Food and Chemical Toxicology 124 (2019) 439–449

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: Risk evaluation for consumers

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ARTICLE INFO

Keywords:
Cadmium
Lead
Mercury
Fish
Aquaculture
Risk assessment

ABSTRACT

Cadmium (Cd), lead (Pb) and mercury (Hg) presence was investigated in the muscle tissue of gilthead seabream and seabass, collected from various aquaculture sites of the Aegean and Cretan Sea as well as from the fish market (fisheries). Risk for the Greek population through consumption of these species was estimated using two approaches: Target Hazard Quotient (THQ) and Hazard Index (HI). All heavy metal levels in the fish tissue were below the established safe limits for consumption. Metal accumulation was found to differ amongst mode of production, species, location and seasonality. Seabass demonstrated higher Hg and lower Cd concentrations than seabream, Hg and Pb seem to be more accumulated in closed seas and Pb values displayed a linear increasing trend from warmer to colder periods. Regression analysis revealed that the main contributing factor to Cd accumulation is species (beta: −0.28, 95%CI: −0.48 to −0.09); lead is predominately affected by seasonality (beta: 0.44, 95%CI: 0.29 to 0.59), Hg accumulation is mainly affected by location (beta: -0.32, 95%CI: −0.61 to −0.03) while wild seabream accumulates greater levels for Hg and Pb than farmed. Risk analysis demonstrated that consumption of the studied species, is safe for all metals (HI $<$ 0.460 and TTHQ $<$ 0.299).

1. Introduction

Heavy metal presence in marine environments has been under investigation globally, due to potential repercussions on the ecosystem and to the human health. Metals such as cadmium (Cd), lead (Pb) and mercury (Hg) can be introduced into marine systems through atmospheric and coastal depositions, although the primary origin of metal pollution is their discharge as industrial waste. Cd, Pb and Hg are non-essential metals, with no known biological role and could be toxic even in traces for both marine organisms and humans (Islam and Tanaka, 2004; Olmedo et al., 2013).

Due to the fact that these contaminants are not bio-degradable, once accumulated by marine organisms, they can reach humans through the food chain, representing an eminent health hazard. Dietary intake constitutes the major route of human exposure to these contaminants (Rodríguez-Hernández et al., 2016; Renieri et al., 2014; Storelli, 2008). Although several metals have been implicated, marine fish have been reported to contain predominantly Cd, Hg and Pb often in levels exceeding the permissible limits (Bosch et al., 2016).

Health benefits of fish consumption have been well established and attributed to its high nutritional value and rich content in essential ω-3 polyunsaturated fatty acids, which play a major cardio-protective role (Domingo, 2016; Castro-González, Méndez-Armenta, 2008). However, long term consumption of fish, even with a low metal burden, could counterbalance its nutritional benefits (Bosch et al., 2016; Storelli, 2008; Copat et al., 2015).

Health risks arising from the toxicity of Cd are mainly kidney and skeletal damages, neurological disorders and endocrine disruption as well as cardiovascular disfunction and carcinogenic effects, as it has been characterized as a carcinogenic to humans by the International Agency for Research on Cancer (IARC). Pb interrupts the activity of enzymes leading to numerous adverse effects such as neurological problems, hematological effects, nephrotoxicity and hypertension in addition to DNA damage. Pb is also characterized as probably carcinogenic to humans (group 2A) by IARC. Toxic effects of Hg and its most toxic form methyl-mercury (MeHg), include reduced neuronal development and immunodeficiency (Bosch et al., 2016; Buha et al., 2018; Renieri et al., 2014; Copat et al., 2015; Gunnar et al., 2007; Derelanko and Hollinger, 2002). Within the framework of human health protection, health advisories such as the World Health Organization (WHO)

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https://doi.org/10.1016/j.fct.2018.12.020
Received 26 November 2018; Received in revised form 11 December 2018; Accepted 14 December 2018
Available online 18 December 2018

and the European Food Safety Authority (EFSA) have established safe consumption levels for these metals, considering metal levels in fish tissue with respect to human intake.

In recent years, many researchers have focused on human dietary exposure to these heavy metals, via fish consumption in Mediterranean countries. Human risk derives not only from the fish metal load, but population dietary habits as well, which vary amongst countries (Olmedo et al., 2013; Kalantzi et al., 2016; Pazi et al., 2017; Copat et al., 2013; Rodríguez-Hernández et al., 2016., Llull et al., 2017; Storelli, 2008; Conti et al., 2012., Copat et al., 2018; Renieri et al., 2014). In Greece, the studies dealing with the issue of human dietary exposure to heavy metals are limited and to the best of our knowledge there are very few studies on metal exposure through consumption of the aforementioned species (Kalogeropoulos et al., 2012; Kalantzi et al., 2016).

Marine fish, and specifically gilthead seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), are widely farmed and consumed in Greece, as an integral part of the Mediterranean diet (Kalantzi et al., 2016). Talking into account that fishing degradation has been reported in the Mediterranean sea (Islam and Tanaka, 2004) as well as globally (Christensen et al., 2014) and that aquacultures' stocks could replenish the consumers' demand, the risk estimation for both fish origins have become essential. Besides that, there have been noted differences in metal levels between farmed and wild (Ferreira et al., 2010).

For the purpose of this study, we collected samples of farmed fish of both species from different aquaculture plants of Greece, as well as wild samples from the local fish market. The specific objectives of the study were: (1) to assess the metal load of Cd, Pb and Hg in fish tissue (2) to estimate the potential health risk for consumers.

2. Materials and methods

2.1. Chemicals – reagents

Cd and Pb standards (100 μg/ml ± 0.5% in 2% HNO_3) for inductively coupled plasma mass spectrometry (ICP-MS) and ICP-MS internal standards of Li, Sc, Y, In, and Tb (100 μg/mL ± 0.5% in 2% HNO_3) were used from Bruker Daltonics Chemical Analysis (USA). Nitric acid (HNO_3) trace SELECT, for trace analysis ≥69%, hydrogen peroxide solution (H_2O_2) for ultratrace analysis ≥30% and hydrochloric acid (HCL) ≥ 37%, trace SELECT, for trace analysis, were purchased from Sigma Aldrich. Type 1 (18.2 MΩ cm at 25 °C) ultrapure water was used (produced by a Direct-Q® Water Purification System). All glassware and polyethylene vials were kept in 10% HNO_3 solution overnight and rinsed thrice with ultrapure water prior to use.

2.2. Sample collection, preparation, and digestion

Fish samples of both species were collected from aquaculture sites as well as the fish market of Heraklion, Crete during the period August 2017–March 2018. All collection sites are located in the Aegean Sea and the Sea of Crete (FAO fishing area 37, subarea 37.3, division 37.3.1). A total of 101 fish of both species namely gilthead seabream (n = 47) and sea bass (n = 54) were collected. More specifically 81 samples (gilthead seabream n = 37, sea bass n = 44) were collected from aquaculture sites and 20 samples from the fish market (10 fish from each species) which were caught in Cyclades and Dodecanese.

There were three distinct periods of collection (months) from fish farms: 21 samples (25.9%) on August (summer), 30 samples (37.0%) on November (autumn) and 30 samples (37.0%) on February–March (winter-early spring).

In the laboratory, length and weight of fish were measured and the condition factor (CF: body weight (g)*100/length^3 (cm) was calculated; samples were labeled and stored at −20 °C until dissection. Upon dissection of the fish dorsal muscle tissue was collected in polyethylene vials and stored at −20 °C until further analysis. Fish muscle tissue was homogenized with liquid nitrogen, and 250 mg wet weight (w.w.) of each sample was weighed and placed in acid-cleaned borosilicate glass vials. 6 ml HNO_3 ≥ 69% 1:1 H_2O was added to each vial and left overnight for pre-digestion. For total dissolution, the predigest with the addition of 0.5 ml H_2O_2 and 1 ml HCl were placed in Teflon digestion vessels, sealed and placed in a high-pressure microwave digestion system. A speedwave MWS- 3+, BERGHOF microwave digestion system with built in, non-contact temperature and pressure measurement was used for the digestion of the samples in PFA Teflon DAP-60 + pressure vessels. Digested samples were stored in borosilicate glass vials at 4 °C until further analysis. Each sample was diluted with ultrapure water up to HNO_3 2% final concentration prior to Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) analysis. All method blanks and spiked samples were prepared using the same protocol. Spiking was conducted before the addition of acids and immediately after homogenization.

2.3. Instrumentation and metal analysis

Analysis of the samples for the determination of heavy metal content was conducted at the shared-access equipment centre, "Industrial Biotechnology", A.N. Bach Institute of Biochemistry, Research Centre of Biotechnology of the Russian Academy of Sciences, Moscow, Russia. The ICP-MS measurements were carried out with a quadrupole ICP-MS instrument Aurora M90 (Bruker Corp., USA), equipped with an autosampler and a MicroMist low flow nebulizer. Quantum software (Bruker Corp., v 3.1 b1433) was used for data collection and processing. Limit of detection (LoD) was defined as 3 times the standard deviation of the blank and LoD values (ng/mg) for each metal were Hg = 0.002, Pb = 0.014 and Cd = 0.002 ng/mg. Limit of Quantification (LoQ) was defined as 10 times the standard deviation and LoQ (ng/mg) values determined for each metal were: Hg = 0.008, Pb = 0.046 and Cd = 0.006 ng/mg. A 20-fold dilution was used for all samples. Each sample was measured 5 times.

2.4. Exposure assessment

For the risk assessment of fish consumption two different approaches were used:

(I) Hazard index (HI) estimated as the ratio of the estimated weekly intake (EWI) to the tolerable weekly intake (TWI) proposed by EFSA [Equation (1)].

$$HI = \frac{EWI}{TWI} \tag{1}$$

The EWI was calculated as follows [Equation (2)]:

$$EWI = \frac{(C_m * WFC)}{BW} \tag{2}$$

where WFC is Weekly Fish Consumption, Cm is the metal concentration in fish tissue (ng/mg), weekly fish consumption for the Greek population is 332 g/week [based on per-capita consumption - live weight equivalent reported by the European Community (EC) = 17.3 kg/year (Eumofa, 2017)] and BW is the average body weight for adult consumer (70 kg).

TWI for Cd is 2.5 μg/kg which replaced the previous value of 7 μg/kg proposed by JEFCA, for MeHg is 1.3 μg/kg BW. Provisional tolerable weekly intake (PTWI) for Pb is 25 μg/kg BW (WHO, 2011).

(II) Target Hazard Quotient estimated as follows based on USEPA method [Equation (3)]:

$$THQ = \frac{(EF * ED * FIR * C)}{RfD * BW * AT} \tag{3}$$

Where EF and ED represent the exposure frequency (365 days/year) and the exposure duration (26 years), respectively; FIR is the fish ingestion rate for the Greek population = 47 gr/day [based on per-capita consumption - live weight equivalent reported by EC = 17.3 kg/year (Eumofa, 2017)], C is the metal concentration (ng/mg) and RfD is the reference oral dose in μg/kg BW/d (0.1 for Hg, 1 Cd and 3.57 for Pb) (USEPA, 2001, 2014); BW is the average body weight for adult consumer (70 kg); AT is the average exposure time (EF*LT) for a lifetime (LT) of 70 years. Total target hazard quotient is the sum of THQ of each metal.

We applied both approaches under 2 risk scenarios: Scenario 1- For not detected metals in fish samples, LoD/2 was imputed for purposes of the statistical analysis and Scenario 2- Only positive values were used for metal concentration in fish muscle tissue. Additionally, we estimated the risk for both modes of fish production (wild and farmed) and for each species separately, as well as in total.

2.5. Statistics

Levels of Cd, Pb and Hg were expressed in the form of mean and standard deviation (SD). Median, 3rd quartile and 90th percentile of heavy metals were also calculated as indicators of exposure, in HI and THQ estimations. Two groups or more than two groups' comparisons were made using non-parametric Mann-Whitney and Kruskal Wallis respectively. Multiple linear regression using log scale values of Cd, Pb and Hg as dependent variable and area (closed vs. open seas), species (Seabass vs. Seabream), collection period (Aug, Sep, Feb–March) and CF as explanatory variables were applied. Bar charts of mean concentrations with 95%CI were used for the graphical representation of heavy metal levels.

Statistical analysis was carried out using IBM SPSS Statistics 24.0 and a level of acceptance of null hypotheses was set at 0.05. A value of 1 was set for HQ, HI and THQ as a margin of (un)safe exposure.

3. Results

A total of 81 fish samples of both species, collected from aquacultures sites located in various regions of Greece, were analyzed. An additional group of 20 fish samples of wild captures of seabass and gilthead seabream from two fishing areas were also collected from the open fish market.

The mean length ± SD of farmed Gilthead seabream (n = 37) was 28.9 ± 2.2 while for seabass (n = 44) 32.1 ± 2.1 cm. Mean weight ± SD of farmed Gilthead was 476.7 ± 136.1 gr and for farmed seabass 403.5 ± 61.9 gr. Mean length and weight of wild fish captures were significantly higher in seabass, 34.4 ± 1.4 cm ($p = 0.001$) and 459.9 ± 44.3 gr ($p = 0.002$) respectively, than farmed ones. Wild seabream had similar dimensions to farmed seabream, with mean length 29.5 ± 0.8 cm and mean weight 473.2 ± 35.5gr. All fish presented CF values above one, indicative of well growth, with seabass fish samples achieving a significantly lower mean CF value (1.23) than seabream (1.92) ($p < 0.001$).

3.1. Metal levels in farmed fish and variance between species

Cadmium was detected (> LoD) in 16 samples (19.8%), lead in 31 (38.3%) and mercury in 74 (91.4%). With respect to Cd, mean measured concentration (> LoD) was 0.004 ± 0.002 ng/mg and there were no significant differences found between the two species ($p = 0.957$). Mean Pb for all farmed fish presented the highest levels among metals 0.137 ± 0.169 ng/mg yet there was no significant differences between species ($p = 0.886$). On the other hand, mean Hg concentration in Seabass: 0.050 ± 0.038 ng/mg was found to be significantly higher than in gilthead seabream: 0.032 ± 0.021 ng/mg ($p = 0.012$).

For not detected metals in fish samples, LoD/2 was imputed for purposes of the statistical analysis. Levels of Cd, Pb and Hg imputed with detection values (LoD/2) are presented as mean concentrations with error-bars (95%CI: confidence intervals) in Fig. 1. Pb levels do not differ significantly between Gilthead Seabream 0.058 ± 0.149 ng/mg w. w. and Seabass 0.056 ± 0.094 ng/mg w. w. ($p = 0.983$). However, mean Cd levels in Gilthead seabream (0.002 ± 0.002 ng/mg w. w.) and seabass (0.001 ± 0.022 ng/mg w. w.), demonstrated a significant difference, as did mean Hg levels in Gilthead seabream (0.029 ± 0.022 ng/mg w. w.) and seabass (0.047 ± 0.038 ng/mg w. w.) with p-values < 0.001 and 0.029 respectively.

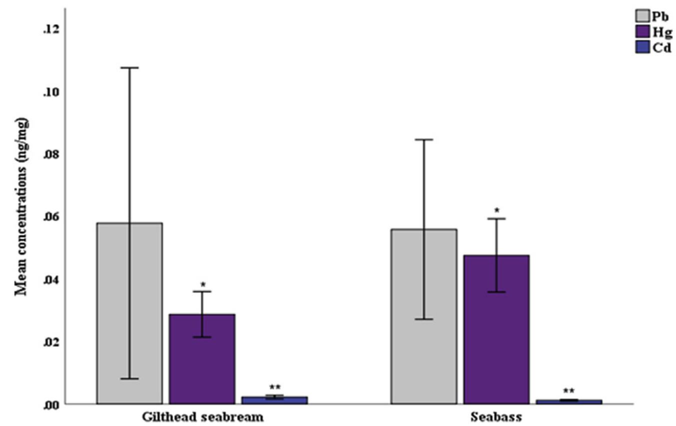

Fig. 1. Mean levels with 95%CIs (95%confidence intervals) of Cd, Pb and Hg in muscle tissue of farmed gilthead seabream and seabass. Levels expressed as ng/mg w. w. (wet weight) *$p < 0.05$, **$p < 0.001$.

It is worth mentioning that levels of Hg and Pb were correlated weakly yet significantly ($r_s = 0.230$, $p = 0.039$) while assessing metal levels in all farmed fish tissues. Partial correlation analysis did not shown any association when species was considered a controlling factor ($p > 0.05$).

3.2. Variance/distribution of metals in farmed fish depending on collection site

Distribution of heavy metals was studied for fish of both species and in total, collected from different fish farm sites and results are presented in Table 1. Based on the obtained results it is clear that there is a site effect in most of the comparisons.

Comparison of Cd levels of Gilthead seabream tissues from different sites revealed significant differences ($p = 0.032$). Samples from Crete and the Saronic Gulf displayed the higher means (0.003 ± 0.002 ng/mg w. w.), while median values were higher in Crete 0.002 ng/mg. Correspondingly, with regard to mercury, mean (0.047 ± 0.020 ng/mg) and median (0.047 ng/mg) values are significantly higher in Crete ($p < 0.001$). Pb followed a similar pattern with previous metals for seabass, presenting significantly higher values in the Crete site (mean Pb: 0.138 ± 0.120, median 0.122 ng/mg) than the other farming sites ($p < 0.001$).

For both species in total, all metal levels were significantly different amongst areas. In further detail, Cd levels in fish tissues were significantly higher in Crete and Saronic Gulf farming sites, with means 0.002 ± 0.002 ng/mg ($p = 0.014$). Fish samples from Crete presented the highest Pb levels 0.119 ± 0.176 (mean) and 0.051 (median) ng/mg w. w. amongst sites ($p < 0.001$), while Crete and NE Aegean samples revealed a significant difference in Hg levels with mean Hg values in fish tissues, estimated at 0.046 ± 0.019 and 0.053 ± 0.058 ng/mg w. w., respectively ($p = 0.006$).

Additional analysis for the location factor was made grouping sampling sites into open and closed seas. Differences in metal levels were also studied between open seas (NE Aeagean and Dodecanese) and closed seas (Crete, Mainland and Saronic Gulf). Lead is significantly higher in closed seas ($p < 0.05$) for farmed fish in total (Fig. 2c), as it is

Table 1
Concentrations of heavy metals in muscle tissue of both fish species collected from different sites, as means and medians (ng/mg w.w.).

Area	Cd		Hg		Pb	
	Mean ± SD	Median	Mean ± SD	Median	Mean	Median
Gilthead seabream						
Crete	0.003 ± 0.002	0.002	0.047 ± 0.020	0.047	0.101 ± 0.221	0.039
Mainland	0.001 ± < 0.001	0.001	0.019 ± 0.008	0.02	0.010 ± 0.006	0.007
Saronic Gulf	0.003 ± 0.002	0.001	0.025 ± 0.009	0.024	0.042 ± 0.092	0.007
NE Aeagean	0.001 ± < 0.001	0.001	0.002 ± 0.001	0.001	0.04 ± 0.057	0.007
Dodecanese	0.002 ± 0.002	0.001	0.010 ± 0.005	0.005	0.025 ± 0.038	0.007
p	**0.032**		< **0.001**		0.134	
Seabass						
Crete	0.001 ± 0.001	0.001	0.046 ± 0.020	0.045	0.138 ± 0.120	0.122
Mainland	0.001 ± 0.001	0.001	0.034 ± 0.028	0.037	0.009 ± 0.005	0.007
Saronic Gulf	0.001 ± 0.001	0.001	0.047 ± 0.024	0.035	0.007 ± < 0.001	0.007
NE Aeagean	0.001 ± 0.001	0.001	0.105 ± 0.021	0.113	0.007 ± < 0.001	0.007
Dodecanese	0.001 ± 0.001	0.001	0.047 ± 0.058	0.022	0.021 ± 0.047	0.007
p	0.859		0.073		< **0.001**	
Total						
Crete	0.002 ± 0.002	0.001	0.046 ± 0.019	0.046	0.119 ± 0.176	0.051
Mainland	0.001 ± < 0.001	0.001	0.029 ± 0.024	0.023	0.009 ± 0.005	0.007
Saronic Gulf	0.002 ± 0.002	0.001	0.032 ± 0.017	0.029	0.031 ± 0.077	0.007
NE Aeagean	0.001 ± < 0.001	0.001	0.053 ± 0.058	0.042	0.024 ± 0.040	0.007
Dodecanese	0.001 ± 0.001	0.001	0.035 ± 0.050	0.019	0.022 ± 0.043	0.007
p	**0.014**		**0.006**		< **0.001**	

for seabass alone ($p < 0.05$) (Fig. 2b). Median mercury level on the other hand, is significantly higher in closed seas for seabream ($p < 0.001$) and in total as well ($p = 0.029$) (Fig. 2b,c).

3.3. Variance/distribution of metals in farmed fish depending on seasonality

Another factor that seemed to affect metal accumulation is seasonality. The study of metal levels in fish tissues of both species, as well as in total, for the different months of collection revealed statistical differences for all heavy metals ($p < 0.001$). Levels of Cd in all fish samples were higher in summer (August) and winter to early spring (February–March) (mean: 0.002 ng/mg w. w.) in comparison to autumn (November) (mean: 0.001 ng/mg w. w.) ($p = 0.002$) (Fig. 3c). The above difference in sampling season was revealed in Seabream ($p < 0.001$) but not in Seabass tissues ($p = 0.620$) (Fig. 3a and b).

The estimated Pb levels for fish samples in total showed a statistically significant increase in winter (February/March) with a mean level of 0.119 ± 0.176 ng/mg w. w. ($p < 0.001$). That same seasonal pattern was presented for each species alone. More specifically, seabass samples presented a mean concentration of 0.138 ± 0.120 ng/mg ($p < 0.001$), while Gilthead seabream a mean of 0.101 ± 0.221 ng/mg w. w. ($p = 0.019$). Conclusively, Pb values seem to have a linear increasing trend when assessing metal levels from warmer (August) to colder (February/March) periods.

Hg levels in Gilthead seabream were significantly higher in February/March (mean: 0.047 ± 0.020 ng/mg) ($p < 0.001$), while during August farming period they were higher in seabass samples (mean: 0.071 ± 0.052) ($p = 0.004$) (Fig. 3 a, b). In the total of samples both summer and winter farming periods have increased Hg levels in fish tissues ($p < 0.001$) as it is presented in Fig. 3 (c).

3.4. Analysis of main effects on heavy metal levels in fish tissues

Multiple linear regression models using log-scaled Pb, Cd, and Hg levels as dependent variables were applied using species, seasonality (from "warmer" periods to "colder" periods), farming areas (closed/open seas) and condition factor of fish.

The analysis revealed that Cd levels seem to have only a species

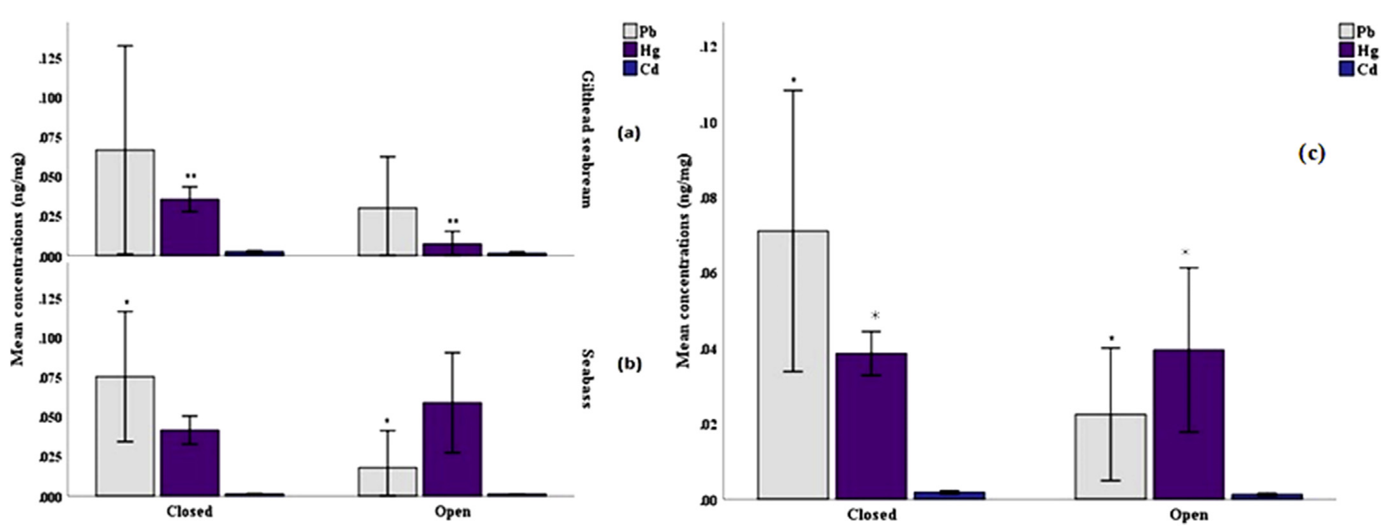

Fig. 2. (a),(b), (c). Comparison of mean levels (ng/mg w. w.) of heavy metals between open and closed seas in gilthead seabream (a), seabass (b) and in total (c). *$p < 0.05$, **$p < 0.001$.

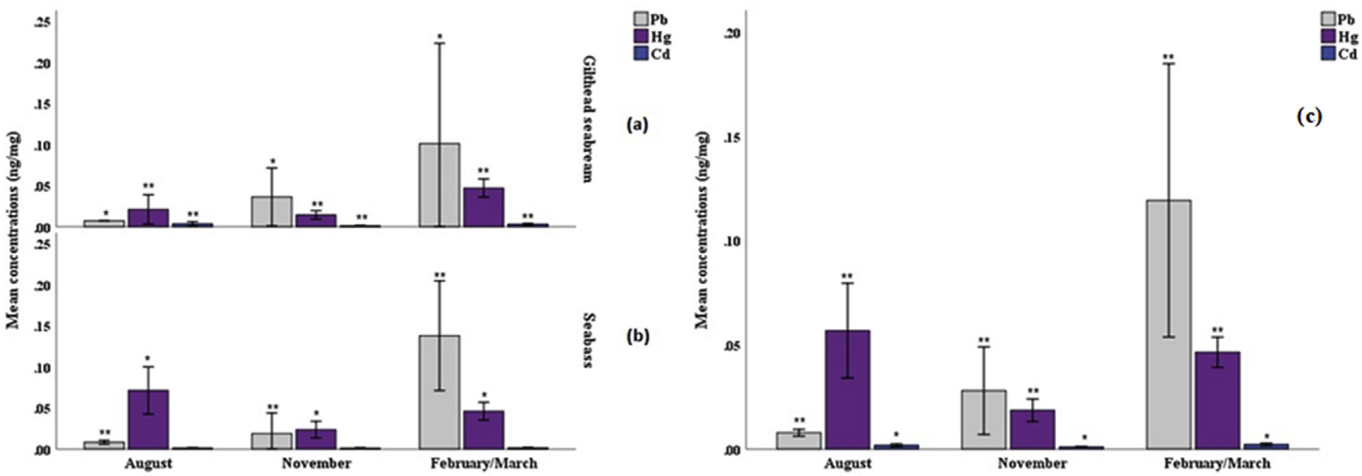

Fig. 3. (a), (b), (c). Comparison of mean levels (ng/mg w. w.) of heavy metals among different months of collection in gilthead seabream (a) and seabass (b) and in total (c). *p < 0.05, **p < 0.001.

Table 2
Adjusted Beta coefficient and 95 CIs using Cd, Pb, and Hg as dependant variables.

Log (Cd)				
From	To	B	95%LL to 95%UL	p
Seabream	Seabass	−0.28	−0.48 to −0.09	0.005
Closed	Open	−0.09	−0.22 to 0.03	0.141
Aug	Nov, Feb–Mar	0.00	−0.07 to 0.08	0.929
CF		−0.14	−0.38 to 0.09	0.233
Log (Hg)				
From	To	B	95%LL to 95%UL	P
Seabream	Seabass	0.18	−0.27 to 0.62	0.437
Closed	Open	−0.32	−0.61 to −0.03	0.032
Aug	Nov, Feb–Mar	0.11	−0.06 to 0.27	0.211
CF		−0.16	−0.71 to −0.40	0.575
Log (Pb)				
From	To	B	95%LL to 95%UL	P
Seabream	Seabass	0.01	−0.40 to 0.42	0.956
Closed	Open	−0.07	−0.33 to 0.20	0.609
Aug	Nov, Feb–Mar	0.44	0.29 to 0.59	< 0.001
CF		−0.17	−0.67 to 0.33	0.491

effect presenting lower levels in Seabass as indicated from the estimates slope (beta: −0.28, 95%CI: −048 to −0.09). Within the same regression model open seas showed a decreasing level of Hg (beta: -0.32, 95%CI: −0.61 to −0.03). Finally, when moving from warmer to colder periods an increasing level was observed for Pb (beta: 0.44, 95%CI: 0.29 to 0.59) (Table 2).

3.5. Distribution of metals in farmed fish and wild fish

For the assessment of differences in the metal load between farmed and wild fish, samples of wild seabass were compared to farmed seabass collected from the same area, namely Dodecanese, while wild seabream from Cyclades was compared to farmed seabass from open seas (N.E. Aegean and Dodecanese).

Wild seabream showed significantly greater levels for Hg and Pb than farmed seabream($p < 0.05$). Similarly, wild seabass presented significantly higher mean values of Pb than farmed (Table 3).

3.6. Dietary intake and risk assessment for humans through fish consumption

For both approaches used for the exposure assessment of the Greek population to heavy metals via fish consumption, HI and THQ, all risk indexes are far below one, which indicates that gilthead seabream and seabass from the Greek seas are safe for consumption (Table 4).

Farmed fish consumption for each species separately as well as in total, poses no risk to humans and more specifically, HI for seabream is slightly lower that for seabass (0.33 vs. 0.46), whereas for both species in total HI is 0.38. The metal that seems to be contributing the most is mercury. Risk of consuming farmed fish (HI = 0.38) is of the same magnitude as in consuming wild fish (HI = 0.32). Matching results are obtained when using the second approach (TTHQ), although the risk is even lower, probably due to the fact that this method estimates exposure for a fragment of time and (ED/LT) and that there are differences between TWI values set by EFSA and RfDs values set by EPA. Again, farmed fish seem to pose an analogous risk to wild ones (TTHQ farmed = 0.22 and TTHQ wild = 0.19).

Table 3
Mean and median values (ng/mg ww.) of Cd, Pb and Hg in the tissues of farmed and wild Gilthead seabream and seabass.

		Farmed			Wild			p
		Mean	SD	Median	Mean	SD	Median	
Gilthead seabream	Cd	0.001	0.001	0.001	0.007	0.008	0.001	0.114
	Hg	0.007	0.010	0.003	0.037	0.022	0.044	**0.008**
	Pb	0.030	0.042	0.007	0.219	0.189	0.185	**0.009**
Seabass	Cd	0.001	0.000	0.001	0.001	0.000	0.001	0.361
	Hg	0.047	0.058	0.022	0.034	0.016	0.035	0.235
	Pb	0.021	0.047	0.007	0.039	0.049	0.021	**0.026**

4. Discussion

4.1. Metal levels in fish muscle tissue

Heavy metal concentrations determined in fish muscle of both species, in all cases, are below the established safe limits for food consumption (EFSA, 2012; FAO, 2011). Existing regulatory limit for Cd in muscle meat of fish is 0.050 mg/kg w. w., while for Pb is 0.3 mg/kg w. w. and 0.5 for Hg mg/kg w. w. Our results are in agreement or comparable with the recent literature on Cd, Hg and Pb concentrations in the muscle tissues of gilthead seabream and seabass, collected from various regions in the Mediterranean Sea (Table 5). To be more specific, Cd levels in fish collected for this study, from the Aegean Sea and the Sea of Crete, were similar or lower than fish collected from adjacent seas (Creti et al., 2010) and somewhat higher than fish collected from the same FAO division (FAO 37.3.1) (Kalantzi et al., 2016). This is the case for Cd in farmed seabass as well; our findings are similar or lower

Table 4
Estimated Hazard Indexes (HIs) and Target Hazard Quotients THQs for each metal in farmed and wild fish under 2 scenarios ([a] (Scenario 1): Imputed values were used,[b] (Scenario 2): Only positive values were used for metal levels).

		HI[e]				THQ[f]			
		HQ50	HQ75	HQ90	HI	THQ50	THQ75	THQ90	TTHQ
Gilthead seabream[a,d]	Cd	0.002	0.009	0.016	0.270	0.000	0.001	0.002	0.160
	Hg	0.106	0.172	0.201		0.073	0.118	0.139	
	Pb	0.001	0.020	0.053		0.000	0.007	0.020	
Gilthead seabream[b,d]	Cd	0.009	0.017	0.033	0.330	0.001	0.002	0.004	0.179
	Hg	0.113	0.175	0.201		0.078	0.121	0.139	
	Pb	0.020	0.047	0.096		0.007	0.018	0.036	
Seabass[a,d]	Cd	0.002	0.002	0.002	0.392	0.000	0.000	0.000	0.260
	Hg	0.131	0.228	0.360		0.090	0.157	0.248	
	Pb	0.001	0.010	0.030		0.000	0.004	0.011	
Seabass[b,d]	Cd	0.008	0.000	0.000	0.460	0.001	0.000	0.000	0.299
	Hg	0.136	0.237	0.402		0.094	0.163	0.277	
	Pb	0.018	0.029	0.058		0.007	0.011	0.022	
Total[a,c,d]	Cd	0.002	0.002	0.009	0.322	0.000	0.000	0.001	0.204
	Hg	0.117	0.185	0.272		0.081	0.127	0.188	
	Pb	0.001	0.013	0.041		0.000	0.005	0.015	
Total[b,c,d]	Cd	0.009	0.013	0.032	0.385	0.001	0.002	0.004	0.224
	Hg	0.131	0.189	0.278		0.090	0.130	0.192	
	Pb	0.020	0.036	0.075		0.007	0.013	0.028	
Wild[a,c]	Cd	0.002	0.002	0.032	0.341	0.000	0.000	0.004	0.189
	Hg	0.139	0.175	0.220		0.096	0.121	0.152	
	Pb	0.006	0.038	0.089		0.002	0.014	0.033	
Wild[b,c]	Cd	0.030	0.035	NE	0.321	0.004	0.005	NE	0.191
	Hg	0.146	0.177	0.226		0.101	0.122	0.156	
	Pb	0.028	0.054	0.095		0.011	0.020	0.035	

[a] Imputed values were used.
[b] Only positive values were used.
[c] Both species.
[d] Farmed, NE: not estimated.
[e] HQ50, HQ75 and HQ90, indices based on median, 3rd quartile and 90[th] percentile of Cd, Hg, Pb concentrations.
[f] THQ50, THQ75 and THQ90, indices based on median, 3rd quartile and 90[th] percentile of Cd, Hg, Pb concentrations.

compared to other studies within the same region (Squadrone et al., 2016; A. Iamiceli et al., 2015; Dalman et al., 2006; G. Dugo et al., 2006; Kalantzi et al., 2016). With regard to Pb in farmed seabream our results are quite similar to the ones obtained by Minganti et al. (2010), yet higher that others (Kalantzi et al., 2016; A. Iamiceli et al., 2015), while for seabass our results are lower than levels reported in other studies (Iamiceli et al., 2015; Dugo et al., 2006; Dalman et al., 2006). Hg levels estimated in our study for both species are within the range reported in the relevant literature (Table 5).

Cd in wild seabream in our study, is lower than values reported by Ersoy and Çelik, 2010 for the same species and the same season of collection. It is worth mentioning that Pb was lower yet close to the maximum level safe for consumption (0.289 ng/mg) and higher than values published by other authors (Olmedo et al., 2013; Minganti et al., 2010; Naccari et al., 2015). Hg levels estimated for wild seabream are within the range reported in the relevant literature (Table 5).

It has been reported by many authors, that metal accumulation in fish tissue is affected by a number of factors such as species, physiologic condition, diet, season, habitat, metal concentration etc. (Renieri et al., 2014; Ferreira et al., 2010; Rodríguez - Hernández et al., 2017; Martignago, R.et al., 2009; Dural et al., 2006; Copat et al., 2018; Storelli et al., 2008; Renieri et al., 2017). We investigated such relationships between the aforementioned factors and metal levels detected in our study and focused our study on muscle tissue, since it is typically the edible part and the primary concern of fish consumption risk assessment. It should also be taken into consideration, that muscle tissue, being the less active metabolically, accumulates metals in lower levels than other tissues (Renieri et al., 2014; Nasyitah Sobihah et al., 2018; Squadrone et al., 2016) however, metals are transported to muscles through other tissues and muscles can serve as indicators of an implemented chronic exposure (Kalantzi et al., 2016).

4.2. *Species specific metal accumulation in fish muscle tissue*

Although both fish species inhabit similar depths (demersal species) and samples of each species were collected from the same farming sites, differences in Hg and Cd levels were revealed. Our results suggest that heavy metal accumulation is species dependant. Mercury levels in seabass muscle tissue exceeded significantly Hg levels in seabream tissue, which is in consistency to results reported by Kalantzi et al. (2016). On the other hand, cadmium mean concentrations in seabass were found to be significantly lower than in seabream muscle tissue. Similar differences have been depicted by numerous authors, attributing species specific metal accumulation to various factors such as natural habitat, diet and feeding behavior, lipid content and metabolic activity among others (Renieri et al., 2014; Ferreira et al., 2010; Storelli et al., 2008; Á. Rodríguez-Hernández et al., 2017; Copat et al., 2018). In the present study, samples of both species were collected from each aquaculture site, exposed to the same environmental metal background, implying an alternate factor liable for different metal accumulation.

A possible interpretation could be the differences in size, condition factor and lipid composition between species. We have determined that seabass fish samples showed a significantly lower mean CF than seabream ones. According to the literature, CF is positively correlated to the total lipid content of fish (Mozsár et al., 2015) and seabass muscle tissue has a lower lipid and higher protein content than seabream (Nasopoulou C et al., 2011; Erkan and Ozden, 2007). Moreover, metal distribution in tissues has been reported to be dependent on the tissue lipid and protein content; some metals are accumulated more in tissues with low fat and high protein content (Kalantzi et al., 2016). It has also been suggested that high lipid content might lead to less accumulated Hg (Bosch et al., 2016). While the concentrations of Cd and Pb were found to positively relate to lipid contents in farmed fish in the work of Y.−W. Qiu et al. (2011) concentrations of Hg did not. Additionally,

Table 5
Heavy metal concentrations in the muscle tissues (expressed as means and range ng/mg ww) of gilthead seabream and seabass, in the Mediterranean sea, reported in the recent literature and this study.

Species		Cd		Pb		Hg			
	season	mean	range	mean	range	mean	range	REGION	REFERENCE
gilthead seabream[a]		bdl		bdl			0.02–0.1	Greece	Kalantzi et al. (2016)
gilthead seabream[a]		< 0.01		< 0.1				Italy	Cretì et al. (2010)
gilthead seabream[a]			< 0.003–0.022		< 0.013–0.139	0.12	0.07–0.16	Italy	Minganti et al., 2010
gilthead seabream[a]	all year	< 0.01		< 0.02				S Adriatic, Tyrrhenian, Ionian Sea	Iamiceli et al., (2015)
gilthead seabream[a]	August	0.003		0.007		0.021		FAO zone 37.3.1	This study
gilthead seabream[a]	November	0.001		0.036		0.014		FAO zone 37.3.1	This study
gilthead seabream[a]	Feb–March	0.003		0.101		0.047		FAO zone 37.3.1	This study
seabass[a]		bdl		bdl			0.06–0.07	Greece	Kalantzi et al. (2016)
seabass[a]	February	< 0.010		< 0.010		0.036		Ligurian Sea	Squadrone et al., (2016)
seabass[a]	all year	< 0.01		< 0.02				S Adriatic, Tyrrhenian, Ionian Sea	Iamiceli et al., (2015)
seabass[a]	all year	< 0.01		0.274				S Adriatic, Tyrrhenian, Ionian Sea	Iamiceli et al., (2015)
seabass[a]	July		0.08–0.13		0.18–0.32			Tyrrhenian Sea	Dugo et al., (2006)
seabass[a]	July		< 0.9–0.11		0.12–0.35			Sea of Sicily	Dugo et al., (2006)
seabass[a]			< 0.01–0.04		< 0.02–0.4			SE Aegean Sea (Turkey)	Dalman et al., (2006)
seabass[a]	August	0.001		0.008		0.071		FAO zone 37.3.1	This study
seabass[a]	November	0.001		0.024		0.024		FAO zone 37.3.1	This study
seabass[a]	Feb–March	0.002		0.019		0.046		FAO zone 37.3.1	This study
seabass[b]	all year						0.017–0.108	Serbia	Djinovic-Stojanovic et al. 2015
seabass[b]		0.002		0.004		0.079		Murcia, Spain	Olmedo et al., (2013)
gilthead seabream[b]	all year						0.017–0.108	Serbia	Djinovic-Stojanovic et al. 2015
gilthead seabream[b]		0.001		0.004		0.037		Murcia, Spain	Olmedo et al., (2013)
gilthead seabream[c]			< 0.004–0.007		< 0.013–0.027	0.54	0.29–0.72	Italy	Minganti et al., 2010
gilthead seabream[c]	summer	0.11		0.16				E. Mediterranean Sea.	Ersoy and Çelik, 2010
gilthead seabream[c]	automn	0.05		0.58				E. Mediterranean Sea.	Ersoy and Çelik, 2010
gilthead seabream[c]	winter	0.2		0.15				E. Mediterranean Sea.	Ersoy and Çelik, 2010
gilthead seabream[c]	spring	0.19		0.19				E. Mediterranean Sea.	Ersoy and Çelik, 2010
gilthead seabream[c]		< 0.09		< 0.02		< 0.06		SE Sicilian coast(FAO zone 37 1.3)	Nacarri et al. (2015)
gilthead seabream[c]		0.073		< 0.02		0.455		SE Sicilian coast(FAO zone 37 1.4)	Nacarri et al. (2015)
gilthead seabream[c]	Feb–March	0.007		0.289		0.037		FAO zone 37.3.1	This study
seabass[c]	Feb–March	0.001		0.039		0.034		FAO zone 37.3.1	This study

[a]farmed.
[b]fish market.
[c]wild, bld: below detection limit.

mercury has been found to be accumulated more in seabream muscle rather than other tissues (Ferreira et al., 2008; Kalantzi et al., 2016) whereas Cd is more accumulated in the gills and kidney (Creti et al., 2010). Therefore we could argue that higher Hg and lower Cd concentrations in seabass found in our study is linked to differences in size and body composition between species and different metal behavior.

Another explanation could be lying in the fact that each species was administered different aquafeed. Fish feeds have been implicated in contributing to the metal load in farmed fish muscle and differences have been attributed to variations in the metal concentrations among feeds (Rodríguez-Hernández et al., 2017; Yildiz, 2008; Creti et al., 2010; N. Nasyitah Sobihah et al., 2018). It has also been reported that cadmium accumulates mainly through the diet (R. Martignago et al., 2009) and this is supported by L.D. Rozon-Ramilo et al., (2011), who suggested that Cd behavior in the fish body is dependent on route of exposure, with diet exposure leading to greater levels that waterborne. Moreover, metals are distributed to different tissues depending on the route of exposure (Ferreira et al., 2008). Accordingly, these factors may contribute to the fact that Cd and Hg levels are diversified between seabream and seabass. It is also worth mentioning, that a recent study has revealed potentially different metal detoxification mechanisms between the two species *in vitro*, suggesting that seabream cells were more sensitive to metals than sea bass cells (P. Morcillo et al., 2018).

4.3. Metal distribution in fish muscle tissue depending on farming site

Distribution of metals in fish is a reflection of metal occurrence in their habitat; micro-element composition and pollution of sea water is of high importance in fish metal bioaccumulation (G. Dugo et al., 2006). Cadmium, lead and mercury end up in marine ecosystems through both natural and anthropogenic origins as well. Aquaculture sites located in the Aegean and the Cretan Sea receive metal loads through atmospheric and onshore waste origins, although metals in aquacultures may also occur from agricultural runoff, antifouling paints on the nets of fish cages, sediment diffusion as well as waste feed and fecal production (Islam and Tanaka, 2004; Alhashemi et al., 2012; J. Castritsi-Catharios et al., 2015; Kalantzi et al., 2016; Weng and Wang, 2014; Ranjbar Jafarabadi et al., 2017). However, metal bioavailability and potential toxicity to organisms is determined by their chemical form, salinity, pH value, hardness (Alonso Castillo et al., 2013) and dissolved metals are more bioavailable (Ranjbar Jafarabadi et al., 2017). The dissolved form is favored in low pH values of seawater and low dissolved oxygen levels.

Our results showed that metal levels vary amongst fish collected from different fish farms. Distribution appears to be site dependent for all metals analyzed and in particular, Pb demonstrates the greatest variance of mean concentrations, with fish from Crete showing the higher values. Pb levels in Crete could be attributed to the existing harbor in the bay where the farming site is located (Pb is often used in coating in shipping activities), aside from the possible Pb release of antifouling in fish cages (Dean et al., 2007). Fish samples collected from the NE Aegean, exhibited the highest mercury levels. The aquaculture site located in the NE Aegean, being close to Dardanelles is affected by the continuous transport of contaminants originated from the Black Sea and in addition it is located near the industrialized Aliaga and Izmir Bays. The mean Hg levels, in fish samples collected from the adjacent Aliga Bay, in 2009 were above the acceptable values in fresh fish according to the FAO limits due to industrial inputs into the Aliaga Bay (I. Pazi et al., 2017). Additionally, a biomonitoring study in microalgae from eastern Aegean coastal areas revealed high Hg levels in algae from the Izmir Bay (Akcali & Kucuksezgin, 2011), while a simulation of the fate of the pollutants, transported from the Dardanelles into the North Aegean Sea by Kopasakis et al. (2012) predicts that the ecosystem may be seriously threatened in the future decades. In addition, the entire Mediterranean Sea is characterized by variations in Hg distribution, creating zones with high mercury concentrations (Damiano et al., 2011). Taken together, higher mean Hg values in the NE Aegean found in our study could be justified. With regard to Cd distribution, Cd was found to be higher in fish collected from Crete and the Saronic Gulf, both fish farms located in closed seas. Higher levels in the Cretan aquaculture site could be explained by agricultural washouts from adjacent farms through the river which flows nearby, taking into account that a major source of Cd release into the environment is the production and use of phosphate fertilizers (McGeer et al., 2011). The Saronic Gulf on the other hand, is burdened by industries and shipyards in addition to urban waste effluents. However the existing biological wastewater treatment plant counterbalances the pollution rates.

Attempting to further elucidate our findings, we also studied differences in metal distribution between samples from open seas (NE Aeagean and Dodecanese) and closed seas (Crete, Mainland and the Saronic Gulf). Lead and mercury are significantly higher in closed seas for farmed fish in total. Closed seas retain a low capacity of water interchange which may affect metals' biochemical cycle and environmental fate which is evident also in a greater scale, for instance, fish from the Atlantic have lower metal levels than their Mediterranean counterparts (Ferreira et al., 2008).

In addition, our results regarding site specific variations, disclose a species effect as well. With respect to Cd levels in gilthead seabream alone, fish from Crete and Saronic Gulf displayed the highest values. Correspondingly, mean mercury values were significantly higher in Crete. For seabass samples, Pb followed a similar pattern with previous metals, presenting significantly higher values in Crete than in the other farming sites.

4.4. Metal distribution in fish muscle tissue depending on seasonality

Seasonality as a modulator of metal accumulation in fish muscle tissue has been illustrated by several authors (Renieri et al., 2014; Giannakopoulou, L., & Neofitou, C., 2014; B. Ersoy, M. Çelik, 2010; Aksu et al., 2011; Cardinal et al., 2011). Our results revealed differences in metal levels among the different farming seasons, suggesting a season effect of metal accumulation.

More specifically, Cd displayed the lowest levels for all fish samples in autumn and higher levels at the end of summer and winter. The above difference in sampling season was also revealed in Seabream but not in Seabass tissues. This is in agreement to the results published by Ersoy, M. Çelik (2010), who reported that the maximum Cd value was detected in Gilthead seabream in winter seasons. This could be explained by differences in fat content among seasons, since it has been shown that sea bream present a substantial fatty acid variation according to the season with the highest fat level observed in October and that the richer in fat of fish muscle, the lower the affinity for certain metals (Cardinal et al., 2011; Grigorakis, 2007). Contrastingly, although (Giannakopoulou & Neofitou, 2014) determined that the seasonality factor is significant for Cd concentrations in muscle tissue of *Pagellus erythrinus*, reported higher Cd values in autumn and the lowest in summer, associating the variations with differences in polluting sources, apart from each fish species characteristics.

Moreover we determined that estimated Pb levels for all fish samples showed a statistically significant increase in winter. That same seasonal pattern was presented for each species alone. Pb values displayed a linear increasing trend when assessing metal levels from warmer to colder periods whereas investigation of differences in fish CF showed a statistically significant decrease in seabass from summer to winter. This is in contrast to the results of Giannakopoulou & Neofitou (2014) who stated that Pb was more concentrated in June for *Pagellus erythrinus*. This could reflect a species effect since we analyzed different fish species.

Furthermore, we observed that for all fish both summer and winter farming periods have increased Hg levels in fish tissues and lower levels are presented in autumn. Hg levels in Gilthead seabream were significantly higher in winter, while during the summer farming period Hg was higher in seabass samples. In the work of Aksu et al. (2011), Hg levels in *Merluccius merluccius* displayed lower concentrations in August and slightly higher in winter that was linked to the fact that the total lipid content increases in fish during colder periods. Possibly, this is another case of species specific variations and differentiated metal accumulation due to body composition.

It must also be taken into consideration that seabream reproduction occurs during autumn, while seabass during the end of the winter and early spring. This suggest variations in metabolic condition, body composition and feeding behavior, all factors affecting metal accumulation (Renieri et al., 2014).

4.5. Analysis of main effects on heavy metal levels in fish tissues

We attempted to clarify the variability of our results by applying linear regression analysis models. Log transform of metals' concentrations was made for normalizing the distribution, while linear regression was applied to avoid biases due to not systematic sampling. The results of the analysis highlighted the main contributing factor to metal accumulation for each metal. Cadmium levels in particular, seem to be subjected only to species effect presenting lower levels in Seabass. This is consistent to what has been in discussed in the respective individual study assessing species specific variations and was related to the fact that the two species differ in lipid composition which is additionally affected by different reproductive periods. Mercury levels on the other hand were mostly affected by location and as depicted by the analysis, open seas present lower levels as closed ones. This could be linked to the fact that closed seas have a slower interchange rate of waters, leading to higher levels of metals in the water, sediment and biota. As revealed by the same model, lead is predominately affected by seasonality and presents higher levels in muscle tissue levels when moving from warmer to colder periods. Again, this could be linked to the seasonal changes in lipid composition of fish and the way it affects this metal's accumulation.

4.6. Comparison of metal distribution between farmed fish and wild fish

Potential variations in the metal load of fish muscle, between farmed and wild fish in the Mediterranean Sea, have been studied by a number of authors, in the light of assessing the hazard of fish consumption (Creti et al., 2010; Ferreiera et al., 2008; Rodríguez-Hernández et al., 2017; Minganti et al., 2010; S. Vizzini et al., 2010; Grigorakis, 2007). Our findings show that wild seabream tissue accumulates greater levels for Hg and Pb than farmed seabream. Our results are in agreement with Ferreira et al. (2008) who reported that wild white seabream showed higher accumulation than cultured ones. It is worth mentioning that farmed fish have higher lipid levels than their wild counterparts (Grigorakis, 2007; Rodríguez-Hernández et al., 2017;

Cirillo et al., 2009; Ferreira et al., 2008a). Moreover out study revealed that, wild seabass tissue presented higher mean values of Pb than farmed ones. Ferreira et al. (2010) established that wild seabass has shown higher metal accumulation than cultivated species and argued that it was a result of feeding behavior, since adult seabass are top predators and their diet may contain higher metal loads than fish feeds, highlighting the importance of diet exposure as the main source of metal accumulation in European seabass. It could also be argued that, higher Pb levels in wild fish are a reflection of breeding patterns. Seabass early life stages are pelagic, occupying more shallow waters that adults (demersal), where temperature could be higher during summer. Higher temperature values usually render fish more susceptible to intoxication (Sfakianakis et al., 2015). Pb detected in wild fish could be a result of early life accumulation. On the other hand, Minganti et al., (2010) found that only Hg showed significant differences between farmed and wild seabream, linking these results to the fact that mollusks the main diet of wild *S. aurata,* and have been found to contain high Hg levels. Diet composition could also affect antioxidant responses of gilthead sea bream as has been reported (Kokou et al., 2017) which could ultimately lead to variations in metal accumulation. Rodríguez-Hernández et al., 2017 reported higher Cd and Hg levels in wild whitefish, yet they could not define a pattern, acknowledging the fact that elements in marine environment have a more complex distribution than organic pollutants, as a result of local anthropogenic inputs, natural sources and hydrological conditions. It has also been suggested that lower metal levels in farmed fish maybe due to the effect of bio dilution because of faster growth rates of farmed fish (Kelly et al., 2008). On the whole, we could suggest that differences in metal levels found in our study between the muscle of farmed and wild fish, are the combined result of different feeding behavior, growth rate and therefore metabolic rate, aside from the effects of waterborne exposure.

4.7. Risk assessment for humans through fish consumption

Our study of potential health risks for Greek consumers revealed that the risk involved in consumption of the studied species is minimal. TTHQ values in all cases were less than 0.3 indicating safe levels of heavy metal dietary intake through fish consumption, since for HQ values lower than 0.1 no hazard exists, while for values between 0.1 and 1.0 the hazard is considered low (Kalogeropoulos et al., 2012). Moreover, taking into account the alternate approach of the HI, deriving as the ratio of the estimated to the tolerable intake, heavy metals measured in the fish consist no threat to humans as well, since the ratios are below 0.5. The highest HI values were estimated for Hg in seabass under the worst case scenario (0.4), yet still remaining on the safe side. Using a conservative approach, we intentionally used the TWI value for methyl-mercury (MeHg instead) of total mercury (THg) (assuming that 100% is MeHg), the most toxic form of THg, as it is estimated that approximately 90% of the total mercury (THg) in fish and shellfish is present in the form of MeHg and that they are the main diet contributor for this metal (EFSA CONTAM Panel, 2012).

Similar results to ours for cadmium, lead and mercury, were reported for fish from the Greek seas, describing low to non-existing risks for Greek consumers for the same (Kalantzi et al., 2016) as well as various other fish species (Kalogeropoulos et al., 2012). In the broader area of the Mediterranean Sea, numerous authors have evaluated the risk of dietary heavy metal intakes, using different hazard estimation approaches (Marti-Cid et al., 2008; Vieira et al., 2011; Conti et al., 2012; Pastorelli et al., 2012; Copat et al., 2013; Ersoy and Celik, 2010; I. Pazi et al., 2017). In a recent study from the western Mediterranean, consumption of lean fish by the Spanish population, although considered safe, contained a higher risk with regard to THg (EWI = 50% of PTWI), than that estimated in our study, while risk using values for MeHg was alarming (EWI = 150% of PTWI) (Llull et al., 2017). Nonetheless, we must take into consideration that the Spanish population consumes fish from other areas f.i. Canary Islands and the Atlantic Ocean in general (Rodríguez-Hernández et al., 2016). On the other hand, the maximum intake values set by European regulations for Hg, Cd and Pb were never exceeded for farmed seabass from Italy (Squadrone et al., 2016). In the Eastern Mediterranean, EWI for Pb and Cd through the consumption of seabream among other species was far below the PTWI (Ersoy and Celik, 2010).

With respect to data obtained with the use of THQ method, some results that raise concern were reported from the Eastern Mediterranean recently. Although the THQ values for Cd, Pb in fish samples collected from the coasts of Turkey were lower than 1.0, the THQ for Hg was higher than 1.0 for most of the samples and the consumption of certain species from Aliaga Bay was considered potentially hazardous to human health due to the Hg concentrations (Pazi et al., 2017). In a comparative study aiming to evaluate changes in the health risk of Italian consumers due to fish and shellfish consumption between 2012 and 2017, the risk for Cd was slightly higher because of higher Cd levels in mollusks but still very low (THQ = 0.007) whereas Pb-THQ obtained in 2017 (0.003) was equal to Pb-THQ in 2012 and Hg-THQ (0.2) in 2017 lower than in 2012 (Copat et al., 2018). Bonsignore et al. (2018) reported high Hg-THQ (≥ 1) for fish, crustaceans, mollusks and echinoderms from the Tuscany coast and warned that TTHQ values suggested that the local population could experience adverse health effects due to local seafood consumption, mainly of demersal and benthic species.

On the whole, studies from the Mediterranean focusing on the health risk resulting from fish consumption report quite diverse data, since it is affected by a number of miscellaneous parameters, such as consumption habits of each population, metal levels in fish, fish species selected and relationships between metals among others. It is noteworthy that several studies have showed that selenium (Se) may hold a protective role against MeHg toxicity whereas others argue that it may even exacerbate MeHg toxicity; in any case it has been signified that the Hg:Se ratio could be a useful tool to better assess the risk linked with fish intake (P. Olmedo et al., 2013; Kalantzi et al., 2016; Renieri et al., 2014). With regard to consumption, an EU report on Consumer Habits Regarding Fishery and Aquaculture Products (Eumofa, 2017) demonstrated the preference fluctuations between wild and farmed fish as well as fresh or processed seafood based on criteria such as country of origin, socioeconomic status and age as well as supply and demand dynamics.

Although we assessed the risk arising from the metals considered to be more toxic in two of the most consumed species, for an under - represented country in the literature such as Greece, we have to acknowledge certain limitations to our study. The dietary exposure to Cd, Pb and Hg is a result not only of fish consumption but other foodstuff as well (various seafood, cereals, milk etc.) which must be factored in risk assessment analysis. It must be underlined however, that seafood is the main contributor to heavy metal dietary intake. We did not include the Hg:Se ratio in our analysis, but we do not consider it likely that it would have affected our results. In addition, although heavy metal adverse effects in humans have been described widely in the literature, long term low dose effects such as those that could be induced by fish consumption, could be the object of further investigation and risk analysis. Furthermore, current established limits on metal exposure have been set talking into consideration the single-stimulus exposure f. i. cadmium, lead or mercury alone, whereas this is far from the truth, since exposure to a combination of stressors usually occurs in real life scenarios. (Kostoff, R. N., Goumenou, M., & Tsatsakis, A.,2018). Acknowledging the fact that it would be nearly impossible to obtain a comprehensive optimization over all potential combinations of toxic stimuli in order to mitigate combination enhancement effects, biomonitoring and epidemiological studies on cumulative exposure remain essential in order to set more realistic exposure limits (Kostoff, R. N., Goumenou, M., & Tsatsakis, A., 2018; Tsatsakis A.M. et al., 2016; Tsatsakis A.M. et a., 2017; Hernandez A.F. & Tsatsakis A.M., 2017; Hernandez A.F. et a., 2013).

5. Conclusions

Cadmium, lead and mercury concentrations determined in the muscle tissue of gilthead seabream and seabass collected from two modes of production, aquaculture as well as fisheries showed levels far below the safe limits for consumption set by authorities. Differences in Hg and Cd levels between species were depicted and more specifically seabass demonstrated higher Hg and lower Cd concentrations, which can be attributed to differences in fish size and body composition as well as different metal behavior, besides potential variations in the metal concentrations among feeds. In addition site specific variations in metal levels were revealed, which can be difficult to read as each metal displayed diverse distribution, however Hg and Pb seem to be more accumulated in closed seas. Seasonality is also a factor that weighs in metal accumulation in fish tissue as indicated by results obtained from this study as Pb values displayed a linear increasing trend from warmer to colder periods. Increased Hg levels in fish tissues for all fish were observed both in summer and winter farming periods while lower levels are presented in autumn. A species effect is evident while assessing seasonality as well, since Hg levels in Gilthead seabream were significantly higher in winter, while during the summer farming period higher Hg levels were detected in seabass. Regression analysis highlighted that the main contributing factor to Cd accumulation is the species; lead is predominately affected by seasonality while Hg accumulation is mainly affected by location. Cd and Pb accumulation patterns could be linked to the seasonal and species-specific changes in lipid composition of fish. Moreover, this study provides evidence of differences in metal levels between fish from the two modes of production which can be linked to different feeding behavior, metabolic rate in combination to effects of waterborne exposure. Finally, health risks for Greek consumers revealed that the risk involved in consumption of the studied species, farmed and wild, is minimal for all metals. Further investigation is essential in order elucidate metal accumulation issues in frequently consumed fish species as well as the development of more comprehensive models for risk assessment of the dietary intake of heavy metals.

Acknowledgments

This study was supported by the Special Research Account of University of Crete (ELKE No 4920).

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.020.

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