abujamara et al., 2014; Anjos et al., 2017	

Tabla 7. Moluscos gasterópodos, crustáceos anfípodos y cnidarios acuáticos nativos de la región sur de América del Sur utilizados en bioensayos, compuestos a los que son expuestos, características de los ensayos (alimentación, tiempo, temperatura, salinidad, pH, oxígeno) y bioindicadores evaluados (fisiológicos/metabólicos, morfológicos, crecimiento y mortalidad) y las referencias correspondientes. d=días, h=horas. na=información no reportada.

Table 7. Native aquatic gastrophod mollusks, amphipods crustaceans and cnidarians to the southern region of South America used in bioassays, compounds to which they are exposed, characteristics of the assays (feeding, time, temperature, salinity, pH, oxygen) and bioindicators evaluated (physiological/metabolic, morphological, biochemical, growth and mortality) and the associated references. D=days, h=hours. Na=unreported information.

Grupo	**Ambien-te**	**Especie**	**Compuesto tóxico**	**Tipo**	**Ensayo**	**Tiempo**	**T (ºC)**	**Sali-nidad**	**pH**	**O2**	**Alimenta-ción**	**Fisiológicos/ metabólicos**	**Morfológicos**	**Bioquímicos**	**Crecimiento**	**Mortalidad**	**Referencias**
Moluscos gasteró-podos	Dulce-acuícola	*Biomphalaria sp.*	Cobre	metal pesado	Micro-cosmos	48h	25	Dulce	7,4	8-9 mg/l	Na					si (LC50)	de Oliveira-Filho et al., 2004
			Metilazinfos/ Acetamiprid y formulado (Assail 70)	plaguicidas	Micro-cosmos	14d	23	Dulce	Na	Na	Lechuga			ROS, TAC, ChE, CE, GST, CAT, SOD glucógeno y proteínas		Si	Cossi et al., 2018; Cossi et al., 2020
			Carbaril	plaguicida	Micro-cosmos	14d	24	Dulce	Na	Na	Lechuga			ROS, TAC, ChE, CE, GST, CAT, SOD y proteínas			Cossi, 2018
		Pomacea sp	Mercurio, Arsénico y Uranio	metales	Micro-cosmos	8 s	23-26	Dulce	Na	Na	Na			[Hg, As, U] GD, H, Excreción			Campoy-Díaz et al., 2018

			Tributilin	biocida	Micro-cosmos	6m	Na	Dulce	Na	Na	Lechuga		SOD, CAT, GSH, LPO, AChE, proteínas en CP, G, HP, GA			Martínez et al, 2017
			Cipermetrina	plaguicida	Microcos mos	14d	20-22	Dulce	Na	Na	sin alimentar	cambios estructura les branquias, pie y glándula digestiva	SOD, CAT, GST, LPO, oxidació n proteínas			Arrighet ti et al., 2018
Crustá-ceos anfípo-dos	Dulce-acuícola	*Hyalella curvispina*	Cadmio y Cobre	metal pesado	Microscos mos	10d	23	Dulce	7,4 - 8,2	6,6-8,3	Ración peces		[Cd] corporal, en agua y sediment os; Glucóge no, proteínas, lípidos, triglicéri dos, glicerol, arginina, LPO, Na/K ATPasa, CAT y SOD	si	Si	Giusto et al., 2012; Giusto & Ferrari, 2014
			Clorpirifos	insecticida	Microscos mos	48h-10d	22	Dulce	na	na	nada-algas		[CPF]ag ua y sediment o		Si	Mugni et al., 2016
			Cromo y Zinc (sedimento natural)	metal pesado	Microscos mos	10d	na	Dulce	8,1 - 8,7	na	Ración peces y lechuga			si	si (LC50)	Peluso et al., 2011
			Sedimento natural	na	Microscos mos	10d	21,2-22,2	Dulce	6,1 - 7,8	5,0-6,4	Lechuga		Materia orgánica	si	Si	Sansiñen a et al., 2018

													sediment o	
Cnidarios	Marina	*Bunodosoma cangicum*	Cobre	metal pesado	Microcosmos	96h (exposición)+6h (reoxigenación)	19-21	33	na	7,5	sin alimentar	Consumo oxígeno	ACAP, SOD, GST, GSH, LPO, ATP	Abujamara et al., 2014
			Cobre/Verapamil	metal pesado/bloqueador canales calcio)	Microcosmos	24h	20	30	na	Na	Na		MXR (células)	Anjos et al., 2017

peces, algas y lechuga. En este caso, los bioindicadores evaluados fueron bioquímicos, como concentración de compuestos en tejidos, agua y sedimentos, reservas energéticas (glucógeno, proteínas, lípidos, entre otras) y niveles de LPO; además de fisiológicos (crecimiento) y poblacionales (mortalidad). También se determinó la curva de letalidad (LC50) (Giusto et al., 2012; Giusto & Ferrari, 2014; Mugni et al., 2016; Peluso et al., 2011; Sansiñena et al., 2018) (Tabla 7).

Los organismos fueron mantenidos en rangos de temperatura entre 11,5 y 30°C (Doyle & Momo, 2009; García et al., 2010;

Sansiñena et al., 2018), pH entre 7,4 y 8,2 (Giusto et al., 2012; Giusto & Ferrari, 2014) y de 5 a 9,1 mg/l de oxígeno disuelto (Doyle& Momo, 2009; García et al., 2010: Sansiñena et al., 2018; Solis et al., 2019). Fueron alimentados con algas o ración para peces (Doyle & Momo, 2009; Dutra et al., 2008; Giusto et al., 2012; Giusto & Ferrari, 2014) (Tablas 6 y 7).

Cnidarios (Filo: Cnidaria, Clase: Anthozoa)

La anémona de mar *Bunodosoma cangicum* se alimenta de peces y animales de pequeño porte; además, es osmoconformadora (Anjos, 2017) (Tabla 1). Ha sido aclimatada durante 15 días y alimentada con pescado, para posteriormente ser utilizada en bioensayos (Abujamara et al., 2014; Anjos et al., 2017) (Tabla 6). *B. cangicum* fue expuesta a concentraciones de cobre desde 24 a 96 horas. Los organismos no fueron alimentados durante los períodos de exposición. Se evaluaron bioindicadores fisiológicos (*i.e.* consumo de oxígeno) y bioquímicos (*i.e.* actividad de enzimas relacionadas al estrés oxidativo, energía en forma de ATP, LPO, capacidad antioxidante contra radicales peroxil y resistencia a xenobióticos (Tabla 7). En general, la temperatura se mantuvo entre 19 y 21°C, salinidad entre 30 y 33 y el oxígeno en 7,5 mg/l (Abujamara et al., 2014; Anjos et al., 2017) (Tablas 6 y 7).

Productores primarios macroscópicos (Orden: Alismatales; Orden: Ulvales)

Los productores primarios macroscópicos que han sido mantenidos en cautiverio corresponden a seis divisiones (por su clasificación taxonómica): Angiospermae (Orden: Alismatales), representada por *Egeria densa*; Pteridophyta (Orden: Salviniales), representada por *Azolla filiculoides*; Magnoliophyta (Ordenes: Alismatales, Cyperales, Myrtales, Saxifragales), representada por *Lemna minuta*, *L. valdiviana*, *Ludwigia pelpoides*, *Myrophyllum aquaticum*, *M. quítense* y *Spirodela intermedia*; Fanerógama (Orden: Apiales), representada por *Hydrocotyle ranuculoides*; todas ellas dulceacuícolas. Las especies marinas están representadas por *Ulva* sp. y *Gracilaria chilensis*, que corresponden a las divisiones Chlorophyta (Orden: Ulvales) y Rhodophyta (Orden: Gracilariales), respectivamente.

E. densa, *M. aquaticum* y *M. quitense* son plantas utilizadas y comercializadas con fines decorativos para acuarios de peces ornamentales (observación personal; Yarrow et al., 2009;

Wikipedia Myriophyllum aquaticum - Wikipedia, la enciclopedia libre). En el ambiente, *E. densa* aporta a la estabilidad de sedimento y la claridad del cuerpo de agua de las zonas donde habita, regulando la cantidad de fitoplancton (Jeppsen et al., 1997; Scheffer et al., 1993). En la naturaleza ha sido hallada en aguas con temperaturas entre 10 y 27,5°C, pH de 6,8 a 8,3 y 79% de saturación de oxígeno (Mazzeo et al., 2003). Otras, como *M. aquaticum*, pueden crecer en forma descontrolada, ya que, en general, no hay otras especies que compitan por los recursos (Wikipedia). Con las especies dulceacuícolas han sido realizados ensayos para determinar los efectos de la alimentación, intensidad de luz, temperatura, turbidez y/o fotoperíodo sobre el crecimiento, por entre 8 y 60 días, con temperatura entre 15 y 35°C y pH entre 5,5 y 7,45 (Fernández y Mujica, 1984; Machado et al., 2020; Neto et al., 2005). Neto et al. (2005) evaluaron el efecto de la temperatura sobre la intensidad de infección del hongo *Fusarium graminearum* sobre *E. densa*; mientras que en otros PPM fue evaluado el efecto de la exposición a metales, agua de efluentes, antibióticos, etcétera, sobre diversos biomarcadores (demanda química de oxígeno, morfología de raíces, clorofila, actividad enzimática, ROS, crecimiento, entre otros) (Cohelo-Gómes et al., 2020; Cook et al., 2014; Herrera et al., 2019; Silva et al., 2013; Valderrama et al., 2013).

Ulva sp. ha sido aclimatada por entre 2 y 10 días a una temperatura de 22 a 24°C, salinidad de 30 y alimentada con medio von Stosch; los factores restantes no fueron reportados (Freire da Costa, 2006; Kokolowicz-Pilatti et al., 2017). Además, fue estudiada su capacidad de remover nitrógeno en forma de amonio en sistemas multitróficos (cría de camarón) a 28°C, salinidad entre 18 y 35, pH entre 8,26 y 8,36 y 7,7 mg/l de oxígeno disuelto (Freire da Costa, 2006). Las células de esta macroalga fueron expuestas a una combinación de cobre y glifosato para evaluar la capacidad de absorción del herbicida en presencia de cobre, durante 24 horas (Trinelli et al., 2013).

Los biomarcadores relevados en esta revisión fueron adicionados a la figura elaborada por Cossi (2018), de acuerdo con el nivel de organización correspondiente (Figura 7).

Discusión

La utilización de macroinvertebrados y productores primarios macroscópicos nativos en Argentina, Brasil, Chile y Uruguay para evaluar características biológicas/ecológicas y los efectos de la exposición a compuestos tóxicos data de las décadas de 1970-1980 (Bond & Buckup, 1983; Cunha-Nalesso, 1988; Fernázdez & Mujica, 1973; Navarro & Winter, 1982; Zaixso et al., 1979). De acuerdo con Oliveira-Filho et al. (2017), los invertebrados son los organismos modelo más utilizados en los ensayos que buscan analizar o evaluar características del estado ambiental. De esta revisión se desprende que las especies empleadas son principalmente dulceacuícolas y marinas, destacándose los CD y PPM en el ambiente dulceacuícola y los MB en el marino. Esto se debe a que, en general, las especies

utilizadas para la realización de ensayos bajo condiciones controladas son seleccionadas por ser representativas de los ambientes que habitan y de fácil acceso en la naturaleza, manipulación y mantenimiento en el laboratorio (Castiglioni et al., 2009; Gonzalez-Baro & Pollero, 1988; Jergentz et al., 2004). Otros son escogidos por presentar importancia comercial, ya sea por su captura en la naturaleza o cultivo en cautiverio, y/o ser consumidos por el hombre (por colecta directa del medio en que habitan o adquiridos en el mercado) (obs. personal; Collins, 1977; Navinta, 2019). En otros casos, como el de la anémona de mar *Bunodosoma cangicum*, se utilizan como modelo de estudio por ser abundantes, sésiles y estar expuestos permanentemente a los cambios que ocurren en el ambiente que habita (de marea, temperatura, salinidad, períodos de exposición al aire) y a la presencia de sustancias tóxicas, sin posibilidad de movilizarse a otras zonas no perturbadas (Abujamara et al., 2014).

Las especies de macroinvertebrados y productores primarios macroscópicos nativos usados como modelos de estudio en la región, en general son de pequeño porte. Las características biológicas/ecológicas son bien conocidas en la mayoría de las especies registradas en esta revisión. En algunos casos, no hay información disponible sobre la estrategia osmorreguladora, como en el caso de *B. darwinianus*, *Biomphalaria* sp., *H. australis*, *H. curvispina*, *A. septentrionalis*, *M. borelli, Mulina edulis, Venus antiqua* y *T. borellianus*, entre otras. Esto pudo deberse también a que esta información no estuvo accesible en los medios explorados.

Cautiverio

En general, luego de realizar las colectas en el ambiente y previo a la experimentación, los organismos son aclimatados para disminuir o eliminar el estrés causado por la manipulación y el cambio de ambiente, en tiempos variables. En otros casos, como el de la almeja amarilla *A. mactroides*, la aclimatación tiene una duración aproximada de una semana donde elimina el alimento, los productos de excreción y los sedimentos pertenecientes a su ambiente natural (Carvalho et al., 2015; Sauco et al., 2013).

Tabla 8. Productores primarios acuáticos nativos utilizados en la región sur de América del Sur con diversos fines (aclimatación, mantenimiento, cultivo y/o experimentación). Se reportan datos de períodos de tiempo, factores abióticos (temperatura, salinidad, pH y oxígeno), alimentación y las referencias correspondientes. a=año, d=días, h=horas, m=meses. Na= información no reportada.

Table 8. Native aquatic primary producers used in the south of South America for various purposes (acclimatization, maintenance, cultivation and/or experimentation). Data on time periods, abiotic factors (temperature, salinity, pH and oxygen), diet and corresponding references are reported. a=year, d=day, g=generations, h=hours, m=months. Na = unreported information

Grupo	Ambiente (especies)	Aclimatación/ ensayos	Duración	T (ºC)	Salinidad	pH	O2	pCO2 (µatm)	Profun-didad(m)	Alimentación	Referencias
Productores primarios macroscópicos	**Dulceacuícola** *E. densa, L. minuta,L. valdiviana, S. intermedia, H. runuculoides, M. quitense*	Aclimatación/Manteni-miento	48h- 2m	19-29	dulce	5,0-8	Na			no; medio Hoagland; medio Clark; medio Steinburg y Altenburger	Fernandez y Mujica, 1973; Caris et al., 2008; Cook, 2014; Harguinteguy et al., 2015; de Souza et al., 2018; Fernándes San Juan et al., 2018; de Souza et al., 2019; Garanzini et al ., 2019; Herrera, 2019; Gomes-Coelho et al., 2020
		Alimentación y/o condiciones de cultivo	14d-21d	20-33	dulce	Na	Na			Medio Hbt, medio Htr, agua corriente; estiércol de gallina	Paisio et al., 2018; Matos, 2019
		Cultivo	Na	25	dulce	Na	Na			Na	Amorim et al., 2017
		Fotoperíodo	8d	25	dulce	7	Na			Solución nutritiva de Clark	Neto et al., 2005
		Intensidad de luz /ácido giberélico/ fotoperíodo/temperatura	Na-15d	24-27	dulce	5,5	Na			Medio Steinburg	Fernandez y Mujica, 1973; Fernandez y Mujica, 1984; Klich et al., 1987
		Temperatura	8d	15-35	dulce	7	Na			Solución nutritiva de Clark	Neto et al., 2005
		Temperatura y turbidez	60d	25-27	Dulce	6,48-7,45	Na			Na	Machado et al., 2020
	Marina *G. chilensis, Ulva* sp.	Aclimatación	2-10d	22-24	30	Na	Na			Medio von Stosch	Freire da Costa, 2006; Kokolowicz-Pilatti et al., 2017
		Absorción Amonio (NH4)	1m	28	18-35	8,26-8,36	7,7 mg /L			Agua cria de camarón	Costa, 2006

	Alimentación	24h	13-17	32	Na	Na	Nirato. Amonio y urea	Chow & de Oliveira, 2008
	Crecimiento	10s	10-18	Mar	Na	Na	Medio SWM-3	Muñoz & Santelices, 1994
	Cultivo	30d-1a	11-20	10-32; mar	7,2-7,8	Na	Medio SFC; agua ambiente; medio Provasoli; aguas de cultivo de peces, ostras y erizos; medio PES	Prieto et al., 1991; Alveal et al., 1997; Meneses et al., 1999; Macchiavello et al., 2001; Abreu et al., 2009; Gallegos-Sánchez et al., 2018
	Densidad cultivo	60d	13-17	34-36	7,9	Na	Agua de cultivo de *Haliotis rufescens*	Macchiavello & Bulboa, 2014
	Exposición a radiación solar	3d	20	Mar	Na	Na	Agua ambiente	Gómez et al., 2005; Molina & Montecino, 1996
	Mantenimiento	Na; 35d	8-17	Na; 32	Na	Na	no; medio von Stock; medio SFC; nitrato de amonio y fosfato disódico	García et al., 2007; Chow & de Oliveira, 2008; Chow et al., 2013; Usandizaga et al., 2018
	Nutrientes	30d	15-17	Na	Na	Na	Agua marina con y sin adición de fertilizante	Usandizaga et al., 2018
	Reproducción	2s	14-16	35	Na	Na	Medio SFC	Guillemin et al., 2014
	Salinidad e intensidad luminosa	60d	15-35	13-15	Na	Na	Medio SFC	Guillermin et al., 2013
	Temperatura	45min-48hs	5-50	Na	Na	Na	no	Cruces et al., 2017

Tabla 9 Productores primarios acuáticos nativos de la región sur de América del Sur utilizados en bioensayos, compuestos a los que son expuestos, características de los ensayos (alimentación, tiempo, temperatura, salinidad, pH, oxígeno) y bioindicadores evaluados (fisiológicos/metabólicos, morfológicos, crecimiento y mortalidad) y las referencias correspondientes. d=días, h=horas, s=semanas. na=información no reportada.

Table 9. Native aquatic primary producers to the southern region of South America used in bioassays, compounds to which they are exposed, characteristics of the assays (feeding, time, temperature, salinity, pH, oxygen) and bioindicators evaluated (physiological/metabolic, morphological, biochemical, growth and mortality) and the associated references. D=days, h=hours,.s=weeks. na=unreported information.

Grupo	Ambiente	Especie	Compuesto tóxico	Tipo	Ensayo	Tiempo	Temperatura	Salinidad	pH	O2	Alimentación	Fisiológicos/ metabólicos	Morfológicos	Bioquímicos	Mortalidad	Referencias
Plantas	Dulce-acuícola	*Azolla filiculoides*	Cobre y Cadmio	Metales	Microcosmos	7d	20-25	dulce	7,5	Na	medio con macro y micronutrientes			Concentración de metales, clorofila		Valderrama et al., 2013 (CHILE)
		Lemna. minuta	Nutrientes	Agua de efluentes de criadero de cerdos	Microcosmos	7d	18-25	dulce	Na	Na				Concentración de nutrientes	Crecimiento (biomasa)	Caris et al., 2008 (Brasil)
			Cromo y Fenol	Metal y compuesto orgánico	Microcosmos	21d	20-28	dulce	Na	Na	medio Hogland			Concentración Cromo y Fenol; LPO; Clorofila, Carotenos		Paisio et al., 2018 (Arg)
			Fenol	Compuesto orgánico	Microcosmos	5d-30d	Na	dulce	Na	Na	Medio Hogland		Raíces	Concentración fenol	Crecimiento	Herrera, 2019 (Arg)
			Fenol+ Peróxido de Hidrógeno	Compuesto orgánico y químico	Microcosmos	48h	Na	dulce	Na	Na	Medio Hogland			Concentración Fenol; actividad enzimas peroxidasas en planta		Herrera, 2019 (Arg)
			Materia orgánica	Agua de Efluente de curtiembre	Microcosmos	6d	25	efluente	7	Na	No	Demanda química de oxígeno				Herrera, 2019 (Arg)

	Agua poluída	Agua de Efluente de criadero de cerdos		20d	Na	Efluen-te + agua corrien te	7-7,13	27,8-108,45 mg/l	No		Concentra-ción de nutrientes	Creci-miento	Antonelo, 2018 (Bra)
Lemna valdiviana	Arséni-co	Meta-loide	Micro-cosmos	7d	Na	dulce	6,5	Na	Medio Clark		Concentra-ción Arsénico	Creci-miento	de Souza et al., 2019 (Bra)
	Arséni-co + Ácido jasmó-nico	Meta-loide y hormona	Micro-cosmos	24h	23-27	dulce	6,6	Na	Medio Clark		Concentra-ción de Arsémico-, Factor de Bioacumul ación; Clorofila, carotenoi-des, LPO, ROS,SOD, CAT,POX, GPX, GR		Coelho-Gomes et al., 2020 (Bra)
	Arsénic o	Meta-loide	Micro-cosmos	168h	23-27	dulce	6,7	Na	Medio Clark, fósforo y nitrógeno		Concentr-ación de Arsénico y fósforo; factor de bioacumu-lación	Creci-miento	de Souza et al., 2018 (Bra)
	Agua efluente cultivo de peces		Meso-cosmos	26d	Na	dulce	na	Na	na	Demanda química de oxígeno	Nutrientes (amonio, fósforo, nitrito, nitrato), sólidos en suspensión		Mohedano, 2004 (Bra)
	Agua poluída	Agua de Efluente de criadero de cerdos	Micro-cosmos	21d	25,4	dulce	na	Na	na	Demanda química de oxígeno	Nutrientes (fósforo, nitrito, nitrato), sólidos en suspensión del agua; contenido proteico plantas	Creci-miento	Tavares et al., 2008 (Bra)

	Oxitetraciclina, Eritromicina, Florfenicol y Flumequina	Antibióticos	Microcosmos	7d	Na	na	na	Na	na		Clorofila; EC50	N.º y área de frondas; crecimiento	Cook, 2014 (Chi)
	Agua poluída	Agua de Efluente ciadero de salmón	Microcosmos	5d	18-22	dulce	na	Na	medio Steinberg modificado por Altenbuger		Proteinas, GST, CAT, GT, Gpx, GSH, GSSG,		Barrera, 2015 (chi)
Schoenoplec-tus californicus	Zinc	Metal	Microcosmos	369d	ambiente	dulce	na	Na	na		Concentración Zinc		Arreghini et al., 2018 (Arg)
	Zinc y Plomo	Metales	Microcosmos	63d	ambiente	dulce	na	Na	na		Concentración de zinc y plomo en sedimento y plantas; factor de Biocon-centración		Arreghini et al., 2017 (Arg)
Spirodela intermedia	Mercurio	Metal	Microcosmos	336h	Na	dulce/ mineral	8,1	Na	nutrientes del agua mineral	Raíces			de la Fournière et al. 2019 (arg)
	Agua poluída	Agua de Efluente de criadero de aves de corral	Mesocosmos	144h	22-24	dulce	na	Na	nutrientes del agua		Concentración de nutrientes	Crecimiento	Basílico et al., 2013 (Arg)
	Agua poluída	Agua de Efluente de criadero de aves de corral	Mesocosmos	6d	Na	na	8-8,55	0-6 mg/l	na		Porcentaje de remoción	Crecimiento	Basílico et al., 2016 (Arg)
	hierro, Zinc, Mangnesio, Cobre, Cromo y Plomo	Metales	Microcosmos	15d	Na	dulce	na	Na	nutrientes del agua		Concentración metales		Miretzky, et al., 2004

	Arséni-co	Meta-loide	Microcos mos	24h	23-27; Na	dulce	Na; 6,5	Na	solución con nutrientes	Raíces	Concentra-ción Arsénico, antocianin a; ROS, LPO; CAT, POX, APX,SOD, GPX, GR; MDA		Silva et al., 2013; da Silva et al., 2017
Ludwigia peploides	Plomo	Metal	Meso-cosmos	18s	Na	dulce	Na	Na	Na	Raíces	Concentra-ción Plomo; Bioacumu-lación		Auguet et al., 2017 (Arg)
	Plomo y Zinc	Metales	Micro-cosmos	4d	15-23	dulce	Na	Na	na		Concentra-ción Plomo y Zinc, Factor Bioconcen -tración		Fernández San Juan et al., 2018 (Arg)
Myriophyll um aquatic um	Agua poluída	Agua de Efluente	Meso-cosmos	30d	17-30	dulce	na	Na	nutrientes del agua			Creci-miento	Souza et al, 2013
	Níquel, Plomo y Zinc	Metales	Micro-cosmos	7d	Na	dulce	na	Na	na		Concentra-ción metales; clorofia y MDA		Harguinteguy et al., 2015
Myriophyll um quitens e	Endo-sulfán	Insecti-cida	Micro-cosmos	24h	9-22	dulce	na	Na	Medio Hoagland		GST, CAT, GR, POD; ROS,		Menone et al., 2008 (Arg); Garanzini et al., 2019 (arg)
	Axistor-bina	Fungici-da	Micro-cosmos	24h	Na	dulce	na	Na	Medio Hoagland		Concentra-ción Axistorbin; Clorofila, POD, GST, CAT, ROS, LPO; fragmentac ión ADN; proteinas		Garanzini y Menone, 2015; (Arg); Garanzini et al., 2019 (Arg)

	Marina	*Ulva* sp.	Cobre y glifosato	metal pesado/agroquí-mico	Micro-cosmos	24h	Na	Na	2-5,5	Na	sin alimentar	[Cu y Glifosato] alga	Trinelli et al., 2013

Sólo en Chile se registraron especies cultivadas con fines comerciales (*A. purpuratus* y *M. chilensis*); con el camarón de río *M. borelli* en Argentina y del camarón *Farfantepenaeus paulensis* en Uruguay (especies potencialmente cultivables), se realizaron estudios con la finalidad de desarrollar estrategias de cultivo (Bond & Buckup, 1983; Vitancurt et al., 2005). Se evaluaron aspectos como el crecimiento, la relación del largo con edad, peso, sexo y temperatura; esto aporta a dichas estrategias ya que permite optimizar

condiciones de cultivo de los organismos. Otras, como el anfípodo *H. curvispina*, el caracol manzana *Pomacea* sp. y *Biomphalaria* sp. han sido cultivados exitosamente con fines experimentales, para evaluar la exposición a sustancias tóxicas como pesticidas (Cossi et al., 2018; García et al, 2010; Martínez et al., 2017; Sansiñena et al., 2018). Mantener cultivos en cautiverio permite disponer de organismos (adaptados a las condiciones de laboratorio) para experimentación, de forma continua, en condiciones controladas y estandarizadas. Esto evita la dependencia de la disponibilidad en la naturaleza y la incertidumbre asociada a la historia de vida de los organismos. Por otro lado, existen aspectos éticos a considerar al tener en cautiverio organismos vivos. En general, es el uso de animales vertebrados el que está contemplado desde el punto de vista ético y legal (CHEA, 2019). En este sentido, el mismo se basa en tres principios básicos, como la reducción, el refinamiento y el reemplazo (Kirk, 2017). La reducción se refiere a que el número de animales utilizado debe ser el mínimo posible; el refinamiento refiere a que las técnicas a emplear no afectan (o lo hagan en menor medida) el bienestar de los organismos; y el reemplazo apunta a evitar el uso de vertebrados (si fuera posible), sustituyéndolos por otros objetos de estudio, como los invertebrados. Actualmente el uso de estos últimos es debatido, ya que también presentan sistema nervioso, en algunos casos con un grado de desarrollo considerable; esto ha llevado a que en algunos países los invertebrados sean integrados al marco legal que los protege. Esto, sumado a que los resultados obtenidos en los trabajos aquí relevados muestran una afectación de los organismos a distintos niveles, puede sugerir que “sienten” y están sufriendo un estrés, aunque en muchos casos no se puede visualizar por comportamiento. Esto quizás pueda considerarse al momento de evaluar el incluir a este grupo dentro de las reglamentaciones de experimentación animal.

La mayoría de los ensayos fueron del tipo microcosmos (i.e. realizados en laboratorio, en volúmenes pequeños); esta simplificación de las condiciones de la naturaleza puede generar cambios que hacen que los resultados no sean directamente extrapolables. Por ello es de vital importancia conjugar ensayos controlados con estudios sobre la historia de vida y modelización matemática que permita trasladar los resultados a la naturaleza (Jergentz et al., 2004).

Replicar el ciclo de vida de los organismos utilizados con fines experimentales es importante ya que evita la colecta de la naturaleza, permite contar con un stock continuo de organismos

de diferentes estadios y disminuye la etapa de aclimatación con un control sobre la historia de desarrollo.

Descripción de condiciones de mantenimiento en cautiverio

Los valores de salinidad y temperatura se mantuvieron en rangos similares a los que ocurren en las zonas donde habitan las diferentes especies (Costa, 2006; Montagna, 2011; Montagna & Collins, 2008; Renzulli & Collins, 2000). Se evaluó en algunos casos su efecto en bivalvos marinos y eurihalinos, moluscos gasterópodos marinos y dulceacuícolas, crustáceos anfípodos y productores primarios (Cancino et al., 2003; Cruces et al., 2017; Doyle & Momo, 2009; Martínez et al., 2008; Montagna, 2011; Navarro et al., 2000; Navarro et al., 2020; Neto et al., 2005; Tamburi et al., 2018). Lo mismo ocurre con la salinidad en decápodos eurihalinos y bivalvos eurihalinos y marinos, donde la misma juega un papel preponderante en la fisiología y ecología de los organismos de esos ecosistemas (Carvalho et al., 2015; Cunha-Nalesso, 1988; Pinoni & Mañanes, 2004).

El pH y el oxígeno fueron las variables con menor cantidad de reportes (Bond & Buckup, 1983; Giaccomin et al., 2014; Macchiavello & Bulboa, 2014; Montagna & Collins, 2005; Núñez et al., 2013; Sansiñena et al., 2018) a pesar de que, en algunos casos, se mencionó su registro (Martínez et al, 2017). Esta falta de información dificulta conocer cuáles son los rangos que pueden tolerar las distintas especies, lo que es de gran importancia al momento de realizar ensayos bajo condiciones controladas. Se sugiere que se reporten de forma rutinaria estas variables que son cruciales para los organismos acuáticos.

En varios ensayos fueron evaluados efectos de factores como alimentación, marcaje, oxígeno, pH, salinidad, sitio de colecta, temperatura y en algunos casos, combinaciones de estos. De acuerdo al tiempo de exposición pueden observarse tres clases de ensayos: los de corto plazo (minutos-horas) donde se determinó el comportamiento de indicadores como tasa de clareo y consumo de oxígeno; los de mediano plazo (días), donde se evaluaron variaciones en actividad enzimática y sobrevivencia de los organismos; y los de largo plazo, en los cuales fueron evaluados factores relacionados al crecimiento, sobrevivencia, cambios a nivel estructural, variaciones en reservas energéticas, entre otros (Carvalho et al., 2015; Cunha-Nalesso, 1988; Doyle & Momo, 2009; Machado et al., 2020; Otegui & Soares-Gomes, 2007; Silva-Castiglioni et al., 2016; Tomasetti et al., 2018). La diferencia entre ellos se debe al tipo de indicadores que se pretende evaluar, que presentan respuestas en escalas de tiempo diferentes; por ejemplo, una variación en el consumo de oxígeno de un organismo se puede observar en períodos de tiempo pequeños (minutos-horas) respecto a cambios en el crecimiento (Jorge et al., 2016; Seuffert & Martin, 2013) o en la capacidad reproductiva. Siempre se debe considerar que las escalas eco-fisiológicas son relativas a los organismos. En este sentido, el tamaño de los organismos es un buen indicador que determina las tasas y patrones de respuestas.

Determinar las condiciones abióticas que promueven el mejor desempeño de los organismos (o qué efectos causan sus variaciones) es de vital importancia ya que, además de brindar información acerca de la biología/ecología de la especie, pueden ser útiles al momento de realizar ensayos de laboratorio. De esta revisión surge que los factores más evaluados han sido la salinidad y la temperatura y solo en dos casos se ha considerado la interacción de dos factores (Magalhaes et al., 2014; Otegui & Soares-Gomes, 2007). La evaluación de efectos de diferentes factores abióticos sobre algunas de estas especies ha sido realizada a nivel internacional con el objetivo de controlar las poblaciones, conocer su biología/ecología o mantener los organismos en cautiverio, como en los casos del caracol *Pomacea* sp., la planta *E. densa* y el mejillón *M. edulis* (Dumee et al., 2015; Haramoto & Ikusima, 1988; Thiábaut et al., 2016; Tomasetti et al., 2018).

Bioensayos

Las especies reportadas en bioensayos poseen características particulares y en general, habitan zonas de descarga de aguas de escorrentía contaminadas por la actividad agrícola y/o la urbanización (de Oliveira et al., 2018; Torres, 2019). En cuanto a la agricultura, en algunos casos estas descargas se dirigen al mar, como ocurre en el Canal Andreoni (La Coronilla, Rocha, Uruguay), pudiendo afectar la fauna asociada a la costa, entre cuyos integrantes se encuentran *E. brasiliensis* y *A. mactroides* (Sauco et al., 2010; Sauco et al., 2013). El uso de estas especies en bioensayos, también se debe a que presentan una elevada abundancia relativa en las zonas que habitan, son de fácil acceso, captura y manipulación.

Los crustáceos artrópodos, como *H. curvispina* y *Daphnia* sp., son los invertebrados más usados en este tipo de ensayos ya que son de pequeño porte y con gran sensibilidad a sustancias tóxicas (Peluso et al., 2011; Spósito et al., 2016). Además, presentan ciclos de vida cortos y requieren un mantenimiento muy sencillo, lo que facilita su uso y cultivo en cautiverio.

El uso de gasterópodos en ensayos de ecotoxicidad surge a partir de la evaluación de los efectos de productos molusquicidas, ya que pueden causar problemas en cultivos (Dumee et al., 2015). El uso del caracol *Biomphalaria* sp. en bioensayos ha sido fomentado debido a que es hospedero intermediario de un parásito (gusano platelminto) que puede afectar al ser humano (Oliveira-Filho et al., 2017). Además, esta especie presenta un elevado potencial reproductivo, un ciclo de vida relativamente corto (eclosión de huevos en pocos días), su mantenimiento en cultivos de laboratorio es sencillo (Cossi et al., 2018) y tolera un amplio rango de condiciones ambientales (Yipp, 1983).

Algunas de las especies nativas relevadas han sido empleadas en bioensayos a nivel internacional. El efecto de la exposición a metales pesados y/o agroquímicos ha sido estudiado en *Mytilus* sp. (Al Subiai et al., 2011; de Boissel et al., 2017; Granmo et al., 1989;

Hagger et al., 2005; Widdows & Johnson, 1988), *Pomacea* sp. (Dumee et al., 2015) y *E. densa* (Maleva et al., 2018), *M. aquaticum* (Turgot & Formin, 2002), *A. filiculoides* (Sela et al., 1989). *Ulva* spp. fue expuesta a pintura antifouling y también ha sido estudiada su capacidad de biorremediación frente a compuestos nitrogenados (en sistemas multitróficos) y exposición a metales (Habaki et al., 2013; Henriques et al., 2017; Pellizzari & Reis, 2011; Sphigel et al., 2018; Turner et al., 2009; Valdés et al., 2018;); esta especie es considerada como un importante biofiltro e indicadora de contaminación en los ambientes que habita (Costa, 2006). Especies de bivalvos del género *Amiantis* han sido expuestas a microplásticos (Naji et al., 2018). Otro organismo utilizado en bioensayos a nivel internacional (y que es una especie nativa en Uruguay) es la macrófita acuática del género *Potamogeton* (Ali et al., 2000; Xu et al., 2012), que además se emplea con fines ornamentales en acuarismo (Kalita et al., 2011).
Los compuestos empleados en los bioensayos están relacionados a actividades antropogénicas, como la agricultura; en esta actividad, parte de los compuestos aplicados (agroquímicos) son arrastrados por aguas de escorrentía hacia cuerpos de agua cercanos, pudiendo perjudicar a especies no blanco, a diferentes niveles (desde efectos a nivel endócrino hasta variaciones en la composición de las comunidades) (Ballesteros et al., 2017; Moresco et al, 2014; Rizo-Patrón et al., 2013; Villar et al., 2014;). En los bioensayos predominaron los insecticidas y herbicidas, que son los más empleados en la actividad agrícola de la región (Choidi Boudet et al., 2015; Jacomin et al., 2006; Mugni et al., 2016). También los efectos de metales como Cadmio, Cobre, Zinc, Hierro, entre otros, han sido muy estudiados, y se registra en la mayoría de los grupos de invertebrados evaluados; esto sucede porque son extensamente usados en actividades como agricultura, industrias, puertos y hasta pueden encontrarse entre desechos domésticos que son vertidos en cuerpos de agua (Giusto & Ferrari, 2014). Evaluar los efectos de compuestos como los hidrocarburos o derivados del petróleo es importante, ya que permite deducir los efectos que pueden producirse al ocurrir un derrame en el mar o cuerpos de agua dulce; además es un campo con escaso estudio (Lavarías et al., 2011). En cuanto a las pinturas antiincrustantes, es fundamental evaluar sus efectos (en algunos casos poco estudiados) ya que muchas veces las partículas quedan incluidas en el sedimento, son ingeridas por organismos filtradores; además, parte de sus componentes pueden ser incorporados por algas (Turner et al., 2009).

Los bioindicadores estudiados correspondieron a cuatro niveles de organización. Estos fueron evaluados de acuerdo con el tiempo de duración del ensayo. Cambios en el consumo de oxígeno, tasa de filtración, excreción y mortalidad son evaluados en ensayos de tiempo acotado (horas), ya que estos parámetros fisiológicos pueden modificarse en períodos de tiempo breves (Jorge et al., 2016; Montagna & Collins, 2008). Los bioindicadores histológicos fueron los menos evaluados, a pesar de ser considerados sensibles y de proporcionar una idea directa y visual de lo que puede causar la exposición de un organismo a un compuesto tóxico (Chiodi Boudet et al., 2015; Román et al., 1992; Saavedra et al., 2012). Los

órganos/tejidos analizados son aquellos involucrados con funciones vitales, como el hepatopáncreas (en crustáceos decápodos), la glándula digestiva (en moluscos gasterópodos) y las branquias. Tanto el hepatopáncreas como la glándula digestiva están relacionados con actividades como síntesis de enzimas digestivas, absorción de nutrientes y eliminación de compuestos tóxicos (detoxificación); en el camarón dulceacuícola *P. argentinus* expuesto a Cadmio, el hepatopáncreas sufrió alteraciones tales como atrofia del epitelio y necropsia (muerte del tejido) (Choidi Boudet et al., 2015). Otras estructuras analizadas fueron las gónadas, branquias y raíces (Herrera et al., 2019; Román et al., 1992; Saavedra et al., 2012).

Los indicadores bioquímicos y poblacionales fueron los más estudiados, siendo evaluados en casi todos los grupos de organismos analizados. Varios estudios mostraron que la exposición a agroquímicos produce estés oxidativo, lo que lleva a un aumento en la actividad de enzimas con capacidad antioxidante, ya que son consideradas una de las principales defensas frente a la presencia de compuestos tóxicos que promueven la producción de especies reactivas de oxígeno (ROS, por su sigla en inglés) (Griboff et al., 2014). Esta variación fue observada en *Biomphalaria* sp. expuesto al insecticida azinfos metil por 14 días, donde también aumentó la producción de ROS; sin embargo, las reservas energéticas como glucógeno y proteínas no variaron; dichas reservas pueden ser afectadas ya que, frente a situaciones de estrés, los organismos pueden consumirlas para obtener energía para lidiar con los procesos metabólicos/fisiológicos afectados (Cossi et al., 2020). En el caso de los bivalvos, esto no siempre ocurre debido a que poseen la capacidad de cerrar sus valvas y disminuir su metabolismo, como ocurrió con *A. trapesalis* expuesto a Zinc, Manganeso y Hierro y combinaciones de estos por 96 horas (de Oliveira et al., 2018).

Los niveles de LPO aumentaron en la anémona *B. cangicum* expuesta a cobre, lo que está relacionado con el daño producido por las ROS, que también aumentaron (Abujamara et al., 2014). La energía también puede ser medida como ATP o NADH, como lo fue en un ensayo donde la almeja amarilla *A. mactroides* fue expuesta a cobre. Se observó una disminución del ATP en la hemolinfa (en los demás tejidos de mantuvieron los niveles pre-exposición), que junto a la disminución de glucosa en el músculo y el aumento de lactato en este y en la hemolinfa, indicaría un aumento de la producción de energía por la vía anaeróbica para mantener los niveles constantes (Giacomin et al., 2014).

Los bioindicadores como la actividad de la enzima Na/K ATPasa y acetilcolinesterasa, los niveles de metalotioneínas, clorofila, osmolaridad, la viabilidad de hemocitos, la resistencia a xenobióticos (MXR, por su sigla en inglés) han sido evaluados menos frecuentemente (Anjos et al., 2017; de Boisel et al., 2017; Harguinteguy et al., 2015; Jorge et al, 2016). La mortalidad como indicador poblacional, es muy utilizado en estos ensayos; en algunos casos para

determinar la concentración letal media del compuesto evaluado (Amin & Comiglio, 2002; Diodato et al., 2019; Peluso et al., 2011; Sauco et al., 2010).

A partir de esta revisión se puede determinar que el estudio de los efectos de tóxicos derivados de la actividad antropogénica presenta un desarrollo importante en la región. Aunque el uso de especies nativas con este fin data de las décadas de 1970-1980, el mayor volumen de estudios comienza casi 20 años después. Los ensayos evalúan los efectos de estos compuestos bajo condiciones de laboratorio (microcosmos), por lo que las extrapolaciones a condiciones realistas deben realizarse con cautela. Por lo tanto, realizar monitoreos en el ambiente (tanto de factores abióticos del agua como de los organismos) sería ventajoso ya que aportaría información para diseñar y realizar ensayos del tipo microcosmos que evalúen los efectos de

compuestos tóxicos bajo condiciones que se asemejen a la realidad de los organismos. Esto además aportaría información que puede contribuir en la conservación tanto de las especies como de los cursos de agua, los cuales muchas veces son utilizados por el hombre para realizar actividades recreativas.

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