UNIVERSIDADE Đ
COIMBRA

Flávio Alexandre Mestre Franco

Mercury bioaccumulation in flatfish species of the Portuguese coast

Dissertação no âmbito do Mestrado em Ecologia orientada pelo Professor Doutor Miguel Ângelo do Carmo Pardal (Universidade de Coimbra) e pelo Doutor João Pedro Martins Coelho (Universidade de Aveiro) apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

Outubro de 2020

Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia
da Universidade de Coimbra

MERCURY BIOACCUMULATION IN FLATFISH SPECIES OF THE PORTUGUESE COAST

Flávio Alexandre Mestre Franco

Dissertação no âmbito do Mestrado em Ecologia orientada pelo Professor Doutor Miguel Ângelo do Carmo Pardal (Universidade de Coimbra) e pelo Doutor João Pedro Martins Coelho (Universidade de Aveiro) apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Outubro de 2020

1 2 9 0

UNIVERSIDADE D
COIMBRA

This thesis was supported by:

Project "RENATURE (CENTRO-01-0145-FEDER-000007) - Valorization of the Natural Endogenous Resources of the Centro Region, funded by the Comissão de Coordenação da Região Centro (CCDR-C) and subsidized by the European Regional Development Fund (FEDER).

Project Xávega 2020 (MAR 2020, 16-01-03-FMP-0008)

Project UC Mar – Transferência de tecnologia e conhecimento produzida na Universidade de Coimbra para a economia do Mar (MAR-01.03.01-FEAMP-0032)

Strategic plan for CFE (UID/BIA/04004/2020), funded by Fundação para a Ciência e Tecnologia (FCT)

Agradecimentos

Esta etapa define o fim de um ciclo na minha vida, que não seria possível sem todas as pessoas que de alguma maneira me ajudaram, incentivaram e estiveram presentes. Assim expresso os meus mais sinceros agradecimentos a todos vós:

Ao meu orientador, Professor Miguel Pardal, pela disponibilidade que mostrou desde o primeiro dia. Esta etapa não foi fácil, mas sinto que escolhi a melhor pessoa para me poder orientar e transmitir ensinamentos tanto pessoais como profissionais que levo comigo para a vida e que me tornaram uma pessoa melhor.

Ao Doutor Pedro Coelho, que sempre esteve presente quando precisei. Apesar de toda a situação que se desenvolveu nos últimos meses, devido ao Covid-19, esteve sempre presente para me apoiar quer via Skype, email, ou whatssap, dando-me todo o suporte necessário para que este trabalho se concluísse. Obrigado por todo o tempo que disponibilizaste e por todas as correções feitas.

Ao Doutor Filipe Martinho por todo apoio e ensinamentos que me deu, principalmente na fase final deste trabalho. Obrigado!

A todos os meus colegas do grupo de investigação, que tive o prazer de conhecer e constatar as excelentes pessoas que são, e que de alguma forma contribuíram para este trabalho. E em especial á Milene e ao Manuel pela alegria e companheirismo naquelas horas/manhãs/dias a abrir peixes. Muito da minha parte prática devo-a a vocês.

Ao Jalves, ao JP e ao Xano pela camaradagem e convivência quotidianas, em que cada almoço se tornava numa infindável tertúlia.

Aos meus companheiros de casa, Daniela, Sara e Super que sempre me apoiaram, quer fosse com concelhos, correção ou companhia naqueles dias em que a escrita estava mais difícil.

À Iolanda por todo apoio que me deu. Foram muitos os dias em que estava mal, nada corria bem, mas tu sempre estiveste lá para me apoiar. Sabes que sem ti nada disto seria possível.

Aos meus avós e tia, que sempre estiveram presentes e preocupados com esta etapa da minha vida.

E finalmente um agradecimento especial aos meus pais e irmã, a quem dedico este trabalho. Sem vocês nada disto seria possível. São para mim uma fonte de inspiração e um modelo a seguir no futuro! Tenho muito orgulho em ser vosso filho.

Mais uma vez, **Muito Obrigado!!**

Contents

Abstract

Fish consumption is highly recommended to prevent health problems, such as cardiovascular diseases, and is a source of vitamin and omega-3. Within fish species with commercial interest, flatfishes are highly consumed in Portugal. Despite their benefits for human health, they may also represent a risk. Flatfishes are benthic fish, which live near the bottom and in close contact with sediments, both marine and in estuaries. Since sediments are repositories of contaminants, such as mercury, there is a possibility of being incorporated in fish and, through its consumption, to humans.

The main goal of this study was to clarify the mercury bioaccumulation processes of the main flatfish species in Portugal: *Pegusa lascaris, Platichthys flesus, Scophthalmus maximus, Scophthalmus rhombus, Solea senegalensis and Solea solea.* The risk for human health from the consumption of these species was evaluated according to legislation adopted by European Food Safety Authority (EFSA), World Health Organization (WHO), and Food and Drug Administration (FDO). The results demonstrated differences between length classes, and a general bioaccumulation tendency in most species, with higher mercury burdens in larger fish. Apart from *P. lascaris,* which revealed higher body burdens than all other flatfish species, mercury concentrations were always below the maximum levels allowed for human consumption (0.5 mg kg^{-1} wet weight). While Hg concentrations were mostly within the threshold for human consumption, the food safety guidelines assessed in this study show in most cases values above recommendations, suggesting some risk associated with the consumption of these fish species. This study highlights the importance of the consumer choice (species, size, and the geographic origin) to minimize health risks associated with fish consumption.

Keywords: Mercury, Flatfishes, Bioaccumulation, Food safety, Estuary and adjacent coastal zone

Resumo

O consumo de peixe é bastante recomendado para a prevenção de problemas de saúde tais como doenças cardiovasculares, e é uma fonte importante de vitaminas e ómega-3. Dentro das espécies com interesse económico, os peixes chatos são bastante consumidos em Portugal. Apesar de trazerem benefícios para a saúde, podem representar também um risco de contaminação. Os peixes chatos são peixes bentónicos que vivem junto ao fundo, em contacto direto com sedimentos marinhos e estuarinos. Os sedimentos são repositórios de diversos contaminantes, como o mercúrio, que podem assim ser incorporados pelos peixes e, pelo seu consumo, afetar a população humana.

Os objetivos principais deste estudo foram então perceber o processo de bioacumulação de mercúrio nas principais espécies de peixes chatos em Portugal: *Pegusa lascaris Platichthys flesus, Scophthalmus rhombus, Scophthalmus maximus Solea senegalensis and Solea solea.* O risco para a saúde proveniente do consumo destas espécies foi avaliado conforme a legislação aplicada pela Autoridade Europeia para a Segurança dos Alimentos (AESA), Organização Mundial da Saúde (OMS) e a "Food and Drug Administration" (FDA).Os resultados demonstraram que existem diferenças entre as classes de tamanho, assim como uma tendência de bioacumulação em quase toda as espécies, evidenciando que peixes maiores têm maiores concentrações de mercúrio. A concentração de mercúrio manteve-se sempre abaixo dos valores estipulado por lei para o mercúrio (0.5 mg kg^{-1} peso fresco), exceto na espécie *P. lascaris* que se destacou das restantes como a espécie de peixe-chato com concentrações mais elevadas. As diretrizes de risco de consumo avaliadas no estudo mostraram em muitos dos casos valores superiores aos aconselhados, sugerindo a existência de risco associado ao consumo excessivo destes peixes. Este estudo frisa a importância da escolha do consumidor, (espécie, tamanho e origem geográfica) para minimizar os riscos para a saúde provenientes do consumo de peixe.

Palavras-chave: Mercúrio, Peixes chatos, Bioacumulação, Segurança-alimentar, Estuários e zonas costeiras adjacentes.

List of figures

List of tables

1.Introduction

1.1-Estuaries

Estuaries are transitional areas between the river and sea waters and they are among the most productive and variable ecosystems in the world (Costanza et al. 1997; Beck et al. 2001; Leitão et al. 2007). These areas play a major role in the life of many fish species including estuarine, marine, and migratory species (Franco et al. 2008). These habitats are characterized by low fish species diversity but high abundances of individual taxa (Baptista et al. 2010; Nyitrai et al. 2012). The major community of fish found in estuaries are marine juvenile and resident (Martinho et al. 2007a). Because it is an interface area between ocean and land, estuaries act like nursery grounds for several species of fish, like the flatfishes (Cabral et al. 2007; Martinho et al. 2007b), migratory routes, preferential feeding areas, and wintering areas due to the low predator pressure (Beck et al. 2001).

Estuaries have diurnal and seasonal variations concerning the environmental variables and, consequently, estuaries are often considered the most naturally stressful of all aquatic systems in the world (Wołowicz et al., 2007). Estuaries create a singular environment due to the mixing of marine water and freshwater, which creates important gradients in water salinity and temperature (Kennish 2002). Special adaptations are needed to deal with these physico-chemical alterations (Wołowicz et al. 2007), to allow the species to survive in this dynamic environment.

These coastal systems are recognized to provide shelter and nursery areas to several fish species with commercial value, including flatfishes (Beck et al. 2001; Martinho et al. 2007b, 2013; Dolbeth et al. 2008; Primo et al. 2013), allowing estuaries to make a greater than average overall contribution to adult populations (Beck et al. 2001). Besides that, estuaries confer high food availability, low predation and are therefore used by fish species during their first years of life (Martinho et al. 2010)

In some species like the flounder and the sole, spawning takes place offshore which leads to the migration of larvae from the continental shelf to coastal areas and estuaries (Martinho et al. 2009). In estuaries, fish have high food availability and low predation mainly due to the high turbidity of the water, and the availability of protection sites (Marchand 1993; Martinho et al. 2007b). So estuaries have all conditions for the rapid growth of the juvenile fish (Beck et al. 2001; Cabral et al. 2007).

Taking into account the strategic location of these areas, estuaries all around the world are exposed to numerous anthropogenic perturbations (Elliott and Whitfield 2011). Estuaries are usually near industrial activities, agricultural fields and are used as communication routes between land and ocean, which affects these ecosystems, generating changes in the structure and dynamics of biotic communities (Kennish 2002; Halpern et al. 2007; Pérez-Ruzafa et al. 2018), mainly due to pollution. This anthropogenic pressure can lead to an increase in eutrophication (Cardoso et al. 2004), pollution, and over-exploitation (Elliott and Hemingway 2002) which leads to a decrease in the abundance of many fish species (Vinagre et al. 2007; Fonseca et al. 2013).

1.2-Flatfishes

One of the most important fish groups with an estuarine life stage is the flatfish (Ramos et al. 2009a). Flatfishes have been present in the human diet for millennia (Gibson 2005) and occur throughout the world seas from the subarctic to the tropics (Pauly 1994) with a wide range of spawning seasons, habitat requirements, and life history strategies (Ramos et al. 2009b). In Portugal, a large number of flatfish species have been reported and many of them have high commercial interest (Teixeira et al. 2009, 2010; Pajuelo and JM 2011). This species richness is higher in Portugal than in Northern Europe, and identical to the Mediterranean because many flatfish species are on our coasts, at their southern or northern distribution limits (Teixeira et al. 2010).

Their body morphology is what allows these fish to be called flatfishes, however, flatfishes start as pelagic, bilaterally symmetrical fishes (Gibson et al. 2015). The flatfishes life cycle begins with the adults migration to shallow waters to spawn (Minami and Tanaka 1992; Ramos et al. 2009a). The majority of species are characterized by having one single spawning period during the year, however, some species of flatfishes have the ability to spawn more than once a year (Gibson et al. 2015). At this stage, after hatching, pelagic larvae are transported inshore to settle in the nurseries of shallow coastal and estuarine habitats (Ramos et al. 2009b; Martinho et al. 2010), where the chances of survival are higher. This migration with larvae being transported from the continental coastal areas to the inshore nursery grounds mostly by oceanic currents (Grioche et al. 2006), can be affected by biological and physical processes like starvation, predation, and water temperature (Chambers and Leggett 1987). Besides that, larval transport is one of the most important factors affecting recruitment (De Pontual et al. 2003). In the nursery areas, larvae suffer a metamorphose, transforming them into immature juveniles with the morphological characteristics of an adult (Campinho et al., 2015; Vaz et al., 2019).

Throughout metamorphosis that may extend to 1 year (Gibson 2005), flatfish larvae spend most of their time in the water column, feeding on planktonic prey (Gibson 2005). During metamorphosis one of the eyes migrates to the opposite side of the head and subsequent pigmentation of the ocular side only occurs (Suzuki and Tanaka 2015). Internally, flatfish organs undergo dramatic morphological, biochemical, and functional development (Dufour et al. 2011). After the metamorphosis phase, flatfishes stay in the nursery areas where they gain size and weight, and afterward migrate to deeper waters, when a certain fish length is attained (Cabral 2003).

The most common flatfish species in Portugal are, *Solea solea* (Linnaeus, 1758)*, Solea senegalensis* (Kaup, 1858)*, Pegusa lascaris* (Risso, 1810)*, Scophthalmus rhombus* (Linnaeus, 1758)*, Scophthalmus maximus* (Linnaeus, 1758), *and Platichthys flesus* (Linnaeus, 1758).

P. lascaris (Risso, 1810), the sand sole, is present from the central part of the North Sea to South Africa, around the Canary Islands, in the Mediterranean, Black, and Azov seas (Pajuelo and JM 2011). The principal sources of food for this species are small marine bivalves; mainly crustaceans such as amphipods, mysids, shrimps, decapods, bivalves, and polychaetes (Froese et al. 2019).

P. flesus (Linnaeus, 1758), the European flounder, occurs in the Northwest Atlantic, from the White Sea to the Mediterranean, Adriatic, and the Black Sea. The principal sources of food for this species are benthic fauna, including small fishes and invertebrates (Froese et al. 2019). Portuguese coasts are considered to be the southern limit of its geographical distribution.

S. maximus (Linnaeus, 1758), usually called turbot, can be found in Northeast Atlantic, throughout the Mediterranean, and along the European coasts to Arctic Circle; also found in most of the Baltic Sea. Adults feed normally on other bottom-living fishes, and, less often, on larger crustaceans and bivalves (Aarnio et al. 1996; Froese et al. 2019).

S. rhombus (Linnaeus, 1758), brill, can be found between the Scandinavian coast and Morocco. It can also be found throughout the Mediterranean and Black Sea (Froese et al. 2019). This species feeds on bottom-living fishes and larger crustaceans (Haynes et al. 2011).

S. senegalensis (Kaup, 1858), the senegalese sole, is distributed along the Atlantic coast, from the English Channel to Senegal, and is less frequent in the

Mediterranean Sea (Froese et al. 2019). They feed on invertebrates (polychaeta, bivalve, and mollusks) and small crustaceans (Froese et al. 2019).

S. solea (Linnaeus, 1758) the common sole, is widely distributed in cold, temperate, and tropical areas (Gibson 2005) from the North Atlantic to Senegal. Adults feed on worms, mollusks, and small crustaceans usually at night (Aarnio et al. 1996; Froese et al. 2019).

Three of our six species (*P. flesus, S. solea, and S. senegalensis*) are marine estuarine-dependent species, which means, that they are born at sea, then migrate into estuaries as juveniles, where they grow into adults before migrating back into the ocean to spawn (Nyitrai et al. 2012).On the other side, *P. lascaris*, *S. maximus,* and *S. rhombus* are marine species, meaning that the spawning occurs offshore, then drift on ocean currents as larvae before settling as juveniles in marine coastal areas to grow into adults before migrating back to spawning grounds.

Flatfishes are good potential indicators for mercury contamination since they spend almost their entire life in the bottom near the sediments, where the concentration of mercury is higher. Besides that flatfish utilize near-shore habitats, where environmental degradation is likely to be the greatest (Kerambrun et al. 2012; Polak-Juszczak 2012). Flatfishes may accumulate this metal by absorbing it through the gills and skin, which is more common in fishes with a high ratio of the skin surface to body mass; also, through diet, since they consume other benthic organisms that contain mercury (Polak-Juszczak 2012).

1.3-The biogeochemical cycle of mercury

Mercury (Hg) is a toxic metal that has been one of the main concerns in recent decades (Bosch et al. 2015) because of its high toxicity, high bioavailability, and persistence (Polak-Juszczak 2017; Fuentes-Gandara et al. 2018; Azaroff et al. 2019; Wang et al. 2019). As a long-range transported pollutant, it can be found in atmospheric, aquatic, and terrestrial systems (Lamborg et al. 2014).

Mercury reaches the ecosystems due to natural processes such as erosion and vulcanism or anthropogenic activities like mining, burning fossil fuels, and gold production (EPA United States 1999; Liu et al. 2012; La Colla et al. 2019). About two-thirds of the mercury existing in the atmosphere and aquatic systems come from human activities while only one third comes from natural sources (Morel et al. 1998). After being released into the ecosystems, mercury reaches marine ecosystems such as estuaries (Mason et al. 2012), where due to its high affinity for suspended particles, settles down and accumulates in sediments (Mieiro et al. 2009). In sediments, mainly due to lack of oxygen, microbiological activity by anaerobic prokaryotes, including sulfate-reducing bacteria (SRB), iron-reducing bacteria (FeRB), and methanogens, convert inorganic Hg into methylmercury (MeHg), which is the most toxic form of Hg (Mieiro et al. 2009; Tavares et al. 2011; Azaroff et al. 2019).

Elemental mercury (Hg^0) composes most of all Hg in the atmosphere (>90%) permitting long-range transport on a global scale (Liu et al. 2012). Elemental mercury can be oxidized in the atmosphere into ionized mercury (Hg^{2+}) which is then transported to Earth surface (by rain), through wet deposition. Once on the Earth surface, Hg2+ reaches the soil and consequently freshwater and aquatic systems (Lamborg et al. 2014).

In the aquatic systems, Hg^{2+} is quickly reduced to Hg^0, and is dispersed around the globe (Lamborg et al. 2014). In addition to that, the methylation of inorganic Hg may occur which leads to the formation of MeHg, which is a potent neurotoxin (Azaroff et al. 2019) as previously mentioned.

Organisms tend to accumulate this toxic element (Hg) at a higher rate than they eliminate it, leading to an increase of concentration through food chains (Biomagnification) (Magalhães et al. 2007; Coelho et al. 2010; Liu et al. 2012; Lyons et al. 2017), and through the lifetime of each individual (Bioaccumulation).

The increase of Hg through the marine food webs starts from the lower trophic level of micro-organisms and plankton to predatory fish and mammals at the top of the food chain (Lyons et al. 2017; Polak-Juszczak 2018). This is why marine fish that occupy higher trophic levels accumulate large amounts of mercury in their muscle, by the process of biomagnification (Bosch et al. 2016). Besides that, mercury is bioaccumulated by fish throughout their lifespan, being substantially higher in older fishes (Polak-Juszczak 2018) (Bioaccumulation).

Consequently, the uptake of mercury by fish results from two different pathways: the surrounding environment (sediments and dissolved phase), or through their diet. Both accumulation pathways can lead to biomagnification, characterized as the passage of the contaminant along the food chain, (Polak-Juszczak 2017), or bioaccumulation if it happens along the life span of each individual.

1.4-Fish as a source of mercury

Due to its high nutrient content and its known benefits for human health, fish consumption is highly recommended (Mergler et al. 2007). Despite providing a healthy source of energy, proteins, vitamins, and nutrients, fish is also a source of contaminants, especially mercury (Hg) (Rosa 2006). Consumption of fish is the major source of mercury in humans since it bioaccumulates in the aquatic food webs (Mergler et al. 2007; Sunderland et al. 2009; Bosch et al. 2015). The amount of mercury ingested depends on the type of fish that is consumed. A diet based mainly on carnivorous fish leads to a higher level of mercury consumption when compared to diets based mainly on noncarnivore fish (Mergler et al. 2007). Furthermore, the fish habitat also affects the amount of mercury ingested, since animals that are long-lived or inhabit areas with high anthropogenic inputs of mercury, like flatfishes that live in estuaries or nearby, may be more prone to bioaccumulate mercury (Lyons et al. 2017).

The ability of Hg to bind to proteins, as well as to amino acids, which are components of muscle tissues, prevents Hg elimination through any cooking or cleaning processes, given that these steps do not destroy muscle tissues, and consequently do not remove the amino acids or proteins to which mercury is bound (Webb et al. 2006; Mergler et al. 2007).

After ingestion, 95% of the consumed Hg is absorbed through the gastrointestinal tract and is distributed through the entire body through the bloodstream, which allows penetration into the central nervous system (CNS) where the effects are most harmful (Rosa 2006; Antunes dos Santos et al. 2016). Human contamination by Hg leads to blurred vision, weight loss, difficulty in locomotion, generalized weakness, taste and vision reduction, tremors and loss of consciousness; high concentrations can ultimately lead to death (Wolfe et al. 1998; Jaishankar et al. 2014).

The ingestion of Hg can lead to the appearance of several disorders both in children and in adults (Mieiro et al. 2016). In cases of pregnancy, the Hg that is present in the bloodstream easily passes to the fetus through the placenta, leading to a deficient development of the nervous system, which may include neurological abnormality and locomotion, speech and mental retardation (Wolfe et al. 1998; Jaishankar et al. 2014).

1.5-Food safety legislation

Fish consumption is regulated worldwide by guidelines, such as European Food Safety Authority (EFSA) (Commission Regulation 2006), United States Food and Drug Administration (FDA) (Evans et al. 2002) and World Health Organization (WHO) (FAO/WHO 2016). For European Union and consequently for Portugal all fishery products may not contain mercury levels above 0.5 mg kg^{-1} wet weight (ww). This maximum level of mercury is applied to most of the consumable fish species, where the levels of mercury vary from 0.01 to 0.5 mg kg^{-1} ww. However, for top predators like tuna, sharks, and swordfish, the maximum level of mercury determined as acceptable for human consumption is 1.0 mg kg^{-1} ww (Commission Regulation 2006).

1.6-Food safety assessment and fish consumption in Portugal

Portugal is one of the countries with the highest fish consumption rates, ranking first in the EU and third in the world (Caetano et al. 2019), only behind Iceland and Japan. In Portugal, the fish consumption is in average 55.9 kg.$person^{-1}$.$year^{-1}$, which is more than double the European Union average (25.1 kg.$person^{-1}$.$year^{-1}$) (EUMOFA 2017). In Portugal, mainly due to is wide coastline and economic zone fishing has always been an important source of livelihood, since coastal communities are quite dependent of fisheries.

Considering the data from Instituto Nacional de Estatística (INE), in the year of 2018, 107,996 tonnes (t) of marine species were captured, from which 869 tonnes (t) represent the total of flatfishes captured. Data from Docapescas revealed that there has been an increase in the capture of flatfishes from 2018 to 2019, generating more than 5.5 million euros in 2018 and 6.3 million euros in 2019 (in the first sales market), demonstrating that they have a high economic value. Considering the information above, it is important to understand the levels of mercury present in the species that are consumed and captured along the Portuguese coast.

1.7-Objectives

Considering the life cycle, ecology, and commercial value of flatfish, the main goal of this study was to better understand the mercury bioaccumulation in the most consumed 6 species of flatfishes in Portugal and calculate the levels of mercury present in them. Taking this into account, the specific objectives of this study were: (1) to evaluate the patterns of bioaccumulation throughout the life cycles of these species; (2) to evaluate the importance of local contamination for the bioaccumulation patterns; (3) to confirm that these species are safe for human consumption.

2.Materials and Methods

2.1-Study site and fish sampling

The fish sampling took place in 3 different estuaries and adjacent areas of the Portuguese coastline, between 2017 and 2020. The sites were from North to South: Aveiro Lagoon, Mondego estuary, and Tagus Estuary. Besides these, other fish samples were obtained from the areas nearby each estuary. The sampling sites were chosen in order to include samples from estuaries with different anthropogenic pressure.

The fish samples were obtained using a 2 m beam trawl with one tickler chain and 5 mm mesh size in the cod end, in the Mondego estuary. In the offshore of each estuary, samples were collected from traditional beach seine fisheries (*arte-xávega*). In this traditional fishery, the boats go up to 2 km from shore deploying the nets into the water and returning to shore. Afterward, the nets are hauled beachwards with the help of mechanical tractors. The beach seine nets used for this study were approximately 280 m long, the central bag was approximately 35 m long, with a stretched mesh size of 22 mm (Cabral et al. 2003). In order to obtain the missing sizes, it was necessary to buy fish from the market.

For our 6 species we assume 3 length classes, that were obtained using the following procedure:

Length Class 1 (LC1): fish smaller than the minimum capture size (Martins and Carneiro 2018);

Length Class 2 (LC2): fish with a length between LC1 and the length corresponding to the double of the age of LC1;

Length Class 3 (LC3): adult fish larger than LC2;

The age of each fish was calculated using the Von Bertalanffy function for each species (Arneri et al. 2001; Teixeira et al. 2009, 2010; Teixeira and Cabral 2010a).

Table 1-Total *lengths (cm) and length class (cm) for each species.*

Length class (cm)	***P. flesus* (cm)**	***S. senegalensis* (cm)**	***S. solea* (cm)**	***P. lascaris* (cm)**	***S. maximus* (cm)**	***S. rhombus* (cm)**
LC1	<22	<24	<24	<24	<30	<30
LC2	22-29	24-31	24-29	24-31	30-40	30-38
LC3	>29	>31	>29	>31	>40	>38

2.2-Laboratory procedures

At the laboratory, all individuals were identified using Martins and Carneiro, 2018. The individuals were measured and weighed and a sample of the muscle of each individual was collected. Muscle samples were frozen for 24h, freeze-dried for 72h, homogenized to a fine powder, and stored dry until further analysis.

The dry weight/wet weight conversion factor (CF) was calculated for the 6 species using the following equation:

CF= (ww1+ww2+ww3) / (dw1+dw2+dw3)

where ww and dw are the wet and dry weight, for each sample respectively.

Total mercury content in each sample was measured by thermal decomposition atomic absorption spectrometry with gold amalgamation using LECO AMA-254 (Advanced Mercury Analyzer). The instrument's operation may be separated into three phases during any given analysis: Decomposition, Collection, and Detection.

In the decomposition phase, samples are placed into a pre-cleaned combustion boat and inserted in a quartz combustion catalytic tube, heating the sample to around 750°C which provides the necessary thermal decomposition of the sample into a gaseous form. The gas is transported to the amalgamator in the Collection phase of the system. The mercury gets held in amalgamator (small glass tube containing gold-plated ceramics) due to the strong affinity of mercury to gold. When all mercury has been collected from the gas phase, the amalgamator is heated to 900 °C, releasing all mercury vapor to the detection system. In the final phase, the Detection phase, the mercury gases are transported to a heated cuvette (120°C) and then quantified by atomic absorption spectroscopy using a light at a wavelength of 253.65 nm, and a silicon UV diode detector.

The limit of detection of this methodology is 0.01 ng. The accuracy and precision of the method were assessed through replicate analysis of certified reference material (CRM) Dorm-4, used as CRM for fish muscle tissue samples. Precision of the method was always better than 10% (n=34), with a recovery efficiency of 86 ± 12.7% (n=146).

2.3-Data analysis

All statistical treatments were performed using the 'R' statistical and programming environment and the packages: "ggpubr", "ggplot2"," tidyverse", "ggpubr", "rstatix" and "dunn.test". Differences in Hg bioaccumulation between the length classes, locations, and species were assessed by non-parametric ANOVA (Kruskal-Wallis) following Dunn's multiple comparisons tests, with a Benjamini-Hochberg Procedure.

Consumption risk assessment was evaluated for the general population (men and women over 19 years). In all calculations, the average consumption of fish of the Portuguese population was 1069g per week (EUMOFA 2017), and 153g per day (Costa et al. 2019). The average body weight for the Portuguese population was considered a weight of 70kg (Costa et al. 2019).

2.4-Food safety assessment

The guidelines created by European food safety authority, the World Health Organization, and the United States Food and Drug Administration were set to assure the safety of Humans. With this in mind, different approaches have been created to determine the potential risk to human health throughout the consumption of different fish species, such as the Provisional Tolerable Weekly Intake (PTWI), Estimated Daily Intake (EDI), Maximum Safe Consumption (MSC_A) and Hazard quotient (HQ) (Costa et al. 2019).

Provisional tolerable weekly intake (PTWI)

The PTWI determines the amount of a contaminant that can be ingested by week over a lifetime without causing health problems, considering not only the contaminant but also the amount of the contaminant ingested and the body weight.

According to EFSA, the maximum PTWI is 4 μg kg^{-1} body weight/week for inorganic mercury (EFSA Scientific Committee 2015).

The PTWI calculation is made according to the following equation:

PTWI = C X AvC/BW

where C (μg g^{-1} wet weight (ww) of fish) is the mean concentration of contaminant in the fish fillet, AvC is the average consumption of fish per week, and BW is the average body weight of the risk group.

Estimated daily intake (EDI)

The estimated daily intake (EDI) is used to calculate the amount of a contaminant that can be ingested per day over a lifetime without causing any health issues (Varol et al. 2019). According to Copat and their colleagues in 2013, the equation reported in previous reports should be calculated (Copat et al. 2013b):

EDI= (IR x C) / BW

where IR is the daily ingestion rate or meal size, C is the metal concentration (mg kg^{-1} ww) and BW is the body weight. The EDI values were compared to the established values of reference doses (RfD), 0.1 μg g^{-1} wet weight of fish for Hg (Costa et al. 2019). This 0.1 μg g^{-1} ww of fish represents the amount of Hg a person of 70 kg may ingest daily, without producing any health effects (Costa et al. 2019).

Maximum Safe Consumption (MSC_A)

This index indicates the maximum amount of fish that a person can eat by day without causing health problems, due to the potential toxicity from a given contaminant. The maximum safe consumption (MSC_A, kg fish ww day^{-1}) is calculated through the following equation (Metian et al. 2013):

MSC_A = bw x RfD / C x 1000

where bw is the average body weight, C (μg g^{-1} ww of fish) is the mean metal concentration, and RfD is the reference dose. For mercury, the RfD has been established at 0.1 μg kg^{-1} bw/day (US EPA 2019).

Hazard quotient (HQ)

The hazard quotient (HQ) allows us to identify the possible chronic non-carcinogenic health risk associated, in this case, with dietary Hg exposure. The Hazard Quotient (HQ) is estimated by the following equation (Copat et al. 2013a).

HQ= EDI / RfD

For this quotient, values below 1 indicate that consumption of these species/size classes represents no danger for health. In cases where the HQ is higher than 1, it is assumed that there is a high probability of long term health effects (Copat et al. 2013a; Jeevanaraj et al. 2019).

All previously approaches, consider different variables, such as the average body weight, the amount of food eaten daily or weekly, the concentration of contaminant (mercury) in the tissue, and the reference dose established for in this case (mercury).

3.Results

3.1-Geographical analysis

Results from the three sampling areas are summarized in Tables 2-4. Overall, all results obtained were below the threshold for human consumption (0.5 mg kg^{-1} ww) established in Europe (Commission Regulation 2006), and are therefore formally considered safe for consumption.

A total of 88 individuals were analyzed from Aveiro, with a length between 12.1 cm and 53 cm (Table 2). The highest mean mercury concentration was observed in *P. lascaris* (0.23 ± 0.13 mg kg^{-1} ww), while the lowest was recorded in P. *flesus* (0.046 ± 0.022 mg kg^{-1} ww). In Aveiro, the highest individual concentration was obtained in *P. lascaris,* with 0.384 mg kg^{-1} ww.

In Figueira da Foz, a total of 100 individuals were analyzed, with a length between 8.9 cm and 43 cm. The highest mean mercury concentration was also observed in *P. lascaris* (0.19 ± 0.15 mg kg^{-1} ww). The lowest mean mercury concentration in this location, in turn, was obtained in *S. solea* (0.046 ± 0.038 mg kg^{-1} ww). The highest overall individual body burden was observed in this sampling area, reaching 0.52 mg kg^{-1} ww (in *P. lascaris*).

Finally, a total of 79 individuals, with a length between 6.5 cm and 48 cm, were analyzed from Lisboa. The highest mean concentrations at this site correspond to *S. solea* (0.074 ± 0.019 mg kg^{-1} ww) and *P. lascaris* (0.073 ± 0.032 mg kg^{-1} ww), while the highest individual concentration was recorded in *P. lascaris* (0.15 mg kg^{-1} ww).The lowest contaminated species in Lisboa was *S. maximus*, with 0.035 mg kg^{-1} ww.

Generally, lower concentrations were observed in Lisboa. Nevertheless, the values were always considered species-specific. Aveiro recorded the maximum mean Hg burden in *S. senegalensis, S. solea*, *P. lascaris* and *S. maximus*, while for *P. flesus* and *S. rhombus* the highest mean concentration was observed in Figueira da Foz.

The results show significant differences between locations. For smaller fish, represented by LC1 class, differences were observed in *S. solea* (H=18.8034, p< 0.001) between Aveiro and Figueira da Foz (p<0.001) with a higher concentration in fish from Aveiro, and in *S. rhombus* (H=6.9222, p=0.03) also between Aveiro and Figueira da Foz (p=0.0135) with a higher concentration in fish from Figueira da Foz. For LC2 differences were found in *S. senegalensis* (H=7.4007, p=0.02), between Figueira da Foz and Lisboa (p=0.0105), with higher mercury concentrations in fish from Figueira da Foz, and in *S. maximus* (H=10.2171, p=0.01) between Aveiro and Lisboa (p=0.0061), with higher concentrations in fish from Aveiro, and Figueira da Foz and Lisboa (p=0.012) with a higher concentration in fish from Figueira da Foz. Differences between locations at LC3 were only observed in *P. flesus* (H=4.2471, p= 0.04), with differences among Aveiro and Figueira da Foz (p=0.04) with a higher concentration in fish from Figueira da Foz (Figure 1).

Table 2-Characteristics of the fish collected in Aveiro.

	P. flesus	***S. senegalensis***	***S. solea***	***P. lascaris***	***S. maximus***	***S. rhombus***
Maximum Hg (mg kg $^{-1}$ ww)	0.12	0.30	0.17	**0.38**	0.096	0.19
Minimum Hg (mg kg $^{-1}$ ww)	0.029	0.040	0.031	0.061	0.038	0.022
Mean Hg (mg kg $^{-1}$ ww)	0.046	0.11	0.089	**0.23**	0.062	0.064
Standard deviation Hg (mg kg $^{-1}$ ww)	0.022	0.063	0.038	0.13	0.018	0.040
Maximum length (cm)	34	38	42	34	48	**53**
Minimum length (cm)	24	21	14	24	**12**	16
N	13	15	26	8	13	13

Table 3- Characteristics of the fish collected in Figueira da Foz.

	P. flesus	***S. senegalensis***	***S. solea***	***P. lascaris***	***S. maximus***	***S. rhombus***
Maximum Hg (mg kg $^{-1}$ ww)	0.37	0.38	0.16	**0.52**	0.12	0.15
Minimum Hg (mg kg $^{-1}$ ww)	0.019	0.009	0.011	0.037	0.042	0.045
Mean Hg (mg kg $^{-1}$ ww)	0.075	0.085	0.046	**0.19**	0.060	0.078
Standard deviation Hg (mg kg $^{-1}$ ww)	0.076	0.086	0.038	0.15	0.025	0.033
Maximum length (cm)	42	38	41	34	**43**	**43**
Minimum length (cm)	**8.9**	25	14	23	**8.9**	21
N	21	18	25	12	9	15

Table 4- Characteristics of the fish collected in Lisboa.

	P. flesus	***S. senegalensis***	***S. solea***	***P. lascaris***	***S. maximus***	***S. rhombus***
Maximum Hg (mg kg $^{-1}$ ww)	-	0.079	0.11	**0.15**	0.063	0.11
Minimum Hg (mg kg $^{-1}$ ww)	-	0.026	0.047	0.030	0.020	0.025
Mean Hg (mg kg $^{-1}$ ww)	-	0.047	**0.074**	**0.073**	0.035	0.054
Standard deviation Hg (mg kg $^{-1}$ ww)	-	0.020	0.019	0.032	0.016	0.028
Maximum length (cm)	-	45	**48**	28	38	40
Minimum length (cm)	-	26	23	**6.5**	13	12
N	-	11	11	33	8	16

3.2-Fish species Hg concentration

P. lascaris was the species that consistently presented the highest concentration of mercury, with a median value above 0.3 mg kg^{-1} ww of mercury for LC3 in Aveiro and Figueira da Foz. On the opposite position, *P. flesus* and *S. maximus* represent the species with the lowest mercury concentration, with median values below 0.1 mg kg^{-1} ww of mercury for the 3 length classes. Some exceptions were observed, however, such as the case of *S. solea.* While in Lisboa this species was the most contaminated, together with *P. lascaris*, in Figueira da Foz it recorded the lowest mean mercury concentration, and in Aveiro it was found to be in the middle of the range (Figure 1).

Statistically differences were observed for all length classes and sites. Our results showed that in fish from Aveiro we have differences in the smaller fish (LC1) (H=15.8304, p <0.0010) between *S. solea* and S. *maximus* (p=0.0120), with *S. solea* presenting a higher mercury concentration. In LC2 differences were found (H=15.0697, p= 0.01) between *S. solea* and *P. flesus* (p=0.030), also *S. solea* present a higher mercury concentration. For LC3 differences were found (H=15.856, p= 0.01) between *S. solea* and *P. lascaris* (p=0.0124), and *P. flesus* and *P. lascaris* (p=0.0015), and in both cases *P. lascaris* present a higher mercury concentration.

Fish from Figueira da Foz show differences (H= 19.9216, p<0.001) between *S. rhombus* and *S. solea* (p=0.0121) for LC1, with S. rhombus presenting a higher mercury concentration. For LC2 (H=14.0071, p=0.02), differences were found between *P. lascaris* and *P. flesus* (p=0.0149) and *S. maximus* and *P. lascaris* (p=0.0153), and in both cases P. lascaris is the species with the higher mercury concentration.

Fish from Lisboa show differences in LC1 (H=14.9821, p<0.001) between *P. lascaris* and *S. rhombus* (p=0.0005), and in LC2 (H=6.8437, p<0.001) between *P. lascaris* and *S. maximus* (p=0.0010) and between *P. lascaris* and *S. solea* (p=0.0151). In all three cases, mercury concentration was higher in *P. lascaris*.

3.3-Effect of fish size in Hg concentration

The possible effect of fish size in the Hg body burden was studied in the three defined size classes. Our results show differences in mercury concentration with size in *S. rhombus* in Aveiro (H=7.1538, p=0.03),(Figure 1) and Lisboa (H=10.6858, p<0.001). Other species only presented significant differences in Figueira da Foz, such as *S. solea*

(H=17.3769, p<0.001, Figure 1) and *P. flesus* (H=10.798, p<0.001, Figure 1). Finally, *P. lascaris* revealed significant differences (H=6.7573, p=0.01, Figure 1) in fish from Lisboa.

Overall, and regardless of statistical significance, different lifespan accumulation tendencies were observed (Figure 1). While some species tend to increase their Hg concentration with size (*P. lascaris* and *S. rhombus*) regardless of location, others evidence site-specific accumulation patterns, increasing in some sites while decreasing in others (*S. solea, S. maximus, S. senegalensis*).

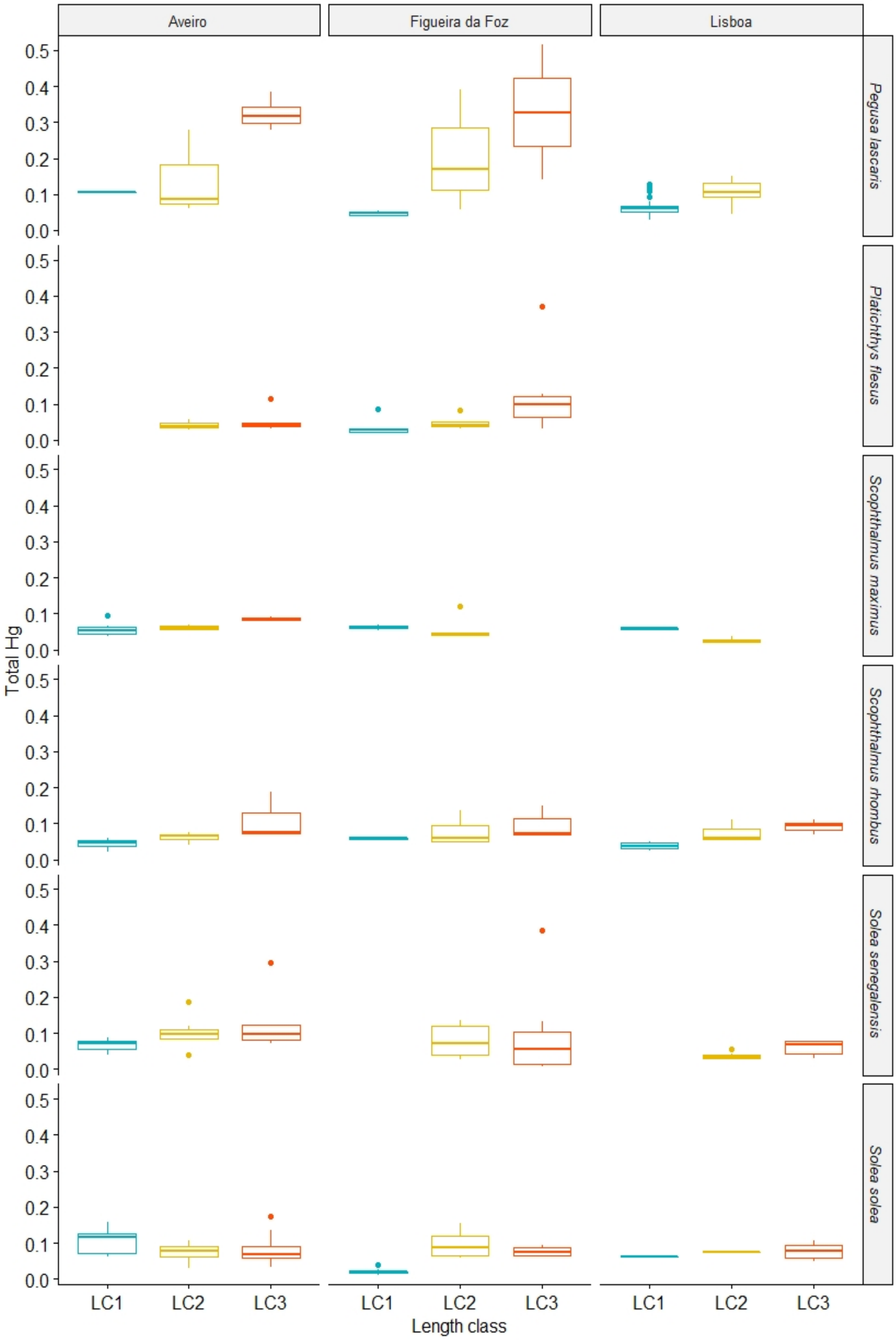

Figure 1- Boxplots of the total amount of mercury present in each species, local and class. The units are mg kg^{-1} ww. The middle line of the boxplots indicates the median value; extremities of the box are the 25th and 75th percentiles; the maximum length of each whisker are minimum and maximum values. Filled circles represent outliers.

3.4-Human risk assessment

Considering the consumption advisories of fish (EFSA Scientific Committee 2015), the average consumption of the Portuguese population for men and women over 19 years (1069g / week), and the assumed average concentration of Hg present in our samples, the Provisional tolerable Weekly Intake (PTWI) indicated that the ingestion of *S. solea, S. senegalensis, P. flesus, S. maximus, and S. rhombus* are safe for human consumption. On the other side, the PTWI for *P. lascaris* exceeds the limit (4 μg kg^{-1} bw/week), in LC3 in Aveiro (Table 5).

Table 5- Provisional tolerable weekly intake for T-Hg through the consumption of different lengths class (cm) in three different locations. Values above the limit are highlighted in bold. The limit for PTWI is 4 μg kg^{-1} bw/week. Units are μg kg^{-1} bw/week.

		P. flesus	***S. senegalensis***	***S. solea***	***P. lascaris***	***S. maximus***	***S. rhombus***
Aveiro	**LC1**		1.02	1.6		0.86	0.67
	LC2	0.63	1.5	1.1	2.2	0.95	0.93
	LC3	0.80	2.04	1.3	**4.9**		1.7
Figueira da Foz	**LC1**	0.53		0.30		0.96	0.88
	LC2	0.75	1.2	1.5	3.0	0.89	1.2
	LC3	1.8	1.4	1.2			1.4
Lisboa	**LC1**				0.97		0.57
	LC2		0.55		1.6	0.53	1.1
	LC3		0.92	1.15			1.4

The estimated daily intake (EDI) index represents the amount of mercury that a person can ingest daily, without the risk of any health effects. The EDI values must be below the established reference dose (RfD), 0.1 μg g^{-1}. Our results showed that almost all specimens have an EDI higher than the RfD. The EDI was lower than the established reference dose, particularly in the smaller fish, such as in *P. flesus* and *S. rhombus* in Aveiro, *S. solea* and *P. flesus* in Figueira da Foz, and *S. senegalensis*, *S. maximus* and *S. rhombus* in Lisboa (**Table *6***).

Table 6 - Estimated daily intake for T-Hg through the consumption of different lengths class (cm) in three different locations. Values above the limit are highlighted in bold. EDI has a maximum level of 0.1 μg kg^{-1} bw/day. Units are μg kg^{-1} bw/day.

		P. flesus	***S. senegalensis***	***S. solea***	***P. lascaris***	***S. maximus***	***S. rhombus***
	LC1		**0.15**	**0.23**		**0.12**	0.096
Aveiro	**LC2**	0.09	**0.23**	**0.16**	**0.31**	**0.14**	**0.13**
	LC3	**0.12**	**0.30**	**0.18**	**0.71**		**0.24**
	LC1	0.076		0.043		**0.14**	**0.13**
Figueira da Foz	**LC2**	**0.11**	**0.17**	**0.21**	**0.43**	**0.13**	**0.17**
	LC3	**0.25**	**0.21**	**0.17**			**0.21**
	LC1				**0.14**		0.082
Lisboa	**LC2**		0.078		**0.23**	0.076	**0.16**
	LC3		**0.14**	**0.16**			**0.20**

The Maximum Safe Consumption (MSCa, g fish ww/day) revealed good agreement with the previous guideline. Smaller fish were considered safer to consume, with less amount of risk (**Table *7***). Since the average fish consumption of the Portuguese population is 153g fish ww/day, only values above this value are considered safe for human consumption.

Table 7- Maximum Safe Consumption for T-Hg through the consumption of different lengths class (cm) in three different locations. Values below average daily consumption of the Portuguese population are highlighted in bold. Units are g fish ww day^{-1}.

		P. flesus	***S. senegalensis***	***S. solea***	***P. lascaris***	***S. maximus***	***S. rhombus***
	LC1		***110***	***67***		***120***	*160*
Aveiro	**LC2**	170	**69**	**94**	**49**	**110**	**120**
	LC3	**140**	**52**	**85**	**22**		**64**
	LC1	200		360		**110**	**120**
Figueira da Foz	**LC2**	**140**	**91**	**72**	**36**	**120**	**93**
	LC3	**61**	**75**	**91**			**74**
	LC1				**110**		190
Lisboa	**LC2**		200		**65**	200	**094**
	LC3		**120**	**0.093**			**77**

The Hazard Quotient (HQ) values are in almost all cases above 1, indicating a high probability for long term health effects (**Table *8***). The higher values of HQ are related to the largest specimens, similarly to what occurs in EDI.

Table 8- The Hazard *Quotient* for T-Hg through the consumption of different lengths class (cm) in three different locations. Values above the limit are highlighted in bold. The limit for HQ is 1 μg kg^{-1} bw/week. Units are μg kg^{-1} bw/week.

		P. flesus	*S. senegalensis*	*S. solea*	*P. lascaris*	*S. maximus*	*S. rhombus*
Aveiro	**LC1**		**1.5**	**2.3**		**1.2**	0.96
	LC2	0.90	**2.2**	**1.6**	**3.1**	**1.4**	**1.3**
	LC3	**1.1**	**2.9**	**1.8**	**7.1**		**2.4**
Figueira da Foz	**LC1**	0.76		0.43		**1.4**	**1.3**
	LC2	**1.1**	**1.7**	**2.1**	**4.3**	**1.3**	**1.6**
	LC3	**2.5**	**2.1**	**1.7**			**2.1**
Lisboa	**LC1**				**1.4**		0.82
	LC2		0.78		**2.4**	0.76	**1.6**
	LC3		**1.3**	**1.6**			**1.9**

4. Discussion

This research is one of the first to quantify mercury accumulation in flatfishes species of commercial interest, in three different estuarine areas and three length classes. In addition, it takes one step forward by evaluating the possible health risk, from the consumption of these six flatfishes species, considering all the established legislation for food safety.

Overall, the results obtained from the six species evaluated in this research show levels of mercury below the established limits (0.5 mg kg^{-1} ww of fish), indicating that all 6 species are in harmony with European food safety legislation (Commission Regulation 2006). While there are no other studies with these species on the Portuguese coast, studies with pelagic species (Vieira et al. 2011; Costa et al. 2019), reported mercury concentrations below those obtained in our study. This higher level of mercury present on flatfishes is an expected result because flatfishes are benthic fishes, living in close contact with sediments, a possible source of contamination (Polak-Juszczak 2012).

4.1-Geographical analysis

The study areas in this research were chosen to illustrate different levels of contamination, resulting from the nearby estuaries. Aveiro lagoon is known as a mercury hot spot because the Hg-rich effluents from a chlor-alkali plant located in the municipality of Estarreja were released, from 1950 to 1994, to the Aveiro lagoon system, leading to the storage of about 33 tons of mercury in the lagoon sediments (Cardoso et al. 2014; Alves et al. 2017). Mondego estuary is pristine for metals (Tavares et al. 2011), has already been used as a non-contaminated system in terms of mercury contamination (Coelho et al. 2006). Tejo estuary is one of the largest estuaries in Europe, and the largest in Western Europe (320 km^2 total area), which means this estuary is under considerable anthropogenic pressure leading to contamination by industrial discharges, urban effluents from Lisbon, and effluents from agriculture.

Considering the different potential levels of contamination of our study areas, it was expected to have a fish mercury contamination gradient from Aveiro (highest) to Figueira da Foz (lowest). This pattern should be clearer in juveniles (LC1) since at this stage flatfishes come near estuaries or even use them as nursery areas and can potentially be more affected by estuarine contamination. This pattern was significant only for *S. solea* but was also visible (but not with statistical significance) in *P. lascaris.*

Unfortunately, no LC1 individuals were analyzed of the other marine estuarine-dependent species (*S. senegalensis* and *P. flesus*) to confirm this pattern.

The absence of a more evident contamination gradient between sites may be related with the historical nature of contamination. Even though Ria de Aveiro suffered from mercury discharges in the past, these have ceased more than 25 years ago. While significant effects of the contamination gradient were still observed in the fish community structure (García-Seoane et al. 2016), higher mercury concentrations are only recorded in an inner area of the Ria de Aveiro, called Laranjo Bay, which is an upstream area (Oliveira et al. 2018). These results may further indicate an improvement in the environmental condition of the Ria de Aveiro, since the differences between the sites (Ria de Aveiro, Figueira da Foz, and Lisboa) were not that noticeable.

The absence of significant differences between sites for other species and larger individuals suggests that the local effect of estuarine residency seems to dilute with fish growth, once the fish enter the marine period.

4.2-Fish species Hg concentration

Some species were not analyzed at all sites because they were absent. In the case of *P. flesus* in Lisboa, no specimens were obtained, because this species southern limit of distribution is the Mondego estuary (Cabral et al. 2007).

Even though all these species bioaccumulate mercury, five of our six species present a low level of mercury contamination, below 0.2 mg kg^{-1} ww of mercury for all three length classes, which is less than half of the concentration allowed by law(Commission Regulation 2006).

P. lascaris stands out from the other species, with the largest individuals presenting significantly higher concentrations, near 0.5 mg kg^{-1} ww in Aveiro and Figueira da Foz (Figure 1). Our results are in agreement with a previous study on Hg accumulation in flatfish, in which *P. lascaris* was also the species with the highest mercury concentration (Bat et al. 2019). In that study, however, mercury concentrations were much lower when compared with present results, but no information on the size of fish was given, which hampers the direct comparison between the two studies.

One possible explanation for the higher Hg concentration in *P. lascaris* is due to intra-specific characteristics. *P. lascaris* is the species with the highest growth coefficient, which may result in a higher feeding rate to address the energy demand and consequently higher mercury intake. All other species have similar growth rates, with the lowest growth coefficient belonging to *P. flesus*, which may explain the lower concentration of mercury (Arneri et al. 2001; Vinagre et al. 2008; Teixeira et al. 2010; Teixeira and Cabral 2010b).

The concentrations obtained in this study for *S. solea*, are in the same range as those recorded in previous studies in the Portuguese coastal waters (Cabral et al. 2001). Other studies performed for this species reveal higher mercury concentration (Llull et al. 2017), however, this study was on the Mediterranean Sea, with a higher water temperature which may increase Hg bioavailability (Dijkstra et al. 2013). Available data for other species comprise similar results for *P. flesus* but higher values for *S. maximus* (Polak-Juszczak 2012)*,* however, the individuals used in that study was captured near an important agricultural and industrial area, and the size range of specimens reported in that study was smaller (29–33 cm). The mercury concentration on *S. senegalensis* is in accordance with other study realized in the Senegalese coast (Diop and Amara 2016), while no other studies were found to compare the mercury concentration of *S. rhombus* obtained in this study.

Overall, the inconsistent comparison with available literature data obtained for all species suggests that mercury accumulation depends on numerous interconnected factors, both exogenous (e.g. location, available food items) and endogenous (sex, lifespan, bioaccumulation rate, physiology) (Payne and Taylor 2010; Polak-Juszczak 2012; Diop and Amara 2016).

4.3-Effect of Fish size in Hg concentration

Previous articles have shown that there is frequently a size dependent increase in mercury levels (Bosch et al., 2015; 2016), which is in accordance with our results in most cases (Figure 1). However, mercury bioaccumulation can be affected by biotic and abiotic factors such as, among others, life cycle, habitat, feeding pattern, age, and size (La Colla et al. 2019), with can lead to different bioaccumulation patterns.

In some species (mainly *P. lascaris* and *S. rhombus*), there was an increasing accumulation pattern of mercury with length classes. In this case, during fish growth the levels of mercury increase, as a result of continuous exposure to contaminants and consequent accumulation with time, as previously demonstrated in other studies (Storelli et al., 2006; 2007,). Moreover, bigger fish can be in a higher trophic level, capturing larger prey to increase the energy uptake, and consequently increase the mercury intake (Teixeira et al. 2009).

However, the opposite behavior was also observed, such as the case of *S. solea* in Aveiro, *S. senegalensis* in Figueira da Foz, and *S. maximus* in Figueira da Foz and Lisboa. In these cases, a decrease in the mercury concentrations with age was observed, which can be explained by a process of detoxification (Siscar et al. 2013) or a growth dilution phenomenon that occurs when the organism's growth is faster than its rate of metal absorption (Tavares et al. 2011). The differences in mercury accumulation between sites and species demonstrate that the bioaccumulation of mercury is species and site-specific. Differences of contamination with size will have a significant impact on the risk associated with the human consumption of flatfishes. The choice between species, sizes and location will influence the consumption of mercury by the consumer, even though the levels of mercury are within current food safety legislation.

4.4-Human risk assessment

In the European Union, the average annual fish consumption is around 25.1 kg/capita. However, in Portugal, the average consumption value is much higher at 55.9 kg / per capita/year. Since fish is so important in the Portuguese diet, it is vital to understand if the flatfishes are a healthy choice.

According to EFSA, the maximum PTWI is 4 μg kg^{-1} bw/week PTWI (EFSA Scientific Committee 2015). Considering the average weight in the Portuguese population (70 kg, Costa et al., 2019), the average fish consumption in Portugal (Costa et al. 2019) and the average Hg concentration for each fish species, length class, and site, the five species are considered safe to eat, except the bigger fish (LC3) of *P. lascaris* from Aveiro, that presents a value above the limit (4.9 μg kg^{-1} bw/week). Taking this into account, a maximum of 860g of *P. lascaris (LC3, Aveiro)* should be consumed to be under 4 μg kg^{-1} bw/week and prevent health issues.

In addition to the PTWI calculations, three other commonly used risk assessment procedures were used to evaluate the risk of mercury contamination from eating flatfishes. The EDI values were in most of the cases above the established values of reference dose 0.1 μg kg^{-1} wet weight of fish for Hg (Costa et al. 2019). Results showed that the consumption of the largest fish sizes leads to a contaminant intake higher than the established limit. Considering this, it's important to make a good choice in fish size, because choosing smaller fish will reduce the amount of mercury ingested.

Considering the average daily consumption of the Portuguese population (153 g of fish), the MSCa results showed to be in accordance with the previous guideline, where the choice of the consumer must consider smaller fish with less amount of mercury. The Hazard Quotient also highlighted some risks associated with the consumption of the largest fish. Considering the previous guidelines, the best choice for human consumption would be *P. flesus* from Aveiro (LC2), *S. senegalensis,* and *S. maximus* from Lisboa (LC2).

It is important to highlight that these high values obtained in the different guidelines are due to the high consumption of fish by the Portuguese citizens (153 g of fish per day), which is more than double what is consumed in the European Union. Besides that, these calculations were made for the exclusive consumption of fish in all meals from each species and size, and therefore the risk is overestimated. However, a balanced diet, alternating between different species of fish and sizes should reduce the possibility of toxicity caused by mercury.

These results highlight the significant role of consumers in the minimization of risk associated with fish consumption. Choosing species like *P. lascaris* will significantly increase the amount of mercury ingested, and consequently, increase the possibility of health risks in the future. Beyond this, taking into account the fish size, choosing between smaller or bigger fish influences the amount of mercury to ingest. Choosing smaller fish will reduce the ingestion of contaminants, and potentially contribute to preventing health issues. This emphasizes that the consumer has an active role in the minimization of the risk from fish consumption, and so society needs to be informed, about the best choices to reduce health problems associated with fish consumption. Besides that, knowing how to physically distinguish the different species of flatfish will be important, so that at the time of purchase it is possible to choose the species with less mercury.

5. Conclusion

The main purpose of this thesis was: to evaluate the patterns of bioaccumulation throughout the life cycles of these species, evaluate the importance of local contamination for the bioaccumulation patterns, and to confirm that these species are safe for human consumption.

Results have shown that these species bioaccumulate mercury in different patterns, with some increasing with age while others reduce their contamination load with growth. Flatfish Hg accumulation has been proven to also be species-specific, highlighting *P. lascaris* as the one with the highest mercury concentrations particularly in the older/bigger fish, when compared with the other five species.

Regarding the effect of local contamination on flatfish Hg accumulation, results did not evidence a clear separation between sites. Such behavior may suggest a reduction of local contamination and an improvement of environmental conditions in historically contaminated estuaries such as Ria de Aveiro and Lisboa.

Regardless of the bioaccumulation pattern, all species reveal concentrations below current legislation, except *P. lascaris* in Figueira da Foz (LC3), which means that these species should be a safe choice for human consumption. However, risk assessment indexes demonstrate that some species and size classes may have some associated risk. Therefore, consumers should aim at a balanced diet with different species and fish sizes to minimize risks of toxicity.

Despite the increase in the control in the levels of mercury, and more preventive environmental legislation the levels of mercury may increase in the future due to the climatic changes since we are facing an increase in the water temperature due to global warming. This event can lead to an increase in the Hg bioavailability a consequently increase the mercury levels in fish. The levels of mercury should be constantly monitored to ensure that these fish remain a safe choice in the Portuguese diet.

References

Aarnio K, Bonsdorff E, Rosenback N (1996) Food and feeding habits of juvenile flounder platichthys flesus (L.), and turbot Scophthalmus maximus L. In the Aland archipelago, northern Baltic Sea. J Sea Res 36:311–320. doi: 10.1016/s1385-1101(96)90798-4

Alves AC, Monteiro MS, Machado AL, Oliveira M, Bóia A, Correia A, Oliveira N, Soares AMVM, Loureiro S (2017) Mercury levels in parturient and newborns from Aveiro region, Portugal. J Toxicol Environ Heal - Part A Curr Issues 80:697–709. doi: 10.1080/15287394.2017.1286926

Antunes dos Santos A, Appel Hort M, Culbreth M, López-Granero C, Farina M, Rocha JBT, Aschner M (2016) Methylmercury and brain development: A review of recent literature. J Trace Elem Med Biol 38:99–107. doi: 10.1016/j.jtemb.2016.03.001

Arneri E, Colella S, Giannetti G (2001) Age determination and growth of turbot and brill in the Adriatic Sea: Reversal of the seasonal pattern of otolith zone formation. J Appl Ichthyol 17:256–261. doi: 10.1046/j.1439-0426.2001.00293.x

Azaroff A, Tessier E, Deborde J, Guyoneaud R, Monperrus M (2019) Mercury and methylmercury concentrations, sources and distribution in submarine canyon sediments (Capbreton, SW France): Implications for the net methylmercury production. Sci Total Environ 673:511–521. doi: 10.1016/j.scitotenv.2019.04.111

Baptista J, Martinho F, Dolbeth M, Viegas I, Cabral H, Pardal M (2010) Effects of freshwater flow on the fish assemblage of the mondego estuary (Portugal): Comparison between drought and non-drought years. Mar Freshw Res 61:490–501. doi: 10.1071/MF09174

Bat L, Şahin F, Öztekn A (2019) Heavy metal contamination of Pleuronectiformes species from Sinop coasts of the Black Sea. Sustain Agri, Food Environ Res 7:150–162. doi: 10.7770/safer-v0n0-art1664

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates. Bioscience 51:633–641. doi: 10.1641/0006-3568(2001)051[0633:ticamo]2.0.co;2

Bosch AC, O'Neill B, Sigge GO, Kerwath SE, Hoffman LC (2015) Heavy metals in marine fish meat and consumer health: A review. J Sci Food Agric 96:32–48. doi:

10.1002/jsfa.7360

Bosch AC, O'Neill B, Sigge GO, Kerwath SE, Hoffman LC (2016) Mercury accumulation in Yellowfin tuna (Thunnus albacares) with regards to muscle type, muscle position and fish size. Food Chem 190:351–356. doi: 10.1016/J.FOODCHEM.2015.05.109

Cabral H (2003) Differences in growth rates of juvenile Solea solea and Solea senegalensis in the Tagus estuary, Portugal. J Mar Biol Assoc United Kingdom 83:861–868. doi: 10.1017/S0025315403007902h

Cabral H, Duque J, Costa MJ (2003) Discards of the beach seine fishery in the central coast of Portugal. Fish Res 63:63–71. doi: 10.1016/s0165-7836(03)00004-3

Cabral HN, Costa MJ, Salgado JP (2001) Does the Tagus estuary fish community reflect environmental changes? Clim Res 18:119–126. doi: 10.3354/cr018119

Cabral HN, Vasconcelos R, Vinagre C, França S, Fonseca V, Maia A, Reis-Santos P, Lopes M, Ruano M, Campos J, Freitas V, Santos PT, Costa MJ (2007) Relative importance of estuarine flatfish nurseries along the Portuguese coast. J Sea Res 57:209–217. doi: 10.1016/j.seares.2006.08.007

Caetano T, Branco V, Cavaco A, Carvalho C (2019) Risk assessment of methylmercury in pregnant women and newborns in the island of Madeira (Portugal) using exposure biomarkers and food-frequency questionnaires. J Toxicol Environ Heal Part A 82:833–844. doi: 10.1080/15287394.2019.1658859

Campinho MA, Silva N, Roman-Padilla J, Ponce M, Manchado M, Power DM (2015) Flatfish metamorphosis: A hypothalamic independent process? Mol Cell Endocrinol 404:16–25. doi: 10.1016/j.mce.2014.12.025

Cardoso PG, Pardal MA, Lillebø AI, Ferreira SM, Raffaelli D, Marques JC (2004) Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. J Exp Mar Bio Ecol 302:233–248. doi: 10.1016/j.jembe.2003.10.014

Cardoso PG, Pereira E, Duarte AC, Azeiteiro UM (2014) Temporal characterization of mercury accumulation at different trophic levels and implications for metal biomagnification along a coastal food web. Mar Pollut Bull 87:39–47. doi: 10.1016/j.marpolbul.2014.08.013

Chambers RC, Leggett WC (1987) Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (Pseudopleuronectes americanus) with a review of variation in other species. Can J Fish Aquat Sci 44:1936–1947. doi: 10.1139/f87-238

Coelho JP, Rosa M, Pereira E, Duarte A, Pardal MA (2006) Pattern and annual rates of Scrobicularia plana mercury bioaccumulation in a human induced mercury gradient (Ria de Aveiro, Portugal). Estuar Coast Shelf Sci 69:629–635. doi: 10.1016/j.ecss.2006.05.027

Coelho JP, Pardal MA, Duarte AC, Rodrigues ET, Pereira ME, Falcão J, Santos H, Reis AT (2010) Mercury bioaccumulation in the spotted dogfish (Scyliorhinus canicula) from the Atlantic Ocean. Mar Pollut Bull 60:1372–1375. doi: 10.1016/j.marpolbul.2010.05.008

Commission Regulation (2006) COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

Copat C, Arena G, Fiore M, Ledda C, Fallico R, Sciacca S, Ferrante M (2013a) Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. Food Chem Toxicol 53:33–37. doi: 10.1016/j.fct.2012.11.038

Copat C, Conti GO, Signorelli C, Marmiroli S, Sciacca S, Vinceti M, Ferrante M (2013b) Risk Assessment for Metals and PAHs by Mediterranean Seafood. Food Nutr Sci 4:10–13. doi: 10.4236/fns.2013.47A002

Costa F, Coelho JP, Baptista J, Martinho F, Pereira ME, Pardal MA (2019) Mercury accumulation in fish species along the Portuguese coast: Are there potential risks to human health? Mar Pollut Bull. doi: 10.1016/j.marpolbul.2019.110740

Costanza R, Arge R, DeGroot R, Farberk S, Grasso M, Hannon B, Limburg K, Naeem S, Neill RVO, Paruelo J, Raskin RG, Sutton P (1997) Costanza et al. - 1997 - The value of the world ’ s ecosystem services and natural capital. Nature 387:253–260. doi: 10.1038/387253a0

De Pontual H, Lagardère F, Amara R, Bohn M, Ogor A (2003) Influence of ontogenetic and environmental changes in the otolith microchemistry of juvenile sole (Solea solea). J Sea Res 50:199–210. doi: 10.1016/S1385-1101(03)00080-7

Dijkstra JA, Buckman KL, Ward D, Evans DW, Dionne M (2013) Experimental and Natural Warming Elevates Mercury Concentrations in Estuarine Fish. PLoS One 8:58401. doi: 10.1371/journal.pone.0058401

Diop M, Amara R (2016) Mercury concentrations in the coastal marine food web along the Senegalese coast. Environ Sci Pollut Res 23:11975–11984. doi: 10.1007/s11356-016-6386-x

Dolbeth M, Martinho F, Viegas I, Cabral H, Pardal MA (2008) Estuarine production of resident and nursery fish species: Conditioning by drought events? Estuar Coast Shelf Sci 78:51–60. doi: 10.1016/j.ecss.2007.11.021

Dufour S, Rousseau K, Kapoor BG (2011) Metamorphosis in fish.

EFSA Scientific Committee (2015) Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. EFSA J 13:3982. doi: 10.2903/j.efsa.2015.3982

Elliott M, Hemingway K (2002) Fishes in Estuaries.

Elliott M, Whitfield AK (2011) Challenging paradigms in estuarine ecology and management. Estuar Coast Shelf Sci 94:306–314. doi: 10.1016/j.ecss.2011.06.016

EPA United States (1999) Mercury Update : Impact on Fish Advisories. EPA, Washingt 823:1–13.

EUMOFA (2017) The EU Fish Market 2017 Edition.

Evans EC, MSN, CRNP (2002) The FDA recommendations on fish intake during pregnancy. J Obstet Gynecol Neonatal Nurs 31:715–720. doi: 10.1177/0884217502239205

FAO/WHO (2016) Evaluation of certain food additives and contaminants. Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives.

Fonseca VF, Vasconcelos RP, Gamito R, Pasquaud S, Gonçalves CI, Costa JL, Costa MJ, Cabral HN (2013) Fish community-based measures of estuarine ecological quality and pressure-impact relationships. Estuar Coast Shelf Sci 134:128–137. doi: 10.1016/j.ecss.2013.02.001

Franco A, Elliott M, Franzoi P, Torricelli P (2008) Life strategies of fishes in European estuaries: The functional guild approach. Mar Ecol Prog Ser 354:219–228. doi: 10.3354/meps07203

Froese R, Pauly D, Editors (2019) Fishbase -http://fishbase.org.

Fuentes-Gandara F, Herrera-Herrera C, Pinedo-Hernández J, Marrugo-Negrete J, Díez S (2018) Assessment of human health risk associated with methylmercury in the imported fish marketed in the Caribbean. Environ Res 165:324–329. doi: 10.1016/j.envres.2018.05.001

García-Seoane E, Coelho JP, Mieiro C, Dolbeth M, Ereira T, Rebelo JE, Pereira E (2016)

Effect of historical contamination in the fish community structure of a recovering temperate coastal lagoon. Mar Pollut Bull 111:221–230. doi: 10.1016/j.marpolbul.2016.07.005

Gibson RN (2005) Flatfishes: Biology and Exploitation.

Gibson RN, Nash RDM, Geffen AJ, van der Veer HW (2015) Flatfishes: Biology and Exploitation, Second Edition. John Wiley & Sons, Ltd

Grioche A, Koubbi P, Sautour B (2006) Ontogenic migration of Pleuronectes flesus larvae in the eastern English Channel. J Fish Biol 51:385–396. doi: 10.1111/j.1095-8649.1997.tb06110.x

Halpern BS, Selkoe KA, Micheli F, Kappel C V. (2007) Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conserv Biol 21:1301–1315. doi: 10.1111/j.1523-1739.2007.00752.x

Haynes PS, Brophy D, de Raedemaecker F, McGrath D (2011) The feeding ecology of 0 year-group turbot Scophthalmus maximus and brill Scophthalmus rhombus on Irish west coast nursery grounds. J Fish Biol 79:1866–1882. doi: 10.1111/j.1095-8649.2011.03128.x

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7:60–72. doi: 10.2478/intox-2014-0009

Jeevanaraj P, Hashim Z, Saliza ·, Elias M, Ahmad ·, Aris Z (2019) Risk of Dietary Mercury Exposure via Marine Fish Ingestion: Assessment Among Potential Mothers in Malaysia. Expo Heal 11:227–236. doi: 10.1007/s12403-017-0270-x

Kennish MJ (2002) Environmental threats and environmental future of estuaries. Environ Conserv 29:78–107. doi: 10.1017/s0376892902000061

Kerambrun E, Henry F, Perrichon P, Courcot L, Meziane T, Spilmont N, Amara R (2012) Growth and condition indices of juvenile turbot, Scophthalmus maximus, exposed to contaminated sediments: Effects of metallic and organic compounds. Aquat Toxicol 108:130–140. doi: 10.1016/j.aquatox.2011.07.016

La Colla NS, Botté SE, Marcovecchio JE (2019) Mercury cycling and bioaccumulation in a changing coastal system: From water to aquatic organisms. Mar Pollut Bull 140:40–50. doi: 10.1016/j.marpolbul.2018.12.051

Lamborg CH, Hammerschmidt CR, Bowman KL, Swarr GJ, Munson KM, Ohnemus DC, Lam PJ, Heimbürger L-E, Rijkenberg MJA, Saito MA (2014) A global ocean inventory of anthropogenic mercury based on water column measurements. Nature 512:65–68. doi: 10.1038/nature13563

Leitão R, Martinho F, Cabral HN, Neto JM, Jorge I, Pardal MA (2007) The fish assemblage of the Mondego estuary: Composition, structure and trends over the past two decades. Hydrobiologia 587:269–279. doi: 10.1007/s10750-007-0688-4

Liu G, Cai Y, O'Driscoll NJ (2012) Environmental chemistry and toxicology of mercury. John Wiley & Sons, Inc.

Llull RM, Garí M, Canals M, Rey-Maquieira T, Grimalt JO (2017) Mercury concentrations in lean fish from the Western Mediterranean Sea: Dietary exposure and risk assessment in the population of the Balearic Islands. Environ Res 158:16–23. doi: 10.1016/j.envres.2017.05.033

Lyons K, Carlisle AB, Lowe CG (2017) Influence of ontogeny and environmental exposure on mercury accumulation in muscle and liver of male Round Stingrays. Mar Environ Res 130:30–37. doi: 10.1016/J.MARENVRES.2017.07.004

Magalhães MC, Costa V, Menezes GM, Pinho MR, Santos RS, Monteiro LR (2007) Intra- and inter-specific variability in total and methylmercury bioaccumulation by eight marine fish species from the Azores. Mar Pollut Bull 54:1654–1662. doi: 10.1016/j.marpolbul.2007.07.006

Marchand J (1993) The influence of seasonal salinity and turbidity maximum variations on the nursery function of the loire estuary (France). Netherlands J Aquat Ecol 27:427–436.

Martinho F, Leitão R, Viegas I, Dolbeth M, Neto JM, Cabral HN, Pardal MA (2007a) The influence of an extreme drought event in the fish community of a southern Europe temperate estuary. Estuar Coast Shelf Sci 75:537–546. doi: 10.1016/j.ecss.2007.05.040

Martinho F, Cabral HN, Marques JC, Leitão R, Neto JM, Pardal MA (2007b) The use of nursery areas by juvenile fish in a temperate estuary, Portugal. Hydrobiologia 587:281–290. doi: 10.1007/s10750-007-0689-3

Martinho F, Dolbeth M, Viegas I, Teixeira CM, Cabral HN, Pardal MA (2009) Environmental effects on the recruitment variability of nursery species. Estuar Coast Shelf Sci 83:460–468. doi: 10.1016/j.ecss.2009.04.024

Martinho F, Dolbeth M, Viegas I, Baptista J, Cabral HN, Pardal MA (2010) Does the flatfish community of the Mondego estuary (Portugal) reflect environmental changes? J Appl Ichthyol 26:843–852. doi: 10.1111/j.1439-0426.2010.01486.x

Martinho F, Van der Veer HW, Cabral HN, Pardal MA (2013) Juvenile nursery colonization patterns for the European flounder (Platichthys flesus): A latitudinal approach. J Sea Res 84:61–69. doi: 10.1016/j.seares.2013.07.014

Martins R, Carneiro M (2018) Manual De Identificação De Peixes Ósseos Da Costa Continental Portuguesa - Principais Características Diagnosticantes. IPMA, I.P

Mason RP, Choi AL, Fitzgerald WF, Hammerschmidt CR, Lamborg CH, Soerensen AL, Sunderland EM (2012) Mercury biogeochemical cycling in the ocean and policy implications. Environ Res 119:101–117. doi: 10.1016/j.envres.2012.03.013

Mergler D, Anderson HA, Chan LHM, Mahaffey KR, Murray M, Sakamoto M, Stern AH (2007) Methylmercury exposure and health effects in humans: A worldwide concern. Ambio 36:3–11. doi: 10.1579/0044-7447(2007)36[3:MEAHEI]2.0.CO;2

Metian M, Warnau M, Chouvelon T, Pedraza F, Rodriguezy Baena AM, Bustamante P (2013) Trace element bioaccumulation in reef fish from New Caledonia: Influence of trophic groups and risk assessment for consumers. Mar Environ Res 87–88:26–36. doi: 10.1016/j.marenvres.2013.03.001

Mieiro CL, Pacheco M, Pereira ME, Duarte AC (2009) Mercury distribution in key tissues of fish (Liza aurata) inhabiting a contaminated estuary - Implications for human and ecosystem health risk assessment. J Environ Monit 11:1004–1012. doi: 10.1039/b821253h

Mieiro CL, Coelho JP, Dolbeth M, Pacheco M, Duarte AC, Pardal MA, Pereira ME (2016) Fish and mercury: Influence of fish fillet culinary practices on human risk. Food Control 60:575–581. doi: 10.1016/j.foodcont.2015.09.006

Minami T, Tanaka M (1992) Life History cycles in flatfish from the Northwestern Pacific, with particular reference to their early life histories. Netherlands J Sea Res 29:35–48. doi: 10.1016/0077-7579(92)90006-Z

Morel FMM, Kraepiel AML, Amyot M (1998) The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Syst 29:543–566. doi: 10.1146/annurev.ecolsys.29.1.543

Nyitrai D, Martinho F, Dolbeth M, Baptista J, Pardal MA (2012) Trends in estuarine fish assemblages facing different environmental conditions: Combining diversity with functional attributes. Aquat Ecol 46:201–214. doi: 10.1007/s10452-012-9392-1

Oliveira VH, Coelho JP, Reis AT, Vale C, Bernardes C, Pereira ME (2018) Mobility versus retention of mercury in bare and salt marsh sediments of a recovering coastal lagoon (Ria de Aveiro, Portugal). Mar Pollut Bull 135:249–255. doi: 10.1016/j.marpolbul.2018.07.035

Pajuelo J, JM L (2011) Validation of age determination methods and growth studies of the sand sole Pegusa lascaris (Soleidae) from the eastern-central Atlantic. Ciencias Mar 37:323–338. doi: 10.7773/cm.v37i3.1826

Pauly D (1994) A framework for latitudinal comparisons of flatfish recruitment. Netherlands J Sea Res 32:107–118. doi: 10.1016/0077-7579(94)90035-3

Payne EJ, Taylor DL (2010) Effects of diet composition and trophic structure on mercury bioaccumulation in temperate flatfishes. Arch Environ Contam Toxicol 58:431–443. doi: 10.1007/s00244-009-9423-7

Pérez-Ruzafa A, Joshua Sanders C, Tuya F, Fowles afowles AE, Rick Stuart-Smith nrmsouthorgau D, Ema S, Fowles AE, Edgar GJ, Stuart-Smith RD, Kirkpatrick JB, Hill N, Thomson RJ, A Strain EM (2018) Effects of Pollution From Anthropogenic Point Sources on the Recruitment of Sessile Estuarine Reef Biota Effects of Pollution From Anthropogenic Point Sources on the Recruitment of Sessile Estuarine Reef Biota. Front Mar Sci 5:417. doi: 10.3389/fmars.2018.00417

Polak-Juszczak L (2012) Bioaccumulation of mercury in the trophic chain of flatfish from the Baltic Sea. Chemosphere 89:585–591. doi: 10.1016/j.chemosphere.2012.05.057

Polak-Juszczak L (2017) Methylmercury in fish from the southern Baltic Sea and coastal lagoons as a function of species, size, and region. Toxicol Ind Health 33:503–511. doi: 10.1177/0748233716685647

Polak-Juszczak L (2018) Distribution of organic and inorganic mercury in the tissues and organs of fish from the southern Baltic Sea. Environ Sci Pollut Res 25:34181–34189. doi: 10.1007/s11356-018-3336-9

Primo AL, Azeiteiro UM, Marques SC, Martinho F, Baptista J, Pardal MA (2013) Colonization and nursery habitat use patterns of larval and juvenile flatfish species in a small temperate estuary. J Sea Res 76:126–134. doi: 10.1016/j.seares.2012.08.002

Ramos S, Ré P, Bordalo AA (2009a) Environmental control on early life stages of flatfishes in the Lima Estuary (NW Portugal). Estuar Coast Shelf Sci 83:252–264.

doi: 10.1016/j.ecss.2009.03.031

Ramos S, Ré P, Bordalo AA (2009b) Environmental control on early life stages of flatfishes in the Lima Estuary (NW Portugal). Estuar Coast Shelf Sci 83:252–264. doi: 10.1016/j.ecss.2009.03.031

Rosa M (2006) Mercúrio e amêijoa: uma relação perigosa? MSc thesis, University of Aveiro

Siscar R, Torreblanca A, Palanques A, Solé M (2013) Metal concentrations and detoxification mechanisms in Solea solea and Solea senegalensis from NW Mediterranean fishing grounds. Mar Pollut Bull 77:90–99. doi: 10.1016/j.marpolbul.2013.10.026

Storelli MM, Giacominelli-Stuffler R, Marcotrigiano GO (2006) Relationship between total mercury concentration and fish size in two pelagic fish species: Implications for consumer health. J Food Prot 69:1402–1405. doi: 10.4315/0362-028X-69.6.1402

Storelli MM, Barone G, Piscitelli G, Marcotrigiano GO (2007) Mercury in fish: Concentration vs. fish size and estimates of mercury intake. Food Addit Contam 24:1353–1357. doi: 10.1080/02652030701387197

Sunderland EM, Krabbenhoft DP, Moreau JW, Strode SA, Landing WM (2009) Mercury sources, distribution, and bioavailability in the North Pacific Ocean: Insights from data and models. Global Biogeochem Cycles 23:1–14. doi: 10.1029/2008GB003425

Suzuki M, Tanaka T (2015) Development and regulation of external asymmetry during flatfish metamorphosis. In: Flatfishes: Biology and Exploitation, 2n edition. pp 171–184

Tavares S, Oliveira H, Coelho JP, Pereira ME, Duarte AC, Pardal MA (2011) Lifespan mercury accumulation pattern in Liza aurata: Evidence from two southern European estuaries. Estuar Coast Shelf Sci 94:315–321. doi: 10.1016/j.ecss.2011.07.002

Teixeira CM, Cabral HN (2010a) Comparative analysis of the diet, growth and reproduction of the soles, Solea solea and Solea senegalensis, occurring in sympatry along the Portuguese coast. J Mar Biol Assoc United Kingdom 90:995–1003. doi: 10.1017/S0025315410000238

Teixeira CM, Cabral HN (2010b) Comparative analysis of the diet, growth and reproduction of the soles, Solea solea and Solea senegalensis, occurring in sympatry along the Portuguese coast. J Mar Biol Assoc United Kingdom 90:995–

1003. doi: 10.1017/S0025315410000238

Teixeira CM, Pinheiro A, Cabral HN (2009) Feeding ecology, growth and sexual cycle of the sand sole, Solea lascaris, along the portuguese coast. J Mar Biol Assoc United Kingdom 89:621–627. doi: 10.1017/S0025315409002562

Teixeira CM, Batista MI, Cabral HN (2010) Diet, growth and reproduction of four flatfishes on the Portuguese coast. Sci Mar 74:223–233. doi: 10.3989/scimar.2010.74n2223

US EPA (2019) Regional Screening Levels (RSLs) - Generic Tables Tables as of: November 2019. In: United States Environ. Prot. Agency. https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables.

Varol M, Kaya GK, Sünbül MR (2019) Evaluation of health risks from exposure to arsenic and heavy metals through consumption of ten fish species. Environ Sci Pollut Res 26:33311–33320. doi: 10.1007/s11356-019-06450-x

Vaz AC, Scarcella G, Pardal MA, Martinho F (2019) Water temperature gradients drive early life-history patterns of the common sole (Solea solea L.) in the Northeast Atlantic and Mediterranean. Aquat Ecol 53:281–294. doi: 10.1007/s10452-019-09688-2

Vieira C, Morais S, Ramos S, Delerue-Matos C, Oliveira MBPP (2011) Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: Intra- and inter-specific variability and human health risks for consumption. Food Chem Toxicol 49:923–932. doi: 10.1016/j.fct.2010.12.016

Vinagre C, Fonseca V, França S, Costa MJ, Ruano M, Cabral H, Vasconcelos RP, Maia A, Reis-Santos P (2007) Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: A multi-metric index and conceptual approach. Sci Total Environ 374:199–215. doi: 10.1016/j.scitotenv.2006.12.048

Vinagre C, Fonseca V, Maia A, Amara R, Cabral H (2008) Habitat specific growth rates and condition indices for the sympatric soles Solea solea (Linnaeus, 1758) and Solea senegalensis Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA-DNA ratio. J Appl Ichthyol 24:163–169. doi: 10.1111/j.1439-0426.2007.01048.x

Wang YL, Fang M Der, Chien LC, Lin CC, Hsi HC (2019) Distribution of mercury and methylmercury in surface water and surface sediment of river, irrigation canal, reservoir, and wetland in Taiwan. Environ Sci Pollut Res 26:17762–17773. doi: 10.1007/s11356-019-05176-0

Webb MAH, Feist GW, Fitzpatrick MS, Foster EP, Schreck CB, Plumlee M, Wong C, Gundersen DT (2006) Mercury concentrations in gonad, liver, and muscle of white sturgeon Acipenser transmontanus in the lower Columbia River. Arch Environ Contam Toxicol 50:443–451. doi: 10.1007/s00244-004-0159-0

Wolfe MF, Schwarzbach S, Sulaiman RA (1998) Effects of mercury on wildlife: A comprehensive review. Environ Toxicol Chem 17:146–160. doi: 10.1897/1551-5028(1998)017<0146:EOMOWA>2.3.CO;2

Wołowicz M, Sokołowski A, Lasota R (2007) Estuaries - A biological point of view. Oceanol Hydrobiol Stud 36:113–130. doi: 10.2478/v10009-007-0025-2