



FACULTAD DE CIENCIAS NATURALES Y EXACTAS

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**PRESENCIA DE METILMERCURIO EN TRUCHAS EN CHILE: SUS
EFECTOS A NIVEL MOLECULAR Y RIESGO PARA LA SALUD HUMANA**

Tesis para optar al grado de Doctor en Ciencias Ambientales

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Dedicatoria

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Resumen

El metilmercurio (MeHg) es un neurotóxico de alta potencia y la forma más persistente de mercurio en el ambiente. Su lipoafinidad favorece la bioacumulación y la biomagnificación en tejidos de organismos acuáticos, lo que resulta en niveles preocupantes en depredadores, incluidos peces y mamíferos, y potencialmente, en humanos. Aunque el mercurio tiene fuentes naturales, como la geotermia y el vulcanismo, las actividades antropogénicas, como la minería y el uso de combustibles fósiles, han incrementado significativamente su biodisponibilidad, particularmente en Latinoamérica y el Caribe, donde la minería es una actividad económica relevante. La presencia de MeHg en diversas especies de peces marinos y dulceacuícolas incrementa el riesgo para la salud humana. Este estudio tiene como objetivo evaluar el riesgo asociado con la acumulación e impactos de MeHg en truchas y su posterior consumo, utilizando modelos de estimación de riesgo y transcriptómica. Se identificó un gradiente de bioacumulación de MeHg en truchas y diferencias en la distribución de mercurio total en agua y sedimento, con niveles más altos en el norte del país. Estos hallazgos permitieron detectar alteraciones en genes involucrados en el metabolismo de la glucosa y ácidos grasos, así como con procesos reproductivos, con diferencias significativas entre truchas machos y hembras. Además, se evaluó el riesgo del consumo de peces para la población humana, adoptando un enfoque ecosistémico de "One Health", que subraya la necesidad de considerar factores demográficos y hábitos alimentarios en lugar de basarse únicamente en una concentración promedio para la estimación del riesgo.

Palabras claves: ecotoxicología acuática, vías metabólicas, factor de riesgo del Hg, biomagnificación, expresión génica.

Abstract

Methylmercury is a highly potent neurotoxin and the environment's most persistent form of mercury. Its lipophilicity promotes bioaccumulation in the tissues of aquatic organisms and biomagnification along the food chain, leading to concerning levels in predators, including fish and mammals, and potentially humans. Although mercury has natural sources, such as geothermal activity and volcanism, anthropogenic activities, such as mining and fossil fuel use, have significantly increased its bioavailability, particularly in Latin America and the Caribbean, where mining is a relevant economic activity. The presence of methylmercury in various marine and freshwater fish species increases the risk to human health. This study aims to assess the risk associated with methylmercury accumulation in trout and its subsequent consumption, using risk estimation models and transcriptomic analysis. Transcriptomics provides a tool for detecting molecular alterations at an early stage, facilitating the anticipation of potential adverse impacts. A gradient of methylmercury bioaccumulation in trout was identified, along with an uneven distribution of total mercury in water and sediment, with higher levels in the northern part of the country. These findings allowed the detection of alterations in genes involved in glucose and fatty acid metabolism, as well as in reproductive processes, with significant differences between male and female trout. Additionally, the risk of fish consumption for human populations was assessed, adopting a "One Health" ecosystem approach, which emphasizes the need to consider demographic factors and dietary habits, rather than relying solely on an average concentration for risk estimation.

Keywords: aquatic ecotoxicology, metabolic pathways, Hg risk factors, biomagnification, gene expression.

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Capítulo I. Introducción general

I.1 Dinámica del mercurio en sistemas fluviales

El mercurio (Hg) es un elemento traza natural que se encuentra en la corteza terrestre, tiene una gran habilidad de transformación, tanto abiótica como biótica, lo que le permite cambiar entre formas volátiles, solubles y orgánicas (Clarkson & Magos, 2006; Lin et al., 2011; UNEP, 2018). Las principales emisiones de Hg provienen de fuentes naturales: volcanes, sedimentos oceánicos, deshielo de glaciares, desgasificación de la corteza e incendios forestales (Asaduzzaman et al., 2019); y fuentes antropogénicas incluyendo la minería, plantas cloro-álcali y consumo de combustibles fósiles (Soe et al., 2022; Streets et al., 2017; Sundseth et al., 2017). Las formas de Hg más comunes en sistemas acuáticos fluviales son tres: inorgánico (Hg(II)), elemental (Hg⁰) y orgánico como metilmercurio (MeHg; Hong et al., 2012).

El Hg⁰ es altamente volátil, por lo que se transporta eficientemente por la atmósfera. El Hg⁰ se oxida a formas de Hg(II), las cuales se incorporan al medio terrestre o acuático a través de deposición húmeda, vinculada a las partículas de lluvia, o mediante deposición seca, asociada a compuestos de materia orgánica. Ya en la superficie se moviliza por arrastre hacia ríos, lagos y océanos, debido al efecto de la lluvia (Driscoll et al., 2013; Liu et al., 2013). En la columna de agua se asocia a materia orgánica y se deposita en el sedimento, donde el Hg (II) es transformado por actividad bacteriana a MeHg (Figura I.1; Lin et al., 2011). Debido a la lipoafinidad del MeHg, este compuesto es bioacumulado en los tejidos de organismos acuáticos y tiene la peculiaridad de ser uno de los escasos metales que se biomagnifica, es decir, que aumenta su concentración al traspasar de un organismo a otro de mayor nivel trófico (Lavoie et al., 2013; Viana et al., 2023). Por lo tanto, en depredadores tope encontramos altos niveles de MeHg, por ejemplo, en peces con alto contenido graso, tales como atún o trucha (Muñoz-Abril et al., 2022; Vieira et al., 2024). Estos peces son altamente consumidos por humanos, convirtiéndose en una de las principales fuentes de

exposición humana a MeHg a escala global (Felix et al., 2022; Jeevanaraj et al., 2019).

Se ha comprobado una relación entre la concentración de Hg en agua y el nivel de actividad antropogénica adyacente a los sitios estudiados, encontrándose bajas concentraciones (0.3 a 8 ngL^{-1}) en sitios considerados prístinos (Hadzi et al., 2015; Ouboter et al., 2012) y niveles significativamente mayores ($450 \text{ } \mu\text{gL}^{-1}$) en sitios influenciados por la minería o descargas industriales (Gutiérrez-Mosquera et al., 2021; Soe et al., 2022; Viana et al., 2023).

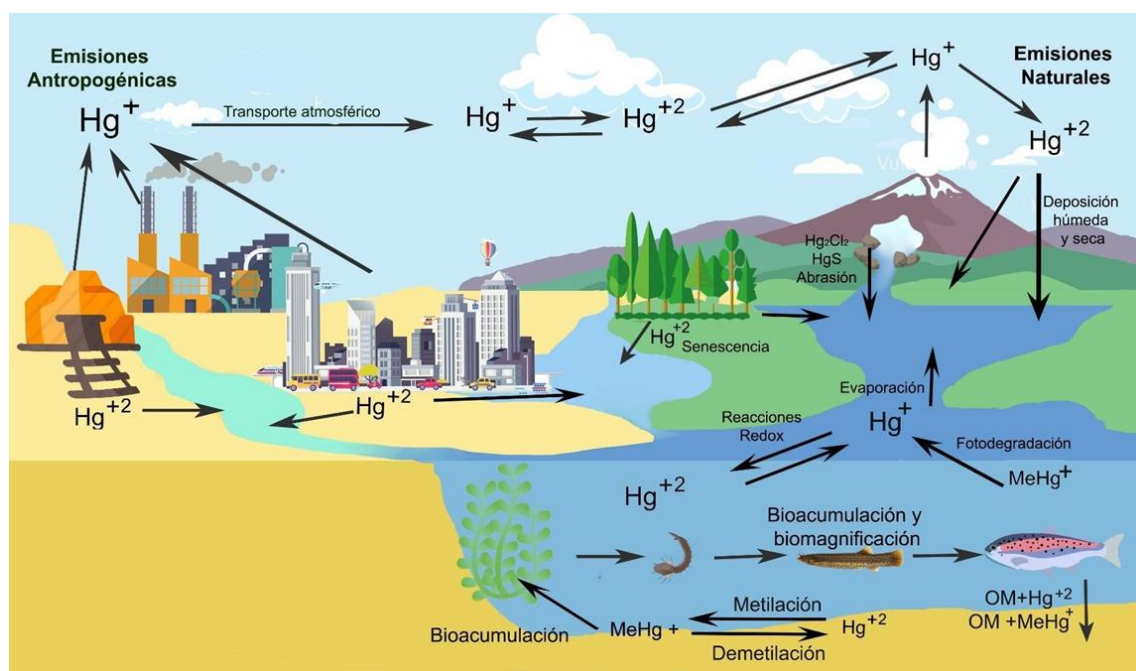


Figura I.1 Ciclo biogeoquímico del Hg incluyendo emisiones naturales y antropogénicas, su transporte, bioacumulación y biomagnificación. OM: materia orgánica.

I.2 Bioacumulación de MeHg en peces y el riesgo para la salud humana

La bioacumulación de MeHg en peces es un fenómeno de gran relevancia tanto ecológica, económica como sanitaria, dado que estos organismos están ampliamente distribuidos en diversos sistemas acuáticos y abarcan una variedad de especies a nivel global. Los peces, debido a su posición media/alta en la cadena alimentaria, contribuyen a incrementar la bioacumulación de Hg, convirtiéndose en un foco de interés en la investigación sobre la contaminación por este metal. Además, los peces son relevantes como bioindicadores de la

salud ambiental debido a su sensibilidad a los cambios en su hábitat. Diversos estudios han evidenciado la presencia de Hg en múltiples especies de peces en todo el mundo, desde aguas dulces hasta ambientes marinos, lo que resalta la ubiquidad de este contaminante (Echevarría et al., 2024; Vergara et al., 2024). Esto plantea serios riesgos para la salud humana, ya que los seres humanos consumen peces como parte esencial de su dieta que son potencialmente nocivos por su alto contenido de Hg.

I.3 Toxicidad del Hg

Los primeros efectos de la acumulación de Hg en humanos fueron narrados en la obra de Plinio El Viejo "Historia Naturalis", donde se evidencia daños neurológicos en mineros de la antigua Roma, debido a la aspiración de gases de Hg. Sin embargo, el caso más reconocido mundialmente es el desastre de Minamata (Japón, 1950-1960), un envenenamiento masivo por MeHg proveniente de los recursos marinos extraídos de la bahía de Minamata, que causó graves consecuencias neurosensoriales en la población, incluso llevando a la muerte (Eto, 2000). Desde entonces, la toxicidad neuronal y sensorial de los compuestos mercuriales ha sido objeto de investigación en humanos y fauna, con un enfoque especial en la salud pública (Hachiya, 2006). Diversos estudios señalan que la exposición a MeHg produce efectos nocivos sobre el funcionamiento del sistema endocrino en distintas especies, desde peces hasta humanos y otros mamíferos (Guzzi et al., 2021; Owoko et al., 2023). Se ha descrito: a) acumulación y daño de las glándulas pituitaria y tiroides, entre otras (Rice et al., 2014); b) citotoxicidad específica en tejidos endocrinos (Guzzi et al., 2021); c) alteración en las concentraciones de hormonas (Soldin et al., 2008); d) interacción con hormonas sexuales (Jasrotia et al., 2021; Rice et al., 2014); y e) regulación positiva o negativa de las enzimas en la vía de la esteroidogénesis en humanos (Lee et al., 2020; Zhu et al., 2000). Estos hallazgos han llevado a considerar al Hg y sus derivados como Disruptores Endocrinos (DE), definidos como "sustancias exógenas que causan efectos adversos en la salud de un

organismo intacto o su progenie, a través de cambios en la función endocrina" (OECD, 2002).

En las últimas décadas, se ha avanzado en el estudio de la toxicidad del MeHg en peces, utilizados como indicadores de la calidad ambiental de los sistemas acuáticos (Okwuosa et al., 2019). Los efectos más conocidos incluyen alteraciones en procesos bioquímicos y reproductivos, los cuales ocurren a concentraciones de entre 0.5 y 1.2 $\mu\text{g g}^{-1}$ peso húmedo (p.h.) de MeHg en músculo (más de 1 $\mu\text{g g}^{-1}$ p.h. en peces de gran tamaño se considera riesgoso para el consumo humano; Canham et al., 2021; Owoko et al., 2023; Zheng et al., 2019). En peces también se ha observado que la acumulación de MeHg puede causar alteraciones en el eje hipotálamo - hipófisis - glándula adrenal y gonadal afectando la síntesis de diversas hormonas esenciales en la reproducción (Rice et al., 2014).

A nivel molecular y celular, se han descrito otros efectos en peces expuestos crónicamente a bajas concentraciones de MeHg, tales como interrupción del metabolismo energético, estrés oxidativo, apoptosis (muerte celular programada), alteración de la respuesta inmune y del metabolismo de los lípidos (Olsvik et al., 2021). A pesar de la importancia del hígado en el ciclo del Hg, la hepatotoxicidad causada por este elemento sigue siendo un área poco explorada. El Hg acumulado por diversos tejidos se secreta hacia la bilis y se reabsorbe parcialmente, regresando al hígado (Hazelhoff & Torres, 2018). La alta movilidad del Hg en el cuerpo se debe a la formación de complejos solubles en agua, principalmente mediante su unión al átomo de azufre de los grupos tiol en residuos de cisteína, como el glutatión, que cumple funciones esenciales en varios procesos bioquímicos (Renu et al., 2021). Esta es la forma en la que el Hg ingresa a las células endoteliales de las barreras hematoencefálica y placentaria (Li et al., 2021; Türker, 2015).

Estudios enfocados en la hepato-transcriptómica en peces expuestos a MeHg (0.5-1.99 mg kg^{-1}), han mostrado alteraciones en la vía de plegamiento de proteínas, en la adipogénesis y el metabolismo de lípidos (Liu et al., 2013). Además, ensayos *in vivo* en peces, junto con ensayos en líneas celulares

hepáticas humanas, mostraron efectos comunes, como la inhibición de la vía de señalización mediada por el receptor de estrógeno (un receptor nuclear), de la beta-oxidación de ácidos grasos y de la cadena de transporte de electrones (Ung et al., 2010).

I.4 Potencial de Chile para el monitoreo de Hg en sistemas fluviales y el uso de truchas invasoras como bioindicadores

Chile presenta una notable diversidad climatológica, geológica e hidrológica, la cual genera un gradiente latitudinal natural a lo largo de su territorio. Las actividades económicas en el país se distribuyen de acuerdo con el potencial productivo de cada región: la minería predomina en el norte, la agricultura en el centro, y la ganadería y silvicultura en el centro-sur, y la salmonicultura en el sur. Además, la presencia de volcanes en el sur de Chile representa una fuente conocida de Hg, lo que, junto con otros factores, modifica el aporte de este contaminante hacia los sistemas acuáticos.

El Hg disponible en el agua se bioacumula y biomagnifica en la cadena trófica acuática, alcanzando altas concentraciones en peces de alto contenido graso. Este es el caso de los salmónidos, que son de mayor tamaño que las especies endémicas y presentan un alto contenido graso (Katikou et al., 2001; Nava et al., 2023). Además, no presentan problemas de conservación en Chile, ya que más bien son considerados especies invasoras (Agustín Iriarte et al., 2005; Camus, 2005). Los salmónidos son especies de interés comercial y de consumo (B. Cid-Aguayo et al., 2021), y tienen una amplia distribución geográfica (Lobos et al., 2020), lo que permite evaluar un gradiente latitudinal de contaminación. Por esta razón, la trucha arcoíris (*Oncorhynchus mykiss*) y la trucha marrón (*Salmo trutta*), son considerados excelentes sujetos para evaluar los efectos del Hg en los sistemas fluviales chilenos.

La Ley de Pesca Recreativa N° 20.256 permite la pesca de ciertas especies de salmónidos entre los meses de septiembre y mayo, dependiendo de la cuenca, y exclusivamente para consumo personal (SERNAPESCA, 2021). No obstante, se ha evaluado la posibilidad de otorgar permisos comerciales debido al potencial

económico y beneficios para algunas comunidades locales (Cid-Aguayo et al., 2020), sin embargo, no se conocen los riesgos de consumir y/o comercializar estos peces. Por lo tanto, este proyecto no solo busca determinar la salud del ecosistema, sino también evaluar el riesgo asociado a la acumulación de Hg en estos organismos y su posterior consumo mediante algoritmos de estimación de riesgo y transcriptómica. Esta última técnica permite detectar alteraciones a nivel molecular en etapas tempranas de exposición a contaminantes, tales como el Hg, anticipándose a posibles escenarios graves. La respuesta molecular ante la presencia de MeHg en peces aún no se ha evaluado en Chile. Estas herramientas moleculares permiten investigar las vías biológicas que este contaminante puede alterar, lo que puede ser extrapolado a especies endémicas, que son más vulnerables a las alteraciones del medio ambiente.

La singular geografía de Chile, extendiéndose por más de 4.000 kilómetros con una notable diversidad climática y una activa cadena volcánica, se combina con una distribución estratégica de actividades económicas a lo largo del territorio: la minería en el árido norte, la agricultura y urbanización en el poblado centro, y la ganadería, silvicultura y salmonicultura en el lluvioso sur. Esta interacción entre geografía y economía genera un claro gradiente latitudinal de contaminación por Hg, incrementándose de norte a sur, donde la actividad volcánica y la salmonicultura intensiva se suman a las fuentes de contaminación. Este gradiente afecta directamente a los ecosistemas acuáticos, evidenciándose en la bioacumulación de Hg en peces como la trucha, lo que representa un riesgo tanto para la salud humana como para la integridad de los ecosistemas dulceacuícolas chilenos.

Este enfoque proporcionará una actualización sobre la situación nacional respecto al problema del Hg, permitiendo asociar las concentraciones de THg y MeHg con los efectos potenciales en la salud de los peces, dado que el Hg es un disruptor endocrino. Además, permitirá extrapolar estos hallazgos a la salud de los sistemas dulceacuícolas y evaluar el riesgo de su consumo para la población humana, todo dentro de la perspectiva ecosistémica de "One Health" (OH; Una sola salud). El concepto de OH implica la convergencia entre la salud humana,

animal y ambiental, abarcando las interconexiones entre estos tres componentes a través de un enfoque multidisciplinario, representado por un sistema biológico y social complejo que involucra múltiples actores, procesos y sus interacciones a lo largo del tiempo, a nivel local, nacional y global (Couto & Brandespim, 2020).

Capítulo II. **Hipótesis y objetivos**

El Hg es un elemento tóxico ubicuo en las cuencas de Chile dada la actividad antrópica como volcánica. Sin embargo, existe un gradiente latitudinal de concentraciones de Hg y MeHg (mayor a menor) en cuencas hidrográficas de Chile, donde las concentraciones más altas causan efectos de disrupción endocrina en truchas, que, en caso de ser consumidas, representan un riesgo para la salud humana de la comunidad local.

II.1. Objetivo general

Determinar el efecto latitudinal en la distribución de concentraciones de Hg y MeHg en cuencas fluviales y su impacto diferencial en la bioacumulación en truchas, sus efectos a nivel molecular, y estimar el riesgo que presenta su consumo para la salud humana.

II.2. Objetivos específicos

- I. Evaluar la distribución del Hg en sistemas fluviales en un gradiente latitudinal. Por medio de la cuantificación de la concentración de Hg total en sedimento y agua de río y MeHg en macroinvertebrados y músculo de truchas, y su relación con marcadores isotópicos de $\delta^{15}\text{N}$ y $\delta^{13}\text{C}$, parámetros fisicoquímicos y las posibles fuentes de contribución de Hg.
- II. Evaluar los efectos a nivel molecular de la bioacumulación de MeHg en truchas, mediante herramientas de transcriptómica para el análisis de la expresión de genes relacionados a mecanismos de disrupción endocrina.
- III. Evaluar el riesgo en la salud humana mediante la asociación entre concentración de MeHg en peces e ingesta por dieta en humanos mediante estimaciones matemáticas.

Capítulo III. Environmental drivers of mercury accumulation and biomagnification across Chilean watersheds

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III.1. Abstract

Methylmercury (MeHg) biomagnification in aquatic food webs is a significant environmental concern, especially in river systems affected by anthropogenic activities. This study investigates the distribution of Total Mercury (THg) and the biomagnification of MeHg across seven Chilean rivers from 21°S to 55°S, spanning various climatic and economic regions. THg concentrations were measured in abiotic (water and sediment) and biotic (benthic macroinvertebrates and trout) matrices, examining the impact of physicochemical factors on MeHg trophic transfer. THg ranged from 0.01 to 0.11 µg/g dry weight in sediments and from 1.72 to 28.93 ng/L in water, with the highest levels in the Northern rivers. MeHg concentrations in benthic macroinvertebrates ranged from 0.001 to 0.021 µg/g, and trout exhibited a broader range from 0.005 to 0.327 µg/g, with higher concentrations in the area impacted by mining and industrial activity. Even in the pristine Robalo River Basin, with minimal human population, MeHg concentrations in brook trout were comparable to those in more impacted sites, suggesting lasting effects from historical gold mining. The MeHg Trophic Magnification Factor (TMF) ranged from 1.40 to 3.41, confirming biomagnification at all sites. TMF was best explained by a positive association with the number of human inhabitants surrounding the basin and a negative association with dissolved oxygen (DO) in the water. The strongest associations for THg in sediment were negative with DO and pH, and positive with Fe and Mg concentrations, while urban and industrial land use, along with Al and Mn, were key drivers positively correlated with THg in water. The findings highlight the complexity of MeHg dynamics and the need for site-specific monitoring and management to mitigate MeHg exposure in aquatic ecosystems.

Keywords: Mercury, Riverine food webs, stable isotope analysis, latitudinal gradient analysis, mercury trophodynamics.

III.2. Introduction

Rivers are key in transporting atmospherically deposited and geologically weathered mercury (Hg) from terrestrial environments to the oceans. Rivers are

a dominant pathway for fluxes of mercury, exporting approximately 11 tons of total Hg (THg) and methylmercury (MeHg) to marine systems per year globally (Gworek et al., 2016; M. Liu et al., 2021).

Changes in physicochemical parameters in aquatic ecosystems, such as organic matter composition, temperature or salinity, and variation in microbial community structure mediate the bioavailability of Hg forms and the transformations of inorganic Hg to highly bioavailable MeHg (Bravo & Cosio, 2020; Tsui & Finlay, 2011). The bioavailability of MeHg refers to the extent to which MeHg is available for uptake by organisms, particularly those at the lowest trophic levels. This form has a high affinity with organic tissue, particularly for protein thiol groups. This strong binding ability disrupts protein function and can lead to various toxic effects (Jeong et al., 2024). Bioaccumulating in different biota and biomagnifying through food webs (Chiang et al., 2021; Yurkowski et al., 2023); these processes lead to concentrations in top predator fish that are up to six orders of magnitude higher than the MeHg present in water (Córdoba-Tovar et al., 2022; McMeans et al., 2015). The concentration of MeHg in an individual organism is a result of several different factors that can be difficult to quantitatively analyze. Stable isotope analysis (SIA) is a valuable tool to assess the source and trophic magnification of contaminants, such as Hg, in aquatic food webs. By analyzing $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$, SIA helps determine whether variations in contaminant levels among top predators result from dietary intake ($\delta^{15}\text{N}$, biomagnification; Layman et al., 2012), habitat use ($\delta^{13}\text{C}$, $\delta^{34}\text{S}$), or other environmental factors (Clayden et al., 2013; Lescord et al., 2019). For example, the regression between THg (or MeHg) in biota and $\delta^{15}\text{N}$ quantifies the average Hg biomagnification in a food web and provides an estimate of the trophic magnification factor (TMF), and this metric can be compared among ecosystems (Páez et al., 2022). Also, it has been determined that TMF can vary across systems due to factors such as water quality, food web structure, and broad-scale gradients like latitude (Lavoie et al., 2013).

Spatial differences in Hg concentrations are detectable in abiotic (sediment and water) and biotic (macroinvertebrates and fish) matrices within and among aquatic systems (Azad et al., 2019; Gutiérrez-Mosquera et al., 2021; Nagorski et al.,

2021). Notably, the variability of Hg levels within fish species is greater than in most matrices, highlighting the complexity of Hg dynamics in these ecosystems. It is also important to note that in higher trophic level aquatic biota, particularly fish muscle, THg is predominantly present as MeHg, often accounting for 80–100% of THg (Bloom, 1992; Karimi et al., 2012). This justifies the use of THg as a proxy for MeHg in higher trophic level biota, and explains why MeHg is commonly measured in upper trophic level organisms, while THg is prioritized in abiotic samples. Understanding the transfer of Hg from water and sediment into biota, the subsequent biomagnification into fish and factors affecting these processes is crucial, especially in the Southern Hemisphere, where we have a major gap of comprehensive Hg fate and transport data (Schneider et al., 2023), and it could inform and enhance environmental policies aimed at reducing Hg concentrations in fish (Wu et al., 2019). In this context, the present study aims to characterize the distribution of MeHg and THg concentrations in abiotic and biotic matrices, and bioaccumulation and trophic magnification factors in riverine food webs along a large latitudinal gradient (21°S to 55°S) in Chile. Effects of climate (latitude), land use and physicochemical characteristics (source of Hg, water quality) were examined. This comprehensive approach will contribute to a better understanding of Hg dynamics and its ecological implications.

III.3. Methodology

III.3.1. Study area and site selection

Chile has a diverse geography and latitudinal gradient (~4,000 km, spanning from 17°30'S to 56°30'S). This extensive range encompasses a variety of landscapes and four distinct freshwater ecoregions (Figure III.1): Atacama, South Andean Pacific Slopes, Valdivian Lakes and Patagonia (Abell et al., 2008). The varied climates across Chile give rise to distinct ecosystems and support a range of primary economic activities, including mining in the northern deserts, agriculture and forestry in the central fertile plains, livestock in the temperate south, and specialized industries like salmon farming in the fjord lands. To capture this heterogeneity, seven catchments were strategically selected for sampling (Table

III.1), with care taken to represent the diverse landscape characteristics and associated economic activities that are possible sources of Hg and prevalent across the country.

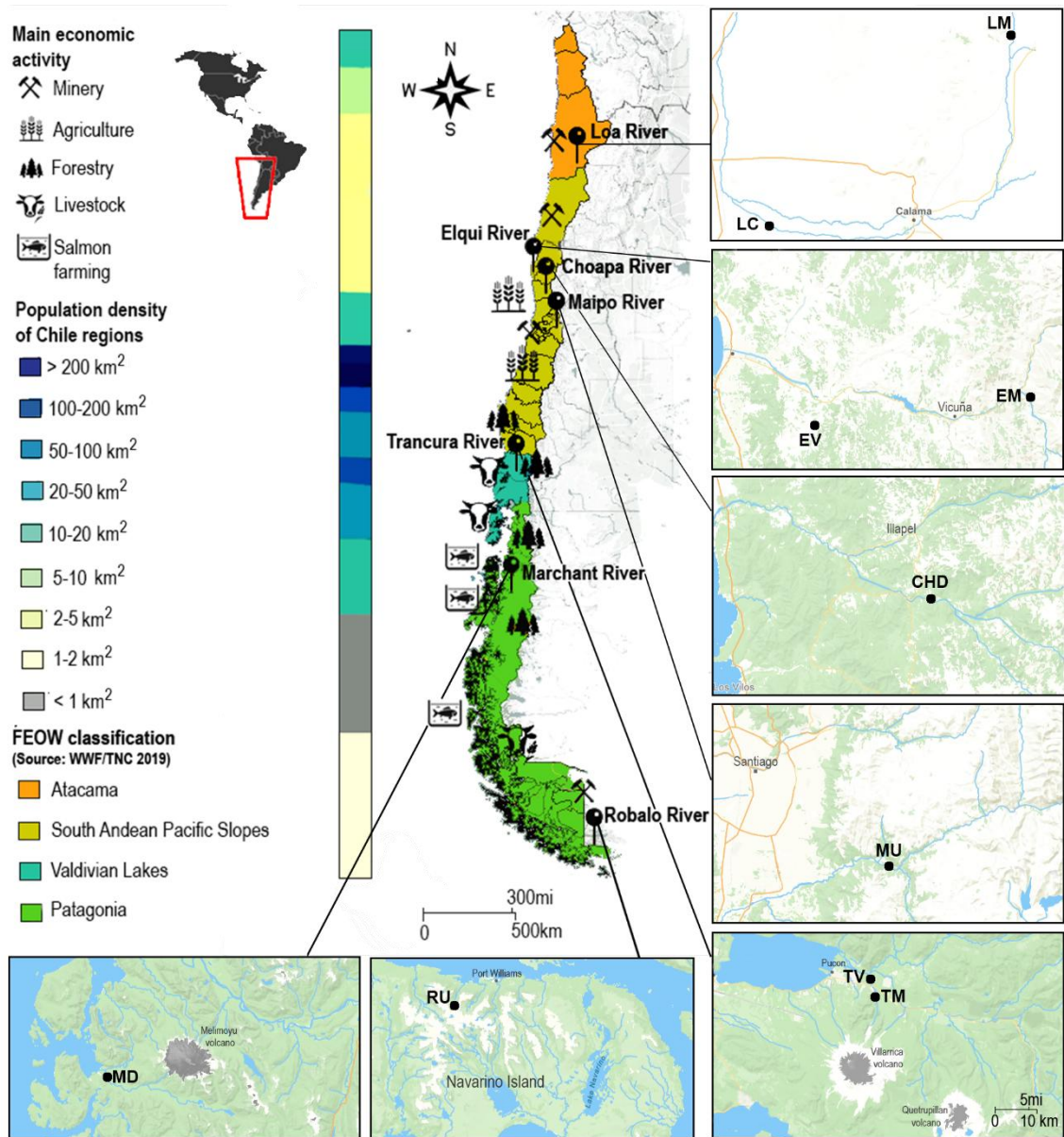


Figure III.1 Map of Chile illustrating the diverse freshwater ecoregion of the world (FEOW) classifications and corresponding population densities across its regions and the seven sampling sites: from Northern Chile, Loa Mountain site (LM) and Loa City site (LC) from Loa River, and Elqui Mountain site (EM) and Elqui Valley site (EV) from Elqui River; from Central Chile, Choapa downstream (CHD; from Choapa River), and Maipo upstream (MU; from Maipo River); Trancura Mountain (TM) and Trancura Valley (TV; from Trancura River), Marchant downstream (MD; from Marchant River) and Robalo upstream (RU; from Robalo

River) from Southern Chile. In addition, population density (Gil & Atria, 2022) and main economic activities are incorporated.

Table III.1 Characteristics of the seven sampled catchments. Total surface, agricultural (Agric.), urban and industrial (U&I), and mining land use (surface is presented in hectares, ha). Number of Inhabitants (Inhab)Source of information: SONAMI (2025) and Dirección General de Aguas, 2025.

Name	Total surface (ha)	Landscape	Agric. (ha)	U&I (ha)	Mining (ha)	Economic activities	Inhab.
Loa River Basin	3,357,000	Aridity stems from its desert climate, terrain arrangement, and high soil salinity.	759	1,990	4,568	Mining Agriculture	445,109
Elqui River Basin	982,600	Flowering desert, coastal steppe shrubland, drier interior steppe shrubland, open pre-cordillera and high Andean steppe.	27,713	343	156	Agriculture Mining	203,421
Choapa River Basin	812,400	Four distinct plant formations: woody steppe shrubland, interior steppe shrubland, Andean shrubland, and high Andean steppe	31,152	172	156	Mining Agriculture	65,144
Maipo River Basin	1,530,400	High Andean steppe, cushion-like plants in arid conditions; shrubland and sclerophyll forest, and deciduous forest.	215,238	58,220	1,966	Urban and industrial Mining	7,112,808
Trancura River Sub-Basin	788,600	Volcanoes and hot springs, southern deciduous forest, Valdivian laurel forest, mixed deciduous forest of the Andes, and high Andean deciduous forest.	602	2,000	0	Tourism Forestry	10,660
Marchant River sub-Basin	86,059	Temperate Andean ecosystems include herbaceous layers, mixed deciduous forests, and evergreen forests.	0	0	0	Natural heritage Tourism Salmon farming	< 100
Robalo River Basin	5,196.24	High Andean habitat. High wind exposure and a rocky substrate, this habitat support the development of cushion plants and lichens.	0	0	0	Natural heritage Gold mining in the past	1,868

III.3.2. Sample collection and preparation

Samples were collected from two sites in each river when possible, one site upstream and one site downstream of anthropogenic activity. In cases where there was no local anthropogenic presence, but a possible volcanic source, samples were collected from two points in the same river: 1) closer to the volcanic source (upstream) and 2) farther away from the volcanic source (downstream). Sites with just one sampling site were due to field difficulties (no trout found or landscape issues). The sites defined for each river upstream to downstream were: from Northern Chile, Loa Mountain site (LM) and Loa City site (LC) from Loa River, and Elqui Mountain site (EM) and Elqui Valley site (EV) from Elqui River; from Central Chile, Choapa downstream (CHD; from Choapa River), and Maipo upstream (MU; from Maipo River); Trancura Mountain (TM) and Trancura Valley (TV; from Trancura River), Marchant downstream (MD; from Marchant River) and Robalo upstream (RU; from Robalo River) from Southern Chile.

To assess the distribution of Hg in abiotic compartments, triplicate sediment samples were collected from the surface layer at each site (top 3 cm). To avoid contamination, a plastic shovel was used to collect the samples, stored in sterilized plastic bags and preserved in cold storage until analysis. Grab water samples were collected in triplicate in 250 mL certified amber bottles (previously sterilized and treated with 20% HCl), at a depth of 1 m below the surface. Before collection, the bottles were rinsed three times with ambient water. Samples for THg analysis were preserved with 0.5% HCl and 0.5% BrCl (USEPA, 1998).

Trout of a standardized size ≥ 9 cm, indicating maturity in trout of at least 1 year (Arismendi et al., 2011; Pepe-Victoriano et al., 2024), were collected at each site. The corresponding Fishing and Animal Care Permits were obtained from SUPBESCA (R.EX. N.º 3443) and Universidad Mayor Animal Care Committee (N.º 4231), for the capture and handling of predatory fish (rainbow, brook trout and brown trout) which were obtained through electro fishing (Halltech Aquatic Research Inc., HT-2000) with a non-lethal electric shock sufficient to immobilize the fish. The established protocols were strictly followed to ensure animal welfare throughout the process. They were processed in the dissection laboratory, with

measurements of total length (± 1 mm), total weight (± 0.001 g), liver weight (± 0.001 g), and gonad weight (± 0.001 g). Muscle tissue was collected for the quantification of MeHg and isotope analysis.

Benthic macroinvertebrates (BMI) were collected at the lowest flow in the rivers, live sorted into major taxonomic groups by physical features (Table S1), grouped in pools of 10 to 20 individuals depending on the taxa, and stored in sterile Whirl-pak® bags (Merck, Chile) at -20 °C. BMI and trout muscle samples were freeze-dried (-50 °C for 24 to 48 hours) at The Regional Center for Studies in Healthy Food (CREAS), Valparaíso, and homogenized in a ceramic mortar.

III.3.3. Mercury analysis

THg was performed on freeze-dried, sieved sediment samples subjected to microwave-assisted digestion using a Milestone model Start D (Soriso BG, Italy). THg detection was performed by CV-AFS at the Environmental Analytical Chemistry Laboratory at the Pontifical Catholic University of Valparaíso, Valparaíso, Chile, following the EPA method 3052 (USEPA, 1996) using a mercury analyzer model Millennium Merlin (PSA Analytical, TM, England). Quality control measures were performed during each run, including certified reference materials for Buffalo River Sediment 2704, Marine sediment PACS-2, and Montana soil 2711a, continuous calibration verifications, system and method blanks, and duplicate samples. Recoveries were on average up to 98%.

The concentration of THg in water samples was measured with CV-AFS (Modell III CVAFS detector, Brooks Rand), following EPA Method 1631 (USEPA, 2002). Bromine monochloride (BrCl) was controlled for Hg contamination, for which $40\text{ }\mu\text{L}$ was added to the water sample (40 mL), allowing it to react for at least 20 minutes. Quality control measures were performed, including a 25 pg THg check standard every five samples; the mean recovery% was $106.8\% \pm 9.4\%$. The detection limit, calculated as the standard deviation of the blanks multiplied by 3.3, was 0.15 pM .

MeHg was quantified in trout muscles and whole BMI in lyophilized and homogenized samples following the EPA method 1630 (USEPA, 1998) with some modifications according to Edmonds et al. (2012) at the Mercury Lab at Acadia

University, Wolfville, Canada. An alkaline digestion of the sample with KOH/MeOH was performed to detect the organic form of Hg, followed by derivatization using an ethylating agent, $\text{NaB}(\text{C}_2\text{H}_5)_4$, for subsequent separation by gas chromatography and detection by cold vapour atomic fluorescence spectrometry (GC/CV-AFS) using a Brooks Rand MERX system. This method has demonstrated good quality standards and high sensitivity. The method detection limit was calculated as 3 times the standard deviation of a repeated blank, assuming a standard 50 mg subsample (MDL was between 0.03 ng/g and 0.10 ng/g; $n = 114$). Quality control measures were performed using fish tissue certified reference materials DORM4 (mean recovery between $80.41\% \pm 3.43\%$ and $102.8\% \pm 15.7\%$; $n = 24$) and DOLT5 (mean recovery between $97.6\% \pm 18.1\%$ and $120.6\% \pm 16.3\%$; $n = 30$). The mean % recovery of a 50 pg MeHg check standard was $110\% \pm 9.0\%$.

III.3.4. Stable Isotope Analysis

Approximately 1.5 mg of homogenized tissue was sent to the UA Stable Isotope Facility (Antofagasta, Chile). Elemental percentages for carbon and nitrogen, as well as stable isotope ratios (SIR; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), were estimated using a Pyrocube elemental analyzer (Elementar, Langenselbold, Germany) connected to a visION continuous-flow isotope ratio mass spectrometer (Elementar). SIR was expressed in δ units, with Vienna Pee Dee Belemnite as the isotopic standard for carbon and air for nitrogen. Different international standards were employed in each batch run to calibrate and assess analytical error using the ionOS software package (Elementar). Certified reference materials USGS40 and USGS41 were utilized for carbon and nitrogen, respectively. Repeated analysis of standards demonstrated that analytical errors (± 1 SD [standard deviation]) were $\pm 0.05\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.1\text{‰}$ for $\delta^{15}\text{N}$. Two calibration standards were applied: 1) sulfonamide (Elementar) and 2) an in-house, matrix-matched standard (rainbow trout) to correct for machine drift.

III.3.5. Mercury trophic magnification factor

The Trophic level (TL) for each organism is calculated from the $\delta^{15}\text{N}$ value using equation 1:

$$TL_{\text{consumer}} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}) / \Delta^{15}\text{N} + \lambda \text{ (Equation 1)}$$

Where λ is the trophic level of the baseline organism (TL = 1 for primary producers and TL = 2 for primary consumers), TL_{consumer} is the trophic level of a given consumer, and $\delta^{15}\text{N}_{\text{consumer}}$ and $\delta^{15}\text{N}_{\text{baseline}}$ are $\delta^{15}\text{N}$ values of a given consumer and the baseline organism, respectively. A trophic discrimination factor of 2.2‰ for $\Delta^{15}\text{N}$ was used for freshwater aquatic organisms based on Blanke et al. (2017). The TMF, calculated as the antilog of the slope of the relationship between logarithm transformed (to base 10) MeHg concentration in wet weight (ww) for which the data were converted from dw to ww using the following Equation according to (Cresson et al., 2017).

$$C_{\text{wet}} = C_{\text{dry}} \times (100 - \% \text{Hg} / 100) \text{ (Equation 2)}$$

Divided by the TL. This represents the average biomagnification per TL through the entire food web and is calculated from the slope of equation 3.

$$\text{TMF} = \text{Log}_{10} \text{ MeHg}_{\text{ww}} / \text{TL} \text{ (Equation 3)}$$

III.3.6. Physicochemical factors and land use, and their relationship with TMF, and THg distribution

To explore the relationships between environmental variables and MeHg bioaccumulation and biomagnification across sites, physicochemical variables data and land use for each river sampled were collected from the Chilean General Directorate of Water data repository (Dirección General de Aguas Gobierno de Chile, 2025) and the Chilean National Mining Society (SONAMI, 2025).

The analysis was conducted to evaluate how the physicochemical and anthropogenic factors of seven rivers modulate the trophic magnification factor (TMF) of MeHg and the distribution of THg in sediment and water. Multivariate methods and generalized regression models were employed. Generalized linear models (GLM) with a Gaussian error distribution and an identity link function were fitted, using the TMF, THg or MeHg as the dependent variable. The physicochemical variables considered as predictors included Conductivity (Cond),

phosphate (PO_4^{3-}), total Nitrogen (NitTot), dissolved oxygen (DO), pH, temperature (T°), aluminium (Al), arsenic (As), boron (Bo), calcium (Ca), chloride (Cl), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), sulfate (SO_4^{2-}); and the landscape variables for surface (ha) of agriculture land use, urban and industrial land use, mining land use, and number of inhabitants (inhab) per river basin in which the sampling sites are.

All possible combinations of up to four predictor variables were explored, avoiding combinations with high collinearity to ensure model robustness. This procedure was automated in R, following a reproducible approach to evaluate a comprehensive set of potential relationships. Each fitted model was assessed using the corrected Akaike Information Criterion for small samples (AICc). Models with $\text{AICc} < -200$, indicative of overfitting or numerical issues, were excluded. For the remaining models, ΔAICc values (difference relative to the best model) and Akaike weights ($w\text{AICc}$) were calculated, providing a measure of relative parsimony for each model (Anderson & Burnham, 2002).

The analysis was done in R software (version 4.4.1), using the *dplyr*, *MuMIn*, *lme4*, *ggplot2* and *writexl* packages. These facilitated the automation of predictor combination generation, GLM fitting, and AICc metric evaluation. The results for diet, TMF, and isotopic signals were consolidated into final tables sorted by parsimony and exported to Excel files for further interpretation and analysis.

III.4. Results

III.4.1. Abiotic water factors

The analysis of the seven rivers reveals several notable deviations from typical freshwater characteristics (Tables S2 and S3). The LC site exhibited extremely high Cond at 7,977 $\mu\text{ohm/cm}$, indicating a significant presence of dissolved salts, and alarmingly elevated As at 1.51 mg/L, a clear health concern (WHO guideline value is 10 $\mu\text{g/L}$ equivalent to 0.01 mg/L; WHO & UNICEF, 2018). Additionally, Cl at 2245.27 mg/L, Na at 811.28 mg/L, and Ca at 288.01 mg/L levels were exceptionally high in the Loa River city site. Along with these high mineral concentrations, DO was observed to be 9.69 mg/L (54.73% Oxygen Saturation, OS). Upstream, LM

site showed a significantly elevated Cond of 2,150.2 $\mu\text{ohm}/\text{cm}$ and a high pH of 8.82, a more alkaline condition. The EV site presented high PO_4^{3-} at 1.25 mg/L, suggesting potential agricultural or sewage contamination. The CHD site displayed a very high DO (133.73% OS) level of 12.13 mg/L and a very high T° of 19.18 $^\circ\text{C}$. The MU site was characterized by a very high SO_4^{2-} concentration of 397.27 mg/L.

III.4.2. Total mercury in abiotic matrices

Figure III.2 summarizes all the THg levels found across the rivers in sediments and water. THg concentrations in sediment ranged from 0.01 $\mu\text{g}/\text{g dw}$ (TV site of Trancura River) to 0.11 $\mu\text{g}/\text{g dw}$ (Loa River, LM site). Overall, no clear latitudinal trend was observed in THg concentrations in sediments, but the northern sites (LM in Loa River and sites from Elqui River) showed significantly higher THg levels in sediment (p value = [0.005, 0.0044]; Table S4). Within sites, THg concentrations in Elqui River sediments were higher in the mountain site than the valley (p value = 0.022).

THg concentrations in water showed wider variability, ranging from 1.72 ng/L (TV site) to 28.93 ng/L (EM site). The Elqui River's mountain site (EM) exhibited significantly higher THg concentrations in water compared to the other rivers (p value = [0.001, 0.050]; Table S5), and there was not a statistical difference between the mountain site and the valley site of Elqui River (Dunn's Pairwise Comparison p-value = 0.476). In this catchment, high THg concentrations were found in both sediments and water, in the mountain region, exceeding those observed in the other rivers. However, concentrations in the valley were much lower. In contrast, the southern rivers (i.e., TV site and MD site) had the lowest THg concentrations in both sediment and water.

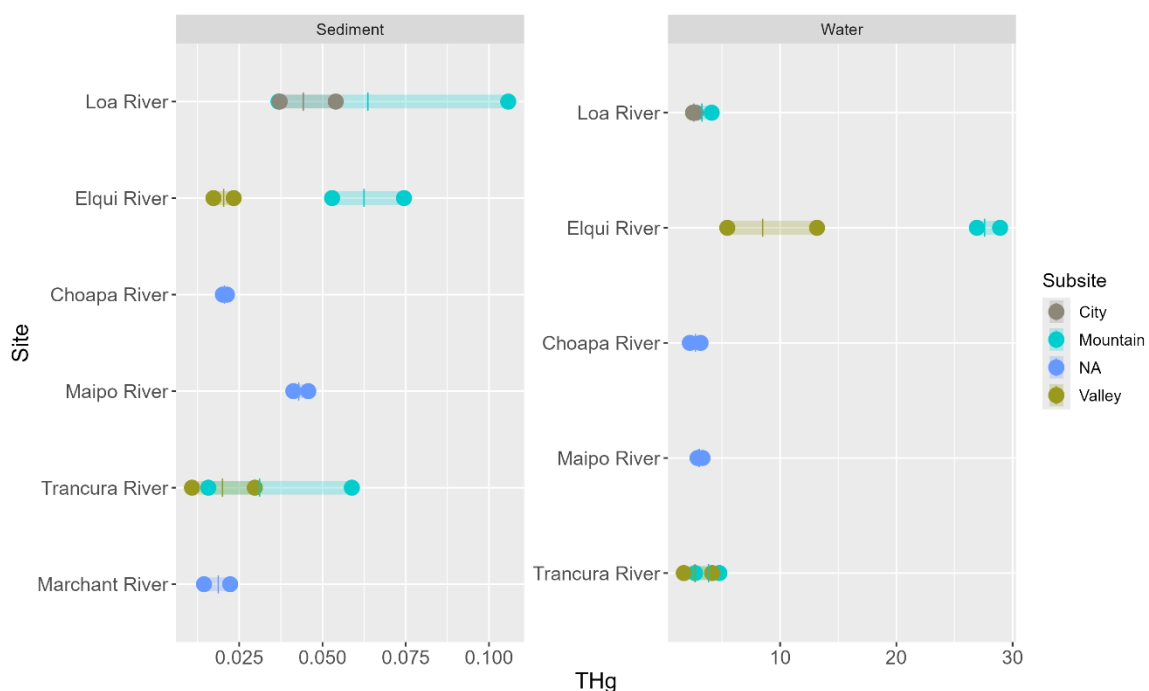


Figure III.2 Concentrations of THg in sediment ($\mu\text{g/g}$ dry weight) and water (ng/L), arranged along a latitudinal gradient, with the lowest latitude at the top. The data include average THg (line), minimum (left dot) and maximum (right dot) values for each river and matrix.

III.4.3. Methylmercury in Biota

Figure III.3 displays MeHg concentrations detected in BMI and trout species along the site gradient. MeHg concentrations in BMI ranged from $0.001 \mu\text{g/g dw}$ (TM site) to $0.021 \mu\text{g/g dw}$ (LC site), with no clear latitudinal trend nor statistical difference (Table S6).

Even though trout were differentially analyzed for MeHg concentrations between biological sexes (Male and female), no significant difference was found in MeHg concentrations between sexes ($p\text{-value} = [0.06, 0.93]$; Table S7). In rainbow trout, concentrations range from $0.005 \mu\text{g/g dw}$ (TM site) to $0.327 \mu\text{g/g dw}$ (LC site) and were found to have a broader distribution compared to the other trout species. Brown trout were only found in the Elqui River and Marchant River, and their concentrations ranged from $0.016 \mu\text{g/g dw}$ (MD site) to $0.066 \mu\text{g/g dw}$ (EM site). Similarly, brook trout in the Robalo River site, where the MeHg concentration for this species was $0.053 \pm 0.1 \mu\text{g/g dw}$.

The highest MeHg concentrations in rainbow trout were observed in the Loa River, particularly in the LC site (p value = 0.029; Table S8). Meanwhile, the TM site showed the lowest MeHg concentrations in rainbow trout compared to the other sites (p value < 0.001).

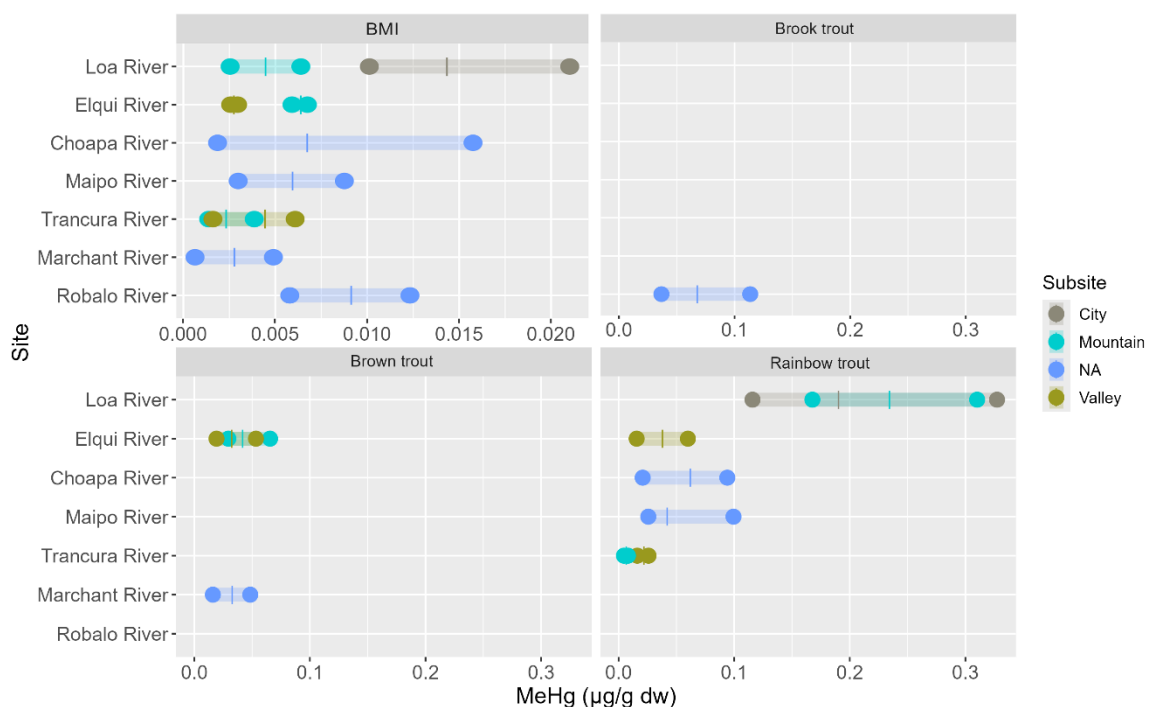


Figure III.3 Concentrations of MeHg (µg/g dw) in benthic macroinvertebrates (BMI) samples and muscle from three species of trout: rainbow trout, brown trout, and brook trout, arranged along a latitudinal gradient with the lowest latitude at the top of the table. The data include average (line), minimum (left dot) and maximum (right dot) values for each river site and species.

III.4.4. Mercury biomagnification spatial fluxes

Figure III.4 shows a clear trend of increasing MeHg concentration with trophic level in food webs of diverse Chilean rivers. The variability in slopes suggests differences in MeHg biomagnification among rivers. TMF values ranged from 1.40 (TM site having the lowest TMF) to 3.41 (LM site, the highest TMF), demonstrating biomagnification across all studied rivers, though with varying intensities. Regression slopes differed significantly between the LM site and almost every other

site (p-value < 0.001; ANCOVA Table S9), except for MU. Comparing the length of the food webs, the LC site, EM site, MU site, and TM site had shorter food webs, compared to the CHD site, MU site and RU site.

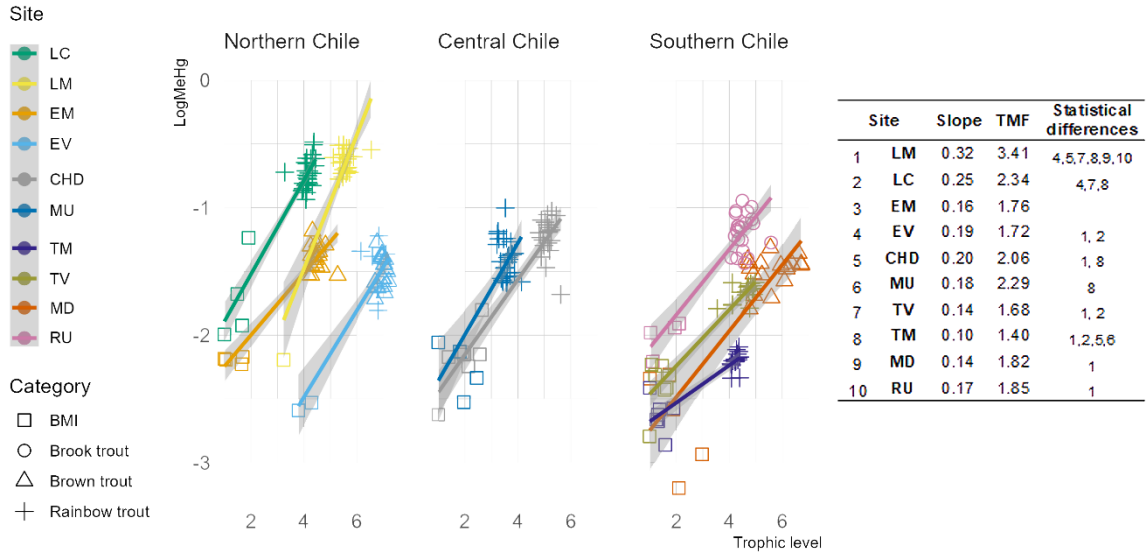


Figure III.4 Relationships between the logarithm of MeHg (LogMeHg) and the trophic level in BMI and trout samples collected from rivers along a latitudinal gradient in Chile. Line colours represent different river sites, and the points, different organisms in the food web. All linear regressions were significant (adjusted R² >0.5, p-value < 0.05, Table S10).

III.4.5. Food web structure

The stable isotope biplots (Figure 5) demonstrated a clear separation of organisms along the $\delta^{15}\text{N}$ axis, which reflects trophic level. The BMI group exhibits a wide range of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, suggesting varied diets and trophic positions. Different BMI orders (e.g., Amphipoda, Coleoptera, Diptera, Ephemeroptera, Odonata, Plecoptera, Trichoptera) were distributed across a range of trophic levels and carbon sources, highlighting the complexity of trophic interactions within this group. The trout had the highest $\delta^{15}\text{N}$ values, thereby occupying the highest trophic position in these food webs. The different trout species (Brown trout, *Salmo trutta*; Brook trout, *Salvelinus fontinalis*; Rainbow trout, *Oncorhynchus mykiss*) appear to occupy slightly different, but overlapping, isotopic niches, potentially indicating some level of trophic specialization.

The size and shape of the dashed polygons, including each site's organisms, reflect the diversity of food sources and trophic pathways contributing to the food web. Differences in polygon size suggest variations in the breadth of the food web's energetic base and the degree of trophic diversity among sites. From Figure 5, it is evident that the food web structure from the southern sites was broader than that from the central and northern sites. The small polygons in the northern sites, LM site, LC site and EV site, showed less diversity compared to the other sites.

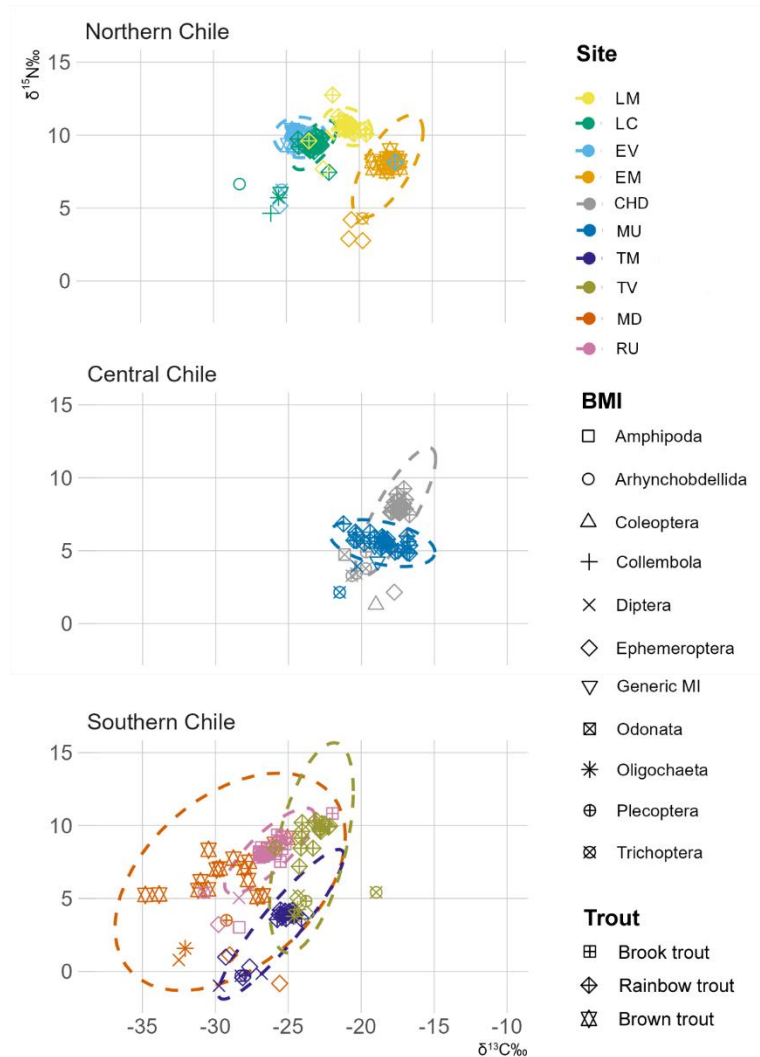


Figure III.5 The isotopic niche ($\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$) of the BMI and trout sampled in this study across the different river sites.

III.4.6. Abiotic factors driving methylmercury biomagnification and THg distribution

A total of 591 (TMF), 1115 (THgsediment) and 392 (THgwater) GLM were evaluated to identify the best predictors of the TMF, THg in sediment and water. The top 5 best models per response variable are presented in Table III.2.

For TMF, the top-ranked model included the number of human inhabitants as the main predictor, showing a positive association with TMF. This model had the lowest AICc (-11.158), the highest model weight ($wAICc = 0.249$), and strongest explanatory power ($R^2 = 0.659$, power = 0.833). The second-best model included DO, which was negatively associated with TMF, and had a slightly higher AICc ($\Delta AICc = 0.711$) and moderately lower support ($wAICc = 0.175$). The model that combined both predictors (Inhabitants + DO) was also competitive ($\Delta AICc = 0.87$, $R^2 = 0.948$, power = 1), indicating potential synergistic effects. Other models involving pH and mining activities were included in the top five but with substantially lower support ($wAICc < 0.07$).

For THg in sediment, the best model included a combination of DO, pH, Fe, and Mg. DO and pH showed negative associations, while Fe and Mg were positively associated with THg concentrations in sediment. This model achieved the lowest AICc (-183.899), the highest model weight ($wAICc = 0.918$), and the strongest explanatory power ($R^2 = 1$, power = 1). Other models, including combinations of Al, Cl, and anthropogenic factors such as agriculture, urban/industrial activities, and temperature, also appeared in the top five, albeit with much lower $wAICc$ values (≤ 0.058), indicating low support.

For THg in water, the top model included urban and industrial development, Al, and Mn, all of which were positively associated with THg concentrations in the water column. This model was strongly supported ($AICc = -144.260$, $wAICc = 0.989$, $R^2 = 1$, power = 1) and outperformed all others by a large margin ($\Delta AICc > 10$), highlighting the critical role of anthropogenic land use and metal availability in influencing THg concentrations in the water column.

Table III.2 Top 5 models selected to explore the relationship between environmental factors and TMF, THg in sediment (THg_{sediment}) and water (THg_{water}). The sign of the “Coef.” indicates if the association is negative (-) or positive (+), and is displayed in the same order as the predictor variables. Bold indicates the best model for each response variable.

Response	Predictors	Coef.	AICc	delta_AICc	wAICc	R2	Power
TMF	Inhabitants	+0.037	-11.158	0	0.249	0.659	0.833
TMF	DO	-0.613	-10.447	0.711	0.175	0.622	0.773
TMF	Inhabitants + DO	+0.028 / -0.446	-10.288	0.870	0.161	0.948	1
TMF	pH	+2.301	-8.360	2.798	0.062	0.491	0.553
TMF	Mining	+0.032	-7.615	3.543	0.042	0.434	0.465
THg_{Sediment}	DO + pH + Fe + Mg	-0.092 / -0.199 / +0.015 /+ 0.006	-183.899	0	0.918	1	1
THg _{Sediment}	DO + pH + Al + Cl	-0.074 / -0.111 / +0.016 / +0.003	-178.383	5.5161	0.058	1	1
THg _{Sediment}	Agriculture + Urban and industrial + PO4 + T°	+0.015 / -0.017 / +0.219 / -0.146	-175.409	8.489	0.013	1	1
THg _{Sediment}	Agriculture + Urban and industrial + DO + Bo	+0.032 / -0.055 / -0.264 /+0.101	-173.444	10.455	0.005	1	1
THg _{Sediment}	T° + Al + Cl + Mn	-0.051 /+0.018 /+0.005 / +0.006	-172.345	11.554	0.003	1	1
THg_{water}	Urban and industrial + Al + Mn	+0.008 / +0.023 / +0.028	-144.260	0	0.976	1	1
THg _{water}	δ ¹³ C + Ca + SO	-0.046 / -0.049 / +0.073	-135.667	8.593	0.013	1	1
THg _{water}	Agriculture + Al + Mn	-0.005 / +0.022 / +0.033	-133.342	10.918	0.004	1	1
THg _{water}	Mining + Al + Mn	+0.006 /+0.022 / +0.033	-132.559	11.700	0.003	1	1
THg _{water}	pH + T° + Ca	-0.634 / -0.054 / -0.039	-132.476	11.783	0.003	1	1

III.5. Discussion

This study investigated the distribution, bioaccumulation and biomagnification of MeHg and THg in seven rivers along a latitudinal gradient in Chile, considering both abiotic and biotic matrices. While the study aimed to characterize MeHg distribution along this latitudinal gradient, the results did not show clear spatial trends in abiotic or biotic matrices. This aligns with other studies that have found that local factors often outweigh broad-scale geographic patterns in determining MeHg concentrations (C. Y. Chen & Evers, 2023; Driscoll et al., 2013).

III.5.1. THg and MeHg distribution

Natural background THg concentrations in unpolluted river waters typically range from below detection limits up to approximately 7 ng/L. For instance, a study of 58 rivers in Maine, USA, reported THg levels ranging from below detection to 7.01 ng/L, with an average of 1.80 ± 1.29 ng/L (Peckenham et al., 2003). Similarly, the Australian and New Zealand Guidelines for Fresh and Marine Water Quality indicate that THg concentrations in freshwater rarely exceed 12 ng/L (Australian & New Zealand Governments, 2018). However, industrial or mining-impacted areas can exhibit much higher concentrations, sometimes exceeding 1,000 ng/L (Pirrone et al., 2010). Notably, the Elqui River Basin, characterized by a total surface of 982,600 ha, exhibited the highest THg levels in water at the EM site (26.93–28.93 ng/L). This significantly surpasses typical background concentrations, as mentioned, and strongly suggests localized contamination. The presence of mining activities (156 ha dedicated to mining) in this basin, and mostly agricultural land use (27,713 ha), could be contributing factors. The EV site within the same basin also showed elevated levels (5.46–13.18 ng/L), potentially influenced by the downstream transport of contaminants. In contrast, the Loa River Basin (3,357,000 ha) showed moderate THg concentrations in water at the LM and LC sites (2–4 ng/L). Despite a larger mining footprint (4,568 ha), the region's arid conditions and potentially distinct geological substrates may influence THg mobility and availability in the water column, resulting in lower observed concentrations compared to the Elqui River. The lower THg in water, despite substantial mining, highlights the

complexity of Hg cycling and the influence of basin-specific characteristics. The Choapa River Basin (812,400 ha), which features diverse vegetation types—including woody steppe shrubland, interior steppe shrubland, Andean shrubland, and high Andean steppe—also showed moderate THg concentrations in water (approximately 2–4 ng/L). Although mining (156 ha) and agriculture (31,152 ha) were present, THg levels did not reach the elevated values seen in the Elqui River. The lowest water THg values were observed in the Trancura River Sub-Basin (1.7–4.2 ng/L). The minimal industrial and mining activities reported in these basins (Trancura: 2,000 ha urban/industrial, 0 ha mining) likely contribute to these levels aligning with natural background concentrations. Similarly, the Maipo River Basin showed moderate THg concentrations (around 2–4 ng/L) despite significant urban and industrial land use (58,220 ha) and some mining activity (1,966 ha). Interestingly, the relationship between land use and THg concentrations in water across the studied basins does not follow a simple gradient. Although mining and agriculture are potential sources of Hg, the variability in THg levels — particularly the low concentrations in the highly mined Loa Basin and moderate levels in the heavily urbanized Maipo Basin — highlights the complex interplay of geological, hydrological, and climatic factors in modulating Hg availability and transport in river systems. This underlines the need for basin-specific assessments rather than assuming uniform responses to anthropogenic pressures.

In sediments, background levels in rivers and lakes typically range from 0.01 to 0.2 µg/g dw (Covelli et al., 2008; USGS, 1996). Moderately contaminated areas, such as industrialized or urban-influenced sites, often show levels between 0.2 and 5.0 µg/g dw, whereas highly contaminated regions, including those affected by gold mining or industrial discharges, can exceed 50 µg/g dw (Hsu-Kim et al., 2013). Extreme cases, like Minamata Bay in Japan, have recorded sediment Hg concentrations over 100 µg/g dw (Harada, 1995). These values illustrate the significant variation in Hg distribution due to both natural sources and human activities (Covelli et al., 2008; Pirrone et al., 2010; USGS, 1996). The average highest THg levels in sediment were found in the Loa River Mountain site (0.06 ± 0.04 µg/g dw) and City site (0.04 ± 0.009 µg/g dw), as well as the Elqui River

Mountain site ($0.06 \pm 0.01 \mu\text{g/g dw}$). These values, while within the lower range of moderate contamination, suggest some degree of THg accumulation. For the Loa River, the historical and ongoing intense mining activity (4,568 ha) within its arid landscape likely contributes to this accumulation in sediments, even if water concentrations are moderate. Similarly, the mining activities in the Elqui River (156 ha), coupled with its diverse landscape, could explain the elevated sediment THg at the Mountain site. The Choapa River site, with mining and agricultural activities, shows lower sediment THg levels ($0.02 \pm 0.0004 \mu\text{g/g dw}$). The Maipo River Basin, despite urban and industrial influence and some mining, also exhibited relatively low sediment THg ($0.04 \pm 0.002 \mu\text{g/g dw}$). The Trancura River sites and Marchant River sub-Basin, with minimal industrial and mining impact, showed the lowest sediment THg concentrations ($0.02\text{--}0.06 \mu\text{g/g dw}$), consistent with less anthropogenic pressure.

In BMI, MeHg concentrations ranged from $0.001 \mu\text{g/g dw}$ at the TM site (located within the Trancura River Sub-Basin, characterized by minimal industrial and mining activity and a landscape of volcanoes and forests) to $0.021 \mu\text{g/g dw}$ at the LC site (situated in the Loa River Basin, a region with significant mining activity and arid conditions). Notably, no statistically significant differences in BMI MeHg concentrations were observed across the sites. This suggests that while local conditions might have influenced MeHg accumulation in BMI, broader geographical factors in this study did not appear to be primary drivers.

Rainbow trout exhibited a wider range of MeHg concentrations compared to other trout species, spanning from $0.005 \mu\text{g/g dw}$ at the TM site to a high of $0.327 \mu\text{g/g dw}$ at the LC site. This finding aligns with the significant mining activities within the Loa River, which are known to enhance Hg bioavailability and its subsequent bioaccumulation in aquatic food webs (Caballero-Gallardo et al., 2022; Kerfoot et al., 2018; Silva et al., 2023). The low MeHg concentrations in rainbow trout at the Trancura Mountain site further support the idea that pristine environments with minimal anthropogenic disturbance tend to have lower MeHg levels in biota.

Brown trout were exclusively found in the Elqui River and the Marchant River. Concentrations in the Elqui River suggest a moderate level of MeHg accumulation,

potentially linked to the mining activities and agricultural land use within this basin. The single data point for brown trout in the Marchant River site, a basin with no reported mining or industrial activity, indicates a lower MeHg concentration, but still similar to more populated areas, although the sample size was very limited.

Even though the Robalo River Basin is considered a pristine area with minimal current human population and industrial activity, the MeHg concentrations in brook trout ($0.053 \pm 0.1 \mu\text{g/g dw}$) were comparable to those observed in rainbow trout from the more impacted sites (CHD and MU; ranging 0.05 to $0.1 \mu\text{g/g dw}$) and in brown trout from Elqui River sites and Marchant River site (EM and MD; ranging 0.01 to $0.08 \mu\text{g/g dw}$). Additionally, basal MeHg concentrations in benthic macroinvertebrates were higher in Robalo than in other basins, suggesting that MeHg availability may be driven more by natural or diffuse sources rather than direct anthropogenic inputs. Elevated precipitation levels in both Robalo and Marchant may promote greater atmospheric mercury deposition, a known contributor to MeHg production in aquatic systems. Therefore, while historical gold mining in the Robalo basin (Bascopé Julio, 2010) could still play a role, the overall MeHg patterns likely reflect a combination of legacy contamination, atmospheric inputs, and local biogeochemical conditions. This highlights the importance of considering both historical and natural drivers when evaluating mercury dynamics, even in areas perceived as undisturbed.

The heterogeneity of THg and MeHg across sites likely reflects the differences in local Hg sources (natural geological deposits vs. mining activities), watershed characteristics (arid vs. more temperate and vegetated), and the scale and type of anthropogenic influences (mining, agriculture, urban/industrial).

III.5.2. Mercury biomagnification spatial fluxes

MeHg, unlike inorganic Hg, is easily taken up by aquatic organisms, primarily through dietary uptake and, to a lesser extent, direct from the water column via gill uptake (de Almeida Rodrigues et al., 2019). The efficiency of MeHg assimilation varies across species and is influenced by factors such as feeding strategies, gut physiology, and metabolic rates (Li et al., 2022). The significant positive relationship between MeHg concentration and trophic level herein reported (Figure

4) further corroborates biomagnification, with TMF values ranging from 1.40 to 3.41 (Wu et al., 2019; Yurkowski et al., 2023). Trout, as top predators, then accumulate MeHg by consuming BMI and/or other fish. This trophic transfer is highly efficient for MeHg due to its strong affinity for organic tissues and its slow rate of depuration (Driscoll et al., 2013).

Although the TMF values were all >1, reinforcing the biomagnification of MeHg in riverine food webs, they varied among rivers, suggesting differences in MeHg transfer efficiency between food webs. These differences were likely due to variations in food web structure and the physiology and behaviour of organisms involved (Chasar et al., 2009; Finley et al., 2016; Lavoie et al., 2013). The isotopic niche width (size and shape of polygons in Figure III.5) varied among rivers, indicating differences in food web complexity and energy flow pathways. In addition, variation in nutrient levels can alter primary productivity and the composition of basal food web resources, consequently impacting MeHg bioaccumulation (Kickbush et al., 2018; Kozak et al., 2021; Lescord et al., 2015). This has been impacting MeHg bioaccumulation (Kozak et al., 2021; Lescord et al., 2015). A similar pattern has been observed with PO_4^{3-} in some wetland studies, where elevated nutrient levels were associated with changes in MeHg dynamics, further supporting the link between nutrient enrichment and mercury bioaccumulation (Kickbush et al., 2018).

III.5.3. Factors driving TMF and THg changes

The GLM/AICc analysis provided important insights into how environmental and land use variables influence Hg dynamics across the studied river systems.

The TMF was best explained by anthropogenic variables, particularly human population (number of inhabitants) and DO, either individually or in combination (Table 2). The top-ranked model, which included both human population and DO, exhibited the highest fit ($R^2 = 0.948$) and maximum statistical power (1.0), suggesting that areas with higher human activity and lower oxygen levels may experience enhanced Hg biomagnification in food webs. These results are consistent with previous studies linking anthropogenic pressures to increased MeHg production and its transfer through food webs (Bravo & Cosio, 2020; Lavoie

et al., 2013). Specifically, low oxygen conditions are known to favour the microbial reduction of Hg(II) to MeHg, which can then be biomagnified as it moves up trophic levels (Bargagli & Rota, 2024; Jonsson et al., 2022). Our findings align with the hypothesis that eutrophication and oxygen depletion in urbanized or industrialized regions can exacerbate MeHg contamination in aquatic ecosystems (Achá et al., 2018). THg in sediments was most strongly associated with physicochemical conditions, particularly the combination of DO, pH, and metal concentrations such as Fe and Mg. This finding reinforces the idea that redox-sensitive processes and sediment geochemistry are key drivers of Hg retention and transformation in benthic environments (Ignatavičius et al., 2022; Tsui & Finlay, 2011). Sediment is a major reservoir for Hg, where it undergoes complex transformations, including methylation under anaerobic conditions (Millard et al., 2023; Zhang et al., 2024; Zhao et al., 2024). Additionally, the relationship between Fe and Hg concentrations is well-documented, as iron oxide minerals can bind Hg, facilitating its retention in the sediment matrix (O'Connor et al., 2019). Our results suggest that regions with altered sediment geochemistry due to anthropogenic influences may have increased Hg bioavailability. Furthermore, models incorporating land use—specifically agriculture, urban and industrial development, and temperature—also ranked highly, suggesting that land-based sources and thermal regimes influence Hg inputs to sediments. This aligns with findings from other studies, which show that agricultural runoff, industrial effluents, and urbanization contribute to elevated Hg levels in sediments (Benoit et al., 2002; X. Chen et al., 2010).

For THg in water, the best-performing model included the proportion of urban and industrial land use alongside Al and Mn concentrations. These results highlight the contribution of point-source pollution and metal mobilization, often linked to mining and urban runoff, as significant factors influencing dissolved Hg concentrations. Mining activities are well-known sources of Hg release into water systems, both directly through effluent discharges and indirectly through sediment resuspension (Caballero-Gallardo et al., 2022; Feng et al., 2019). The mobilization of metals such as Al and Mn can influence Hg speciation in the water column, enhancing its solubility and potential for methylation. Manganese and iron oxides, for instance,

can adsorb inorganic forms of Hg and alter redox conditions that favour the formation of MeHg (Skylberg, 2011). While Al is less directly involved in redox cycling, it can modify pH and ionic strength, indirectly affecting Hg mobility and complexation (Gilmour et al., 2013; J. Liu et al., 2023). Our findings indicate that urban and industrial areas, especially those affected by mining, are key hotspots for Hg contamination. Models incorporating mining activity and major ions (e.g., Cl^- , SO_4^{2-} , Ca^{2+}) performed moderately well, suggesting contributions from both natural weathering and anthropogenic sources. Major ions play a critical role in Hg dynamics, as shifts in water chemistry can alter Hg binding to particulates and its downstream transport (Hsu-Kim et al., 2013).

Environmental and anthropogenic variables—particularly human population, dissolved oxygen, land use, and metal concentrations—emerged as strong predictors of Hg behaviour in these river systems. The alignment of the land use vector with highly impacted sites further highlights the ecological consequences of anthropogenic pressures. This is especially apparent in heavily mined basins like the Loa River, where geochemical and land use factors converge to promote Hg bioavailability and trophic transfer.

Overall, these results underscore the multifactor nature of Hg contamination, shaped by both direct (e.g., mining) and indirect (e.g., nutrient enrichment, landscape alteration) drivers. They reinforce previous work suggesting that catchment-scale variables are critical in determining Hg fate and effects (Hsu-Kim et al., 2013; Schartup et al., 2015), and underscore the need for integrated watershed management to mitigate Hg risks in freshwater ecosystems.

III.6. Conclusions

This study highlights the considerable variability in the distribution, bioaccumulation and biomagnification of THg and MeHg across seven rivers in Chile, shaped predominantly by local environmental conditions and anthropogenic influences, particularly historical and active mining. Rather than following a latitudinal gradient, Hg concentrations were more closely linked to watershed-specific characteristics, including land use, water chemistry, and redox-sensitive processes. Elevated Hg levels in the Elqui River point to localized contamination, while other regions

displayed concentrations within natural background levels. Notably, even in pristine areas like the Robalo River Basin, which has minimal current human activity, MeHg concentrations in brook trout were comparable to those observed in more populated sites, highlighting the long-lasting effects of past mining activities on Hg availability in the food web. Evidence of MeHg bioaccumulation in benthic macroinvertebrates and trout, especially pronounced in the mining-influenced Loa River, underscores the potential for food web transfer and ecological risk. The role of water quality—particularly temperature, nutrients, and oxygen levels—was evident in shaping food web structure and MeHg dynamics, likely through effects on microbial methylation processes. The strong association between low oxygen levels and increased MeHg biomagnification further supports the hypothesis that eutrophication and oxygen depletion exacerbate Hg contamination. Stable isotope analysis further revealed complex food web interactions and varying energy sources, influencing Hg transfer efficiency across trophic levels. These findings reinforce the need for site-specific monitoring and management of Hg contamination and call for further research into Hg speciation, source tracing, and microbial methylation pathways to better inform mitigation strategies and protect aquatic ecosystems and human health. Overall, this research emphasizes the multifactor nature of Hg contamination, with both direct (e.g., mining) and indirect (e.g., nutrient enrichment, landscape alteration) drivers, highlighting the need for integrated watershed management approaches to mitigate Hg risks in freshwater systems.

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III.8. Author contributions

- Elvira G. Vergara: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

- Celine Lavergne: Conceptualization, Methodology, Resources, Validation, Writing – review & editing.
- Gabriel Rambaldi: Investigation, Validation, Writing – review & editing.
- Nelson J. O’Driscoll: Conceptualization, Investigation, Methodology, Resources, Validation, Writing – review & editing.
- Karen A. Kidd: Conceptualization, Validation, Writing – review & editing.
- Manuel Bravo-Mercado: Investigation, Methodology, Resources.
- Sonnia Parra-Vargas: Investigation, Methodology, Resources.
- Borja Bernales-Santolaya: Investigation, Validation, Writing – review & editing.
- Gustavo Chiang: Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Validation, Writing – review & editing.
- Paulina Bahamonde: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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III.7. References

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Capítulo IV. **Sex-dependent gene expression responses to methylmercury bioaccumulation in wild rainbow trout (*Oncorhynchus mykiss*)**

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IV.1. Abstract

Methylmercury (MeHg) is a persistent environmental contaminant known to impair endocrine, metabolic, and immune functions in aquatic organisms. While South America is a major contributor to global anthropogenic Hg emissions, largely from artisanal gold mining and industrial activities, little is known about sex-specific molecular responses to MeHg bioaccumulation in its wild trout populations. This study investigated the effects of MeHg on hepatic mRNA expression and physiological condition in wild rainbow trout (*Oncorhynchus mykiss*) from three Chilean watersheds spanning a gradient of MeHg bioaccumulation in trout muscle. Transcriptomic analyses were conducted using RNA sequencing and validated via quantitative PCR (qPCR) for selected genes. Results revealed extensive sex-dependent transcriptional responses. qPCR confirmed consistent expression patterns for key biomarkers such as *zona pellucida*, *peroxiredoxin-like 2B*, *renin*, and *estrogen receptor β* , reinforcing their potential as indicators of MeHg exposure. Females with high MeHg levels (0.93-1.55 $\mu\text{g/g}$ dry weight) exhibited predominant transcriptomic downregulation, particularly in genes associated with lipid metabolism and cellular homeostasis. In contrast, males at high MeHg (0.84-1.35 $\mu\text{g/g}$ dry weight) levels showed pronounced upregulation in nucleotide metabolism, RNA processing, and oxidative stress pathways. These transcriptomic responses were reflected in morphometric indices: individuals from high MeHg sites, particularly males, exhibited lower hepatosomatic index and condition factor, indicating compromised physiological condition. This study provides the first evidence of sex-specific transcriptomic and physiological effects of MeHg bioaccumulation in wild trout from Chile. These findings underscore the importance of incorporating sex as a biological variable in ecotoxicological assessments and support the use of integrative molecular-physiological approaches for monitoring contaminant impacts in freshwater ecosystems.

Key words: Methylmercury, Endocrine disruption, Hepatic transcriptomics, Metabolism pathways, Aquatic ecotoxicology, Freshwater ecosystems

IV.2. Introduction

Mercury (Hg) is a persistent and globally distributed environmental contaminant that poses significant risks to ecosystems and human health (UNEP, 2018). In Latin America and the Caribbean, anthropogenic emissions make this region the second-largest emitter of Hg into the atmosphere (Sundseth et al., 2017). In 2015 alone, the LAC region was responsible for 20.5% of global anthropogenic Hg air emissions, largely driven by small-scale gold mining, which contributed 354.3 tons, followed by industrial activities (66.4 t) and intentional use (20.21 tons, including product waste) (UNEP, 2018). Moreover, South America accounted for 53% of the estimated 1,220 tons of Hg released globally from artisanal gold mining into aquatic and terrestrial environments (Canham et al., 2021). Once the Hg in the atmosphere is transformed and deposited into aquatic environments by dry and wet deposition, afterward Hg undergoes microbial methylation, forming methylmercury (MeHg), a highly toxic and bioavailable compound that biomagnifies through food webs (Driscoll et al., 2013).

Although Chile is not a major contributor to mercury emissions from artisanal gold mining, it remains vulnerable to Hg pollution due to industrial activity and atmospheric deposition from transboundary sources. Its diverse freshwater ecosystems, particularly in southern regions, may serve as sensitive indicators of MeHg bioaccumulation and biomagnification. Despite these vulnerabilities, comprehensive national monitoring of Hg contamination in biota is still limited, underscoring the importance of this study in providing baseline data on MeHg dynamics and biological responses in native fish populations.

The effect of MeHg in wildlife, particularly fish, impacts at multiple biological levels (Morcillo et al., 2017). Fish accumulate MeHg through bioaccumulation and biomagnification, leading to physiological disruptions. MeHg bioaccumulation interferes with vitellogenin synthesis, a process essential for egg development in female fish, contributing to reproductive impairments (Bera et al., 2022; DeBofsky et al., 2018). Alterations in energy metabolism, including glucose and lipid metabolism, have also been observed, affecting growth and survival in female fathead minnows (Sandheinrich & Wiener, 2011).

Importantly, the level of MeHg bioaccumulation is intrinsically linked to the generation of reactive oxygen species (ROS; Antunes dos Santos et al., 2018; Fujimura & Usuki, 2020). As MeHg accumulates in tissues, it disrupts cellular redox balance, promoting ROS formation and inducing oxidative stress, cellular damage, and inflammation (Aschner et al., 2007; Sasaki et al., 2023). This oxidative stress triggers apoptosis, or programmed cell death, in tissues such as the brain, gills, and liver (Choi et al., 2010; Loh et al., 2006; Neto et al., 2020). Additionally, MeHg compromises immune function, increasing susceptibility to disease and impairing the capacity to mount an effective immune response (Nyland et al., 2011; Yin et al., 2024).

The liver, an organ involved in detoxification and metabolism, plays a central role in the physiological response to MeHg bioaccumulation. As a primary site for xenobiotic processing, the liver accumulates MeHg, leading to a series of toxic effects (Peng et al., 2016). This accumulation disrupts hepatic function, including enzyme inhibition (Alqahtani et al., 2023), mitochondrial dysfunction (Mieiro et al., 2015), oxidative stress (Desjardins et al., 2024; Mozhdeganloo et al., 2015), and histological changes (Zulkipli et al., 2021).

Rainbow trout (*Oncorhynchus mykiss*) is widely regarded as a bioindicator species for assessing Hg contamination in aquatic ecosystems, largely due to its extensive distribution across diverse freshwater habitats (MacCrimmon, 2011). This broad distribution facilitates comprehensive spatial assessments of Hg pollution. The species' adaptability to various environments has led to its introduction beyond its native range, further enhancing its utility in environmental monitoring. Its ability to adapt to varying environmental conditions enhances its reliability as an indicator of Hg contamination. As a secondary or tertiary consumer, rainbow trout accumulate MeHg through the consumption of invertebrates and smaller fish (Sinclair et al., 2024). This trophic position and lipid-rich tissue facilitate MeHg bioaccumulation and biomagnification, resulting in higher MeHg concentrations in tissues (Iliopoulou-Georgudaki & Kotsanis, 2001). Studies on MeHg accumulation often assume sex-based differences (Hanson et al., 2008; Madenjian et al., 2011). However, emerging evidence indicates that

molecular responses to MeHg bioaccumulation can vary significantly between males and females (Liu et al., 2013). This variation may result from differences in hormonal regulation, metabolic pathways, and gene expression patterns (Blencowe et al., 2022; Christianto et al., 2021; Robison et al., 2008). Genes involved in biotransformation and detoxification, such as cytochrome P450 enzymes, may show sex-specific expression patterns, affecting MeHg metabolism and elimination rates (Alqahtani et al., 2023; Williams et al., 1986). Transcriptomic approaches, such as RNA sequencing (RNASeq), enable the identification of sex-specific gene expression changes linked to MeHg bioaccumulation (Richter et al., 2011, 2014). Analyzing the transcriptome of male and female rainbow trout exposed to MeHg can reveal differentially expressed genes and pathways contributing to sex-specific responses.

Despite the growing understanding of sex-specific impacts of MeHg, comprehensive national monitoring of Hg contamination and its detailed biological effects in wild Chilean biota remains critically limited. Furthermore, while rainbow trout have been extensively studied as a bioindicator, the vast majority of this knowledge originates from the Northern Hemisphere, which often presents distinct landscapes and climatic conditions compared to the diverse environments found in the Southern Hemisphere. This geographical disparity highlights a crucial gap in our understanding of MeHg dynamics in different biogeographical contexts. This study addressed this significant knowledge gap by providing the first evidence of sex-specific transcriptomic and physiological responses to MeHg bioaccumulation in wild rainbow trout from South America. Our focus on Chilean watersheds, characterized by unique environmental factors, contributed new and essential knowledge regarding MeHg dynamics in a different biogeographical context. Using transcriptomic approaches, this study aimed to identify sex-specific genetic responses and biomarkers for Hg bioaccumulation. Identifying these biomarkers will improve methods for assessing MeHg toxicity in aquatic organisms. Ultimately, the findings from this work will enhance understanding of MeHg toxicity, inform environmental monitoring, and support the development of mitigation strategies. These results underscore the importance of including sex

as a biological variable in environmental risk assessments and support the development of biomarker-informed monitoring strategies that integrate molecular, physiological, and ecological health indicators.

IV.3. Methodology

IV.3.1. Study Area

To assess MeHg distribution in trout muscle, three river catchments were selected (Figure 1): the Loa River (21°25'S, 70°03'W – Mild Desert Climate) in the arid north, the Choapa River (31°37'S, 71°33'W – Temperate Mediterranean Climate) in the semi-arid central zone, and the Trancura River (39°20'S, 71°34'W – Temperate Oceanic Climate) in the temperate south. These sites differ in climate, land use, and potential MeHg sources, allowing for a comparative analysis of MeHg bioaccumulation.

The Loa River Basin covers a total surface area of 3,357,000 hectares. This basin is characterized by an arid landscape due to its desert climate, specific terrain arrangement, and high soil salinity. Land use within the basin includes agriculture (759 ha), urban and industrial development (1,990 ha), and mining (4,568 ha), with mining being a predominant activity. The Loa River Basin supports a human population of 445,109 inhabitants, and the primary economic activities are mining and agriculture (M. Dirección General de Aguas, 2004b).

The Choapa River encompasses a total surface area of 812,400 hectares. The basin's landscape presents diverse plant formations, including woody steppe shrubland, interior steppe shrubland, Andean shrubland, and high Andean steppe. Land use distribution consists of agriculture (31,152 ha), urban and industrial areas (172 ha), and mining (156 ha). The Choapa River Basin's main economic activities are mining and agriculture, supporting a human population of 65,144 inhabitants (M. Dirección General de Aguas, 2004a).

The Trancura River covers a total surface area of 788,600 hectares. The landscape in this sub-basin is defined by the presence of volcanoes and hot springs, alongside various forest types such as southern deciduous forest, Valdivian laurel forest, mixed deciduous forest of the Andes, and high Andean deciduous forest. Land use is predominantly characterized by agriculture (602 ha)

and urban/industrial areas (2,000 ha), with minimal mining activity (0 ha). The primary economic activities within the Trancura River Sub-Basin are tourism and forestry, supporting a human population of 10,660 inhabitants (M. de O. P. Dirección General de Aguas Gobierno de Chile, 2025; Proyectos y Estudios Año 2050 Ltda, 2016).



Figure IV.1. Geographic location of the sampling sites.

IV.3.2. Sample collection

Each River was sampled during the spring/summer. Rainbow trout of a standardized size ≥ 9 cm, indicating maturity in salmonids of at least 1 year (Arismendi et al., 2011), were collected at each site by electrofishing (Halltech Aquatic Research Inc., HT-2000). The corresponding Fishing and Animal Care Permits were obtained from SUPBESCA (R.EX. N°3443 and N°4231). The established protocols were strictly followed to ensure animal welfare throughout

the process. Fifteen female and 15 male rainbow trout were to be collected at each site. Samples of liver were collected for molecular studies, flash frozen in liquid nitrogen, and kept at -80 °C in the laboratory until processed. Fish were measured for weight (instrument readability ± 0.001 g), length (instrument readability ± 1 mm), gonad weight (instrument readability ± 0.001 g), and liver weight (instrument readability ± 0.001 g).

IV.3.3. MeHg analysis

MeHg was quantified in trout muscles in lyophilized and homogenized samples following the EPA method 1630 (USEPA, 1998) with some modifications according to Edmonds et al. (2012) at the Mercury Lab at Acadia University, Wolfville, Canada. An alkaline digestion of the sample with KOH/MeOH was performed to detect the organic form of mercury, followed by derivatization using an ethylating agent, $\text{NaB}(\text{C}_2\text{H}_5)_4$, for subsequent separation by gas chromatography and detection by cold vapour atomic fluorescence spectrometry (GC/CV-AFS). This method has demonstrated good quality standards and high sensitivity. Quality control measures were performed using fish tissue-certified reference materials DORM4 (mean recovery 102.8% $\pm$ 15.7%) and DOLT5 (mean recovery 120.6% $\pm$ 16.3%). The mean % recovery of a 50pg MeHg check standard was 110% $\pm$ 9.0%.

IV.3.4. Nucleic Acid Extraction

Liver tissue (52 samples) was homogenized with a Scilogex D160 Handheld Homogenizer (Scilogex, LLC, USA). Total RNA was extracted using TRIzol® RNA Isolation Reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. RNA concentration (~ 500 ng. μL^{-1}) and A260/A280 ratio (ratio above 1.8) were assessed using a SPECTROstar Nano Spectrophotometer (BMG Labtech, Ortenberg, Germany). RNA integrity was verified with a Qubit Fluorometer (Invitrogen, Carlsbad, California, USA) selecting samples with an integrity quotient (IQ) above 7, and agarose bleach gel electrophoresis at 1%. After quality control, 36 samples (six per sex and site) were

chosen and stored at -80°C for subsequent RNASeq and qPCR gene expression analysis.

IV.3.5. RNA Sequencing (RNASeq)

Initial quantities of total RNA ($20\ \mu\text{L}$, $\sim 100\ \text{ng}/\mu\text{L}^{-1}$) were sent to Novogene (<https://en.novogene.com/>) for the preparation of 36 independent Illumina sequencing libraries using the Total RNA Prep with Ribo-Zero Plus™ (Illumina) kit. Sequencing was performed using the Illumina NovaSeq 6000 platform. The data were processed using a standardized pipeline (Conesa et al., 2016). Read quality was assessed with FastQC and MultiQC, and low-quality reads were trimmed using Trimmomatic. Trimmed reads were aligned to the reference genome (GCF_013265735.2_USDA_OmykA_1.1) using HISAT2. Differential expression genes (DEGs) analysis was conducted with DESeq2 (Love et al., 2014) in R, filtering lowly expressed genes (counts >10). Normalization and variance stabilization were performed via rlog transformation, followed by exploratory analyses (PCA, scatterplots). DESeq2 was used to identify differentially expressed genes highlighting genes with significant log2 fold changes ($\text{Log}_2\text{FC} > 2$, and $\text{padj} < 0.05$; Kolde, 2022, Blighe et al., 2024).

IV.3.6. Gene Ontology (GO) terms and KEGG pathway enrichment analysis

To explore functional enrichment patterns in gene expression, the DAVID Gene Functional Classification Tool (v6.8; Huang et al., 2007) was used on DEG identified from RNASeq data in wild rainbow trout (*Oncorhynchus mykiss*). RNASeq feature counts were analyzed separately for females and males and further divided by sites with medium and high MeHg bioaccumulation. Gene lists representing DEG for each sex and bioaccumulation level were submitted to DAVID using official gene symbols as input. Functional classification was based on GO terms, KEGG pathways, and protein domain annotations. DAVID groups genes into annotation clusters based on shared biological function using a kappa-statistic-based similarity metric and hierarchical clustering (Sherman et al., 2022). Enrichment scores for each cluster were calculated from the geometric mean of

modified Fisher's Exact Test (EASE) p-values, highlighting biologically meaningful themes among the DEG in a sex- and exposure-specific manner.

IV.3.7. Real-time Quantitative PCR (qPCR)

Primers for qPCR were sourced considering the literature and RNASeq data, and designed using Primer3 (biotools.umassmed.edu/bioapps/primer3_www.cgi; Rozen & Skaletsky, 2000), and later synthesized by Life Technologies (Invitrogen), targeting 120-300 bp sequences related to sex differentiation, steroid signaling, mercury-impacted reproduction, and metal bioaccumulation (more specifications in Table S1). *Ub* and *RPL8* served as housekeeping genes for transcript normalization. Primer specificity was validated through conventional PCR and visualized with 1% agarose gel electrophoresis using SYBR safe staining. The efficiency percentages of each selected primer were determined by qPCR calibration curves and are displayed in Table S1 (Supplementary material). The qPCR was conducted to quantify mRNA abundance through three MeHg levels. Complementary DNA (cDNA) was synthesized from the extracted RNA using the AffinityScript qPCR cDNA Synthesis Kit (Aligent, Santa Clara, USA), following the manufacturer's protocol. The qPCR analysis was performed utilizing PowerTrack™ SYBR™ Green Master Mix (Thermo Fisher, Waltham, Massachusetts, USA), and the QuantStudio 3 Real-Time PCR System (Thermo Fisher, Waltham, Massachusetts, USA), providing precise amplification and detection.

Each 10 µL reaction mixture consisted of 1 µL of cDNA template (~100 ng/µL) 5 µL of Master Mix, 0.8 µL each of forward and reverse primers specific to the target genes (10µM), and nuclease-free water to achieve the final volume. To ensure statistical robustness, three replicates were included for each sample, with six samples analyzed per site and sex. One no-template control (NTC) was included per reaction plate to verify the absence of contamination.

Normalized gene expression levels were calculated using the $\Delta\Delta C_q$ method (Livak & Schmittgen, 2001). Bioinformatics tools from SRPlot (Tang et al., 2023) were utilized to process and analyze the resulting qPCR data. To examine the distribution of the gene expression data, Shapiro-Wilk test was performed on the

data for each group (site and sex) to test for normality. Since the data did not meet the assumption of normality, non-parametric methods were chosen for further analysis. A Kruskal-Wallis Test (non-parametric one-way ANOVA) followed by Dunn's test was performed to compare gene expression between each site, with Bonferroni correction applied to adjust for multiple comparisons and reduce the likelihood of Type I errors. (Figures 1S and 2S). A significance level of $\alpha = 0.05$ was used for all statistical tests.

IV.3.8. Morphological endpoints

Morphological endpoints were used to calculate the condition factor (K), gonadosomatic index (GSI), and liver somatic index (LSI) as follows:

$$K = 100 \times (\text{body weight} / \text{length}^3),$$

$$\text{GSI} = 100 \times (\text{gonad weight} / \text{body weight}), \text{ and}$$

$$\text{LSI} = 100 \times (\text{liver weight} / \text{body weight}).$$

These physiological indices allow us to relate overall fish condition and energy allocation to the observed transcriptomic responses.

IV.3.9. Statistical Analysis

Statistical analyses were conducted using R version 4.4.1. Before analysis, the Shapiro-Wilk test was applied to assess normality within each group. For datasets that did not meet the assumptions of normality, non-parametric tests were used throughout.

For morphological indices (K, GSI and LSI), group comparisons across sites were conducted using the Kruskal-Wallis test, followed by Dunn's post hoc test with Bonferroni correction to adjust for multiple comparisons.

For qPCR gene expression data, normalized relative expression was calculated using the $\Delta\Delta\text{C}_q$ method (Livak & Schmittgen, 2001). Group differences in gene expression across sites and sexes were analyzed using Kruskal-Wallis tests, followed by Dunn's test with Bonferroni correction when appropriate (Figures S1 and S2).

Additionally, a Wilcoxon Test was performed to assess the similarities between RNASeq and qPCR derived fold changes ($\log_2FC$) values for genes analyzed by both platforms, validating concordance across molecular dataset.

All statistical tests were two-tailed, with a significance threshold of $\alpha = 0.05$. Data visualization and figure generation were performed using ggplot2 and ggpubr.

IV.4. Results

IV.4.1. MeHg concentrations

Table IV-1 summarizes MeHg concentrations in rainbow trout collected from three river systems—Loa, Choapa, and Trancura—arranged along a latitudinal gradient and representing distinct levels of mercury bioaccumulation. For each site, MeHg concentrations are reported separately for female and male fish, including the mean, minimum, and maximum values ($\mu\text{g/g}$ dry weight; dw). In the Loa River, classified as a High bioaccumulation site, MeHg levels were highest, with females showing a mean of $1.25 \mu\text{g/g}$ dw (ranging from 0.93 to $1.55 \mu\text{g/g}$ dw) and males slightly lower, averaging $1.08 \mu\text{g/g}$ (range: 0.84 – $1.35 \mu\text{g/g}$ dw). The Choapa River, representing a Medium bioaccumulation category, showed intermediate concentrations, with female trout averaging $0.27 \mu\text{g/g}$ dw (0.17 – $0.40 \mu\text{g/g}$ dw) and males $0.32 \mu\text{g/g}$ dw (0.10 – $0.47 \mu\text{g/g}$ dw). In contrast, the Trancura River, designated as a Low bioaccumulation site, had the lowest MeHg concentrations observed, with both females and males averaging just $0.03 \mu\text{g/g}$ dw and maximum values not exceeding $0.04 \mu\text{g/g}$ dw.

While females tended to have slightly higher MeHg levels than males at some sites, statistical analyses revealed no significant differences in MeHg concentrations between sexes (Figure S1). Consequently, male and female data were pooled for each river system to simplify interpretation and classification. Based on the resulting pooled concentrations, we established three distinct bioaccumulation categories—Low (Trancura), Medium (Choapa), and High (Loa)—which reflect consistent spatial trends in MeHg accumulation across the latitudinal gradient for the following transcriptomic analysis.

Table IV.1 MeHg concentrations in rainbow trout, arranged along a latitudinal gradient. The data includes average ($\mu\text{g/g}$ wet weight), minimum (Min) and maximum (Max) values for each river and species.

Site	Bioaccumulation classification	MeHg Female			MeHg Male		
		Mean	Min	Max	Mean	Min	Max
Loa	High	1.25	0.93	1.55	1.08	0.84	1.35
Choapa	Medium	0.27	0.17	0.40	0.32	0.10	0.47
Trancura	Low	0.03	0.02	0.04	0.03	0.03	0.04

IV.4.2. RNA Sequenciation

From a total of 39,000 genes detected, a substantial number exhibited differential expression across all comparisons (Figure IV.2).

In females transitioning from low to medium MeHg levels (Female Low/Medium), a notable trend of upregulation was observed, with 1536 genes showing increased expression compared to 909 downregulated genes. Conversely, males in the same comparison (Male Low/Medium) exhibited a slightly higher number of downregulated genes (514) relative to upregulated genes (429). When comparing low to high MeHg levels, females (Female Low/High) now displayed a greater number of downregulated genes (1532) than upregulated genes (1207). In contrast, males in the Low/High comparison (Male Low/High) showed a higher count of both downregulated (1485) and upregulated (1637) genes, with a more pronounced difference in the number of upregulated genes. Females in comparison with Medium/High MeHg bioaccumulation levels continued to show a predominance of downregulated genes (1727) over upregulated genes (1549), while males (Medium/High) exhibited a striking decrease in DEGs, showing less upregulated genes (429) compared to downregulated genes (514).

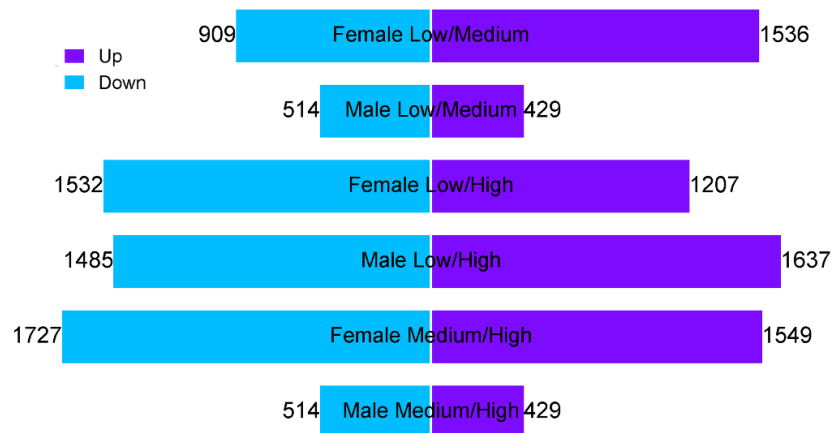


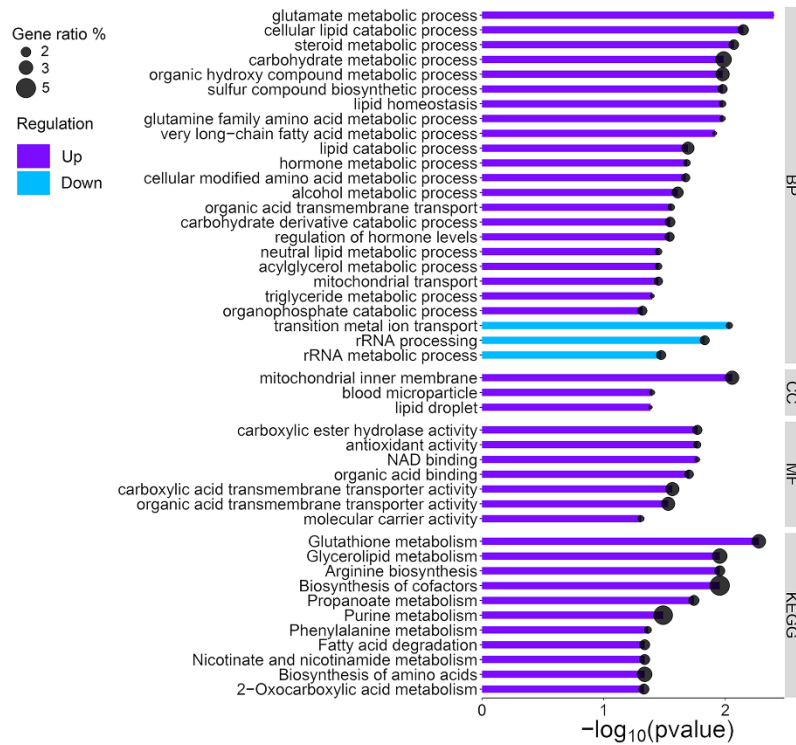
Figure IV.2 Number of differentially expressed genes (DEGs) in each comparison, categorized by sex and MeHg bioaccumulation level. Genes are classified as either "upregulated" (increased mRNA expression, represented in purple) or "downregulated" (decreased mRNA expression, represented in cyan) in the medium/high MeHg exposure groups relative to the low MeHg exposure groups.

IV.4.3. Pathway and gene ontology analysis

Female trout

Gene ontology analysis of medium MeHg levels in female trout (Figure IV.3a) led to the significant enrichment of several terms, with a general trend of upregulation. Enriched biological processes (BP) were largely metabolic, including those related to lipids, carbohydrates, amino acids, and hormones. The mitochondrial inner membrane and blood microparticles were enriched cellular components (CC). Enriched molecular functions (MF) included enzyme activities (e.g., hydrolase, antioxidant) and transport functions. KEGG pathway analysis highlighted the enrichment of various metabolic pathways, such as glycolysis/gluconeogenesis and amino acid biosynthesis. Female trout with higher MeHg levels (Figure IV-3b), enriched BP shifted towards regulatory functions and cellular localization. The ribosomal subunit was enriched in CC, and MF terms related to catalytic and regulatory activities were prominent. KEGG pathways showed enrichment in metabolism and the p53 signaling pathway, with a greater proportion of downregulated genes compared to medium MeHg bioaccumulation.

a) Medium MeHg level in Female trout



b) High MeHg level in Female trout

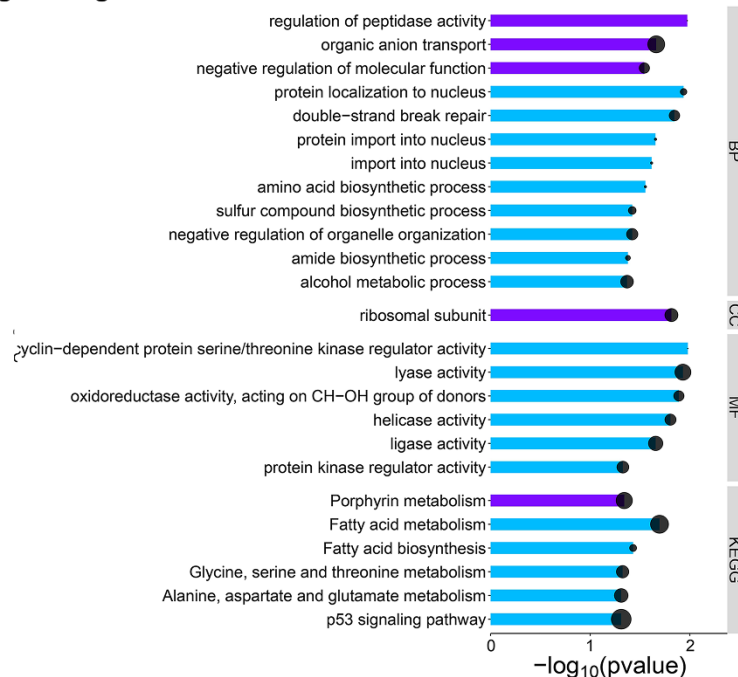
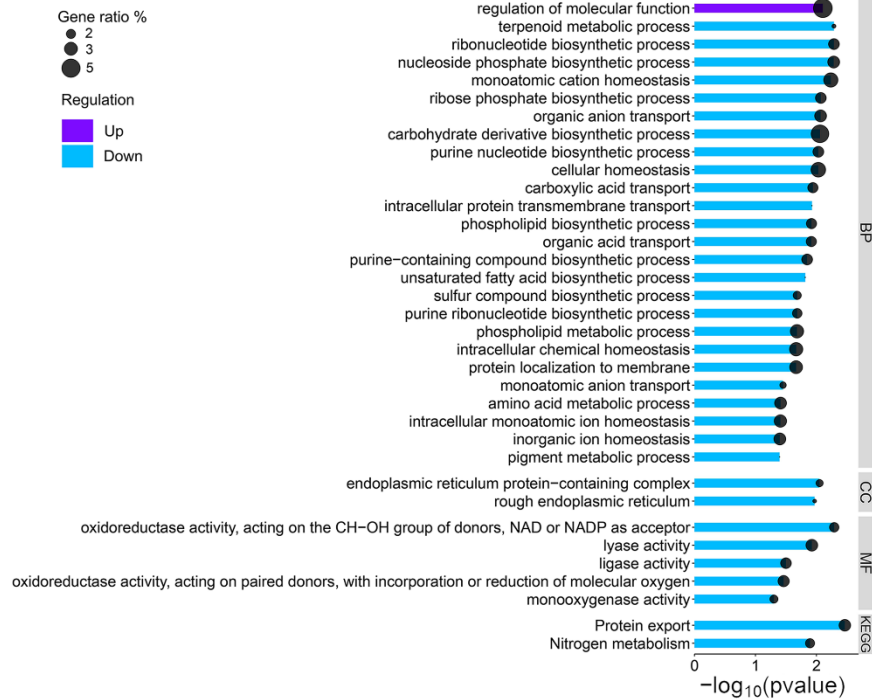


Figure IV.3 Most relevant terms ($\text{padj} = [0.001, 0.05]$; Fold enrichment > 2; Gene Ratio > 1 %) influenced by MeHg bioaccumulation in female rainbow trout with medium and high MeHg levels. Bar color indicates the direction of gene expression change.

Male trout

Medium MeHg levels in male trout (Figure IV.4a) resulted in the significant enrichment of a wide array of BP, primarily downregulated, encompassing metabolism (e.g., terpenoid, ribonucleoside, phospholipid), transport (e.g., organic anion, carboxylic acid), homeostasis (e.g., monoatomic ion, cellular, intracellular chemical), and cellular organization (e.g., protein localization to membrane, endoplasmic reticulum-related). Correspondingly, enriched MF included oxidoreductase, lyase, and ligase activities, as well as protein export. KEGG pathway analysis highlighted protein export and nitrogen metabolism. Conversely, high MeHg bioaccumulation in male trout (Figure IV-4b) led to the enrichment of BP related to upregulation (e.g., of molecular function, catalytic activity, proteolysis), catabolism (e.g., pyridine nucleotide, purine-containing compound, carbohydrate), stress response (e.g., response to oxidative stress, positive regulation of apoptotic process/programmed cell death), and nucleotide metabolism. Enriched MF shifted to peroxidase, peptidase inhibitor/regulator, methyltransferase, and protein phosphatase regulator activities, along with metal cluster binding and catalytic activity acting on RNA. KEGG pathway analysis revealed enrichment in carbon metabolism, biosynthesis of amino acids, and pathways involved in sugar and nucleotide interconversions.

a) Medium MeHg level in Male trout



b) High MeHg level in Male trout

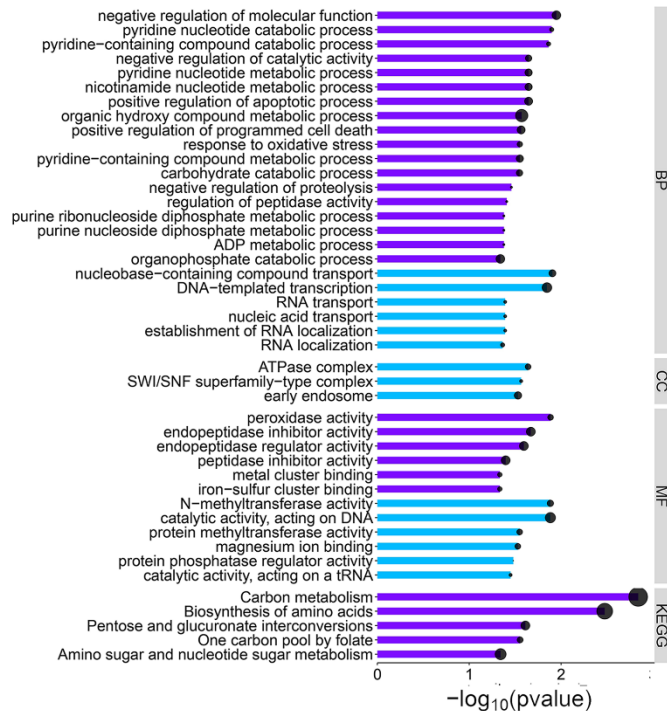


Figure IV.4 Most relevant terms ($padj = [0.001, 0.05]$; Fold enrichment > 2; Gene Ratio > 1 %) influences by MeHg bioaccumulation in male rainbow trout with medium and high MeHg levels. Bar color indicates the direction of gene expression change.

IV.4.4. Differential Gene Expression in response to MeHg (RT-qPCR)

Figure IV.5 shows how gene expression patterns are influenced by the level of MeHg bioaccumulation. As MeHg bioaccumulation increases from low (green) to medium (yellow) and high (red), the magnitude of gene expression changes becomes more pronounced. Distinct sex-specific differences in gene expression are evident across all MeHg bioaccumulation levels. While both sexes exhibit a range of up- and downregulated genes, the degree and direction of change vary significantly between females and males. Taking a fold change (FC) threshold of ± 1 into account, the genes estrogen receptor α (*era*), phosphoribosyl pyrophosphate amidotransferase (*ppat*), and metallothionein b (*mtb*) showed statistically significant differences between bioaccumulation levels but did not exhibit substantial changes in expression in either female or male trout.

In female trout as bioaccumulation increases to medium, the gene coding for zona pellucida (*zp*) exhibits significant upregulation ($\log_2FC = 1$), which is further enhanced at the higher bioaccumulation level ($\log_2FC = 1.5$). This upregulation is statistically significant between low and high levels ($p = 0.026$). Female trout genes coding for peroxiredoxin like 2B (*prxl2b*) and renin (*ren*) show significant downregulation at both medium (*prxl2b* $\log_2FC = -0.9$ and *ren* $\log_2FC = -1.2$) and higher MeHg levels (*prxl2b* $\log_2FC = -1.3$ and *ren* $\log_2FC = -1.1$).

In male trout, starting at the medium MeHg bioaccumulation level, genes coding for *prxl2b* and *ren* also show significant downregulation, in medium (*prxl2b* $\log_2FC = -1.2$ and *ren* $\log_2FC = -1.6$) MeHg levels. At the high bioaccumulation level, the gene coding for estrogen receptor β (*erb*) exhibits significant upregulation ($\log_2FC = 1.3$) compared to low and medium levels ($p = 0.005$). Similarly, *mtb* is also significantly upregulated at high MeHg levels in males ($\log_2FC = 1$; $p = 0.008$).

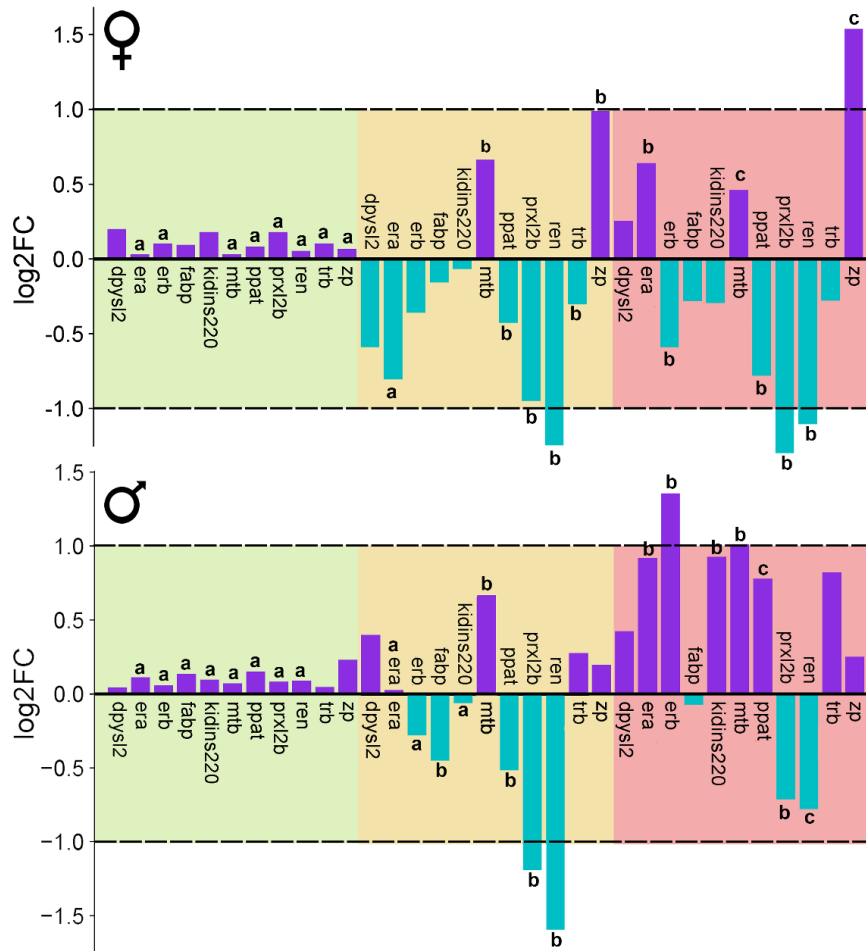


Figure IV.5 Gene expression response to MeHg levels (Low-Green, Medium-Yellow and High-Red), separated by sex (female ♀ and male ♂). Values in purple bars are up-regulation, and values in blue bars are down-regulation. The horizontal dashed lines at values 1 and -1 represent a threshold for biologically significant changes. N = 6 per site and sex. The letter on top and below the bars represents the statistical difference between sites by gene.

IV.4.5. Trout condition

In female trout (Figure IV.6), individuals from the high MeHg bioaccumulation group exhibited significantly higher K values compared to those from the medium bioaccumulation group ($p = 0.031$), but there is no significant difference with the low MeHg group. Although differences in HSI were not statistically significant between groups, there was a trend toward higher HSI values in the low MeHg group ($p = 0.096$; HSI close to 1 and over 1), and lower HSI values in the high MeHg group (HSI close to 0.5).

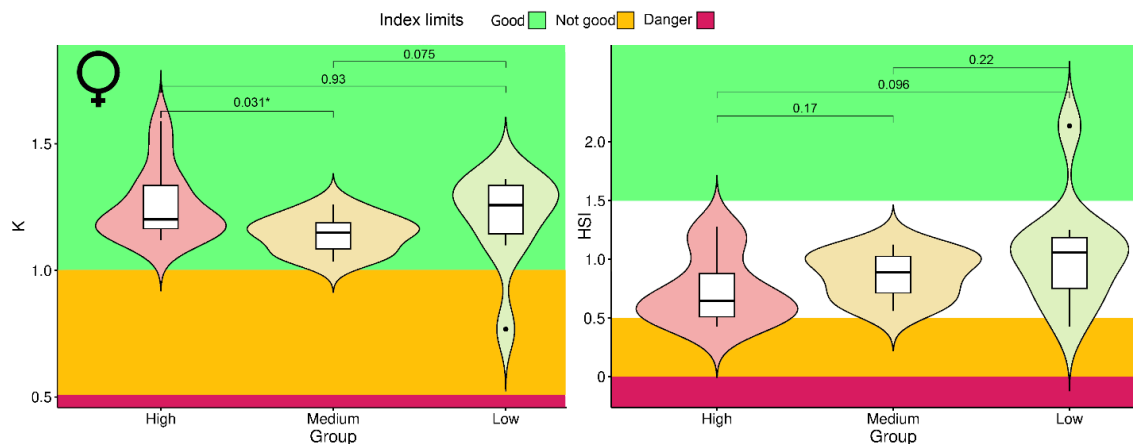


Figure IV.6 Distribution, median, and interquartile range of K (left) and HSI (right) in female trout across the MeHg bioaccumulation groups: High, Medium, and Low). Background colors represent health index limits: green = Good, yellow = Not good, red = Danger. Statistical comparisons between groups are shown with corresponding p-values; * indicates significance at $p < 0.05$.

In male trout (Figure IV.7), both K and HSI showed significant differences across MeHg bioaccumulation levels. Males from the low ($p = 0.0029$) and high ($p = 0.0027$) MeHg group had significantly higher K values than those from the medium group. Similarly, to the females, HSI values were significantly higher in the low bioaccumulation groups compared to the high group ($p = 0.036$), but also the medium MeHg bioaccumulation group was statistically higher than the high MeHg group ($p = 0.049$).

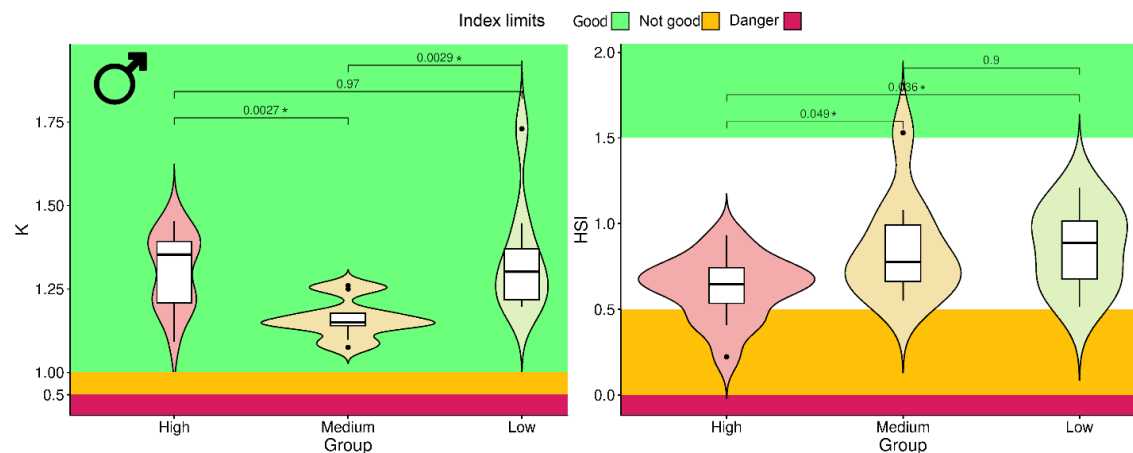


Figure IV.7 Distribution, median, and interquartile range of K (left) and HSI (right) in male trout across three MeHg bioaccumulation groups: High, Medium, and Low. Background colors represent health index limits: green = Good, yellow = Not good, red = Danger.

Statistical comparisons between groups are shown with corresponding p-values; * indicates significance at $p < 0.05$.

IV.5 Discussion

The findings from the RNASeq analysis suggest differential effects of MeHg bioaccumulation on gene expression, with notable variations based on both sex and bioaccumulation level. The large number of DEGs across comparisons highlights the transcriptome's sensitivity to MeHg, suggesting that even moderate levels of bioaccumulation can lead to measurable molecular alterations. Importantly, these transcriptomic changes were accompanied by shifts in physiological indices, establishing a link between molecular responses and apical biological outcomes.

In general, Females exhibited more DEGs than males across comparisons, particularly at the medium MeHg levels, where upregulated genes were more prominent. Whereas males showed predominantly downregulated genes at high MeHg bioaccumulation levels. This diverging pattern suggests that females may engage in an active metabolic strategy, while males might initiate a more robust compensatory or stress-response mechanism at higher toxicant levels (Zhang et al., 2012). The greater transcriptomic responsiveness in females at moderate bioaccumulation, particularly in pathways associated with lipid metabolism and cellular homeostasis, could be attributed to their increased energy demands for reproduction, which are especially sensitive to toxicant interference (Sun et al., 2020). Conversely, the marked transcriptional activation observed in males at high MeHg levels may reflect the engagement of detoxification and survival pathways, albeit with potentially higher physiological costs (Richter et al., 2014; Ruszkiewicz et al., 2019; Unoki et al., 2018).

Enrichment analysis revealed that MeHg bioaccumulation affects core metabolic and detoxification-related pathways, such as carbon metabolism, amino acid biosynthesis, and oxidative stress responses. In these analyses, it is also observed a sex-specific difference. Enrichment in down-regulated pathways, suggesting a metabolic suppression strategy in female trout at high MeHg levels. However, at high MeHg levels in male trout, upregulation in apoptosis-related,

mitochondrial, and antioxidative pathways suggests activation of damage control and repair mechanisms (Bassler et al., 2019; Bernhoft, 2012; Shilling et al., 2010). These results align with previous studies showing MeHg inhibits enzymes involved in metabolic pathways, such as those related to glutathione synthesis and antioxidant defense, impairing the liver's ability to neutralize reactive oxygen species (ROS) and other toxic metabolites (Desjardins et al., 2024; Farina et al., 2011). The involvement of the p53 signaling pathway in females may indicate a stress-related response possibly linked to DNA repair or cell death (Danzmann et al., 2016; Phalen et al., 2017). These effects establish the liver as a primary target organ for MeHg toxicity in fish, highlighting its role in assessing MeHg-related health risks.

Although differences in gene expression were observed for several genes between sexes (e.g., *era*, *ppat*, and *mtb* in females and males), it was important to avoid overinterpreting these results. In this study, genes significantly differentially expressed were considered only if they showed an absolute $\log_2$ fold change ($\log_2FC$) ≥ 1 and an adjusted p-value (FDR) < 0.05 . These thresholds helped ensure that only biologically and statistically meaningful changes were interpreted, while genes below these cutoffs were not considered relevant. (McCarthy & Smyth, 2009).

As for the validation of the RNASeq results through qPCR, from the total of five genes tested (*kidins220*, *dpysl2*, *ppat*, *prx2b* and *ren*), three of them showed to be consistent with the RNASeq results (*ppat*, *prx2b* and *ren*; Table S1). Also, the qPCR results showed to be slightly underestimated compared to RNASeq. This is consistent with previous study that says that 85% of genes showed consistent expression measurements between RNASeq and qPCR, but a small subset exhibited inconsistencies, often attributed to gene characteristics like smaller size or lower expression levels (Everaert et al., 2017).

The targeted qPCR results confirm sex-specific and MeHg-dependent expression of key genes. In females, *zp* was significantly upregulated at medium and high MeHg levels, indicating MeHg-induced reproductive disruption, consistent with previous findings on oocyte maturation impairment (Klaper et al., 2006, 2008).

Genes associated with oxidative stress and blood pressure regulation—*prxl2b* and *ren*—were downregulated in both sexes, supporting their potential as universal biomarkers of MeHg bioaccumulation.

In males, the upregulation of estrogen receptor β (*erb*) at high MeHg levels may reflect endocrine disruption through feminization or altered steroid signalling (Jarque et al., 2015; Tinant et al., 2021). Similarly, increased expression of *kidins220*, involved in synaptic plasticity and neuroprotection, may indicate a systemic neuroendocrine stress response, suggest indirect neurotoxicity signalling through liver-mediated pathways (e.g., oxidative stress, or metal detoxification; Arévalo et al., 2010; Neubrand et al., 2012), or reflect the liver's role in producing neuroactive molecules or hormones such as estrogen or cortisol (Giovanini et al., 2025).

The sex-specific gene expression patterns observed in this study were reflected by physiological changes, particularly in the hepatosomatic index (HSI), a widely used indicator of energy reserves, liver function, and overall health (Dekic et al., 2016). HSI values above 1.5% are typically associated with healthy fish populations, while values below 0.5% may reflect nutritional depletion or liver dysfunction (Dane & Şişman, 2023; Saha & Thomas, 2020). However, in contaminated environments, elevated HSI can also result from hepatomegaly due to increased detoxification activity, complicating interpretation (Budi et al., 2024). In this study, trout from high MeHg sites (both females and males) exhibited HSI values approaching 0.5%, suggesting compromised liver function and possible nutritional stress. In contrast, fish from low MeHg sites had HSI values closer to 1%, which, although suboptimal, do not indicate severe physiological stress. These HSI patterns are consistent with transcriptomic results. Trout from high MeHg environments showed a general downregulation of genes related to metabolism and energy production, particularly in males, indicating impaired hepatic function and reduced metabolic capacity (Coimbra et al., 2018). This molecular suppression aligns with the lower HSI values observed, reinforcing the interpretation of liver dysfunction under elevated MeHg exposure. Conversely, females showed more upregulated genes in metabolic pathways, suggesting a

sex-specific capacity for metabolic compensation that may explain their relatively higher HSI values despite similar contaminant exposure. Importantly, male trout more frequently fell into HSI-based ecological health categories such as “Not good” or “Danger,” and also exhibited stronger transcriptomic suppression in key metabolic and detoxification pathways. This reinforces the notion of sex-specific vulnerability, with males showing a lower physiological and molecular capacity to cope with MeHg stress. These findings align with previous studies documenting MeHg’s negative effects on growth, reproduction, and population dynamics in aquatic vertebrates (Depew et al., 2012; Morcillo et al., 2017), and highlight the value of integrating molecular biomarkers with morphometric indices like HSI for robust environmental health assessments.

The K-index, a proxy for overall body condition, showed less consistent patterns. While significant differences were detected between MeHg exposure levels, no clear trend emerged that could be directly linked to transcriptomic outcomes. Nevertheless, all trout displayed K-values above 1, suggesting generally adequate body condition and energy stores across populations, with no evidence of starvation or critical stress (Amara et al., 2007; Selleslagh & Amara, 2013).

IV.6 Conclusions

Understanding what constitutes healthy or concerning values for morphometric indices is essential for fisheries management and conservation, especially when evaluating environmental stressors such as contaminant exposure. In this study, we observed sex-specific and dose-dependent transcriptomic and physiological differences in wild rainbow trout (*Oncorhynchus mykiss*) from Chilean freshwater ecosystems in relation to varying levels of MeHg bioaccumulation.

Using integrated RNASeq and qPCR analyses, we found that moderate to high MeHg tissue concentrations were associated with notable changes in gene expression—particularly in pathways related to metabolism, oxidative stress, and endocrine function. In females, high MeHg levels corresponded with stronger transcriptional downregulation in genes linked to lipid metabolism and cellular homeostasis, potentially reflecting an energy-conserving strategy, possibly influenced by reproductive demands. In contrast, males exhibited marked

upregulation of genes involved in detoxification and stress-response pathways, suggesting a more reactive metabolic profile that may come with increased energetic costs.

These molecular patterns were paralleled by physiological changes, especially in the HIS. Trout from sites with higher MeHg levels—particularly males—tended to show lower HSI values, which may reflect altered liver function or depleted energy reserves. While these findings do not establish causation, the observed concordance between MeHg levels, gene expression changes, and morphometric responses points to potential physiological stress linked to contaminant exposure. Notably, several genes—including *zp*, *prxl2b*, *ren*, and *erb*—displayed consistent expression shifts aligned with MeHg bioaccumulation patterns and may serve as promising candidates for future biomonitoring of sublethal contaminant effects in freshwater systems.

In conclusion, this work provides the first evidence of sex-specific transcriptomic and physiological responses to MeHg bioaccumulation in wild rainbow trout from South America. These findings underscore the importance of including sex as a biological variable in environmental risk assessments and support the development of biomarker-informed monitoring strategies that integrate molecular, physiological, and ecological health indicators. Given the growing anthropogenic pressure on freshwater ecosystems, such integrated approaches are essential for effective environmental assessment and conservation planning.

IV.7. References

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Capítulo V. **Risk map of human intake of mercury through fish consumption in Latin America and the Caribbean**

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Risk map of human intake of mercury through fish consumption in Latin America and the Caribbean

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Mercury (Hg) is a persistent pollutant highly bioaccumulated in the aquatic environment through the food chain reaching high concentration levels in the tissues of predator fishes. Among the relevant sources of anthropogenic mercury emissions in Latin America and the Caribbean (LAC), mining is one of the most important along with soil erosion due to deforestation and agricultural activities where pesticides are intensively used. Several reports have demonstrated an association between a fish-based diet with elevated Hg levels in the blood and neurotoxic effects in humans. In this systematic review with quantitative analysis data from 92 articles were compiled, providing evidence of Hg concentration in fishes that are commonly consumed in LAC. An assessment was conducted using three indices for health risk: the Minamata Initial Assessment (MIA), the Target Hazard Quotient (THQ), and the Meals per Week (MPW) index. Of the 410 fish species studied, 5.4% had concentrations above $0.95 \mu\text{g g}^{-1}$ of wet weight (ww), which is the recommended limit for Total Hg (THg) ingestion through fish consumption according to the MIA index, regardless of the water habitat (i.e., marine or freshwater). Additionally, the Target Hazard Quotient (THQ) calculation indicated high-risk values ($\text{THQ} \geq 1$) in 15 out of the 19 countries studied, and very high-risk values ($\text{THQ} \geq 10$) were obtained from Hg concentrations measured in 5 fish species inhabiting watersheds in Trinidad and Tobago, Suriname and Peru. Finally, recommendations on fish consumption were made based on the MPW index. This study reveals the need for updated biomonitoring studies of Hg concentrations in fish to perform more accurate human health risk analyses.

KEYWORDS

health risk assessment, mercury, Latin America and the Caribbean, fish consumption, mercury intake

V.1. Abstract

Mercury (Hg) is a persistent pollutant highly bioaccumulated in the aquatic environment through the food chain reaching high concentration levels in the tissues of predator fishes. Among the relevant sources of anthropogenic mercury emissions in Latin America and the Caribbean (LAC), mining is one of the most important along with soil erosion due to deforestation and agricultural activities where pesticides are intensively used. Several reports have demonstrated an association between a fish-based diet with elevated Hg levels in the blood and neurotoxic effects in humans. In this systematic review with quantitative analysis data from 92 articles were compiled, providing evidence of Hg concentration in fishes that are commonly consumed in LAC. An assessment was conducted using three indices for health risk: the Minamata Initial Assessment (MIA), the Target Hazard Quotient (THQ), and the Meals per Week (MPW) index. Of the 410 fish species studied, 5.4% had concentrations above $0.95 \mu\text{g g}^{-1}$ of wet weight (ww), which is the recommended limit for Total Hg (THg) ingestion through fish consumption according to the MIA index, regardless of the water habitat (i.e., marine or freshwater). Additionally, the Target Hazard Quotient (THQ) calculation indicated high-risk values ($\text{THQ} \geq 1$) in 15 out of the 19 countries studied, and very high-risk values ($\text{THQ} \geq 10$) were obtained from Hg concentrations measured in 5 fish species inhabiting watersheds in Trinidad and Tobago, Suriname and Peru. Finally, recommendations on fish consumption were made based on the MPW index. This study reveals the need for updated biomonitoring studies of Hg concentrations in fish to perform more accurate human health risk analyses.

Keywords: Health risk assessment, mercury, Latin America and the Caribbean, fish consumption, mercury intake

V.2. Introduction

Mercury (Hg), in its various chemical forms, is widely distributed in the environment. It is found in different ecosystems due to its many natural sources such as volcanic emissions, soil and rock erosion, and wildfire, but also due to anthropogenic activities such as fossil fuel burning and mining extraction (Pirrone et al., 2010). According to the UN Environment (2019). Latin America and the Caribbean (LAC) in 2015 released 20.5% of global anthropogenic Hg air emissions, of which small-scale gold mining contributed to 354.3 t of Hg emissions, followed by the industry sectors (66.4 t of Hg) and intentional use (including product waste; 20.21 t of Hg). In addition, South America alone accounted for 53% of the estimated 1,220 t of Hg released globally from small-scale gold mining to aquatic and terrestrial environments in 2015 (Canham et al., 2021).

Aquatic ecosystems are one of the most relevant environments for the cycling of Hg deposited from the atmosphere since its methylation is carried out mainly by benthic microorganism transformations that produce methylmercury (MeHg), an organic form of Hg with higher toxicity (Al-Sulaiti et al., 2022). More than 85–90% of the Hg found in fish tissues is MeHg and its concentration depends on its trophic position, age, and size (Grieb et al., 1990; Southworth et al., 1995). Higher MeHg is found in those predator species with the highest trophic position in the food chain (Lavoie et al., 2013).

In an ecotoxicological report from Brazil, it was found that top predator fish, which are locally consumed, present high levels of Total mercury, THg, ($1.2\text{--}2.1\ \mu\text{g g}^{-1}$ wet weight, ww; Hacon et al., 2020), well above the risk threshold level of $0.95\ \mu\text{g g}^{-1}$ ww according to MIA recommendations (MIAs in the Caribbean, 2017). This and other studies (Rice et al., 2014; Gribble et al., 2015) evidence that in LAC humans are exposed to risky levels of Hg and its associated harmful effects through a diet based on fish consumption. Furthermore, reported evidence obtained in LAC indicates associations between consumption of marine and freshwater fishes displaying high levels of Hg (THg and MeHg) concentrations

and detectable amounts of this metal in human hair (Bravo et al., 2019; Valdelamar-Villegas and Olivero-Verbel, 2020).

It is widely known that human exposure to high levels of MeHg/THg is associated with neurotoxicity (Castoldi et al., 2001). One of the most well-known cases of high-scale poisoning with fish and seafood contaminated with MeHg was the Minamata disaster in the 1950s at Minamata Bay in Japan. This compound was generated as a by-product in reaction chambers for manufacturing acetaldehyde and discharged directly to the bay, resulting in fish and seafood bioaccumulation that later was consumed by the local inhabitants. The intake of contaminated marine products produced a syndrome called Minamata disease, with numerous severe neurological and physical sequelae on the muscle, sight, and hearing systems (Eto, 2000; Hachiya, 2006). Besides these high exposure levels to MeHg, chronic exposure to lower levels can cause hepatic diseases and reproductive toxicity (Tan et al., 2009; Rice et al., 2014). In 2013, the Minamata Convention was created, and to date, 137 countries have adopted the agreement, of which 20 are countries from LAC (Coulter, 2016). Therefore, it is of major importance to implement a risk assessment method that can be applied in decision-making and the generation of environmental regulations in vulnerable countries such as those of LAC. This literature review and meta-analysis seeks to assess the risk of Hg exposure and consequently health hazards due to fish consumption in LAC by gathering available data from THg levels in fish and fish consumption rates per country.

V.3. Methods

V.3.1. Literature review

This systematic review with a quantitative analysis focus was conducted following the PRISMA guideline (Figure V.1; Page and Left, 1996). A literature search on peer-reviewed journal articles and published reports documenting THg and/or MeHg concentrations in fish in LAC countries was conducted on Web of Science¹ and Google Scholar² according to Sheehan et al. (2014). Studies published from 2005 until 2024 were selected. Exceptionally some reports published before 2005

were included since for some countries there was no information after this year (Table V-1).

Studies were first selected based on the title and abstract screening, followed by full-text review and exclusion criteria. Studies that did not state if the concentrations were dry weight (dw) or wet weight (ww) were excluded. In addition, a secondary search was made on FishBase (Froese and Pauly, 2023) to determine if the fish species were human-consumed and exclude reports on non-consumed species. When the same species or site, was reported on multiple articles, the most recently published was chosen.

Table V.1 Keywords and concepts used in the bibliographic search and their connectors.

"mercury" OR "methylmercury" OR "mercury AND methylmercury"
"fish" OR "shark"
"Latin America" OR "Central America" OR "South America" OR "Amazon" OR "Amazonian" OR "Andes" OR "Andean" OR "Country name" (Example Argentina, Brasil, etc)
Combined terms: 1 AND 2 AND 3

Data were classified by fish species, country, sample size, habitat (marine or freshwater environment), and fish diet (herbivore, omnivore, and carnivore; Supplementary Table S1). In addition, the classification considered the type of mercury species measured (THg and/or MeHg) and the analytical methods used. All data reported in dw were converted to ww using the Equation 1, as described by Cresson et al. (2017).

$$C_{wet} = C_{dry} \times \left(\frac{100 - \%H}{100} \right) \quad (1)$$

Where, C_{wet} and C_{dry} correspond to the substance concentration of interest measured in wet and dry tissue respectively, and %H is the percentage of humidity in wet tissues (~80% for most of the species).

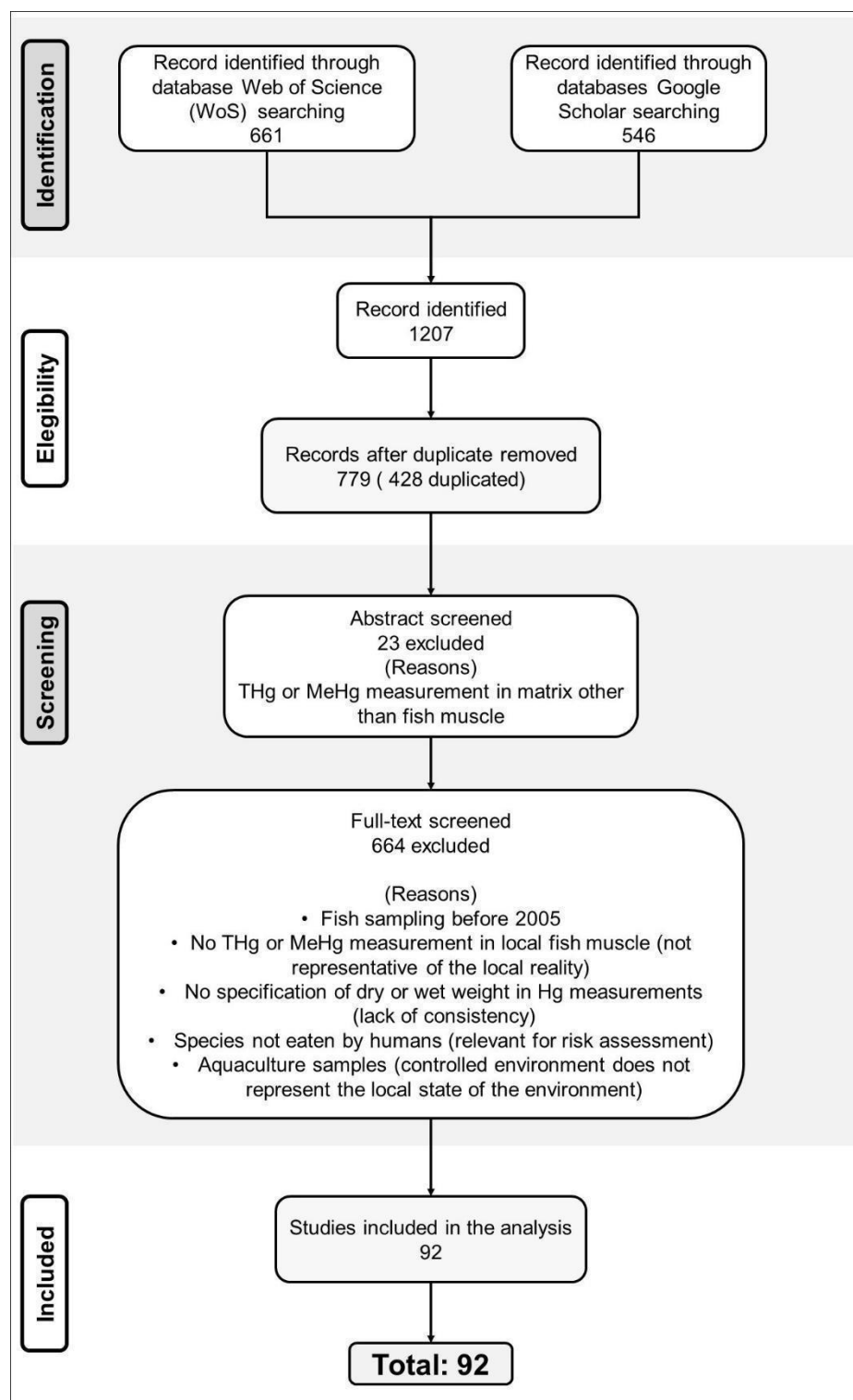


Figure V.1 Schema of the literature search on peer-reviewed journal articles and published reports documenting THg and/or MeHg concentrations in fish in Latin American and Caribbean countries.

V.3.2. Mercury risk assessment

As previously mentioned, data were filtered by (1) fish species that are consumed by humans, and (2) THg and/or MeHg concentration quantified in the muscle that corresponds to consumed fish tissue. Additional data was obtained for human health risk assessment such as average body weight by sex and average fish consumption per country and day. Average body weight was obtained from the Eglitis dataset (Eglitis, 2021) for adult women (BWW) and men (BWM) from each country analyzed. Average fish consumption per country and day (FCR) was estimated from the “Fish and seafood consumption per capita” dataset via www.ourworldindata.org and the National Oceanic and Atmospheric Administration (NOAA, 2014) which are expressed in kg per year.

The Target hazard quotient (THQ) is a non-carcinogenic index that assesses the health risk from food consumption by residents and is calculated based on Salazar-Camacho et al. (2022):

$$THQ = \frac{EF \times ED \times FCR \times CHg}{RfD \times BW \times AT} \times 10^{-3} \quad (2)$$

In Equation 2, EF corresponds to the exposure frequency (365 days/year); ED is the total duration of fish consumption, considering an average human life of 70 years (Roser et al., 2013); FCR is the fish consumption rate (g per day), and CHg is the MeHg concentration expressed as $\mu\text{g g}^{-1}$ (ww). Due to some reports included in the survey informing of THg instead of MeHg concentrations, a MeHg conversion factor has been applied according to the Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories (USEPA, 2011) which assumes that 95% of the Hg concentration measured in fish tissue is MeHg. BW is human body weight (kg); AT is the average time of food consumption ($EF \times 70$ years); and RfD is the reference dose which is the daily dosage that enables an individual to sustain a long-term level of exposure without experiencing any harmful effects (USEPA, 2011). A RfD of $0.1 \mu\text{g per kg per day}$ was used, according to EPA’s guidelines (USEPA, 2001).

Recommendations for fish consumption per week (MPW) were calculated with Equation 3, based on (Vieira et al., 2021):

$$MPW = \frac{(RfD \times BW) \times 7 \text{ days}}{(Fish \text{ meal size (g)} \times C_{Hg}) / 1000} \quad (3)$$

We have considered a fish meal size of 226.8 g of fish per week based on a 2000 cal diet according to the Dietary Guidelines for Americans (2015–2020). Body weight (BW) was obtained from the Eglitis data set from 2021 and is based on the BW average for the adult population in each country analyzed according to sex (BWW: women's body weight and BWM: men's body weight).

V.3.3 Data analysis

THg concentration is presented as median $\pm$ range ($\mu\text{g g}^{-1}$ ww, Supplementary Figure S1). The Mood's median test was used to estimate statistical differences ($p = 0.05$) in THg concentrations among dietary preferences, habitat type (marine or freshwater), and countries (Supplementary Table S2). Further analyses were applied (Mood's pairwise median tests) for comparison between aquatic environments (S3), diet preferences (S4), and countries (S5). The analysis was made using the R-packages (R version 4.1.2). Species showing a THg content $>0.95 \mu\text{g g}^{-1}$ (ww) were analyzed to determine their distribution according to the habitat (marine or freshwater) and diet preferences (carnivores, detritivores, herbivores, or omnivores). With these classifications, a Sankey plot was built using the Sankey Network function from the networkD3 R-package (Allaire et al., 2017).

To compare the risk (THQ) differences between women and men, a Mann–Whitney U-test was performed (Supplementary Table S6). To observe the geographic distribution of the Hg exposure risk by fish consumption among inhabitants of LAC countries, the obtained risk values for women and men were mapped using the geographic coordinates reported in the respective paper of origin. For this, the rgdal (Bivand et al., 2023) and ggplot2 (Wickham, 2016) R-

packages (R Core Team, 2021) were used. All plots resulting from the information gathered were created with ggplot2 and R-packages (R version 4.1.2).

V.4 Results

V.4.1 Analysis of fish species from LAC representing a risk for human consumption according to their Hg content

Studies concerning THg bioaccumulation in human-consumed fishes were found in around 55% of LAC countries including, from north to south: Mexico, Cuba, Jamaica, Belize, Anguilla, Nicaragua, Costa Rica, Colombia, Trinidad and Tobago, Venezuela, Suriname, Ecuador, Brazil, Peru, Bolivia, Chile, Paraguay, Uruguay and Argentina with a total data set of 1,046 samples. Countries reporting a higher number of fish species in which Hg bioaccumulation was measured (Table V.2) were Brazil (n = 194) and Mexico (n = 58).

Table V.2 Median and range of the THg concentration ($\mu\text{g g}^{-1}$ ww) in fish muscle by country and number of species (n), and the proportion of fish species with median concentrations $\geq 0.95 \mu\text{g g}^{-1}$ ww.

Country	THg [Min, Max]	Number of species (n)	% THg $\geq 0.95 \mu\text{g g}^{-1}$ ww
Anguilla	0.070 [0.004, 0.174]	8	0
Argentina	0.094 [0.007, 0.597]	15	0
Belize	0.355 [0.165, 0.545]	2	0
Bolivia	0.093 [0.007, 2.063]	12	8.5
Brazil	0.150 [0.002, 3.570]	194	7.1
Chile	0.056 [0.002, 0.800]	33	0
Colombia	0.094 [0.004, 2.530]	44	3.4
Costa Rica	0.032 [0.006, 0.065]	7	0
Cuba	0.034 [0.020, 0.218]	2	0
Ecuador	0.0330 [0.010, 1.260]	18	5.6
Jamaica	0.047 [0.002, 0.143]	16	0
Mexico	0.059 [0.004, 0.500]	58	0
Nicaragua	0.022 [0.004, 0.164]	7	0
Paraguay	0.374 [0.138, 0.921]	4	0
Peru	0.094 [0.004, 2.800]	40	2.7
Suriname	0.215 [0.040, 2.800]	17	25.8
Trinidad and Tobago	0.034 [0.006, 2.218]	15	13.3
Uruguay	0.214 [0.024, 1.310]	12	8.3
Venezuela	0.314 [0.070, 1.600]	17	11.8

A total of 410 fish species were analyzed from studies reporting THg and/or MeHg levels in muscle. THg concentrations in freshwater fish range from 0.003 to 3.570 $\mu\text{g g}^{-1}$ ww; meanwhile marine fish range from 0.002 to 2.218 $\mu\text{g g}^{-1}$ ww (Supplementary Table S1). Most of the species were found in freshwater ecosystems; specifically, rivers (45%), lakes (7.4%), reservoirs (1.3%), and wetlands (1.5%). A lower number of species were from marine ecosystems and were distributed as follows: species from coastal environments (36.9%), coastal/oceanic environments (3.7%), oceanic species (2%), and estuaries (2.2%). The distribution of the fish species in the water column is mainly benthopelagic (45.6%) and benthic (36.4%), and a smaller portion is pelagic (18%).

The distribution of the species in the water column does not have a significant impact on the THg (Mood's median test, p-value = 0.058), contrary to the habitat (freshwater or marine, Mood's median test, p-value = 3.63e^{-8}) and aquatic environment where we found significant differences between the Coastal, Coastal/Oceanic and River fish (Mood's median test, p-value <0.001; Supplementary Tables S2, S3).

There are significant differences in THg content in fish muscle between species with different diet preferences. Carnivorous fish had significantly higher THg content than detritivores, herbivores, or omnivorous fishes (Mood's median test p-value <0.001, Supplementary Tables S2, S4). No differences were detected within the last three groups, regardless of the habitat. Fish species showing THg concentrations above MIA's standard ($\geq 0.95 \mu\text{g g}^{-1}$ ww) mostly correspond to carnivore species.

The Sankey plot (Figure V.2) shows a subsample of those fish species with a THg concentration $\geq 0.95 \mu\text{g g}^{-1}$ ww (5.4% of the data). This includes 44 fish species from 9 countries. According to MIA's the remaining samples are considered to have concentrations that are safe to be consumed unrestrictedly ($< 0.22 \mu\text{g g}^{-1}$ ww, 70.9%) or to be consumed in a moderated diet ($0.22\text{--}0.95 \mu\text{g g}^{-1}$ ww, 23.7%).

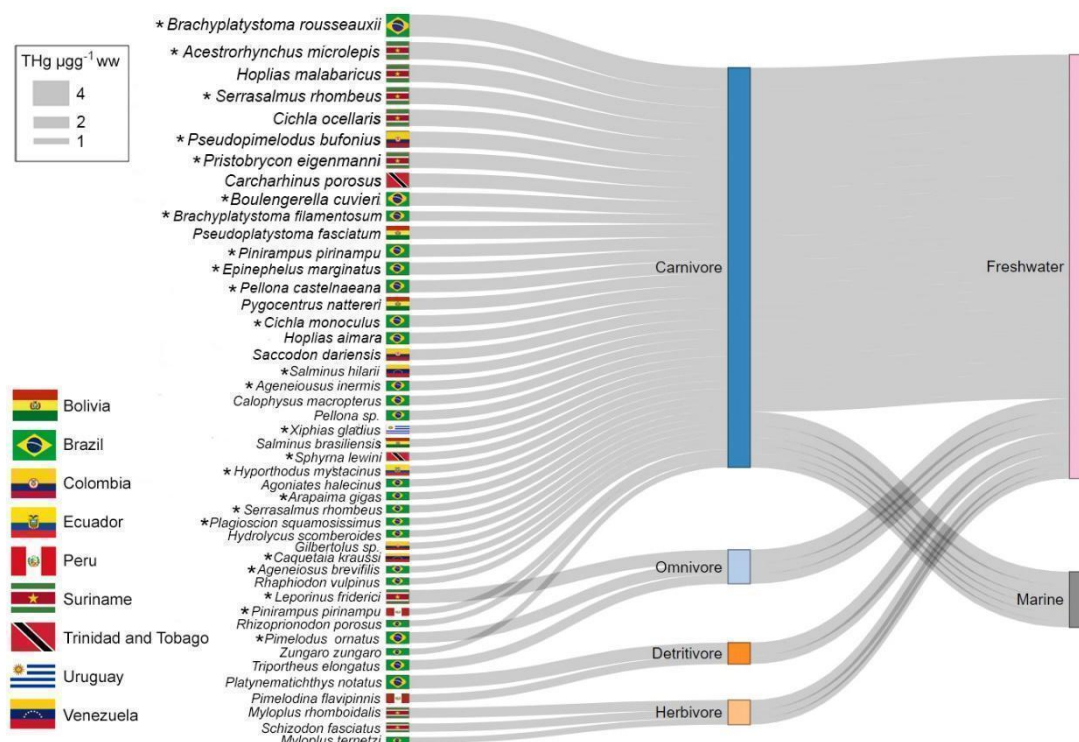


Figure V.2 Sankey diagram representing the distribution of THg concentrations in fishes from LAC. THg concentration ranges are represented by the width of grey ribbons. To the right, bars indicate the distribution between water habitats (marine or freshwater, respectively). Food preferences (carnivorous, omnivorous, detritivore, or herbivore) are indicated by colored bars in the middle. *: highlighted those species most consumed. Only species displaying THg concentrations above MIA's regulations ($\geq 0.95 \mu\text{g g}^{-1} \text{ ww}$) were considered in this diagram.

The plot showed that carnivorous species present the largest range and highest concentrations of THg (0.954 to $3.570 \mu\text{g g}^{-1} \text{ ww}$), independently of the water habitat. However, three omnivorous (1.560 to $2.140 \mu\text{g g}^{-1} \text{ ww}$), two detritivores (1.325 and $2.131 \mu\text{g g}^{-1} \text{ ww}$), and three herbivores (1.000 to $1.630 \mu\text{g g}^{-1} \text{ ww}$) also displayed THg concentrations that might deserve attention since most of the recommendations declare not to intake THg concentrations above $0.95 \mu\text{g g}^{-1} \text{ ww}$.

On the other hand, specimens showing high THg concentrations, and considered hazardous for human consumption (1.2 to $1.7 \mu\text{g g}^{-1} \text{ ww}$) by the regulation of FAO and WHO (2018) were five marine species and 31 freshwater species, were predators *C. porosus* ($2.218 \mu\text{g g}^{-1} \text{ ww}$) a marine fish from Trinidad and Tobago,

and the freshwater *B. rousseauxii* ($3.570 \mu\text{g g}^{-1} \text{ ww}$) from Brazil the highest THg concentration.

Brazil, Bolivia, Ecuador, Colombia, Peru, Suriname, Trinidad and Tobago, Uruguay, and Venezuela showed the fish species with the highest Hg levels. Belize, Paraguay, and Venezuela had the highest median values of THg concentration on fish muscle ($> 0.3 \mu\text{g g}^{-1} \text{ ww}$; Table 2). Statistical tests showed significant differences in the THg median between countries (Mood's median test, $p\text{-value} < 0.001$, Supplementary Table S2).

V.4.2. Human risk analysis of mercury intake by fish consumption

In Figure V.3, 77.2–79.3% of the total species studied showed to have a THQ < 1 , from which 68.7–69.2% showed THg concentrations lower than $0.22 \mu\text{g g}^{-1} \text{ ww}$, and 18.9–20.5% present concentrations above this value without exceeding the threshold value of $0.95 \mu\text{g g}^{-1} \text{ ww}$. Of the remaining species with THQ values ≥ 1 , only 5.6% have THg levels $\geq 0.95 \mu\text{g g}^{-1} \text{ ww}$. From this subset, only 0.8–0.9% have THQ values ≥ 10 . The group of species with THg concentrations above the MIA's recommended levels ($\geq 0.95 \mu\text{g g}^{-1} \text{ ww}$) were found in Brazil, Bolivia, Ecuador, Colombia, Peru, Suriname, Trinidad and Tobago, Uruguay, and Venezuela and should not be recommended for frequent human consumption. The distribution of the THQ index in the 19 LAC countries is in Supplementary Figure S2.

The results of risk estimation for fish consumption are displayed in Figure V.4, showing that THg concentrations $\geq 0.95 \mu\text{g g}^{-1} \text{ ww}$ and THQ ≥ 1 were from Brazil, Uruguay, Perú, Suriname, Trinidad and Tobago, and Venezuela. However, some species with THg concentrations below $0.95 \mu\text{g g}^{-1} \text{ ww}$ also show a THQ ≥ 1 in almost every analyzed country (Supplementary Table S1). Finally, considering all the species studied, the distribution by country of those showing a THQ ≥ 1 remained as follows: Anguilla (4 species), Argentina (2 species), Belize (1 species), Brazil (77 species), Chile (6 species), Colombia (16 species), Ecuador (9 species), Jamaica (2 species), Mexico (7 species), Paraguay (1), Peru (16 species), Suriname (12 species), Trinidad and Tobago (3 species), Uruguay (2

species), and Venezuela (10 species). The list of fish species is in Supplementary Table S1.

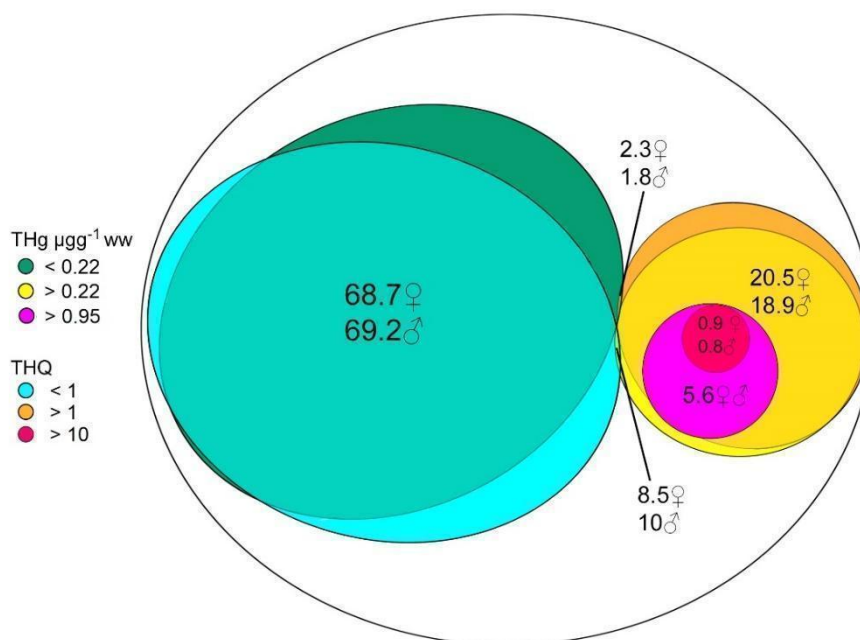


Figure V.3 Venn diagram summarizing the distribution of THg concentration (range THg < 0.22 $\mu\text{g g}^{-1} \text{ ww}$; 0.22 < THg < 0.95 $\mu\text{g g}^{-1} \text{ ww}$; and THg < 0.95 $\mu\text{g g}^{-1} \text{ ww}$) and its interaction with Target Hazard Quotient (THQ) ranges (THQ < 1; 1 > THQ > 10; and THQ > 10) in the total species assessed in this study. The data is presented in percentages and is sex-specific, indicated as ♂ (men) and ♀ (women).

There is a slight trend difference in the risk estimation regarding women (excluding lactating or pregnant women) and men, with women the group displaying higher THQ values, however, no statistical differences between both groups were found (Figure 4, Mann–Whitney U-test, p-value = 0.131, mean difference 0.020, Supplementary Table S6).

The countries estimated to have the greatest health risk were Brazil, Colombia, Peru, Suriname, and Venezuela due to more than 1/3 of the species studied from these countries presenting a THQ ≥ 1 (n = 77 from 203, 16 from 46, 16 from 43, 12 from 18, and 10 from 17, respectively). Fewer species contributed to very high THQ values (THQ > 10) namely *A. microlepis*, *H. malabaricus*, *S. rhombeus*, *C. ocellaris*, *P. eigenmanni*, and *L. friderici* from Suriname; *C. porosus* from Trinidad and Tobago; *P. flavipinnis* and *P. pirinampu* from Peru; and *B. rousseauxii* from Brazil.

Additionally, considering that a $THQ \geq 1$ is considered hazardous for human health, the frequency plot shows that most studied fish species have no risk for adult human consumption (68.7% for women and 69.2% for men).

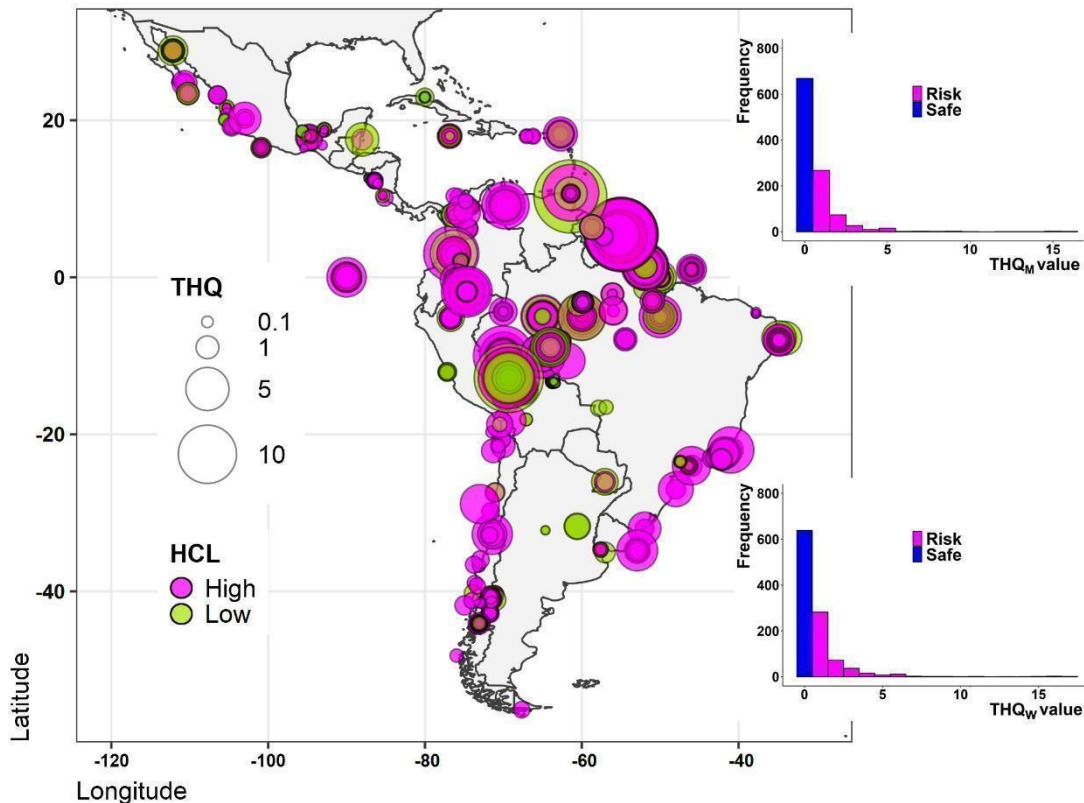


Figure V.4 Geolocation of the health risk estimation related to total mercury (THg) contaminated fish consumption from LAC. The adult human consumption level (HCL) of fish is indicated by color, where fish with a high consumption rate are those of high commercial interest (magenta), while fish with a low consumption rate are those of the local diet (green). Circle sizes indicate the average Target Hazard Quotient (THQ) value, associated with a fish species. The bar graphs illustrate the frequency of risky THQ values (≥ 1) and safe THQ values (< 1) for the men population (THQM, up) and women population (THQW, down).

V.4.3 Meals per week recommendations according to Hg intake

The latest Dietary Guidelines for Americans from the U.S. Department of Agriculture and U.S. Department of Health and Human Services advise eating around 226.8 g of fish per week, based on a 2,000-calorie diet USDA and HHS (2020). This recommendation would be acceptable for 70.9% of the data collected

in the present study based on the MPW estimation. MPW < 1 (29.06%) estimation corresponded in most of the cases to concentrations >0.22 µg g⁻¹ ww THg, which is recommended to eat once a month or to avoid in the species with levels higher than 0.95 µg g⁻¹ ww (MIAs in the Caribbean, 2017). Only 71.7% of the MPW lower than 1 had a THQ above 1. In Supplementary Table S1 there is a recommendation chart for those species to avoid consuming, keep consuming with moderation (good choice), and encourage their consumption (best choice) based on their THg levels and THQ values obtained during this study. This classification is based on the MPW index, where less than 1 MPW is recommended to avoid, 1 or 2 MPW is a good choice, and more than 2 MPW is the best choice. Specifications are described by country.

V.5 Discussion

Fisheries are one of the most important economic activities in LAC countries, and in some of these nations, fish is a significant component of the population's diet. Consequently, it is essential to evaluate the risk of mercury intake through this food source. In this study, THQ values were obtained to perform the risk assessment associated with fish consumption in LAC countries. These values varied widely across species, ecosystems, and populations from different countries, highlighting the need for region-specific assessments considering consumption rates, body weight, and cultural preferences. The human risk analysis of Hg intake by fish consumption indicated that ~70% of the fish analyzed were considered safe and non-risky for human consumption. However, it is recommended to avoid consuming fishes from some genera, such as *Acestrorhynchus*, *Brachyplatystoma*, *Cichla*, *Hoplias*, *Myloplus*, *Pinirampus*, *Pseudoplatystoma*, *Pygocentrus*, and *Salminus* due to the occurrence of high THg concentrations in some countries.

Due to Hg biomagnification across trophic levels, the highest THg levels were found in carnivore species which is consistent with previous studies (Kidd and Batchelar, 2011; Lavoie et al., 2013; Chiang et al., 2021). The same species had a significant contribution to the estimated risk showing a correlation between

elevated THg concentrations and THQ values above 1. Nevertheless, in some cases, low THg levels caused high THQ values. This was observed in Peru where despite most species having safe THg concentrations ($<0.95 \mu\text{g g}^{-1} \text{ ww}$), the THQ values were above 1. This is due to the high rate of fish consumption by the Peruvian population (68.6 g day^{-1}) compared for example to Argentina whose fish species have similar THg levels to Peru's, but its consumption rate is among the lowest in LACs (15.6 g day^{-1}) leading to safe THQ values. This exemplifies that regardless of having safe THg concentrations, the fish consumption rates ($> 65 \text{ g day}^{-1}$) and body weight ($>76 \text{ kg}$ per adult woman and $> 80 \text{ kg}$ per adult man) are important factors in determining the safety of human fish consumption. On the contrary, in countries like Anguilla, Cuba, Nicaragua, and Costa Rica we observed a direct correlation between THg concentrations and THQ levels, indicating that the amount of fish consumption and body weight distribution in these countries does not alter this correlation. Most of their fish species have acceptable THg levels, and THQ values lower than 1. Due to the variety of species, ecosystems, and demographic factors, we have found in this a wide range of THQ values (THQW = 0.006–17.008; THQM = 0.006–15.934), slightly different from those reported in other places in the world like Augusta Bay in southern Italy (THQ = 0.31–15.8; Bonsignore et al., 2013); Azores archipelago in Portugal (THQ = 0.1–8.6; Vieira et al., 2021) or Atrato River basin at the west of Colombia (THQ = 0.09–21.6; Salazar-Camacho et al., 2022), reflecting the importance of this kind of assessment to be specific for different parts of the world. It is interesting to highlight the low value of the lower limit of the reported ranges of the THQ for men and women in Chile, which is several indices of magnitude smaller than the lower limits of the ranges reported in the other regions described above. This could suggest that there is greater variability in the risk associated with fish consumption for the Chilean population.

There are several approaches to estimating the risk associated with food consumption contaminated with Hg. If only the MIAs in the Caribbean (2017) recommendations were considered, 5.35% of the total fish species would be recommended to avoid their consumption. On the other side, regarding the U.S.

EPA guidelines, in which a THQ value above 1 is considered risky for human consumption, the percentage of fish species to be avoided increased to 22.56% for adult women and 20.45% for adult men, depending on average body weight, rates of fish consumption, and also considering the RfD and other variables like a cooking adjustment factor that would alter the MeHg concentration in the fish muscle depending on if it is raw or cook (USEPA, 2011). Finally, Vieira et al. (2021) employed the MPW variable and categorized the recommendations as choices to avoid, good choices, and best choices. These authors were based on the Joint Expert Committee on Food Additives (JECFA) recommendations for the maximum amount of fish meals per week. The JECFA recommendations also consider body weight, RfD, and THg concentrations in fish, in addition to the quantity and frequency of the consumption of fish species. According to these antecedents and our MPW calculation, 29.06% of the fish species from our study should be avoided, similar to the percentages obtained using the THQ.

It is important to highlight that this study only considers a general estimation of fish consumption by country and does not consider regional differences within the country. For geographical reasons, fish consumption in coastal living populations is higher than in non-coastal communities. Therefore, in an ideal scenario, the risk analysis should be performed by geographical areas sharing features like frequency of fish consumption and other demographic variables. However, it is difficult to carry out an approach with this level of precision to design public policies, regulations, and health safety guidelines.

The nutritional benefits of fish consumption are undeniable. A diet that frequently includes fish provides proteins, energy, and nutrients like vitamin D, selenium (Se), omega-3/n-3 long-chain, and polyunsaturated fatty acids (n-3 LC-PUFA). However, Hg contamination related to anthropogenic activities has raised concerns about healthy options for fish consumption. Nevertheless, Se is known to provide defense against the toxicity of different metals such as Hg. Se and Hg exhibit a strong affinity for binding to each other, thereby diminishing each other's toxic effects (Gochfeld and Burger, 2021). Studies must consider then measuring Se content to calculate the real toxicity of fish consumption. In addition, (FAO and

WHO, 2018) made a risk/benefit analysis between Hg and omega-3 intake which made recommendations on the frequency of fish consumption based on Hg and omega-3 concentrations.

Reducing the risks of Hg poisoning is not as simple as avoiding fish entirely, but optimizing fish choices. Fish lower on the food chain should be more likely to be consumed than those on the high trophic level. There is a need for updated information to help people make rational decisions about their choices for fish consumption. In terms of public health, some actions should be considered to mitigate the risks of mercury exposure: (a) Surveillance and monitoring of mercury levels in fish and the population through consumption patterns; (b) Establishing safety limits and applying regulations; (c) Risk communication: to provide information about the risks associated to fish consumption with high Hg content and provide the recommended intake per week; (d) Nutritional interventions, education and training for the safe consumption of fish; (e) Exposure assessment, conduct epidemiological studies and evaluate possible health effects.

Most of the countries considered in this study do not have updated data on levels of Hg contamination in their fish species. This is a matter of concern since mining is one of the most important economic activities in Latin America (Alimonda, 2015) and one of the most relevant Hg contamination sources (Chalker and Esdaile, 2018). Although most of the global fishery and aquaculture activities are centered on marine species (FAO, 2020), only one-third of the data found during this research belonged to marine ecosystems. For instance, *Trachurus murphyi*, *Engraulis ringens*, and *Strangomera bentincki* make up 83% of Chilean fishery exports (SUBPESCA, 2020; OECD, 2021), and only two of these species were found in the studies analyzed.

To compare Hg pollution with other countries, East and Southeast Asia, a major responsible for Hg emissions from mining and industry (Soe et al., 2022) has a similar average of Hg bioaccumulation in fish as some LAC countries (Park et al., 2011; Jeevanaraj et al., 2019; Shalini et al., 2021; Soe et al., 2022).

Finally, the study identified some limitations to obtaining more accurate and representative estimations. Some issues were the outdated demographic and

environmental information and the data's homogenization. Almost half of the countries from LACs have no records of Hg pollution in fish, and some of them, like Nicaragua and Bolivia, were from fish samples from 30 or 20 years ago. Furthermore, this study only addressed the adult population. Consequently, in more vulnerable demographic groups, such as children and pregnant/lactating people, the risk could be underestimated. This topic is crucial, because along with damage from acute or chronic exposure to the brain, lungs, kidneys, and immune system (Tan et al., 2009; Rice et al., 2014; Guzzi et al., 2021), Hg exposure also relates to neuropsychological decline (Corral et al., 2013). Jacobson et al. (2015) found a link between Hg in pregnant women's hair and lower children's IQ. Specifically, for each part per million (ppm) increase in a mother's hair mercury, a child's IQ drops by 0.7 points. This evidence has proved the urge to enhance the development of local Hg monitoring systems in fish that are most consumed in LAC countries.

V.6 Conclusion

In many countries, there is a dearth of recent information on the mercury content in fish, as highlighted in this review. The rise in mining activities, deforestation, and other environmental issues in LAC necessitates an improvement in environmental regulations to prevent health problems among the population. Differences in the distribution of mercury emissions, population behavior, and demographic characteristics make it unfeasible to regulate under the standards of other countries. Therefore, we propose utilizing strategies that consider various parameters and incorporate local data to ensure a more precise risk assessment analysis.

V.7 Author contributions

EV: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. FP: Conceptualization, Formal analysis, Methodology, Visualization,

Writing – review & editing. LZ: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – review & editing. KF: Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. PB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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V.10 Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

V.11 Publisher's note

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V.12 Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2024.1470683/full#supplementary-material>

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Capítulo VI. **Discusión final**

Los resultados de esta tesis revelan el impacto del MeHg en los ecosistemas fluviales de Chile, integrando evidencia ambiental, molecular y de riesgo para la salud humana. A lo largo de un gradiente latitudinal que abarca siete cuencas hidrográficas, se confirmó la bioacumulación y biomagnificación de MeHg en truchas (*Oncorhynchus mykiss*), especialmente en zonas con actividad minera o vulcanismo activo, como lo demuestra la alta concentración de Hg en sedimentos y organismos acuáticos en los ríos Loa y Elqui. Esta distribución espacial sugiere una fuerte influencia de las condiciones geológicas y antrópicas sobre la movilización y disponibilidad del Hg en estos sistemas. En particular, el vulcanismo activo y la actividad minera pueden liberar Hg en formas que favorecen su transformación en MeHg por comunidades microbianas presentes en sedimentos anóxicos (Driscoll et al., 2013; Ullrich et al., 2001). La variabilidad observada entre cuencas indica que el riesgo ecológico y humano asociado al MeHg no es homogéneo, sino que depende del contexto hidrogeológico y del uso del suelo. Este hallazgo refuerza la necesidad de implementar estrategias de monitoreo y gestión adaptativas, que consideren las particularidades ecológicas y socioambientales de cada cuenca.

La trucha arcoíris demostró ser un bioindicador efectivo, revelando diferencias marcadas en la acumulación de MeHg entre sitios y la transferencia trófica del contaminante a lo largo de la red alimentaria acuática. Su utilidad como especie centinela para la vigilancia ambiental en ecosistemas dulceacuícolas, particularmente en regiones con presiones naturales y antrópicas, se ve reforzada por la capacidad de integrar datos ambientales con análisis genómicos y morfométricos (Chiang et al., 2021; Córdoba-Tovar et al., 2022; McMeans et al., 2015). Este enfoque permite no solo caracterizar la presencia del contaminante, sino también evaluar sus efectos biológicos en organismos clave.

Desde una perspectiva ecotoxicológica, el estudio identificó valores elevados del factor de biomagnificación trófica (TMF) con valores mayores a uno en todas las cuencas estudiadas, lo que confirma la biomagnificación de MeHg a lo largo de

la red trófica acuática (Yurkowski et al., 2023). Este patrón, consistente incluso en cuencas con diferencias marcadas en clima, uso de suelo y presión antrópica, subraya la ubicuidad y persistencia del MeHg como contaminante. El TMF, que representa el incremento promedio de la concentración de un contaminante por cada nivel trófico, es una métrica esencial en toxicología ambiental. Valores mayores a 1, como los observados en este estudio (rango: 1.40 a 3.41), indican que el MeHg se biomagnifica, acumulándose en concentraciones crecientes a medida que asciende en la cadena alimentaria. Estos valores se encuentran dentro del rango reportado en otros sistemas fluviales contaminados por Hg a nivel internacional (Kidd et al., 2011; Negrazis et al., 2022). La biomagnificación es particularmente preocupante para contaminantes como el MeHg debido a su alta afinidad por tejidos lipídicos y su baja tasa de eliminación biológica, lo que facilita su acumulación en organismos de vida larga o en depredadores tope, incluyendo humanos que consumen frecuentemente estos peces (Felix et al., 2022; Porto et al., 2024).

Para comprender los variables ambientales explican la distribución y biomagnificación del mercurio, se utilizaron modelos lineales generalizados (GLM) con selección por AICc. Este análisis reveló que tanto las condiciones del agua como el impacto humano influyen en los THg en agua y sedimentos, y en el factor de biomagnificación trófica (Driscoll et al., 2013; Eagles-Smith et al., 2016; Lavoie et al., 2013; S. Li et al., 2022).

El TMF fue mejor explicado por variables relacionadas con el impacto humano, particularmente la densidad poblacional y el DO, ya sea individualmente o en combinación. El modelo con mayor ajuste ($R^2 = 0.948$) indicó que cuencas más densamente pobladas y con menores concentraciones de DO presentan un mayor potencial de biomagnificación del MeHg en las redes tróficas. Esto es consistente con estudios previos que han demostrado que la eutrofización y la hipoxia favorecen la metilación microbiana de Hg inorgánico, aumentando así su transferencia trófica (Bravo & Cosio, 2020; Jonsson et al., 2022; Bargagli & Rota, 2024).

Para el THg en sedimentos, los predictores más relevantes fueron el DO, el pH y las concentraciones de metales como Fe y Mg, lo que destaca la influencia de la geoquímica redox en la retención y transformación del Hg en ambientes bentónicos (Zhang et al., 2024; Ignatavičius et al., 2022). El hierro, en particular, tiene un rol clave en estos procesos, ya que sus óxidos pueden retener Hg en el sedimento, aunque también pueden facilitar su liberación bajo condiciones anóxicas (O'Connor et al., 2019).

En el caso del THg en el agua, el modelo más robusto incluyó la proporción de uso de suelo urbano-industrial y las concentraciones de aluminio (Al) y manganeso (Mn). Estos resultados resaltan la importancia de las fuentes puntuales de contaminación, como la actividad minera y el escurrimiento urbano, en la movilización de Hg. Al y Mn pueden influir en la solubilidad y especiación del Hg, afectando su transporte y disponibilidad para la metilación (Skylberg, 2011; Liu et al., 2023). Aunque el Al no participa directamente en reacciones redox, puede modificar el pH y la fuerza iónica, lo que indirectamente afecta la movilidad del Hg (Gilmour et al., 2013).

Asimismo, los modelos que incorporaron variables relacionadas con la actividad minera y la presencia de iones mayores (como Cl^- , SO_4^{2-} y Ca^{2+}) mostraron un desempeño moderado, sugiriendo que tanto el medio natural como las fuentes antrópicas contribuyen a la dinámica del mercurio. Estos iones pueden modular el comportamiento del Hg en el agua, alterando su afinidad por partículas y su transporte a lo largo del sistema fluvial (Hsu-Kim et al., 2013).

En conjunto, estos resultados subrayan que la biomagnificación del MeHg y la distribución del THg están fuertemente influenciadas por una combinación de presiones antrópicas (p. ej., urbanización, minería) y condiciones ambientales (p. ej., oxígeno disuelto, pH, metales traza). Esto refuerza la importancia de incorporar variables de uso de suelo y geodinámica de cuenca en las evaluaciones de riesgo por contaminación con mercurio. En contextos como el norte de Chile, donde convergen actividad minera, urbanización y condiciones naturales extremas, este tipo de enfoque es clave para el diseño de estrategias de monitoreo y mitigación.

A nivel molecular, se evidenciaron alteraciones en la expresión génica hepática de truchas en respuesta a la bioacumulación de MeHg. Las hembras mostraron una tendencia a la supresión transcriptómica, afectando rutas relacionadas con el metabolismo lipídico y la homeostasis celular, lo que podría estar asociado a demandas energéticas reproductivas (Sun et al., 2020). En contraste, los machos activaron genes vinculados al estrés oxidativo y la detoxificación, incluyendo receptores de estrógeno (*erb*) y genes neuroprotectores (*kidins220*). Esta respuesta diferencial sugiere un mecanismo compensatorio ante la mayor exposición al tóxico (Josifova et al., 2016; Roy et al., 2024; Wojnarowski et al., 2022). Estos resultados concuerdan con investigaciones previas que han detectado alteraciones a nivel molecular en las interacciones de biomoléculas y vías bioquímicas, así como cambios en el funcionamiento celular y en procesos celulares clave como la respuesta inmune y el metabolismo de lípidos (Brandão et al., 2015; Olsvik et al., 2021; Ung et al., 2010). Los análisis de enriquecimiento funcional confirmaron que la exposición a MeHg afecta vías metabólicas esenciales como el metabolismo del carbono, la biosíntesis de aminoácidos y la respuesta al estrés oxidativo, con una activación de procesos apoptóticos en niveles elevados de bioacumulación (Bernhoft, 2012; Farina et al., 2011; Desjardins et al. 2024). La corroboración de los resultados transcriptómicos mediante qPCR para genes clave (*zp*, *ren*, *prxl2b* y *erb*) destaca su potencial como biomarcadores para monitorear la exposición a MeHg en peces silvestres. Estas respuestas moleculares se reflejaron en indicadores morfométricos, donde truchas de sitios con alta carga de MeHg presentaron índices hepatosomáticos (HSI) bajos y, en algunos casos, valores de condición corporal (K) que caían en categorías de salud "no buena" o "peligro" (Budi et al., 2024; Pham & Nguyen, 2019).

Desde una perspectiva de salud humana, el análisis de riesgo basado en la ingesta estimada de peces subraya la necesidad de restringir la frecuencia de consumo en ciertos casos, especialmente para poblaciones vulnerables como niños y mujeres embarazadas. La presencia de niveles elevados de MeHg en tejidos musculares de peces provenientes de algunas cuencas representa un

riesgo real de exposición crónica a través de la dieta, con potenciales efectos neurológicos y en el desarrollo, tal como ha sido documentado en diversos contextos internacionales (Guzzi et al., 2021; Lee et al., 2020; Muñoz et al., 2023; Souza-Araujo et al., 2022). Estos hallazgos refuerzan la necesidad de un enfoque preventivo, integrando datos ambientales, biológicos y sociales bajo el marco de "Una sola salud" (One Health), que considere no solo las concentraciones promedio de contaminantes, sino también factores demográficos, hábitos de consumo, características ecosistémicas y aspectos culturales ligados al consumo de productos pesqueros.

A pesar de los riesgos asociados, es fundamental reconocer los beneficios nutricionales del consumo de pescado, particularmente por su contenido de ácidos grasos omega-3, como el ácido docosahexaenoico (DHA) y el ácido eicosapentaenoico (EPA). Estos compuestos son esenciales para la salud humana, con efectos beneficiosos en el desarrollo neurológico y la reducción de la inflamación (Lange, 2020; Nikolajeva et al., 2024). La evidencia científica sugiere que, si bien el MeHg presenta un riesgo, los beneficios de consumir pescado rico en omega-3 pueden contrarrestar algunos de los efectos negativos del Hg (Ricketts et al., 2020). En este contexto, la implementación de sellos de seguridad alimentaria para productos pesqueros se presenta como una estrategia valiosa. Estos sellos, análogos a certificaciones orgánicas o de pesca sustentable, podrían indicar que el pescado ha sido sometido a controles de calidad ambiental y toxicológica, contribuyendo a decisiones de compra más informadas y protegiendo la salud de los consumidores. La trazabilidad geográfica del producto y el monitoreo periódico de contaminantes serían componentes integrales de este sistema, coordinado con autoridades sanitarias y organismos ambientales.

Capítulo VII. Conclusión general

Esta tesis constituye la primera evaluación integrada del riesgo ecológico y humano asociado a la bioacumulación de MeHg en truchas de sistemas fluviales chilenos, combinando evidencia ambiental, molecular y de salud pública. A lo largo de un gradiente latitudinal que abarca cuencas con distintos niveles de presión antrópica y características geológicas, se demostró que la trucha arcoíris (*Oncorhynchus mykiss*), como especie invasora ampliamente distribuida, y propone como un bioindicador eficaz de exposición a MeHg en Chile. Esta especie permitió detectar patrones espaciales de biomagnificación y gradientes de exposición, así como evaluar efectos fisiológicos y transcriptómicos con sensibilidad diferencial entre sexos biológicos.

Mediante el análisis transcriptómico y morfométrico, se evidenció que la exposición a MeHg afecta procesos celulares clave, como el metabolismo lipídico, el estrés oxidativo, la señalización endocrina y la apoptosis. Se identificó al hígado como un órgano blanco altamente sensible y se propusieron un conjunto de genes candidatos (como *zp*, *ren*, *prxl2b*, *erb*) como biomarcadores moleculares de exposición en truchas. Los resultados resaltan la importancia de considerar el sexo biológico como una variable crítica en estudios de toxicología ambiental, debido a la distinta susceptibilidad y estrategias fisiológicas observadas entre machos y hembras.

Desde una perspectiva ecosistémica, los modelos predictivos mostraron que la distribución del THg en agua y sedimento, así como el grado de biomagnificación trófica (TMF), están fuertemente modulados por variables fisicoquímicas (p. ej., oxígeno disuelto, pH, metales traza) y por factores asociados al uso de suelo (densidad poblacional, urbanización, actividad minera). Estos hallazgos subrayan la necesidad de incorporar parámetros fisicoquímicos y presiones antrópicas en las evaluaciones de riesgo, destacando la relevancia del contexto hidrogeológico y de la dinámica de cuenca para comprender los procesos de metilación, transporte y acumulación del Hg.

A nivel de salud pública, los resultados del análisis de riesgo sugieren que, en ciertas cuencas del norte de Chile, los niveles de MeHg en peces superan

umbrales recomendados para el consumo frecuente, especialmente en grupos vulnerables como niños y mujeres embarazadas. Por tanto, se propone que las evaluaciones de riesgo incorporen no solo los niveles promedio de contaminantes, sino también la especie consumida, su origen geográfico y la frecuencia de consumo real, de acuerdo con hábitos culturales y prácticas locales.

Además, se plantea la necesidad de avanzar hacia esquemas de certificación sanitaria y trazabilidad de productos pesqueros de agua dulce y marinos, que permitan informar a los consumidores sobre la seguridad alimentaria del pescado, protegiendo tanto la salud humana como la sustentabilidad de los ecosistemas.

En conjunto, esta investigación aporta evidencia científica para el desarrollo de herramientas de vigilancia ambiental y estrategias de gestión del riesgo por Hg en ecosistemas acuáticos chilenos. La metodología propuesta, que integra análisis ecológicos, moleculares y de salud humana bajo un enfoque ecosistémico e interdisciplinario, es replicable en otros sistemas fluviales del país y del mundo, y constituye un aporte relevante al enfoque de Una Sola Salud (One Health), que vincula la salud ambiental, animal y humana en contextos de cambio global.

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