Universidade de Lisboa

Faculdade de Farmácia

Mercury Isotopic Characterization in Fish from Aquitanian Lakes: Potential Application on Mercury Source Tracing

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Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

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Co-Orientadores: Doutora Cristina Carvalho e Doutora Mathilde Monperrus

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Resumo

O mercúrio é um poluente ubíquo na Natureza, existindo na atmosfera, no solo e nos meios aquáticos, incluindo os meios lacustres, onde é acumulado e alvo de diversas reações, entre elas a alquilação, maioritariamente realizada por bactérias anaeróbias, cujo produto é o metilmercúrio. O metilmercúrio é acumulado no plâncton e devido à sua elevada afinidade pelos grupos sulfidrilo das proteínas citosólicas, é bioacumulado ao longo da cadeia alimentar, atingindo elevadas concentrações em peixes predadores. O consumo de peixe é a maior fonte de exposição de metilmercúrio para os humanos. De acordo com o conhecimento atual, o mercúrio é acumulado principalmente nos rins e no fígado, uma vez que estes são órgãos depuradores. Por outro lado, o metilmercúrio é principalmente acumulado no músculo e no cérebro.

A maior parte do metilmercúrio ingerido a partir do peixe é absorvido no trato gastrointestinal e após a formação de um complexo com a L-cisteína, consegue mimetizar a metionina e atravessar a barreira hematoencefálica. Assim, este composto atinge concentrações no cérebro, 3 a 6 vezes superiores às do sangue, levando a neurotoxicidade.

Num estudo feito pela ANSES - Agência de segurança alimentar de França em 2011, foi verificada a existência de elevadas concentrações de mercúrio em determinadas espécies de peixes dos lagos Aquitâneos,levando à proibição da pesca nos lagos Hourtin-Carcans e Lacanau.

O projeto ***C**ontamination des **L**acs **AQ**uitains et impacts **H**umains* (CLAQH) surge no seguimento destes acontecimentos e com o intuito de ser compreendida a extensão da poluição por mercúrio nos lagos Aquitâneos, a sua origem e distribuição nos diferentes ecossistemas, o impacto que pode ter na população humana e a perceção de risco para os consumidores. As tarefas estão divididas entre diferentes grupos, sendo que o objetivo deste trabalho específico foi rastrear a transferência do mercúrio ao longo da cadeia trófica por análise isotópica. Para isso, foram pescados dos lagos Hourtin-Carcans, Lacanau, Casaux-Sanguinet e Parentis-Biscarrose, as espécies *Abramis brama, Esox Lucius, Perca fluviatilis, Sander lucioperca, Procambarus clarkii* e *Corbicula flumínea* entre Setembro e Novembro de 2015.

O elemento mercúrio tem diversos isótopos estáveis, entre eles, ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg e ^{204}Hg, cuja abundância natural é de 0,15%, 9,97%, 16,87%,

23,10%, 13,18%, 29,86% e 6,87%, respetivamente. O fracionamento isotópico tem a ver com a ocorrência de variações na composição isotópica natural do elemento. A análise isotópica do mercúrio permite rastrear as fontes de mercúrio por forma a reduzir a exposição das populações a este composto neurotóxico. Quando submetidos a reações de transformação ou troca, todos os isótopos de mercúrio são sujeitos a fracionamento dependente da massa (MDF). Por outro lado, o fracionamento independente da massa (MIF) é principalmente verificado nos 2 isótopos ímpares de mercúrio (^{199}Hg e ^{201}Hg) e pensa-se que este último fenómeno ocorra devido a diferenças na paridade do isótopo ao invés da sua massa.

Os diferentes isótopos de um elemento têm ligeiras diferenças na sua massa, o que faz com que os isótopos participem em processos físicos e reações bioquímicas com eficiências diferentes. Este fenómeno é denominado de fracionamento dependente da massa e é a forma mais comum de fracionamento, sendo uma ferramenta útil na obtenção de informação acerca de processos físicos e reações químicas aos quais os elementos foram expostos. Este parâmetro é comummente representado por δ^{XXX}Hg.

Observa-se o fracionamento independente da massa quando é observado um desvio na composição isotópica esperada pelo fracionamento dependente da massa. Normalmente, este parâmetro é representado por Δ^{XXX}Hg. Pensa-se que o fracionamento independente da massa ocorra devido a ligeiras diferenças na interação entre o núcleo destes isótopos e a sua nuvem eletrónica, o que vai afetar algumas características de certos isótopos, nomeadamente o *bonding.* Estes processos ocorrem essencialmente nos isótopos de massa ímpar.

Diferentes estudos, compilados por Blum et al., 2014, indicam que variações negativas no fracionamento dependente da massa são observadas em amostras atmosféricas e sedimentos enquanto que variações positivas são observadas em amostras colhidas de humanos e material geológico. Peixes de água doce apresentam uma grande variação desde valores negativos a positivos de MDF. Valores positivos de fracionamento independente da massa são observados em peixes e invertebrados enquanto que valores negativos são observados e amostras atmosféricas e vegetação. Em suma, processos que contribuam para a absorção e manutenção do mercúrio na cadeia alimentar originam valores positivos de Δ^{199}Hg. Por outro lado, processos responsáveis pela libertação do mercúrio para a atmosfera estão associados a valores negativos de Δ^{199}Hg.

Os isótopos de mercúrio de massa atómica par são úteis para a rastreabilidade do metilmercúrio durante transferências tróficas e bioacumulação, uma vez que estes fracionam principalmente através de reações fotoquímicas. É sugerido que na mesma cadeia alimentar é observado um enriquecimento isotópico, sendo verificado um valor positivo de $\delta^{202}Hg$. Em geral, na mesma cadeia alimentar, os predadores possuem valores de $\delta^{202}Hg$ superiores às presas.

A assinatura MIF permite identificar fontes específicas de mercúrio como antropogénicas derivadas da indústria ou atividade mineira. Permite ainda o seguimento da fonte de exposição para os organismos uma vez que à luz do conhecimento atual, o fracionamento independente da massa não foi observado em processos biológicos.

A assinatura isotópica do mercúrio permite também o melhor conhecimento do ciclo biogeoquímico deste elemento, uma vez que diversos processos de transformação causam um aumento na assinatura isotópica do mercúrio. Em meios aquáticos o mercúrio pode ser transformado por processos biológicos e não biológicos tal como oxidação/redução e metilação/desmetilação, entre outros. Em geral, processos de oxidação e de metilação provocam um incremento em $\Delta^{199}Hg$ enquanto que processos de redução e desmetilação provocam uma diminuição no valor deste parâmetro.

Neste trabalho, a composição isotópica do mercúrio foi determinada em diferentes níveis tróficos do ecossistema existente em alguns lagos Aquitâneos, para os quais também foram determinados a concentração total de mercúrio, a composição isotópica do carbono e azoto ($\delta^{13}C$ e $\delta^{15}N$) e o cálculo das posições tróficas a partir da composição isotópica do azoto.

Os valores de $\delta^{202}Hg$ variaram de -0,93‰ a 0,60‰. Os 2 maiores lagos (Hourtin-Carcans e Casaux-Sanguinet) apresentaram valores positivos enquanto que nos 2 lagos de menores dimensões (Lacanau e Parentis-Biscarrosse) os valores foram negativos. No geral, os valores de $\delta^{202}Hg$ variam muito pouco mas verifica-se uma ligeira tendência crescente de amêijoa-asiática (*Corbicula flumínea)* para lagostim-vermelho *(Procambarus clarkii)* para brema *(Abramis brama)* para lúcio *(Esox Lucius)* para perca *(Perca fluviatilis)* para lucioperca *(Sander lucioperca).*

O rácio $\Delta^{199}Hg/\Delta^{201}Hg$ obtido foi aproximadamente 1.3, o que indica que o mercúrio bioacumulado nestas amostras resulta de reações de desmetilação.

Os valores do MIF apresentaram uma variação superior (0,11‰ a 3,84‰). A *Corbicula fluminea* apresenta valores inferiores aos encontrados em *Procambarus clarkii* e *Abramis brama. Esox Lucius, Perca fluviatilis* e *Sander lucioperca* apresentaram os valores superiores de Δ^{199}Hg.

Para caraterizar melhor a transferência de mercúrio ao longo das cadeias alimentares existentes nestes lagos, é necessária a realização de estudos posteriores com uma amostragem mais completa.

Palavras-chave: Mercúrio; Metilmercúrio; Isótopos estáveis; Nível trófico; Lagos.

Abstract

Mercury (Hg) is an ubiquitous contaminant with a severe impact in public health. In general, humans are mainly exposed to this metal due to fish and seafood consumption where high concentrations of this element can be found due to the bioaccumulation and biomagnification processes occurring in the water environments.

A previous study driven by ANSES showed high mercury concentrations on fish from the Aquitanian lakes and this work integrates a new research program that aims at determining the origin of Hg pollution to the biota living in these lakes.

The species analysed by CVG-MC-ICP-MS include clams, crayfish, bream, pike, perch and pike-perch collected from September to November 2015 in Hourtin-Carcans, Lacanau, Casaux-Sanguinet, and Parentis-Biscarrose lakes.

Hg is involved in several transformations due to environmental and biological processes that influence its stable isotope composition. Therefore, Hg isotopic mass dependent (MDF) and mass independent fractionation (MIF) are powerful tools to identify the major sources and processes involved in Hg bioaccumulation in foodwebs.

In the samples analysed, δ^{202}Hg showed a wide range (-0.93‰ to 0.60‰). In general, δ^{202}Hg is increased from clam to crayfish to bream to pike to perch to pike-perch. MIF had a wider range (0.11‰ to 3.84‰) and clam showed the smallest value. Pike, perch and pike-perch displayed the highest Δ^{199}Hg value. In these samples, the mercury bioaccumulated results principally of MeHg demethylation (Δ^{199}Hg/Δ^{201}Hg = 1.3).

Additionally, the stable isotopic composition of Hg was related to the trophic levels of the species calculated by using δ^{13}C, δ^{15}N.

The results obtained are a good contribution for the understanding of the mercury biogeochemical cycle in the Aquitanian lakes although there is a need for analysis of samples such as sediments and prey fish to conclude about the trophic transferences of mercury existing in the Aquitanian lakes.

Keywords: Mercury; Methylmercury; Stable isotope; Trophic guild; Freshwater lake; Lakes.

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Tiago Caetano

Abbreviations

Hg **-** Mercury

DIRECT – Acronym of Project: *DIversité et Risques liés à la méthilation du MErcure et son transfert vers la Chaine Trophique*

ANSES - *Agence Nationale de Sécurité sanitaire de l'alimentation, de l'Environnement et du travail* (National Agency for Food Safety, Environment and Labour)

CLAQH - Acronym of Project: *Contamination des Lacs AQuitains et impacts Humains*

Hg^0 - Elemental mercury

Hg^{2+} - Mercuric mercury

MeHg - Methylmercury

-SH - sulfhydryl groups

EtHg – Ethylmercury

BBB – Blain blood barrier

CNS - Central Nervous System

US FDA - United States Food and Drug Administration

JECFA - Joint FAO/WHO Expert Committee on Food Additives

PTWI - Provisional Tolerable Weekly Intake

RfD - Reference Dose

WHO - World Health Organization

NRC - National Research Council

USA - United States of America

MDF – Mass Dependent Fractionation

MIF – Mass Independent Fractionation

SML – Surface mixed layer

DCE - *Directive Cadre sur L'Eau*

MC-ICP-MS - Multi-Collector - Inductively Coupled Plasma – Mass Spectrometry

Tl - Thallium

CV – Cold vapour

CVG – Cold vapour generator

CVG-MC-ICP-MS - Multi-Collector - Inductively Coupled Plasma – Mass Spectrometer coupled to a cold vapor generator

2SD – 2 times the Standard deviation value

$SnCl_2.2H_2O$ - Stannous chloride dihydrate

HCl - Hydrochloric acid

TPi - Trophic Positions

[THg] – Total Mercury Concentration

HC – Referred to Hourtin-Carcans lake

L - Referred to Lacanau lake

CS – Referred to Casaux-Sanguinet lake

PB – Referred to Parentis-Biscarrosse lake

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1. Introduction

Mercury is considered by the World Health Organization among the top 10 chemicals of “major public health concern” (1). In general, humans are mainly exposed to Hg due to fish and seafood consumption. High concentrations of mercury can be found in fish from lakes exposed to Hg pollution (2). Thus, studying Hg in fishing environments is mandatory for better understanding the Hg pollution origin and for Hg tracking in the trophic chain (3).

Mercury is the only metal that is liquid at room temperature. It’s the 80th element of the periodic table, belonging to the transition metals’ group and is a non-essential metal. Mercury is ubiquitous, it occurs naturally in the environment but over the last century, human activities contributed to its concentration increasing over three-fold (4). Traditionally it was used in thermometers, barometers, inks, fungicides and had a therapeutic usage on obstipation, syphilis and wound healing (merbromin, also known as Mercurochrome) (5). Nowadays, despite the efforts made to reduce its utilization, it is still used in dental amalgams, fluorescent lamps and in industrial processes such as gold mining, batteries, chloralkali industries and as a preservative of some vaccines. These industrial processes account for over 75% of worldwide mercury consumption (5–7).

1.1 Project Contextualization

In 1964, the water law for the management of the aquatic environments and a water policy was instituted. It has evolved to the Water Policy Directive (2000/60 / EC) and has been adjusted with the guidelines resulting from research and development. Their objectives are to achieve good ecological and chemical status for water bodies. The daughter directive 2013/39/EU thus classifies 41 priority substances for which member states must comply with environmental quality standards. Mercury is one of them.

In 2010, the research project **DIRECT** “Les microorganisms sulfato-réducteurs colonisant les racines de macrophytes aquatiques: “**DI**versité et **R**isques liés à la méthilation du M**E**rcure et son transfert vers la **C**haine **T**rophique” studied the risks

linked with mercury methylation and its transfer through the trophic chain on Cazaux-Sanguinet lake. A mercury level close to the regulated limits for consuming was found on pike fish (8).

In Autumn 2011, under the pressure of the fishing association of Hourtin, 25 Kg of fish were collected from the lake Hourtin-Carcans for analysis. The results showed mercury concentrations over 1 mg total $Hg.kg^{-1}$ fresh weight in Pike (*Esox lucius*) and 1.4 mg total $Hg.kg^{-1}$ fresh weight in Pike-perch (*Sander lucioperca*). These concentrations are over the limit recommendations established by European Union (0.5 mg total $Hg.kg^{-1}$ fresh weight for Pike and 1 mg total $Hg.kg^{-1}$ fresh weight for Pike-sander. Because of these facts, in July 2012, the consumption of Pike-perch from Hourtin-Carcans lake was forbidden. The geographic location and characteristics of these lakes are further presented at the sections 1.5.1 and 3.1.

In parallel, the Regional Health Agency of Nouvelle Aquitaine (Agence Régionale de Santé – ARS – de Nouvelle Aquitaine), communicated this incident to the National Agency for Food Safety, Environment and Labour (Agence Nationale de Sécurité sanitaire de l'alimentation, de l'Environnement et du travail – ANSES) and this agency ordered an additional study regarding the mercury contamination on fish from the principal Aquitanian lakes. Following this study, where fish from the Aquitanian lakes (Hourtin-Carcans and Lacanau) and *Landais* lakes (Cazaux-Sanguinet, Parentis-Biscarrosse and Aureilhan) were analysed, the results showed that regulated Hg levels are surpassed on Pike-perch from Hourtin-Carcans and Lacanau Lake. Therefore, the prohibition of fish consumption was also extended to Lacanau lake.

The understanding the extension of Hg pollution in the Aquitanian lakes, the Hg distribution on the different ecosystems, the origin of the Hg found in fish species, the impact that this pollution could have in the human population and the risk perception of the fish consumers, were the objectives of the CLAQH "**C**ontamination des **L**acs **AQ**uitains et impacts **H**umains" project. The work described in this report was carried out in the frame of CLAQH. All the tasks are divided between different work groups and this piece of work is focused on the origin of the Hg pollution and tracing the trophic transfer of this element.

1.2 Global Mercury Cycle

Mercury appears in the environment in three distinct forms: elemental mercury (Hg^0), mercuric mercury (Hg^{2+}) and organomercurials, essentially methylmercury (MeHg) (Figure 1.1) (6,9).

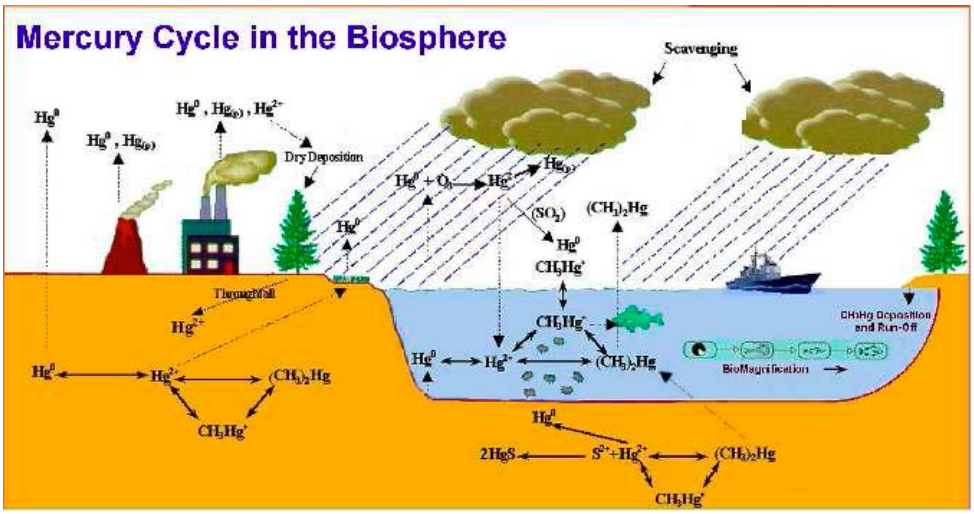

Figure 1.1 - Global Mercury cycle in the biosphere (from European Communities, 2001)

Hg^0 is the dominant form of mercury in the atmosphere. Due to its volatility and the residence time of around 1 year, it could be transported through the air to areas located far away from the emission points. In the atmosphere, Hg^0 can be oxidised to Hg^{2+} which is followed by dissolution in rain, aerosols or snow. This process leads to mercury deposition on the Earth's surface. (10)

In aquatic systems, Hg^{2+} can be once again reduced, by microorganisms or abiotically, to Hg^0 and return to the atmosphere. Alternatively, Hg^{2+} may be transformed, mainly by anaerobic bacteria into alkylated forms of mercury, being MeHg the most relevant one (10).

MeHg accumulates in phytoplankton and, due to its high liposolubility and high affinity to bind the sulfhydryl groups (-SH) of cytosolic proteins, it undergoes bioaccumulation and biomagnification through the food chain, reaching high concentrations in long-lived, predatory fish (11).

1.2.1 Hg transference mechanisms in biota

The bioaccumulation of Hg in the aquatic food chain was firstly demonstrated after the Minamata outbreak in Japan in the 1950s (2). A factory manufacturing acetaldehyde used an inorganic compound of mercury as a catalyst to this process was used and discharged together with MeHg to the environment since during the synthetic process some inorganic Hg was converted to MeHg. At the time, it was hard to believe that the Hg released in a large ocean bay could be bioaccumulated in such extent and that the fish would carry levels of MeHg that could be dangerous to human health. However, it is estimated that during all the years that the factory was working, from 1932 to 1968, 456 tons of total Hg was discharged and of these, around 1 ton corresponded to MeHg (12).

Later on, it was shown (13) that, in aquatic environments, when increasing on trophic level, the ratio of MeHg increases towards THg (inorganic Hg + organic Hg). The relative ratios of MeHg are 15% to phytoplankton, 30% to zooplankton and 95% to fish. Thereby, in the sediments and in the water column, Hg is found majorly in the inorganic form. Then, after direct exposure of phytoplankton and bacteria, organic mercury is successively bioaccumulated and biomagnified through the chain food and in fish it reaches a high ratio (13,14).

As described in the literature if fish are exposed to Hg via direct or trophic routes, this element will accumulate in different organs. After direct exposure, the gills and the muscle are the organs where Hg accumulates more. On the other hand, after trophic exposure, the gills are weakly contaminated and the most concentrated organs are liver, brain and muscle (15,16).

The Hg metabolism in living organisms in general remains elusive. According to the actual knowledge, the Hg form is also important in the organ distribution. MeHg accumulates preferentially in the brain and in the muscle while inorganic Hg accumulates in a more extensive way in the kidneys and in the liver as these are the depurator organs (15,17).

1.3 Toxicokinetics and Health effects of Hg compounds

The toxicity of Hg depends on the specie to which exposure occurs and also on the dose and duration of that exposure. This element is considered as neurotoxic, teratogenic, nephrotoxic, immunotoxic and cardiotoxic and the symptoms may intensify, become irreversible and eventually lead to death. In general, the toxicity of Hg compounds decreases in the following order: MeHg > EtHg > Hg^{2+} (12,18,19). Ethylmercury is essentially present in Thimerosal, used as a preservative for pharmaceutical preparations and is principally toxic for the central nervous system (CNS) (2).

1.3.1 Mercury vapour

Absorption of elemental mercury in the GI tract is low, thus in its metallic state Hg is not of great toxic concern. However, metallic mercury is volatile releasing mercury vapour (Hg^0) which is extensively absorbed through the lungs (>80%) and reaches systemic circulation. Most of Hg^0 reaches the Central Nervous System and easily crosses the blood-brain barrier. Inside the CNS, Hg^0 is oxidized to Hg^{2+} and exerts the toxic effects to the brain cells (1,20). Hg^{2+} is mostly excreted via the urine (2) and can cause selective damage to the kidneys with special incidence in the proximal tubule. Hg^{2+} is also immunotoxic (1,2).

With the exception of workers occupationally exposed to mercury vapour (Hg^0), the major source of exposure to this Hg form is its release from dental amalgams (2).

1.3.2 Methylmercury

Most of the MeHg ingested from fish (95%) is absorbed in the gastrointestinal tract. MeHg hits its maximum concentration in the blood 6 hours after food exposure (12). About 30h after ingestion, MeHg is distributed to all tissues and about 5% is found in the blood and 10% in the brain. In the liver, MeHg can be conjugated to glutathione and is excreted through the bile. However, it suffers extensive enterohepatic

circulation, with only 1% of MeHg being converted to Hg^{2+} by microflora and excreted through the faeces (1,20). The half-life period of MeHg varies from 40 to 80 days (1,20).

MeHg forms a complex with L-cysteine which mimicries methionine (Figure 1.2) and can cross the brain blood barrier (BBB) via the neutral amino acid transport system. This allows MeHg to accumulate in the brain, where it can reach concentrations 3 to 6 times greater than in the blood (11,20). Thus, the CNS is the main target organ for MeHg and the neurotoxic effects of MeHg are particularly serious if exposure takes place during fetal development or at early life (21). Experimental data showed that MeHg can affect processes extremely important to neurodevelopment, such as the migration of neuronal cells from the site of germination to the site of function by interacting with elements of the cytoskeleton. Also, MeHg interferes with interneural contacts, i.e., interferes with the formation of dendrites and axons. MeHg induces mitotic arrest and caspase-dependent apoptosis on the developing CNS, by altering the expression of several developmental regulators and in genes involved in the regulation of cell growth and proliferation (11).

In Minamata outbreak, the lower number of males offspring at birth, suggested that MeHg is also toxic for the reproductive system (20,22). MeHg compounds are teratogenic (22,23) and are classified as possible carcinogens to humans (group 2B)

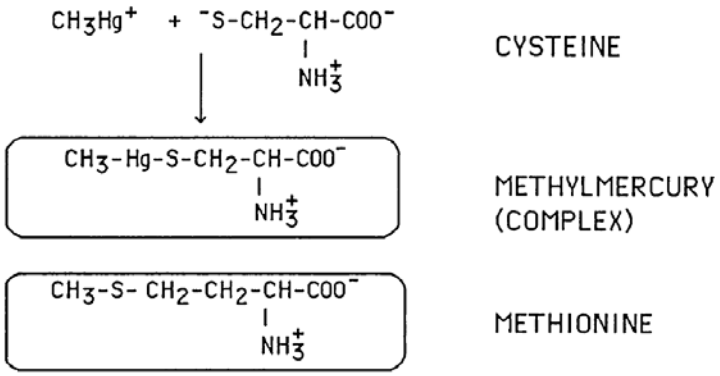

Figure 1.2 - Formation of a complex of MeHg cathion and L-cysteine similar to methionine's structure (adapted from Clarkson, et al., 2006)

Even in the absence of symptoms in the mother, maternal exposure to MeHg can have irreversible effects on the neurobehavioral development of children due to multiple deficits in neurons and glia including motor disturbances (ataxia and trembling), dysesthesia, decreased IQ, impaired visual perception and speech (11,22,23).

1.4 Normative values

Different organizations established normative values for limiting the levels of Hg and/or MeHg on fish and seafood and recommended ingestion levels, for assuring that the population is not exposed to high levels of this toxic element. The normative values for Hg vary depending on the organization and the underneath risk assessment.

For fish and seafood, the established limits for the maximum Hg and MeHg are the following. Codex Alimentarius (a collection of internationally recognized standards, codes of practice, guidelines, and other recommendations relating to foods, food production, and food safety), sets the limits for the maximum methylmercury levels in foods for fresh non-predatory fish of 0.5 mg/kg and for fresh predatory fish of 1.0 mg/kg. The European Community Legislation allows, with some exceptions, 0.5 mg/kg in fishery products. Japan allows up to 0.4 mg of mercury per kg of fish, or 0.3 mg/kg to methylmercury. The United States Food and Drug Administration (US FDA) established a limit of 1 mg of MeHg / kg of finfish and shellfish (1).

Regarding recommended ingestion levels, in 2003, The Joint FAO/WHO Expert Committee on Food Additives (JECFA), which also evaluates chemical contaminants in the food supply reduced the provisional tolerable weekly intake (PTWI) of MeHg from 3.3 µg/kg body weight to 1.6 µg/kg body weight for children, pregnant women and women at their fertile age, which represents a 0.23 µg/kg body weight tolerable daily intake (TDI). For total mercury, JECFA established a PTWI of 5 µg/kg body weight. The US EPA has established a reference dose (RfD) of 0.3 µg mercuric chloride/kg body weight and 0.1 µg MeHg/kg body weight (1).

The normal mean concentration of mercury in total blood in humans is, according to WHO (World Health Organization), between 5 and 10 µg/L in populations that usually eat fish. NRC (National Research Council) identifies the normal mean concentrations of mercury 2 µg/L for populations with little or no fish consumption in the USA (United States of America) (1).

The reference dose established for assessing risks associated with MeHg exposures do not have Se in account and are based on effects that were observed in a population consuming a Se-rich diet. Based on the observation that Selenium is an antagonist of Hg toxicity, a new risk assessment criterion was developed by Ralston et al., 2016 (24), the Se health benefit value. By using this new criterion one can predict effects of MeHg exposures from seafood consumption and in the case of pregnant and

breastfeeding women, it is now possible to do a better advising regarding seafoods that should be limited and those that are beneficial to consume (24).

1.5 The lake environments

The aquatic environments such as the lakes are considered freshwater reservoirs. The lakes are fed by many water sources including the water sheds that can be submitted to anthropogenic pressures such as agriculture and industry that are pollution originators and so, the drained and the flowing water can carry some micropollutants such as Hg.

The lakes have a high specific richness that depends on the lake and the surrounding area dimensions. The lake and the surrounding area will define the ecological niches diversity. Therefore, the biggest the lake and the surrounding area are, the more species the lake will contain (25,26).

1.5.1 The Aquitanian lakes

The great Aquitanian lakes are in the southwest of France, along the Atlantic coast and isolated of the ocean by the dunes. The lakes are in Gironde and Landes departments and both are part of Aquitaine Region (Figure 1.3). From north to south, the more important lakes from this region are Hourtin-Carcans, Lacanau, Cazaux-Sanguinet and Parentis-Biscarrosse, that are shown on Figure 3.1.

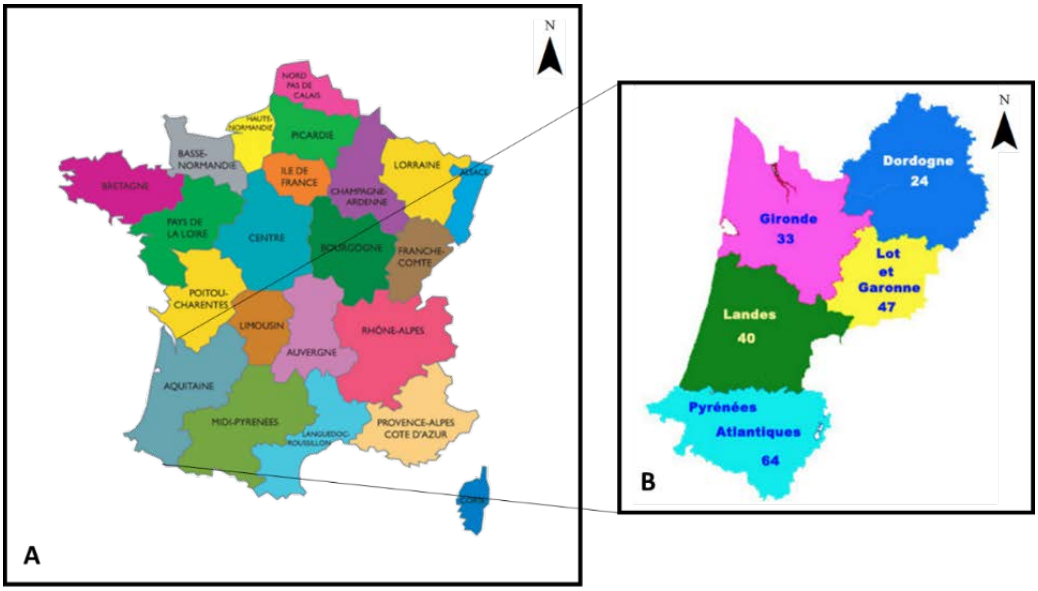

Figure 1.3 - Map of France's Regions (A) and Aquitaine Region including the Departments (B).

From 75 to 80% of the lakes' surrounding area are covered with maritime pine forests. Therefore, the wood industry is very present in this area. There are also big agriculture fields and the fishing tradition is also present in this region. At the Aquitaine coast, the tourism economy is also very important, being the Cazaux-Sanguinet the most developed lake for leisure and tourism. A considerable area of this lake is also reserved for military activity. In Landes there is the second French place where oil was discovered and remains the most important sub-lacustrine deposit in France. It is, more precisely, located very close to the Parentis-Biscarrosse lake (8,27). The lakes' characteristics are more extensively presented at section 3.1 .

1.5.2 Principal biota present in the lakes

According to Irz et al. and Argillier et al., the Aquitanian lakes host a cyprinicole population showing strong affinities to that found in specific zones of the rivers. The fish ecosystem is mainly constituted by pike-perch (*Sander lucioperca*), pike (*Esox lucius*) and perch (*Perca fluviatilis*) as predatory species and bream (*Abramis brama*) as detritivore (25,26).

The aquitanian lakes also host clam and crayfish. The predominant specie of crayfish used to be *Austropotamobius pallipes* (White legged crayfish) as it is native of this region. Nowadays, the most common specie is *Procambarus clarkii* (Louisiana crayfish), an invasive species that is more adapted to pollution. Crayfish diet is based on small invertebrates (worms, molluscs, insect larvae), amphibian tadpoles, fish, plants (terrestrial or aquatic), decaying animals and plants. Juveniles are generally more carnivorous than vegetarians, unlike adults (28).

Asiatic clam (*Corbicula fluminea*) is the predominant mollusc specie in this environment although it's an invasive species. *C. fluminea* adapts to many conditions of life, it is not very sensitive to the hydrogen potential (pH) and quite eurythermal. It can tolerate a salinity rate of 5 to 8 ‰ and temperatures between 2 and 30 °C. Asiatic clam food habits are based on phytoplankton (29).

On Figure 1.4 the above-mentioned species are represented.

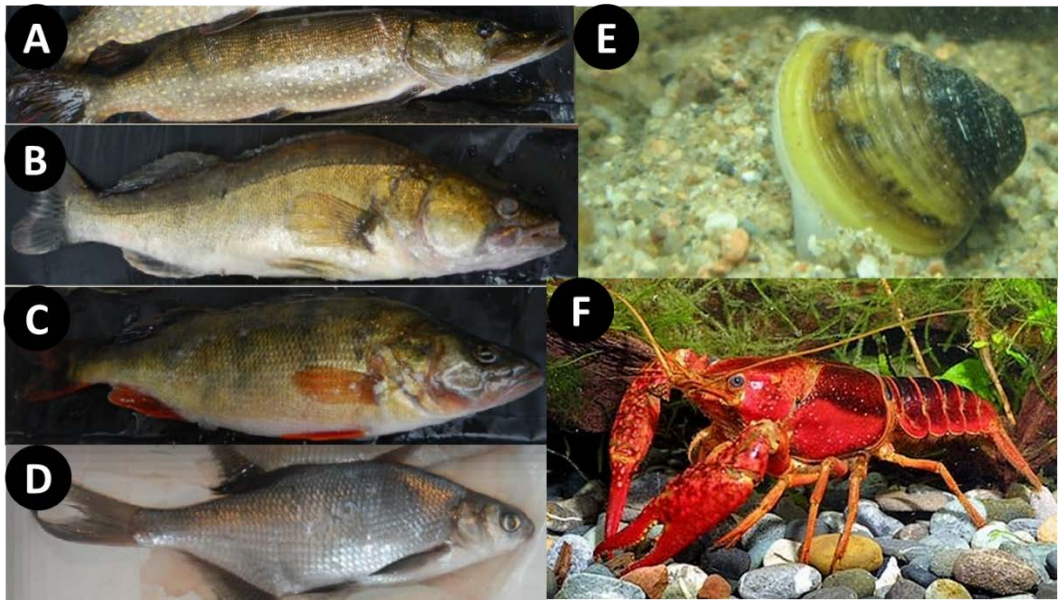

Figure 1.4 - Images of the most representative biota species of the Aquitanian lakes. (A) Pike, *Esox lucius*; (B) Pike-perch, *Sander lucioperca*; (C) Perch, *Perca fluviatilis*; (D) Bream, *Abramis brama*; (E) Asian corbicula, *Corbicula fluminea*; (F) Louisiana crayfish, *Procambarus clarkii*.

1.6 Mercury isotopic fractionation

Determination of the total Hg levels is very useful for studying bioaccumulation and biomagnification although with this determination it is not possible to track the origin of mercury. Instead, Hg isotopic fractionation allows to trace sources of Hg in sensitive environments (30).

Due to the recent developments of mass spectrometry technology, it is possible to access information embedded at the isotopic level of many elements, such as Hg (31).

Mercury stable isotopes open the door to trace Hg pollution source, tracking Hg biogeochemical processes in the environment and its metabolic behaviour in the environment. As shown by Jackson, 2001 (32), the natural lake environment and the processes related with Hg cycling do not destroy or mask Hg isotopic composition. It also allows the tracing of Hg sources due to the fact that in general different Hg sources are related with distinct isotopic profiles (33,34).

Isotopic signature is defined as the ratio of the natural abundances of particular isotopes and is a powerful tool that can provide information about the metal(loids), such as, their origin, ways of transport, degradation mechanisms and metabolism. In isotopic fractionation, Hg is an element that has several stable isotopes ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg which natural abundance are approximately 0.15%, 9.97%, 16.87%, 23.10%, 13.18%, 29.86% and 6.87%, respectively (31,35). It is due to the faster breakage of the bonds with the lighter isotopes and lower atomic mass that fractionation exists (33,34). In mercury, some factors such as organo-metallic complexing, volatility and potential for multiple redox states suggest that despite the small differences in relative atomic masses, mercury isotopic fractionation may be significant (34). It is also common to observe mass dependent stable isotope variations which magnitude is a function of the relative differences of the involved isotope masses ($\Delta m/m$) in mercury as well as in other non-radiogenic elements (34).

During transformation in other Hg species or exchange reactions, all Hg isotopes experience mass dependent fractionation (MDF). On the other hand, mass independent fractionation (MIF) is mainly experienced with the two odd mercury isotopes (^{199}Hg and ^{201}Hg) and it is thought to be generally caused by differences in isotope parity instead of mass (36). The proposed theories for MIF are better explored in the next subsections.

1.6.1 Mass dependent fractionation (MDF)

The different isotopes of an element have slight differences in mass. Due to that, the different isotopes participate in physical processes and (bio)chemical reactions with slightly different efficiencies. This phenomenon is referred to as isotope fractionation and in general the extent is function of the extent to which an element takes part in physical processes or chemical reactions (37).

Since isotope variations in nature are small, the results are reported as per mil (‰) deviations (δ) relative to a reference standard.

$$\delta^{xxx}Hg(‰) = \left\{\left[\frac{^{xxx}Hg/^{198}Hg_{sample}}{^{xxx}Hg/^{198}Hg_{NIST3133}}\right] - 1\right\} \times 1000 \quad (1)$$

where xxx is the mass of a Hg isotope between 199 and 204 amu. MDF is usually reported as δ^{202}Hg (‰) (35). δ values can be both negative or positive what means that the isotope is lighter or heavier, respectively, relative to the Hg isotopic standard. This standard is very important for making inter-laboratory evaluation of precision and accuracy of absolute measurements and to make data comparison. The standard could be a standard with certified isotopic ratios or a standard calibrated against a certified isotopic reference material. NIST-3133 shows low matrix effects and due to that it is universally adopted as a Hg isotope reference standard.

Mass dependent fractionation is the most common form of fractionation and is a powerful tool for providing information about the physical processes and chemical reactions to which the elements were exposed (37). Mercury MDF occurs during biological processes, as it is induced by diverse processes such as metabolic processes, species transformation, transport, vaporization and volatilization (3). It is shown that MDF occurs through all biotic, dark abiotic and photochemical transformations such as methylation, demethylation of Hg, Hg reduction, evaporation, volatilization, adsorption and diffusion, as shown on Figure 1.5 (30).

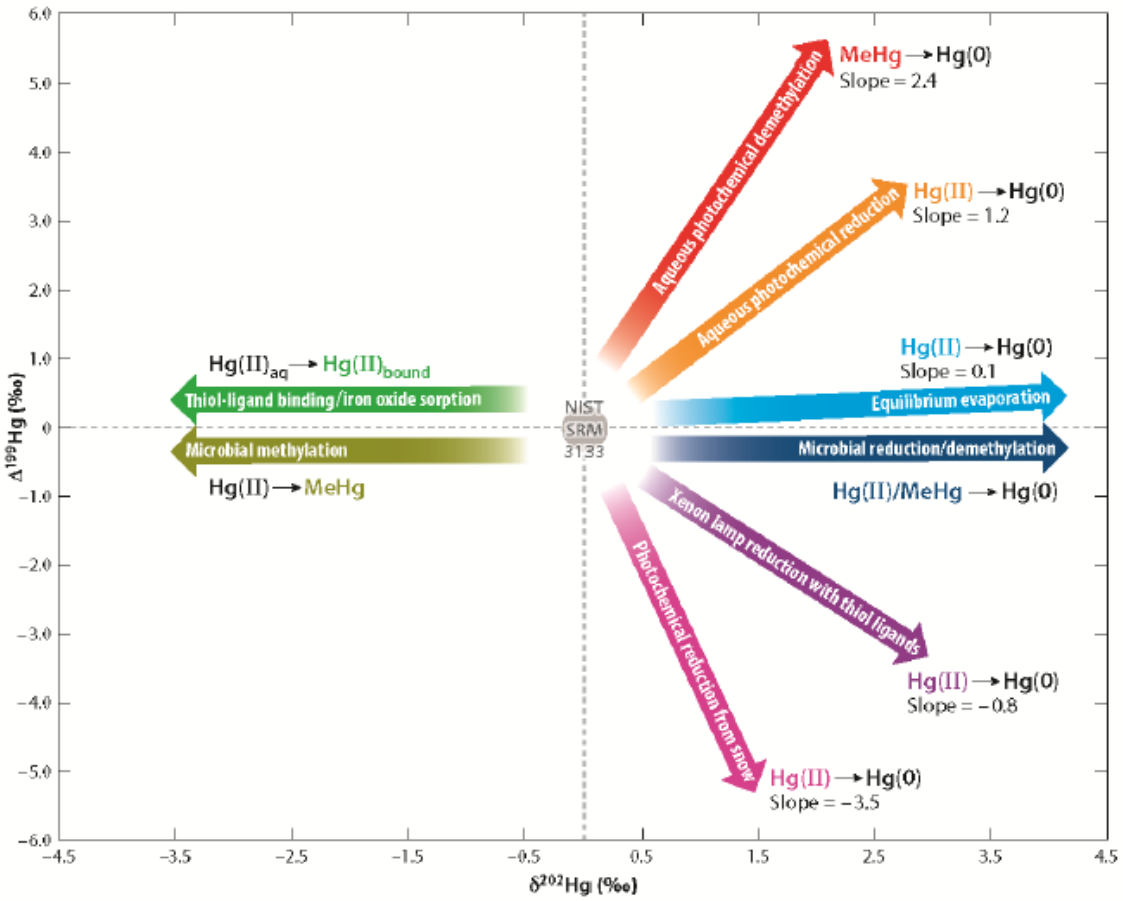

Figure 1.5 - Overview of the general patterns in Hg isotope fractionation (Blum et al., 2014)

1.6.2 Mass independent fractionation (MIF)

Mass independent fractionation is observed when the isotope fractionation is not characterized by a linear relationship between the magnitude of the effect established and the difference in mass between the isotopes, i.e., elements undergoing mass independent fractionation show distinct pattern that differs from that predicted by mass dependent fractionation (37).

Mercury MIF is commonly reported as per mil (‰) deviations (Δ) relative to the measured isotope ratio from the theoretical ratio predicted by MDF, i.e., is the difference between the measured δ^{xxx}Hg and the theoretically predicted δ^{xxx}Hg (35) as:

$$\Delta^{xxx}Hg(‰) \approx \delta^{xxx}Hg - (\delta^{202}Hg \times \beta_{xxx}) \quad (2)$$

where xxx is the mass of the Hg isotope and β_{xxx} is the kinetic or equilibrium fractionation factor appropriate for that isotope.

MIF is usually reported as $\Delta^{199}Hg$ and $\Delta^{201}Hg$. The $\Delta^{199}Hg/\Delta^{201}Hg$ ratio may also be used to quantify the reduction and degradation extent of inorganic Hg ($\Delta^{199}Hg/\Delta^{201}Hg$=1.00) or MeHg ($\Delta^{199}Hg/\Delta^{201}Hg$=1.36) (38). The mechanisms that induce MIF are not fully known but there are a lot of studies indicating that MIF does not occur during biological processes, i.e., MIF signature is preserved during trophic transfer, what makes it a useful tool for characterizing and identifying Hg sources (3).

MIF is thought to occur due to subtle differences in the interaction between the nucleus of these isotopes and the electron cloud, which will affect the characteristics of certain isotopes such as bonding. The proposed processes proposed for MIF are the magnetic isotope effect (MIE) and nuclear volume effect (NVE), that occur firstly in the odd mass isotopes (38).

1.6.3 Mercury isotopes in the environment

It is reported that in natural samples, it is observed a large variation in Hg isotope ratios as expressed on Figure 1.6 (30,35).

Depending on several factors, MDF is reported to vary differently, as it is shown on the XX' axis of Figure 1.6, according to a review made by Blum et al., 2014, MDF ($\delta^{202}Hg$) is reported to vary largely. Mostly negative MDF variations are observed in atmospheric samples and sediments, whereas mostly positive variations are seen on samples collected from humans and geological materials. Freshwater fish show a large variation from negative to positive MDF values (30).

MIF is reported to vary differently, as is shown on YY' axis of Figure 1.6. Large positive variations are observed in fish and invertebrates and mostly negative MIF ($\Delta^{199}Hg$) variations are observed in atmospheric samples and vegetation (30). Absorption and preservation of Hg during the food chain gives a final positive MIF. In contrast, processes that release Hg to the atmosphere during photoreduction could represent the negative MIF values (35).

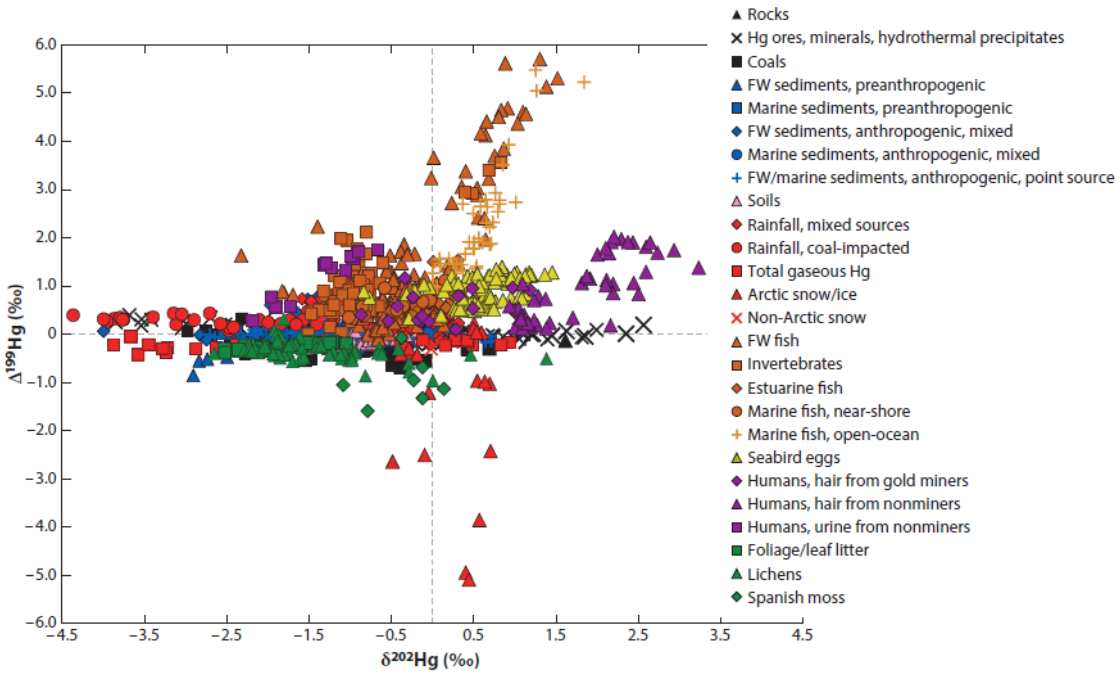

Figure 1.6 - Global range of $\delta^{202}Hg$ and $\Delta^{199}Hg$ from environmental samples (Blum et al., 2014).

The odd-mass number Hg isotopes are very useful as a tracer for monitoring MeHg during trophic transfer and bioaccumulation since they fractionate primarily via photochemical reactions (38). It is suggested that within the same food web, there is observed an isotopic enrichment due to mercury bioaccumulation, being verified a positive $\delta^{202}Hg$ (36). Generally, in trophic chains, predators exhibit higher MDF values than the preys.

As shown by Bergquist and Blum (2007), the natural samples from aquatic systems show both MDF and MIF of Hg isotopes. After measuring fish samples from several lakes, these authors observed that MDF increases with the Hg concentration and fish size. They also observed that MIF varies from lake to lake but remains constant in the same lake (31). Das et al., 2009, after analysing food chains at different trophic levels from a single lake, concluded that between trophic levels and MIF values, there is a striking correspondence (39). This fact was also shown by Perrot et al., 2012 after studying the pelagic food web of lake Baikal, in Russia (36).

In vivo studies conducted by Feng, et al., 2015, where fish were dietary exposed to both MeHg and inorganic Hg. After a period that is shorter to MeHg-enriched food than to inorganic Hg-enriched food, both MDF and MIF in fish tend to be close to the MDF and MIF expressed by the food (40).

Senn, et al., 2010, analysed carbon, nitrogen and mercury stable isotopes to study MeHg sources and trophic transfer in the Northern Gulf of Mexico. The results showed that coastal fish species displayed lower MDF and MIF values when comparing to oceanic fish species. These observations showed that these food webs are not connected, as confirmed by $\delta^{15}N$ signatures (41).

Overall, both MDF and MIF values are keys on quantifying and understanding Hg pathways and bioaccumulation in aquatic systems.

Combining the actual knowledge about the rate constants for various fractionation reactions and the characteristic range of isotopic composition in distinct environments, it makes Hg isotopes a new powerful tool for identifying Hg pollution sources, tracking of mercury biogeochemical processes and tracking metabolic behaviour in living organisms.

1.6.3.1 Identification of mercury pollution sources

MIF isotope signature of Hg is a powerful tool for identifying specific Hg sources such as anthropogenic from industry or mining and for tracing Hg exposure sources to living organisms (33). Sediments are an important reservoir of Hg anthropogenic pollution in aquatic systems, being a key element to investigate temporal and spatial variations of Hg sources (30). Perrot et al., 2010 observed that fish samples with higher Hg concentration show $\Delta^{199}Hg$ similar to that found in the sediments, whereas the less contaminated fish display higher MIF than the sediment. It is suggesting that the more contaminated fishes have bioaccumulated Hg from feeding sources associated with the contamination of sediment (42).

1.6.3.2 Tracking of mercury biogeochemical processes

Hg isotope ratios can be also useful for better understanding the biogeochemical cycle of Hg. Through the biogeochemical cycle, Hg is affected by diverse transformation processes that can cause a shift in the isotopic signature (30). Once in aquatic environments, Hg can undergo transformation by biological and non-biological processes, such as oxidation/reduction and methylation/demethylation, amongst others (16). Hg isotopic signatures have been extensively used to study

biogeochemical processes in the aquatic environments, such as lakes (36,39,43) and coasts and seas (41,44).

Blum et al., 2013 proposed a model for Hg biogeochemical processes in aquatic environments (Figure 1.7). These authors suggest that after Hg^{2+} deposition from precipitation, in the surface mixed layer of the ocean (SML), it can undergo photochemical or microbial reduction to Hg^0, microbial methylation or even transported to greater depths (the pycnocline region) attached to particles. From the mentioned processes, just the transport to greater depths is not associated to an increase in MIF signature. In sum, Hg^{2+} and MeHg with higher positive MIF are propagated through the epipelagic food web and on the mesopelagic (deeper) food web, the accumulated MeHg shows lower positive MIF (44).

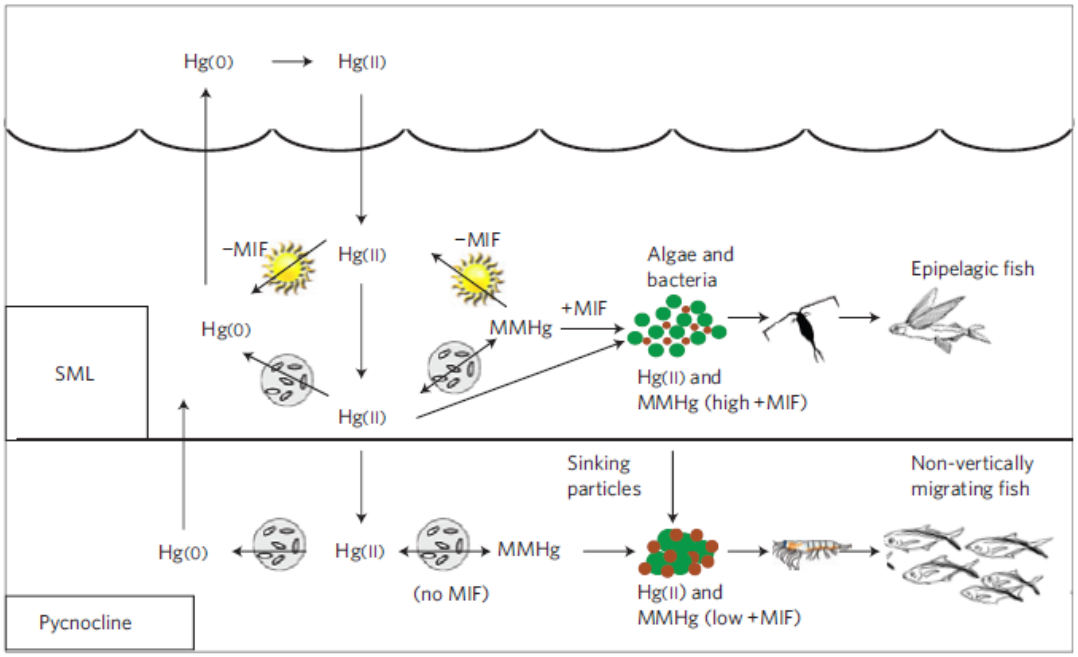

Figure 1.7 - Schematic representation of a simplified model for Hg cycling between atmosphere, SML, pycnocline and marine food web (Blum et al., 2013).

2. Objectives

The main objective of this work is to better understand the origin of Hg pollution to the fish living in the Aquitanian lakes by isotopic analysis since it is impossible to track Hg origins just by total mercury concentration determination. To meet the goal, six different species, of which, four are fish, one is crayfish and another one is a mollusc were analysed in a MC-ICP-MS for Hg isotopic composition determination.

3. Materials and methods

3.1 The Aquitanian lakes

The sand dunes along the Aquitaine coastline form a physical barrier for the streams, draining the plain of the Gascogne Lands – a french natural region of around 1.5 million hectares located in three departments (Gironde, Landes and Lot de Garonne) around the Arcachon Bay and dominated by a pine forest. These retained waters create the permanent freshwater reservoirs at the foot of the dunes, all along the coast. The only exception is the Arcachon bay that is opened towards the ocean (Figure 3.1). These lakes are shallow (less than 23m). Starting from the north to the south, the first lake is Hourtin-Carcans (Gironde) which extends over a maximum length of 18km and a maximum width of 5km and has a surface area of 62 km^2 what makes it the largest natural lake on the metropolitan France, although its maximum depth is only 10m. The southern part of Hourtin-Carcans lake is connected to the northern part of the Lacanau lake (Gironde) through a channel built in the years 1868-1870. This lake has a 20km^2 surface area and a maximum depth of 8.5m. Lacanau lake outlets in the Arcachon basin. On the south of Arcachon basin, the first lake is Cazaux-Sanguinet (Landes) with an area of 58km^2 and 23m depth, being the most profound of the four lakes. In the south, this lake is connected to Parentis-Biscarrosse (Landes) that has a 35km^2 surface area with a maximum depth of 20.5m (27). The water residence time is longer for Casaux-Sanguinet lake (1587 days) than to Parentis-Biscarrosse (372 days) (8). According to OCDE, the four lakes are classified in different

trophic levels: Hourtin-Carcans, eutrophic; Lacanau, mesotrophic; Cazaux-Sanguinet, oligo-mesotrophic; Parentis-Biscarrosse, meso-eutrophic. These classifications are done according to the physicochemical data (phosphorus, nitrogen, chlorophyll a and depth of Secchi) (45). The main characteristics of the four studied lakes are summarized in Table 3.1.

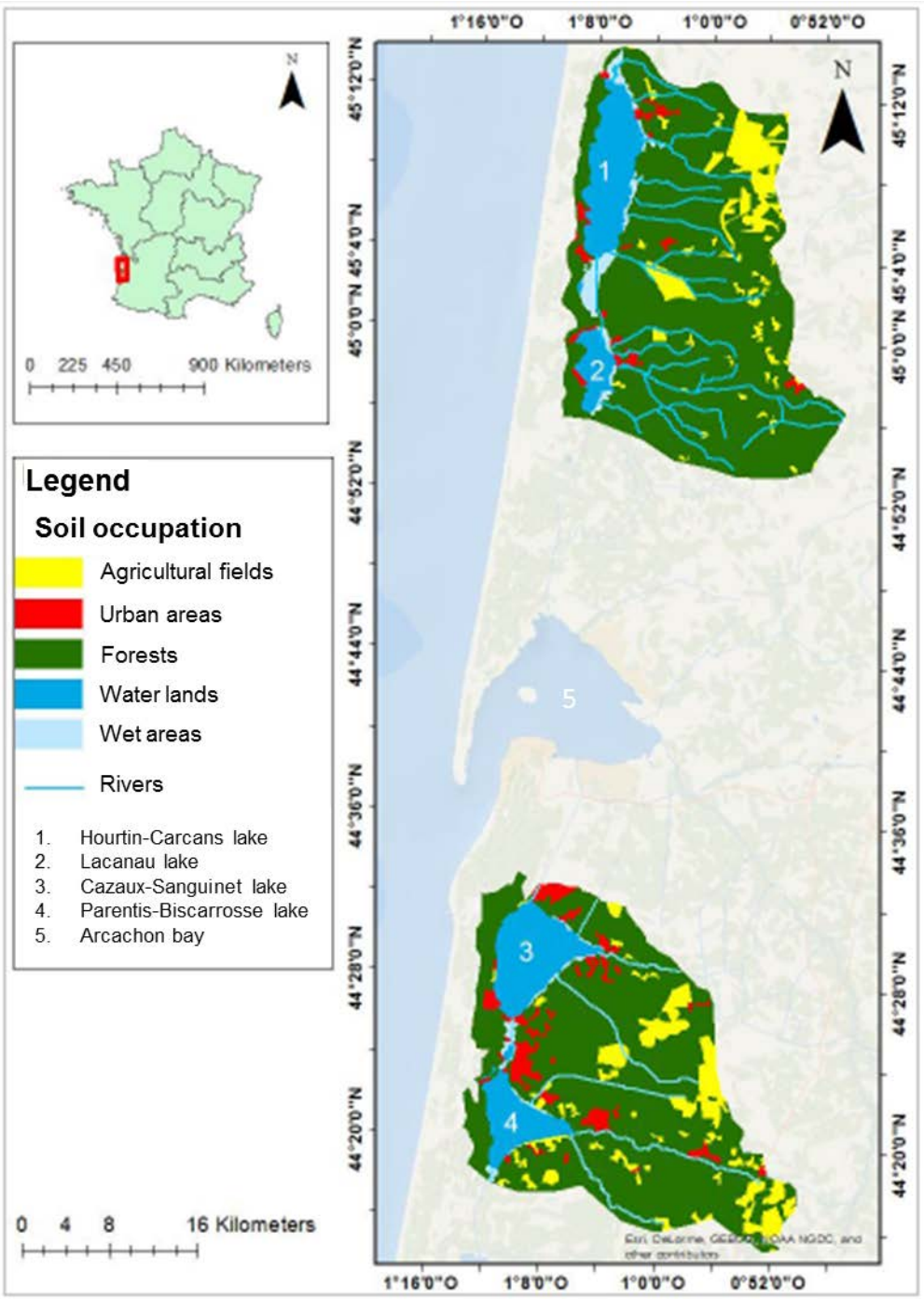

Figure 3.1 - Map of the location of the great aquitaine lakes and the close lands occupation (adapted from Canderon, 2016).

Table 3.1 - Main characteristics of the studied lakes.

Lakes	Surface area (km^2)	Maximum depth (m)	Water residence time (years)	Water volume (million m^3)	Trophic level	Substrate
Hourtin-Carcans	62	11	1.8	210	Eutrophic	Sand
Lacanau	20	8	0.4	53	Meso-eutrophic	Sand
Cazaux-Sanguinet	58	23	4	500	Oligotrophic	Sand
Parentis-Biscarosse	36	20.5	1	240	Eutrophic	Sand

3.2 Sampling

In order to avoid the seasonal effect "intra and inter-lakes", fish samples were taken over a short period, from September to November 2015 in the scope of the CLAQH project. Several types of fishing were used to provide a representative sampling of each lake and species. To obtain a good statistical representation of the mercurial concentration and mercurial isotopic fractionation of each link of the trophic network, a minimum number of 5 individuals was collected. However, in order to avoid unnecessary large-scale fish harvesting, the collected fish were harvested from 3 complementary fishing methods: the carnivores were recovered from the fishing competitions in agreement with the local fishermen's associations; in the Landais lakes (Cazaux-Sanguinet and Parentis-Biscarrosse), the fishing campaign was carried out within the framework of the DCE (Directive Cadre sur L'Eau) and the incomplete sampling on the lakes of Gironde (Hourtin-Carcans and Lacanau) was supplemented by a net fishing with a professional.

The Table 4.1 summarizes the samples' characteristics (scientific name, trophic guild, total mercury concentration, mean mass of the individuals of each specie living in the same lake and the number of individuals analysed) analysed in the scope of this study, for mercury isotopic analysis. The concentration of total Hg was determined on the study previously realized in the same scope of this project and is reported in the final report (Azaroff, 2016) (46).

3.3 Trophic position calculation

The organisms were analysed for carbon and nitrogen isotope ratios by Sophie Gentès (Université de Bordeaux) in the aim of CLAQH project and gently ceded for describing the food web structure in each lake. The analysis were done in a Thermo Scientific Delta V advantage isotope ratio mass spectrometer at La Rochelle University isotopic platform (France). The isotopic values are expressed as the standard $\delta^{15}N$ and $\delta^{13}C$ notation in parts permillage (‰) relative to the international reference material standards (atmospheric N_2 for nitrogen and Vienna Pee Dee Belemnite for carbon), using the standard equation (47):

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = [R_{Sample}/R_{Standard})/R_{Standard}] \times 1000 \quad (3)$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

The relatively constant $\delta^{15}N$ enrichment between each trophic level is an important tool for interfering the position of an organism in a food web (48).

As described by Bergamino et al., 2011, $\delta^{15}N$ signatures were converted to trophic positions (TP), using the following equation:

$$TP_i=[(\delta^{15}N_i - \delta^{15}N_{pc})/3.4] + 2 \quad (4)$$

Where TP_i is the average trophic position of species I, $\delta^{15}N_i$ represents the average $\delta^{15}N$ of species I, $\delta^{15}N_{pc}$ represents the average $\delta^{15}N$ of primary consumers, 3.4 is the mean $\delta^{15}N$ trophic enrichment occurring per trophic level (49) and 2 is the trophic position of the baseline organism (Clam, in this case).

3.4 Sample preparation

For mercury isotopic analysis, the biologic samples analysed (fish muscle, mollusc and crayfish muscle), were digested to assure total mineralization with nitric acid and oxygen peroxide by using HPA-S (Anton Paar).

0.2 – 0.9g of freeze dried fish muscle were precisely weighted and predigested with 1-5 mL of Nitric acid (HNO_3) InstrA overnight at room temperature and then digested by adding 0.5-2 mL Hydrogen peroxide (H_2O_2) UltraPure (depending on the

Hg content) using HPA (High Pressure Asher) (Anton Paar) as represented on Figure 9 (40,50).

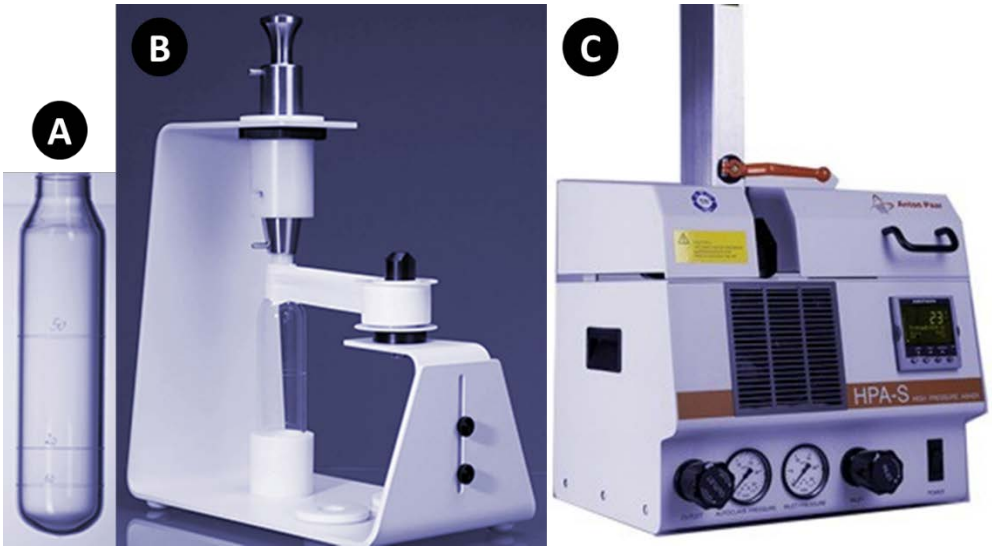

Figure 3.2 – (A) Quartz vial for HPA digestion (Anton Paar) (B) HPA tubes closing apparatus (Anton Paar) (C) HPA-S (Anton Paar).

3.5 Mercury isotopic analysis

Nowadays, MC-ICP-MS (Multi-Collector - Inductively Coupled Plasma – Mass Spectrometry) (Figure 3.3) is the most used technology for Hg isotope ratios measurements due to the high precision (<0.2‰) and sensitivity. It consists on an ion source, a mass analyser and a mass unit. The first is a high-temperature plasma that is able to ionize elements with high ionization potential. The mass analyser reduces the kinetic energy of the ions and separates them according to their mass. Like this, the ion beam is focused when entering the mass spectrometer. The last unit consists on a collector array that measures ion beams of different isotopes at the same time, what leads to an acceptable precision for the ratios (33).

Figure 3.3 - Nu Plasma II - Multi-Collector - Inductively Coupled Plasma – Mass Spectrometer (Nu Instruments).

The most used introduction system is the cold-vapour generator (CVG) as it relies on a continuous generation of Hg^0 vapour that creates a steady and continuous signal (Figure 3.4) (33).

For mass bias correction, both sample-standard bracketing and elemental spike are used. The first consists on a dry Thallium (Tl) aerosol generation using a desolvating nebulizer and simultaneous mixture with the Hg vapour generated in the CVG. Then, the both elements are introduced into the plasma and analysed at the same time (Figure 3.4). Sample-standard bracketing consists on the measurement of a Hg isotopically certified standard (usually NIST 3133) before and after sample analysis. This approach allows the correction of the fact that mass spectrometers favour the transmission of heavier isotopes prior to lighter isotopes (33,51).

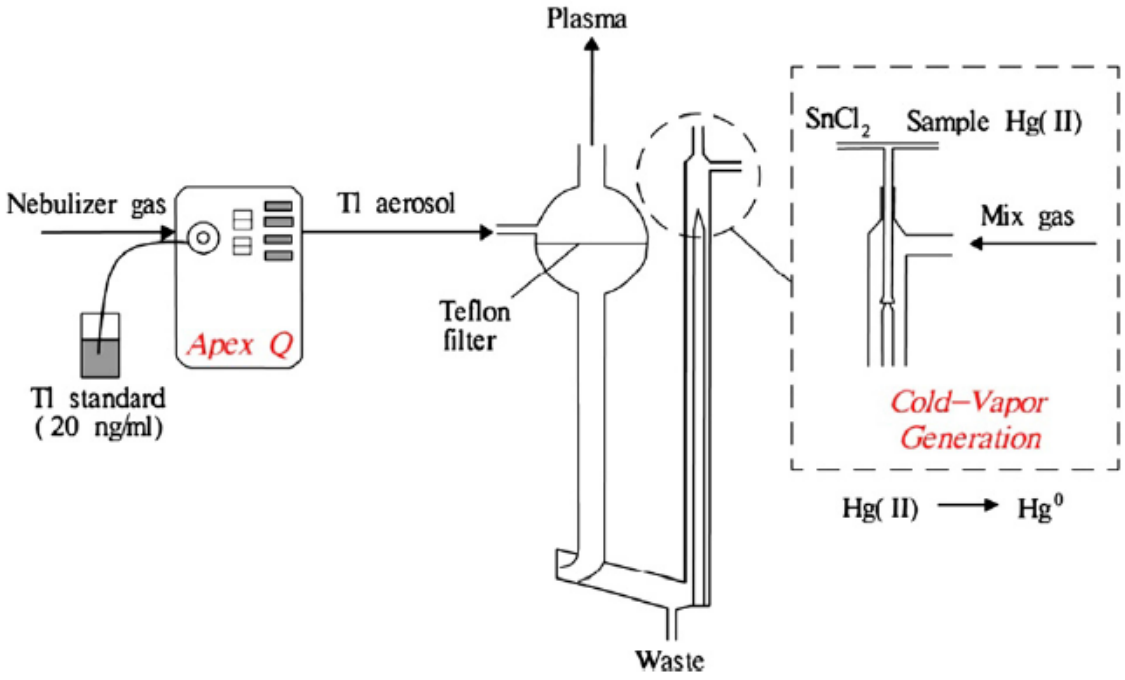

Figure 3.4 - Schematic introduction system for Hg stable isotope analysis using cold vapour generation and thallium simultaneous injection (Adapted from Fouscher and Hintelmann, 2006)

The samples were analysed using a Nu Plasma II Multi-Collector - Inductively Coupled Plasma – Mass Spectrometer coupled to a cold vapor generator (Nu Instruments) – CVG-MC-ICP-MS. Total Hg isotopic composition was measured for the six most abundant stable Hg isotopes (^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg) relative to the bracketing standard NIST SRM-3133. For mass bias correction using the exponential law, NIST SRM-997 thallium solution was introduced using a DSN-100 desolvation nebulizer system from Nu Instruments and analysed the two isotopes (^{205}Tl and ^{203}Tl). Russell-correlation was used to calculate fractionation factor (*f*), according to the following equation:

$$f = \log_{1.009864}\left[\frac{\left(\frac{^{205}Tl}{^{203}Tl}\right)_{measured}}{2.38714}\right] \quad (5)$$

and Hg isotopic ratios were recalculated using the same correlation:

$$\left(\frac{^{xxx}Hg}{^{198}Hg}\right)_{true} = \frac{\left(\frac{^{xxx}Hg}{^{198}Hg}\right)_{measured}}{\left(\frac{xxx}{197.966743}\right)^{f}} \quad (6)$$

Where xxx is the mass of Hg isotope.

MDF ($\delta^{xxx}Hg$) was calculated as presented on 1.6.1.

MIF ($\Delta^{xxx}Hg$) was calculated as presented on 1.6.2.

Analytical uncertainty of the method was determined as the largest 2SD (2*Standard deviation) corresponding to the sample or to the measurement of the UM-Almadén secondary standard during each session ($\delta^{202}Hg$ = -0.48 ± 0.20, $\Delta^{199}Hg$ = −0.03 ± 0.16, n = 27) (Table 3.2) previously characterized (40,52).

As shown on Table 3.2, the long-term reproducibility is assured on this method. The UM Almadén results obtained for the different Hg isotopes are in accordance with the previous results (40,52). The results obtained for the NIST 1947 validate the accuracy of the method for these analyses.

Table 3.2 - Hg isotopic ratios measurements of standards (UM-Almadén and NIST 1947) and comparison with previous published results. Results expressed as mean value ± 2SD. SD means standard deviation. "NR" means not reported. "N" means the number of samples analysed.

	Samples	$^{204}\delta$ ‰	$^{202}\delta$ ‰	$^{201}\delta$ ‰	$^{200}\delta$ ‰	$^{199}\delta$ ‰	$^{201}\Delta$ ‰	$^{200}\Delta$ ‰	$^{199}\Delta$ ‰	N
UM ALMADÉN	This study	-0.74 ± 0.29	-0.48 ± 0.20	-0.41 ± 0.17	-0.23 ± 0.16	-0.15 ± 0.16	-0.05 ± 0.07	0.01 ± 0.10	-0.03 ± 0.16	27
	Feng. 2015	-0.80 ± 0.23	-0.52 ± 0.16	-0.42 ± 0.14	-0.26 ± 0.09	-0.16 ± 0.06	-0.03 ± 0.06	0.00 ± 0.05	-0.03 ± 0.06	31
	Epov et al. 2008	NR	-0.51 ± 0.17	-0.42 ± 0.15	-0.23 ± 0.13	-0.14 ± 0.08	-0.04 ± 0.07	0.02 ± 0.08	-0.01 ± 0.08	34
NIST 1947	This study	1.29 ± 1.26	0.98 ± 0.88	3.05 ± 2.19	0.58 ± 0.42	3.12 ± 2.84	2.31 ± 2.11	0.09 ± 0.05	2.87 ± 2.83	7
	NIST Certificate	1.66 ± 0.07	1.20 ± 0.07	5.09 ± 0.18	0.69 ± 0.09	5.62 ± 0.25	4.17 ± 0.28	0.09 ± 0.02	5.31 ± 0.29	NR

The one-way ANOVA post-hoc Tukey statistical test was used for the MDF and the MIF values of the species living in the same lake. The results of the test are expressed on Annex 2 and represented on Figure 4.1 and Figure 4.3.

3.5.1 Reagents preparation

A 3% $SnCl_2{\cdot}2H_2O$ (Stannous chloride dihydrate) in 1N HCl (Hydrochloric acid) solution was prepared and used in a CVG to convert Hg(II) to Hg(0).

All the blanks, standards, samples and rinse solutions for MC-ICP-MS analysis were prepared in 10% HNO_3 and 2% HCl solution.

4. Results

4.1 Mercury stable isotopic compositions and total mercury concentration

In this study the range of $\delta^{202}Hg$ and $\Delta^{199}Hg$ values were evaluated in the different fauna cohabiting in the same lake and also in the same species living in different lakes.

It was observed an increase of total Hg concentration in biota from north to south, being the northern lakes (Hourtin-Carcans and Lacanau) more contaminated with this metal (Figure 4.1). The total Hg concentration was taken into account to see if there was a trend between this parameter and the Hg isotopic analysis (Figure 4.1 and Figure 4.3).

The overall results for Hg isotopic compositions of the analysed samples are shown on Table 4.1. Following, the results of MDF (represented as $\delta^{202}Hg$) and MIF (represented as $\Delta^{199}Hg$) will be presented in separate since these parameters give different types of information about sources and transformation processes of Hg in the Aquitanian lakes. Total mercury concentration and stable isotopic analysis of carbon and nitrogen were used in this study for better interpretation of the stable isotopic analysis of mercury data.

Some fish samples were chosen for plotting [THg] against fish mass and no relationship was found between these 2 variables (Annex A1).

Table 4.1 - Number of analyses (N), arithmetic mean values of total mercury concentration and Hg isotopic composition (reported as δ and Δ versus NIST 1947) of biota samples collected from the 4 sampling sites - Hourtin-Carcans (HC), Lacanau (L), Casaux-Sanguinet (CS) and Parentis-Biscarosse (PB). Error deviations are reported as SD and 2SD for [THg] and mercury isotopic compositions, respectively.

Lake	Specie	Scientific name	N	Trophic guild	[THg] (mg.kg^{-1} dw)	Mass (g)	δ^{204}Hg ‰	δ^{202}Hg ‰	δ^{201}Hg ‰	δ^{200}Hg ‰	δ^{199}Hg ‰	Δ^{204}Hg ‰	Δ^{201}Hg ‰	Δ^{199}Hg ‰
HC	Bream	*Abramis brama*	4	Omnivorous	0.48 ± 0.08	496 ± 410	-0.02 ± 0.10	0.05 ± 0.11	1.01 ± 0.13	0.11 ± 0.12	1.46 ± 0.26	-0.11 ± 0.11	1.01 ± 0.13	1.45 ± 0.25
	Pike	*Esox Lucius*	4	Carnivorous	3.85 ± 1.25	2 000 ± 474	0.04 ± 0.20	0.07 ± 0.19	1.99 ± 0.38	0.12 ± 0.16	2.66 ± 0.54	-0.07 ± 0.12	1.94 ± 0.34	2.64 ± 0.54
	Perch	*Perca fluviatilis*	5	Carnivorous	5.46 ± 0.92	1 660 ± 372	0.26 ± 0.15	0.30 ± 0.14	2.54 ± 0.71	0.24 ± 0.09	3.15 ± 0.83	-0.18 ± 0.10	2.32 ± 0.64	3.07 ± 0.80
	Pike-perch	*Sander lucioperca*	1	Carnivorous	6.24	2 750	0.21	0.28 ± 0.88	2.06 ± 2.19	0.15 ± 0.42	2.60 ± 2.84	-0.21	1.85 ± 2.11	2.52 ± 2.83
	Crayfish	*Procambarus clarkii*	3	Omnivorous	0.476 ± 0.059	NA	0.12 ± 0.56	0.09 ± 0.34	1.37 ± 0.61	0.12 ± 0.14	1.79 ± 0.59	-0.02 ± 0.12	1.30 ± 0.42	1.76 ± 0.54
L	Bream	*Abramis brama*	4	Omnivorous	0.73 ± 0.24	1 468 ± 212	-0.31 ± 0.28	-0.12 ± 0.26	0.98 ± 0.39	-0.02 ± 0.11	1.44 ± 0.31	-0.13 ± 0.12	1.07 ± 0.24	1.47 ± 0.28
	Pike	*Esox Lucius*	5	Carnivorous	2.16 ± 0.83	2 415 ± 452	-0.52 ± 0.44	-0.29 ± 0.35	1.46 ± 0.91	-0.11 ± 0.24	2.21 ± 0.99	-0.09 ± 0.22	1.79 ± 0.67	2.28 ± 0.91
	Perch	*Perca fluviatilis*	5	Carnivorous	1.96 ± 1.50	910 ± 421	-0.55 ± 0.38	-0.31 ± 0.21	1.39 ± 0.77	-0.13 ± 0.15	2.17 ± 0.90	-0.08 ± 0.17	1.62 ± 0.65	2.25 ± 0.86
	Pike-perch	*Sander lucioperca*	2	Carnivorous	3.73 ± 1.53	3 750 ± 1 909	-0.16 ± 0.81	-0.05 ± 0.47	1.96 ± 1.18	0.00 ± 0.13	2.71 ± 0.96	-0.08 ± 0.10	2.00 ± 0.83	2.72 ± 0.84
	Clam	*Corbicula fluminea*	5	Filter-feeder	0.117 ± 0.019	NA	-1.34 ± 0.22	-0.81 ± 0.17	-0.55 ± 0.23	-0.32 ± 0.09	-0.03 ± 0.21	-0.13 ± 0.07	0.06 ± 0.15	0.17 ± 0.18
	Crayfish	*Procambarus clarkii*	5	Omnivorous	0.260 ± 0.065	NA	-0.55 ± 0.32	-0.32 ± 0.24	0.77 ± 0.31	-0.13 ± 0.13	1.37 ± 0.25	-0.06 ± 0.22	1.01 ± 0.20	1.45 ± 0.22
CS	Bream	*Abramis brama*	5	Omnivorous	0.23 ± 0.11	1 146 ± 496	0.08 ± 0.32	0.10 ± 0.17	1.38 ± 0.53	0.13 ± 0.08	1.91 ± 0.54	-0.08 ± 0.19	1.30 ± 0.42	1.88 ± 0.51
	Pike	*Esox Lucius*	5	Carnivorous	1.92 ± 0.48	2 220 ± 396	0.05 ± 0.19	0.08 ± 0.16	2.28 ± 0.52	0.10 ± 0.11	3.06 ± 0.54	-0.06 ± 0.09	2.22 ± 0.41	3.04 ± 0.50
	Perch	*Perca fluviatilis*	4	Carnivorous	3.01 ± 0.86	1 006 ± 308	0.29 ± 0.12	0.24 ± 0.11	2.67 ± 0.17	0.18 ± 0.04	3.51 ± 0.56	-0.06 ± 0.10	2.49 ± 0.09	3.45 ± 0.53
	Pike-perch	*Sander lucioperca*	3	Carnivorous	2.83 ± 0.24	3 650 ± 589	0.54 ± 0.12	0.51 ± 0.16	3.06 ± 0.46	0.35 ± 0.22	3.73 ± 0.45	-0.21 ± 0.13	2.67 ± 0.34	3.61 ± 0.41
PB	Bream	*Abramis brama*	5	Omnivorous	0.28 ± 0.09	395 ± 46	-0.38 ± 0.41	-0.21 ± 0.28	0.63 ± 0.54	-0.06 ± 0.08	1.09 ± 0.43	-0.07 ± 0.19	0.79 ± 0.34	1.14 ± 0.39
	Pike	*Esox Lucius*	5	Carnivorous	1.46 ± 0.33	2 460 ± 451	-0.46 ± 0.14	-0.26 ± 0.08	0.78 ± 0.14	-0.06 ± 0.08	1.26 ± 0.06	-0.07 ± 0.13	0.97 ± 0.08	1.33 ± 0.05
	Perch	*Perca fluviatilis*	5	Carnivorous	1.79 ± 0.79	860 ± 192	-0.45 ± 0.17	-0.24 ± 0.15	0.87 ± 0.15	-0.07 ± 0.22	1.36 ± 0.20	-0.10 ± 0.14	1.05 ± 0.06	1.41 ± 0.14
	Pike-perch	*Sander lucioperca*	5	Carnivorous	2.07 ± 0.26	1 890 ± 284	-0.33 ± 0.34	-0.15 ± 0.22	0.97 ± 0.22	-0.03 ± 0.19	1.45 ± 0.23	-0.10 ± 0.15	1.08 ± 0.11	1.49 ± 0.19
	Crayfish	*Procambarus clarkii*	5	Omnivorous	0.117 ± 0.021	NA	-0.44 ± 0.29	-0.27 ± 0.12	0.38 ± 0.29	-0.22 ± 0.13	0.57 ± 0.28	-0.04 ± 0.18	0.58 ± 0.23	0.64 ± 0.28

4.1.1 Comparison between lakes

4.1.1.1 Mass dependent fractionation of mercury and total mercury levels

MDF of Hg ($\delta^{202}Hg$) in biota is shown on Table 4.1. Mean $\delta^{202}Hg$ varied from -0.81 ± 0.17‰ to 0.51 ± 0.16‰, for clam from lake L and for pike-perch from lake CS, respectively. This means that pike-perch is richer in heavier isotopes than clam, which is 2 TPi lower (results shown on 4.2 Isotopic analysis and trophic positions). Moreover, total mercury concentration in pike-perch is one order of magnitude higher than in clam (Table 4.1). On both CS and HC lakes, the $\delta^{202}Hg$ is positive. On L and PB $\delta^{202}Hg$ values are negative, meaning that on CS and HC the biota is more enriched in heavier isotopes than the same species living in L and PB.

In a study done in 2016, sediments from these lakes were analysed to determine the total Hg concentration (53). The highest concentrations of MeHg were present in the surface organic sediments. The particulate MeHg concentrations were 4.30 $\mu g.kg^{-1}$ for Carcans-Hourtin Lake, 3.75 $\mu g.kg^{-1}$ for Lacanau, 1.25 $\mu g.kg^{-1}$ for Cazaux-Sanguinet, and 1 $\mu g.kg^{-1}$ for Parentis Biscarrosse (53). It is showing the trend of Hg accumulation in these Aquitanian lakes from south to north.

By the analysis of the total mercury on the fish, it is seen that in this study, one individual of bream, a non-predatory fish, is surpassing the normative value, displaying 5.7 mg Hg/kg On the other hand, the perches and pike-perches, considered predatory fishes, are surpassing the normative value established by ANSES (Saisine nº2012-SA-0066), are surpassing the normative value on Hourtin-Carcans, Lacanau and Cazaux-Sanguinet lakes. The more contaminated fish sample was collected from Hourtin-Carcans lake and shows a mercury concentration that surpasses the normative value for non-predatory fish and even for predatory fish. The pike-perches that surpass the normative values are found in the same lakes and the more concentrated value is obtained once again from the Hourtin-Carcans lake with a 6.3 mg/kg Hg concentration (46).

The existence of the mercury contamination gradient of the fish muscles between the 4 lakes is well related with the concentrations of MeHg found in the sediments (46,53).

From Figure 4.1, seen a good correlation between [THg] and δ^{202}Hg, unlike it was seen on Perrot et al., 2010.

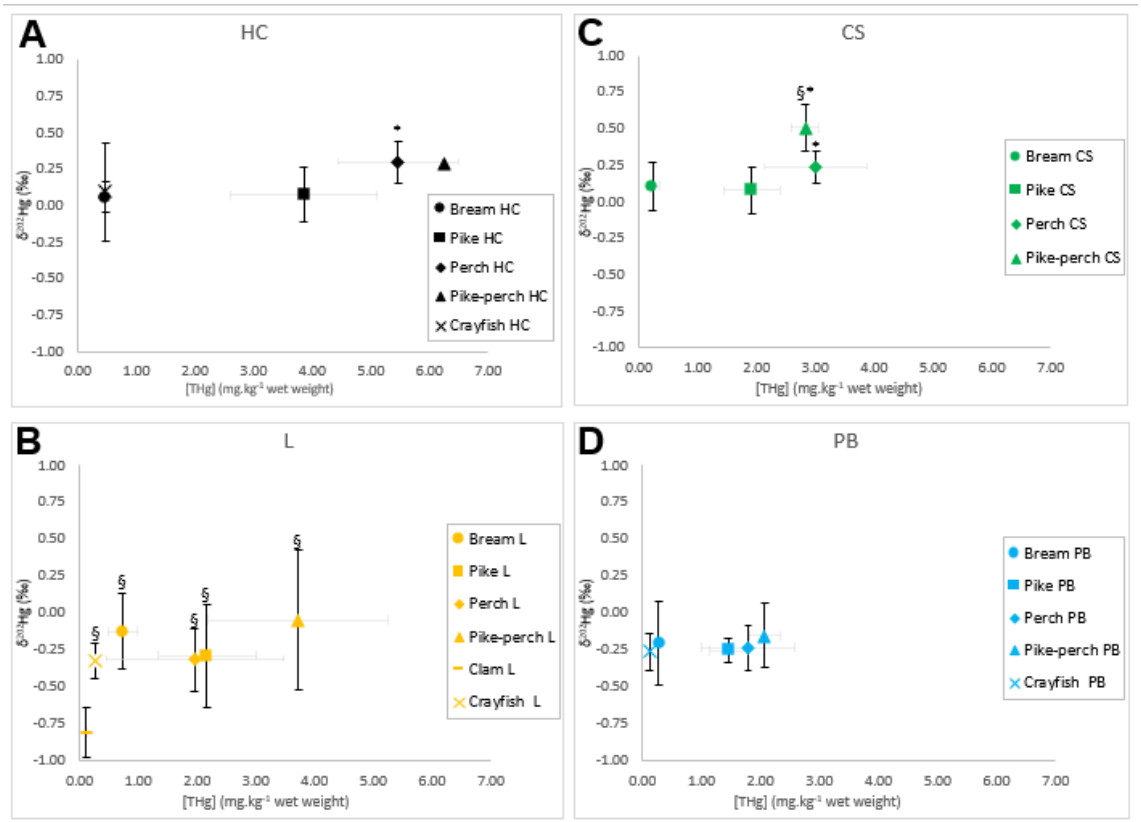

Figure 4.1 - Representation of δ^{202}Hg values variation with total mercury concentration for biota samples from: A - Hourtin-Carcans (HC); B – Lacanau (L); C - Casaux-Sanguinet (CS) and D - Parentis-Biscarosse (PB). Error bars for δ^{202}Hg show 2SD and for [THg] show SD. The § means that the δ^{202}Hg value is statistically different ($p < 0.05$) of the sample with lower value (clam or crayfish for all the lakes except CS). The * means that the δ^{202}Hg is statistically different from the precedent sample. The detailed statistical results are shown in Annex 2.

When comparing the lakes on Figure 4.1, in general, all the fish species and crayfish show almost the same δ^{202}Hg. Although, on CS lake, pike-perch shows the slightly higher δ^{202}Hg. Crayfish, pike, bream and perch have different distributions on each lake. Otherwise, on L and PB, the southern lakes, crayfish shows a similar δ^{202}Hg to this shown on all the fish species. Crayfish and bream are both omnivorous and in the different lakes both show similar δ^{202}Hg values and THg concentration.

Despite the fact that bream for Lacanau is showing the higher overall weight of the individuals of this specie, the MDF is statistically similar to the other fish species and crayfish.

Perrot et al., 2010 and 2012 (36,42) also analysed δ^{202}Hg for perch from lake Baikal and Bratsk water reservoir, both in Russia. On the both sampling sites, δ^{202}Hg is close to -0.5‰ that is similar to the value found in our study for the smaller lakes (L and PB), where the THg concentration is closer to the found in the lakes analysed by these authors.

4.1.1.2 Mass independent fractionation of mercury and total mercury levels

When plotting Δ^{199}Hg vs Δ^{201}Hg it is possible to infer the reaction that occurred in the mercury bioaccumulated. A slope of 1.0 is consistent with inorganic mercury photoreduction, whereas a slope of 1.3 is associated with MeHg demethylation (31,35). The results obtained in this study (Fig. 4.2) fit in a 1.3 slope linear regression, meaning that in mercury bioaccumulated in this biota results principally of MeHg demethylation. This is in agreement with other studies in biota (36,42)

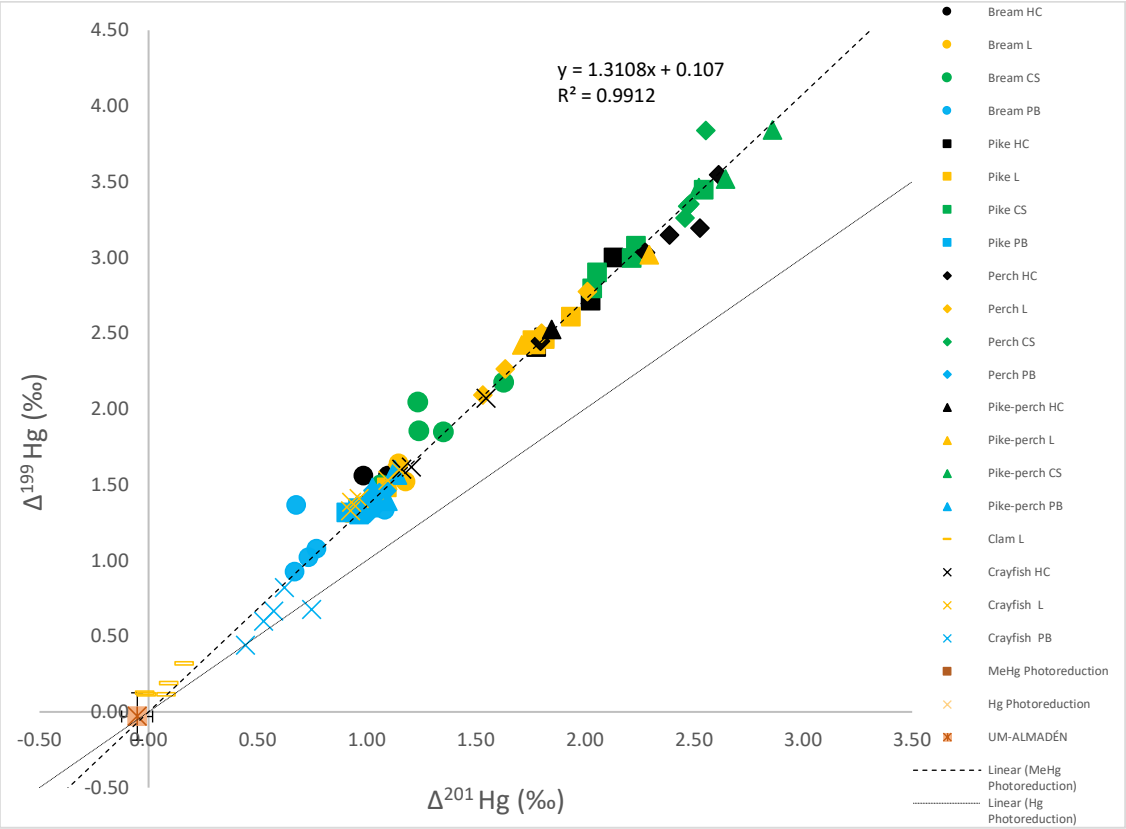

Figure 4.2 - Δ^{199}Hg versus Δ^{201}Hg for all biota sample analyses in this study. The dotted line represents the theoretic line for Hg photoreduction. The dashed line represents the theoretic line for MeHg photoreduction.

The regression did not consider the samples from PB lake as their lowest content in mercury was increasing the slope.

Odd isotopes of Hg in the analysed biota exhibit MIF, as shown on Table 4.1. Mean Δ^{199}Hg vary from 0.17 ± 0.18‰ to 3.61 ± 0.41‰, n=67, corresponding to L clam and CS pike-perch, respectively (Table 4.1 and Figure 4.3).

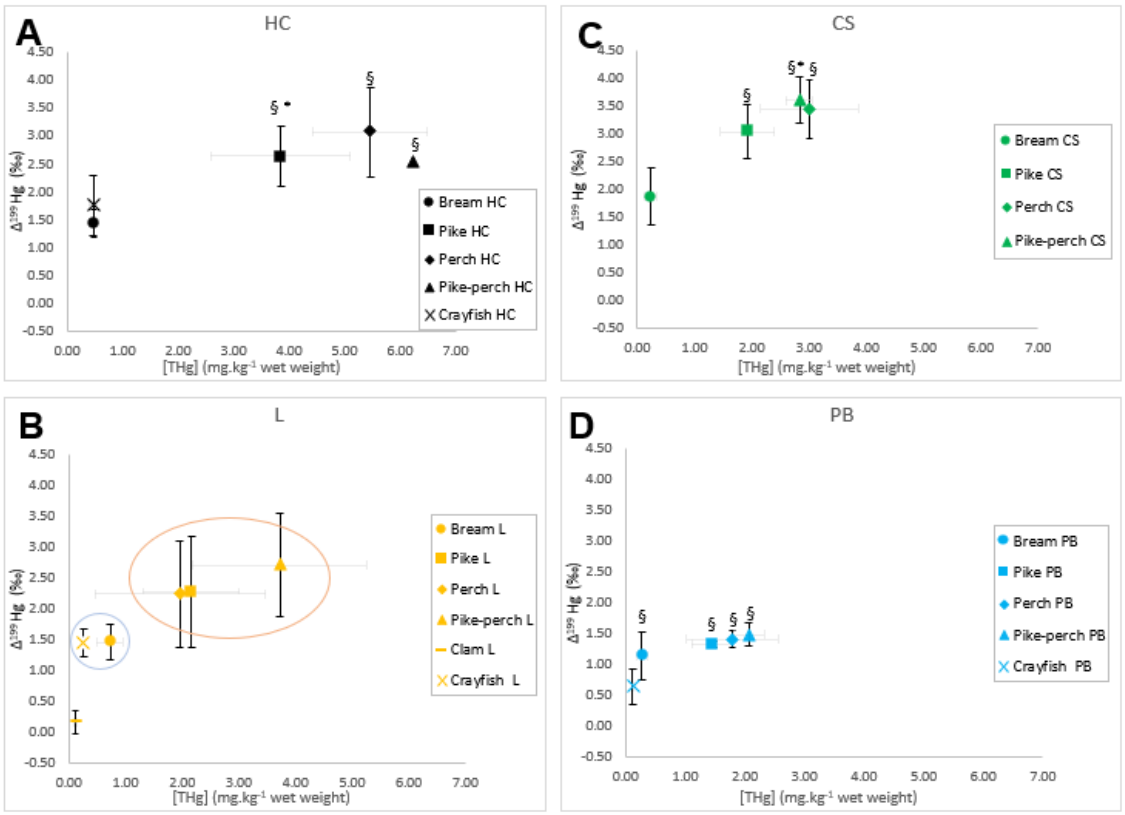

Figure 4.3 - Δ^{199} Hg plotted against wet weight total mercury concentration for biota samples from: A - Hourtin-Carcans (HC); B – Lacanau (L); C - Casaux-Sanguinet (CS) and D - Parentis-Biscarosse (PB). Error bars for Δ^{199} Hg show 2SD and for [THg] show SD. The § means that the Δ^{199}Hg value is statistically different ($p < 0.05$) of the sample with lower value (clam or crayfish for all the lakes except CS). The * means that the Δ199Hg is statistically different from the precedent sample. In the case of the figure B, the circles are grouping the statistically significant. The detailed statistical results are shown in Annex 2

When comparing the 4 lakes (Figure 4.3), it is possible to see that on HC, L and CS, pike, perch and pike-perch showed similar Δ^{199}Hg of around 3‰ although in general pike has lower THg levels than perch and pike-perch. Bream and crayfish have similar Δ^{199}Hg of around 1.5‰ and [THg]. Although clams were only analysed for Lacanau lake, this specie displays the lowest MIF and [THg] values amongst the species analysed. PB was the less contaminated lake and pike, perch and pike-perch also showed similar MIF values, however, with lower magnitude. When analysing just the MIF parameter, these 2 groups of biota seem to make part of the same trophic chain, although, the MDF parameter (Figure 4.1), in each group, is not increasing substantially. It means that for the establishment of the trophic chains in each lake, more studies are needed analysing a bigger number of samples of the same specie living in the same lake and also biota from the base of the chain food, such as biofilms, periphyton and microalgae are needed.

When analysing Δ^{199}Hg for bream and crayfish, one concludes that for 2 of the 3 lakes where the two species were analysed, they are showing similar values, what means that they may have similar food sources. Perch, pike and pike-perch are the three carnivorous and in each lake, except for CS, the 3 species showed similar Δ^{199}Hg higher than bream, crayfish and clam that have distinct nutritional habits (Table 4.1 and Figure 4.3).

The MIF verified for HC pike-perch can be lower since only one individual was analysed in this study. Therefore, the analysis of more individuals is needed to make a better estimation on this value. Additionally, in this lake, the mean mass of the perches analysed is higher than for the other lakes Table 4.1.

4.1.1.3 Mass dependent and independent fractionation of mercury

In this study, the most representative lake for trophic chain analysis is Lacanau since this is the only lake where Hg was analysed isotopically in all the species of this study (bream, pike, perch, pike-perch, clam and crayfish) (Table 4.1).

In Lacanau, the lower MDF was obtained for clam (-0.81 ± 0.17‰) and increases to With this data, it seems that the fish species analysed are not in the same trophic chain. Concerning MIF, clam is the specie with lower Δ^{199}Hg (0.17 ± 0.18‰) and also lower [THg] followed by crayfish and bream at the same level (around 1.5‰)

and similar [THg] and by pike, perch and pike-perch (around 2.5‰). On that lake, perch and pike show similar [THg], lower than pike-perch (Figure 4.3 and Table 4.1).

When analysing both MDF and MIF for Lacanau, one can conclude that $\delta^{202}Hg$ is quite similar between all the species and that $\Delta^{199}Hg$ is different between crayfish and bream (~1.50‰) and perch, pike and pike-perch (~2.50‰), what lets us to suppose that these species are not in the same trophic chain or in the case they are, they do not seem to be direct predator-prey and their nutritional habits might be more complex. Although, $\delta^{15}N$ analyses show that each previously mentioned group is on the same trophic level, what makes this theory that they belong to the same trophic chain unfeasible. More results are needed, including the analysis of the same biota on each lake, to confirm the trophic chains in these lakes and allow the comparation between lakes. It is also needed to analyse more individuals from each lake to decrease the standard deviation between individuals. As referred by Perrot et al., 2012, (36) there is a marked difference on the pelagic and coastal trophic chain of the same lake (36). Although the Aquitanian lakes are much smaller than this analysed by the above-mentioned authors, it is important to also analyse these differences between the coastal and pelagic microenvironments.

Perrot et al., 2010 and 2012 (36,42) also analysed $\Delta^{199}Hg$ for perch from lake Baikal and Bratsk water reservoir, both in Russia. $\Delta^{199}Hg$ obtained for the less contaminated sampling site is similar to the one found in PB, the less contaminated lake from our study. However, Perrot et al., verified that MIF is decreasing from the more contaminated lake to the less contaminated lake and on our study, it was observed the opposite.

4.1.2 Comparison between species

In this subsection, the results relative to each specie will be displayed grouped.

As shown on table 4.1 and Figure 4.4, $\Delta^{199}Hg$ is positive for all the samples and the $\Delta^{199}Hg/\Delta^{201}Hg$ ratio is very close to 1.3 (Figure 4.2). In general, $\Delta^{199}Hg$ is increasing in all species from PB to L to HC to CS (Figure 4.4). On HC and L, for all the species, $\Delta^{199}Hg$ is very similar, meaning that on both the northern lakes, that are connected, the origin of Hg could be common. Nevertheless, the southern CS and PB lakes, are also connected but displayed very different MIF signatures.

The analysis of the δ^{202}Hgshows that all the values for L and PB lakes are negative and for CS and HC are positive (Table 4.1 and Figure 4.4). Clam was not represented in the figure since this specie was analysed in just one lake.

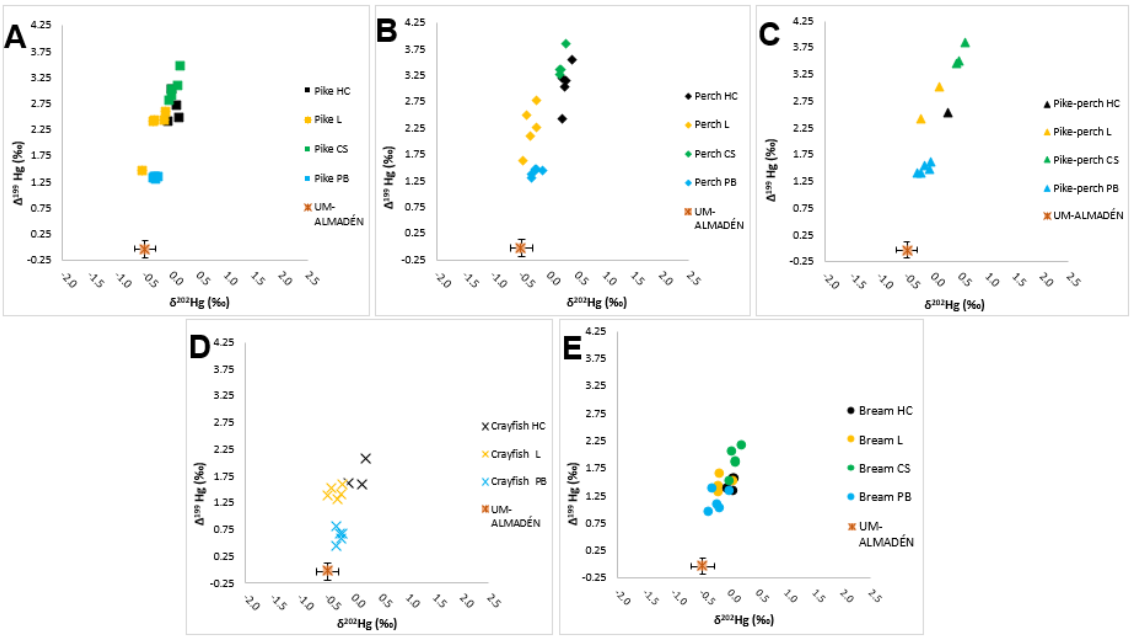

Figure 4.4 - Δ^{199}Hg plotted against δ^{202}Hg for pike (A), perch (B), pike-perch (C), crayfish (D) and bream (E) in order to compare the Hg isotopic signature of between species. UM-ALMADÉN represents the error bars (2SD) for each individual sample.

4.2 Isotopic analysis and trophic positions

Carbon and nitrogen isotopic analysis are a good tool for completing the information given by mercury mass dependent and independent fractionation. It is a useful tool for studying bioaccumulation and biomagnification of Hg in food webs and is a good complement for MDF of Hg isotopes that is suggested to trace Hg bioaccumulation and excretion in fish (42,54). With this in view, the results of δ^{13}C and δ^{15}N that were gently ceded by Sophie Gentès are displayed in Table 4.2.

Table 4.2 - Carbon and Nitrogen isotopic composition and trophic positions of biota samples collected in the lakes Cazaux-Sanguinet (CS), Hourtin-Carcans (HC), Lacanau (L) and Parentis-Biscarrosse (PB). Values represented as mean value (‰) ± 2SD. N represents the number of individuals analysed. NA means not analysed. NC means not calculated. The trophic position for HC biota was not calculated due to inexistence of $\delta^{15}N$ for clam (Gentès, S. 2017 non-published results).

Lake		Scientific name	N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Trophic Position
Casaux-Sanguinet	Bream	*Abramis brama*	5	-23,35 ± 1.51	12.82 ± 0.72	3.37
	Pike	*Esox Lucius*	5	-22.83 ± 0.67	14.23 ± 0.37	3.78
	Perch	*Perca fluviatilis*	5	-22.59 ± 0.68	14.09 ± 0.31	3.74
	Pike-perch	*Sander lucioperca*	5	-22.88 ± 0.56	15.17 ± 0.40	4.06
	Crayfish	*Procambarus clarkii*	2	-24.35 ± 1.77	9.73 ± 0.27	2.46
	Clam	*Corbicula fluminea*	5	-25.75 ± 0.32	8.17 ± 0.18	2.00
Hourtin-Carcans	Bream	*Abramis brama*	5	-21.70 ± 0.76	8.83 ± 0.87	NC
	Pike	*Esox Lucius*	5	-19.92 ± 1.11	10.37 ± 1.10	NC
	Perch	*Perca fluviatilis*	5	-18.93 ± 0.90	10.30 ± 0.87	NC
	Pike-perch	*Sander lucioperca*	1	-18.14	12.61	NC
	Crayfish	*Procambarus clarkii*	5	-19.51 ± 1.15	5.73 ± 0.44	NC
	Clam	*Corbicula fluminea*	0	NA	NA	NC
Lacanau	Bream	*Abramis brama*	5	-26.49 ± 1.63	8.99 ± 1.16	2.39
	Pike	*Esox Lucius*	5	-24.04 ± 0.49	11.91 ± 0.60	3.25
	Perch	*Perca fluviatilis*	5	-22.77 ± 0.70	11.43 ± 0.67	3.11

	Pike-perch	*Sander lucioperca*	2	-24.00 ± 2.12	12.82 ± 1.24	3.52
	Crayfish	*Procambarus clarkii*	5	-23.08 ± 2.13	8.67 ± 0.64	2.30
	Clam	*Corbicula fluminea*	5	-26.96 ± 2.66	7.66 ± 2.92	2.00
Parentis-Biscarrosse	Bream	*Abramis brama*	5	-22.82 ± 1.79	16.10 ± 0.28	3.27
	Pike	*Esox Lucius*	5	-21.08 ± 0.59	18.29 ± 0.39	3.92
	Perch	*Perca fluviatilis*	5	-22.62 ± 0.18	17.78 ± 0.10	3.77
	Pike-perch	*Sander lucioperca*	5	-22.92 ± 0.37	18.47 ± 0.21	3.97
	Crayfish	*Procambarus clarkii*	5	-22.37 ± 0.67	14.12 ± 1.05	2.69
	Clam	*Corbicula fluminea*	5	-25.97 ± 0.33	11.77 ± 0.21	2.00

$\delta^{13}C$ values vary from -26.96 to -18.14 and in general there is a relative similarity for all the samples (Table 4.2), indicating similar habitat and/or food sources. Overall, the fish and crayfish living in the same lake showed very similar $\delta^{13}C$ values and clam seems to be a slightly outlier.

On the four lakes, the six species analysed showed consistent differences for $\delta^{15}N$ values (Table 4.2) depending on the species and geographic origin. When analysing $\delta^{15}N$ values on the different lakes, despite the similarity, this parameter increases from HC to L to CS to PB, what means that there is an increasing trend from north to south, that is opposite to the level of contamination. On all the four lakes clam shows the lowest $\delta^{15}N$ value, followed by crayfish, bream, perch, pike, and pike-perch showing the higher value.

Based on $\delta^{15}N$ results, the trophic positions (TPi) were estimated and the results are shown on Figure 4.5 and on Table 4.2. The method used for TPi calculation is explained on 3. Materials and methods. In this method, a $\delta^{15}N$ baseline correction is done. As HC clam were not analysed, it was not possible to estimate the TPi for this lake. For the 3 lakes where TPi is calculated, one can see that this parameter

increases along with $\delta^{15}N$. On the southern lakes (CS and PB), the TPi values estimated for each specie is almost the same.

According to the TPi results expressed on Figure 4.5, it is possible to categorize biota in 3 different Trophic Guilds. On the 1st trophic guild should be the periphyton that is the baseline food for the whole trophic chain. Clam, crayfish and bream are part of the 2nd trophic guild and on the 3rd trophic guild perch, pike and pike-perch are found. From the organisms on the 2nd trophic guild, clam is a filter-feeder and crayfish and bream are both omnivorous (Table 4.1). Perch, pike and pike-perch, all on the 3rd trophic guild are carnivorous.

Both the $\delta^{13}C$ and $\delta^{15}N$ obtained in this study are in agreement with the ones obtained for Gentès et al., 2013, were clam, crayfish, bream, perch, pike and pike-perch were analysed in the same lakes. Perrot et al., 2010 and 2012 also analysed these parameters for perch from lake Baikal and Bratsk water reservoir, both in Russia. For $\delta^{15}N$, the values obtained are similar. For $\delta^{13}C$, on Bratsk water reservoir the values are similar and lake Baikal is showing higher values (-14.0 ‰). In our study $\delta^{13}C$ is lower on the highly contaminated lakes and on the case of Perrot´s studies, the trend was inverse.

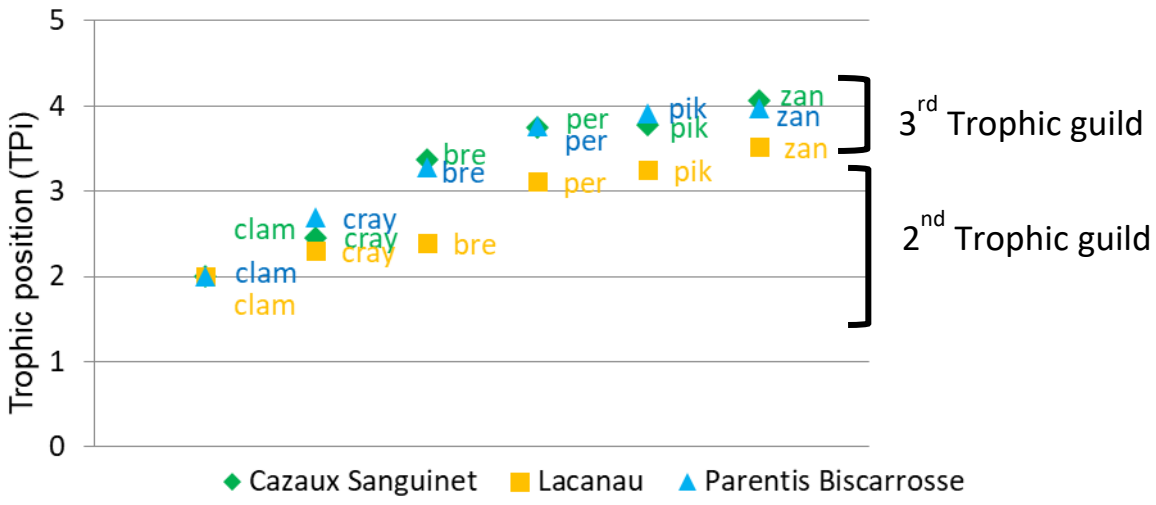

Figure 4.5 - Trophic position of biota samples collected in the lakes Cazaux-Sanguinet (CS), Hourtin-Carcans (HC), Lacanau (L) and Parentis-Biscarrosse (PB). Clam (clam), crayfish (cray), bream (bre), perch (per), pike (pik) and pike-perch (zan). TPi are calculated using the equation described by Bergamino et al., 2011. (Gentès, S. 2017 non-published results).

5. Conclusions

This work is one of the tasks of the CLAQH project and allowed to open new perspectives.

Relevant differences of mass dependent fractionation values were not observed in each of the lakes sampled, although, clam is showing the lower δ^{202}Hg value and only for CS lake, pike-perch is showing the statistically significant higher value. Total mercury concentration also is, in general, increasing from clam to crayfish to bream to pike to perch and to pike-perch.

One can assume that the mercury accumulated in the analysed biota results principally of MeHg demethylation since the Δ^{199}Hg / Δ^{201}Hg ratio is 1.3.

When analysing the mass independent fractionation values, there could be 3 groups of samples with increasing Δ^{199}Hg. (1) clam, (2) crayfish and bream and (3) pike, perch and pike-perch, what automatically lets us to assume that these 3 groups represent different trophic chains. Although, when taking MDF into account, one can see that this parameter is not increasing in each group. On PB, the less contaminated lake, the increasing trend of the referred groups is maintained, although with lower values.

Lacanau lake was the only lake where all the species were analysed, what makes it the most representative lake for trophic chain analysis. MDF is lower in clam and similar in to crayfish, bream, pike, perch and pike-perch. MIF is also lower in clam, followed by crayfish and bream showing similar value and the other 3 fish species with higher Δ^{199}Hg. Although, with the data available at this moment, it is not possible to surely conclude about the trophic chains verified in this study.

When comparing the different species, it is possible to see that for bream and crayfish (the less contaminated species), both MDF and MIF display a lower range, in the different lakes, what can mean that these species have more restricted nutritional habits than the 3 carnivorous fish species.

The results obtained in this study are in agreement with the fact that the lakes HC and L are connected, since in both lakes, the isotopic signature of mercury for each specie is almost the same.

When analysing the $\delta^{15}N$ results, and so, TPi, one can find 2 trophic guilds in these lakes. The lower includes crayfish and bream and the higher including perch, pike and pike-perch.

The results obtained in this study are a solid base for future tasks involved in the CLAQH project that will allow to better understand the entry points for Hg in the trophic chain of the Aquitanian lakes and why is there a northern trend for Hg contamination in these lakes.

6. Future perspetives

The results present in this work raised some questions related with the entry points of mercury in these Aquitanian lakes, its distribution along the different lakes and also its bioaccumulation along the food webs. To achieve these conclusions, some points need to be further analysed.

First of all, it is important to analyse all the biota samples for each lake and with a good representativeness (a minimum number of samples collected and when possible, with similar characteristics between them in terms of weight and length). It is also important to analyse isotopically for Hg the sediments to conclude about the entry points of this element in these lakes and also, its distribution in each lake. With these results and also the isotopic analysis of mercury for the organisms of lower trophic levels, such as plants, biofilm, periphyton and microalgae it will be possible to track the Hg isotopic pattern along the trophic chain.

When interpreting Hg stable isotopes patterns, the water quality parameters including light penetration and pH in each lake are important factors to take in account, because these parameters influence the reactions occurring in each lake and so, both MDF and MIF.

The future analyses of Hg isotopic fractionation on different fish tissues will allow a better understanding of the processes of metabolization and biomagnification in the food web.

For risk management on the human populations, it would be important to analyse isotopically human samples such as hair or blood from the population that directly consumed the fish from these lakes to see if the isotopic signature is maintained in the humans that have much more complex nutritional habits.

Further studies are needed on aquatic systems for better understanding the modifications on MDF and MIF of mercury along the trophic chains. It is still not clarified what is the MDF shift between trophic levels and it seems to be dependent on several factors such as animal species, age and mercury concentration.

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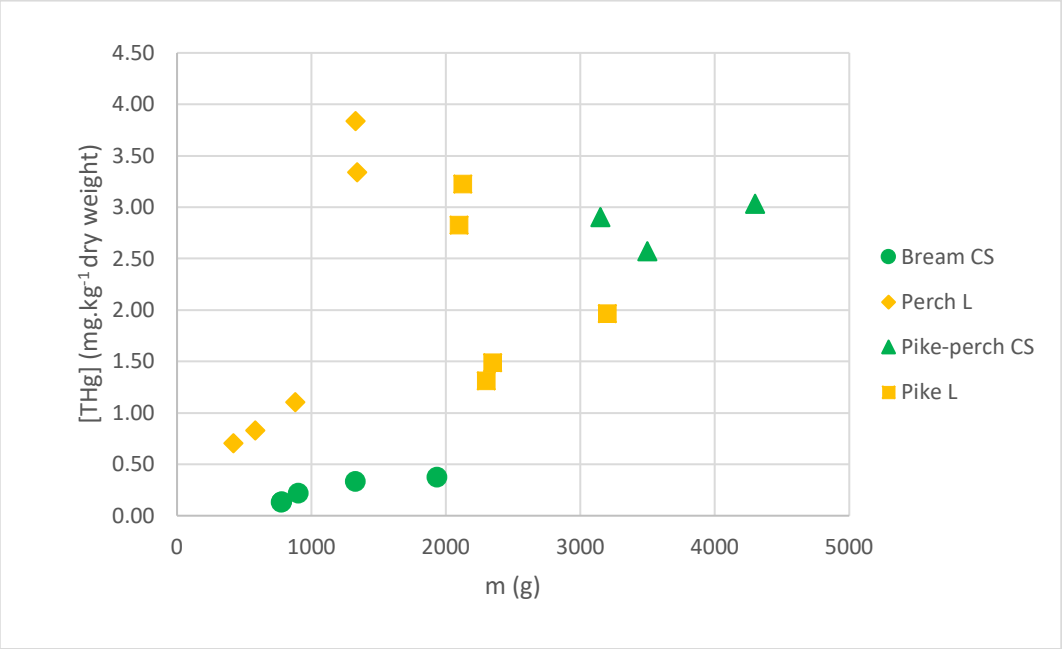
8. Annexes

A1. [THg] (mg.kg^{-1} dry weight) plotted against fish mass (g) for Bream CS, Perch L, Pike-perch CS and Pike L.



A2. Results of One-way ANOVA post-hoc Tukey test for the results shown on Figure 4.1 and Figure 4.3.

Statistical tests for Mass Dependent Fractionation

Hourtin-Carcans lake

Cell No.	Var1	{1} .09498	{2} .05480	{3} .07441	**{4} .29541**	{5} .28006
1	Crayfish		0.980396	0.998499	0.088107	0.488396
2	Bream	0.980396		0.998317	0.019993	0.283935
3	Pike	0.998499	0.998317		0.033629	0.362720
4	Perch	0.088107	0.019993	0.033629		0.999889
5	Pike-perch	0.488396	0.283935	0.362720	0.999889	

Lacanau lake

Cell No.	**NewVar**	{1} -.8097	{2} -.3225	{3} -.1239	{4} -.2887	{5} -.3144	{6} -.0500
1	Clam		0.000279	0.000147	0.000197	0.000252	0.000157
2	Crayfish	0.000279		0.275336	0.998529	0.999999	0.191574
3	Bream	0.000147	0.275336		0.466406	0.315867	0.986623
4	Pike	0.000197	0.998529	0.466406		0.999622	0.310844
5	Perch	0.000252	0.999999	0.315867	0.999622		0.216451
6	Pike-perch	0.000157	0.191574	0.986623	0.310844	0.216451	

Casaux-Sanguinet lake

Cell No.	**NewVar**	{1} .10497	{2} .07743	{3} .23660	{4} .50769
1	Bream		0.940657	0.098472	0.000225
2	Pike	0.940657		0.038605	0.000211
3	Perch	0.098472	0.038605		0.002551
4	Pike-perch	0.000225	0.000211	0.002551	

Parentis-Biscarrosse lake

Cell No.	**NewVar**	{1} -.2650	{2} -.2054	{3} -.2565	{4} -.2359	{5} -.1532
1	Crayfish		0.843492	0.999899	0.986701	0.342872
2	Bream	0.843492		0.902913	0.984171	0.896070
3	Pike	0.999899	0.902913		0.996433	0.418267
4	Perch	0.986701	0.984171	0.996433		0.625728
5	Pike-perch	0.342872	0.896070	0.418267	0.625728	

Statistical tests for Mass Independent Fractionation

Hourtin-Carcans lake

Cell No.	Var7	{1} 1.7625	{2} 1.4498	{3} 2.6446	{4} 3.0730	**{5} 2.5249**
1	Crayfish		0.644942	0.014337	0.000539	0.229379
2	Bream	0.644942		0.000847	0.000171	0.043962
3	Pike	0.014337	0.000847		0.255080	0.995802
4	Perch	0.000539	0.000171	0.255080		0.470068
5	Pike-perch	0.229379	0.043962	0.995802	0.470068	

Lacanau lake

Cell No.	**NewVar**	{1} .17026	{2} 1.4479	{3} 1.4690	{4} 2.2822	{5} 2.2513	{6} 2.7200
1	Clam		0.000164	0.000182	0.000146	0.000146	0.000146
2	Crayfish	0.000164		0.999998	0.004256	0.006038	0.001070
3	Bream	0.000182	0.999998		0.009132	0.012667	0.001776
4	Pike	0.000146	0.004256	0.009132		0.999984	0.546498
5	Perch	0.000146	0.006038	0.012667	0.999984		0.475275
6	Pike-perch	0.000146	0.001070	0.001776	0.546498	0.475275	

Casaux-Sanguinet lake

Cell No.	**NewVar**	{1} 1.8828	{2} 3.0392	{3} 3.4471	{4} 3.6050
1	Bream		0.000218	0.000201	0.000201
2	Pike	0.000218		0.115866	0.035783
3	Perch	0.000201	0.115866		0.837944
4	Pike-perch	0.000201	0.035783	0.837944	

Parentis-Biscarrosse lake

Cell No.	**NewVar**	{1} .63896	{2} 1.1420	{3} 1.3293	{4} 1.4076	{5} 1.4862
1	Crayfish		0.000142	0.000132	0.000132	0.000132
2	Bream	0.000142		0.136038	0.016723	0.001732
3	Pike	0.000132	0.136038		0.836387	0.268877
4	Perch	0.000132	0.016723	0.836387		0.833719
5	Pike-perch	0.000132	0.001732	0.268877	0.833719	

The values on the central cells are the p values. If $p < 0.05$, the variables are considered statistically different (displayed in red).