Original Research

Total Mercury in Aquaculture Fish

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Abstract

Three fish species from aquaculture cages located along the coast of Tenerife island (Canary Islands, Spain) were analyzed: gilthead bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), and rainbow trout (*Oncorhynchus mykiss*). Cold vapor atomic absorption spectrometry was used to determine total mercury in 120 fish muscle samples. 0.037±0.04 mg/kg, 0.082±0.15 mg/kg, and 0.023±0.025 mg/kg wet weight of Hg were found for sea bass, gilthead bream, and rainbow trout, respectively. Due to these low Hg levels, the consumption of these species of aquaculture fish contributes very little to total Hg dietary intake.

Keywords: mercury, aquaculture, sea bass, gilthead bream, rainbow trout, cold vapor atomic absorption spectrometry

Introduction

Many types of environmental pollutants (organic compounds, pesticides, radionuclides, and metals) reach coastal waters [1-3] where fish farms are generally established.

Fish farming is the fastest-growing part of the world's food-producing sector. In 2004, the Food and Agriculture Organization of the United Nations (FAO) estimated that aquaculture production represented 32.4% of the total world production of fish, crustaceans, mollusks, and other aquatic animals [4]. Due to limited natural resources, continued growth in aquaculture will be needed to address the world's future dietary fish demand. FAO estimates that, by 2030, an additional 37 million tons of fish will be needed annually to maintain current consumption levels, due to world population growth.

At a local level, the Canary Islands have developed infrastructure to produce more than 10,000 tons of farmed fish that are mainly exported to European markets. Marine fish farming has become an important emerging industry in our islands. The main types of fish being farmed in the Canary Islands are gilthead bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), and rainbow trout (*Oncorhynchus mykiss*).

Mercury (Hg) is the contaminant that regulatory agencies focus on most carefully when considering the effects of fish intake on human health [5]. Hg is recognized as one of the most hazardous environmental pollutants. It is estimated that approximately 10,000 tons of this metal are released every year into the environment as a consequence of human activities [6-10].

Once introduced in aquatic environments, inorganic Hg is methylated by micro-organisms producing organic species of Hg like methyl-mercury (MeHg), a compound that can easily be absorbed by aquatic organisms. Many marine species are often used as bio-indicators of mercury environmental pollution [9, 11-15]. Quite recently, Mieiro et al. used sea bass (*Dicentrarchus labrax*) as a bioindicator of environmental Hg contamination [16].

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The primary source of human exposure to environmental mercury is through seafood consumption. Outbreaks of methylmercury poisoning occurred in Japan (Minamata, Niigata) during the 1950s due to industrial discharges of mercury into rivers and coastal waters. Mercury levels in fish in Minamata Bay were over 10 μg/g in 1961, though the levels decreased to an average of 0.5 μg/g after 1969 [17].

The kidneys, the central nervous system, and the thyroid glands are recognized mercury target organs. Symptoms of acute poisoning include severe abdominal pains, vomiting, and diarrhea. Chronic exposure results in soreness, swelling, bleeding, and ulceration of the gums, tongue and oral mucosa with anemia, edema, and body wasting, ending in death. Chronic mercury poisoning may be accompanied by mental disturbances due to degeneration of nerve tracts and may result in blindness, weakness, loos of coordination, and coma. Mercury is also known to be responsible for "acrodynia" (also known as the "pink disease") [7, 18].

In Europe, Hg concentrations in foods are monitored by European Commission Regulation (EC) 1881/2006 [19]. The limit is set at 0.5 $mg \cdot kg^{-1}$ wet weight in fishery products, except for some species with a high throphic level, where this level is raised to 1.0 $mg \cdot kg^{-1}$.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) defines the PTWI (Provisional Tolerable Weekly Intake) as an endpoint used for food contaminants such as heavy metals with cumulative properties. The PTWI value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods. The JECFA established a PTWI for mercury of 1.6 μg/kg body weight based on two epidemiological studies that investigated the relationship between maternal exposure to mercury and impaired neurodevelopment in their children [20, 21].

A previous study measuring total diet Hg in the Canary Islands [10] found that 97% of dietary mercury comes from the intake of fish and fishery products. Due to growing concern about increased concentrations of mercury in fish tissue, the Spanish Health Ministry has begun to control levels of Hg in domestic and imported fish and fishery products. Therefore, the aim of this study was to comparatively evaluate the total mercury (THg) in the muscle tissue of three of the most commercially important species of fish farmed in Tenerife island (gilthead bream and sea bass from sea water aquaculture cages, and rainbow trout from freshwater aquaculture cages).

Materials and Methods

Samples: A total of 120 samples of gilthead bream, sea bass, and rainbow trout were collected (40 samples from each specie) from three different marine farms (one located on the north coast of Tenerife, one located on the south coast and one located inland in fresh water) (Fig. 1). Samples were collected from March 2009 to June 2010. Samples were taken from fish that were 12 months old and weighed between 350-450 g.

Reagents: Milli-Q deionized water, HSO_4 and HNO_3 Merck p.a. acids, Hg standard solution 1.000 $mg \cdot l^{-1}$ (Fisher, El Paso, Texas, USA), certified for EAA and Acationox

Fig. 1. The variability of mercury levels in marine species.

Table 1. Recovery study in fish by cold vapor atomic absorption spectrometry.

Sample	n[a]	Hg Added (mg/g)	Recovered (mg/g)	Standard Deviation	Recovery (%)	Coefficient of variation
Thumus thynnus	10	0.1	0.152	0.0035	97.1	1.9
Scomber colias	10	0.1	0.137	0.0099	94.6	3.6

[a]Number of samples

Table 2. Mercury (Hg) (mg/kg wet weight) concentrations obtained for the different species and sampling points.

	Northern sampling point		Southern sampling point		Freshwater sampling point
	Sea bass *Dicentrarchus labrax* (n=20)	Gilthead bream *Sparus aurata* (n=20)	Sea bass *Dicentrarchus labrax* (n=20)	Gilthead bream *Sparus aurata* (n=20)	Rainbow trout *Oncorhynchus mykiss* (n=40)
Mean [Hg]	0.029	0.060	0.046	0.106	0.023
SD	0.029	0.043	0.044	0.192	0.025
Max [Hg]	0.060	0.170	0.100	0.690	0.070
Min [Hg]	BDL	BDL	BDL	BDL	BDL

BDL – below analytical detection limit

detergent (Sherwood St. Louis, MO, USA), 2% solution for the washing of the whole glass labware. All plastic materials used for the storing and treating of the samples were cleaned with a solution of 5% HNO_3 for 24 hours, followed by three washes with Milli-Q water.

Apparatus

A Spectrophotometer 4100 ZL Zeeman Perkin Elmer, with a hydride generator, Fias 400 injection system and automatic sampler (AS 90, Perkin Elmer) was used.

Analytical Procedure

Samples were taken from the aquaculture farms to the laboratory in a cooler and stored at -4ºC. Fish were cleaned with Milli-Q water, all vital organs removed, and the muscles dissected and weighed. Each sample consisted of a sliced edible fillet that was analyzed in triplicate. 0.35-0.50 g of fish sample was placed into a screw cup digestion vessel and 10 ml of H_2SO_4 95-97% HNO_3 65% (1:1) were added. The vessel was sealed with a cap. Before wet digestion, samples were kept at 40ºC for 10 hours. Once the degasification flasks were cold, the solution was filtered using Albert 240 paper and adjusted to 50 ml volume with HNO_3 solution (1.5%). To avoid cross-contamination between the samples, single used plastic tools were used to transfer all materials. Although total Hg determination can be performed by applying a number of different analytical methods, the most widely used technique (cold vapor atomic absorption spectrometry (AAS)) was applied in this study due to its simplicity and high sensitivity [22, 23]. Limit of detection (LOD) was set at 0.04 and limit of quantification (LOQ) at 0.125. The recovery study used a 1,000 ppm certified Hg standard solution (Fisher) to check the procedure (Table 1).

Statistical Analysis

All results were tested for normality with the Kolmogorov-Smirnow model. The homogeneity of variance was tested with the Levene test. Since data did not show a normal distribution, the following statistical tests were used: a non-parametric Kruskal-Wallis test, which allows discrimination of individual samples with significantly different results, and the Mann-Whitney U-test, to establish whether there were significant differences between sample groups.

Results and Discussion

Mean values, standard deviations, and ranges for all the analyzed samples are shown in Table 2. The highest mean concentrations of Hg were detected in gilthead bream (*Sparus aurata*) (0.082±0.15 $mg \cdot kg^{-1}$ w.w.), the second highest Hg concentration was found in sea bass (*Dicentrarchus labrax*) (0.037±0.04 $mg \cdot kg^{-1}$ w.w.) and, finally, the lowest Hg content was detected in the fresh water species rainbow trout (*Oncorhynchus mykiss*) (0.023±0.025 $mg \cdot kg^{-1}$ w.w) (Table 2). The variability of mercury levels in the marine species (gilthead bream and sea bass) is much greater than in freshwater species (rainbow trout), as shown in Fig. 1, especially in the case of gilthead bream. All samples showed mercury levels well below legal limits, with none of the samples approaching the Hg maximum tolerance limit fixed for fishery products in Europe (0.5 $mg \cdot kg^{-1}$) [19].

It is well known that mercury levels generally increase with fish size, weight, and age [24-28]. As this study is based on young fish (12-month-old) with low weights (between 350-450 g), total mercury concentrations are low.

Table 3. Comparison of total Hg concentrations in fish species from other studies.

Zone	Fish species	Total Mercury concentration Mean (mg·kg^{-1} w.w)	Reference
Canary islands	Gilthead bream	0.082	This study
	Rainbow trout	0.023	
	Sea bass	0.037	
Poland	Smocked mackerel	0.050	37
	Smoked sprat	0.025	
	Smoked herring	0.068	
	Smoked Baltic salmon	0.071	
	Smoked Norwegian salmon	0.039	
	Smoked Norwegian salmon farmed	0.045	
	Salted herring fillets	0.048	
	Marinated herring fillets	0.052	
	Fried mackerel in vinegar	0.052	
Persian Gulf	Largetooth flounder	0.028	38
	Spotfin flathead	0.039	
	Japanese threadfin bream	0.039	
	Greater lizardfish	0.043	
	Giant seacatfish	0.045	
	Elongate sole	0.028	
Mediterranean Sea	Albacore	1.17 (range: 0.84-1.45)	31
	Bluefin tuna	1.18 (range: 0.16-2.5)	
	Bluefin tuna	1.02	30
Tyrrhenian Sea	Bluefin tuna (average length: 140.5±27.1 cm, average weight: 56.4±34.0 kg)	0.61 (range: 0.07-1.76)	32
Turkey, Izmir Bay (Eastern Aegean)	Annular sea bream	0.091	26
	Red mullet	0.0631	
Greater North Sea	Ray	0.039	39
	Dogfish	0.61	
	Sole	0.088	
USA	Pacific cod	0.17	25
	Silver hake	0.009-0.253	28
	Bluefish	0.195-1.217	
	Bowfin fillet	0.742-1.77	5
Canada	Marlin	1.43	18
	Salmon	0.04	
	Rainbow trout	0.037	
	Tuna	0.36	
	Silver pomfret	0.047	
Malaysia	Giant toadfish	0.012	40
	Catfish	0.112	
	Croacker	0.030	

Table 3. Continued.

Zone	Fish species	Total Mercury concentration Mean (mg·kg^{-1} w.w)	Reference
Malaysia	Anchovy	0.024	40
	Spotted scat	0.017	
	Stingray	0.241	
	Sardine	0.042	
	Striped eel catfish	0.014	
	Short-bodied mackerel	0.449	
	Scad	0.039	41
	Narrow-barred Spanish mackerel	0.038	
	Black pomfret	0.113	
	Long tail tuna	0.500	
	Greasy grouper	0.032	
	Chacunda gizzard shad	0.086	
	Yellow-banded scad	0.063	
	Easter little tuna	0.046	
	Delagoa threadfish bream	0.087	
	Giant perch	0.098	
	Sardine	0.004	
Turkey	Canned rainbow trout	0.026	42
Portugal (Ría de Aveiro)	Sea bass muscle	0.06±0.06 Organic Hg mg/kg dry weight 0.01±0.01 Inorganic-Hg mg/kg dry weight	16

Although no significant differences in the THg concentrations were found between species, marine aquaculture species (gilthead bream and sea bass) show higher mercury concentrations than freshwater aquaculture species (rainbow trout). As in previous studies [29], our results suggest that freshwater fish farms, located inland, are able to bring safer products to market than their marine counterparts, due to higher pollution levels in sea water. Furthermore, the inland aquaculture farms in Tenerife island obtain their water supply from an elevated aquifer located above any population centers. This translates into a high water quality, and the high quality of the water used in inland aquaculture farms translates into in a higher quality of fish.

Significant differences among the THg levels have been observed for the different sampling points. The highest Hg concentration and variability were found in the southern fishery, while the inland fishery and the northern cages presented lower variability. Different weather conditions in the sampling locations could explain these differences. Northern Tenerife and inland areas experience higher rainfall and less isolation than the southern area of the island (Fig. 2).

Table 3 compares our results with those published by other authors for fish. Total Hg levels in the analyzed farmed fish species, especially sea bass and rainbow trout,

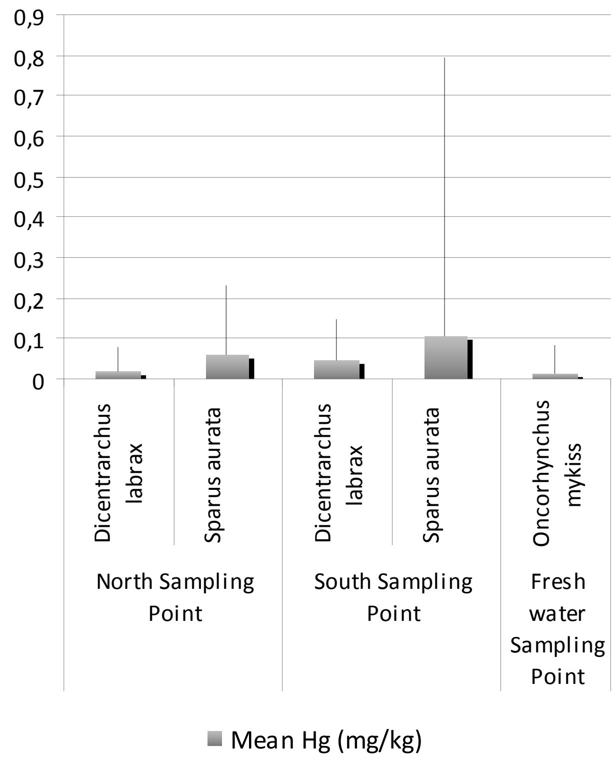

Fig. 2. Mean Hg concentrations by species and sampling points.

are generally lower than those observed for larger predatory fish species and fatty fish species [30, 31], but very close to the average contents observed for smoked fish products [37] and big bluefin tuna from the Tyrrhenian Sea [32]. For rainbow trout, our results are lower than those found in Canada [18].

Fatty fish (albacore tuna, bluefin tuna, marlin, swordfish) have presented in some studies with very high Hg concentrations, in many cases exceeding the maximum (1 mg·kg^{-1}) allowed by the European Legislation for these fatty species (European Commission 2006). Fatty fish from the Mediterranean Sea are known to be the most polluted in the world [24, 30-33]. Recently, Storelli et al. detected that 20% of fresh tuna samples in the Tyrrhenian Sea exceeded the standard for Hg [32]. Furthermore, in Korea, mackerel, tuna and squid have also been found to be major contributors to the total Hg intake [34].

Due to the concern about dietary exposure to neurotoxic Hg [35], different species of blue and fatty fish have been cited recently by the Spanish Agency for Food Safety and Nutrition (AESAN) as fish species to avoid during pregnancy and infancy [36]. None of the fish species presented in this study are included in this restriction list, so its consumption could be promoted during these stages of life.

For an adult eating a 50 g daily portion of gilthead bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), or rainbow trout (*Oncorhynchus mykiss*), the amount of THg ingested would be 4.1 µg Hg/day (28.7 µg Hg/week), 1.85 µg Hg/day (12.95 µg Hg/week), and 1.15 µg/Hg (8.05 µg Hg/week), respectively. Considering the provisional tolerable weekly intake (PTWI) of 1.6 µg Hg/kg body weight or 112 µg Hg/week for a person of 70 Kg body weight, a 50 g daily portion of gilthead bream, sea bass, or rainbow trout would contribute 25.62%, 11.56%, and 7.18%, respectively, to the PTWI. These contributions are not negligible and should be added to other Hg food inputs in total diet studies.

Conclusions

Fish from aquaculture and fish farms in the Canary Islands generate safe foods from the perspective of mercury concentrations, and no health implications or risks for human consumers should be expected. Nevertheless, the type of fish, the frequency of consumption, and the meal size are essential issues when evaluating the risks derived from fish Hg.

The species-specific information provided by this study may be used by consumers to make informed decisions on which fish to consume. The consumption of non predators and small fish species like gilthead bream, sea bass, and rainbow trout with lower Hg levels than big fatty fish species could be promoted to minimize the risks associated with neurotoxic fish mercury.

Since the Hg in fish comes from the sea, marine pollution management is essential to effectively prevent health risks. Fish farming industries should be located in areas with low contaminated waters like the north coast or inland of our island.

Future studies should focus on the heavy metal distribution and accumulation tissue-specific patterns while correlating metal levels with length, weight, and age.

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